



Ecological Connectivity of Kimberley Marine Communities

Oliver Berry^{1,8}, Jim Underwood^{2,8}, Kathryn McMahon^{3,8}, Mike Travers^{4,8}, Zoe Richards^{5,6,8}, Glenn Moore^{5,8}, Udhi Hernawan^{3,8}, Joseph DiBattista^{6,8}, Richard Evans^{7,8}, James Gilmour^{2,8}

¹CSIRO, Crawley, Western Australia

²Australian Institute of Marine Science, Townsville, Queensland

³Edith Cowan University, Joondalup, Western Australia

⁴Fisheries Division, Department for Primary Industries and Regional Development, Hillarys, Western Australia

⁵Western Australian Museum, Perth, Western Australia

⁶Curtin University, Bentley, Western Australia

⁷Department of Biodiversity, Conservation and Attractions, Kensington, Western Australia

⁸Western Australian Marine Science Institution, Perth, Western Australia

WAMSI Kimberley Marine Research Program

Final Report

Project 1.1.3

August 2017



western australian
marine science institution



Department of Biodiversity,
Conservation and Attractions



Department of
Primary Industries and
Regional Development



Curtin University

Mayala
Community



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.

Ownership of Intellectual property rights

Unless otherwise noted, copyright (and any other intellectual property rights, if any) in this publication is owned by the Western Australian Marine Science Institution, CSIRO, Australian Institute of Marine Science, Edith Cowan University, The University of Western Australia, Western Australia Department of Primary Industries and Regional Development, Western Australian Museum, Curtin University and the Western Australian Department of Biodiversity, Conservation and Attractions.

Copyright

© Western Australian Marine Science Institution

All rights reserved.

Unless otherwise noted, all material in this publication is provided under a Creative Commons Attribution 3.0 Australia License. (<http://creativecommons.org/licenses/by/3.0/au/deed.en>)



Legal Notice

The Western Australian Marine Science Institution advises that the information contained in this publication comprises general statements based on scientific research. The reader is advised and needs to be aware that such information may be incomplete or unable to be used in any specific situation. This information should therefore not solely be relied on when making commercial or other decision. WAMSI and its partner organisations take no responsibility for the outcome of decisions based on information contained in this, or related, publications.

Front cover images (L-R)

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

Image 2: Powerful currents are a feature of the Kimberley marine environment. Whirlpool in Sunday Strait (Image: Kathryn McMahon)

Image 3: Humpback whale breaching (Image: Pam Osborn)

Image 4: Coral platform exposed at low tide. Bathurst Island Buccaneer Archipelago (Image: Kathryn McMahon)

Year of publication: August 2017

Metadata: <http://catalogue.aodn.org.au/geonetwork/srv/eng/metadata.show?uuid=fb1d80bf-6ef2-4150-9479-22b4240435a7>

Citation: Berry O, Underwood J, McMahon K, Travers M, Richards Z, Moore G, Hernawan U, DiBattista J, Evans R, Gilmour J (2017) Ecological Connectivity of Kimberley Marine Communities. Executive Summary Report of Project 1.1.3 prepared for the Kimberley Marine Research Program, Western Australian Marine Science Institution, Perth, Western Australia, 16pp.

Author Contributions: All authors contributed to the drafting of this text.

Corresponding author and Institution: Oliver Berry, CSIRO Oceans and Atmosphere. oliver.berry@csiro.au

Funding Sources: This project was funded (commissioned) by the Western Australian Marine Science Institution as part of the WAMSI Kimberley Marine Research Program, a \$30M program with seed funding of \$12M provided by State government as part of the Kimberley Science and Conservation Strategy. The Program has been made possible through co-investment from the WAMSI Joint Venture partners and further enabled by data and information provided by Woodside Energy Ltd.

Competing Interests: The commercial investors and data providers had no role in the data analysis, data interpretation, the decision to publish or in the preparation of the manuscript. The authors have declared that no competing interests exists.

Kimberley Traditional Owner agreement: This research was enabled by the Traditional Owners through their advice, participation and consent to access their traditional lands.

Acknowledgements: We are grateful to all WAMSI staff for their assistance, and in particular Kelly Waples, Stuart Field and Kim Friedman for providing advice and constructive criticism throughout the project. Many other people and organisations contributed to the success of this project, and we thank them sincerely. We especially thank the following Indigenous communities. The Bardi and Jawi Niimidiman Aboriginal Corporation, the Bardi Jawi Rangers and the Bardi Jawi Traditional Owners and specifically Daniel Oades, Damon Pyke, Azton Howard, Chris Sampi, Kevin George, Kevin Ejai, Kevin Dougal, Tasha Stumpagee, Phillip McCarthy, Peter Hunter, Zac Ejai, Paul Davey and Trevor Sampi. The Mayala people, specifically Sandy, Alec and Janella Isaacs. The Wunambal Gaambera Aboriginal Corporation, Traditional Owners, Unguu Rangers and Tom Vigilante. The Dambimangari Aboriginal Corporation, Dambimangari Traditional Owners and the Dambimangari Rangers. Thanks also to the Bioinformatic pipeline development: Bernd Gruber (University of Canberra); Oceanographic modelling: Ming Feng, Dirk Slawinski (CSIRO); SNP marker development and genotyping: Andrzej Kilian (Diversity Arrays Technology); Field assistance: Sam Moyle and Fiona Webster; Kimberley Marine Research Station for facilities and logistical support: James Brown, Michael Flynn, Scott Whitlam, Duncan Smith and Erin McGinty; and advice on project development: Karen Miller (AIMS).

Collection permits/ethics approval: SF008440, SF009910, SC001362 (Western Australian Department of Parks and Wildlife); 2485 2085, 2344 (Western Australian Department of Fisheries)

Contents

- 1 EXECUTIVE SUMMARY..... I**

 - 1.1 FINE SCALE: THE EXTENT OF CONNECTIVITY DIFFERS AMONG SPECIES..... I**
 - 1.2 FINE SCALE: POPULATION BOUNDARIES ARE SHARED BETWEEN SOME TAXA..... I**
 - 1.3 FINE SCALE: SOME SITES ACT AS LINKS BETWEEN OTHERWISE ISOLATED REGIONS..... II**
 - 1.4 FINE SCALE: KING SOUND, SUNDAY STRAIT ARE BARRIERS TO DISPERSAL IN SOME SPECIES..... II**
 - 1.5 BROAD SCALE: THE INSHORE AND OFFSHORE KIMBERLEY ARE POORLY CONNECTED..... II**
 - 1.6 BROAD SCALE: CONNECTIVITY BETWEEN THE KIMBERLEY AND NEIGHBOURING BIOREGIONS DIFFERS AMONG SPECIES II**
 - 1.7 CRYPTIC GENETIC DIVERSITY EXISTS IN THE BROADCAST SPAWNING CORAL III**

- 2 IMPLICATIONS FOR MANAGEMENT..... III**
- 3 KEY RESIDUAL KNOWLEDGE GAPS IV**

 - 3.1 HABITAT FORMING: TWO SPECIES OF CORALS IV**
 - 3.2 HABITAT FORMING: TWO SPECIES OF SEAGRASSES V**
 - 3.3 HARVESTED: A LARGE GASTROPOD (TROCHUS) V**
 - 3.4 REEF-DWELLING: A CORAL REEF FISH..... V**
 - 3.5 HARVESTED: A DEMERSAL FISH V**

- 4 REPORT STRUCTURE 1**
- 5 COMMUNICATION..... 1**

 - 5.1 STUDENTS SUPPORTED 1**
 - 5.2 JOURNAL PUBLICATIONS 1**
 - 5.3 SUBMITTED MANUSCRIPTS 1**
 - 5.4 PRESENTATIONS..... 2**
 - 5.5 OTHER COMMUNICATIONS ACHIEVEMENTS..... 2**
 - 5.6 KNOCK ON OPPORTUNITIES CREATED AS A RESULT OF THIS PROJECT 2**
 - 5.7 KEY METHODS FOR UPTAKE 2**

- 6 APPENDIX..... 3**

 - APPENDIX 1: QUESTIONS OUTLINED IN THE KIMBERLEY MARINE RESEARCH PROGRAM SCIENCE PLAN 3**



1 Executive Summary

The overarching objective of KMRP Project 1.1.3 (Ecological Connectivity of Kimberley Marine Communities) was to provide the first estimates of ecological connectivity (demographic inter-dependence) across multiple spatial scales for a suite of representative marine organisms from the Kimberley. The full report for this project is structured as five individual sub-reports each focusing on different representative marine organisms. Here, we summarise the key findings of these sub-reports. Further summary and synthesis of the key findings for the entire project provided in sub-report 1.1.3a (Synthesis).

The key findings of this study are summarized in Figure 1:

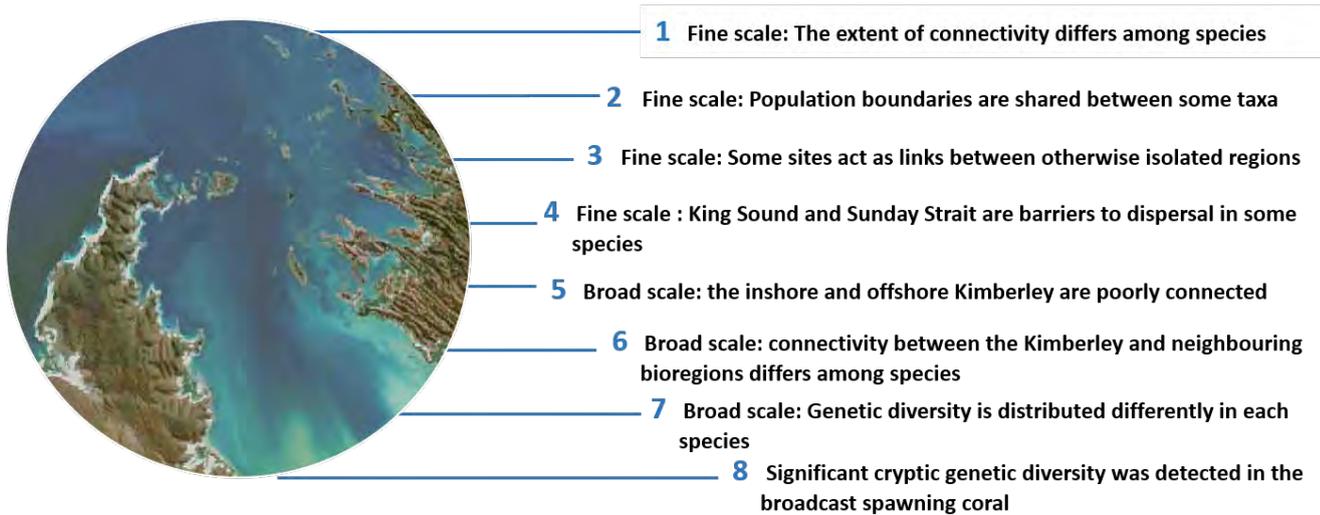


Figure 1. Key findings of KMRP Ecological Connectivity Project 1.1.3.

1.1 Fine scale: The extent of connectivity differs among species

Despite experiencing a common set of environmental conditions, the extent of ecological connectivity differed among the focal organisms, and not always in predictable ways. Habitat forming organisms (coral, Report 1.1.3.1; seagrass, Report 1.1.3.2) typically exhibited the most localised population structure, with evidence for limitations to routine dispersal evident on scales of 10s of kilometres or less. In the remaining organisms (fishes, Report 1.1.3.4a; trochus, Report 1.1.3.3), population structure was weaker or not detectable, and limits to dispersal were evident on scales of 80 to several 100s kilometres. Some of these results were unexpected. For example, the seagrass with floating seeds had finer scale genetic structure compared with the seagrass with sinking seeds, and similarly, the pelagic spawning fish also had finer scale genetic structure compared to the benthic spawning fish. Further, the mollusc with a short larval duration exhibited the lowest level of genetic structure of all taxa. **Clearly, expectations of realised connectivity based on simple life history characteristics are unreliable, and patterns therefore need to be assessed on a species by species basis.**

1.2 Fine scale: Population boundaries are shared between some taxa

Major population boundaries were identified in several taxa, notably the habitat-forming corals (Report 1.1.3.1), and seagrasses (Report 1.1.3.2), and the pelagic spawning fish (Report 1.1.3.4b), but not the mollusc (Report 1.1.3.3), nor the damselfish (Report 1.1.3.4a). **Broadly, the divisions in seagrasses, corals and fish were between the Dampier Peninsula and Buccaneer Archipelago sites**, but the specific positions and breadths of the boundaries differed for individual taxa. For example, in *T. hemprichii*, the seagrass with

buoyant seeds, the northern Buccaneer Archipelago sites were differentiated from those in the southern Buccaneer Archipelago and Dampier Peninsula, whereas both the broadcast spawning and brooding corals exhibited a strong division between the Dampier Peninsula and the Buccaneer Archipelago. A division also exists in the fish, *L. carponotatus*, but it occurred as a broad transition zone in which the genetic composition changes across a distance of c. 40km at the tip of the Dampier Peninsula from the Kimberley bioregion signature to the Pilbara/Canning bioregion signature (see also Major Finding 1.6). In contrast, ***T. niloticus* forms a single highly-mixed genetic unit within the Dampier Peninsula and Buccaneer Archipelago**, suggesting considerable exchange of larvae occurs throughout this region.

1.3 Fine scale: Some sites act as links between otherwise isolated regions

Although restricted connectivity was detected in the region of Sunday Strait and the Dampier Peninsula for corals (Report 1.1.3.1), seagrasses (Report 1.1.3.2), and *L. carponotatus* (Report 1.1.3.4b), exchange of genes across this barrier over multiple generations occurs through the important stepping-stones at Tide Rip, Mermaid and Bedford Islands for corals and seagrass. For *L. carponotatus* a similar transition zone was detectable between Tallon Island and Emeriau Point (Dampier Peninsula).

1.4 Fine scale: King Sound, Sunday Strait are barriers to dispersal in some species

The region at the mouth of King Sound is characterised by the largest tropical tidal range and the fastest tidal currents in the world including the input of massive volumes of freshwater in a highly turbid plume from the Fitzroy catchment in the wet season; a time when propagules from many of these species are in the plankton. These extreme environmental conditions appear to restrict connectivity. Coupled with the finding of a highly divergent population of *I. bruggemanni* on the western side of Dampier Peninsula, these results demonstrate that **the tip of Dampier Peninsula is an important intra-specific genetic barrier for various marine taxa with range of life histories**.

1.5 Broad scale: The inshore and offshore Kimberley are poorly connected

The species of corals (Report 1.1.3.1) and trochus (Report 1.1.3.3) that were sampled over broader scales at the offshore reefs of Rowley Shoals, Scott Reef, and Ashmore Reef showed that these **inshore Kimberley reef populations are highly divergent from the offshore 'oceanic' reef populations, strongly indicating that these regions are ecologically and evolutionary independent**. This likely reflects the limited hydrodynamic connectivity between these reefs, but in addition, genetic patterns suggest **strong environmental differences between these regions has driven local adaptation in these species**.

1.6 Broad scale: connectivity between the Kimberley and neighbouring bioregions differs among species

The species that were sampled across the broader north-west coast of Australia exhibited some consistencies in their broad-scale patterns of connectivity. The seagrass *T. hemprichii* (Report 1.1.3.2) and the damselfish *P. milleri* (Report 1.1.3.4a) exhibited a sharp discontinuity between the Kimberley and Pilbara, indicating negligible exchange, and probably reflecting discontinuous habitat between these regions. In contrast, Pilbara and Kimberley populations of *L. carponotatus* (Report 1.1.3.4b), exhibited only weak genetic distinctiveness. Furthermore in *L. carponotatus*, the transition zone between Kimberley and Pilbara genetic groups occurred at Sunday Strait rather than corresponding to the Pilbara and Kimberley Bioregions like *T. hemprichii* and *P. milleri*. *Lutjanus carponotatus* samples from the Northern Territory were weakly genetically distinct from those in the Kimberley, but it is unclear whether this represents limited demographic exchange, or incomplete sampling in the intervening region.

The preliminary otolith geochemistry results (Chapters 1.1.3.4c) generally concur with the findings of the genetic companion studies of the two fish species (Chapters 1.1.3.4a,b), and add support to their conclusions that the movement of both species between the Kimberley, Pilbara and Gascoyne bioregions is restricted. This

preliminary result should be considered cautiously as the margin otolith microchemistry only tells part of the story (adult phase) and additional core samples (larval and post-larval phase) will need to be analysed to allow interpretation of population connectivity. Furthermore, 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 compared to the more enclosed and inshore marine environment where *P. milleri* samples were collected within the western Gulf of Shark Bay. **Broad scale: Genetic diversity is distributed differently in each species**

Within the Dampier Peninsula – Buccaneer Archipelago region, some organisms (coral, Report 1.1.3.1; seagrass Report 1.1.3.2) exhibited large variation between sites in amount of genetic diversity observed, whereas others (fishes, Report 1.1.3.4a; trochus, Report 1.1.3.3) exhibited similar amounts of diversity at each site. Across the broader north-west coast of Australia, species varied significantly in their distributions of genetic diversity. Populations of the seagrass *T. hemprichii* from the Kimberley exhibited significantly lower genetic diversity than those in the Pilbara. In contrast, in the damselfish *P. milleri*, genetic diversity was highest in the Kimberley and declined progressively with latitude towards the Gascoyne bioregion. In the stripey snapper, *L. carponotatus*, levels of genetic diversity were consistent across the entire north-west coast. These contrasting results likely reflect: 1) differences in population size; 2) differences in connectivity between regions (physical and environmental); and 3) differences in colonisation history of the different regions. Further, multiple hotspots (i.e. areas with high genetic diversity or unique variants) were identified at particular sites for coral and seagrass (e.g. West Montalivet for *I. brueggemanni* and Bedford Island south for *H. ovalis*), and these are discussed further in the specific taxon reports.

1.7 Cryptic genetic diversity exists in the broadcast spawning coral

Four genetically distinct, but morphologically cryptic, genetic lineages were detected in the *A. aspera* collection (Report 1.1.3.1), strongly suggesting that these lineages are reproductively isolated, even though they look the same and live side by side, and thus likely **represent unique evolutionary significant units and/or unrecognised species**.

2 Implications for Management

This research has highlighted commonalities and disparities in patterns of connectivity among taxa representing a range of trophic levels and life histories. Many of these findings have important implications for management of Kimberley marine ecosystems. Threats to these ecosystems include local anthropogenic impacts such as overfishing, tourism, industrial development and oil spills, as well as the impacts of climate change, which operates over broader spatial scales and longer time-frames. The resilience of marine ecosystems to these threats depends critically on how they affect ecological processes such as connectivity, which promote population persistence and regeneration. Management strategies that protect healthy sources of recruits and maintain the exchange of adaptive genes will nurture resilience in marine ecosystems. To this end, below we summarise how the patterns of connectivity identified in this project would best inform management of Kimberley marine ecosystems.

- 1. To protect hard corals, the crucial habitat forming organisms of coral reef ecosystems and also seagrass, an important food source for dugongs and turtle, and a nursery habitat for fishes, marine protected areas and indigenous protected areas need to incorporate strategies that account for the spatial dispersal of these organisms.** Protected areas that are large enough to encompass routine dispersal distances of corals (10–20 km), and are spaced at similar distances, will not only maintain self-replenishment, but also aid recovery after disturbance through connectivity between protected areas.

2. **Corals and seagrasses of Buccaneer Archipelago and Dampier Peninsula need to be managed as demographically independent populations.** Furthermore, negligible exchange between the inshore Kimberley and the offshore coral reefs and neighbouring bioregions means that populations of the inshore Kimberley are reliant on standing genetic variation as the basis of adaptation to climate change or other disturbances.
3. **Current estimates of species diversity in corals are likely to be substantial underestimates.**
The cryptic *Acropora* coral lineages detected here reveal that current assessments of the diversity of hard coral species in the Kimberley are likely substantial underestimates and further integrated taxonomic research is needed to clarify species diversity patterns in all taxon groups.
4. **Management of *T. niloticus* on the Dampier Peninsula and Buccaneer Archipelago should treat the region as being effectively a single stock on the ecological timeframes relevant to harvest management.**
Over-harvested sites within this region will be replenished with recruits from neighbouring sites within years, assuming they exist, and allowing for the slow growth of the species.
5. **Management of *T. niloticus* at offshore oceanic reefs should treat each oceanic shoal as being effectively isolated on the ecological timeframes relevant to harvest management.** Recruitment from outside will not replenish over-harvested stocks at these locations. Occasional recruits may be drawn from other offshore shoals, but will contribute to genetic diversity not offset over-harvest. Supplementation of populations should recognise that coastal *T. niloticus* populations may be mal-adapted to oceanic conditions.
6. **The Kimberley and Pilbara bioregions exchange few recruits in seagrasses and reef-obligate damselfishes, and therefore operate largely independently on the ecological timeframes relevant to management.**
7. **Demographic exchange between the Kimberley and Pilbara/Canning bioregions in the harvested stripey snapper, *L. carponotatus*, occurs in a broad transition zone located near the Sunday Strait. The distinctiveness of the Shark Bay *L. carponotatus* samples from all other bioregions indicates that the Gascoyne management boundary is not supported because sites north of Shark Bay have greater affinities to sites in the Pilbara Bioregion. This information should be considered within management arrangements.**
8. **Genetic differentiation between samples of *L. carponotatus* from the Kimberley and Northern Territory may represent limited demographic exchange between these separately-managed stocks, but to be confirmed this requires further samples from the intermediate region.**

3 Key Residual Knowledge Gaps

3.1 Habitat Forming: Two Species of Corals

- Further integrated taxonomic study that includes micro-morphological examination of the *Acropora aspera* lineages in tandem with investigations of reproductive biology is required to resolve species boundaries within the Kimberley *A. aspera* complex.
- In both the spawning and brooding species, this study indicated a lack of cross-shelf connectivity between the southern inshore Kimberley and Ashmore Reef. There was only one exception to this regional scale divergence; *I. brueggemanni* corals from the most northern site sampled, West Montalivet (Bonaparte Archipelago), exhibited genetic affinities with Ashmore Reef. The current study should be extended to include more populations from the central and northern Kimberley to evaluate if there is a higher degree of cross-shelf connectivity in the central or northern Kimberley.

- The study of *I. brueggemanni* corals indicated that the population on the far west side of Dampier Peninsula (Kooljaman) was very divergent from the other inshore Kimberley populations and was characterised by the lowest gene diversity of all sites, suggesting that it is a small and isolated population that may be vulnerable to local extinction. Further comparative studies on other species of coral are needed to clarify if this result is reflective of a wider trend.
- This study indicated that the dispersal of both brooded and broadcast spawned larvae is restricted between of the Buccaneer and Dampier systems across the Sunday Strait. We hypothesize that Tide Rip and Mermaid Islands do however provide important stepping stones facilitating genetic exchange across this barrier. Further examinations are needed to determine the diversity and extent of subtidal reef communities in the vicinity of these islands which present themselves as important transition habitats.

3.2 Habitat Forming: Two Species of Seagrasses

- Increasing the understanding of genetic connectivity of these species outside of the main study area, east into the northern Kimberley, south into the rest of Canning marine bioregion, and more extensively into the Pilbara region.
- Developing a better understanding of the significance of dugong foraging as a mechanism for dispersing seagrasses with dormant seeds (e.g. *H. ovalis*, *H. uninervis*).

3.3 Harvested: A Large Gastropod (Trochus)

This investigation had a limited geographic scope in comparison to the broad Indo-Pacific range of *T. niloticus*, capturing the south-westernmost part of its range. Indeed, even within the Kimberley region, the region of high density in the Buccaneer Archipelago is disjunct from other high density populations in Australia, Indonesia and on offshore atolls. The broad distribution of *T. niloticus* in the tropical Indo-Pacific incorporating a diversity of reef types and hydrodynamic conditions means that it is unlikely that the spatial scale of genetic structure observed here will be reflected throughout its range. Considering the economic and cultural significance of the species to many people, a broader investigation of population structure in *T. niloticus* and its biophysical drivers deserves consideration.

3.4 Reef-dwelling: A Coral Reef Fish

- *Pomacentrus milleri* is a useful model for small reef-dependent species. However, this study has only examined a fraction of the species' range. *Pomacentrus milleri*'s range extends into the Northern Territory and New Guinea. The extent of connectivity between *P. milleri* in Western Australia and other regions is unknown.
- Although the results presented here have revealed evidence for geographically structured adaptive diversification in *P. milleri*, the specific environmental drivers have not been identified.
- *Pomacentrus milleri* shares a life history with many small reef-dependent fish species. It is anticipated that this would be reflected in comparable population genetic structure in similar species, but this hypothesis requires empirical testing.

3.5 Harvested: A Demersal Fish

- Genetic differentiation between samples of Stripecy Snapper from the Kimberley and NT may represent limited demographic exchange between these currently separately managed stocks. Further sampling from the intermediate region is needed to confirm this.
- Ocean currents are likely to play a significant role in distributing the larvae of Stripecy Snapper. Models of hydrodynamic processes throughout NWA are available, however it would be useful to evaluate how well these models predict the observed genetic structure in Stripecy Snapper, since that would provide confidence that the models accurately reflect biological processes and therefore may be applied to other bioregions and/or species.

- In contrast, the transition zone identified around the Dampier Peninsula that separates the Kimberley from the Pilbara/Canning populations is likely to be influenced by the extreme tidal flushing at the head of King Sound, rather than ocean currents. A fine-scale hydrodynamic model for this region was prepared by WAMSI Kimberley Project 2.2.7 (M. Feng, CSIRO, pers. comm.). It would be useful to test whether this model can account for the observed genetic structure in this highly dynamic zone that supports harvest of numerous fishes.
- Evidence for temporal variation in population structure was revealed through the analysis of historically collected samples. For these temporal samples we explored the reason for their observed divergence and were able to exclude at least one mechanism of DNA degradation. This result may therefore represent a real shift in allele frequencies over time, potentially indicative of changing patterns of larval connectivity. However, since we did not sample these exact locations again, it's unclear whether the pattern is wholly temporal or also has a spatial component. Additional sampling at these historical sites is required to resolve this question.

4 Report Structure

The full report for WAMSI project 1.1.3 is structured as an executive summary, six individual sub-project reports that focus on different marine organisms, and a synthesis report, which provides an overview and regional perspective through summarising the key findings for each sub-report, and the broader management implications these have for the region and the State. The following sub-reports are included as separate documents:

- 1.1.3a [Ecological Connectivity in Kimberley Marine Communities: a Synthesis Report](#)
- 1.1.3.1 [Population connectivity and genetic diversity in brooding and broadcast spawning corals in the Kimberley](#)
- 1.1.3.2 [Population genetic diversity, structure and connectivity of two seagrass species, *Thalassia hemprichii* and *Halophila ovalis* in the Kimberley](#)
- 1.1.3.3 [Isolation of oceanic and coastal populations of the harvested mother-of-pearl shell *Tectus niloticus* in the Kimberley](#)
- 1.1.3.4a [Genomic Connectivity in a Tropical Reef Fish from the Kimberley, Pilbara and Gascoyne Bioregions of Western Australia](#)
- 1.1.3.4b [Population connectivity of the Stripey Snapper *Lutjanus carponotatus* along the ecologically significant coast of Northwestern Australia](#)
- 1.1.3.4c [Population connectivity of two reef fish species in northwestern Australia using otolith geochemistry: a pilot study](#)

5 Communication

5.1 Students supported

Mr Udhi Hernawan was supported in the completion of his PhD with field and laboratory resources from this project for his work on seagrass (1.1.3.2). The Kimberley work on *Thalassia hemprichii* forms one chapter in his dissertation, which was submitted in July 2016. The analysis on *T. hemprichii* in this report was undertaken by Mr Hernawan.

5.2 Journal publications

Hernawan U, van Dijk K, Kendrick G, Feng M, Biffin E, Lavery P, McMahon K. (2017) Historical processes and contemporary ocean currents drive genetic structure in the seagrass *Thalassia hemprichii* in the Indo-Australian Archipelago. *Molecular Ecology* 26, 1008-1021.

Joseph DiBattista, Michael Travers, Glenn Moore, Richard Evans, Stephen Newman, Ming Feng, Rebecca Gorton, Samuel Moyle, Thor Saunders, Oliver Berry (2017) Seascape genomics reveals fine-scale patterns of dispersal for a reef fish along the ecologically divergent coast of Northwestern Australia. *Molecular Ecology* 26, 6206-6223.

Richards ZT and O'Leary M (2015) The coralline algal cascades of Tallon Island fringing reef, NW Australia. *Coral Reefs*, 34(2), 595-595

5.3 Submitted manuscripts

Hernawan U, van Dijk K, Kendrick G, Feng M, Berry O, Kavazos C, McMahon K. Extreme ocean currents and habitat characteristics drive genetic divergence in a tropical seagrass. In review *Molecular Ecology*

Hernawan U, van Dijk K, Kendrick G, Feng M, Biffin E, Lavery P, McMahon K. Historical processes and contemporary ocean currents drive genetic structure in the seagrass *Thalassia hemprichii* in the Indo-Australian Archipelago. In review *Molecular Ecology*

5.4 Presentations

- Sarah Hearne, Alison Blyth, Jennifer McIlwain, Michael Travers, Richard Evans, Kate Trinajstic (2017) Connectivity of fishes from the Kimberley region, Western Australia, using otolith geochemistry. Australian Society of Fish Biology annual conference held in Albany, WA, 21-23 July 2017.
- Oliver Berry, Michael Travers, Richard Evans, Glenn Moore, Ming Feng, Udhi Hernawan, Bernd Gruber (2017) Complex ocean currents promote adaptive diversification and lower dispersal in a tropical reef fish from north-western Australia. Australian Marine Science Association Annual Conference, Darwin, July 2- 7, 2017.
- Joseph DiBattista, Michael Travers, Glenn Moore, Richard Evans, Stephen Newman, Ming Feng, Rebecca Gorton, Samuel Moyle, Thor Saunders, Oliver Berry (2017) Genomics reveals fine-scale patterns of dispersal for a reef fish along the ecologically significant coast of Northwestern Australia. Australian Marine Science Association Annual Conference, Darwin, July 2- 7, 2017.
- Oliver Berry, Glenn Moore, Zoe Richards, Udhi Hernawan, Bernd Gruber, Michael Travers (2017) Genomic evidence for isolation of offshore and coastal populations of *Trochus* in the Kimberley. Australian Marine Science Association Annual Conference, Darwin, July 2- 7, 2017.
- Oliver Berry, Jim Underwood, Kathryn McMahon, Zoe Richards, Mike Travers, Glenn Moore, Udhi Hernawan, Joey DiBattista, James Gilmour (2016) Ecological Connectivity of Kimberley Marine Communities: Lunch and Learn session, Department of Parks and Wildlife, Kensington.
- Zoe Richards (2016) Some like it HOT! Hard coral diversity of the Kimberley, NW Australia. Presented to five research institutions in Japan (Fisheries Research Agency, Tokyo Institute of Technology; University of Miyazaki; Sesiko Marine Station; University of the Ryukyus) under a JSPS short term fellowship awarded to Dr Richards.
- Zoe Richards (2016) High cryptic diversity, taxonomic uncertainty and the risk of silent extinctions in corals. International Society for Coral Reefs Symposium, Honolulu.
- Jim Underwood (2016) Genomics of spawning corals in the Kimberley. AMSA snapchat
- Oliver Berry, Jim Underwood, Kathryn McMahon, Zoe Richards, Mike Travers, Glenn Moore, Udhi Hernawan, Joey DiBattista, James Gilmour (2015) Genetic Connectivity in an Extreme Marine Environment. Society for Australian Systematic Biologists Annual Conference, Fremantle.
- Oliver Berry, Jim Underwood, Kathryn McMahon, Zoe Richards, Mike Travers, Glenn Moore, Udhi Hernawan, James Gilmour (2015) Ecological Connectivity in the Kimberley. WAMSI Dredging and Kimberley Nodes Symposium, Library of Western Australia, Perth.
- Jim Underwood, Zoe Richards, James Gilmour, Oliver Berry (2015) Genetic connectivity and cryptic diversity in corals of the Kimberley. Society for Australian Systematic Biologists Annual Conference, Fremantle.
- Kathryn McMahon, Udhi Hernawan, Gary Kendrick, Korjent van Dijk, Paul Lavery, Oliver Berry, Mike Travers, Jim Underwood (2015). Genetic connectivity of the seagrass *Thalassia hemprichii* in the Kimberley and Pilbara. Australian Marine Sciences Association, Geelong, Australia.
- Udhi Hernawan, Kathryn McMahon, Gary Kendrick, Korjent van Dijk, Paul Lavery (2015). Coastal and Estuarine Research Federation, Oregon, Portland, USA. So near, yet so far: Genetic connectivity of the seagrass *Thalassia hemprichii* in tropical Australia.
- Kathryn McMahon (2015). Molecular ecology of seagrasses: tools for conservation and management. University of Jogjakarta, Natural resources from local to global conference. 2015. Invited speaker.
- Kathryn McMahon (2015). Management and conservation of valuable seagrass ecosystems. Indonesian Institute of Sciences.
- Kathryn McMahon (2015). What we know about connections in seagrasses: Long-distance dispersal, millennial movements and emerging patterns in NW WA. ECU Research Week
- Udhi Hernawan, Kathryn McMahon, Gary Kendrick, Korjent van Dijk, Paul Lavery (2015). Predictors of genetic structure in marine organisms in the Indo-Australian Archipelago: Generalisable patterns and a seagrass-case study. ECU Research Week.
- Udhi Hernawan, Kathryn McMahon, Gary Kendrick, Korjent van Dijk, Paul Lavery. Genetic connectivity of a tropical seagrass in an extreme environment: It is not just going with the flow. ECU Postgraduate Symposium.
- Udhi Hernawan, Kathryn McMahon, Gary Kendrick, Korjent van Dijk, Paul Lavery, Oliver Berry, Mike Travers, Jim Underwood (2015). Going with the Flow: Ecological Connectivity of the seagrass *Thalassia hemprichii* in the Kimberley and North West Cape, Western Australia. WAMSI Kimberley Symposium.

5.5 Other communications achievements

WA Science Network - Kimberley reef life considered on a microscopic level - <http://www.sciencewa.net.au/topics/fisheries-a-water/item/3545-kimberley-reef-life-considered-on-a->

[microscopic-level](#)

Oliver Berry, Kathryn McMahon, Jim Underwood (2016) Going with the Flow. *Kimberley Tides Newsletter*. Department of Parks and Wildlife and Department of Fisheries

KMRP 1.1.3 Summary (July 2016) – Ecological Connectivity in the Kimberley Marine Communities <https://indd.adobe.com/view/23b0943a-6eff-4499-bd39-f94ba9d1cf12>

5.6 Knock on opportunities created as a result of this project

Proposal for postdoctoral position at AIMS for J. Underwood to work on a collaborative project (with AIMS, Curtin University and Parks and Wildlife among others) to further coral genetics, particularly in the northern Kimberley where MPA's exist and to address questions of reef resilience.

Proposal for ARC Linkage Grant led by Z. Richards to work on coral biodiversity and resilience in the Kimberley.

Through this project additional genetic connectivity work has been funded as part of a collaboration between ECU and Parks and Wildlife, to investigate further the genetic connectivity of the seagrass *H. ovalis* through the Pilbara. This will allow increasing the scope of the existing beyond the Kimberley and link with previous work by McMahon in the southern Pilbara.

A project on connectivity in the stripey snapper (*L. carponotatus*) across its entire Australian range between Western Australia and Queensland has been initiated through collaborations with researchers at James Cook University. Those researchers are seeking to generate a compatible dataset so that it can be combined with the data generated for this project.

5.7 Key methods for uptake

Lunch and Learn presentation at Parks and Wildlife (August 2016) www.wamsi.org.au/ecological-connectivity and meeting with Node Leader and KMRP Advisory Group to discuss management needs and application.

An open presentation was made at Parks and Wildlife followed by an in-depth discussion with relevant managers on the KMRP Advisory Committee that was used to communicate the key findings and their application by managers and planners as well as to inform and improve the management implications sections of this report.

6 Appendix

Appendix 1: Questions outlined in the Kimberley Marine Research Program Science Plan

<p>Key Questions</p> <p>Informed Response</p>
<p>How do macro-tidal systems influence ecological connectivity of key taxa?</p> <p>In the taxa where comparisons could be made (seagrasses and fishes) connectivity was lower in the macro-tidal and topographically complex Kimberley than in the less tidal and topographically complex Pilbara.</p> <p>Within the Kimberley, organisms generally responded to oceanographic conditions in taxon-specific manners that broadly corresponded to their larval life-history and an isolation-by-distance pattern, but there were some exceptions.</p> <p>We conclude that further detailed oceanographic data is needed in tandem with more information about life-history and larval duration times in order to more fully understand connectivity in complex macro-tidal systems such as the Kimberley.</p>
<p>What is the extent of fine scale connectivity within and between coastal reefs (up to 100 km)?</p> <p>In the Kimberley, with the exception of <i>T. niloticus</i>, which occurs as one large interbreeding population, population structure was evident between sites separated by more than a few kilometres in all species. However, the magnitudes of genetic difference between sites varied significantly among taxa, indicating that some species (corals, seagrasses) were relatively isolated, and their population structure reflected major hydrodynamic or topographic barriers, whereas the fishes experienced high levels of connectedness among sites largely reflecting isolation-by-distance effects.</p>
<p>What is the extent of larger scale connectivity within and between coastal and offshore reefs?</p> <p>Genetic subdivision (and hence some limitation to dispersal) was observed in all taxa with the exception of <i>T. niloticus</i> within the coastal Kimberley.</p> <p>Inshore and offshore Kimberley populations are highly divergent for 3/3 taxa examined and the inshore Kimberley populations are also highly divergent from populations in the Pilbara for 3/4 taxa examined.</p>
<p>What are the dispersal distances of key taxa?</p> <p>The maximum detectable scale of genetic structure, which indicates the routine dispersal distances, was taxon dependant. Habitat forming organisms (coral, seagrass) typically exhibited the most localised population structure, with evidence for limitations to routine dispersal evident on scales of 10s of kilometres or less. In the remaining organisms (fishes, trochus), population structure was weaker or not detectable, and limits to dispersal were evident on scales of 80 to several 100s kilometres (See Figure 5).</p>
<p>Are proposed management areas sufficient for ecological connectivity to support populations of key taxa?</p> <p>The Dampier Peninsula and Buccaneer Archipelago are not included in any of the existing or proposed Kimberley Marine Protected Areas. The Bonaparte Archipelago which was superficially sampled in our broad-scale study is included in the proposed North Kimberley Marine Park.</p> <p>The Montalivet Island group (where a putative genetic diversity hotspot is hypothesised to occur for <i>I. bruggemanni</i>) is designated as a General Use Area. This designation offers little change in management strategy, and thus without sanctuary zoning, offers little benefit to coral reef populations.</p> <p><i>T. niloticus</i> populations at Scott Reef, which are genetically and demographically independent from coastal populations and from the populations at Rowley Shoals do not benefit from existing spatial management as there is no protection from harvesting.</p> <p>In the case of the targeted fish <i>L. carponotatus</i>, existing fishery management does not recognise the separation of Kimberley and Pilbara populations detected in this research. The existing separate management of Northern Territory and Kimberley <i>L. carponotatus</i> is supported by the observed genetic differentiation</p>

between these regions, but analysis of additional intermediate sites is required to better characterise the relationships between these stocks.

What are the influences of major disturbance?

Although we did not directly address this question – the major disturbance events likely to impact our study are cyclonic waves, coral bleaching, flood events and the input of sediment and nutrients. With the exception of *T. niloticus* it appears that disturbances greater than 10km in scale are likely to impact more than one relatively demographically discrete unit.

How will climate change affect dispersal patterns of key taxa?

Rapid climate change may reduce population sizes and genetic diversity through recurrent disturbance. If climate change leads to changes in the hydrodynamic regime, then this could affect dispersal patterns unless species can respond behaviourally to the changes.

How can genetic data be best incorporated into emerging oceanographic models for the region to provide more robust and detailed inferences about patterns of connectivity throughout north-west WA?

Genetic observations can be used to evaluate how well oceanographic models represent biological processes like connectivity. Such evaluations potentially validate models, which then can be generalised to other species or locations.

In this study we show (for corals, *T. niloticus* and *P. milleri*) that a simple measure of distance provides a better explanation of the observed patterns of connectivity than a fine-scale oceanographic model that does not incorporate larval behaviour. That result, along with our observation that life-history roughly predicts levels of connectivity in some of our taxa, indicates that if models are going to provide “more robust inferences” they need to include larval behaviour.

Better predictions of connectivity in the Kimberley are also likely to result from:

- Development of particle tracking (connectivity) models that better match the spatial scale of management as well as the scale of genetic analysis;
- Incorporation of additional biophysical data into predictive models of connectivity (e.g. redundancy analysis).

What role does the Kimberley play in the maintenance of systems outside of the region?

Based on the results of this study, and acknowledging that sampling of outside regions was incomplete, the Kimberley appears to be a largely a self-contained system for most taxa. It is not likely to play a major role in the maintenance of systems outside the region over ecological timescales with the exception of *L. carponotatus* which does have a degree of exchange with both the Northern Territory, and to a lesser extent, the Pilbara. The inshore Kimberley has negligible role in maintaining populations on oceanic shoals and vice versa on an ecological timescale.

How is the condition of the Kimberley influenced by external biological and anthropogenic influences?

Marine communities in the inshore Kimberley are likely to be profoundly influenced by dynamic environmental conditions at a local scale leading to a strong selective pressure and the observed pattern of high population differentiation in species.

Harvesting has the potential to impact *T. niloticus* stocks at offshore atolls, while non-sustainable fishing for *L. carponotatus* could result in impacts to Kimberley stocks of this recreationally targeted species. Anthropogenic impacts like oil spills or development are likely to have lasting impacts due to the fragmented nature of populations so recovery will be slow.



Ecological Connectivity of Kimberley Marine Communities: *Synthesis Report*

Zoe Richards^{1,2,8}, Oliver Berry^{3,8}, Jim Underwood^{4,8}, Kathryn McMahon^{5,8}, Mike Travers^{6,8}, Glenn Moore^{1,8}, Udhi Hernawan^{5,8}, Joseph DiBattista^{2,8}, Richard Evans^{7,8}, James Gilmour^{4,8}

¹Western Australian Museum, Perth, Western Australia

²Curtin University, Bentley, Western Australia

³CSIRO, Crawley, Western Australia

⁴Australian Institute of Marine Science, Townsville, Queensland

⁵Edith Cowan University, Joondalup, Western Australia

⁶Fisheries Division, Department for Primary Industries and Regional Development, Hillarys, Western Australia

⁷Department of Biodiversity, Conservation and Attractions, Kensington, Western Australia

⁸Western Australian Marine Science Institution, Perth, Western Australia

WAMSI Kimberley Marine Research Program *Synthesis Report*

Project 1.1.3a

August 2017



western australian
marine science institution



Department of Biodiversity,
Conservation and Attractions



Department of
Primary Industries and
Regional Development



Mayala
Community



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.

Ownership of Intellectual property rights

Unless otherwise noted, copyright (and any other intellectual property rights, if any) in this publication is owned by the Western Australian Marine Science Institution, CSIRO, Australian Institute of Marine Science, Edith Cowan University, The University of Western Australia, Western Australia Department of Primary Industries and Regional Development, Western Australian Museum, Curtin University and the Department of Biodiversity, Conservation and Attractions.

Copyright

© Western Australian Marine Science Institution

All rights reserved.

Unless otherwise noted, all material in this publication is provided under a Creative Commons Attribution 3.0 Australia License. (<http://creativecommons.org/licenses/by/3.0/au/deed.en>)



Legal Notice

The Western Australian Marine Science Institution advises that the information contained in this publication comprises general statements based on scientific research. The reader is advised and needs to be aware that such information may be incomplete or unable to be used in any specific situation. This information should therefore not solely be relied on when making commercial or other decision. WAMSI and its partner organisations take no responsibility for the outcome of decisions based on information contained in this, or related, publications.

Front cover images (L-R)

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

Image 2: Powerful currents are a feature of the Kimberley marine environment. Whirlpool in Sunday Strait (Image: Kathryn McMahon)

Image 3: Humpback whale breaching (Image: Pam Osborn)

Image 4: Coral platform exposed at low tide. Bathurst Island Buccaneer Archipelago (Image: Kathryn McMahon)

Year of publication: August 2017

Metadata: <http://catalogue.aodn.org.au/geonetwork/srv/eng/metadata.show?uuid=fb1d80bf-6ef2-4150-9479-22b4240435a7>

Citation: Richards Z, Berry O, Underwood J, McMahon K, Travers M, Moore G, Hernawan U, DiBattista J, Evans R, Gilmour J (2017) Ecological Connectivity of Kimberley Marine Communities. Synthesis Report of Project 1.1.3 prepared for the Kimberley Marine Research Program, Western Australian Marine Science Institution, Perth, Western Australia, 17pp.

Author Contributions: All authors contributed to the drafting of this text.

Corresponding author and Institution: Oliver Berry, CSIRO Oceans and Atmosphere. oliver.berry@csiro.au

Funding Sources: This project was funded (commissioned) by the Western Australian Marine Science Institution as part of the WAMSI Kimberley Marine Research Program, a \$30M program with seed funding of \$12M provided by State government as part of the Kimberley Science and Conservation Strategy. The Program has been made possible through co-investment from the WAMSI Joint Venture partners and further enabled by data and information provided by Woodside Energy Ltd.

Competing Interests: The commercial investors and data providers had no role in the data analysis, data interpretation, the decision to publish or in the preparation of the manuscript. The authors have declared that no competing interests exists.

Kimberley Traditional Owner agreement: This research was enabled by the Traditional Owners through their advice, participation and consent to access their traditional lands.

Acknowledgements: We are grateful to all WAMSI staff for their assistance, and in particular Kelly Waples, Stuart Field and Kim Friedman for providing advice and constructive criticism throughout the project. Many other people and organisations contributed to the success of this project, and we thank them sincerely. We especially thank the following Indigenous communities. The Bardi and Jawi Niimidiman Aboriginal Corporation, the Bardi Jawi Rangers and the Bardi Jawi Traditional Owners and specifically Daniel Oades, Damon Pyke, Azton Howard, Chris Sampi, Kevin George, Kevin Ejai, Kevin Dougal, Tasha Stumpagee, Phillip McCarthy, Peter Hunter, Zac Ejai, Paul Davey and Trevor Sampi. The Mayala people, specifically Sandy, Alec and Janella Isaacs. The Wunambal Gaambera Aboriginal Corporation, Traditional Owners, Unguu Rangers and Tom Vigilante. The Dambimangari Aboriginal Corporation, Dambimangari Traditional Owners and the Dambimangari Rangers. Thanks also to the Bioinformatic pipeline development: Bernd Gruber (University of Canberra); Oceanographic modelling: Ming Feng, Dirk Slawinski (CSIRO); SNP marker development and genotyping: Andrzej Kilian (Diversity Arrays Technology); Field assistance: Sam Moyle and Fiona Webster; Kimberley Marine Research Station for facilities and logistical support: James Brown, Michael Flynn, Scott Whitlam, Duncan Smith and Erin McGinty; and advice on project development: Karen Miller (AIMS).

Collection permits/ethics approval: SF008440, SF009910, SC001362 (Western Australian Department of Parks and Wildlife); 2485 2085, 2344 (Western Australian Department of Fisheries)

Contents

1	INTRODUCTION	1
1.1	OBJECTIVE OF THE PROJECT	1
1.2	RATIONALE	1
2	BACKGROUND	1
3	METHODS	3
3.1	FOCAL TAXA	3
3.2	APPROACH: POPULATION GENETICS, GENOMICS AND OTOLITH GEOCHEMISTRY	4
3.2.1	<i>Broad Scale Study</i>	4
3.2.2	<i>Fine Scale Study</i>	4
3.3	GENETIC AND GEOCHEMISTRY ANALYSES	5
4	KEY FINDINGS	7
4.1	FINE SCALE: THE EXTENT OF CONNECTIVITY DIFFERS AMONG SPECIES	7
4.2	FINE SCALE: POPULATION BOUNDARIES ARE SHARED BETWEEN SOME TAXA	7
4.3	FINE SCALE: SOME SITES ACT AS LINKS BETWEEN OTHERWISE ISOLATED REGIONS	8
4.4	FINE SCALE: KING SOUND, SUNDAY STRAIT ARE BARRIERS TO DISPERSAL IN SOME SPECIES	8
4.5	BROAD SCALE: THE INSHORE AND OFFSHORE KIMBERLEY ARE POORLY CONNECTED	8
4.6	BROAD SCALE: CONNECTIVITY BETWEEN THE KIMBERLEY AND NEIGHBOURING BIOREGIONS DIFFERS AMONG SPECIES	9
4.7	BROAD SCALE: GENETIC DIVERSITY IS DISTRIBUTED DIFFERENTLY IN EACH SPECIES	9
4.8	CRYPTIC GENETIC DIVERSITY EXISTS IN THE BROADCAST SPAWNING CORAL	9
5	OVERARCHING IMPLICATIONS FOR MANAGEMENT	11
6	OUTCOMES AND BENEFITS	13
7	CONCLUSION	13
8	REFERENCES	14



1 Introduction

1.1 Objective of the Project

The overarching objective of KMRP Project 1.1.3 (Ecological Connectivity of Kimberley Marine Communities) was to provide the first estimates of ecological connectivity across multiple spatial scales for a suite of model/priority marine organisms in the Kimberley. More specifically, this project aimed to provide species-specific estimates of realised connectivity at a reef-scale (<1 km), inter-reef scale (1-100 km) and inter-region scale (100+ km) through genetic analyses of seven key animals and plants with contrasting dispersive life histories that are representative of common taxa.

The goals of this Synthesis Report are:

- I. To synthesise the major findings and conclusions of WAMSI Project 1.1.3 – Ecological Connectivity of Kimberley Marine Communities; and
- II. To place this information in local and regional contexts to benefit environmental planning and resource management.

1.2 Rationale

For most marine organisms the ocean environment provides the potential for widespread dispersal via oceanic currents, tides and wind. However, depending on the interplay between the biology of an organism and its physical environment, the *potential* for dispersal may be radically different from the *realised* dispersal. The realised connectivity between populations (i.e. the actual numbers of individuals that move between distant populations and survive to reproduce) determines the distribution and abundance of marine organisms and is especially important in the face of disturbances. For example, if the scale of an impact (e.g. over-harvesting) is larger than the routine distances of larval dispersal, then recovery is likely to be slow. Similarly, disturbances may be more significant if they impact populations that act as principal sources of larval recruitment. Therefore, to effectively manage marine resources in the Kimberley and neighbouring bioregions it is crucial to develop a realistic understanding of the extent of connectivity and to highlight the important sources of larvae that maintain healthy populations and supplement recovery after disturbance.

2 Background

The Kimberley marine bioregion (located in northwest Western Australia) is a remote, sparsely populated and poorly studied area characterised by extreme tidal ranges and strong tidal currents (Figure 1). The region is the subject of growing scientific interest because of its near-pristine state and unique biota (Wilson 2013). It is also subject to increasing interest from industry and tourism, which has motivated the establishment of strategically placed marine reserves for the management of regional biodiversity at the State and Federal levels (D.E.P. 2016; D.P.a.W. 2016).

To support these and other management strategies, there is a growing need to understand the environmental drivers that underpin the distribution and abundance of biodiversity in this bioregion. While some recent research has focused on characterising diversity of marine life in the Kimberley (e.g. Jones *et al.* 2014; Richards *et al.* 2015; Travers *et al.* 2012), spatial processes, including ecological and genetic connectivity, are important yet neglected areas of research among the biota of the inshore Kimberley (Kendrick *et al.* 2016; Underwood *et al.* 2013).

Obtaining an understanding of ecological connectivity within marine systems is fundamental to the design of effective management strategies, such as marine protected areas and regulations for the sustainable harvest of fishery resources (Magris *et al.* 2014; Ovenden *et al.* 2015). In practice however, connectivity is spatio-temporally complex, and detailed studies across multiple scales are needed to reveal the way biogeography, life-history and environment interact in individual taxa. For example, if dispersal is primarily local, recruits

produced from afar are unlikely to contribute to the local recovery of populations after a disturbance.

Nevertheless, occasional recruitment can still be important for maintaining genetic diversity over evolutionary time. Therefore, to manage marine systems effectively, it is important to develop an understanding of this multifaceted nature of connectivity.

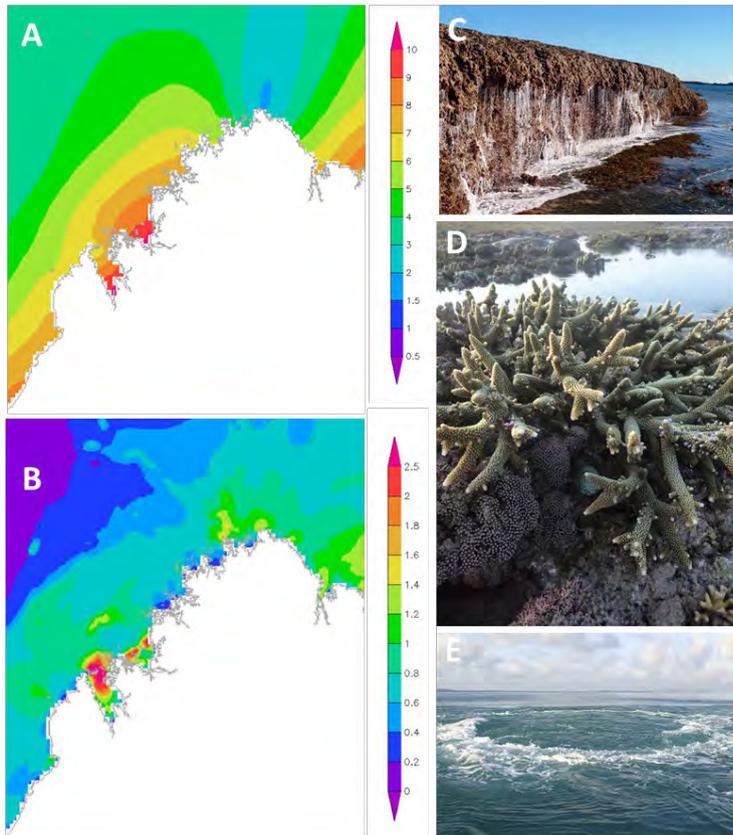


Figure 1. The dynamic Kimberley environment. A. Mean tidal range (m); B. Mean tidal current speed (m/s); C. Tallon (Jalan) Island Cascades at low tide; D. *Acropora aspera* exposed at low tide; E. Large eddy formed by the fast flowing outgoing tide in the Buccaneer Archipelago. Tidal imagery courtesy of the National Tidal Centre.

The inshore Kimberley provides a new frontier for connectivity studies because of the unique and dynamic tidal regime and often harsh environmental conditions (Figure 1). At some inshore locations tidal amplitudes reach nearly 12 metres during spring tides. This means at low tide, intertidal organisms can be exposed to direct sunlight for up to 3 hours at a time (Richards *et al.* 2015). Further, strong tidal currents interact with heterogeneous benthic topography to cause complex, unpredictable and powerful hydrodynamic conditions.

It is unclear how such a unique hydrodynamic regime influences dispersal of marine larvae in the Kimberley. Conceivably it could enhance dispersal, but equally, it could act as a disruptive barrier to dispersal. For example, under maximal tidal velocity (2.5 m/s), a passive propagule could potentially be transported more than 50 kilometres from a natal reef patch during a single (six hour) tide. Alternatively, the influence of recirculating eddies and retention zones created by complex reef topographies or simply the returning tide may result in propagules being retained close to their natal reef (e.g. James *et al.* 2002). These local hydrodynamic effects are potentially made more complex by regional-scale currents and wind-driven effects as well as intermittent influences by cyclones (Radford *et al.* 2014). Exploring these disparate influences on connectivity with a range of model taxa that feature different life history traits will provide new information of direct relevance to conservation planning and resource management in the Kimberley.

3 Methods

3.1 Focal Taxa

Seven organisms (two hard corals, two seagrasses, a mollusc and two fishes) were chosen as models for exploring connectivity in the Kimberley at both fine and broad scales (Figure 2). These species were selected as they were either:

- I. Important habitat forming species;
- II. Harvested species; or
- III. Representative of key trophic levels that may serve as a useful indicator for more vulnerable species.

Focal taxa were also selected according to a range of life history traits that may be influenced by different hydrodynamic processes such as:

- IV. Brooded larvae with short pre-settlement durations;
- V. Spawned larvae with longer pre-settlement durations;
- VI. Demersal egg layer with short pelagic larval duration; and (iv) Sexual reproduction with propagules (seeds) that are dispersed in the water column (floating) or in the sediment (negatively buoyant).



Figure 2. Major functional role, life history, and expected scale of dispersal in target species. PLD = Pelagic larval duration and refers to the average period of larval competency.

3.2 Approach: Population Genetics, Genomics and Otolith Geochemistry

Connectivity is difficult to directly measure for most marine organisms because their dispersal largely occurs during a microscopic planktonic phase, and the scale of movement is potentially very large. A spatial analysis of genetic structure is a widely used “indirect” method for inferring ecological connectivity. Where genetic differences are recorded between sampling individuals or sites, it indicates that dispersal between those sites is also limited to some extent. A useful attribute of genetic analysis is its ability to infer average realised connectivity over multiple generations from a single sample in time. However, this means that inferences about demographic connectivity (i.e. the relative contribution of immigrants and emigrants to total recruitment within a generation) based on genetic analyses need to be made carefully and recognise that history and non-equilibrium population dynamics can also influence genetic structure (Lowe & Allendorf 2010).

Measurement of chemicals embedded in the constantly growing earbones (otoliths) of fishes can also provide information on their movements because unique chemical signatures reflect specific locations that the fish inhabit during their lifetime. Unlike genetic methods, otolith geochemistry is a “direct” method for inferring ecological connectivity in that it provides insight into within-generation movements for fishes at larval, juvenile and adult stages. The combination of longer-term inference from genetic techniques and short-term inference from otolith geochemistry can provide a high level of detail on the movement patterns of fishes. Regrettably, equivalent techniques are not available for other marine organisms. Approach: Sampling design

We employed a hierarchical sampling design, whereby an intensive fine scale study located in the southern Kimberley was nested within a regional study that included sites in the broader Kimberley as well as neighbouring bioregions.

3.2.1 *Broad Scale Study*

Samples were collected opportunistically at 67 sites in the mid-north Kimberley, Pilbara, Gascoyne, and Northern Territory through collaboration with other WAMSI projects, and /or with other research programs (e.g. AIMS offshore atoll research program; WAM Museum Woodside Collection Project; Department of Fisheries WA and NT research programs; Figure 3a). Details of the sample sites for each species can be found in taxon specific reports.

3.2.2 *Fine Scale Study*

The geographic focus for the fine scale study was the complex archipelago of rocky islands and semi-submerged reefs that form the Dampier Peninsula and Buccaneer Archipelago in the southern Kimberley (Figure 3b). The region falls within the southern portion of the Kimberley bioregion, but it also adjoins the King Sound and Canning bioregions. Wherever possible a common sampling design was used which involved collecting samples from 20-50 individuals of each species from up to 26 sites. Sites were generally 200-300 m² and were separated by 1-15 km. Details of the sample sites for each species can be found in taxon specific reports.

3.3 Genetic and Geochemistry Analyses

Samples obtained from a total of 5009 individuals and 157 sites were genotyped using either single nucleotide polymorphism DNA markers (SNPs; corals, mollusc, fishes) or microsatellite DNA markers (seagrass) (Table 1). Wherever possible, taxa were sampled at the same geographic location. SNP genotyping is a state of the art method for population genomic analysis. Its application in the majority of the taxa studied here represents a significant advance over previous connectivity studies on coral reefs due to its increased power to characterise relationships among sites. Fish otolith geochemical analyses were undertaken to provide individual life-histories of fishes by recording the chemical signatures of the environment at larval, juvenile and adult stages as proxies for changes in habitat (environment). 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

Table 1. Total number of sites sampled and individuals genotyped for each focal taxa.

Species	Sites	Individuals	Markers
<i>A. aspera_C</i>	14	388	2894
<i>I. brueggemanni</i>	17	1093	2125
<i>T. hemprichii</i>	17	749	16
<i>H. ovalis</i>	11	407	9
<i>T. niloticus</i>	17	514	5428
<i>P. milleri</i>	28	842	4472
<i>L. carponotatus</i>	53	1016	4468
Total	157	5009	19412

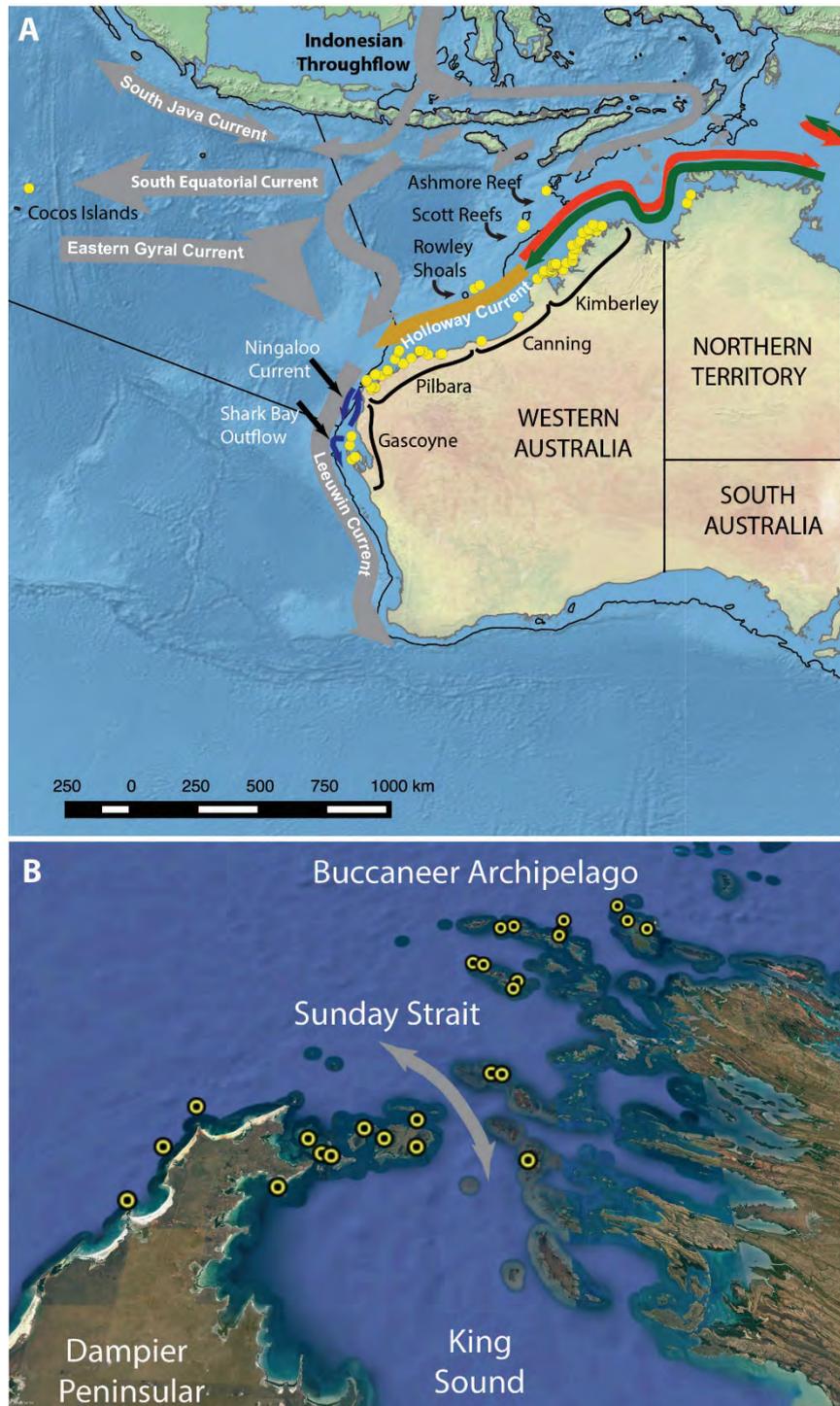


Figure 3. Map of the study sites where genetic samples were collected. A) The broad-scale study; B) The fine scale study. Depicted on panel A are the major surface currents in the Indo- Australian region (adapted from D'Adamo *et al.* 2009; Domingues *et al.* 2007; Schiller 2011; Sprintall *et al.* 2002). Red, green and amber coloured lines indicate flow direction in summer, winter and autumn, respectively.

4 Key Findings

The key findings of this study are summarized in Figure 4:

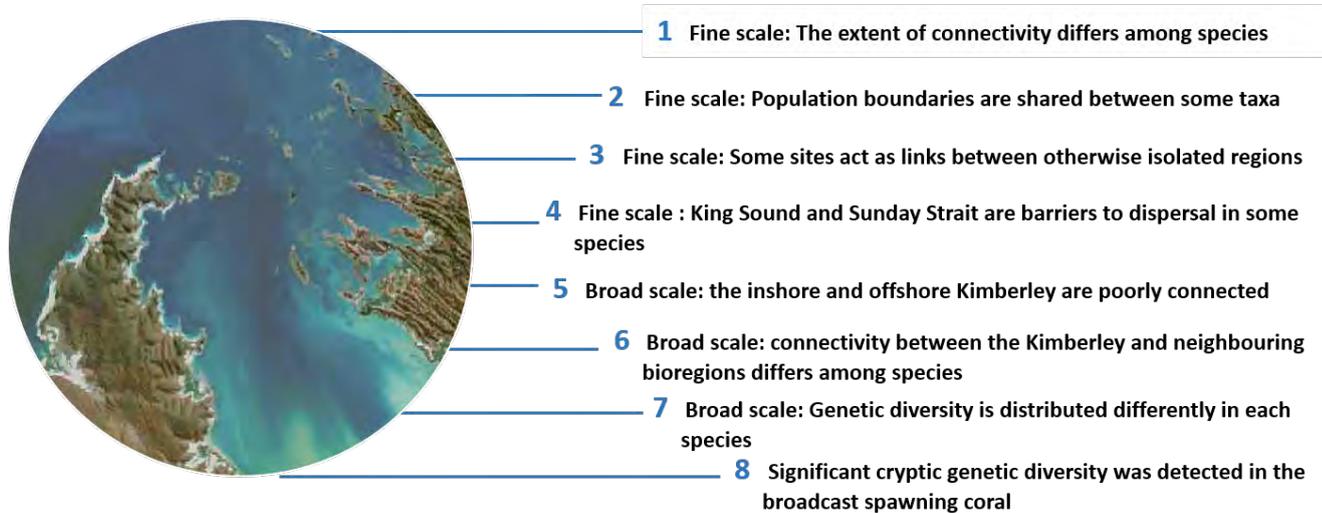


Figure 4. Key findings of KMRP Ecological Connectivity Project 1.1.3.

4.1 Fine scale: The extent of connectivity differs among species

A key finding of this study is that despite experiencing a common set of environmental conditions, the extent of ecological connectivity differed among the focal organisms, and not always in predictable ways. Habitat forming organisms (coral, Report 1.1.3.1; seagrass, Report 1.1.3.2) typically exhibited the most localised population structure, with evidence for limitations to routine dispersal evident on scales of 10s of kilometres or less. In the remaining organisms (fishes, Reports 1.1.3.4a and b; trochus, Report 1.1.3.3), population structure was weaker or not detectable, and limits to dispersal were evident on scales of 80 to several 100s kilometres (Figure 5). Some of these results were unexpected. For example, the seagrass with floating seeds had finer scale genetic structure compared with the seagrass with sinking seeds, and similarly, the pelagic spawning fish also had finer scale genetic structure compared to the benthic spawning fish. Further, the mollusc with a short larval duration exhibited the lowest level of genetic structure of all taxa (Figure 5). **Clearly, expectations of realised connectivity based on simple life history characteristics are unreliable, and patterns therefore need to be assessed on a species by species basis.**

4.2 Fine scale: Population boundaries are shared between some taxa

Major population boundaries were identified in several taxa, notably the habitat-forming corals (Report 1.1.3.1), and seagrasses (Report 1.1.3.2), and the pelagic spawning fish (Report 1.1.3.4b), but not the mollusc (Report 1.1.3.3), nor the damselfish (Report 1.1.3.4a). Broadly, the divisions in seagrasses, corals and fish were between the Dampier Peninsula and Buccaneer Archipelago sites, but the specific positions and breadths of the boundaries differed for individual taxa (Figure 6). For example, in *T. hemprichii*, the seagrass with buoyant seeds, the northern Buccaneer Archipelago sites were differentiated from those in the southern Buccaneer Archipelago and Dampier Peninsula (Figure 6A), whereas both the broadcast spawning and brooding corals exhibited a strong division between the Dampier Peninsula and the Buccaneer Archipelago (Figure 6B). A division also exists in the fish, *L. carponotatus*, but it occurred as a broad transition zone in which the genetic composition changes across a distance of c. 40km at the tip of the Dampier Peninsula from the Kimberley bioregion signature to the Pilbara/Canning bioregion signature (see also Major Finding 6). In contrast, *T.*

niloticus forms a single highly-mixed genetic unit within the Dampier Peninsula and Buccaneer Archipelago, suggesting considerable exchange of larvae occurs throughout this region. Section 4.4 below further evaluates the reasons why a barrier may exist between Buccaneer Archipelago and Dampier Peninsula.

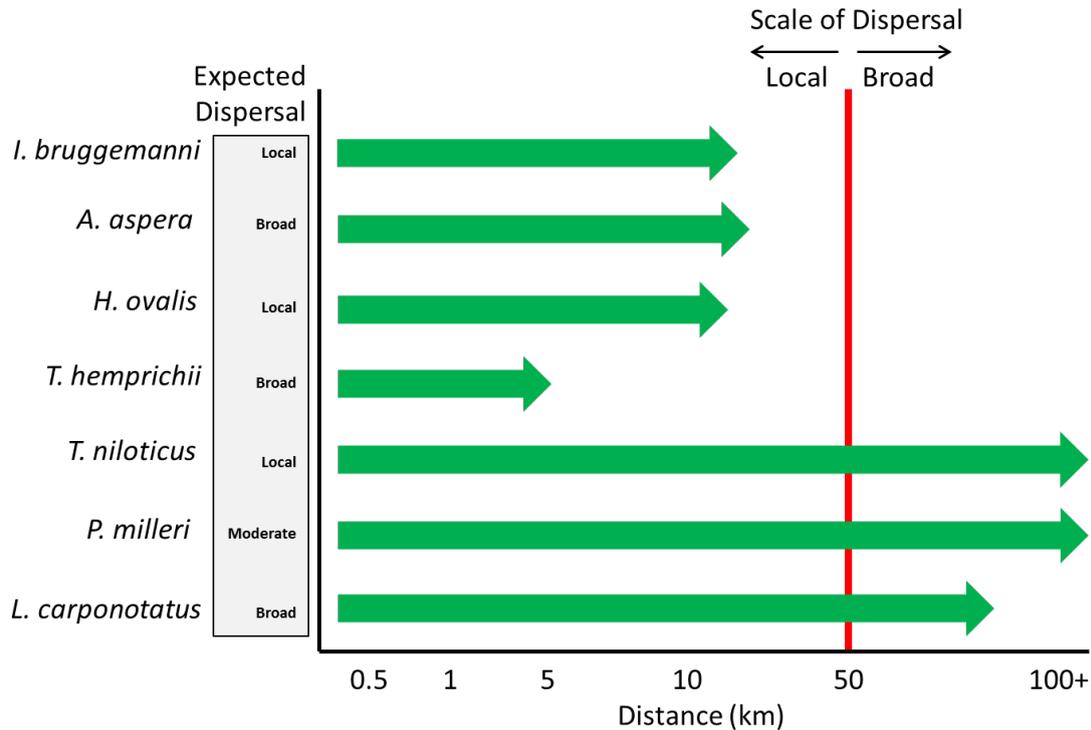


Figure 5. Expected and realised scale of connectivity of focal species in the Dampier Peninsula and Buccaneer Archipelago. The maximum detectable scale of genetic structure (green arrow) is based on spatial autocorrelation analyses and the genetic correlation coefficient (r) between individuals.

4.3 Fine scale: Some sites act as links between otherwise isolated regions

Although restricted connectivity was detected in the region of Sunday Strait and the Dampier Peninsula for corals (Report 1.1.3.1), seagrasses (Report 1.1.3.2), and *L. carponotatus* (Report 1.1.3.4b), exchange of genes across this barrier over multiple generations occurs through the important stepping-stones at Tide Rip, Mermaid and Bedford Islands for corals and seagrass. For *L. carponotatus* a similar transition zone was detectable between Tallon Island and Emeriau Point (Dampier Peninsula).

4.4 Fine scale: King Sound, Sunday Strait are barriers to dispersal in some species

The region at the mouth of King Sound is characterised by the largest tropical tidal range and the fastest tidal currents in the world including the input of massive volumes of freshwater in a highly turbid plume from the Fitzroy catchment in the wet season; a time when propagules from many of these species are in the plankton. These extreme environmental conditions appear to restrict connectivity. Coupled with the finding of a highly divergent population of *I. bruggemanni* on the western side of Dampier Peninsula, these results demonstrate that the tip of Dampier Peninsula is an important intra-specific genetic barrier for various marine taxa with range of life histories.

4.5 Broad scale: The inshore and offshore Kimberley are poorly connected

The species of corals (Report 1.1.3.1) and trochus (Report 1.1.3.3) that were sampled over broader scales at the offshore reefs of Rowley Shoals, Scott Reefs, and Ashmore Reef showed that these inshore Kimberley reef populations are highly divergent from the offshore 'oceanic' reef populations, strongly indicating that these regions are ecologically and evolutionary independent. This likely reflects the limited hydrodynamic

connectivity between these reefs (Figure 7), but in addition, genetic patterns suggest strong environmental differences between these regions has driven local adaptation in these species.

4.6 Broad scale: connectivity between the Kimberley and neighbouring bioregions differs among species

The species that were sampled across the broader northwest coast of Australia exhibited some consistencies in their broad-scale patterns of connectivity. The seagrass *T. hemprichii* (Report 1.1.3.2) and the damselfish *P. milleri* (Report 1.1.3.4a) exhibited a sharp discontinuity between the Kimberley and Pilbara, indicating negligible exchange, and probably reflecting discontinuous habitat between these regions. In contrast, Pilbara and Kimberley populations of *L. carponotatus* (Report 1.1.3.4b), exhibited only weak genetic distinctiveness. Furthermore in *L. carponotatus*, the transition zone between Kimberley and Pilbara genetic groups occurred at Sunday Strait rather than corresponding to the Pilbara and Kimberley Bioregions like *T. hemprichii* and *P. milleri*. *Lutjanus carponotatus* samples from the Northern Territory were weakly genetically distinct from those in the Kimberley, but it is unclear whether this represents limited demographic exchange, or incomplete sampling in the intervening region.

The otolith geochemistry results (Chapters 1.1.3.4c) generally concur with the findings of the genetic companion studies of the two fish species (Chapters 1.1.3.4a, b), and add support to their conclusions that the movements of both species are restricted between the Kimberley, Pilbara and Gascoyne management bioregions. This preliminary result should be considered cautiously as the margin otolith microchemistry only reveals movements in the adult phase and additional core samples will need to be analysed to allow interpretation of population connectivity during larval and post-larval phases. Furthermore, 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 (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.

4.7 Broad scale: Genetic diversity is distributed differently in each species

Within the Dampier Peninsula – Buccaneer Archipelago region, some organisms (coral (Report 1.1.3.1), seagrass (Report 1.1.3.2)) exhibited large variation between sites in amount of genetic diversity observed, whereas others (fishes (Report 1.1.3.4a and b), trochus (Report 1.1.3.3)) exhibited similar amounts of diversity at each site. Across the broader northwest coast of Australia, species varied significantly in their distributions of genetic diversity. Populations of the seagrass *T. hemprichii* from the Kimberley exhibited significantly lower genetic diversity than those in the Pilbara. In contrast, in the damselfish *P. milleri*, genetic diversity was highest in the Kimberley and declined progressively with latitude towards the Gascoyne bioregion. In the stripey snapper, *L. carponotatus*, levels of genetic diversity were consistent across the entire northwest coast. These contrasting results likely reflect: 1) differences in population size; 2) differences in connectivity between regions (physical and environmental); and 3) differences in colonisation history of the different regions. Further, multiple hotspots (i.e. areas with high genetic diversity or unique variants) were identified at particular sites for coral and seagrass (e.g. West Montalivet for *I. brueggemanni* and Bedford Island south for *H. ovalis*), and these are discussed further in the specific taxon reports.

4.8 Cryptic genetic diversity exists in the broadcast spawning coral

Four genetically distinct, but morphologically cryptic, genetic lineages were detected in the *A. aspera* collection (Report 1.1.3.1), strongly suggesting that these lineages are reproductively isolated, even though they look the same and live side by side, and thus likely represent unique evolutionary significant units and/or unrecognised species.

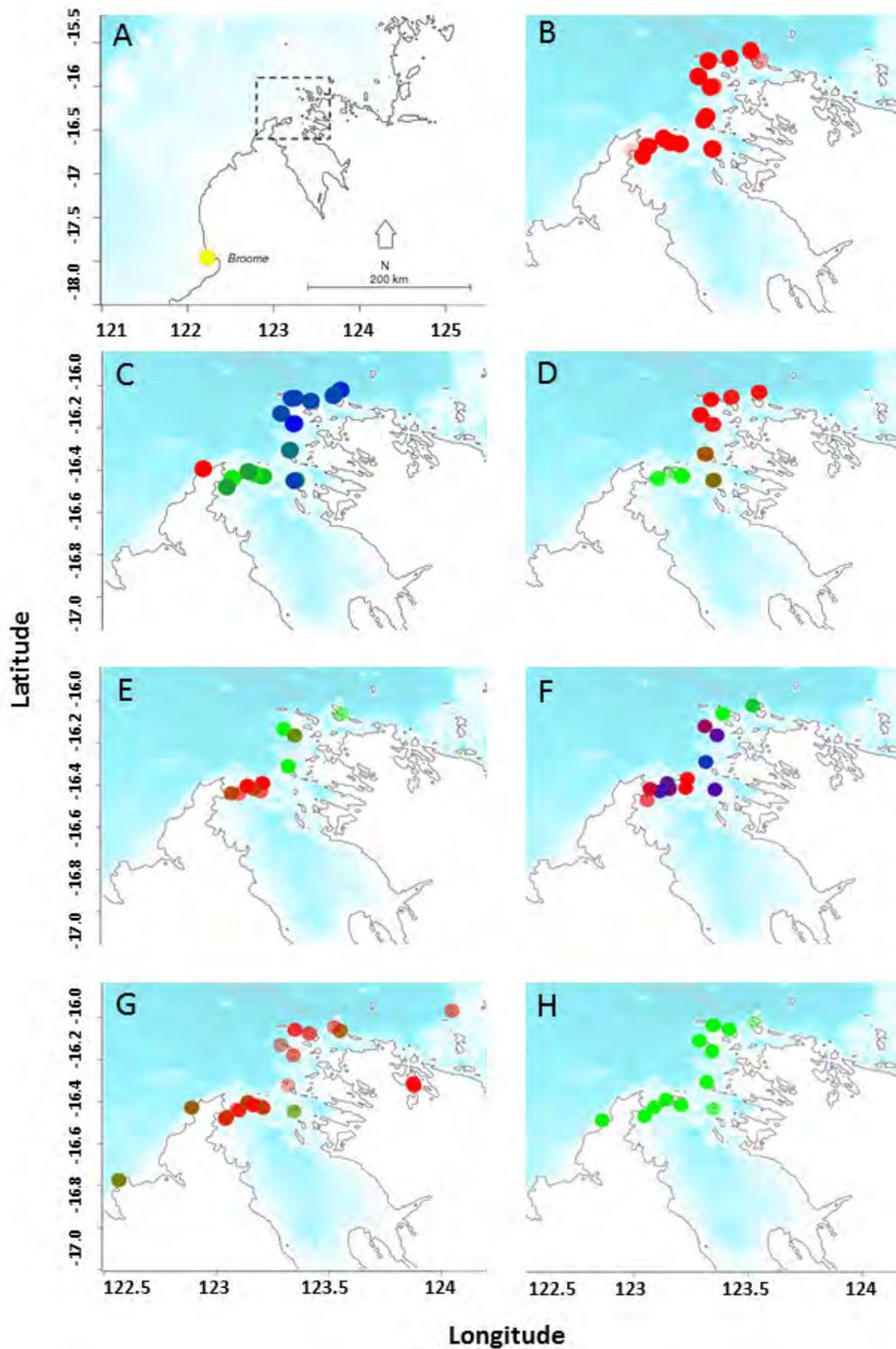


Figure 6. Population structuring within the southern Kimberley. A) The region where fine-scale analysis was conducted; B) A single interconnected population exists for the harvested mollusc *T. niloticus*; C) Three population clusters were evident in the brooding coral *Isopora brueggemanni*; D) Two population clusters were evident in the broadcast spawning coral *Acropora aspera* but zones of admixture occurred in Sunday Strait; E) Two population clusters were evident in the seagrass *Halophila ovalis*; F) Three population clusters were detected for *Thalassia hemprichii*; G) Two population clusters were evident in the pelagic spawning fish *L. carponotatus* but one transitioned to the other across the study region; H) A single population was detected for the demersal nesting reef fish *P. milleri*.

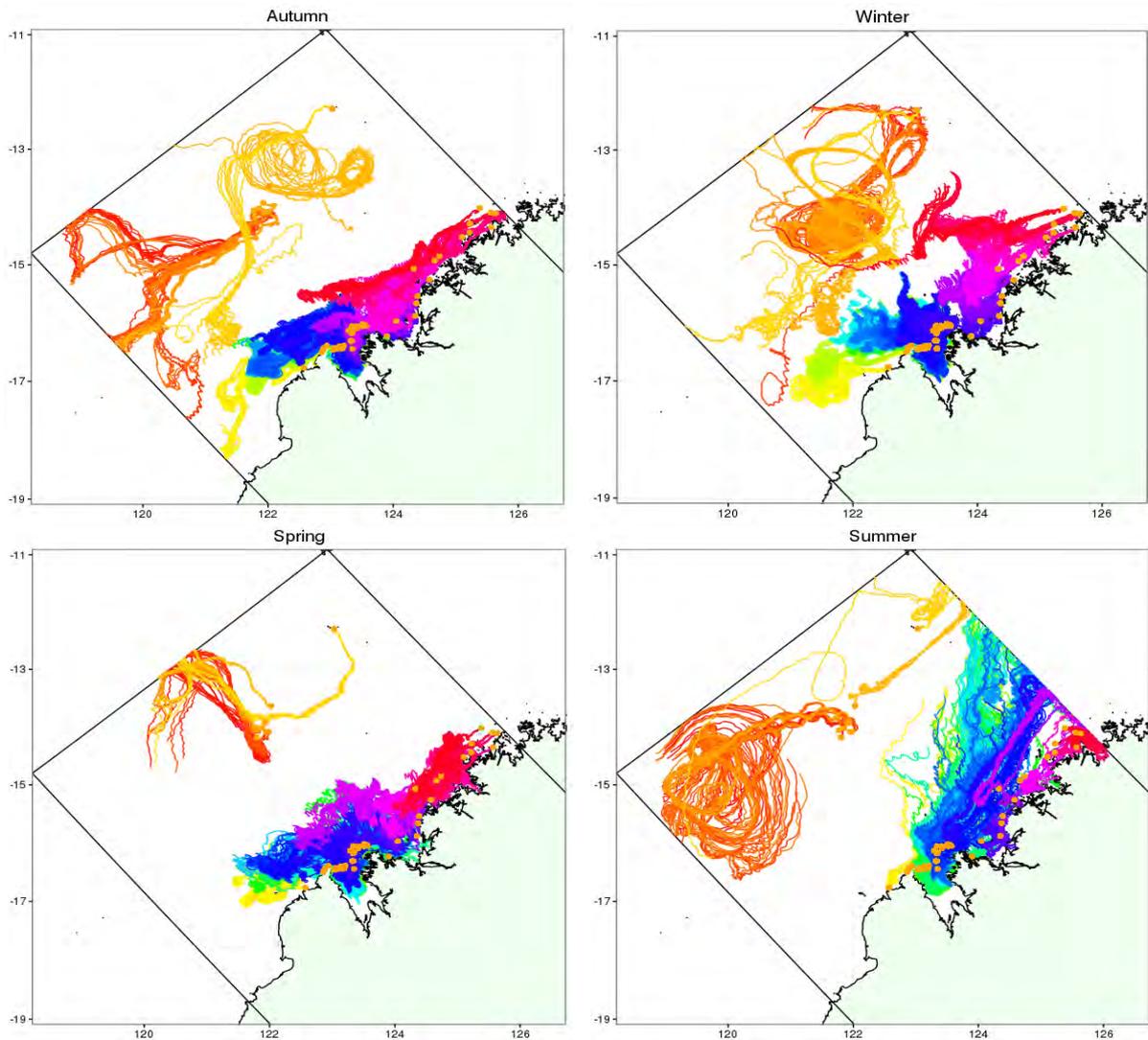


Figure 7. Seasonal particle tracking plots. Indicated are the modelled particle trajectories for passive particles based on 40 days pelagic larval duration. Orange circles represent sampling sites, with particles from each site designated by a unique colour. Data courtesy of Ming Feng (CSIRO; WAMSI Kimberley Project 2.2.7), and plots courtesy Dirk Slawinski (CSIRO).

5 Overarching Implications for Management

This research has highlighted commonalities and disparities in patterns of connectivity among taxa representing a range of trophic levels and life histories. Many of these findings have important implications for management of Kimberley marine ecosystems. Threats to these ecosystems include local anthropogenic impacts such as overfishing, tourism, industrial development and oil spills, as well as the impacts of climate change, which operates over broader spatial scales and longer time-frames. The resilience of marine ecosystems to these threats depends critically on how they affect ecological processes such as connectivity, which promote population persistence and regeneration. Management strategies that protect healthy sources of recruits and maintain the exchange of adaptive genes will nurture resilience in marine ecosystems. To this end, below we summarise how the patterns of connectivity identified in this project would best inform management of Kimberley marine ecosystems. Following this, we provide answers to the original questions posed in the KMRP Agreement for the Ecological Connectivity 1.1.3 project (Appendix 1):

1. **To protect hard corals, the crucial habitat forming organisms of coral reef ecosystems and also seagrass, an important food source for dugongs and turtle, and a nursery habitat for fishes, marine protected areas and indigenous protected areas need to incorporate strategies that account for the spatial dispersal of these organisms.** Protected areas that are large enough to encompass routine dispersal distances of corals (10–20 km), and are spaced at similar distances, will not only maintain self-replenishment, but also aid recovery after disturbance through connectivity between protected areas.
2. **Corals and seagrasses of Buccaneer Archipelago and Dampier Peninsula need to be managed as demographically independent populations.** Furthermore, negligible exchange between the inshore Kimberley and the offshore coral reefs and neighbouring bioregions means that populations of the inshore Kimberley are reliant on standing genetic variation as the basis of adaptation to climate change or other disturbances.
3. **Current estimates of species diversity in corals are likely to be substantial underestimates.** The cryptic *Acropora* coral lineages detected here reveal that current assessments of the diversity of hard coral species in the Kimberley are likely substantial underestimates and further integrated taxonomic research is needed to clarify species diversity patterns in all taxon groups.
4. **Management of *T. niloticus* on the Dampier Peninsula and Buccaneer Archipelago should treat the region as being effectively a single stock on the ecological timeframes relevant to harvest management.** Over-harvested sites within this region will be replenished with recruits from neighbouring sites within years, assuming they exist, and allowing for the slow growth of the species.
5. **Management of *T. niloticus* at offshore oceanic reefs should treat each oceanic shoal as being effectively isolated on the ecological timeframes relevant to harvest management.** Recruitment from outside will not replenish over-harvested stocks at these locations. Occasional recruits may be drawn from other offshore shoals, but will contribute to genetic diversity not offset over-harvest. Supplementation of populations should recognise that coastal *T. niloticus* populations may be mal-adapted to oceanic conditions.
6. **The Kimberley and Pilbara bioregions exchange few recruits in seagrasses and reef-obligate damselfishes, and therefore operate largely independently on the ecological timeframes relevant to management.**
7. **Demographic exchange between the Kimberley and Pilbara/Canning bioregions in the harvested stripey snapper, *L. carponotatus*, occurs in a broad transition zone located near the Sunday Strait. The distinctiveness of the Shark Bay *L. carponotatus* samples from all other bioregions indicates that the Gascoyne management boundary is not supported because sites north of Shark Bay have greater affinities to sites in the Pilbara Bioregion.** This information should be considered within management arrangements.
8. **Genetic differentiation between samples of *L. carponotatus* from the Kimberley and Northern Territory may represent limited demographic exchange between these separately-managed stocks, but to be confirmed this requires further samples from the intermediate region.**

6 Outcomes and Benefits

This project provides the first estimates of ecological connectivity for a range of animals and plants in the Kimberley marine bioregion. This data set indicates the region is largely demographically and genetically independent from neighbouring bioregions, but further research is needed to examine the relationships with the central and northern Kimberley and the broader biogeographic relationships with Indonesia. New empirical data generated in this project can be considered in the design of marine protected areas especially in the size required to protect self-sustaining populations. The new information also informs management of fishery stocks and will benefit future risk assessments for numerous species of high interest to state management agencies (Department of Parks and Wildlife, Department of Fisheries [WA]) because of their habitat-forming nature and/or commercial and indigenous harvest.

This project has also improved links and collaboration in marine science between State and Commonwealth agencies, universities, industry and indigenous rangers and communities (Bardi Jawi and Mayala) in Western Australia. The approach used in this project can serve as a template for investigating ecological connectivity in other bioregions throughout Western Australia (e.g. Pilbara), while the results can form the basis for developing hypotheses about levels of connectivity in other bioregions.

7 Conclusion

Population “connectivity” depends on the magnitude of immigration and migration within and between populations and has the potential to profoundly influence the resilience of communities to natural and anthropogenic disturbances. When coupled with the distribution of biological communities, patterns of connectivity provide meaningful justification for marine protected area design and other resource management decisions.

Results of this study suggest for all taxa examined (with the exception of *T. niloticus*) movement and gene flow in the southern Kimberley is limited to scales of less than ~ 20km. There are important hotspots of genetic diversity along with transition zones which act as conduits of gene flow and dispersal between otherwise isolated reefs. The macro-tidal conditions experienced in the Dampier Peninsula – Buccaneer Archipelago are largely a barrier to the immigration of larvae from outside the Kimberley bioregion and in the case of *A. aspera* may have led to a high level of cryptic speciation. Moreover the Kimberley bioregion and some areas within it is largely a demographically independent system, requiring targeted management to safeguard its unique marine resources.

By delivering the first region-specific and multi-species assessment of connectivity we have provided an empirical basis for planning and managing the regional network of Kimberley marine parks and reserves, and significantly improved the knowledge base for environmental planning and impact and risk assessments by other groups.

8 References

- D'Adamo ND, Fandry C, Buchan S, Domingues C (2009) Northern sources of the Leeuwin Current and the 'Holloway Current' on the North West Shelf. *Journal of the Royal Society of Western Australia* 92, 53-66.
- D.E.P. (2016) Marine. <http://www.environment.gov.au/topics/marine>
- D.P.a.W. (2016) Marine Environment. <https://www.dpaw.wa.gov.au/management/marine/>
- Domingues CM, Maltrud ME, Wijffels SE, Church JA, Tomczak M (2007) Simulated Lagrangian pathways between the Leeuwin Current System and the upper-ocean circulation of the southeast Indian Ocean. *Deep-Sea Research Part II -Topical Studies in Oceanography* 54, 797-817.
- James MK, Armsworth PR, Mason LB, Bode L (2002) The structure of reef fish metapopulations: modelling larval dispersal and retention patterns. *Proceedings of the Royal Society B-Biological Sciences* 269, 2079-2086.
- Jones D, Bryce C, J F, G M (2014) Marine Biodiversity of the Kimberley 1880s-2009. In: *Records of the Western Australian Museum*.
- Kendrick GA, Wilkinson SP, Friedman K, et al. (2016) Strategic marine ecological research priorities for CALM Act marine parks and reserves 2016–2021. *Conservation Science of Western Australia* 10.
- Lowe WH, Allendorf FW (2010) What can genetics tell us about population connectivity? *Molecular Ecology* 19, 3038-3051.
- Magris RA, Pressey RL, Weeks R, Ban NC (2014) Integrating connectivity and climate change into marine conservation planning. *Biological Conservation* 170, 207-221.
- Ovenden JR, Berry O, Welch DJ, Buckworth RC, Dichmont CM (2015) Ocean's eleven: a critical evaluation of the role of population, evolutionary and molecular genetics in the management of wild fisheries. *Fish and Fisheries* 16, 125-159.
- Radford B, Babcock R, Van Niel K, Done T (2014) Are cyclones agents for connectivity between reefs? *Journal of Biogeography* 41, 1367-1378.
- Richards ZT, Garcia RA, Wallace CC, Rosser NL, Muir PR (2015) A Diverse Assemblage of Reef Corals Thriving in a Dynamic Intertidal Reef Setting (Bonaparte Archipelago, Kimberley, Australia). *Plos One* 10(2): e0117791.
- Schiller A (2011) Ocean circulation on the North Australian Shelf. *Continental Shelf Research* 31, 1087-1095.
- Sprintall J, Wijffels S, Chereskin T, Bray N (2002) The JADE and WOCE I10/IR6 Throughflow sections in the southeast Indian Ocean. Part 2: velocity and transports. *Deep-Sea Research Part II-Topical Studies In Oceanography* 49, 1363-1389.
- Travers MJ, Potter IC, Clarke KR, Newman SJ (2012) Relationships between latitude and environmental conditions and the species richness, abundance and composition of tropical fish assemblages over soft substrata. *Marine Ecology Progress Series* 446, 221-241.
- Underwood JN, Wilson SK, Ludgerus L, Evans RD (2013) Integrating connectivity science and spatial conservation management of coral reefs in northwest Australia. *Journal for Nature Conservation* 21, 163-172.
- Wilson B (2013) *Biogeography of the Australian North West Shelf: Environmental Change and Life's Response Elsevier*.



Population connectivity and genetic diversity in brooding and broadcast spawning corals in the Kimberley

Jim Underwood^{1,5}, Zoe Richards^{2,3,5}, Oliver Berry^{4,5}, James Gilmour^{1,5}

¹Australian Institute of Marine Science, Townsville, Queensland

²Western Australian Museum, Perth, Western Australia

³Curtin University, Bentley, Western Australia

⁴CSIRO, Crawley, Western Australia

⁵Western Australian Marine Science Institution, Perth, Western Australia

WAMSI Kimberley Marine Research Program Executive Summary

Subproject 1.1.3.1

August 2017



western australian
marine science institution



Department of Biodiversity,
Conservation and Attractions



Department of
Primary Industries and
Regional Development



Mayala
Community



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.

Ownership of Intellectual property rights

Unless otherwise noted, copyright (and any other intellectual property rights, if any) in this publication is owned by the Western Australian Marine Science Institution, CSIRO, Australian Institute of Marine Science, Edith Cowan University, The University of Western Australia, Western Australia Department of Primary Industries and Regional Development, Western Australian Museum, Curtin University and the Western Australian Department of Biodiversity, Conservation and Attractions.

Copyright

© Western Australian Marine Science Institution

All rights reserved.

Unless otherwise noted, all material in this publication is provided under a Creative Commons Attribution 3.0 Australia License. (<http://creativecommons.org/licenses/by/3.0/au/deed.en>)



Legal Notice

The Western Australian Marine Science Institution advises that the information contained in this publication comprises general statements based on scientific research. The reader is advised and needs to be aware that such information may be incomplete or unable to be used in any specific situation. This information should therefore not solely be relied on when making commercial or other decision. WAMSI and its partner organisations take no responsibility for the outcome of decisions based on information contained in this, or related, publications.

Front cover images (L-R)

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

Image 2: Pink *Seriatopora hystrix* nestled into *Isopora brueggemanni*; both brooding corals from the intertidal zone of Longitude Island in the Buccaneer Archipelago. (Image: Jim Underwood)

Image 3: Humpback whale breaching (Image: Pam Osborn)

Image 4: *Isopora brueggemanni*, a brooding reef-builder in the intertidal zone of Irvine Island. (Image: Jim Underwood)

Year of publication: August 2017

Metadata: <http://catalogue.aodn.org.au/geonetwork/srv/eng/metadata.show?uuid=fb1d80bf-6ef2-4150-9479-22b4240435a7>

Citation: Underwood J, Richards Z, Berry O, Gilmour J (2017) Population connectivity and genetic diversity in brooding and broadcast spawning corals in the Kimberley. Report of Project 1.1.3 - Project 1.1.3.1 prepared for the Kimberley Marine Research Program, Western Australian Marine Science Institution, Perth, Western Australia, 48pp.

Author Contributions: All authors contributed to the drafting of this text.

Corresponding author and Institution: Oliver Berry, CSIRO Oceans and Atmosphere. oliver.berry@csiro.au

Funding Sources: This project was funded (commissioned) by the Western Australian Marine Science Institution as part of the WAMSI Kimberley Marine Research Program, a \$30M program with seed funding of \$12M provided by State government as part of the Kimberley Science and Conservation Strategy. The Program has been made possible through co-investment from the WAMSI Joint Venture partners and further enabled by data and information provided by Woodside Energy Ltd.

Competing Interests: The commercial investors and data providers had no role in the data analysis, data interpretation, the decision to publish or in the preparation of the manuscript. The authors have declared that no competing interests exists.

Kimberley Traditional Owner agreement: This research was enabled by the Traditional Owners through their advice, participation and consent to access their traditional lands.

Acknowledgements: We are grateful to all WAMSI staff for their assistance, and in particular Kelly Waples, Stuart Field and Kim Friedman for providing advice and constructive criticism throughout the project. Many other people and organisations contributed to the success of this project, and we thank them sincerely. We especially thank the following Indigenous communities. The Bardi and Jawi Niimidiman Aboriginal Corporation, the Bardi Jawi Rangers and the Bardi Jawi Traditional Owners and specifically Daniel Oades, Damon Pyke, Azton Howard, Chris Sampi, Kevin George, Kevin Ejai, Kevin Dougal, Tasha Stumpagee, Phillip McCarthy, Peter Hunter, Zac Ejai, Paul Davey and Trevor Sampi. The Mayala people, specifically Sandy, Alec and Janella Isaacs. The Wunambal Gaambera Aboriginal Corporation, Traditional Owners, Unguu Rangers and Tom Vigilante. The Dambimangari Aboriginal Corporation, Dambimangari Traditional Owners and the Dambimangari Rangers. Thanks also to the Bioinformatic pipeline development: Bernd Gruber (University of Canberra); Oceanographic modelling: Ming Feng, Dirk Slawinski (CSIRO); SNP marker development and genotyping: Andrzej Kilian (Diversity Arrays Technology); Field assistance: Sam Moyle and Fiona Webster; Kimberley Marine Research Station for facilities and logistical support: James Brown, Michael Flynn, Scott Whitlam, Duncan Smith and Erin McGinty; and advice on project development: Karen Miller (AIMS).

Collection permits/ethics approval: SF008440, SF009910, SC001362 (Western Australian Department of Parks and Wildlife); 2485 2085, 2344 (Western Australian Department of Fisheries)

Executive Summary

This study utilised next generation sequencing to explore patterns of ecological connectivity and genetic diversity among populations of two species of staghorn coral in the Kimberley; the brooding *Isopora brueggemanni* and broadcast spawning *Acropora aspera*. These two species display the common reproductive modes in hard corals which create the crucial three dimensional structures that provide the habitat and protection that is the foundation of coral reef ecosystems. Both species are listed as “vulnerable” on the IUCN Red List of Threatened Species based on the general estimates of reef degradation within their range as well as their inherent susceptibility to bleaching and disease.

Samples of *I. brueggemanni* (n=612) and *A. aspera* (n=563) were collected across three hierarchical spatial scales. At broad (inter-regional) scales, sites were separated by 100s of kilometres and included the offshore site of Ashmore Reef, as well as inshore reefs of the Bonaparte Archipelago in the central Kimberley, and the Buccaneer Archipelago and Dampier Peninsula in the southern Kimberley. At the intermediate (inter-reef) scale, detailed sampling was undertaken at the Buccaneer Archipelago and Dampier Peninsular, where multiple sites were separated by distances of kilometres to tens of kilometres. At the local (within-reef) scale, sampling allowed for estimates of genetic structure over distances of tens to 100s of metres. Analyses revealed considerable genetic structure within both species at all three scales.

For the brooder, *I. brueggemanni*, 2,125 SNPs revealed three discrete genetic clusters over broad scales; Ashmore Reef in the north, Kooljaman in the far west of the Dampier Peninsula, and the southern inshore Kimberley. At the intermediate scale, the observed level of genetic structure in *I. brueggemanni* indicated that connectivity over more than 20 km is generally rare. More specifically, Dampier and Buccaneer corals formed two genetic groups, but with geographically intermediate Islands of Mermaid and Tide Rip sharing genetic affinities with both groups. Therefore, these two islands appear to be important stepping stones for maintaining occasional connectivity and genetic exchange across the Sunday Strait. At fine scales, significant differentiation was detected between subsites, and colonies separated by less than 500 metres were more closely related than those further apart, indicating that most brooded larvae recruit within a few hundred metres of their natal colony. A general attenuation of gene diversity was detected with increasing latitude, indicating that effective population sizes are larger, and genetic connections to exogenous sources are stronger, in populations in the central region compared with those in the south.

For the broadcast spawner, initial genetic analysis of the entire *A. aspera* collection using a subset of SNPs revealed the presence of four lineages that were genetically distinct but morphologically cryptic. The large magnitude of genetic differentiation among these lineages indicated these lineages are reproductively isolated, even though they look the same and live side by side. The subsequent analyses of population connectivity used 2,894 SNPs to focus on the most abundant and widespread lineage, *Acropora asp-c* (n = 322). Consistent with a greater propensity for widespread dispersal in the broadcast spawned larvae compared with the brooded larvae, the overall amount of genetic subdivision in the *Acropora asp-c* lineage ($F_{ST} = 0.101$) was half that of *I. brueggemanni* ($F_{ST} = 0.230$). Nevertheless, the pattern of geographic structure evident in *Acropora asp-c* was similar to *I. brueggemanni*, with four discrete genetic clusters detected over broad scales among Ashmore Reef, central Kimberley, Buccaneer Archipelago and Dampier Peninsula. At intermediate scales, genetic patterns in *Acropora asp-c* corals also matched those found in *I. brueggemanni*; spawned larvae rarely disperse more than 35 km while corals from Tide Rip and Mermaid Islands exhibited affinities to both the Dampier and Buccaneer clusters. Lastly, at fine scales, relatedness was relatively high among corals separated by less than 500 metres, indicating that many spawned larvae recruit back to their natal reef patch. Levels of gene diversity within the *Acropora asp-c* lineage appeared to be greater in the central Buccaneer Archipelago, and attenuated to west and east from this centre, suggesting that these are the largest and most well connected populations of this species in the region.

The oceanographic model supported the broad scale genetic patterns, with no evidence of any inter-regional connectivity via ocean currents between the offshore and inshore reefs. However, a more biologically realistic oceanographic model is required to properly capture the complex fine-scale hydrodynamics in this region.

Implications for management

The key finding from this study is that ecological connectivity among populations of both the brooding coral and broadcast spawning coral is restricted to the scale of reef or reef patch, with few larvae dispersing more than 35 kilometres from their natal reef patch. This finding has important ramifications for the managers, policy makers and custodians of coral reefs of the Kimberley. Specifically, it implies that locally produced recruits are crucial to the persistence of coral populations, and recovery after disturbance will rarely be supplemented through the input of larvae from locations that are more than a few tens of kilometres away. Therefore, if the intention of Marine Protected Areas (MPA's) and Indigenous Protected Areas (IPA's) is to protect hard corals, they must consider the importance of local recruitment for population maintenance, recovery and adaptation to environmental change by ensuring the maintenance of connectivity networks among reef patches by positioning multiple sanctuaries over scales of less than a few tens of kilometres.

Further specific management considerations include:

- Exchange of genes between the inshore Kimberley and the offshore coral reefs is negligible meaning that inshore populations will rely on maintenance of standing genetic variation to recover from and adapt to natural and anthropogenic impacts.
- The Dampier Peninsula and Buccaneer Archipelago need to be managed as demographically independent systems, with the important consideration that Tide Rip and Mermaid Islands provide stepping stones of genetic exchange that likely augments population resilience and adaptation over multiple generations.
- For the brooding coral, the west coast of Dampier Peninsula appears to support a small, isolated, and genetically unique population that is demographically independent from populations east of the Dampier Peninsula.
- The high genetic diversity at the central Kimberley site of West Montalivet in the Bonaparte Archipelago indicates that these reefs are important reservoirs of genetic variation and have strong connections with other populations, making them priorities for conservation.
- The discovery of four genetically divergent lineages within *Acropora aspera* means that morphological assessments of biodiversity of hard corals in the Kimberley are likely substantial underestimates. Additionally, the effective population size of each lineage will be much smaller, and consequently more vulnerable to disturbance, than expected if assessments are based on distribution of the single morphospecies.

The details of this report are currently subject to a journal publication process. For more information contact the author: Dr Jim Underwood, Australian Institute of Marine Science J.Underwood@aims.gov.au.



Population genetic diversity, structure and connectivity of two seagrass species, *Thalassia hemprichii* and *Halophila ovalis* in the Kimberley

Kathryn McMahon^{1,3}, Udhi Hernawan^{1,3}, Kathryn Dawkins^{1,3}, Kor-Jent van Dijk^{2,3},
Michelle Waycott^{2,3}

¹Edith Cowan University, Joondalup, Western Australia

²University of Adelaide, Adelaide, South Australia, Australia

³Western Australian Marine Science Institution, Perth, Western Australia

WAMSI Kimberley Marine Research Program

Final Report

Subproject 1.1.3.2

August 2017



western australian
marine science institution



Department of Biodiversity,
Conservation and Attractions



Department of
Primary Industries and
Regional Development



Mayala
Community



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.

Ownership of Intellectual property rights

Unless otherwise noted, copyright (and any other intellectual property rights, if any) in this publication is owned by the Western Australian Marine Science Institution, CSIRO, Australian Institute of Marine Science, Edith Cowan University, The University of Western Australia, Western Australia Department of Primary Industries and Regional Development, Western Australian Museum, Curtin University, and the Western Australian Department of Biodiversity, Conservation and Attractions.

Copyright

© Western Australian Marine Science Institution

All rights reserved.

Unless otherwise noted, all material in this publication is provided under a Creative Commons Attribution 3.0 Australia License. (<http://creativecommons.org/licenses/by/3.0/au/deed.en>)



Legal Notice

The Western Australian Marine Science Institution advises that the information contained in this publication comprises general statements based on scientific research. The reader is advised and needs to be aware that such information may be incomplete or unable to be used in any specific situation. This information should therefore not solely be relied on when making commercial or other decision. WAMSI and its partner organisations take no responsibility for the outcome of decisions based on information contained in this, or related, publications.

Front cover images (L-R)

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

Image 2: The seagrass *Thalassia hemprichii* growing around Jalan Island, Sunday Islands, Kimberley, WA (Image: Kathryn McMahon)

Image 3: Humpback whale breaching (Image: Pam Osborn)

Image 4: The seagrass *Halophila ovalis* growing around Aloon Island, Sunday Islands, Kimberley, WA (Image: Kathryn McMahon)

Year of publication: August 2017

Metadata: <http://catalogue.aodn.org.au/geonetwork/srv/eng/metadata.show?uuid=fb1d80bf-6ef2-4150-9479-22b4240435a7>

Citation: McMahon K, Hernawan U, Dawkins K, van Dijk K, Waycott M (2017) Population genetic diversity, structure and connectivity of two seagrass species, *Thalassia hemprichii* and *Halophila ovalis* in the Kimberley. Report of Project 1.1.3 - Project 1.1.3.2 prepared for the Kimberley Marine Research Program, Western Australian Marine Science Institution, Perth, Western Australia, 46pp.

Author Contributions: All authors contributed to the drafting of this text.

Corresponding author and Institution: Oliver Berry, CSIRO Oceans and Atmosphere. oliver.berry@csiro.au

Funding Sources: This project was funded (commissioned) by the Western Australian Marine Science Institution as part of the WAMSI Kimberley Marine Research Program, a \$30M program with seed funding of \$12M provided by State government as part of the Kimberley Science and Conservation Strategy. The Program has been made possible through co-investment from the WAMSI Joint Venture partners and further enabled by data and information provided by Woodside Energy Ltd.

Competing Interests: The commercial investors and data providers had no role in the data analysis, data interpretation, the decision to publish or in the preparation of the manuscript. The authors have declared that no competing interests exists.

Kimberley Traditional Owner agreement: This research was enabled by the Traditional Owners through their advice, participation and consent to access their traditional lands.

Acknowledgements: We are grateful to all WAMSI staff for their assistance, and in particular Kelly Waples, Stuart Field and Kim Friedman for providing advice and constructive criticism throughout the project. Many other people and organisations contributed to the success of this project, and we thank them sincerely. We especially thank the following Indigenous communities. The Bardi and Jawi Niimidiman Aboriginal Corporation, the Bardi Jawi Rangers and the Bardi Jawi Traditional Owners and specifically Daniel Oades, Damon Pyke, Azton Howard, Chris Sampi, Kevin George, Kevin Ejai, Kevin Dougal, Tasha Stumpagee, Phillip McCarthy, Peter Hunter, Zac Ejai, Paul Davey and Trevor Sampi. The Mayala people, specifically Sandy, Alec and Janella Isaacs. The Wunambal Gaambera Aboriginal Corporation, Traditional Owners, Uunguu Rangers and Tom Vigilante. The Dambimangari Aboriginal Corporation, Dambimangari Traditional Owners and the Dambimangari Rangers. Thanks also to the Bioinformatic pipeline development: Bernd Gruber (University of Canberra); Oceanographic modelling: Ming Feng, Dirk Slawinski (CSIRO); SNP marker development and genotyping: Andrzej Kilian (Diversity Arrays Technology); Field assistance: Sam Moyle and Fiona Webster; Kimberley Marine Research Station for facilities and logistical support: James Brown, Michael Flynn, Scott Whitlam, Duncan Smith and Erin McGinty; and advice on project development: Karen Miller (AIMS).

Collection permits/ethics approval: SF008440, SF009910, SC001362 (Western Australian Department of Parks and Wildlife); 2485 2085, 2344 (Western Australian Department of Fisheries)

Contents

- EXECUTIVE SUMMARY I**
- INTRODUCTION I
- INSIGHTS ON LOCAL-SCALE PATTERNS IN THE KIMBERLEY I
- INSIGHTS ON BROAD-SCALE PATTERNS II
- PROCESSES INFLUENCING POPULATION GENETIC STRUCTURE, CONNECTIVITY AND DIVERSITY II
- IMPLICATIONS FOR MANAGEMENT III**
- KEY RESIDUAL KNOWLEDGE GAPS..... III**
- 1 INTRODUCTION 1**
- 1.1 SEAGRASSES 1
- 1.2 GENETIC CONNECTIVITY 1
- 1.3 THE KIMBERLEY 2
- 1.4 RESEARCH QUESTIONS 2
- 2 MATERIALS AND METHODS 3**
- 2.1 GENERAL APPROACH 3
- 2.2 SPECIES SELECTED 3
- 2.3 SITES SAMPLED..... 4
- 2.4 SAMPLE COLLECTION 5
- 2.5 DNA EXTRACTION 6
- 2.6 GENOTYPING 6
- 2.6.1 *Halophila ovalis* 6
- 2.6.2 *Thalassia hemprichii* 6
- 2.7 GENETIC ANALYSIS 7
- 2.7.1 *Clonality and diversity* 7
- 2.7.2 *Genetic differentiation and structure* 7
- 2.7.3 *Spatial autocorrelation*..... 7
- 2.7.4 *Genetic connectivity* 8
- 2.8 DRIVERS OF GENETIC DIFFERENTIATION: SPATIAL DISTANCE, OCEANOGRAPHIC CONNECTIVITY AND ENVIRONMENT..... 8
- 2.9 GENETIC RESILIENCE 9
- 3 RESULTS..... 10**
- 3.1 *HALOPHILA OVALIS*..... 10
- 3.1.1 *Genetic diversity* 10
- 3.1.2 *Population genetic differentiation and structure* 11
- 3.1.3 *Spatial autocorrelation*..... 13
- 3.1.4 *Genetic connectivity* 13
- 3.1.5 *Drivers of genetic differentiation*..... 15
- 3.2 *THALASSIA HEMPRICHII* 15
- 3.2.1 *Genetic diversity* 16
- 3.2.2 *Population genetic differentiation and structure* 17
- 3.2.3 *Spatial autocorrelation*..... 20
- 3.2.4 *Genetic connectivity* 20
- 3.2.5 *Drivers of genetic differentiation*..... 22
- 3.3 *GENETIC RESILIENCE OF SEAGRASS POPULATIONS IN THE SOUTH WESTERN KIMBERLEY* 25

4	DISCUSSION	27
4.1	GENETIC CONNECTIVITY	27
4.1.1	<i>Fine-scale</i>	27
4.1.2	<i>Broad-scale</i>	27
4.2	GENETIC DIVERSITY	28
4.2.1	<i>Fine-scale</i>	28
4.2.2	<i>Broad scale</i>	29
4.3	DRIVERS OF GENETIC CONNECTIVITY	29
4.4	RECOMMENDATIONS FOR MANAGEMENT	30
5	REFERENCES.....	32
6	ACKNOWLEDGEMENTS	35
7	DATA AVAILABILITY	35
8	COMMUNICATION	35

Executive Summary

Introduction

The ecological connectivity of seagrasses, an important benthic habitat in coastal waters, was assessed in the Kimberley, where the marine bioregions, Canning, King Sound and Kimberley meet. Seagrasses provide food resources and habitat for a variety of organisms, stabilize sediments and can store considerable quantities of carbon. In the tropics they provide food for a number of endangered and significant fauna, particularly the dugong and green turtle. There is very little data on seagrasses in the Kimberley. We have some understanding of the biodiversity, but not detailed information of the spatial distribution, population biology or ecology of most species. A body of work is developing through this and other WAMSI research on seagrasses (Projects 2.2.4) in the Sunday Islands, an area with large populations of seagrass.

Two seagrass species were selected, *Thalassia hemprichii* (turtle grass) and *Halophila ovalis* (paddle weed) as they are the most common species across the study region, have contrasting dispersal strategies and represent key ecological values. *Halophila ovalis*, is a small, fast-growing species with a colonizing life-history strategy and is commonly consumed by dugong. Seeds are negatively buoyant, therefore have a low dispersal potential and studies to date have shown limited connectivity and high levels of differentiation over small spatial scales. In contrast, *Thalassia hemprichii* is a large habitat forming species with a persistent life-history strategy and is a favoured food source of green turtles. It produces buoyant fruits that have the potential to disperse over a period of 7-10 days while the fruits remain buoyant. Its sister species in the Caribbean has been documented to disperse over 350 km. Both species are currently under investigation in other projects across Australia and Indonesia, led by the authors of this report, allowing us to compare the patterns found in the Kimberley to Indonesia and more broadly across Australia.

Insights on local-scale patterns in the Kimberley

Population structure and connectivity

There were some clear differences between the two species: populations of *H. ovalis* were more genetically distinct (measured by F_{ST}), but both species showed spatial genetic structure. There were two clear population clusters but the split among populations was slightly different for each species. For *T. hemprichii* the northern Buccaneer Archipelago sites were outliers, separated from the remaining sites further south. Sites in Northern King Sound acted as a stepping-stone between Bedford Island and the Sunday Islands. For *H. ovalis* the sites in the Sunday Islands group separated from those in the Buccaneer Archipelago, and Northern King Sound acted as a genetic stepping-stone. For *T. hemprichii* the majority of dispersal and connectivity occurs over 5 km, indicating that dispersal outside of meadows is rare, whereas for *H. ovalis* it occurs over 20 km. This is in contrast to our predictions that *T. hemprichii*, which has buoyant fruits, would have a greater dispersal distance compared to *H. ovalis*, which has non-buoyant seeds that are released into the sediment. These distances over which individuals are most closely related can be used to indicate the appropriate size of spatial management units.

Genetic diversity

The genetic diversity at sites was defined by the clonal diversity (number of clones) and genetic diversity (allelic diversity and heterozygosity). Clonal diversity was generally higher for *T. hemprichii* compared to *H. ovalis*, while allelic diversity and heterozygosity was much lower in *T. hemprichii* compared to *H. ovalis*. Both species had hotspots and coolspots of genetic diversity, but these sites did not overlap. Overall we ranked the genetic resilience of each site. *T. hemprichii* was generally shown to have higher resilience overall compared with *H. ovalis*, although the sites of strong resilience were different for each species.

Insights on broad-scale patterns

Population structure and connectivity

We examined broad scale patterns in genetic structure for *T. hemprichii* across the Indo-Australian Archipelago by combining this data with that of Hernawan et al. (in press). Western Australian populations in the Kimberley and Pilbara group together, and are separated from four other strongly supported clusters in the Indonesian Archipelago. Interestingly the Australian Territory of Cocos Keeling Island is more closely related to Javanese populations than the Australian populations, most likely driven by oceanographic connectivity of the South East Equatorial Current. Kimberley populations are quite isolated, as the strongest paths of migration are from Indonesia to the Pilbara. There is no stepping stone pattern from Indonesia, to the Kimberley and then the Pilbara, which can be potentially attributed to historical isolation of the Kimberley populations or isolation by oceanography.

Genetic diversity

The Coral triangle in Indonesia is the centre of the range for *T. hemprichii*, and like many other marine species the genetic diversity was greatest here and declined with increasing distance from this location. The outliers to this pattern were the Kimberley populations, which were closer spatially to the center of the range than the Pilbara populations but had a much lower genetic diversity

Processes influencing population genetic structure, connectivity and diversity

Using oceanographic and particle transport modeling, an oceanographic connectivity metric was generated which was the average number of particles or seeds exchanged between sites. Oceanographic distance, the probability of particles dispersing on currents between sites, more consistently supported the patterns of genetic differentiation than spatial distance for both species. There was a significant but small effect of isolation by distance for *T. hemprichii* only, while both species showed significant genetic differentiation with oceanographic distance. This was investigated further for *T. hemprichii* in a more sophisticated analysis where multiple factors were considered simultaneously. Overall, the patterns of genetic differentiation were best explained by a combination of oceanographic connectivity mediated by environmental conditions. The environmental characteristic that best explained these patterns was sediment type. It is possible that the type of sediment may influence the success of recruitment and survival of the dispersing seeds.

However, most dispersal for *T. hemprichii* is occurring over distances of 5 km, thus, despite the clear potential for long-distance dispersal in this species, the extreme tidal environment of this region does not appear to be promoting dispersal, but restricting it. Dispersal distances are much lower than has been observed in the sister species in the Caribbean. Likewise, the population differentiation of *H. ovalis* is best explained by oceanographic distance, but in this case the distances dispersed are further than predicted based on the ability of non-buoyant seeds to disperse. However, if the plants fragment, which often occurs following grazing by dugongs, the fragments, with seeds attached, could float in the water column and disperse, and fragments are viable for about 8 days. Due to the dormant seeds, biotic vectors such as dugongs can also disperse *H. ovalis*, and seeds are viable after passing through these animals. Fragmentation and biotic dispersal are mechanisms that need to be investigated further for understanding connectivity in *H. ovalis*.

Implications for management

Based on the findings of genetic connectivity in the two seagrass species, routine dispersal distances that maintain populations are in the order of 5-20km, with connectivity over larger distances occurring less frequently. Therefore marine reserve systems need to account for this scale in order to protect these processes, particularly in the instance of recovery from disturbance. These areas should be replicated across the two main population groups that show limited interaction, in the Sunday Islands and Buccaneer Archipelago (northern part for *T. hemprichii*). Ideally, the placement of protected areas should also consider sites that are well connected to other sites, so have a greater chance of contributing to recovery. Additionally, sites with a higher genetic diversity have a greater potential to adapt to change, or recover from disturbance. With significant changes in the marine environment occurring currently due to global change, the genetic resilience matrix we present in this study could be used when considering site selection. Although the patterns of genetic connectivity and diversity were somewhat different between the two seagrass species, there were some areas that filled most of these criteria, particularly Hal's Pool and Riptide Island.

Key residual knowledge gaps

- Increasing the understanding of genetic connectivity of these species outside of the main study area, east into the northern Kimberley, south into the rest of Canning marine bioregion and more extensively into the Pilbara region.
- Developing a better understanding of the significance of dugong foraging as a mechanism for dispersing seagrasses with dormant seeds (e.g. *H. ovalis*, *H. uninervis*).



1 Introduction

1.1 Seagrasses

Seagrasses are clonal, marine flowering plants that form critical habitat in coastal waters. They are found in all continents except Antarctica, where they provide significant ecosystem services including: primary productivity; a food source for critically endangered fauna such as dugong and green turtles; habitat for many marine flora and fauna including commercially and recreationally important species; sediment stabilization; and carbon storage (Orth et al. 2006). Seagrasses are considered a 'biological group' as they have not evolved from a single lineage, but from four independent evolutionary events between 35 to 65 million years ago (den Hartog 1970, Les et al. 1997, Janssen & Bremer 2004). The grouping is based on their shared traits, which allow them to survive while submerged in saline water. Despite their ancient origins, the species diversity of seagrasses is relatively low, with only 72 species currently recognised based on Short et al. (2011), although the number of species in some genera is debated. Generally most species have broad distributions (Waycott et al. 2004, Waycott et al. 2014).

Globally, seagrasses are threatened with 29% of the known areal extent lost, and since 1990 the loss rate has increased from 0.9% per year to 7% per year, comparable to those reported for mangroves, coral reefs and tropical rainforests (Waycott et al. 2009). Seagrasses are exposed to multiple anthropogenic threats, but are most vulnerable to urban, agricultural and industrial run-off and development, including dredging (Grech et al. 2012). Based on these significant threats and associated losses, conservation and management of seagrass habitat is critical. However, the best way to monitor, manage and conserve seagrass habitats is not clear, due to the variation in the species life-history traits, form of seagrass meadows and the multiple pressures they are exposed to (Kilminster et al. 2015). Effective management of our seagrass communities requires an understanding of these sources of variation. Among the most poorly understood aspects of variation among seagrasses are their genetic diversity and the connectivity within species, which can significantly affect their resilience (Hughes & Stachowicz 2004, Engelhardt et al. 2014, Salo et al. 2015).

1.2 Genetic connectivity

Genetic connectivity or gene flow can be defined as the proportion of newly immigrant genes moving into a given population (*sensu* Endler 1977) or alternatively N_m , the absolute number of individuals exchanged between populations per generation (Wright 1951). This is different to demographic connectivity which is a measure of the relative contributions of dispersal versus local recruitment to population growth (Lowe & Allendorf 2010). In many plant species, most of the seeds will not disperse far, remaining within the meadow they originated in (e.g. Sherman et al. 2016). Thus, they will contribute to demographic connectivity and maintenance of the local population through the addition of new recruits. If the seeds are dormant and a seedbank develops, this provides a mechanism for ongoing local recruitment through time. A seedbank also provides for resilience to the meadow, allowing recovery following disturbance (Unsworth et al. 2015). Dispersal beyond the original meadows by seeds that eventually recruit may establish new populations and/or facilitate genetic connectivity, evidenced by gene flow.

Population resilience, genetic divergence, adaptation and speciation are all influenced by gene flow among populations. Genetic connectivity data can provide insights into both historical population isolation (e.g. Alberto et al. 2008), as well as more contemporary connectivity processes (e.g. Serra et al. 2010). It can also be used to inform restoration and conservation actions (e.g. Evans et al. 2014) including the identification of genetically depauperate populations, isolated populations and the resilience of populations to withstand or recover from disturbance. For example the ability of a seagrass meadow to recover from complete loss such as from a cyclone is dependent on the migration of individuals from adjacent, persistent meadows. In this case, understanding the genetic connectivity between meadows and the spatial distance over which this occurs is critical to predicting the recovery potential of a meadow. This movement and dispersal of seagrasses can occur via sexually produced propagules such as fruits and via vegetative fragments (McMahon et al. 2014). Genetic data can be used to

estimate migration rates for sexual propagules including the direction and magnitude of dispersal. However, discerning the dispersal of vegetative fragments is more challenging. A potential vegetative fragment dispersal could be identified through the presence of shared MLGs among meadows, but disentangling this from growth due to long-lived clones is difficult (McMahon et al. 2014).

The level of gene flow among populations is primarily dependent on interactions between the mode of reproduction, the mobility of individuals and their propagules (Lowe et al. 2004), and local hydrodynamic conditions. Seagrasses have a variety of reproductive strategies due in part, to the polyphyletic nature of the group across four independent lineages (Les et al. 1997) and the various adaptations for underwater sexual reproduction and dispersal. Reproduction strategies include clonal and sexual, and there are different dispersal strategies for pollen, fruits and other propagules such as viviparous seedlings (Kendrick et al. 2012). Therefore, the magnitude of genetic connectivity is likely to vary among species due to these different reproductive modes. The magnitude of genetic connectivity is also likely to vary across the distributional range of a species as the historical and contemporary environmental processes, which also influence gene flow, vary in space.

1.3 The Kimberley

The Kimberley coast on the Australian North West Shelf is rich in biodiversity (Wilson 2013), one of the least human-impacted regions in world (Halpern et al. 2008), but one of the most poorly understood. The coast is highly complex with thousands of islands subjected to an extreme tidal range, up to 11m, the world's largest tropical tides (Wilson 2013). Currents around the islands are multidirectional and can exceed 1 ms^{-1} , producing spectacular ocean conditions including whirlpools and extreme standing waves (Cresswell & Badcock 2000, Wilson 2013, Lowe et al. 2015). It is not clear whether these large tidal currents and associated eddies would enhance or limit dispersal between populations. The local currents are heavily influenced by tide, which override the broader scale, outer continental shelf currents (Condie & Andrewartha 2008).

There is a critical need to understand the ecology of the region due to increases in human activity including petroleum exploration and tourism and traditional, commercial and recreational fisheries. Fauna such as dugongs and green turtles, which reside in the Kimberley, rely on seagrasses for food. Dugongs exclusively feed on seagrass whereas green turtles feed on both seagrass and seaweeds. A number of surveys have documented seagrass species distribution, with larger meadows observed in the western Kimberley (Wells et al. 1995), and currently a range of seagrass monitoring programs are underway throughout the Kimberley, from Roebuck Bay, through the Sunday Islands and east to Woobinbeye Bay (Jackson et al. 2015, Environment 2016, Kimberley 2016). Current research is investigating the significance and drivers of seagrass primary productivity in the western Kimberley, as well as seagrass and turtle grazing interactions (Gary Kendrick and Mat Vanderklift, personal communication). An improved understanding of genetic connectivity, presently limited in the region, will inform the design of effective management strategies, such as marine protected areas and inform on recovery potential of seagrass meadows following any large-scale loss.

1.4 Research questions

This project aims to assess the patterns and drivers of genetic connectivity of two seagrass species in the western Kimberley. The key objectives are to:

- Characterise genetic connectivity at multiple spatial scales in two seagrass species with contrasting dispersal strategies. This will provide species-specific estimates of realised connectivity at the reef-scale (hundreds of metres), inter-reef scale (kilometres-tens of kilometres) and where possible through collaborations with other studies, inter-region scale (tens-hundreds of kilometres);
- Examine the relationship between the potential drivers of genetic connectivity (spatial distance, oceanographic distance, dispersal mode and environment) and genetic connectivity or differentiation;
- Characterise population genetic diversity for two species with contrasting life-history strategies across a number of sites in the western Kimberley, and from this develop an index of genetic resilience; and

- Based on these findings, provide recommendations for the management of seagrass species in the Kimberley.

2 Materials and Methods

2.1 General approach

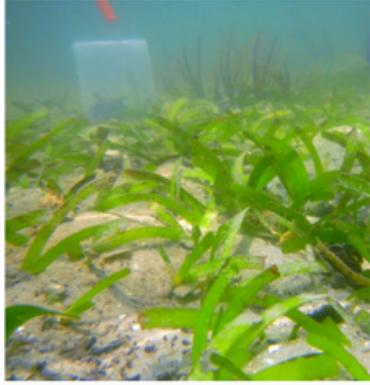
A population genetic approach was used to assess the realized connectivity of seagrass meadows across a range of scales, 5-80 km.

2.2 Species selected

Two seagrass species were selected for inclusion in this study, *Halophila ovalis* and *Thalassia hemprichii* due to the presence across the study region, the contrasting dispersal strategies and the ecological values they provide (Table 1). *Halophila ovalis*, is a small, fast-growing species with a colonizing life-history strategy that forms enduring and transitory meadows (Kilminster et al. 2015). It has a broad Indo-Pacific distribution and is found in most habitats, from the intertidal to deep water (Waycott et al. 2004). It is commonly consumed by dugong (Lanyon et al. 1989). It has small seeds, of which 8-20 develop in single fruits. These are attached at the sediment surface to the rhizome of the plant. The seeds are negatively buoyant, and therefore have a low dispersal potential. Dispersal could occur through the water column by movement of rhizome fragments, with or without fruits attached, through movement of seeds in the sediment by bedload transport or by biotic dispersal with dugongs or potentially birds as vectors (McMahon et al. 2014). Fragments of the genera *Halophila* are known to remain viable for up to 8 days (Hall et al. 2006). Despite these varied dispersal strategies the studies to date have shown limited connectivity among sites and high levels of differentiation over small spatial scales (McMahon et al. 2016, van Dijk et al. in review). The timing of sexual reproduction in the Kimberley is not well understood.

Thalassia hemprichii, is a large habitat forming species with a persistent life-history strategy that forms enduring meadows (Kilminster et al. 2015). It also has a broad Indo-Pacific distribution and is found in a variety of habitats from the intertidal to shallow subtidal, but not deep water (Waycott et al. 2004). This species is a favoured food source of green turtles (Bjorndal 1980) and is regularly grazed in the region. It produces buoyant fruits that have the potential to disperse over a period of 7-10 days while the fruits remain buoyant (Lacap et al. 2002). It's sister species in the Caribbean has been documented to disperse over 350 km (van Dijk et al. 2009). It is possible that vegetative fragments could also disperse if they break from the parent plant, and re-establish in other areas however, the viability time of fragments is not known. Sexual reproduction has been observed in the Kimberley from September to January (A. Z. Perez, personal communication).

Table 1. Features of the two focal seagrass species.

Feature	<i>Thalassia hemprichii</i>	<i>Halophila ovalis</i>
		
Distribution	Tropical Indo-Pacific	Indo-Pacific
Life-history	Persistent	Colonising
Meadow type	Enduring	Enduring & transitory
Reproductive biology	Dioecious	Dioecious
Timing of flowering	Kimberley: Sept – Feb No detailed study	Kimberley: Unknown Tropics can be all year round
Fruit dispersal properties	Buoyant: 2-7 d Fruits dehisce, seeds released Seeds not buoyant, viable 5-10 d Seeds settle & recruit: Prob unknown	Fruits in or on sediment Usually released into sediment Seeds negatively buoyant Dormant and viable for up to 2 yr. Biotic dispersal possible
Vegetative fragment dispersal	Assumed neutrally buoyant Viability time unknown	Assumed neutrally buoyant Viability time up to 8 days Fruits can be transported with fragments

2.3 Sites sampled

This study focused on the western Kimberley in Western Australia where three marine bioregions, Canning, King Sound and Kimberley meet. We focused in three main areas, Sunday Island Group, Buccaneer Archipelago and northeastern King Sound (Figure 1, Table 2). We predicted that there would be more connection within each region than between regions, and that the northeastern King Sound would be a link between the Sunday Islands Group and the Buccaneer Archipelago. Seven sites were sampled in the Sunday Islands Group, four in the Buccaneer Archipelago and two sites in the northeastern King Sound but both species were not collected at each site (Table 2). A number of additional sites were included *ad hoc* to broaden the scope of the study and relied on collaborations with other projects.

Table 2. Location of seagrass sampling sites. Coordinates are based on the WGS 84 grid system. Note that site numbers are the same across all taxa in the 1.1.3 study for ease of comparison.

Site No	Sub-region	Population	<i>Thalassia hemprichii</i>	<i>Halophila ovalis</i>
3	Buccaneer Archipelago	Bathurst Is.	-16.04164 123.52317	
4	Buccaneer Archipelago	Irvine Is.		-16.06437 123.55263
7	Buccaneer Archipelago	Longitude Is.	-16.06936 123.39378	
8	Buccaneer Archipelago	Bedford Is. North	-16.13672 123.29884	-16.13460 123.30029
9	Buccaneer Archipelago	Bedford Is. South	-16.16476 123.34789	-16.16484 123.34796
10	North-eastern King Sound	Riptide Is./Gregory Is.	-16.31016 123.31913	-16.31123 123.31959
11	North-eastern King Sound	Mermaid Is.	-16.44516 123.35142	
12	Sunday Island Group	Sunday Is. –north, Maleny	-16.39642 123.21020	-16.39106 123.20641
13	Sunday Island Group	Sunday Is. –south east, Janinko	-16.42537 123.19805	-16.42949 123.19780
14	Sunday Island Group	Hal’s Pool, Ngooroodool	16.41813 123.16699	-16.41819 123.16698
15	Sunday Island Group	Tallon Is., Jalan	-16.40182 123.13517	-16.40387 123.13906
16	Sunday Island Group	Jackson Is., Aloon	-16.44053 123.10225	-16.44052 123.10226
17	Sunday Island Group	Noyon	-16.43792 123.06940	-16.43844 123.06958
19	Sunday Island Group	Shenton Bluff, Ardinoogoon	-16.48246 123.04702	
20		Woobinbeye Creek		-14.15289 126.53329
21		Cocos Keeling	-12.19832 96.84287	

2.4 Sample collection

A site was defined as a circular area of 50 m diameter. At each site, 50 samples were collected based on randomly generated bearings and distances along the bearing. These were located using compasses and transect tapes. Each sample was separated by a minimum of 2 m and if no seagrass was present at the randomly allocated position, it was collected from the next closest patch of seagrass. If the seagrass was distributed in such a way that this sampling design was not possible, then samples were collected randomly within a similar area. The GPS position of each sample was recorded.

Each sample consisted of a seagrass ramet with 1–3 connected shoots. Samples were stored in seawater at ambient temperature until processing. For *H. ovalis* apical meristems and young leaves were extracted from each sample, and for *T. hemprichii* the young part of the leaves without epiphytes were extracted. All extracted samples were cleaned and stored in silica gel to preserve the DNA within 8 hours of collection. A herbarium voucher specimen of each species from each site was also created.

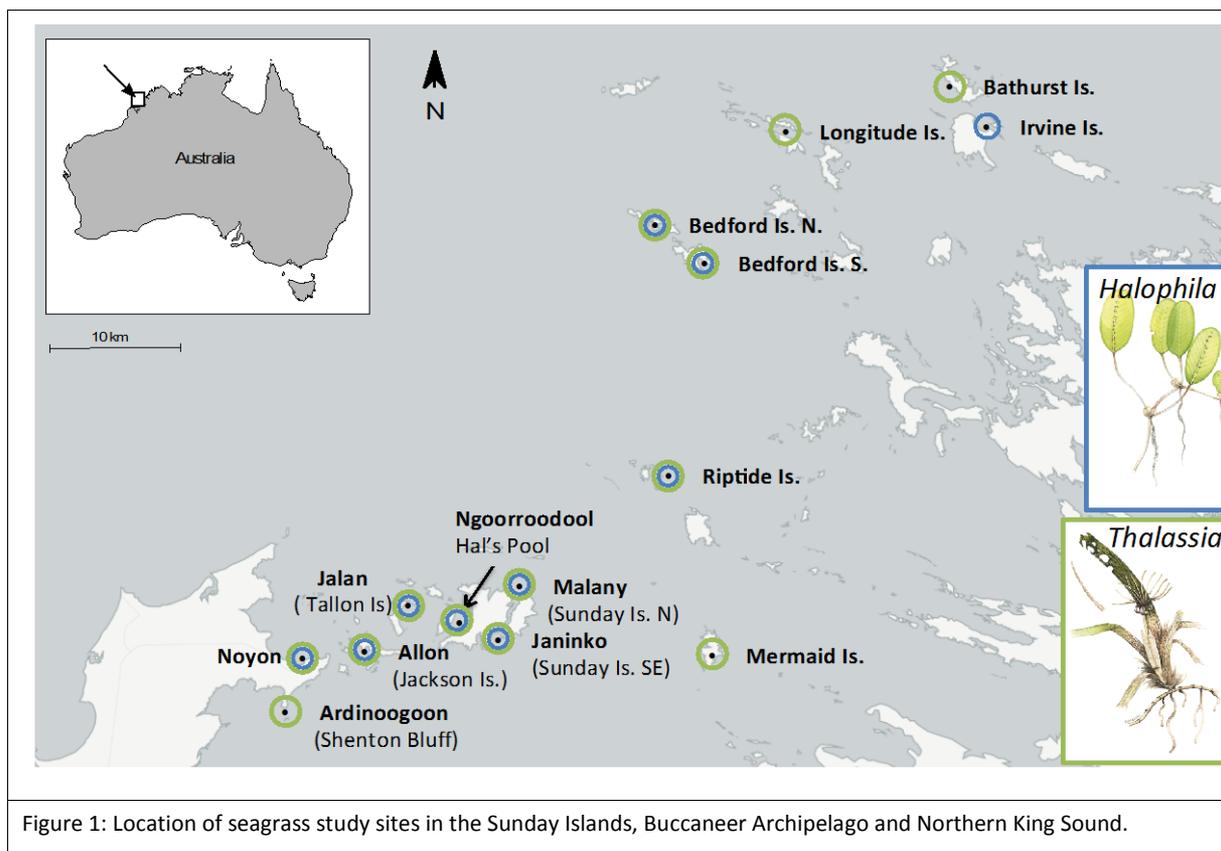


Figure 1: Location of seagrass study sites in the Sunday Islands, Buccaneer Archipelago and Northern King Sound.

2.5 DNA extraction

DNA was extracted from 2–3 leaf pairs, growing tips and/or shoots of silica-dried plant material. All extractions were performed using AGRF extraction service (www.agrf.org.au).

2.6 Genotyping

2.6.1 *Halophila ovalis*

Forty-six to 48 samples from each site were analysed. Genotyping was conducted using 12 species-specific microsatellite markers developed by Xu et al. (2010) and van Dijk (unpublished), of which 7 (Hpo34-11 alleles, Ho31-4 alleles, Hpo55-8 alleles, Ho20-8 alleles, Ho51-6 alleles, Ho8-10 alleles, Hpo46-5 alleles) amplified consistently and were informative. The number of alleles per locus ranged from 4–11. Fluorescently labelled primers were amplified in multiplex reactions using QIAGEN Type-it microsatellite PCR Kit and 0.1–1 ng of DNA template following manufactures guidelines. Fragment analysis by capillary separation was performed at the GGF (Georgia Genomic Facility, USA, <http://dna.uga.edu>) with GGF's size standard 500 ROX. Microsatellite alleles were scored with the Microsatellite plugin in Geneious R7 version 7.1.7 (Biomatters, Auckland, New Zealand). One site, Mermaid Island, was removed from the analysis as the majority of loci did not amplify, despite repeated trials, and those that did amplify, more than two peaks were present. This may indicate that the samples collected at this site were from another taxa or contained polyploids, which impacted the amplification of the loci. The most easterly site, Woobinbeye Creek consistently did not amplify at the locus Ho8.

2.6.2 *Thalassia hemprichii*

Forty-eight samples from each site were analysed. Genotyping was conducted on 16 microsatellite markers developed by van Dijk et al. (2014) and Wainwright et al. (2013): Thh5-5 alleles, Thh34-4 alleles, Thh15-6 alleles, TH66-3 alleles, TH37-7 alleles, TH73-5 alleles, TH43- 6 alleles, Thh8-5 alleles, TH34-8alleles, Thh41-4 alleles, TH52-9 alleles, TH07-4 alleles, Thh29-4 alleles, Thh1-4 alleles, Thh36-4 alleles and Thh3-3 alleles. Multiplex PCR reactions with fluorescently labelled primers were run, analysed and scored as described for *H. ovalis*.

2.7 Genetic analysis

For *Halophila*, genotyping errors were tested with duplicate samples from each population, where the DNA was extracted in a separate reaction. The duplicate samples consistently generated the same results. In addition, for both species, genotyping errors and the presence of null alleles were tested using a maximum likelihood approach implemented in ML-NULLFREQ with 100,000 randomizations (Kalinowski & Taper 2006). This has been shown to be the overall best performing method for null allele detection (Dąbrowski et al. 2015). We tested for linkage disequilibrium across multiple loci based on the standardized index of association (r_D) accounting for different sample sizes using the package POPPR (Kamvar et al. 2014). Departure from Hardy-Weinberg Equilibrium (HWE) was based on the inbreeding coefficient (F_{IS}) calculated in GENETIX 4.05 (Belkhir et al. 2004).

2.7.1 Clonality and diversity

Multilocus genotypes (MLGs) were determined using the POPPR package in R (Kamvar et al. 2014) and expressed as clonal richness ($R = \text{MLG}-1/N-1$), where N stands for the number of samples tested. Samples with missing data were excluded from this to increase confidence in the detection of MLGs. Clone mates were removed from further analyses, so that only one representative of each MLG was included. Genotypic diversity was estimated by allelic richness (average number of alleles per locus) and private alleles (alleles found only at a single site), which were estimated from a standardised number of MLGs (*H. ovalis*: 11, *T. hemprichii*: 28) using rarefaction in HP-Rare (Kalinowski 2005). The genetic diversity including unbiased expected heterozygosity (H'_{exp}) and observed heterozygosity (Hobs) was calculated using GENETIX 4.05.2. Kinship or internal relationship among individuals within a site was calculated using the software Storm (Frasier 2008).

2.7.2 Genetic differentiation and structure

Genetic differentiation was estimated using the descriptor F_{ST} and G_{ST} . Since mutation rates can affect F_{ST} , particularly with highly polymorphic markers, such as microsatellites, then using F_{ST} can lead to bias in estimating genetic differentiation, usually resulting in an underestimation of genetic differentiation. Wang (2015) showed that the mutational effects on F_{ST} can be examined by correlating G_{ST} and H_S across loci. If the correlation (r_{GH}) is highly negative and significant then the F_{ST} is likely to be biased and should not be used (Wang 2015). We examined the r_{GH} using the program CoDiDi and for *Halophila* found a significant and highly negative relationship, raising concern on the use of F_{ST} . However, for *Thalassia* we found a positive relationship ($r_{GH} = 0.554$) suggesting that in our dataset the F_{ST} is not affected by mutation rate, and is thus a reliable measure of genetic differentiation. Pair-wise genetic differentiation was estimated in GenoDive.

Population structure was examined using a Bayesian assignment test in STRUCTURE v2.3.4 (Pritchard et al. 2000). This allows us to identify the number of panmictic clusters (K) among the populations. We set the number of panmictic clusters (K) to be tested from K=1 to K=16, with burn-in=100.000 and replications after burn-in = 1.000.000. We performed 20 iterations for each K value. Determining the “true” K was based on Evanno et al. (2005) from STRUCTUREHARVESTER (<http://taylor0.biology.ucla.edu/structureHarvester/>) (Earl & vonHoldt 2012). CLUMPP V1.1.2 (Jakobsson & Rosenberg 2007) was then employed to align the multiple replicate analysis of the appropriate K. DISTRUCTv1.1 (Rosenberg 2004) was then used to visualize the population structure. The STRUCTURE analysis was conducted on two datasets, (1) all populations within the Sunday Is. and Buccaneer Archipelago, and then (2) all the Kimberley populations beyond the Sunday and Buccaneer Archipelago.

2.7.3 Spatial autocorrelation

Spatial autocorrelation among individuals was assessed in GenAlEx using individual genetic distances and individual spatial distances based on the allelic frequencies following Smouse and Peakall (1999). The test of spatial autocorrelation was based on random permutations and the confidence around this was determined from bootstrapping. Spatial distance categories (km) were set with endpoints of 0.01, 0.025, 0.05, 5, 10, 15, 20, 25, 30, 35, and 45 for *Halophila* and the same for *Thalassia* but with an extra category of 60.

2.7.4 Genetic connectivity

Genetic connectivity was assessed based on the pattern of gene flow indicated by the relative number of migrants per generation \widehat{Nm} (Alcala et al. 2014). This measure is based on the complementary function of both F_{ST} and D . To calculate \widehat{Nm} , we used the function `divMigrate` of the `diversity` package in R (Keenan et al. 2013). For *Thalassia*, as mutation rate does not affect the F_{ST} (based on the `rGH`), we calculated \widehat{Nm} across all loci, but for *Halophila* as there was an indication that F_{ST} was biased and affected by mutation rate we should interpret the network with caution. Visualization of the gene flow was built with the `qgraph` package (Epskamp et al. 2012). The network graph was then analysed to extract four network parameters relating to connectivity for each site. These were: Total strength, sites with the strongest connections; Closeness, sites that are most connected to other sites; Betweenness, the number of shortest connections between two sites that go through the site of interest; and Transitivity, the extent to which adjacent sites are connected to each other. Closeness and betweenness are calculated as a 'cost' instead of 'connection strength', thus it represents the cost needed to connect nodes (higher closeness and betweenness imply a higher degree of isolation) (Barrat et al. 2004, Csardi & Nepusz 2015).

2.8 Drivers of genetic differentiation: spatial distance, oceanographic connectivity and environment

Isolation by distance is a straightforward analysis of connectivity that correlates genetic distance with geographic distance, this was assessed with a paired Mantel test; the pair-wise F_{ST} matrix was compared against the spatial distance matrix. The spatial distance was the shortest distance by water and was calculated in Google Earth. This was plotted as $F_{ST}/(1 - F_{ST})$ by the distance measure.

Oceanographic connectivity was assessed using a biophysical dispersal model based on the Regional Ocean Modelling System (ROMS - M. Feng, unpublished project report) with a 2 km resolution. The model was nested within the Ocean Forecasting for Australia Model 3 (OFAM3) simulation (Yu et al. 2012) and forced by 3-hourly meteorological measures derived from Kobayashi et al. (2015). The model simulation occurred from 2009 to 2012. Hourly sea surface current velocities (0-5 m) were extracted from the model output and used for particle tracking simulations. A total of 100 particles were seeded in each seagrass sampling site and a 4th-order Runge-Kutta sub-time-stepping scheme was used to update the particle locations every hour (Feng et al. 2010) using the random walk effect of $1 \text{ m}^2 \text{ s}^{-1}$. For *Halophila*, as the reproductive period was unknown and dispersal by water is most likely facilitated through rhizome fragments which could be released at any time of year, particles were released throughout the year at 3-day intervals. The probability of a particle being at a particular site was estimated over 8 days, the known viability time of fragments (Hall et al. 2006). In contrast, for *Thalassia* the particle release period was set as the austral spring-summer (September-January) as this is the known fruiting season and the particles were tracked for 7 days based on the potential dispersal duration of the seagrass fruits (Lacap et al. 2002). The grid size for tracking the particles from each sampling site was set to 500m x 500m. Connectivity among sampling sites was estimated as the average number of particles released from site i that were tracked to be in site j , based on 48 simulation replicates in each year of the 4-year time period. The oceanographic connectivity matrix was visualized using the package `qgraph` (Epskamp et al. 2012). A Mantel test was used to test the relationship between the pair-wise oceanographic distance derived from this output and the pair-wise F_{ST} matrix.

For *Thalassia* only we used an additional approach to simultaneously examine the combined effects of genetic distance, oceanographic distance and environment on the patterns of genetic differentiation. Only *Thalassia* was assessed due to less clonality in this species, and hence more individuals at the site level and more sites. Variation partitioning based on partial redundancy analysis (partial-RDA) was used to determine the relative contribution of geographic distance (GD), oceanographic connectivity (OC) and environmental factors based on habitat characteristics (EN) in explaining genetic differentiation (GS). As this analysis required both the response and explanatory variables to be single or multicolumn numeric matrices, we transformed the 'raw' data of GS, GD, OC, and EN into new data frames suitable for the analysis. For GS we performed a principal coordinate analysis (PCoA) on the linearized G_{ST} (Rousset 1997) and a new data matrix was constructed from the positive axes. The

matrix for GD was constructed from a principal coordinate neighbourhood matrix (PCNM) on the pairwise geographic distances, using the first four, out of eight PCNM variables, as these did not display colinearity with GS. For the OC data frame, the pairwise matrix of oceanographic connectivity was transformed into a weighted, directed network based on graph theory using the igraph package in R (Csardi & Nepusz 2015). Four parameters were calculated from this network: (i) strength -defined as the total amount of the weighted connection coming into and out from a sampling site (higher strength indicates higher degree of connectivity), (ii) closeness -defined as the degree to which a site is connected to other sites in a network, (iii) betweenness -the number of shortest connections between two sites that go through the site of interest, and (iv) transitivity, defined as the extent to which the adjacent sites of a site are connected to each other. For calculating closeness and betweenness, the package treats the connection weights as 'cost' instead of 'connection strength', thus it represents the cost needed to connect nodes (higher closeness and betweenness imply a higher degree of isolation) (Barrat et al. 2004, Csardi & Nepusz 2015). The network parameters indicated that Bathurst Island and Longitude Island were oceanographically isolated from the other sites. These network parameters were used for the seascape genetic analysis by running a principal component analysis (PCA) on the centred and scaled values of the network parameters. We constructed the OC data frame based on the first 3 PCA axes representing 90% of the variance. For the EN data frame, the categorical variables (sediment type, habitat type, and the presence of corals) were transformed into dummy variable and combined with the numerical data (water depth and number of other seagrass species). Then, a correspondence analysis (CA, unconstrained ordination) was performed on the transformed environmental data. The ordination plot showed that all sites, with the exception of Bathurst Island and Longitude Island, clustered together, indicating that these two sites were different to the remainder. The variable most responsible in driving the environmental differentiation was sediment type. We constructed the EN data frame based on the first 3 CA axes from the correspondence analysis representing 96% of the variance of the data.

Finally, the basic formula performed in the partial RDAs was ' $GS \sim GD + OC + EN$ '. The analysis decomposed the variation in the response variable GS into components accounted for by the explanatory variable GD, OC and EN. We calculated the adjusted- R^2 to determine the amount of variation attributed to each explanatory variable controlling the effect of the other variables (the conditional effect) and without controlling the effect of the other ones (the marginal effect), and the shared fraction of variation by any combination of explanatory variables (Peres-Neto et al. 2006). This approach is more robust to decompose spatially structured genetic variation than a Mantel test and its derived forms (Legendre & Fortin 2010, Guillot & Rousset 2013, Meirmans 2015). We used the package vegan in R to perform the variation partitioning analysis (Oksanen et al. 2015).

2.9 Genetic resilience

We propose that the resilience of seagrasses to human impacts or natural disturbances can be predicted from a number of genetic measures, and a few studies have confirmed that increased diversity leads to a greater resistance to disturbance (Hughes & Stachowicz 2004). Within seagrasses and other clonal plants the genetic diversity of a meadow is determined by the clonal richness (the number of unique genotypes present) and the genetic variation within these genotypes. We have measured the genetic variation as the allelic richness (the average number of alleles per loci) and the heterozygosity (average number of heterozygotes in the population). Genetic theory predicts that populations with a higher allelic richness have a greater potential to adapt to pressures over generations, and that higher levels of heterozygosity within a population give the population a better capacity to recover from a disturbance immediately following the event (Lowe et al. 2004). The clonal richness of a population implies the relative contribution of vegetative vs. sexual reproduction to maintenance of the population. If clonal richness is low, then vegetative growth is the main process allowing for population growth or meadow expansion and the likelihood of recovery from a seed bank is low. However, if clonal richness is moderate to high then there is a greater likelihood of recovery of the population from sexual reproduction. This is an important point if a meadow is completely lost, as the genetic data implies that there is potential for recovery of the meadow from a seedbank for those species that do develop one, therefore potential pathways of recovery can be predicted. This data is a snapshot in time, and we do not know how these measures of genetic

diversity vary over time. Populations of dynamic species such as *H. ovalis* and *H. uninervis* can fluctuate in abundance, including recruitment and mortality of genets over time, therefore the genetic diversity of a population is not necessarily stable. Sampling at different time points will inform on the stability of this genetic state, and how this data should be incorporated from a management perspective. Accepting this limitation, we have used these three predictions to rank the genetic resilience of seagrass meadows in the Pilbara. We used a relative scale of clonal richness, allelic richness and heterozygosity within species, ranking the higher values as relatively more resilient.

3 Results

3.1 *Halophila ovalis*

Over the entire study area, a total of 51 alleles were observed across 7 microsatellite loci from 365 samples from which 149 MLGs were detected. Six of the seven loci were not in Hardy-Weinberg equilibrium as is common in clonal plants (Sinclair et al. 2014), and for the one locus that was in Hardy-Weinberg equilibrium, null alleles were detected, but at very low frequencies (0.006), therefore this locus was kept in for further analysis. The test for linkage disequilibrium across multiple loci showed a reasonably large and significant standardised index of association ($\bar{r}d = 0.404$, $p=0.001$), indicating a high chance of association between loci (Agapow & Burt 2001). This is common in clonal plants that have high levels of clonality (Meloni et al. 2013).

3.1.1 Genetic diversity

The number of unique genotypes detected (MLGs) was low, 149 out of 365 samples analysed. This resulted in low to moderate clonal richness (R) among sites, ranging from 0.05 to 0.75 (Table 3). Four sites had 6 or less MLGs and 31 was the maximum number of clone mates found at Sunday Is S. The entire population was not in Hardy-Weinberg equilibrium ($F_{IS}=0.312$, $p<0.001$) and this was driven by four sites in particular, Bedford South ($F_{IS}=0.395$), Hal's Pool ($F_{IS}=0.706$), Tallon Island ($F_{IS}=0.209$) and Noyon ($F_{IS}=0.536$), where strong and statistically significant inbreeding was detected (Table 3). A few sites also showed very high levels of inbreeding, but these were not significant, probably due to the low number of individuals in the population (e.g. Irvine Island and Woobinbeye Creek). There were moderate to high levels of linkage disequilibrium at most sites. A number of sites also had a very high level of relatedness, particularly Bedford Island North and South and Hal's Pool.

The number of alleles detected at a site ranged from 9 at Irvine Island and Woobinbeye Creek to 28 at Sunday Island North, and allelic richness from 1.29 at Irvine Island to 3.01 at Noyon. All sites had some private alleles. Heterozygosity varied among sites, 0.048 at Irvine Island to 0.543 at Sunday Island South for the observed heterozygosity (Table 3).

Table 3. Genetic statistics for *Halophila ovalis*. N-number of samples analysed, G-number of multilocus genotypes, G_{Max}-maximum number of clone mates assigned to one MLG, R-Clonal richness, nA-number of alleles, Ar-allelic richness standardized to n=11 (*=not standardized due to low number), pA-private allelic richness, Re-internal relatedness, Ho-observed heterozygosity, Hexp- expected heterozygosity, F_{IS}-Inbreeding coefficient (*=significant, p<0.05), LD-linkage disequilibrium. Grey shading indicates a low number of individuals and less certainty in genetic statistics.

No	Code	Population	N	G	G _{Max}	R	nA	Ar	pA	Re	Ho	Hexp	F _{IS}	LD
4	II	Irvine Is.	41	3	22	0.05	9	1.29*	0.03	0.29	0.048	0.124	0.667	0.04
8	BN	Bedford Is. – north	43	20	5	0.45	14	1.56	0.26	0.72	0.193	0.215	0.103	0.01
9	BS	Bedford Is. – south	41	19	12	0.45	27	2.62	0.18	0.78	0.353	0.578	0.395*	0.62*
10	RI	Riptide Is.	42	32	3	0.75	24	1.83	0.19	0.11	0.317	0.324	0.022	0.23*
12	SN	Sunday Is. – north	26	18	6	0.68	28	2.18	0.24	0.44	0.357	0.374	0.046	0.11*
13	SS	Sunday Is. – south	42	5	31	0.09	19	2.45*	0.11	0.27	0.543	0.533	-0.020	0.55*
14	HP	Halls Pool	35	11	10	0.29	25	2.79	0.05	0.62	0.195	0.641	0.706*	0.66*
15	TI	Talon Is.	40	18	4	0.61	23	2.29	0.14	-0.23	0.371	0.468	0.209*	0.37*
16	JI	Jackson Is.	29	4	24	0.11	15	2.02*	0.16	0.43	0.357	0.2072	0.104	0.61*
17	NY	Noyon	40	13	14	0.31	28	3.01	0.10	-0.17	0.330	0.695	0.536*	0.83*
20	WC	Woobinbeye Creek	28	6	17	0.18	9	1.50*	1.5*	-0.29	0.111	0.162	0.333	-0.06
		OVERALL	365	149	24	0.39	51	2.2	0.15	0.32	0.338	0.722	0.312*	0.40*

3.1.2 Population genetic differentiation and structure

Overall we detected significant genetic differentiation, global F_{ST} 0.380 ± 0.04. There was strong and significant genetic differentiation among most sites, compared pair-wise (Table 4). There were no significant differences between some sites in the Sunday Island group, including Sunday Island North and South, Sunday Island South with Hal's Pool, Sunday Island North with Jackson Island and Hal's Pool with Noyon (F_{ST} ranging from 0.005-0.081). Of those sites with more than ten individuals, where we are more confident of the patterns, the greatest genetic differentiation was between Bedford Island North and Sunday Island North (F_{ST} = 0.521), Bedford Island North and Talon Island (F_{ST} = 0.459) and Riptide Island with Sunday Island North (F_{ST} = 0.475).

These patterns in genetic differentiation were supported by STRUCTURE analysis where two genetic groups (K=2) were best supported (Figure 2). The individuals in these two groups tended to associate based on populations, with the northern and eastern Irvine Island, Bedford Island sites and Riptide Island grouping together, and the remaining Sunday Island sites forming a separate cluster. However, there was some mixture of genetic groups among sites, and some admixture within individuals. When including the additional *Halophila* site, Woobinbeye Creek, it grouped with the Buccaneer Archipelago site.

Table 4. Genetic differentiation statistics for *Halophila ovalis*. Top matrix is $G'_{ST(NEI)}$ and bottom matrix in F_{ST} . Bold values indicate significant differentiation. Bold site codes indicate most confidence due to the higher sample number. For site codes refer to Table 3.

	II	BN	BS	RI	SN	SS	HP	TI	JI	NY	WC
II	-	0.475	0.207	0.401	0.717	0.637	0.426	0.638	0.716	0.335	0.676
BN	0.371	-	0.218	0.395	0.676	0.593	0.429	0.619	0.688	0.385	0.703
BS	0.191	0.139	-	0.222	0.398	0.329	0.329	0.378	0.406	0.121	0.387
RI	0.306	0.256	0.138	-	0.636	0.532	0.396	0.570	0.632	0.251	0.534
SN	0.601	0.521	0.265	0.476	-	0.005	0.176	0.303	0.070	0.319	0.655
SS	0.529	0.448	0.234	0.387	0.005	-	0.053	0.119	0.221	0.172	0.610
HP	0.361	0.299	0.122	0.269	0.124	0.081	-	0.151	0.183	0.029	0.475
TI	0.519	0.459	0.248	0.407	0.192	0.097	0.107	-	0.252	0.215	0.648
JI	0.624	0.554	0.302	0.492	0.079	0.184	0.168	0.187	-	0.283	0.687
NY	0.285	0.260	0.088	0.162	0.212	0.141	0.050	0.141	0.223	-	0.443
WC	0.583	0.569	0.279	0.394	0.513	0.478	0.361	0.508	0.575	0.331	-

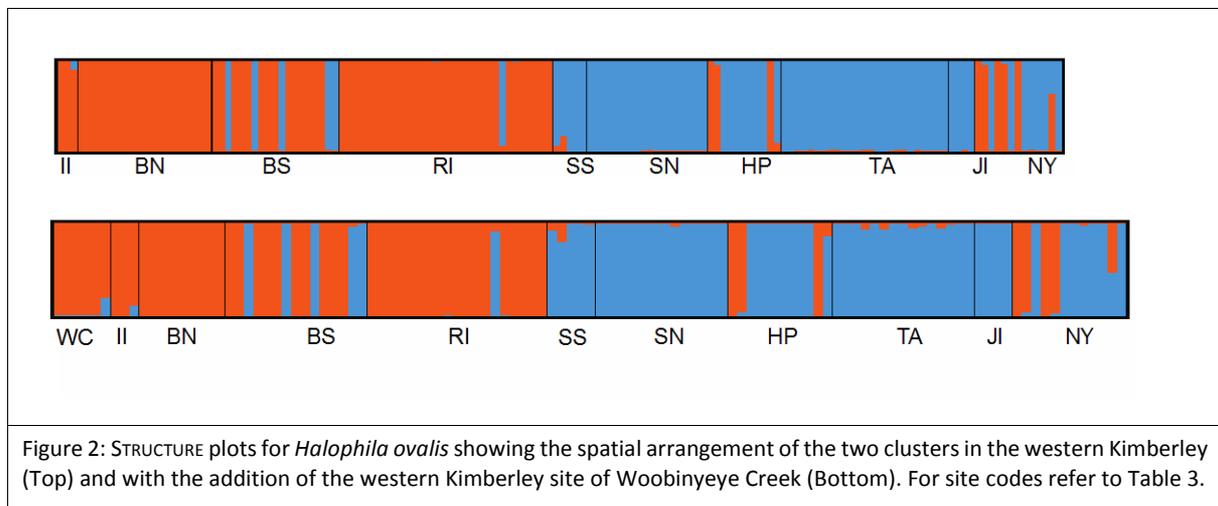
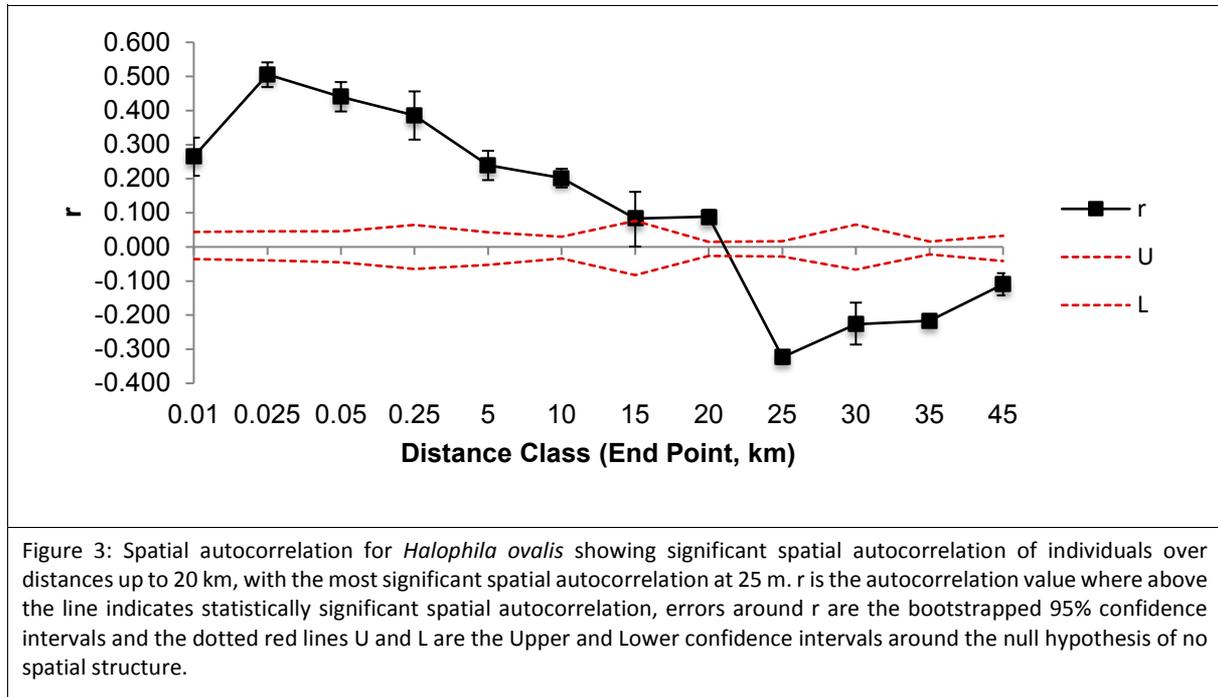


Figure 2: STRUCTURE plots for *Halophila ovalis* showing the spatial arrangement of the two clusters in the western Kimberley (Top) and with the addition of the western Kimberley site of Woobinyeye Creek (Bottom). For site codes refer to Table 3.

3.1.3 Spatial autocorrelation

Significant spatial autocorrelation was detected among individuals, maximizing around 25 m, and then declining over distances up to 10 km (Figure 3). Around 15-20 km very low but significant spatial autocorrelation was detected, and after 20 km there was no significant spatial autocorrelation.



3.1.4 Genetic connectivity

Only sites with > 10 individuals were included in this network analysis as it was based on the population estimates of F_{ST} and D . The most significant migration was detected among sites in the Sunday Islands group, Noyon and Hal's Pool, in both directions, and Sunday Is North to Hal's Pool (Figure 4). There was also evidence of significant migration between the intermediate site Riptide and the Bedford Is North in the Buccaneer Archipelago, moving in a northerly direction (Figure 4).

The sites with the strongest connections are Hal's Pool and Noyon (total strength). These sites, with the addition of Bedford Island South were the most connected to other sites (highest closeness, transivity and lowest cost of betweenness). Bedford Island North was the least connected to other sites (Table 5).

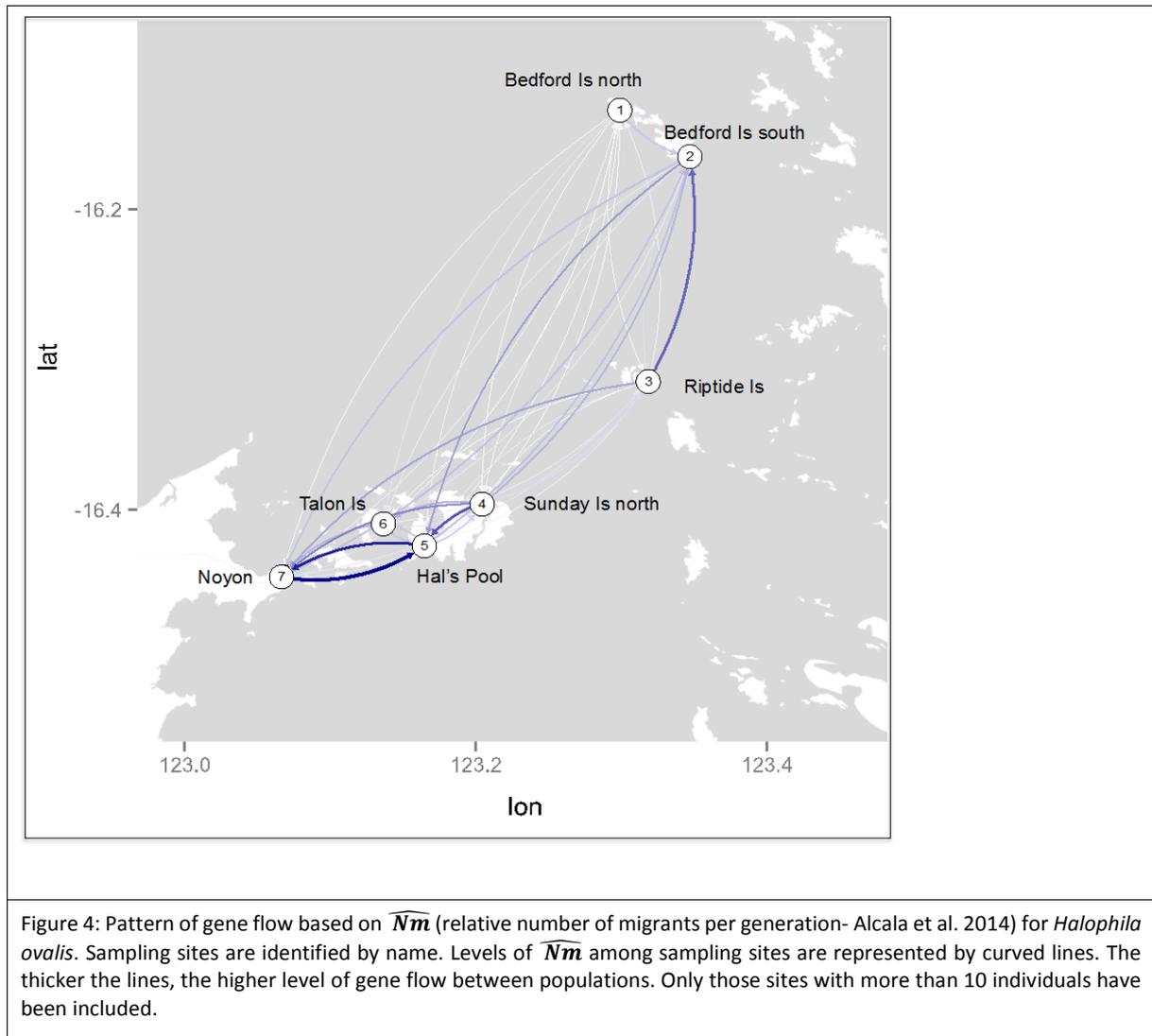
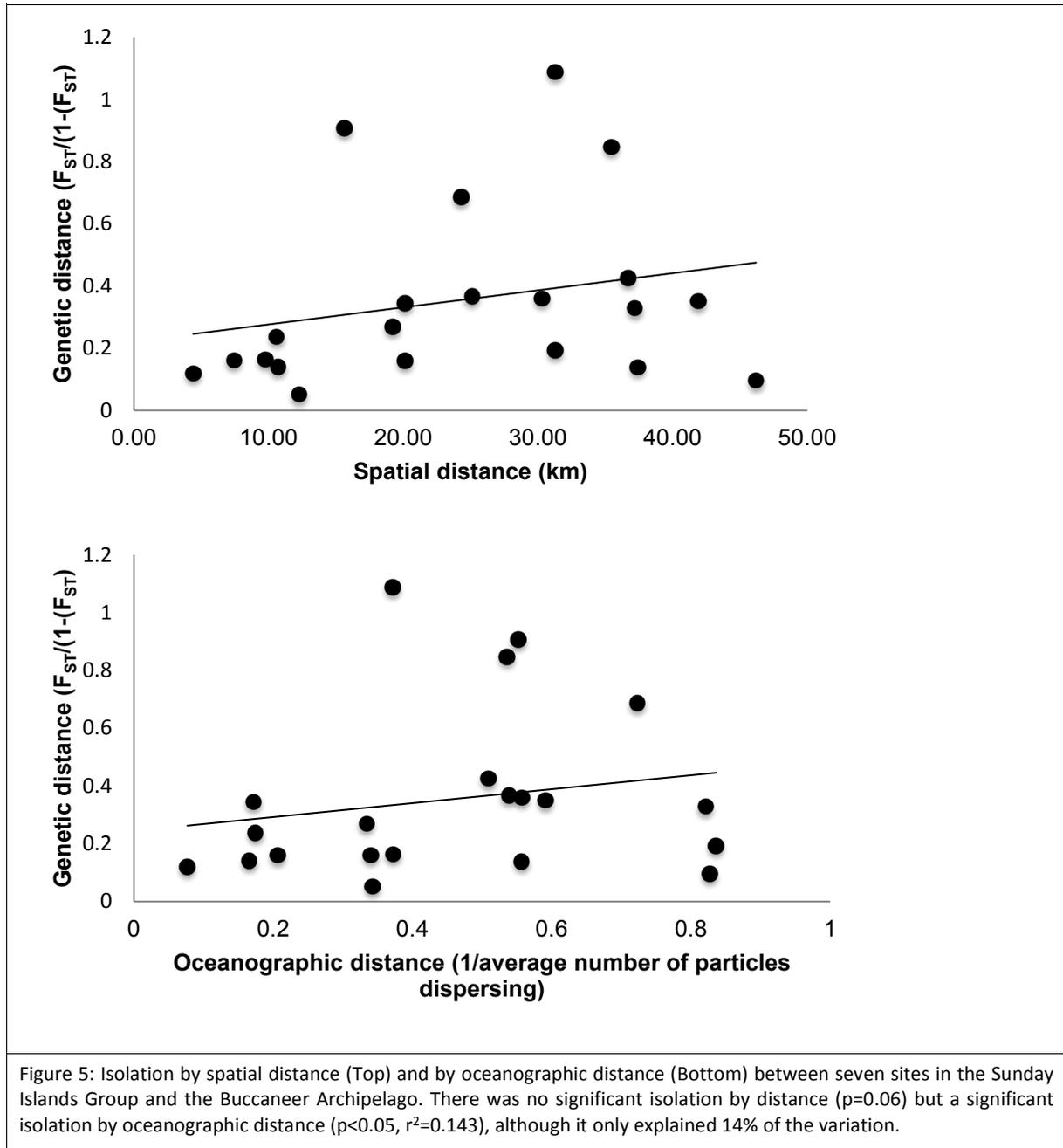


Table 5. Network parameters based on the network of relative migration rates between sites.

	Total strength	Closeness	Transitivity	Betweenness
BN	0.65	3.78	0.78	21
BS	2.62	2.46	1.03	0
RI	1.59	4.18	0.74	8
SN	2.36	3.25	0.62	7
HP	3.96	2.87	1.00	0
TI	1.54	4.46	0.92	5
NY	3.83	2.22	0.99	0

3.1.5 Drivers of genetic differentiation

Only sites with more than 10 individuals were included in this analysis. Genetic differentiation was not significantly related to spatial distance as assessed by a Mantel test ($r=0.384$, $p=0.06$, Figure 5), but it was related to oceanographic distance ($r=0.378$, $p<0.05$)(Figure 5).



3.2 *Thalassia hemprichii*

Over the entire study area, a total of 65 alleles were observed across 16 microsatellite loci from 380 MLGs detected from 653 samples. ML-NULLFREQ did not detect scoring errors in all loci (estimate of genotyping error $\beta < 0.001$). Six of the sixteen loci (Thh34, Thh15, TH73, TH43, Thh1, and Thh3) were not in Hardy-Weinberg equilibrium, as indicated by the heterozygote deficits and significant inbreeding coefficients. ML-NULLFREQ indicated the presence of null alleles in those loci, although the average frequency was relatively low

(Thh34=0.145, Thh15=0.097, TH73=0.115, TH43=0.133, Thh1=0.116, and Thh3=0.120). When these loci were removed, some sites still showed heterozygote deficits, thus this is likely attributed to biological factors, such as inbreeding and the Wahlund effect (reduction in observed heterozygosity due to subpopulation structures), rather than technical issues like the presence of null alleles (Dharmarajan et al. 2013). We retained all loci for further analysis. The test for linkage disequilibrium across multiple loci showed a small standardised index of association ($\bar{r}_d = 0.0217$, $p=0.001$), indicating a low chance of association between loci (Agapow & Burt 2001).

3.2.1 Genetic diversity

The number of MLGs detected at each site ranged from a minimum of 5 at Shenton Bluff to 44 at Mermaid Island. Consequently the clonal richness (R) varied greatly from 0.09 to 0.94 (Table 6). Shenton Bluff had low clonal richness with a maximum of 29 clone mates, seven sites had moderate clonal richness (Bathurst Is, Longitude Is, Bedford Is N, Sunday Is N, Sunday Is S, Talon Is, Noyon) and the remaining had relatively high clonal richness. Significant inbreeding was detected at five sites (Bedford Is N, Riptide Is, Sunday Is S, Halls Pool, Noyon) and an excess of heterozygotes was observed at four sites (Bathurst Is, Longitude Is, Talon Is, Shenton Bluff). A number of sites also showed a high level of relatedness (Bathurst Is, Bedford Is S, Sunday Is N, Noyon, Shenton Bluff).

The total number of observed alleles (n_A) ranged from 21 (Shenton Bluff) to 36 (Mermaid Island), while allelic richness (A_R) ranged from 1.47 (Bedford Island – North) to 1.84 (Talon Island and Mermaid Island). Private alleles were observed at some sites, with the highest frequency at Sunday Is N and Talon Is. The highest observed heterozygosity (H_o) was found at Longitude Island (0.291), with the lowest at Noyon (0.092). Most sites in the Buccaneer Archipelago exhibited significant excess of heterozygotes (negative value of F_{IS}), except Bedford Island-South. In the Sunday Island group and mainland, significant excess of heterozygotes was only detected at Talon Island (Table 6).

Table 6. Genetic statistics for *Thalassia hemprichii*. N-number of samples analysed, G-number of multilocus genotypes, G_{Max} -maximum number of clone mates assigned to one MLG, R-Clonal richness, nA-number of alleles, Ar-allelic richness standardized to n=28 (*=not standardized due to low number), pA-private allelic richness, Re-internal relatedness, Ho-Observed heterozygosity, He expected heterozygosity, F_{IS} -Inbreeding coefficient (*=significant, $p < 0.05$), LD-linkage disequilibrium. Grey shading indicates a low number of individuals and less certainty in genetic statistics.

No	Population	Abb.	N	G	G_{Max}	R	nA	Ar	pA	Re	Ho	Hexp	F_{IS}	LD
3	Bathurst Is.	BAT	30	14	6	0.48	24	1.5	0	0.73	0.232	0.168	- 0.408*	0.06*
7	Longitude Is.	LI	48	23	12	0.49	30	1.83	0.06	0.56	0.291	0.216	- 0.357*	0.10*
8	Bedford Is. – north	BN	48	23	7	0.47	24	1.47	0	0.59	0.133	0.133	-0.004	-0.03
9	Bedford Is. –south	BF	48	37	4	0.77	28	1.66	0.01	0.68	0.120	0.139	0.138*	-0.02
10	Riptide Is.	GI	48	43	2	0.94	31	1.82	0.03	0.40	0.199	0.211	0.059*	-0.01
11	Mermaid Is.	MI	48	44	2	0.91	36	1.84	0.09	0.44	0.215	0.196	- 0.097*	0.06*
12	Sunday Is. –north	SN	48	27	5	0.57	27	1.56	0.12	0.65	0.130	0.131	0.009	-0.01
13	Sunday Is. –south	SI	47	20	6	0.43	27	1.58	0.05	0.52	0.119	0.132	0.105*	0.01
14	Halls Pool	HP	48	32	6	0.66	27	1.61	0.07	- 0.01	0.104	0.171	0.399*	0.00
15	Talon Is.	TI	48	18	16	0.36	31	1.84	0.12	0.26	0.208	0.180	- 0.162*	-0.01
16	Jackson Is.	JI	48	33	3	0.68	31	1.73	0.08	0.54	0.135	0.141	0.047	0.00
17	Noyon	NY	48	17	16	0.36	26	1.59	0	0.65	0.092	0.107	0.143*	-0.03
19	Shenton Bluff*	SB	48	5	29	0.09	21	1.31*	0.06*	0.76	0.175	0.133	-0.366	-0.23
20	Cocos Keeling Is.	CK	48	44	2	0.91	32	1.76	0.01	0.01	0.240	0.218	- 0.102*	-0.01
	OVERALL													

3.2.2 Population genetic differentiation and structure

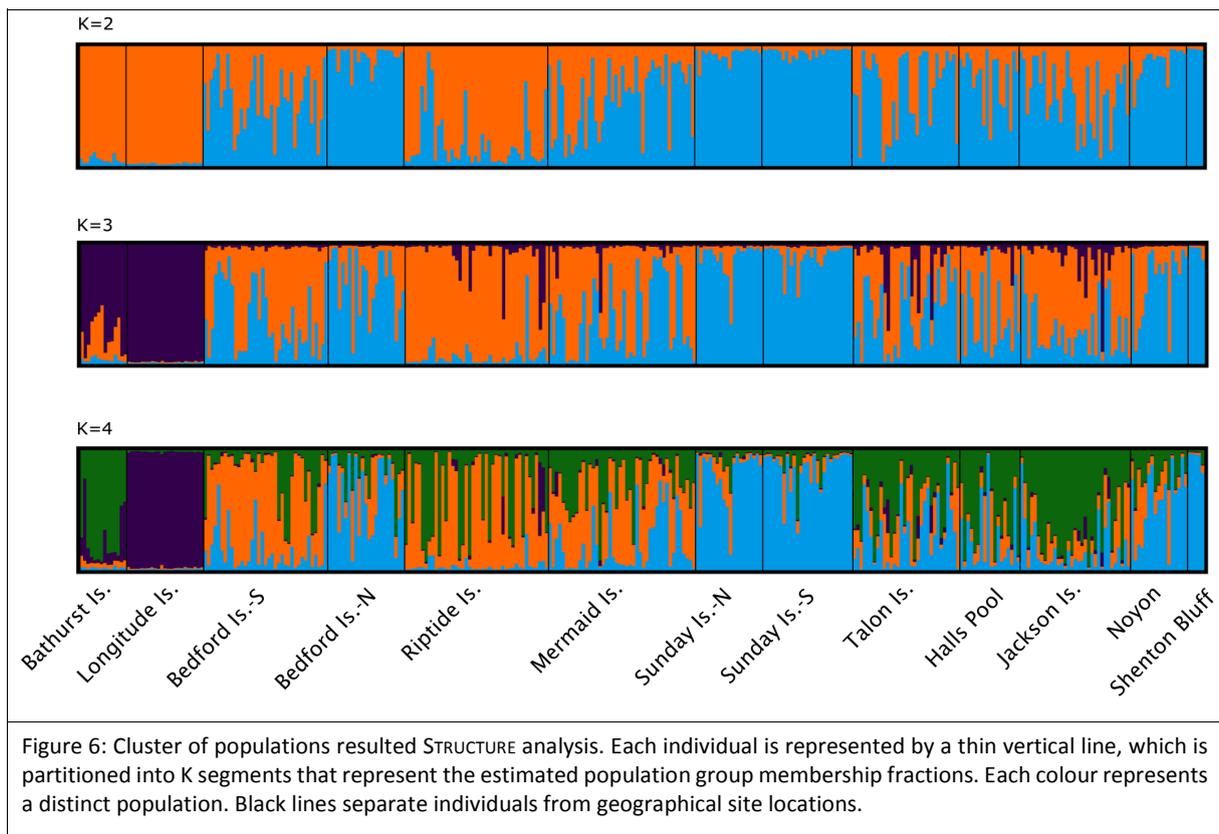
Overall, we detected significant genetic differentiation among the sampling sites (global F_{ST} 0.201, $p=0.001$). Pairwise F_{ST} between sampling sites varied by more than an order of magnitude (0.011 between two Sunday Island populations; to 0.336 between Longitude Island and Bedford Island-North) (Table 7). All pairwise F_{ST} were significantly greater than zero ($p < 0.01$), except between the two Sunday Island populations ($p=0.071$). The highest genetic differentiation was found in populations that were separated by only 14 km (Longitude Island and Bedford Island-North, $G_{ST}=0.336$, whereas more distant sampling sites such as (Bedford Island-South and Noyon) had an F_{ST} of 0.05.

Bayesian probability assignment conducted in STRUCTURE revealed a spatial pattern of genetic differentiation (Figure 6). Model evaluation with the deltaK method (Evanno et al. 2005) indicated two to four populations were best supported, of which $K=3$ had the highest support. At $K=2$, individuals sampled from Bathurst Island and Longitude Island were relatively uniformly assigned with high probability to one cluster. Individuals from the

remaining sampling sites were either assigned strongly to the other cluster or exhibited high admixture between the two clusters. At $K=3$, individuals sampled from Bathurst and Longitude Islands formed a distinct and uniform cluster. Individuals from the remaining sites were either strongly assigned to one cluster (Sunday Island and Noyon), or were highly admixed between the two remaining clusters. At $K=4$, individuals from Bathurst Island became distinct from those collected at Longitude Island, but the clustering pattern of the remaining individuals did not change significantly (Figure 6).

Table 7. Genetic differentiation statistics for *Thalassia hemprichii*. Top matrix is $G'_{ST(NEI)}$ and bottom matrix in F_{ST} . Bold values indicate statistically significant differentiation.

	BAT	LI	BN	BS	RI	MI	SN	SS	HP	TI	JI	NY	SB	CK
B	-	0.22	0.237	0.132	0.078	0.135	0.197	0.189	0.096	0.079	0.095	0.187	0.271	0.492
L	0.353	-	0.329	0.265	0.191	0.216	0.259	0.262	0.208	0.227	0.226	0.287	0.305	0.486
B	0.390	0.495	-	0.001	0.149	0.13	0.082	0.091	0.102	0.075	0.139	0.122	0.07	0.556
B	0.239	0.431	0.001	-	0.074	0.054	0.057	0.058	0.08	0.056	0.055	0.038	0.133	0.529
R	0.134	0.321	0.247	0.136	-	0.048	0.13	0.145	0.044	0.055	0.054	0.099	0.176	0.451
M	0.228	0.359	0.22	0.102	0.092	-	0.07	0.08	0.065	0.077	0.073	0.054	0.114	0.485
S	0.337	0.416	0.151	0.107	0.221	0.124	-	0.073	0.083	0.067	0.078	0.042	0.051	0.553
S	0.322	0.411	0.167	0.109	0.237	0.139	0.071	-	0.106	0.066	0.111	0.084	0.056	0.554
H	0.171	0.348	0.181	0.149	0.083	0.122	0.152	0.186	-	0.03	0.029	0.079	0.126	0.506
T	0.145	0.367	0.142	0.111	0.100	0.141	0.13	0.126	0.057	-	0.041	0.086	0.115	0.510
J	0.177	0.377	0.242	0.105	0.099	0.133	0.144	0.199	0.056	0.081	-	0.047	0.16	0.525
N	0.32	0.434	0.215	0.070	0.160	0.09	0.079	0.154	0.135	0.157	0.085	-	0.13	0.562
S	0.412	0.429	0.125	0.224	0.258	0.176	0.092	0.099	0.197	0.189	0.265	0.237	-	
C	0.499	0.491	0.561	0.533	0.456	0.489	0.558	0.561	0.512	0.517	0.530	0.569		-



3.2.3 Spatial autocorrelation

Significant spatial autocorrelation was detected, with maximum levels at 10 m, then a slight drop up to 50 m and then a decline down to 5 km where very low spatial autocorrelation was detected (Figure 7). Beyond 5 km no spatial autocorrelation was detected.

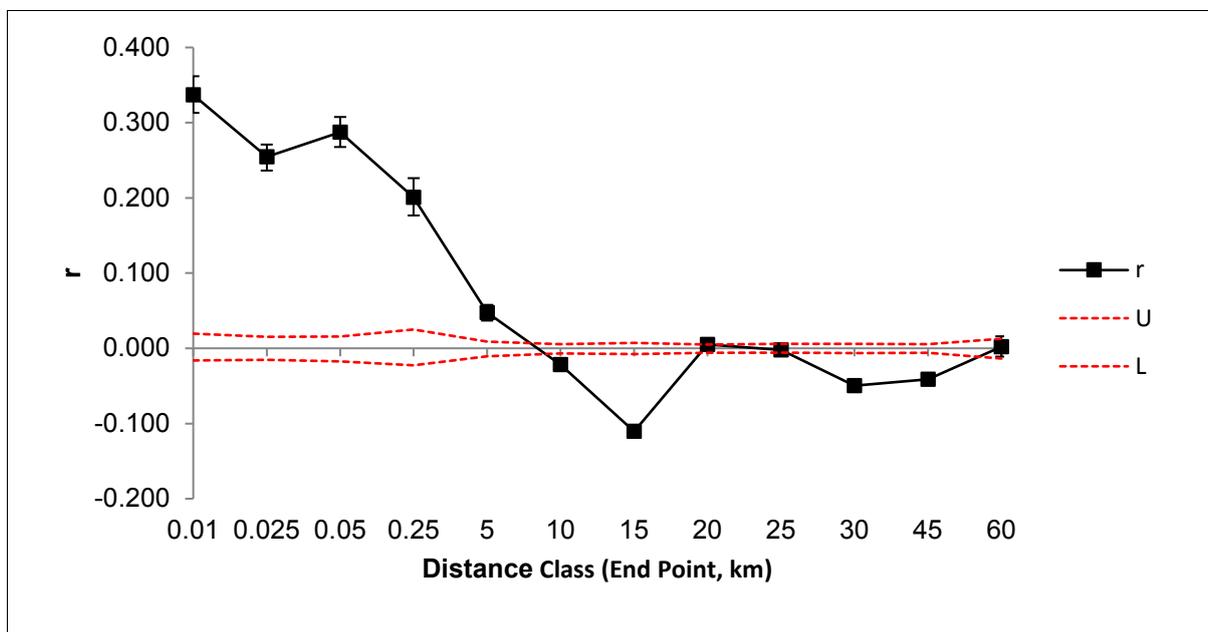


Figure 7: Spatial autocorrelation of *T. hemprichii* individuals among sites with significant autocorrelation over distances up to 5 km, with the most significant spatial autocorrelation at 10 m. r is the autocorrelation value where above the line indicates statistically significant spatial autocorrelation, errors around r are the bootstrapped 95% confidence intervals and the dotted red lines U and L are the Upper and Lower confidence intervals around the null hypothesis of no spatial structure.

3.2.4 Genetic connectivity

The relative number of migrants (\widehat{Nm}) among the seagrass populations ranged from 0.014 to 1 with meadows 12-14 km apart (Longitude Island and the two Bedford Island populations) less connected than ones 30-50 km apart such as Bedford Island-North and Talon Island (Figure 8). Gene flow was asymmetrical, predominantly in a southwestward direction, from the Buccaneer Archipelago to the Sunday Island group. The highest level of gene flow ($\widehat{Nm} = 1$) was observed from Sunday Island-South to Sunday Island-North. Low levels of gene flow were detected from Bathurst Island and Longitude Island to all other sites, suggesting that the two populations were relatively isolated from the other populations (Figure 8).

Hal's Pool, Talon Is and Jackson Is have the strongest connections, but Bedford Island North, Sunday Is North and South and Noyon have the most connections between other sites (Table 8).

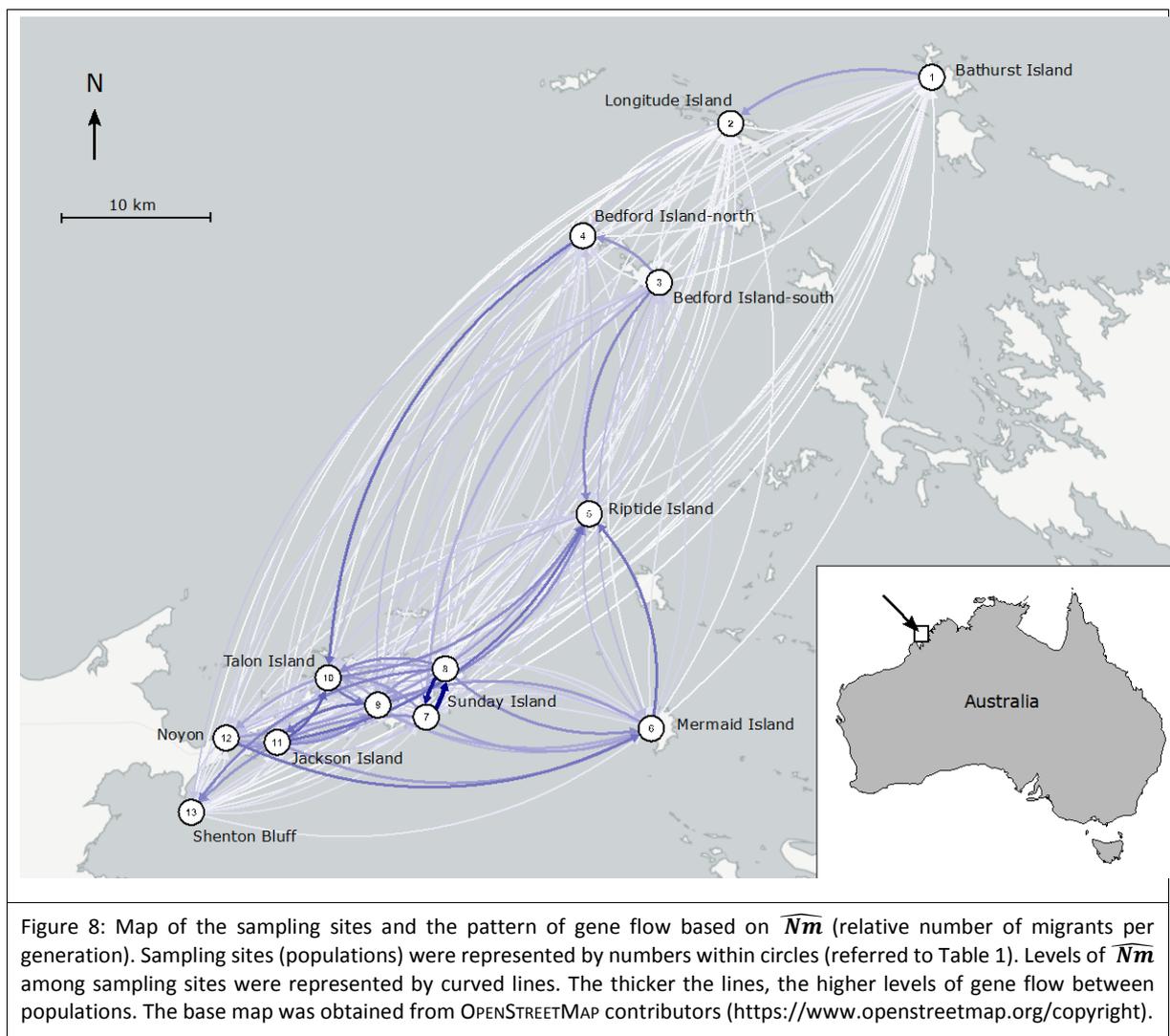


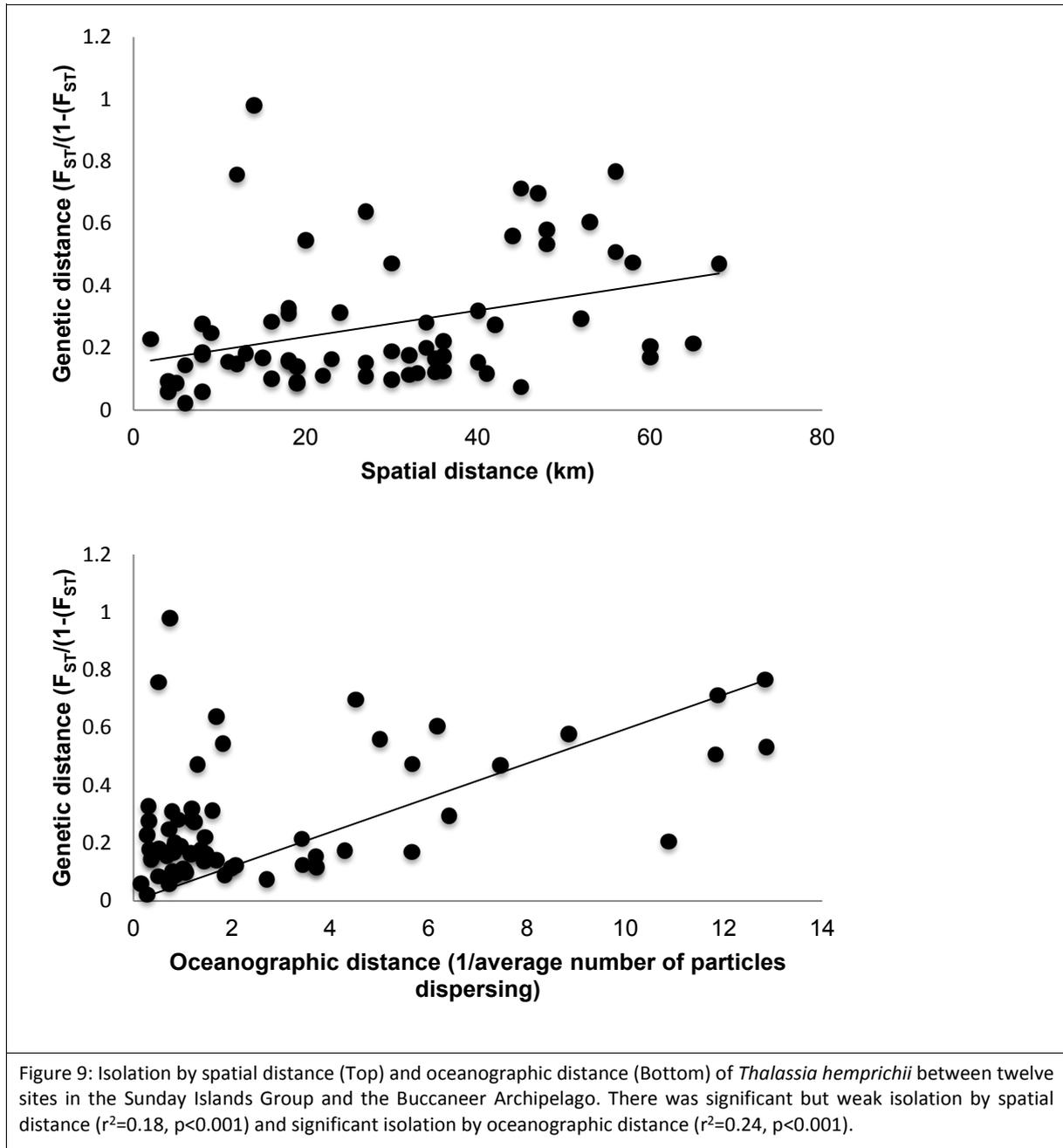
Table 8. Network parameters based on the network of relative migration rates between sites.

	Total strength	Closeness	Transitivity	Betweenness
BAT	4.70	0.74	1.31	50
LI	10.26	0.71	0.70	33
BN	19.68	0.35	1.12	0
BS	15.96	0.78	0.95	1
RI	20.19	0.70	1.06	15
MI	16.53	0.66	0.96	0
SN	18.46	0.34	0.83	11
SS	20.32	0.37	1.03	1
HP	27.29	0.59	0.93	21
TI	25.31	0.61	0.87	12
JI	22.04	0.42	0.80	4
NY	17.13	0.36	1.07	2

3.2.5 Drivers of genetic differentiation

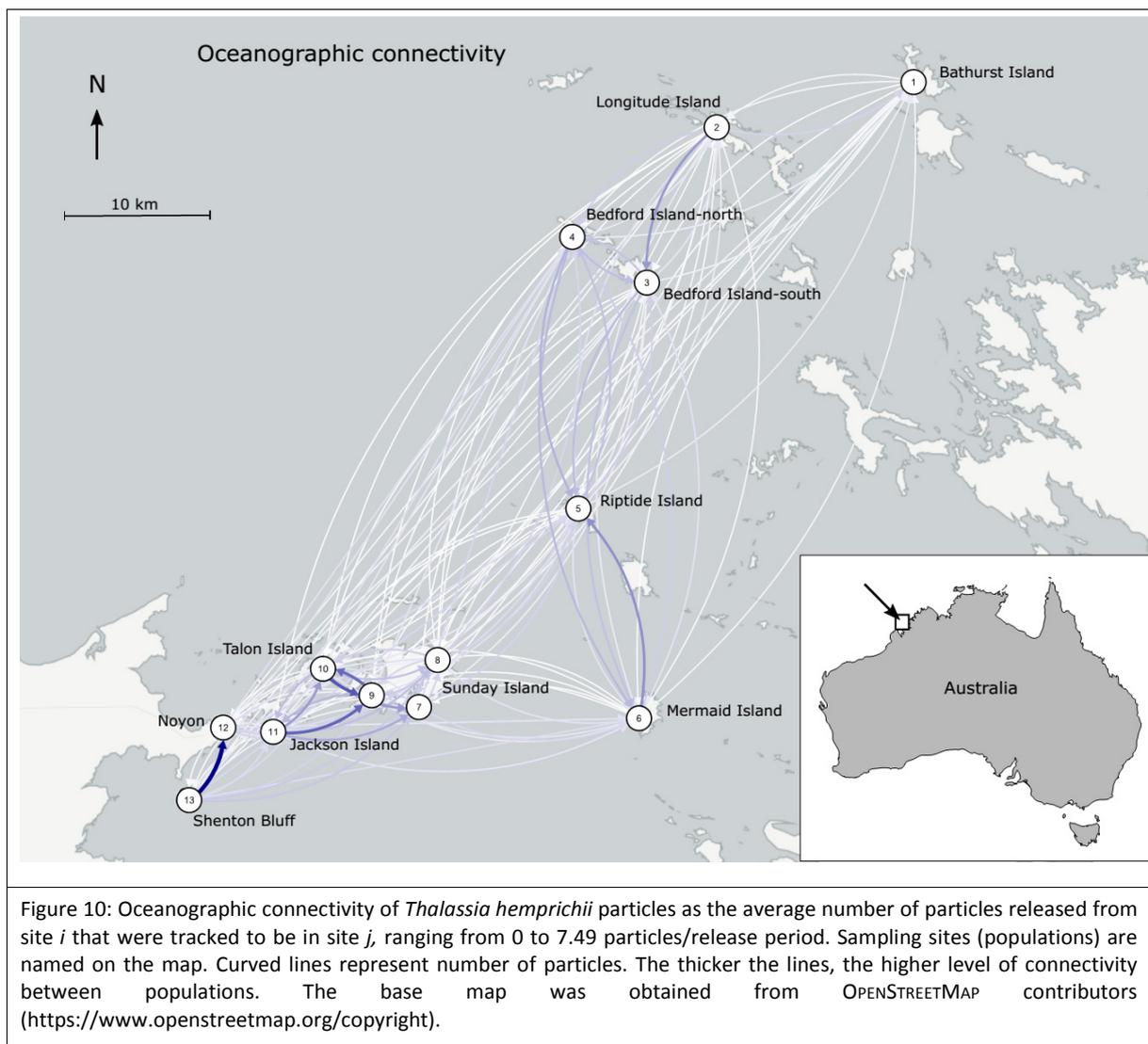
Isolation by distance

Genetic differentiation was significantly but weakly related to spatial distance as assessed by a Mantel test ($r^2=0.18$, $p<0.001$), and more strongly and significantly related to oceanographic distance ($r^2=0.24$, $p<0.001$, Figure 9).



Oceanographic connectivity

The oceanographic connectivity based on *T. hemprichii* particles showed the strongest connection between Shenton Bluff and Noyon, followed by connectivity among a number of Sunday Is sites as well as connectivity from Mermaid Is, up to Riptide and then the Bedford Is and Longitude Is (Figure 10).

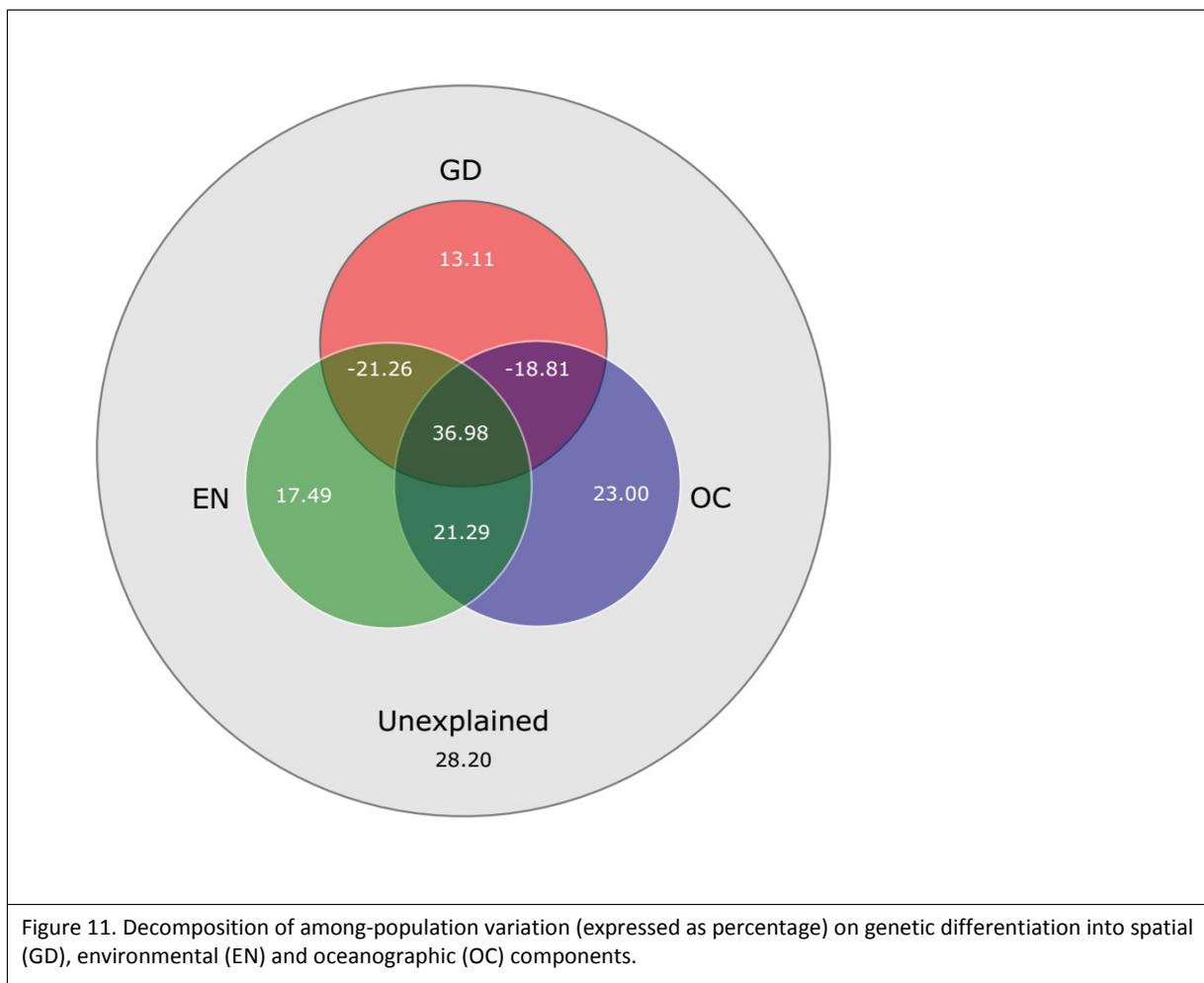


Spatial distance, oceanographic distance and environment

The variance partitioning analysis revealed that oceanographic connectivity was the most significant driver of genetic differentiation, followed by environmental factors (Table 9, Figure 11). The marginal effect of oceanographic connectivity and environmental factors that were significant, accounted for 62.5% and 54.5% of the variation in genetic differentiation among the seagrass populations, respectively (Table 9). In contrast, geographic distance accounted for a smaller proportion of the variation (10%) and the effect was not significant ($p=0.292$). About a third of total variation (28.2%) was not explained by any of the variables. When each individual effect was conditionally estimated by controlling the other explanatory variables, the effects became non-significant ($p>0.05$), therefore oceanographic connectivity and environment do not explain the genetic differentiation independently but in combination.

Table 9. Variation partitioning on among-population variation of genetic differentiation into components accounted for the explanatory variables: GD (geographic distance), OC (oceanographic connectivity) and EN (environmental factors). Fraction of variation is expressed as a percentage from R^2_{adj} values. df_{mod} : degrees of freedom of model; df_{res} : degrees of freedom of residuals

	R^2_{adj} (%)	df_{mod}	df_{res}	F	p-value
Marginal					
EN	54.51	3	9	5.793	0.025
OC	62.46	3	9	7.655	0.002
GD	10.02	4	8	1.334	0.292
Residual	28.19				
Conditional					
EN (OC + GD)	17.49	3	2	2.034	0.278
OC (EN + GD)	23.00	3	2	2.359	0.297
GD (OC + GD)	13.11	4	2	1.697	0.338



The shared fraction among the three explanatory variables explained 36.98% of the total variation, while the shared fraction between oceanographic connectivity and environmental factors explained 21.29% of the total variation. The shared fractions could not be tested for significance as they had zero degree of freedom. There were two negative values associated with the shared fractions, i.e. between geographic distance and environmental factors (-21.26) and between the geographic distance and oceanographic connectivity (-18.81) indicating either: (i) strong, direct and opposing effects of the explanatory variables on the response variable, or (ii) correlations between the explanatory variables (Legendre & Legendre 1998).

3.3 Genetic resilience of seagrass populations in the south western Kimberley

Overall, *T. hemprichii* had more genetically resilient sites than *H. ovalis*, three sites had a high genetic resilience compared to none, and only one site had a low genetic resilience, compared to two for *H. ovalis* (Figure 12). For *T. hemprichii* the sites that showed the greatest genetic resilience were Mermaid Is, Riptide Is and Longitude Island, however different processes drove this high resilience. In some cases a high clonal diversity combined with a high allelic diversity, or a moderate clonal diversity combined with a high allelic diversity and heterozygosity (Figure 12).

		Adapt to pressure over generations (based on allelic diversity)	Recover from declines (based on heterozygosity)	Recover from complete loss (based on genotypic diversity)	OVERALL GENETIC RESILIENCE
Woobinbeye Creek		●	●	●	●
Bathurst Island		●	●	●	●
Irvine Island		●	●	●	●
Longitude Island		●	●	●	●
Bedford Island North		●	●	●	●
Bedford Island North		●	●	●	●
Bedford Island South		●	●	●	●
Bedford Island South		●	●	●	●
Riptide Island		●	●	●	●
Riptide Island		●	●	●	●
Mermaid Island		●	●	●	●
Sunday Island North		●	●	●	●
Sunday Island North		●	●	●	●
Sunday Island South		●	●	●	●
Sunday Island South		●	●	●	●
Hall's Pool		●	●	●	●
Hall's Pool		●	●	●	●
Talon Island		●	●	●	●
Talon Island		●	●	●	●
Jackson Island		●	●	●	●
Jackson Island		●	●	●	●
Noyon		●	●	●	●
Noyon		●	●	●	●
Shenton Bluff		●	●	●	●

 *Thalassia hemprichii*  *Halophila ovalis*

Figure 12: A summary of genetic resilience of both species across sites in the Kimberley.

4 Discussion

4.1 Genetic Connectivity

4.1.1 Fine-scale

We predicted that the species *T. hemprichii* would show connectivity over larger distances than *H. ovalis* due to the potential dispersal of its buoyant fruits. However, this was not the case, as there was no significant spatial autocorrelation beyond 5 km for *T. hemprichii* compared to 20 km in *H. ovalis*. This indicates that in general the population growth of *T. hemprichii* meadows is sustained by recruitment from within meadows and from those up to 5 km away, whereas for *H. ovalis* meadows up to 20 km away contribute to population growth. However, migration and STRUCTURE analysis provide evidence for dispersal events over larger distances, up to 35 km for *T. hemprichii*, between the Bedford Islands in the Buccaneer Archipelago with the Sunday Islands group. These analyses predict genetic connectivity over multiple generations, and imply that these larger dispersal distances are less common. In contrast, the spatial autocorrelation, as well as STRUCTURE and migration analysis for *H. ovalis* identified similar distances of connectivity, around 20 km, and hence may occur more commonly for *H. ovalis*.

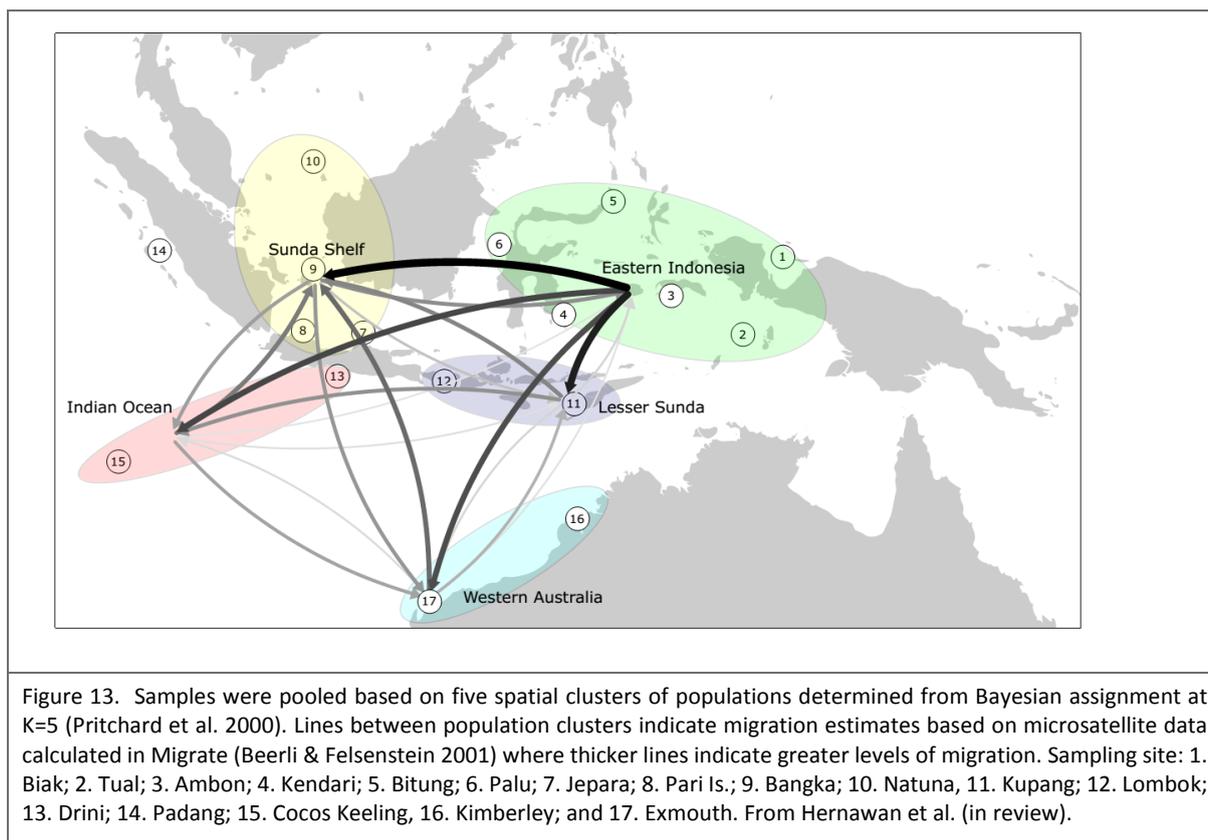
These slight differences in patterns of connectivity identified different dispersal barriers. For *T. hemprichii*, there was a clear barrier within the Buccaneer Archipelago, between the Bedford Islands, and both Longitude Is and Bathurst Is. In contrast, the barrier was between the Buccaneer Archipelago and the Sunday Islands Group for *H. ovalis*. For both species there was evidence that Riptide Is provided a stepping-stone between the Buccaneer Archipelago and the Sunday Islands.

The patterns and directionality of gene flow as identified in the migration analysis was complex, and varied between species. There was a dominant southward dispersal for *T. hemprichii* from Bedford Islands to the Sunday Islands, but a dominant northward dispersal from Sunday and Mermaid Islands to Riptide Island. The only clear directionality for *H. ovalis* was a northward dispersal from Riptide to Bedford Islands.

Network analysis allows identification of sites that are highly connected or disconnected. Hal's Pool and Noyon was a highly connected site for both species. For *H. ovalis* one other site had strong connections (Bedford Is South) and for *T. hemprichii* five other sites (Talon, Jackson, Bedford N, Sunday N and S Islands). These connectivity patterns can help inform decisions on the location of protected areas, as these sites are important for connectivity to other sites.

4.1.2 Broad-scale

The Kimberley data was included in a larger study, a broad scale analysis of patterns in genetic structure for *T. hemprichii* across the Indo-Australian Archipelago (Figure 13) (Hernawan et al. in review). Western Australian populations in the Kimberley and Pilbara group together, and are separated from four other strongly supported clusters in the Indonesian Archipelago (Figure 13). Interestingly the Australian Territory of Cocos Keeling Island (15 in Figure 13) is more closely related to Javanese populations (13 in Figure 13) than to the Australian populations, most likely driven by oceanographic connectivity of the South East Equatorial Current. Kimberley populations are quite isolated, as the strongest paths of migration are from Indonesia to the Pilbara. There is not a stepping stone pattern from Indonesia, to the Kimberley and then the Pilbara, potentially attributed to historical isolation of the Kimberley populations or isolation by oceanography.



4.2 Genetic diversity

4.2.1 Fine-scale

In clonal plants, genetic diversity is related to clonality, such as how many unique individuals are present, as well as the composition of alleles within and among these individuals. The clonal richness was much greater in *T. hemprichii* ($R=0.59$, all samples pooled), more unique individuals were detected compared to *H. ovalis* ($R=0.39$). Only one population was highly clonal for *T. hemprichii* (Shenton Bluff) whereas four were for *H. ovalis*, less than 10 unique individuals were detected at a site, and these sites were not included in further population genetic analysis. These low levels of clonal richness indicate that sexual reproduction is not important for maintaining these populations, and clonal growth is the main mechanism for population growth. Previous studies (McMahon et al. 2016, van Dijk et al. in review) have not documented such high levels of clonality, although within clonal species it is common for populations to vary in their clonal richness from very low to very high diversity (Widén et al. 1994). In fact, clonality is considered advantageous in stressful environments. A low clonal richness could be expected at the edge of a species range or in marginal habitat where populations may be recruitment or dispersal limited. This was observed for *T. hemprichii* at Shenton Bluff with a very small and sparse meadow, and may be the case for *H. ovalis* at Sunday Is South and Woobinbeye Creek, where the meadow was also very sparse. However, in the remaining *H. ovalis* sites with low clonal richness, there was an abundant meadow.

Despite the high clonality of *H. ovalis*, the genetic diversity measured by allelic richness and heterozygosity was greater in *H. ovalis* compared to *T. hemprichii*. It is predicted that highly clonal organisms will have higher allelic richness and heterozygosity over time, and this may explain the elevated levels in *H. ovalis* (Balloux et al. 2003). An alternate hypothesis, is that the low allelic richness and heterozygosity of *T. hemprichii* in this area is due to the historical founder effects, and the low connectivity with adjacent regions as demonstrated above (Figure 13).

The patterns in genetic diversity also varied between species. For *T. hemprichii* the meadows with the greatest diversity were at Longitude, Riptide and Mermaid Islands, whereas for *H. ovalis* they were at Noyon and Bedford Is South. The spatial arrangement of genetic diversity can also be used to identify locations for spatial

management.

4.2.2 Broad scale

Once again, the Kimberley data was included in a larger study, a broad scale analysis of patterns in genetic structure for *T. hemprichii* across the Indo-Australian Archipelago (Figure 14)(Hernawan et al. in review), and in the Pilbara (McMahon et al. 2016). Indonesia is the centre of the range for *T. hemprichii*, as well as the centre of biodiversity for many marine organisms. Genetic diversity was greatest in Indonesia in the heart of the coral triangle, and declined away from this heart, reducing to minimums at the range edge (Figure 14) (Hernawan et al. in review). The outliers to this pattern were the Kimberley populations, which were closer to the center of the range than the Pilbara populations but had a much lower genetic diversity (orange dots in Figure 14).

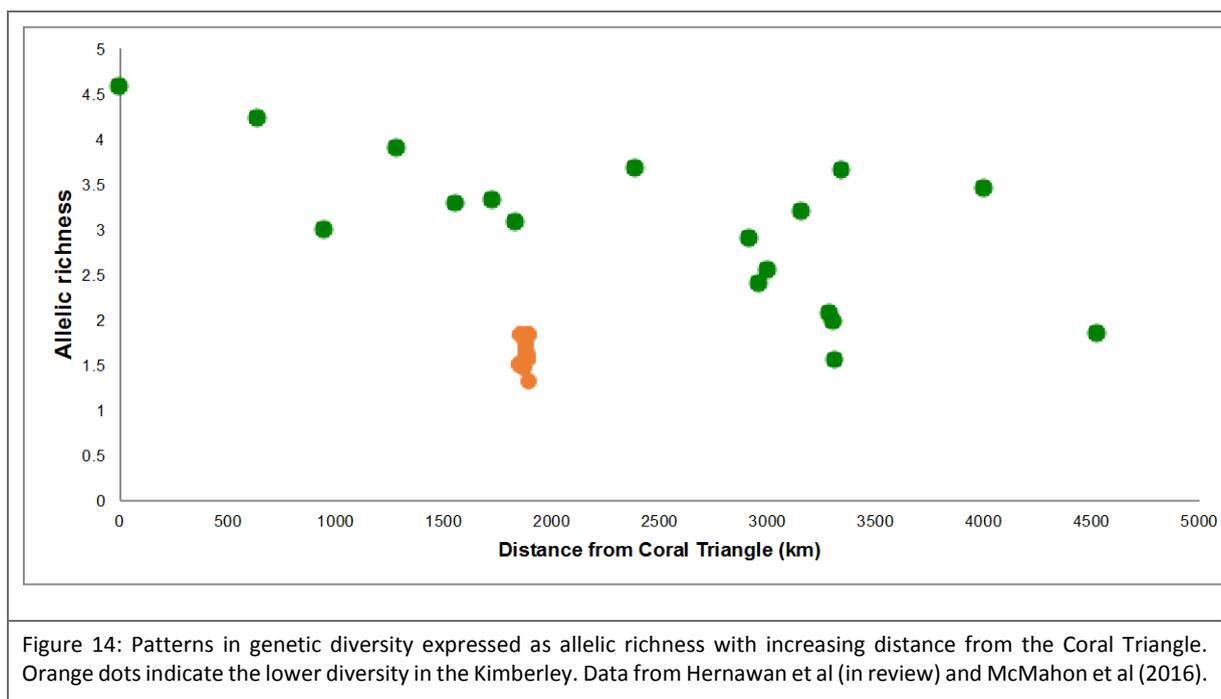


Figure 14: Patterns in genetic diversity expressed as allelic richness with increasing distance from the Coral Triangle. Orange dots indicate the lower diversity in the Kimberley. Data from Hernawan et al (in review) and McMahon et al (2016).

4.3 Drivers of genetic connectivity

Genetic connectivity is influenced by the biological traits of an organism, particularly its dispersal potential and how this interacts with the environment. At local scales (<100 km), the scale of this study, significant genetic structure and variable patterns in connectivity are found in seagrasses. Isolation by distance is not always significant, and patterns of connectivity are influenced most by local currents, wind and tide, and not by the predominant oceanographic currents (McMahon et al. in review). In this study there was evidence of significant but weak isolation by spatial distance for *T. hemprichii* and isolation by oceanographic distance for *H. ovalis* and *T. hemprichii*. It is unlikely that one factor alone would influence connectivity patterns and for *T. hemprichii* where we had more populations to assess we examined spatial distance, oceanographic distance as well as the environmental conditions to identify what best explained the patterns of genetic connectivity. Oceanographic distance combined with environmental characteristics best explained the patterns in genetic distance between sites, and there was no longer a significant effect of spatial distance. The environmental condition that was most important was sediment type, which may influence the success of recruitment and survival of the dispersing seeds.

For *H. ovalis* oceanographic distance best explained the genetic differentiation between populations. *H. ovalis* does not have buoyant seeds, rather they are negatively buoyant and usually fall into the sediment where they can disperse via sediment movement (McMahon et al. 2014). However, water currents can passively transport the vegetative fragments of *H. ovalis* and biotic vectors such as dugongs, which feed in the study area, can

facilitate dispersal of seeds. In fact, the germination rate of *H. ovalis* seeds is greater after passing through a dugong's digestive system (Tol et al. 2015). This mechanism may explain the greater size of related populations in *H. ovalis* (20 km) compared to *T. hemprichii*.

Overall, the very strong tidal currents in the region do not appear to promote greater spatial scales of connectivity. The sister species of *T. hemprichii* in the Caribbean, which has an almost identical dispersal strategy can successfully disperse over 300 km (van Dijk et al. 2009), in contrast to the distances of 35 km over the ~ 100 km area in this study. The complex seascape of the Kimberley with many islands, large tides and strong tidally driven eddies may promote entrainment within the seascapes features.

4.4 Recommendations for management

Protected areas are a common approach in spatial planning. Based on the findings of genetic connectivity in the two seagrass species, routine dispersal distances that maintain populations are in the order of 5-20km, with connectivity over larger distances occurring less frequently. Therefore protected areas need to be at this scale to protect these processes, and spaced at similar distances to enable recovery from disturbance. These areas should be replicated across the two main population groups that show limited interaction, in the Sunday Islands and Buccaneer Archipelago (northern part for *T. hemprichii*). Ideally, the placement of protected areas should also consider sites that are well connected to other sites, so have a greater chance of contributing to recovery. Additionally, sites with a higher genetic diversity have a greater potential to adapt to change, or recover from disturbance. With significant changes in the marine environment occurring currently due to global change, the genetic resilience matrix (Figure E2) we present in this study could be used when considering site selection. Although the patterns of genetic connectivity and diversity were somewhat different between the two seagrass species, there were some areas that filled most of these criteria, particularly Hal's Pool and Riptide Island (Table 10).

Table 10: A summary of the key attributes of genetic connectivity and diversity across all sites sampled in this study. This information can be used for spatial management to aid decisions in the location of protected areas.

Population	<i>Thalassia hemprichii</i>				<i>Halophila ovalis</i>				TOTAL BOTH SPECIES
	Connection	Stepping stone	Genetic diversity	TOTAL	Connection	Stepping stone	Genetic diversity	TOTAL	
Bathurst Is.				0				nd	0
Irvine Is.				nd				0	0
Longitude Is.			X	1				nd	1
Bedford Is. North	X			1				0	1
Bedford Is. South				0	X		X	2	2
Riptide Is./Gregory Is.		X	X	2		X		1	3
Mermaid Is.			X	1				nd	1
Sunday Is. –north	X			1				0	1
Sunday Is. –south east, Janinko	X			1			X	1	2
Hal's Pool, Ngoorroodool	X			1	X		X	2	3
Tallon Is., Jalan	X		X	2				0	2
Jackson Is., Aloon	X			1				0	1
Noyon				0	X		X	2	2
Shenton Bluff, Ardinoogoon				0				nd	0

5 References

- Agapow P, Burt A (2001) Indices of multilocus linkage disequilibrium. *Molecular Ecology Notes* 1:101–102
- Alberto F, Massa S, Manent P, Diaz-Almela E, Arnaud-Haond S, Duarte CM, Serrao EA (2008) Genetic differentiation and secondary contact zone in the seagrass *Cymodocea nodosa* across the Mediterranean-Atlantic transition region. *Journal of Biogeography* 35:1279–1294
- Alcala N, Goudet J, Vuilleumier S (2014) On the transition of genetic differentiation from isolation to panmixia: What we can learn from G(ST) and D. *Theoretical Population Biology* 93:75–84
- Balloux F, Lehmann L, de Meeu's T (2003) The population genetics of clonal and partially clonal diploids. *Genetics* 164:1635–1644
- Barrat A, Barthélemy M, Pastor-Satorras R, Vespignani A (2004) The architecture of complex weighted networks. *Proceedings of the National Academy of Sciences of the United States of America* 101:3747–3752
- Berli P, Felsenstein J (2001) Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. *Proceedings of the National Academy of Sciences of the United States of America* 98:4563–4456
- Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F (2004) GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome, Populations, Interactions, CNRS UMR. Université de Montpellier II, Montpellier, France
- Bjorndal KA (1980) Nutrition and grazing behaviour of the green turtle, *Chelonia mydas*. *Marine Biology* 56:147–154
- Condie S, Andrewartha J (2008) Circulation and connectivity on the Australian North West Shelf. *Continental Shelf Research* 28:1724–1739
- Cresswell G, Badcock K (2000) Tidal mixing near the Kimberley coast of NW Australia. *Marine and Freshwater Research* 51:641–646
- Csardi G, Nepusz T (2015) Package “igraph”, Network Analysis and Visualization. version 1.0.1. <http://igraph.org/r/>.
- Dąbrowski M, Bornelöv S, Kruczyk M, Baltzer N, Komorowski J (2015) “True” null allele detection in microsatellite loci: a comparison of methods, assessment of difficulties and survey of possible improvements. *Molecular Ecology Resources* 15:477–488
- den Hartog C (1970) *Sea-grasses of the world*. North Holland Publishing Company, Amsterdam
- Dharmarajan G, Beatty W, Rhodes O (2013) Heterozygote deficiencies caused by a Wahlund effect: Dispelling unfounded expectations. *The Journal of Wildlife Management* 77:226–234
- Earl DA, vonHoldt BM (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources* 4:359–361
- Endler JA (1977) *Geographic variation, speciation and clines*. Princeton Press, New Jersey
- Engelhardt KAM, Lloyd MW, Neel MC (2014) Effects of genetic diversity on conservation and restoration potential. at individual, population, and regional scales. *Biological Conservation* 179:6–16
- Environment (2016) Bardi Jawi Seagrass Monitoring. Accessed 30th May. <https://www.environment.gov.au/indigenous/ipa/declared/bardijawi.html>
- Epskamp S, Cramer AOJ, Waldorp LJ, Schmittmann VD, Borsboom D (2012) qgraph: Network Visualizations of Relationships in Psychometric Data. *Journal of Statistical Software* 48:1–18
- Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* 14:2611–2620
- Evans SM, Sinclair EA, Poore AGB, Steinberg PD, Kendrick GA, Verges A (2014) Genetic diversity in threatened *Posidonia australis* seagrass meadows. *Conservation Genetics* 15:717–728
- Feng M, Slawinski D, Beckley L, Keesing J (2010) Retention and dispersal of shelf waters influenced by interactions of ocean boundary current and coastal geography. *Marine and Freshwater Research* 61:1259–1267
- Fraser T (2008) STORM: software for testing hypotheses of relatedness and mating patterns. *Molecular Ecology Resources* 8:1263–1266
- Grech A, Chartrand-Miller K, Erftemeijer P, Fonseca M, McKenzie L, Rasheed M, Taylor H, Coles R (2012) A comparison of threats, vulnerabilities and management approaches in global seagrass bioregions. *Environmental Research Letters* 7:8
- Guillot G, Rousset F (2013) Dismantling the Mantel tests. *Methods in Ecology and Evolution* 4:336–344
- Hall LM, Hansiak D, Virnstein RW (2006) Fragments of the seagrasses *Halodule wrightii* and *Halophila johnsonii* as potential recruits in Indian River Lagoon, Florida. *Marine Ecology Progress Series* 310:109–117
- Halpern BS, Walbridge S, Selkoe KA, Kappel CV, Micheli F, D'Agrosa C (2008) A Global Map of Human Impact on Marine Ecosystems. *Science of the Total Environment* 319:948–952
- Hernawan U, van Dijk K, Kendrick G, Feng M, Biffin E, Lavery P, McMahon K (in review) Historical processes and contemporary ocean currents drive genetic structure in the seagrass *Thalassia hemprichii* in the Indo-Australian Archipelago. *Molecular Ecology*

- Hughes AR, Stachowicz JJ (2004) Genetic diversity enhances the resistance of a seagrass ecosystem to disturbance. *PNAS* 101:8998-9002
- Jackson M, Bayliss P, Waina N, Adams J, Cheinmora R, Cheinmora L, Warren R, T V, Wilcox C, Stevens A, Hodge J, Kennett R (2015) Mangguru (marine turtles) and Balguja (dugong) Monitoring Project: Field Trip Report, Wunambal Gaambera country, WA, 21-28 August 2013. North Australian Indigenous Land and Sea Management Alliance Limited
- Jakobsson M, Rosenberg NA (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics* 23:1801-1806
- Jannsen T, Bremer K (2004) The age of major monocot groups inferred from 800+ rbcL sequences. *Botanical Journal of the Linnean Society* 146:385-398
- Kalinowski S, Taper M (2006) Maximum likelihood estimation of the frequency of null alleles at microsatellite loci. *Conservation Genetics* 7:991-995
- Kalinowski ST (2005) HP-RARE 1.0: a computer program for performing rarefaction on measures of allelic richness. *Molecular Ecology Notes* 5:187-189
- Kamvar ZN, Tabima JF, Gruenwald NJ (2014) Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ* 2:e281
- Keenan K, McGinnity P, Cross TF, Crozier WW, Prodoehl PA (2013) diveRsimy: An R package for the estimation and exploration of population genetics parameters and their associated errors. *Methods in Ecology and Evolution* 4:782-788
- Kendrick GA, Waycott M, Carruthers TJB, Cambridge ML, Hovey R, Krauss SL, Lavery PS, Les DH, Lowe RJ, Vidal OMI, Ooi JLS, Orth RJ, Rivers DO, Ruiz-Montoya L, Sinclair EA, Statton J, van Dijk JK, Verduin JJ (2012) The central role of dispersal in the maintenance and persistence of seagrass populations. *Bioscience* 62:56-65
- Kilminster K, McMahon K, Waycott M, Kendrick GA, Scanes P, McKenzie L, O'Brien KR, Lyons M, Ferguson A, Maxwell P, Glasby T, Udy J (2015) Unravelling complexity in seagrass systems for management: Australia as a microcosm. *Science of the Total Environment* 534:97-109
- Kimberley E (2016) Broome Community Seagrass Monitoring. Accessed 30th May. <http://www.envirokimberley.org.au/kimberley-conservation-campaigns-projects/conservation-campaigns/>
- Kobayashi S, Ota Y, Harada Y (2015) The JRA-55 reanalysis: General specifications and basic characteristics. *Journal of the Meteorological Society of Japan* 93:5-48
- Lacap CDA, Vermaat JE, Rollon RN, Nacorda HM (2002) Propagule dispersal of the SE Asian seagrasses *Enhalus acoroides* and *Thalassia hemprichii*. *Marine Ecology Progress Series* 235:75-80
- Lanyon J, Limpus C, Marsh H (1989) Dugongs and turtles; grazers in the seagrass system. In: Larkum AWD, McComb AJ, Shepherd SA (eds) *Biology of seagrasses*. Elsevier, Amsterdam
- Legendre P, Fortin M (2010) Comparison of the Mantel test and alternative approaches for detecting complex multivariate relationships in the spatial analysis of genetic data. *Molecular Ecology Resources* 10:831-844
- Legendre P, Legendre L (1998) *Numerical Ecology*. Developments in Environmental Modelling 20. Elsevier
- Les DH, Cleland MA, Waycott M (1997) Phylogenetic studies in Alismatidae, II: Evolution of marine angiosperms (seagrasses) and hydrophily. *Syst Bot* 22:443-463
- Lowe AJ, Harris SA, Ashton SA (2004) *Ecological Genetics; Design, Analysis and Application*. Blackwells
- Lowe R, Leon A, Symonds G, Falter J, Gruber R (2015) The intertidal hydraulics of tide-dominated reef platforms. *Journal of Geophysical Research: Oceans* 120:4845-4868
- Lowe WH, Allendorf FW (2010) What can genetics tell us about population connectivity? *Molecular Ecology* 19:3038-3051
- McMahon K, Hernawan U, van Dijk K, Waycott M, Biffin E, Evans R, Lavery P (2016) Genetic variability of seagrass in NW Australia. Western Australian Marine Science Institution, Dredging Science Node, Perth
- McMahon K, Sinclair E, Sherman C, van Dijk K, Hernawan U, Verduin J, Waycott M (in review) Genetic connectivity in Australian seagrasses. In: Larkum A, Ralph P, Kendrick G (eds) *Conservation and management of Australian Seagrasses*. Elsevier
- McMahon K, van Dijk K-j, Ruiz-Montoya L, Kendrick GA, Krauss SL, Waycott M, Verduin J, Lowe R, Statton J, Brown E, Duarte C (2014) The movement ecology of seagrasses. *Proceedings of the Royal Society B-Biological Sciences* 281:20140878
- Meirmans P (2015) Seven common mistakes in population genetics and how to avoid them. *Molecular Ecology* 24:3223-3231
- Meloni M, Reid A, Caujape-Castells J, Marrero A, Fernández-Palacios J, Mesa-Coelo R, Conti E (2013) Effects of clonality on the genetic variability of rare, insular species: the case of *Ruta microcarpa* from the Canary Islands. *Ecology and Evolution* 3:1569-1579
- Oksanen J, Blanchet F, Kindt R (2015) *vegan: Community Ecology Package*. R package version 2.3-0.
- Orth RJ, Carruthers TJB, Dennison WC, Duarte CM, Fourqurean JW, Heck KLJ, Hughes AR, Kendrick GA,

- Kenworthy WJ, Olyarnik S, Short FT, Waycott M, Williams SL (2006) A global crisis for seagrass ecosystems. *Bioscience* 56:987-996
- Peres-Neto P, Legendre P, Dray S, Borcard D (2006) Variation partitioning of species data matrices: estimation and comparison of fractions. *Ecology* 87:2614–2625
- Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics* 155:945-959
- Rosenberg NA (2004) DISTRUCT: a program for the graphical display of population structure. *Molecular Ecology Notes* 4:137-138
- Rousset F (1997) Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics* 145:1219–1228
- Salo T, Reusch TBH, Bostrom C (2015) Genotype-specific responses to light stress in eelgrass *Zostera marina*, a marine foundation plant. *Marine Ecology Progress Series* 519:129-140
- Serra IA, Innocenti AM, Di Maida G, Calvo S, Migliaccio M, Zambianchi E, Pizzigalli C, Arnaud-Haond S, Duarte CM, Serrao EA, Procaccini G (2010) Genetic structure in the Mediterranean seagrass *Posidonia oceanica*: disentangling past vicariance events from contemporary patterns of gene flow. *Molecular Ecology* 19:557-568
- Sherman CDH, York P, Smith T, Macreadie P (2016) Fine-scale patterns of genetic variation in a widespread clonal seagrass species. *Marine Biology* 163:82
- Short FT, Polidoro B, Livingstone SR, Carpenter KE, Bandeira S, Bujang JS, Calumpang HP, Carruthers TJB, Coles RG, Dennison WC, Erftemeijer PLA, Fortes MD, Freeman AS, Jagtap TG, Kamal AHM, Kendrick GA, Kenworthy WJ, La Nafie YA, Nasution IM, Orth RJ, Prathep A, Sanciangco JC, van Tussenbroek B, Vergara SG, Waycott M, Zieman JC (2011) Extinction risk assessment of the world's seagrass species. *Biological Conservation* 144:1961-1971
- Sinclair EA, Krauss SL, Anthony J, Hovey R, Kendrick GA (2014) The interaction of environment and genetic diversity within meadows of the seagrass *Posidonia australis* (Posidoniaceae). *Marine Ecology Progress Series* 506:87-98
- Smouse P, Peakall R (1999) Spatial autocorrelation analysis of individual multiallele and multilocus genetic structure. *Heredity* 82:561-573
- Tol SJ, Jarvis JC, Coles RC, York PY, Congdon BC (2015) Tropical seagrass seed dispersal by marine megaherbivores. Poster presentation from Australian Marine Sciences Association. Deakin University, Geelong
- Unsworth RKF, Collier CJ, Waycott M, McKenzie LJ, Cullen-Unsworth LC (2015) A framework for the resilience of seagrass ecosystems. *Marine Pollution Bulletin* 100:34-46
- van Dijk JK, van Tussenbroek BI, Jimenez-Duran K, Judith Marquez-Guzman G, Ouborg J (2009) High levels of gene flow and low population genetic structure related to high dispersal potential of a tropical marine angiosperm. *Marine Ecology Progress Series* 390:67-77
- van Dijk K-J, Mellors J, Waycott M (2014) Development of multiplex microsatellite PCR panels for the seagrass *Thalassia hemprichii* (Hydrocharitaceae). *Applications in Plant Sciences* 2:1400078
- van Dijk KJ, McMahon K, Waycott M (in review) Genetic diversity, structure and connectivity of the seagrass *Halophila ovalis* in a highly grazed marine embayment, Moreton Bay, Australia. *PLOS ONE*
- Wainwright BJ, Arlyza IS, Karl SA (2013) Isolation and characterization of twenty-four polymorphic microsatellite loci for the tropical seagrass, *Thalassia hemprichii*. *Conservation Genetics Resources* 5:939-941
- Wang J (2015) Does GST underestimate genetic differentiation from marker data? *Molecular Ecology* 24:3546–3558
- Waycott M, Duarte CM, Carruthers TJB, Orth RJ, Dennison WC, Olyarnik S, Kenworthy WJ, Short FT, Williams SL (2009) Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Proceedings of the National Academy of Sciences in press*:12377-12381
- Waycott M, McMahon KM, Lavery PS (2014) *A Guide to Temperate Southern Seagrasses*. CSIRO, Melbourne, p108, ISBN13 9780864437266
- Waycott M, McMahon KM, Mellors JE, Calladine A, Kleine D (2004) *A Guide to Tropical Seagrasses of the Indo-West Pacific*. James Cook University, Townsville, p 72, ISBN10 0864437269
- Wells FE, Hanley JR, Walker DI (1995) *Marine Biological Survey of the Southern Kimberley*. Western Australian Museum, Perth
- Widén B, Cronberg N, Widén M (1994) Genotypic diversity, molecular markers and spatial distribution of genets in clonal plants. In: Soukupová L, Marshall C, Hara T, Herben T (eds) *Plant clonality; biology and diversity*. Opulus Press, Uppsala, Sweden
- Wilson B (2013) *The Biogeography of the Australian North West Shelf: Environmental change and life's response*. Elsevier Inc, San Diego, USA
- Wright S (1951) The genetical structure of populations. *Annals of Eugenics* 15:323–354
- Xu NN, Yu S, Zhang JG, Tsang PKE, Chen XY (2010) Microsatellite primers for *Halophila ovalis* and cross-amplification in *H. minor* (Hydrocharitaceae). *American Journal of Botany* 97:E56-E57
- Yu Y, Liu H, Lin P (2012) A quasi-global 1/10o eddy-resolving ocean general circulation model and its preliminary results. *Chinese Science Bulletin* 57:3908-3916

6 Acknowledgements

For funding and logistical support we are grateful to the Western Australian Marine Science Institution (WAMSI), and in particular Kelly Waples, Stuart Field and Kim Friedman who provided feedback, advice and encouragement. We also gratefully acknowledge those who collected or assisted us with collections including the Kimberley Ecological Connectivity team, O. Berry, J. Underwood, M. Travers, Z. Richards, G. Moore, as well as, J Gilmour, J.P. Hobbs, A.Z. Perez, G Kendrick, F. Webster, S. Moyle, A. Isaac, S. Isaac, T. Stumpagee, T. Vigilante, M. Jackson and the Wunambal Gaambera rangers. In addition, we thank the Bardi Jawi community, rangers and the traditional owners, the Kimberley Land Council (Daniel Oades), One Arm Point PBC (Damon Pyke), Cygnet Bay Pearls (James Brown, Erin McGinty, Ali McCarthy, Duncan Smith) for assisting with field logistics in the Kimberley. Finally M. Feng for oceanographic connectivity modelling. K. McMahon was co-supported by the CRN G100379 project of the Department of Education and Training, Collaborative Research Network Program (Funding Agreement CRN2011:5, Edith Cowan University and University of Western Australia) during this project and U. Hernawan by an ECU International Postgraduate Research Scholarship.

7 Data Availability

Data associated with this research is available on the Edith Cowan University Data Access Portal at: <http://dx.doi.org/10.4225/75/58d1f02d5ac30>

8 Communication

- 8.1 Mr Udhi Hernawan was supported with field and laboratory resources from this project. The Kimberley work on *Thalassia hemprichii* forms one chapter in his dissertation, which was submitted in July 2016. The analysis on *T. hemprichii* in this report was undertaken by Mr Hernawan.
- 8.2 One journal publication has been accepted, and one is in review, see below.
- 8.3 No technical reports have been produced.
- 8.4 Manuscripts accepted and in review.
 - One specifically on the patterns of genetic connectivity for *T. hemprichii* (Hernawan, U, van Dijk, KJ, Kendrick, GA, Feng, M, Berry, O, Kavazos, C, McMahon, K. Extreme ocean currents and habitat characteristics drive genetic divergence in a tropical seagrass. (In review Molecular Ecology),
 - and one on the broader patterns from Indonesia to the Pilbara, in WA, including the Kimberley (Hernawan U, van Dijk K, Kendrick G, Feng M, Biffin E, Lavery P, McMahon K. Historical processes and contemporary ocean currents drive genetic structure in the seagrass *Thalassia hemprichii* in the Indo-Australian Archipelago. (Accepted, Molecular Ecology).
- 8.5 The following conference presentations were made during this project
 - Australian Marine Sciences Association, Geelong, Australia. 2015. Genetic connectivity of the seagrass *Thalassia hemprichii* in the Kimberley and Pilbara. **Kathryn McMahon**, Udhi Hernawan, Gary Kendrick, Korjent van Dijk, Paul Lavery, Oliver Berry, Mike Travers, Jim Underwood.
 - Coastal and Estuarine Research Federation, Oregon, Portland, USA. 2015. So near, yet so far: Genetic connectivity of the seagrass *Thalassia hemprichii* in tropical Australia. **Udhi Hernawan**, Kathryn McMahon, Gary Kendrick, Korjent van Dijk, Paul Lavery.
 - University of Jogjakarta, Natural resources from local to global conference. 2015. Invited speaker. Molecular ecology of seagrasses: tools for conservation and management. **Kathryn McMahon**
 - Indonesian Institute of Sciences, 2015. Management and conservation of valuable seagrass ecosystems. **Kathryn McMahon**
 - ECU Research Week 2015. What we know about connections in seagrasses: Long-distance dispersal, millennial movements and emerging patterns in NW WA. **Kathryn McMahon**

- ECU Research Week 2015. Predictors of genetic structure in marine organisms in the Indo-Australian Archipelago: Generalisable patterns and a seagrass-case study. **Udhi Hernawan**, Kathryn McMahon, Gary Kendrick, Korjent van Dijk, Paul Lavery.
- ECU Postgraduate Symposium 2015. Genetic connectivity of a tropical seagrass in an extreme environment: It is not just going with the flow. **Udhi Hernawan**, Kathryn McMahon, Gary Kendrick, Korjent van Dijk, Paul Lavery.

8.6 The following poster presentations were made during this project

- WAMSI Kimberley Symposium 2015. Going with the Flow: Ecological Connectivity of the seagrass *Thalassia hemprichii* in the Kimberley and North West Cape, Western Australia. Udhi Hernawan, Kathryn McMahon, Gary Kendrick, Korjent van Dijk, Paul Lavery, Oliver Berry, Mike Travers, Jim Underwood

8.7 Other communications achievements

- 8.8 Through this project additional genetic connectivity work has been funded as part of a collaboration between ECU and Parks and Wildlife, to investigate further the genetic connectivity of the seagrass *H. ovalis* through the Pilbara. This will allow increasing the scope of the existing beyond the Kimberley and link with previous work by McMahon in the southern Pilbara.



Isolation of oceanic and coastal populations of the harvested mother-of-pearl shell *Tectus niloticus* in the Kimberley

Oliver Berry^{1,5}, Zoe Richards^{2,3,5}, Glenn Moore^{2,5}, Udhi Hernawan^{4,5}

¹CSIRO, Crawley, Western Australia

²Western Australian Museum, Perth, Western Australia

³Curtin University, Bentley, Western Australia

⁴Edith Cowan University, Joondalup, Western Australia

⁵Western Australian Marine Science Institution, Perth, Western Australia

WAMSI Kimberley Marine Research Program Executive Summary

Subproject 1.1.3.3

August 2017



western australian
marine science institution



Department of Biodiversity,
Conservation and Attractions



Department of
Primary Industries and
Regional Development



Mayala
Community



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.

Ownership of Intellectual property rights

Unless otherwise noted, copyright (and any other intellectual property rights, if any) in this publication is owned by the Western Australian Marine Science Institution, CSIRO, Australian Institute of Marine Science, Edith Cowan University, The University of Western Australia, Western Australia Department of Primary Industries and Regional Development, Western Australian Museum, Curtin University, and the Western Australian Department of Biodiversity, Conservation and Attractions.

Copyright

© Western Australian Marine Science Institution

All rights reserved.

Unless otherwise noted, all material in this publication is provided under a Creative Commons Attribution 3.0 Australia License. (<http://creativecommons.org/licenses/by/3.0/au/deed.en>)



Legal Notice

The Western Australian Marine Science Institution advises that the information contained in this publication comprises general statements based on scientific research. The reader is advised and needs to be aware that such information may be incomplete or unable to be used in any specific situation. This information should therefore not solely be relied on when making commercial or other decision. WAMSI and its partner organisations take no responsibility for the outcome of decisions based on information contained in this, or related, publications.

Front cover images (L-R)

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

Image 2: Small biopsy samples were taken non-lethally from the foot of trochus (*Tectus niloticus*) during fieldwork in the Kimberley and on offshore atolls. (Image: Zoe Richards, Curtin University)

Image 3: Humpback whale breaching (Image: Pam Osborn)

Image 4: Trochus (*Tectus niloticus*) is a harvested mollusc that is abundant on some intertidal reefs in the Kimberley, and throughout the Indo-Pacific. (Image: Zoe Richards, Curtin University)

Year of publication: August 2017

Metadata: <http://catalogue.aodn.org.au/geonetwork/srv/eng/metadata.show?uuid=fb1d80bf-6ef2-4150-9479-22b4240435a7>

Citation: Berry O, Richards Z, Moore G, Hernawan U (2017) Isolation of oceanic and coastal populations of the harvested mother-of-pearl shell *Tectus niloticus* in the Kimberley. Report of Project 1.1.3 - Project 1.1.3.3 prepared for the Kimberley Marine Research Program, Western Australian Marine Science Institution, Perth, Western Australia, 27pp.

Author Contributions: All authors contributed to the drafting of this text.

Corresponding author and Institution: Oliver Berry, CSIRO Oceans and Atmosphere. oliver.berry@csiro.au

Funding Sources: This project was funded (commissioned) by the Western Australian Marine Science Institution as part of the WAMSI Kimberley Marine Research Program, a \$30M program with seed funding of \$12M provided by State government as part of the Kimberley Science and Conservation Strategy. The Program has been made possible through co-investment from the WAMSI Joint Venture partners and further enabled by data and information provided by Woodside Energy Ltd.

Competing Interests: The commercial investors and data providers had no role in the data analysis, data interpretation, the decision to publish or in the preparation of the manuscript. The authors have declared that no competing interests exists.

Kimberley Traditional Owner agreement: This research was enabled by the Traditional Owners through their advice, participation and consent to access their traditional lands.

Acknowledgements: We are grateful to all WAMSI staff for their assistance, and in particular Kelly Waples, Stuart Field and Kim Friedman for providing advice and constructive criticism throughout the project. Many other people and organisations contributed to the success of this project, and we thank them sincerely. We especially thank the following Indigenous communities. The Bardi and Jawi Niimidiman Aboriginal Corporation, the Bardi Jawi Rangers and the Bardi Jawi Traditional Owners and specifically Daniel Oades, Damon Pyke, Azton Howard, Chris Sampi, Kevin George, Kevin Ejai, Kevin Dougal, Tasha Stumpagee, Phillip McCarthy, Peter Hunter, Zac Ejai, Paul Davey and Trevor Sampi. The Mayala people, specifically Sandy, Alec and Janella Isaacs. The Wunambal Gaambera Aboriginal Corporation, Traditional Owners, Uunguu Rangers and Tom Vigilante. The Dambimangari Aboriginal Corporation, Dambimangari Traditional Owners and the Dambimangari Rangers. Thanks also to the Bioinformatic pipeline development: Bernd Gruber (University of Canberra); Oceanographic modelling: Ming Feng, Dirk Slawinski (CSIRO); SNP marker development and genotyping: Andrzej Kilian (Diversity Arrays Technology); Field assistance: Sam Moyle and Fiona Webster; Kimberley Marine Research Station for facilities and logistical support: James Brown, Michael Flynn, Scott Whitlam, Duncan Smith and Erin McGinty; and advice on project development: Karen Miller (AIMS).

Collection permits/ethics approval: SF008440, SF009910, SC001362 (Western Australian Department of Parks and Wildlife); 2485 2085, 2344 (Western Australian Department of Fisheries)

Executive Summary

This report focuses on “trochus” or “mother of pearl shell” *Tectus niloticus*, which is a large harvested gastropod mollusc common on intertidal reefs in the Kimberley and the wider Indo-Pacific. This species was selected as a model for a study of connectivity in molluscs because it has a short larval life-history (3-5 days), which is typical of species whose recruitment is primarily local and they are prone to over-harvest. Over-harvest of *T. niloticus* has been documented throughout its range, and it’s been argued that placement of reserves adjacent to harvested regions would be an effective way to sustain the species. However, the unique complexity and power of the Kimberley hydrodynamic environment potentially enlarges the scale of recruitment and/or creates spatially complex dynamics that may be relevant to harvest management in the region.

T. niloticus is also unusual because it is present on both coastal Kimberley reefs and oceanic atolls at the edge of the Australian continental shelf margin. Oceanic and coastal reefs have profoundly different faunal diversity, and biogeographers have speculated on whether this can be attributed to their pronounced environmental differences or to hydrodynamic isolation. However, the scarcity of species common to both environments has meant that this hypotheses remains untested. Oceanic populations are also harvested by Indonesian artisanal fishers, and there is a need to understand how oceanic reefs depend on recruitment and genetic variation from reefs elsewhere.

Samples from 514 *T. niloticus* individuals were collected from 16 “coastal” sites in the Dampier Peninsular and Buccaneer Archipelago as well as the “oceanic” sites the Rowley Shoals and Scott Reef. We employed a genotype-by-sequencing approach to characterise genetic diversity within and between these sampling sites. Custom bioinformatics pipelines were developed to analyse this large dataset. After quality control filtering, 5,428 single nucleotide polymorphisms (SNPs) were available for analysis.

Insights into broad-scale genetic structure between coastal and oceanic sites

Significant genetic sub-division was evident between the oceanic sites (the Rowley Shoals and Scott Reef) and the coastal sites (distances c. 500 and 300 km respectively). Significant genetic sub-division was also evident between the two oceanic sites (distance c. 400km), but it was approximately 25% of the magnitude of the oceanic – coastal sites comparison. Evidence for significant adaptive genetic differences between the coastal and oceanic sites was indicated by the presence of a sub-set of highly divergent “outlier” genetic loci.

This means that oceanic *T. niloticus* populations are genetically and demographically independent from coastal populations and from each other. The closer affinity of oceanic populations to each other than to coastal populations reflects irregular connectivity on evolutionary timescales under the influence of the Indonesian Flow-Through and derivative currents. Different environmental conditions on oceanic and coastal reefs are also driving adaptive divergence between *T. niloticus* populations.

Insights into fine-scale genetic structure within the coastal Kimberley

Negligible genetic sub-division was evident among the Dampier Peninsular-Buccaneer Archipelago coastal sites (distances \leq 75km), and what sub-division was recorded could not be attributed to geographic distance nor modelled oceanographic connectivity.

T. niloticus inhabiting reefs on the Dampier Peninsular and Buccaneer Archipelago form a single highly-mixed genetic unit, and are highly demographically inter-dependent. This is likely due to their high and continuous reproductive output in combination with the extreme hydrodynamic mixing experienced in the region.

Implications for management at a broad-scale

Management of *T. niloticus* at the Rowley Shoals, Scott Reef, and other oceanic shoals should treat each as being effectively isolated on the ecological timeframes relevant to harvest management. Recruitment from outside will not replenish over-harvested stocks even within tens of years. Occasional recruits will be drawn from other offshore shoals, and possibly Indonesia, and will contribute genetic diversity rather than offsetting over-harvest. Potential supplementation of populations should recognise that coastal *T. niloticus* populations may be mal-adapted to oceanic conditions.

Implications for management at a fine-scale

Management of *T. niloticus* on the Dampier Peninsular and Buccaneer Archipelago should treat the region as being effectively a single stock on the ecological timeframes relevant to harvest management. Over-harvested sites within this region will be replenished with recruits from neighbouring sites within years, assuming they exist, and allowing for the slow growth of the species.

Residual knowledge gaps

This investigation had a limited spatial scope in comparison to the broad Indo-Pacific range of *T. niloticus*, capturing the south-westernmost part of its range. Indeed, even within the Kimberley region, the region of high density in the Buccaneer Archipelago is disjunct from other high density populations in Australia, Indonesia and on offshore atolls. The broad distribution of *T. niloticus* in the tropical Indo-Pacific incorporating a diversity of reef types and hydrodynamic conditions means that it is unlikely that the spatial scale of genetic structure will be reflected throughout its range. Considering the economic and cultural significance of the species to many people, a broader investigation of population structure in *T. niloticus* and its biophysical drivers deserves consideration.

Please Note:

The details of this report are currently subject to a journal publication process. For more information contact the author: Dr Oliver Berry, CSIRO Oceans and Atmosphere. oliver.berry@csiro.au.



Genomic Connectivity in a Tropical Reef Fish from the Kimberley, Pilbara and Gascoyne Bioregions of Western Australia

Oliver Berry^{1,6}, Mike Travers^{2,6}, Richard Evans^{3,6}, Glenn Moore^{4,6}, Udhi Hernawan^{5,6}

¹CSIRO Oceans and Atmosphere, Crawley, Western Australia

²Fisheries Division, Department for Primary Industries and Regional Development, Hillarys, Western Australia

³Department of Biodiversity, Conservation and Attractions, Kensington, Western Australia

⁴Western Australian Museum, Perth, Western Australia

⁵Edith Cowan University, Joondalup, Western Australia

⁶Western Australian Marine Science Institution, Perth, Western Australia

WAMSI Kimberley Marine Research Program Executive Summary

Subproject 1.1.3.4a

August 2017



western australian
marine science institution



Department of Biodiversity,
Conservation and Attractions



Department of
Primary Industries and
Regional Development



Mayala
Community



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.

Ownership of Intellectual property rights

Unless otherwise noted, copyright (and any other intellectual property rights, if any) in this publication is owned by the Western Australian Marine Science Institution, CSIRO, Australian Institute of Marine Science, Edith Cowan University, The University of Western Australia, Western Australia Department of Primary Industries and Regional Development, Western Australian Museum, Curtin University and the Western Australian Department of Biodiversity, Conservation and Attractions.

Copyright

© Western Australian Marine Science Institution

All rights reserved.

Unless otherwise noted, all material in this publication is provided under a Creative Commons Attribution 3.0 Australia License. (<http://creativecommons.org/licenses/by/3.0/au/deed.en>)



Legal Notice

The Western Australian Marine Science Institution advises that the information contained in this publication comprises general statements based on scientific research. The reader is advised and needs to be aware that such information may be incomplete or unable to be used in any specific situation. This information should therefore not solely be relied on when making commercial or other decision. WAMSI and its partner organisations take no responsibility for the outcome of decisions based on information contained in this, or related, publications.

Front cover images (L-R)

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

Image 2: Mike Travers (Dept Fisheries) collects samples from the damselfish *Pomacentrus milleri* on a reef platform at Sunday Island in the Kimberley (Image: Oliver Berry)

Image 3: Humpback whale breaching (Image: Pam Osborn)

Image 4: Juvenile *Pomacentrus milleri* (Image: Western Australian Museum)

Year of publication: August 2017

Metadata: <http://catalogue.aodn.org.au/geonetwork/srv/eng/metadata.show?uuid=fb1d80bf-6ef2-4150-9479-22b4240435a7>

Citation: Berry O, Travers M, Evans R, Moore G, Hernawan U (2017) Genomic Connectivity in a Tropical Reef Fish from the Kimberley, Pilbara and Gascoyne Bioregions of Western Australia. Report of Project 1.1.3 - Project 1.1.3.4a prepared for the Kimberley Marine Research Program, Western Australian Marine Science Institution, Perth, Western Australia, 36pp.

Author Contributions: All authors contributed to the drafting of this text.

Corresponding author and Institution: Oliver Berry, CSIRO Oceans and Atmosphere. oliver.berry@csiro.au

Funding Sources: This project was funded (commissioned) by the Western Australian Marine Science Institution as part of the WAMSI Kimberley Marine Research Program, a \$30M program with seed funding of \$12M provided by State government as part of the Kimberley Science and Conservation Strategy. The Program has been made possible through co-investment from the WAMSI Joint Venture partners and further enabled by data and information provided by Woodside Energy Ltd.

Competing Interests: The commercial investors and data providers had no role in the data analysis, data interpretation, the decision to publish or in the preparation of the manuscript. The authors have declared that no competing interests exists.

Kimberley Traditional Owner agreement: This research was enabled by the Traditional Owners through their advice, participation and consent to access their traditional lands.

Acknowledgements: We are grateful to all WAMSI staff for their assistance, and in particular Kelly Waples, Stuart Field and Kim Friedman for providing advice and constructive criticism throughout the project. Many other people and organisations contributed to the success of this project, and we thank them sincerely. We especially thank the following Indigenous communities. The Bardi and Jawi Niimidiman Aboriginal Corporation, the Bardi Jawi Rangers and the Bardi Jawi Traditional Owners and specifically Daniel Oades, Damon Pyke, Azton Howard, Chris Sampi, Kevin George, Kevin Ejai, Kevin Dougal, Tasha Stumpagee, Phillip McCarthy, Peter Hunter, Zac Ejai, Paul Davey and Trevor Sampi. The Mayala people, specifically Sandy, Alec and Janella Isaacs. The Wunambal Gaambera Aboriginal Corporation, Traditional Owners, Uunguu Rangers and Tom Vigilante. The Dambimangari Aboriginal Corporation, Dambimangari Traditional Owners and the Dambimangari Rangers. Thanks also to the Bioinformatic pipeline development: Bernd Gruber (University of Canberra); Oceanographic modelling: Ming Feng, Dirk Slawinski (CSIRO); SNP marker development and genotyping: Andrzej Kilian (Diversity Arrays Technology); Field assistance: Sam Moyle and Fiona Webster; Kimberley Marine Research Station for facilities and logistical support: James Brown, Michael Flynn, Scott Whitlam, Duncan Smith and Erin McGinty; and advice on project development: Karen Miller (AIMS).

Collection permits/ethics approval: SF008440, SF009910, SC001362 (Western Australian Department of Parks and Wildlife); 2485 2085, 2344 (Western Australian Department of Fisheries)

Executive Summary

This sub-report focuses on Miller's damselfish, *Pomacentrus milleri*, which is an obligate reef-dwelling fish endemic to the north-west coast of Australia between Perth and Arnhemland in the Northern Territory.

Pomacentrus milleri was selected as a model because it is ecologically representative of a large group of small coral reef fishes that are abundant throughout tropical Australia and the wider Indo-Pacific. It is also a demersal nester (lays eggs in nests), has a relatively short pelagic larval duration (c. 20 days), and adults permanently reside on reefs. These characteristics suggest that *P. milleri* populations should be highly responsive to local environmental and hydrodynamic conditions, implying lower connectivity between reefs and predominantly short-range recruitment relative to organisms with longer dispersal phases and larger home ranges. However, the unique complexity and power of the Kimberley hydrodynamic environment potentially enlarges the scale of connectivity and/or creates more spatially complex linkages than would be expected elsewhere (Wilson 2013). *Pomacentrus milleri* was also selected as a model because it is moderately common along sub-tropical and tropical parts of the Western Australian coast. This permitted, for the first time in Western Australia, a detailed genomic examination of broad-scale population connectivity in a marine organism.

Samples *P. milleri* individuals were collected from the Dampier Peninsula and Buccaneer Archipelago, and the Bonaparte Archipelago and north Kimberley. In addition, samples from the Pilbara and Gascoyne bioregions¹ were included to provide a regional context for the Kimberley results. We employed a genotype-by-sequencing approach to characterise genetic diversity within and between these sampling sites. Custom bioinformatics pipelines were developed to analyse this large dataset. After quality control filtering, 4,472 single nucleotide polymorphisms (SNPs) were available for analysis.

Broad-scale genetic structure between Kimberley and elsewhere

The three major bioregions sampled (Kimberley, Pilbara, Gascoyne) were all genetically differentiated from each other. The relationships between bioregions followed a strong isolation-by-distance pattern, but with the Pilbara and Gascoyne comparatively more connected to each other than the Kimberley to the Pilbara. This likely reflects the more continuous reef habitat between the Pilbara and Gascoyne. The magnitude of the genetic differentiation observed indicates that *P. milleri* populations from each bioregion exchange few recruits and are effectively demographically independent.

Fine-scale genetic structure within the Kimberley

Some sites sampled within the Dampier Peninsula-Buccaneer Archipelago (≤ 75 km apart) were genetically differentiated from each other, but the magnitude of this differentiation was low. Shenton Bluff, Bowlun (Dampier Peninsula) and Longitude Island (Buccaneer Archipelago) were consistently differentiated from other sites, but most sites were either undifferentiated or weakly differentiated without a clear geographic basis. Sites from the north Kimberley were genetically differentiated from all sites in the Dampier Peninsula-Buccaneer Archipelago region (≤ 400 km apart). Genetic differentiation between sites was largely attributable to distance between sites rather than modelled hydrodynamic connectivity. A signal of relatedness between individuals decayed to c. 350km, indicating the approximate scale of regular demographic exchange. These observations are consistent with movement and gene flow being limited on spatial scales less than hundreds of kilometres.

¹ These samples were made available through collaboration with Richard Evans at the Department of Parks and Wildlife, and historical samples from The Western Australian Department of Fisheries.

The Kimberley is less connected than the Pilbara

The pattern of isolation-by-distance observed in the Kimberley was not replicated within the Pilbara. Only 22% of pairwise comparisons between Pilbara sites were genetically differentiated, whereas in the Kimberley 78% of pairwise comparisons between sites were genetically differentiated. These observations are consistent with more extensive movement occurring between reefs in the Pilbara than the Kimberley.

Evidence for local adaptation

Pomacentrus milleri is subject to directional selection across its sampled range. Adaptation under the influence of directional selection was detected among sites within the Kimberley, but not within the Pilbara. This likely reflects the greater habitat and hydrodynamic variation and complexity in the Kimberley than the Pilbara.

Genetic diversity is highest in the Kimberley

We observed a marked decline in genetic diversity from north to south (Kimberley to Gascoyne). This likely reflects higher densities of *P. milleri* in the north, and potentially a prevailing southwards current leading to asymmetric gene flow southwards.

Implications for Management

- The Kimberley and Pilbara bioregions exchange few recruits and should be considered as largely independent on the ecological timeframes relevant to management.
- With a greater level of genetic diversity, Kimberley populations of *P. milleri*, are likely to be more resilient to environmental and anthropogenic stresses than those in the Pilbara. However, once impacted, the recovery potential of populations in the Kimberley is reduced as a consequence of more restricted movements between reefs. By implication, sanctuary zones in the Kimberley should be more closely spaced than elsewhere in the Pilbara to provide for optimal protection and management of *P. milleri* and similar species.
- Pilbara populations of *P. milleri* are likely to recover from small scale localised impacts through recruitment from other reefs in the Pilbara with which they are highly connected. However, lower genetic diversity in this region means that resilience to impacts may be lower, relative to Kimberley populations.
- Gascoyne populations of *P. milleri* are potentially the most vulnerable among those sampled due to reduced genetic diversity and greater isolation.

Residual knowledge gaps

- *Pomacentrus milleri* is a useful model for small reef-dependent species. However, this study has only examined a fraction of the species' range. *Pomacentrus milleri*'s range extends into the Northern Territory and New Guinea. The extent of connectivity between *P. milleri* in Western Australia and other regions is unknown.
- Although the results presented here have revealed evidence for geographically structured adaptive diversification in *P. milleri*, the specific environmental drivers have not been identified.
- *Pomacentrus milleri* shares a life history with many small reef-dependent fish species. It is anticipated that this would be reflected in comparable population genetic structure in similar species, but this hypothesis requires empirical testing.

Scientific Abstract

Complex ocean currents promote adaptive diversification and lower dispersal in a tropical reef fish from north-western Australia.

Two important goals of biological conservation are to identify regions of high evolutionary novelty, and to manage them at appropriate spatial scales. Characterising these attributes is a technical challenge, particularly in the marine environment where sampling and observation may be difficult. In poorly studied regions, population genomic approaches potentially offer opportunities to simultaneously examine spatial processes as well as contemporary evolutionary diversification. Here we show that a common damselfish from north-western Australia exhibits more spatial genetic structure and greater putative adaptive genetic diversity in a macro-tidal region than a meso-tidal region. Using genome scans consisting of 4,472 SNP loci applied to 847 samples of the damselfish *Pomacentrus milleri*, we detected marked genetic sub-division between the macro-tidal Kimberley bioregion (up to 12 metre tides) and the meso-tidal Pilbara and Gascoyne bioregions (range of spring tides 1-5 metre). Individually, these bioregions also differed in the extent of population sub-division; spatial autocorrelation was detectable over several hundred kilometres in the Kimberley, but undetectable in the Pilbara. This implies, paradoxically, that the substantially stronger currents in the Kimberley promote shorter range dispersal than in the Pilbara, possibly because larval retention zones are created by the region's complex bathymetry, and currents are predominantly tidal rather than along-shore. The Kimberley also exhibited significantly more neutral genetic diversity than the other bioregions, as well as 108 putatively adaptive outlier loci, whereas no outlier loci were detected elsewhere. We conclude that the Kimberley bioregion likely represents an important source of evolutionary novelty in *P. milleri*, and that optimal management of this and similar species would occur on smaller spatial scales than elsewhere in north-western Australia.

Please Note:

The details of this report are currently subject to a journal publication process. For more information contact the author: Dr Oliver Berry, CSIRO Oceans and Atmosphere. oliver.berry@csiro.au



Population connectivity of the Stripey Snapper *Lutjanus carponotatus* along the ecologically significant coast of northwestern Australia

Joseph DiBattista^{1,6}, Mike Travers^{2,6}, Oliver Berry^{3,6}, Glenn Moore^{4,6}, Richard Evans^{5,6}, Ming Feng^{3,6}, Stephen Newman^{2,6}

¹Curtin University, Bentley, Western Australia

²Fisheries Division, Department for Primary Industries and Regional Development, Hillarys, Western Australia

³CSIRO, Crawley, Western Australia

⁴Western Australian Museum, Perth, Western Australia

⁵Department of Biodiversity, Conservation and Attractions, Kensington, Western Australia

⁶Western Australian Marine Science Institution, Perth, Western Australia

WAMSI Kimberley Marine Research Program

Final Report

Subproject 1.1.3.4b

August 2017



western australian
marine science institution



Department of Biodiversity,
Conservation and Attractions



Department of
Primary Industries and
Regional Development



Mayala
Community



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, unspoiled 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.

Ownership of Intellectual property rights

Unless otherwise noted, copyright (and any other intellectual property rights, if any) in this publication is owned by the Western Australian Marine Science Institution, CSIRO, Australian Institute of Marine Science, Edith Cowan University, The University of Western Australia, Western Australia Department of Primary Industries and Regional Development, Western Australian Museum, Curtin University and the Western Australian Department of Biodiversity, Conservation and Attractions.

Copyright

© Western Australian Marine Science Institution

All rights reserved.

Unless otherwise noted, all material in this publication is provided under a Creative Commons Attribution 3.0 Australia License. (<http://creativecommons.org/licenses/by/3.0/au/deed.en>)



Legal Notice

The Western Australian Marine Science Institution advises that the information contained in this publication comprises general statements based on scientific research. The reader is advised and needs to be aware that such information may be incomplete or unable to be used in any specific situation. This information should therefore not solely be relied on when making commercial or other decision. WAMSI and its partner organisations take no responsibility for the outcome of decisions based on information contained in this, or related, publications.

Front cover images (L-R)

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

Image 2: Stripey snapper. (Image: DBCA)

Image 3: Humpback whale breaching (Image: Pam Osborn)

Image 4: Stripey snapper (Image: DBCA)

Year of publication: August 2017

Metadata: <http://catalogue.aodn.org.au/geonetwork/srv/eng/metadata.show?uuid=fb1d80bf-6ef2-4150-9479-22b4240435a7>

Citation: DiBattista J, Travers M, Berry O, Moore G, Evans R, Feng M, Newman S (2017) Population connectivity of the Stripey Snapper *Lutjanus carponotatus* along the ecologically significant coast of northwestern Australia. Report of Project 1.1.3 - Project 1.1.3.4b prepared for the Kimberley Marine Research Program, Western Australian Marine Science Institution, Perth, Western Australia, 41pp.

Author Contributions: All authors contributed to the drafting of this text.

Corresponding author and Institution: Oliver Berry, CSIRO Oceans and Atmosphere. oliver.berry@csiro.au

Funding Sources: This project was funded (commissioned) by the Western Australian Marine Science Institution as part of the WAMSI Kimberley Marine Research Program, a \$30M program with seed funding of \$12M provided by State government as part of the Kimberley Science and Conservation Strategy. The Program has been made possible through co-investment from the WAMSI Joint Venture partners and further enabled by data and information provided by Woodside Energy Ltd.

Competing Interests: The commercial investors and data providers had no role in the data analysis, data interpretation, the decision to publish or in the preparation of the manuscript. The authors have declared that no competing interests exists.

Kimberley Traditional Owner agreement: This research was enabled by the Traditional Owners through their advice, participation and consent to access their traditional lands.

Acknowledgements: We are grateful to all WAMSI staff for their assistance, and in particular Kelly Waples, Stuart Field and Kim Friedman for providing advice and constructive criticism throughout the project. Many other people and organisations contributed to the success of this project, and we thank them sincerely. We especially thank the following Indigenous communities. The Bardi and Jawi Niimidiman Aboriginal Corporation, the Bardi Jawi Rangers and the Bardi Jawi Traditional Owners and specifically Daniel Oades, Damon Pyke, Azton Howard, Chris Sampi, Kevin George, Kevin Ejai, Kevin Dougal, Tasha Stumpagee, Phillip McCarthy, Peter Hunter, Zac Ejai, Paul Davey and Trevor Sampi. The Mayala people, specifically Sandy, Alec and Janella Isaacs. The Wunambal Gaambera Aboriginal Corporation, Traditional Owners, Uunguu Rangers and Tom Vigilante. The Dambimangari Aboriginal Corporation, Dambimangari Traditional Owners and the Dambimangari Rangers. Thanks also to the Bioinformatic pipeline development: Bernd Gruber (University of Canberra); Oceanographic modelling: Ming Feng, Dirk Slawinski (CSIRO); SNP marker development and genotyping: Andrzej Kilian (Diversity Arrays Technology); Field assistance: Sam Moyle and Fiona Webster; Kimberley Marine Research Station for facilities and logistical support: James Brown, Michael Flynn, Scott Whitlam, Duncan Smith and Erin McGinty; and advice on project development: Karen Miller (AIMS).

Collection permits/ethics approval: SF008440, SF009910, SC001362 (Western Australian Department of Parks and Wildlife); 2485 2085, 2344 (Western Australian Department of Fisheries)

Contents

- CONTENTS..... I**
- EXECUTIVE SUMMARY I**
- IMPLICATIONS FOR MANAGEMENT AT A BROAD- AND FINE-SCALE II**
- RESIDUAL KNOWLEDGE GAPS II**
- 1 INTRODUCTION 1**
- 2 MATERIALS AND METHODS 2**
 - 2.1 STUDY AREA AND SAMPLE COLLECTION.....2
 - 2.2 DNA EXTRACTION8
 - 2.3 REDUCED REPRESENTATION SNP GENOTYPING8
 - 2.4 SNP CALLING8
 - 2.5 SNP QUALITY CONTROL FILTERING8
 - 2.6 POPULATION GENETIC STATISTICS.....8
 - 2.7 MODEL-BASED CLUSTERING ANALYSIS9
 - 2.8 DISCRIMINANT ANALYSIS OF PRINCIPLE COMPONENTS (DAPC).....9
 - 2.9 DETERMINANTS OF GENETIC DIFFERENTIATION.....9
 - 2.10 SPATIAL AUTOCORRELATION AND IBD.....10
- 3 RESULTS..... 10**
 - 3.1 GENETIC DIVERSITY10
 - 3.2 GENETIC SUBDIVISION.....11
 - 3.3 MODEL-BASED CLUSTERING ANALYSIS13
 - 3.4 DISCRIMINANT ANALYSIS OF PRINCIPLE COMPONENTS (DAPC).....14
 - 3.5 DETERMINANTS OF GENETIC DIFFERENTIATION.....17
 - 3.6 SPATIAL AUTOCORRELATION AND IBD.....17
- 4 DISCUSSION AND CONCLUSIONS 20**
 - 4.1 GENETIC DIVERSITY IS HIGHEST AT RANGE LIMITS20
 - 4.2 BROAD-SCALE SUBDIVISION ACROSS NWA20
 - 4.3 FINE-SCALE CONNECTIVITY ACROSS NWA.....22
- 5 REFERENCES..... 23**
- 6 ACKNOWLEDGEMENTS 26**
- 7 DATA AVAILABILITY 26**
- 8 APPENDICES..... 27**
 - APPENDIX 1. A COMPARISON OF SNP TYPE.....27
 - APPENDIX 2. PRINCIPAL COMPONENT ANALYSIS28
 - APPENDIX 3. STRUCTURE HARVESTER ANALYSES.....30
 - APPENDIX 4. LINEAR MODEL RANKING.....31
 - APPENDIX 5. CORRELATION BETWEEN PAIRWISE GENETIC DISTANCE, GEOGRAPHIC, AND ENVIRONMENTAL DISTANCES.....32



Executive Summary

This report focuses on the widespread and abundant Stripey Snapper (*Lutjanus carponotatus*), which is an important recreationally targeted lutjanid in coastal waters throughout the Central Indo-Pacific realm, including the coast of northwestern Australia (NWA) south to Shark Bay. This species was selected as a model to represent numerous broadcast pelagic spawning reef-associated fish species with relatively long pelagic larval durations (PLD for *L. carponotatus* ~ 37 days). The Stripey Snapper is among the top five targeted inshore fish species by recreational anglers in NWA and is managed according to a full Ecosystem Based Fisheries Management (EBFM) approach in which the sustainability of targeted fish species are assessed within the bioregional boundaries defined under the Integrated Marine and Coastal Regionalisation of Australia classification scheme (IMCRA, Commonwealth of Australia, 2006). The adoption of a widespread sampling regime in this study allowed us to explore the potential influences of extreme gradients in coastal hydrodynamics, such as tidal driven currents, water turbidity, and seasonal freshwater outflow from the Northern Territory (NT) southwards through the Kimberley, Canning, Pilbara, Ningaloo and Shark Bay Bioregions of Western Australia (WA). This study fills gaps in understanding of both broad-scale marine connectivity in NWA and fine-scale connectivity within the Kimberley Bioregion to be addressed against a background of rapid coastal development supporting the mineral and petrochemical industries. Such development has the potential to directly impact the biodiversity and productivity of nearshore marine ecosystems via dredging, construction, pollution, shipping, and other indirect pressures associated with increased human populations such as fishing.

One thousand and sixteen Stripey Snapper samples were collected from 3 locations in the NT, 29 locations in the coastal Kimberley Bioregion, 17 locations in the Pilbara and Canning bioregions, and 2 locations in the Shark Bay Bioregion (full dataset). In order to focus on only the best sampled populations ($N \geq 20$), 895 Stripey Snapper individuals were considered from 1 location in the NT, 11 locations in the coastal Kimberley bioregion, 17 locations in the Pilbara and Canning bioregions and 2 locations in the Shark Bay Bioregion (reduced dataset). Samples were genotyped via a genotype-by-sequencing method which, after quality filtering, yielded 4,402 polymorphic Single Nucleotide Polymorphism (SNP) loci that met Hardy-Weinberg equilibrium and linkage equilibrium expectations. A number of genetic analyses were repeated with a subset of outlier loci ($N = 66$ SNPs) that are putatively under directional selection.

Insights into broad and fine-scale connectivity

Significant genetic sub-division was evident between the Shark Bay Bioregion and all locations of the North West Shelf, including northern Ningaloo, and NT in most cases. A significant genetic 'transition zone' was evident across a geographic distance of < 80km across the tip of the Dampier Peninsula, near the entrance to King Sound, which marks the border of the Kimberley and Canning marine bioregions. There was evidence for an isolation-by-distance (IBD) effect overall and within the Pilbara, but isolation-by-distance was not evident among samples from the Kimberley Bioregion. Northern Ningaloo and the Pilbara exchange few recruits with Shark Bay and are effectively demographically independent, while the regions north of Shark Bay probably exchange recruits through a stepping stone process. Some tests support the genetic sub-division between NT and the adjacent Kimberley bioregion (i.e. pairwise F_{st} , STRUCTURE), whereas with other tests the evidence is equivocal (i.e. DAPC). Modelling the effects of barriers to dispersal, environmental attributes, and geographic distance on genetic differentiation in this species revealed that all three factors had strong effects, but in most cases, these effects could not be distinguished from each other because of strong correlations among them.

The genetic 'transition zone' in the Kimberley coincides with the Sunday Strait, which experiences the largest tropical tidal range and fastest tidal currents in the world. Here dispersal and realised gene flow is more limited than elsewhere throughout the range of this species, suggesting a possible zone of retention based on local hydrodynamic effects. Results from the spatial autocorrelation analysis showed local scale dispersal within the coastal Kimberley is at a scale of 300 km, except in the transition zone where it was only 80 km. Two

hydrodynamic models for the area now highlight a degree of retention within King Sound, which will likely be relevant to identifying the underlying process that may explain the reduced gene flow northward or southward from the transition zone.

Only 63% of pairwise comparison between Pilbara sites were genetically differentiated, whereas in the Kimberley 92% of pairwise comparisons between sites were genetically differentiated. This suggests that the Kimberley is less connected than the Pilbara. These observations are consistent with more extensive movement occurring between reefs in the Pilbara than the Kimberley.

Implications for management at a broad- and fine-scale

The level of inter-state genetic sub-division revealed in this study suggests that the current separate State and Territory based management arrangements for Stripey Snapper stocks in WA and the NT are likely to be appropriate, although there is a wide gap in sampling coverage between the Kimberley and the NT. The collection of additional samples between these two regions should be a priority. Based on this single broadcast spawning reef fish species, while the intra-state spatial genetic sub-division supports the separate fisheries management arrangements for the Gascoyne and North Coast bioregion stocks, the inclusion of the Ningaloo Bioregion within the Gascoyne coast of the State based fisheries management boundaries is not supported. The potential for demographic separation of Kimberley and Pilbara/Canning populations, including the genetic transition zone, should be taken into consideration for future management initiatives and reviews of management arrangements.

At a Kimberley Bioregional scale, management of Striper Snapper should be treated over this broad area as effectively being a single stock over the ecological timeframes relevant to harvest management. Samples collected from within the gazetted and proposed Kimberley marine parks suggest that at a fine scale, dispersal of Stripey Snapper between parks in the North Kimberley and the South-Western Kimberley is likely. However, the transition zone identified around the Dampier Peninsula that separates the Kimberley from the Pilbara/Canning populations should be recognised by managers of coastal resources along these coasts as a region of ecological significance.

Residual knowledge gaps

Genetic differentiation between samples of Stripey Snapper from the Kimberley and NT may represent limited demographic exchange between these currently separately managed stocks. Further sampling from the intermediate region is needed to confirm this and potentially refine the area of transition.

Ocean currents are likely to play a significant role in distributing the larvae of Stripey Snapper. Models of hydrodynamic processes throughout NWA are available (see Condie & Andrewartha 2008), however it would be useful to evaluate how well these models predict the observed genetic structure in Stripey Snapper, since that would provide confidence that the models accurately reflect biological processes and therefore may be applied to other bioregions and/or species. This analysis is currently in development (O. Berry unpublished).

In contrast, the transition zone identified around the Dampier Peninsula that separates the Kimberley from the Pilbara/Canning populations is likely to be influenced by the extreme tidal flushing at the head of King Sound, rather than ocean currents. A fine-scale hydrodynamic model for this region was prepared by WAMSI Kimberley Project 2.2.7 (M. Feng, CSIRO, pers. comm). It would be useful to test whether this model can account for the observed genetic structure in this highly dynamic zone that supports harvest of numerous fishes.

Evidence for temporal variation in population structure was revealed through the analysis of historically collected samples. For these temporal samples we explored the reason for their observed divergence and were able to exclude at least one mechanism of DNA degradation (Appendix 1). This result may therefore represent a real shift in allele frequencies over time, potentially indicative of changing patterns of larval connectivity.

However, since we did not sample these exact locations again, it's unclear whether the pattern is wholly temporal or also has a spatial component. Additional sampling at these historical sites is required to resolve this question.

1 Introduction

Coastal ecosystems are some of the richest and most productive environments on the planet and yet are often at higher risk to anthropogenic threats (i.e. fishing, tourism, coastal development) than ecosystems further from shore. Many marine species inhabiting coastal ecosystems have restricted home ranges and do not migrate as adults (Cowen & Sponaugle 2009), it is therefore the free-living, dispersive larval stage that instead enables connection between sites. As a direct consequence of this larval stage, nearshore marine species exist in a system of interconnected populations influenced by the vagaries of currents, larval behaviour, and recruitment dynamics (e.g. Treml *et al.* 2015). Some species will therefore operate as closed demographic units on small spatial scales (within a few kilometres), whereas others may remain connected over hundreds of kilometres. Coastal ecosystems can also be topographically complex, which makes predicting connectivity among the network of populations difficult given the environmental variability among sites (for review see Burgess *et al.* 2014).

The proliferation of next-generation sequencing (NGS) approaches that enable high-throughput Single Nucleotide Polymorphism (SNP) discovery and genotyping (Andrews *et al.* 2016) now provides a means to quantify connectivity within coastal ecosystems with much greater resolution. The isolation of thousands of SNP markers across the genome can parse neutral processes, such as genetic drift (Riginos & Liggins 2013), from natural selection, which may drive phenotypic divergence between populations inhabiting different ecological environments (Nosil *et al.* 2009; also see Rellstab *et al.* 2015). Ease of access to environmental data derived from satellite imagery also provides a great opportunity to examine how geography and environment further influence genetic structure, including identifying shared barriers to larval dispersal and significant sources of larval recruits (Balkenhol *et al.* 2009; Wang & Bradburd 2014).

The coast of NWA provides an emerging frontier for implementing these new genomic tools under a management framework, given its diverse and extreme environmental conditions. There are several bioregional classifications for this coast including the Provinces and Ecoregions of Spalding *et al.* (2007) and the Provincial and Meso-scale Bioregions of the Integrated Marine and Coastal Regionalisation of Australia (IMCRA) of the Commonwealth of Australia (2006). As the fisheries resources along this coast are largely managed according to the IMCRA Meso-scale Bioregions, we follow these bioregions and highlight the potential implications of the results of our study in relation to bioregional and management boundaries (see Fig. 1 for overview). The NWA coast spans six marine bioregions (*sensu* Commonwealth of Australia 2006). The tropical Anson Beagle and Kimberley bioregions in particular hosts more than 2,633 islands (i.e. Buccaneer and Bonaparte Archipelagos), a diverse assemblage of fish and corals (Travers *et al.* 2010; Moore *et al.* 2014; Richards *et al.* 2015), highly turbid water, seagrass meadows and mangrove forests (e.g. Duke 2006), and a strong tidal regime (range ~11 meters) that likely impacts larval exchange (Thackway & Cresswell 1998; also see Wilson 2014). Reef faunal communities in the Kimberley display heterogeneous composition within the bioregion, as well as differentiation from adjacent bioregions (Travers *et al.* 2010; Wilson 2014); only a few studies have assessed genetic variation here (sea turtles: Waayers & Fitzpatrick 2013; fish: Horne *et al.* 2011, 2012, 2013; Veilleux *et al.* 2011). The Canning Bioregion to the west is characterized by moderately clear water that becomes turbid during spring tides and a tidal range up to 9m. This coast contains a wide variety of landforms with the shore principally composed of long sandy beaches (Thackway & Cresswell, 1998). The Pilbara Bioregion has tides from 1 to 5m, with water clarity ranging from highly turbid at inshore sites to clearer at offshore sites (i.e. Montebello Islands), with extensive seagrass and macroalgal meadows interspersed between the many islands in the region (Wilson *et al.* 2010; Evans *et al.* 2014, McLean *et al.* 2016). It also harbours a diverse and abundant fish and coral fauna (McLean *et al.* 2016; Travers *et al.* 2010; Hutchins 2001). The Ningaloo Bioregion to the southwest covers the entire Ningaloo Reef and is characterised by low tidal (~1m), fringing reefs adjacent to large lagoons with clear oligotrophic water regularly driven through the system by high-energy waves (Zhang *et al.* 2011). Shark Bay to the south has high cliffs, fringing reefs and low relief sandy shorelines (within Shark Bay), with intermittent but significant freshwater input from river outflows and the largest coverage of tropical and temperate seagrass meadows in WA (Walker 1990). The common

feature of the various bioregional classifications and other quantitative fish assemblage studies is the pronounced faunal break in the Cape Leveque region at the northern tip of the Dampier Peninsula and at the Northwest Cape of Australia near Ningaloo Reef (Fig. 1A; Spalding *et al.* 2007; Travers *et al.* 2010, Thackway & Cresswell 1998). Few studies have investigated connectivity among these six distinct but ecologically important Australian coastal ecosystems (Johnson & Joll 1993; Johnson *et al.* 1993; Veilleux *et al.* 2011), and none to our knowledge focus on inshore fishes and include comprehensive sample coverage.

Here we evaluate genetic connectivity of the Stripey snapper, *Lutjanus carponotatus* (Richardson, 1842), across all six of the aforementioned bioregions using a genotyping-by-sequencing approach. *Lutjanus carponotatus* is abundant on inshore and mid-shelf reefs from Shark Bay to Bargara, Queensland, but also found more broadly in turbid waters from India through to the Indo-West Pacific. We here focus on this “indicator species” given its importance in recreational fisheries (Kritzer 2004), its ecological function as a macrofaunal predator, and the fact that its larval settlement behaviour is similar to other predatory species of commercial importance (e.g. *Plectropomus* sp.; Quéré and Leis 2010). A recent genetic survey of *L. carponotatus* on the Great Barrier Reef using mitochondrial markers found complete admixture within and between inshore islands at a scale of 800 km (Evans *et al.* 2010). A companion study based on the same species and molecular markers in WA identified a comparable scenario of complete admixture in this region, although it was strongly differentiated from the Great Barrier Reef populations (Veilleux *et al.* 2011). Both of these studies failed to separate evolutionary from ecological patterns of gene flow, which SNPs, applied here, may resolve.

We performed a genome-wide survey of *L. carponotatus* among 51 sites along the extensive ~3,000 km coast of NWA to compare broad-scale patterns of genomic divergence among bioregions that differ in reef composition, environmental conditions, and oceanographic current regimes. By using thousands of SNP loci as our proxy for realised dispersal we were able to further partition genetic divergence into the component that departs from neutral expectations (i.e. outlier loci) when comparing sites that are subject to different environmental conditions. We also performed a fine-scale investigation within the Kimberley Bioregion to identify barriers to larval dispersal.

2 Materials and Methods

2.1 Study area and sample collection

Tissue samples of *L. carponotatus* (Fig. 1 and Fig. 2) were collected from 51 coastal sites across NWA from the Anson Beagle (now referred to as NT) through the Kimberley, Canning, Pilbara, Ningaloo and Shark Bay bioregions of WA. In total, 1,016 samples were collected across 13° of latitude and 17° of longitude of tropical Australian coastline (also see Table 1) and immediately preserved in 95% ethanol. The majority of sampling was undertaken in 2014 and 2015, however, historic muscle tissue samples collected in 2002 and frozen at -80°C were obtained from four sites (Cape Bossut, Cape Keraudren, Cape Preston, Locker Point). An indirect test with these historical samples gave negative results for DNA degradation (see Appendix 1 and population genetic statistics methods for more details).

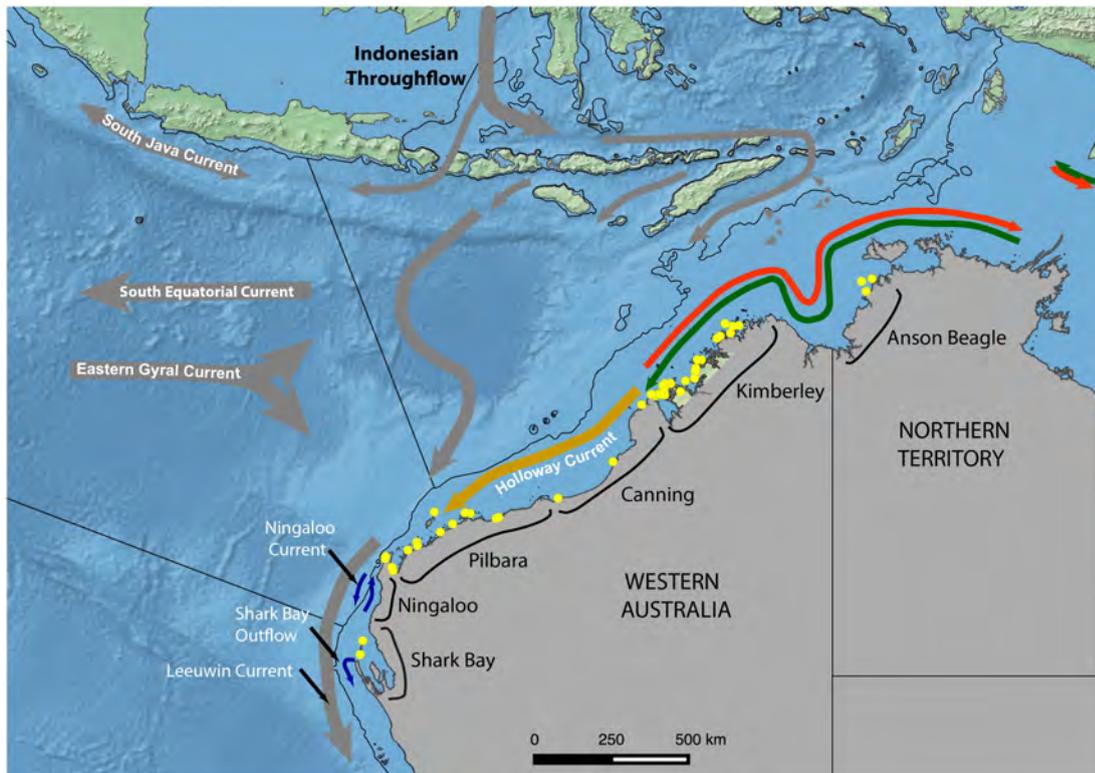


Fig. 1 Map of sampling sites (yellow dots) for *L. carponotatus* in NWA. The dominant current affecting the outer shelf of the Kimberley, Canning and Pilbara Bioregions is the Holloway Current, which flows south-west along the shelf margin from May to September due to the prevailing winds. The dominant current affecting the Ningaloo and Shark Bay Bioregions is the Leeuwin Current (adapted from Sprintall *et al.* 2002; Domingues *et al.* 2007; D'Adamo *et al.* 2009; Schiller 2011). Red, green, and amber coloured lines indicate flow direction in summer, winter, and autumn, respectively. The 220 m isobath is indicated by the curved black line that follows the shoreline of NWA.

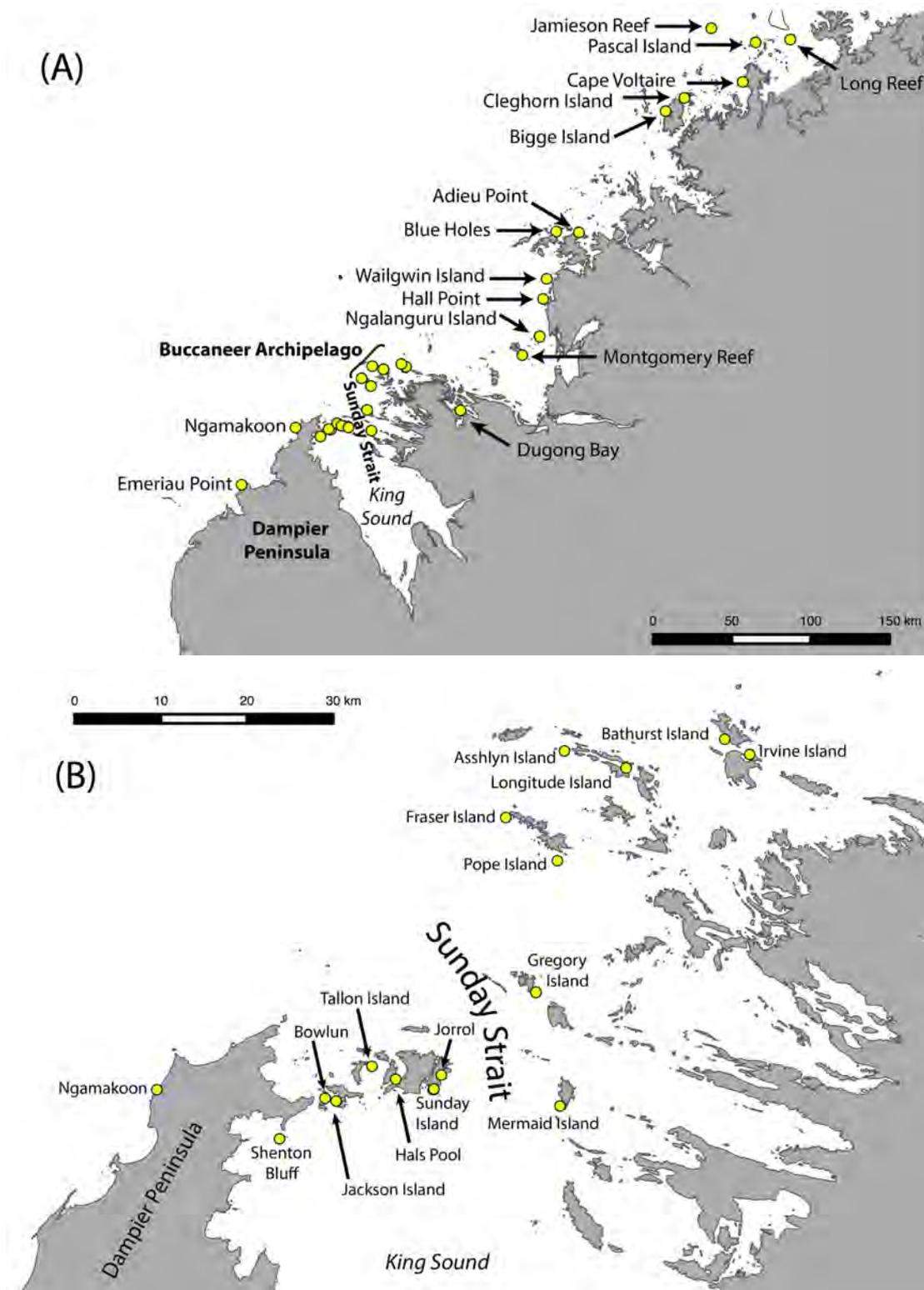


Fig. 2 Sampling sites in the Kimberley management region (A) and sites surrounding the Sunday Strait and King Sound.

Table 1. Site, region, sample size (N), and molecular metrics (Na = number of alleles; Ho = observed heterozygosity; He = expected heterozygosity; Fis = Inbreeding coefficient) for *L. carponotatus* based on 4,402 SNP loci.

Site	Fisheries Management Area	MEOW Province	MEOW Ecoregion	IMCRA Bioregion	N	Na	Ho	He	Fis
Bass Reef	NT	Sahul Shelf	Bonaparte	Anson Beagle	13	1.833	0.208	0.219	0.034
Point Blaze	NT	Sahul Shelf	Bonaparte	Anson Beagle	10	1.784	0.205	0.218	0.040
Sail City	NT	Sahul Shelf	Bonaparte	Anson Beagle	2	1.385	0.195	0.158	-0.246
Long Reef	Kimberley	Sahul Shelf	Bonaparte	Kimberley	28	1.939	0.224	0.229	0.023
Pascal Island	Kimberley	Sahul Shelf	Bonaparte	Kimberley	15	1.868	0.217	0.224	0.024
Jamieson Reef	Kimberley	Sahul Shelf	Bonaparte	Kimberley	12	1.829	0.205	0.218	0.041
Cape Voltaire	Kimberley	Sahul Shelf	Bonaparte	Kimberley	49	1.970	0.214	0.229	0.058
Cleghorn Island	Kimberley	Sahul Shelf	Bonaparte	Kimberley	9	1.763	0.243	0.225	-0.071
Bigge Island	Kimberley	Sahul Shelf	Bonaparte	Kimberley	43	1.968	0.222	0.231	0.037
Blue Holes	Kimberley	Sahul Shelf	Bonaparte	Kimberley	30	1.938	0.200	0.222	0.081
Adieu Point	Kimberley	Sahul Shelf	Bonaparte	Kimberley	22	1.912	0.211	0.224	0.046
Wailgwin Island	Kimberley	Sahul Shelf	Bonaparte	Kimberley	19	1.892	0.207	0.220	0.045
Hall Point	Kimberley	Sahul Shelf	Bonaparte	Kimberley	22	1.921	0.207	0.224	0.059
Ngalanguru Island	Kimberley	Sahul Shelf	Bonaparte	Kimberley	17	1.877	0.225	0.227	0.004
Montgomery Reef	Kimberley	Sahul Shelf	Bonaparte	Kimberley	4	1.580	0.216	0.198	-0.100
Dugong Bay	Kimberley	Sahul Shelf	Bonaparte	Kimberley	21	1.905	0.207	0.223	0.056
Bathurst Island	Kimberley	Sahul Shelf	Bonaparte	Kimberley	3	1.495	0.221	0.183	-0.203
Irvine Island	Kimberley	Sahul Shelf	Bonaparte	Kimberley	8	1.845	0.348	0.262	-0.256
Fraser Island	Kimberley	Sahul Shelf	Bonaparte	Kimberley	7	1.711	0.207	0.210	-0.006
Longitude Island	Kimberley	Sahul Shelf	Bonaparte	Kimberley	5	1.638	0.204	0.202	-0.031
Asshlyn Island	Kimberley	Sahul Shelf	Bonaparte	Kimberley	3	1.517	0.215	0.190	-0.144
Pope Island	Kimberley	Sahul Shelf	Bonaparte	Kimberley	4	1.579	0.209	0.195	-0.085

Population connectivity of the Stripey Snapper Lutjanus carponotatus along the ecologically significant coast of northwestern Australia

Gregory Island	Kimberley	Sahul Shelf	Bonaparte	Kimberley	2	1.421	0.227	0.173	-0.315
Mermaid Island	Kimberley	Sahul Shelf	Bonaparte	Kimberley	5	1.633	0.237	0.210	-0.123
Jorrol	Kimberley	Sahul Shelf	Bonaparte	Kimberley	14	1.863	0.209	0.221	0.035
Hal's Pool	Kimberley	Sahul Shelf	Bonaparte	Kimberley	14	1.850	0.267	0.239	-0.086
Tallon Island	Kimberley	Sahul Shelf	Bonaparte	Kimberley	28	1.938	0.240	0.234	-0.013
Jackson Island	Kimberley	Sahul Shelf	Bonaparte	Kimberley	7	1.717	0.224	0.215	-0.047
Bowlun	Kimberley	Sahul Shelf	Bonaparte	Kimberley	3	1.503	0.224	0.187	-0.197
Shenton Bluff	Kimberley	Sahul Shelf	Bonaparte	Kimberley	41	1.962	0.208	0.226	0.068
Ngamakoon	Kimberley	NW OZ Shelf	EX to BRM	Canning	23	1.923	0.229	0.229	0.003
Emeriau Point	Kimberley	NW OZ Shelf	EX to BRM	Canning	30	1.946	0.241	0.236	-0.009
Cape Bossut	Kimberley	NW OZ Shelf	EX to BRM	Canning	30	1.945	0.267	0.241	-0.071
Cape Keraudren	Pilbara	NW OZ Shelf	EX to BRM	Canning	30	1.949	0.271	0.245	-0.073
Depuch Island	Pilbara	NW OZ Shelf	EX to BRM	Pilbara	27	1.940	0.218	0.228	0.036
West Moore	Pilbara	NW OZ Shelf	EX to BRM	Pilbara	25	1.937	0.218	0.230	0.044
Gidley Island	Pilbara	NW OZ Shelf	EX to BRM	Pilbara	27	1.937	0.199	0.223	0.084
Rosemary Island	Pilbara	NW OZ Shelf	EX to BRM	Pilbara	27	1.937	0.206	0.225	0.068
Cape Preston	Pilbara	NW OZ Shelf	EX to BRM	Pilbara	30	1.950	0.255	0.239	-0.043
Passage Island	Pilbara	NW OZ Shelf	EX to BRM	Pilbara	26	1.930	0.197	0.222	0.091
Montebello North	Pilbara	NW OZ Shelf	EX to BRM	Pilbara (Offshore)	26	1.935	0.198	0.221	0.082
Montebello South	Pilbara	NW OZ Shelf	EX to BRM	Pilbara (Offshore)	24	1.922	0.210	0.226	0.053
Thevenard Islands	Pilbara	NW OZ Shelf	EX to BRM	Pilbara	25	1.930	0.199	0.222	0.086
Paroo Shoal	Pilbara	NW OZ Shelf	EX to BRM	Pilbara	26	1.931	0.213	0.226	0.049
Locker Point	Gascoyne	NW OZ Shelf	EX to BRM	Pilbara	24	1.926	0.256	0.239	-0.050
Bay of Rest	Gascoyne	NW OZ Shelf	EX to BRM	Pilbara	28	1.940	0.203	0.222	0.070
Roberts Island	Gascoyne	NW OZ Shelf	EX to BRM	Pilbara	27	1.938	0.201	0.224	0.090
Tantabiddi	Gascoyne	NW OZ Shelf	Ningaloo	Ningaloo	24	1.917	0.200	0.221	0.075

Milyering	Gascoyne	NW OZ Shelf	Ningaloo	Ningaloo	25	1.927	0.206	0.223	0.057
Bernier Island	Gascoyne	WC OZ Shelf	Shark Bay	Shark Bay	25	1.901	0.193	0.218	0.088
Dorre Island	Gascoyne	WC OZ Shelf	Shark Bay	Shark Bay	27	1.902	0.194	0.218	0.089

Abbreviations: Northern Territory (NT), North West Australia (NW OZ), Buccaneer Archipelago (BA); Broome (BRM); Exmouth (EX); Sunday Islands (SI). Management Area refers to current State based fisheries management areas; Marine Ecoregions of the World (MEOW) derived from Spalding et al (2007) nested Ecoregions within Provinces; Integrated Marine and Coastal Regionalisation of Australia (IMCRA) meso-scale Bioregions derived from Commonwealth of Australia (2006).

2.2 DNA Extraction

DNA was extracted from tissue samples using 96-well plates according to the salt extraction method described by (Cawthorn et al. 2011), followed by purification with Zymo ZR-96 DNA Clean and Concentrator kits (Zymo Research, California, USA).

2.3 Reduced Representation SNP Genotyping

Downstream SNP genotyping was done using a modified DArTseq™ protocol (Grewe *et al.* 2015), which is a proprietary method for reduced representation genomic library preparation and NGS (Kilian *et al.* 2012; Cruz *et al.* 2013). In our case, genomic DNA was digested with two restriction enzymes (PstI-SphI and PstI-NspI) instead of one in order to generate more SNP loci. PCR conditions consisted of an initial denaturation step at 94 °C for 1 min followed by 30 cycles of 94 °C for 20 sec, 58 °C for 30 sec, and 72 °C for 45 sec, with a final extension step at 72 °C for 7 min. After PCR, equimolar amplification products from each sample were pooled and applied to a cBot (Illumina) bridge PCR followed by sequencing on an Illumina HiSeq2500. The sequencing (single read) was run for 77 cycles.

2.4 SNP Calling

Read assembly, quality control, and SNP calling was done using DArT PLD's software DArTsoft14, a program that produces scoring consistency derived from technical sample replicates (i.e. samples processed twice, from DNA library preparation to SNP calling). Testing for Mendelian distribution of alleles in these populations facilitated selection of technical parameters discriminating true allelic variants from paralogous sequences. A total of 17,007 SNP loci were identified during this process.

2.5 SNP Quality Control Filtering

Following SNP genotyping, additional quality control (QC) steps were performed to the 17,007 loci identified prior to genetic analyses: 1) rare alleles (frequency < 0.05) and highly variable loci (heterozygosity > 0.75) were removed, 2) loci with coverage less than 20X and greater than 200X were removed, and 3) individuals with more than 1% missing data were removed. Following these filtering steps, we were left with 5,094 loci. To comply with Hardy-Weinberg Equilibrium (HWE) and Linkage Disequilibrium (LD) expectations, we chose to exclude loci out of HWE in greater than 10 populations and loci that exhibited LD in greater than 5 populations. Testing for HWE made use of custom R scripts implemented within the R packages SNPAssoc (González *et al.* 2007) and pegas (González *et al.* 2007; Paradis 2010). Testing for LD made use of custom R scripts implemented within the R packages doParallel (Calaway *et al.* 2014) and Adegenet (Jombart 2008). After all the outlined filtering steps, we were left with 4,402 loci sampled at 51 sites. We additionally attempted filtering SNP loci using a number of different values for HWE, LD, and QC (+/- 15% of threshold), which did not impact the overall outcome (data not shown); we therefore only present data based on the outlined selection criteria. The resulting genind file was converted to other program specific input files using PGDSPIDER version 2.0.5.1 (Lischer & Excoffier 2012). Downstream genetic analyses were performed with all samples from all collection sites (full dataset) or with only those populations with $N \geq 6$ or $N \geq 20$ individuals collected (reduced dataset) to mitigate the effects of low sample size, where appropriate.

2.6 Population genetic statistics

F_{ST} , F_{IS} , and genetic diversity metrics (percentage of polymorphic loci, average number of alleles, observed and expected heterozygosity) were estimated using Genodive version 2.0 (Meirmans & Van Tienderen 2004). The significance of pairwise F_{ST} values was tested by 10,000 permutations. In order to compare the relative abundance of SNPs that may be under divergent selection, we performed outlier scans between all pairs of sites using Outflank version 0.1 (Whitlock & Lotterhos 2015). The approach implemented in Outflank is based on an improved method for deriving the null distribution of population differentiation for neutral loci. It results in fewer false positive than other outlier tests, which appear to be influenced by the effects of demographic

history (Lotterhos & Whitlock 2015). We ran Outflank with 5% left and right trim for the null distribution of F_{ST} , minimum heterozygosity for loci of 0.1, and a 5% false discovery rate (q-value). Sixty-six SNPs under putative directional selection were identified. These loci were removed from downstream analyses unless otherwise noted.

It should also be noted that individuals collected in the Pilbara bioregion in 2002 appeared genetically distinct from individuals collected in the same bioregion in 2015. We therefore compared SNP type (i.e. transition versus transversion) for a subsample of those individuals ($N = 30$) collected in 2002 and 2015, respectively, to assess whether deamination (C/T transitions) or genetic damage could explain the genetic divergence between the older samples (2002) versus the newer samples (2015; see Appendix 1).

2.7 Model-Based Clustering analysis

To explore genetic structure across sampling sites, a clustering analysis was performed with STRUCTURE version 2.3.4 (Pritchard *et al.* 2000) using locations with $N \geq 20$ individuals both with and without *a priori* information of the geographic origin of each sample. The analyses were run on the CSIRO Accelerator Cluster “Bragg” under the admixture model with correlated allele frequencies, a burn-in of 200,000 MCMC iterations, followed by 500,000 iterations for each run (Falush *et al.* 2003). The number of K (putative populations) ranged from one to eight and 20 replicate analyses were run for each value of K. Although we sampled more than eight sites, we found that $K > 8$ was not necessary to identify the optimal number of clusters (data not shown). The number of clusters was inferred by comparing the $\ln \Pr (X|K)$ among different values of K. The value of K for which $\ln \Pr (X|K)$ was highest or reached a plateau was selected as the most parsimonious number of populations in our sample. The *ad hoc* statistic ΔK (Evanno *et al.* 2005) was also considered. After the initial set of runs, this process was repeated with only the identified outlier loci to assess the extent of natural selection on genetic differentiation (see above).

2.8 Discriminant Analysis of Principle Components (DAPC)

We employed Discriminant Analysis of Principle Components (DAPC) implemented in the R package Adegenet to identify and describe genetic groups present within our data. Initially the k-means algorithm was employed to evaluate all potential clusters (K) in the data. For this analysis we retained all principle components and then evaluated the Bayesian information content (BIC) for all values of K. A linear discriminant analysis was then conducted based on 338 retained principle components (N individuals divided by 3) identified as optimal based on the `optim.a.score` command, and 50 discriminant functions retained ($N-1$ populations) to describe the clusters evident in the data. For this analysis we did not restrict the number of clusters to the number identified in the `find.clusters` analysis. All analysis was repeated on the neutral and the outlier dataset.

2.9 Determinants of genetic differentiation

We used an information-theoretic approach (Anderson 2008) to determine the factors that influence genetic differentiation in this particular species of snapper. This method ranks alternative models according to empirical evidence versus excluding models (Correa & Hendry 2012). Sample sites with $N < 6$ were excluded from the analysis given the uncertainty in F_{ST} estimates when based on low sample size (Willing *et al.* 2012). Environmental and geographical variables were included in the model selection process and each model was ranked based on their evidence ratio and posterior probability. Environmental factors were extracted from freely available ocean climate layers (MARSPEC, Sbrocco & Barber 2013; Bio-ORACLE, Tyberghein *et al.* 2012) and included 43 variables considered likely to influence fitness of larval fishes (e.g. sea surface salinity, sea surface temperature, nutrient load, bathymetry, tidal range). Because many of these variables were correlated, we reduced them into a single composite variable (*env*) by extracting the first component of a Principal Component Analysis (PCA), which accounted for 70% of the variability within the dataset based on the eight most influential factors extracted from Draftsman plots (see Appendix 2). Geographical factors included the Euclidean distance between sites (*Geo*) and the presence of three putative barriers to larval dispersal. Although

the northern and western coasts of Australia have been classified and re-classified according to a number of marine biogeographical boundaries (e.g. Fox & Beckley 2005; Spalding *et al.* 2007; Thackway & Cresswell 1998), we follow the marine ecoregions of the world (MEOW) of Spalding *et al.* (2007), which utilises the most recent quantitative data on marine fishes in this region. The ecoregional units we specifically test are the NT, Bonaparte Coast, Exmouth to Broome, and Ningaloo to Shark Bay. Note that although MEOW classification does not contain a NT ecoregion, we have included it in this analysis as there were limited samples through the north-eastern sector of the Bonaparte Coast, and the NT fisheries are managed separately to WA. The common feature of the various bioregional classifications and other quantitative fish assemblage studies is the pronounced faunal break in the Cape Leveque region at the northern tip of the Dampier Peninsula (Fig. 1A; Spalding *et al.* 2007; Travers *et al.* 2010; Thackway & Cresswell 1998). All barriers were considered independently and in combination (*barrier1_2*, *barrier1_3*, *barrier2_3*, and *barrier1_2_3*). Barriers were modeled as a factor with 1 to 3 levels (number of barriers), where sites in the same level were on the same side of the barrier, and sites in different levels were on different sides of any of the three barriers. Overall, 67 models were fitted using both linear models (lm) and linear mixed effect models (lmer). Linear mixed effects models included site ID as a random effect in order to compensate for the fact that pairwise F_{ST} values are not independent among sites. For each model, the sample size-corrected Akaike information criterion (AIC) was computed as $AICc = AIC + 2K(K + 1)/(n - K - 1)$, where $AIC = -2\log\text{-likelihood} + 2K$ (K = number of parameters in model, n = number of observations). Models were then ranked based on increasing $AICc$ and further interpretation based on model probabilities (w) and evidence ratios (Anderson 2008).

2.10 Spatial autocorrelation and IBD

GenAlEx version 6.502 (Peakall & Smouse 2006) was also used to quantify spatial autocorrelation for all sites with $N \geq 6$ within the Kimberley cluster (0 to 256 km, $N = 266$), within the Pilbara cluster (0 to 426 km, $N = 391$), and within the transition zone between the two clusters (0 to 148 km, $N = 193$). We conducted a multiple distance class spatial autocorrelation rather than conventional correlograms to accommodate uneven sample sizes and distances typical of reef topography (see Peakall *et al.* 2003). Geographic distances between sites were calculated based on the shortest across-water distance with a minimum water depth of 1m. These estimates were calculated with the Marmap R package (Pante & Simon-Bouhet 2013) and based on the GEBCO 2014 30-second bathymetry available from the British Oceanographic Data Centre.

We applied Mantel tests to evaluate the relationship between linearised F_{ST} ($F_{ST}/(1-F_{ST})$) and distance. This analysis was based on 9999 permutations of the data calculated with the vegan R package (Oksanen *et al.* 2007). Mantel tests were applied to the entire dataset as well as the Kimberley and Pilbara sites separately.

3 Results

3.1 Genetic diversity

A summary of the principal statistics (number of individuals per site, percentage of polymorphic loci, average number of alleles, observed and expected heterozygosity, and F_{IS}) obtained for 1,016 individual samples from 51 locations in NWA are presented in Table 1. Based on the observed heterozygosity, genetic diversity was significantly higher in the southwestern bioregions (Canning, Pilbara, and Shark Bay) than the northeastern bioregions (NT and Kimberley; t-test: $t = -4.19$ and $P < 0.001$). Moreover, observed heterozygosity was only weakly correlated with the latitude of each site ($R^2 = 0.159$), suggesting that it is a bioregional effect versus a direct distance effect. F_{IS} values were mostly positive in the SW regions, whereas in the northern region they were mostly negative, suggesting a greater amount of inbreeding in the Pilbara versus the Kimberley. F_{IS} values were similarly only weakly correlated with the latitude of each site ($R^2 = 0.137$). This result based on F_{IS} values may also be an artefact of sampling, whereby Kimberley samples were collected by non-selective traps and Pilbara samples were collected in a more selective manner (i.e. speargun). Note that the only negative F_{IS} values in the Pilbara were the historic samples collected by traps. We also identified 66 outlier loci using Outflank, which represent a small proportion of the overall 4,402 SNP loci identified.

3.2 Genetic subdivision

Patterns of pairwise genetic differentiation are summarized in Fig. 2, revealing small but significant genetic differences among most sampling locations (i.e. 424 out of 496 tests significant), which suggests restrictions in gene flow between geographically distant (e.g. NT and Shark Bay) but even in some cases, neighbouring sites *within* bioregions as little as a few kilometres apart. The historical samples collected from sites in the Pilbara in 2002 consistently exhibited higher levels of genetic differentiation from those collected in 2014 and 2015 (Fig. 2). Pairwise differentiation was greater in the Kimberley (92% pairwise comparisons significant) than the Pilbara (63% pairwise comparisons significant)(Fig. 2).

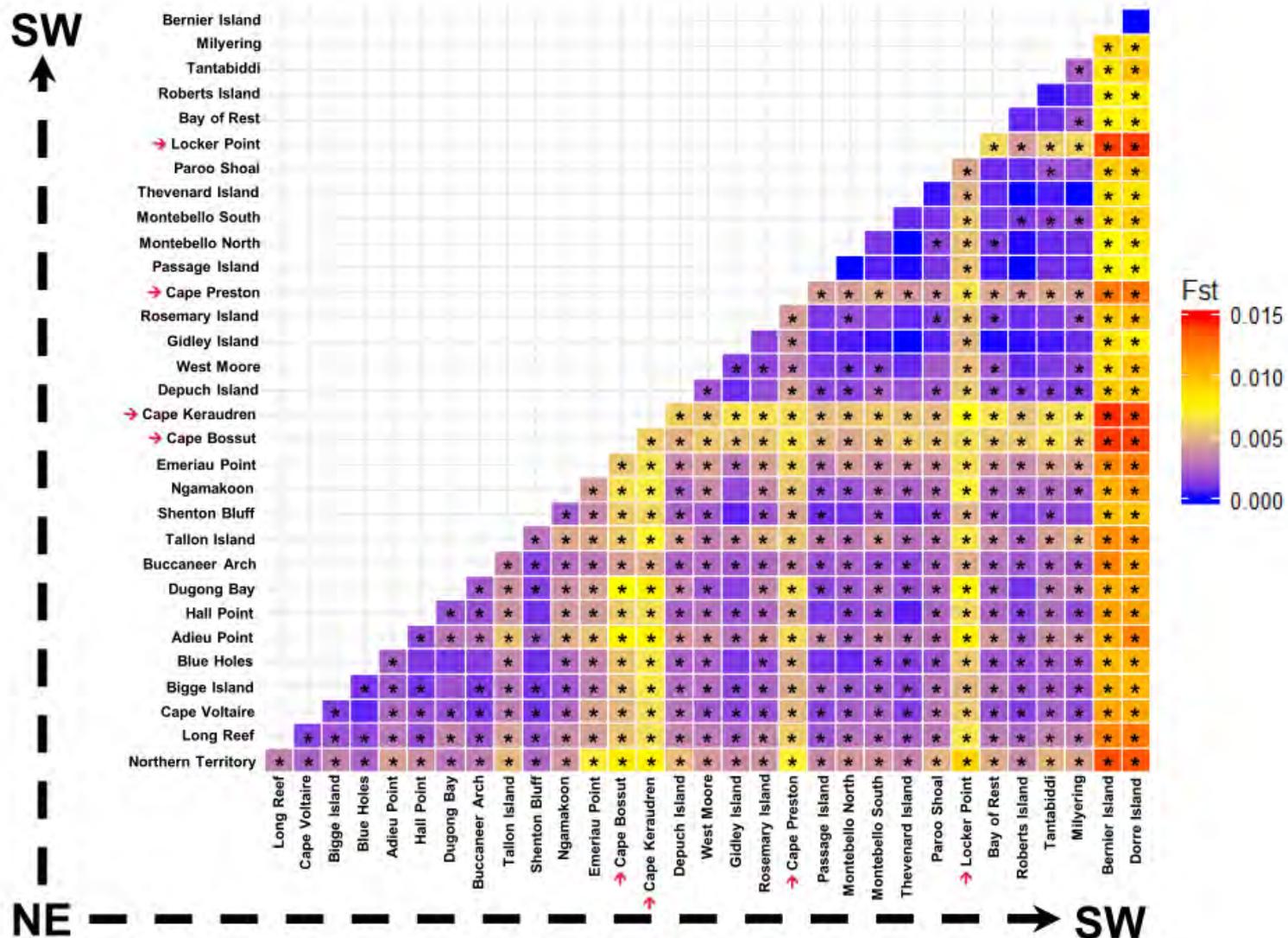
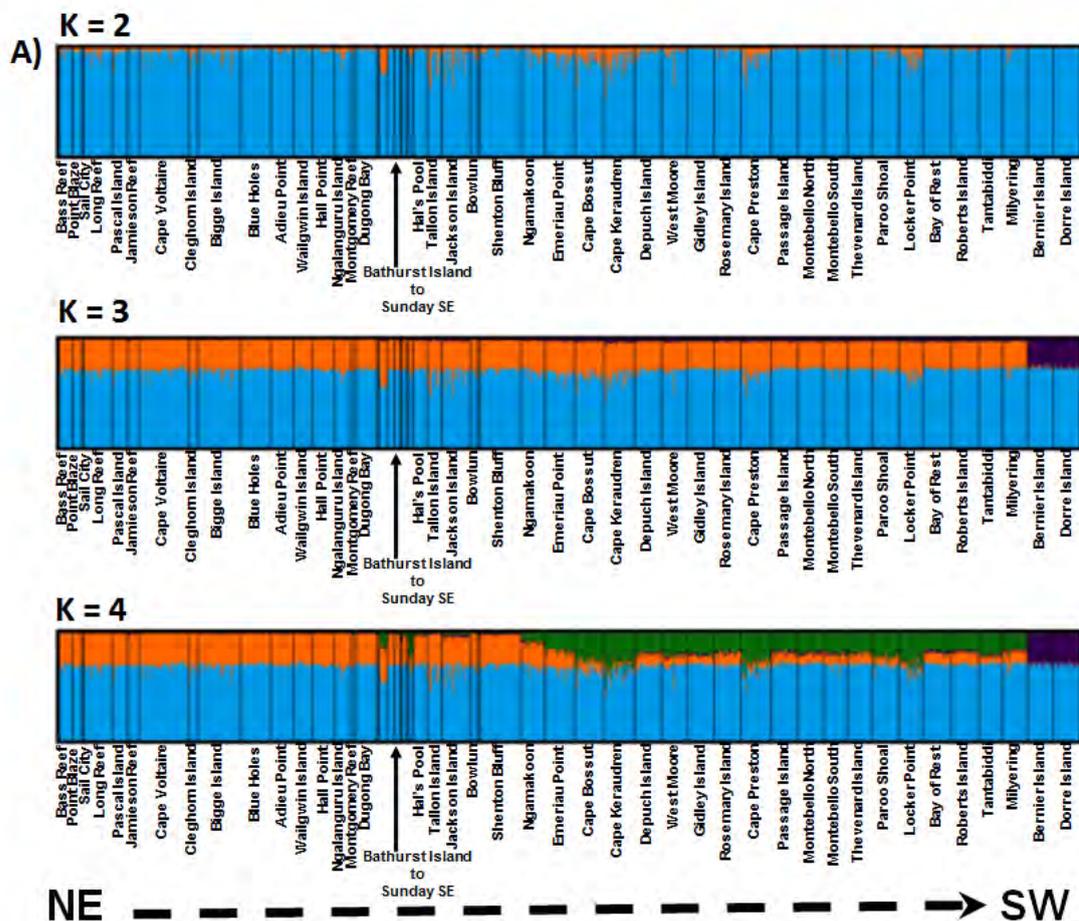


Fig. 2 Heatmap of pairwise F_{ST} values for *L. carponotatus* populations with 20 or more individuals in NWA based on 4,402 SNP loci. *indicates significant difference after Narum correction ($P < 0.0074$). The four historical sample sites (i.e. 2002) are indicated by small, red arrows.

3.3 Model-Based Clustering analysis

Bayesian clustering analysis suggested $K = 2$ populations as the most parsimonious partitioning of individuals based on the metric ΔK (Evanno *et al.* 2005; also see Appendix 3, $\Delta K = 910.944$). For clarity, we also present $K = 3$ and $K = 4$ (Fig. 3). The primary split corresponds to the boundary between Shark Bay and Ningaloo Bioregions with northern Ningaloo grouping with all locations of the North West Shelf towards NT. Also note a significant genetic ‘transition zone’ across a distance of < 80km in the region at the tip of the Dampier Peninsula, near the entrance to King Sound (i.e. Dugong Bay to Emeriau Point). The pattern remains the same whether we consider all 4,402 SNP loci (Fig. 3A) or only the 66 outlier loci (Fig. 3B), which may be subject to strong directional selection. Note that the NT sites and sites in the Buccaneer Archipelago with small sample sizes are pooled for inclusion in the outlier analysis.



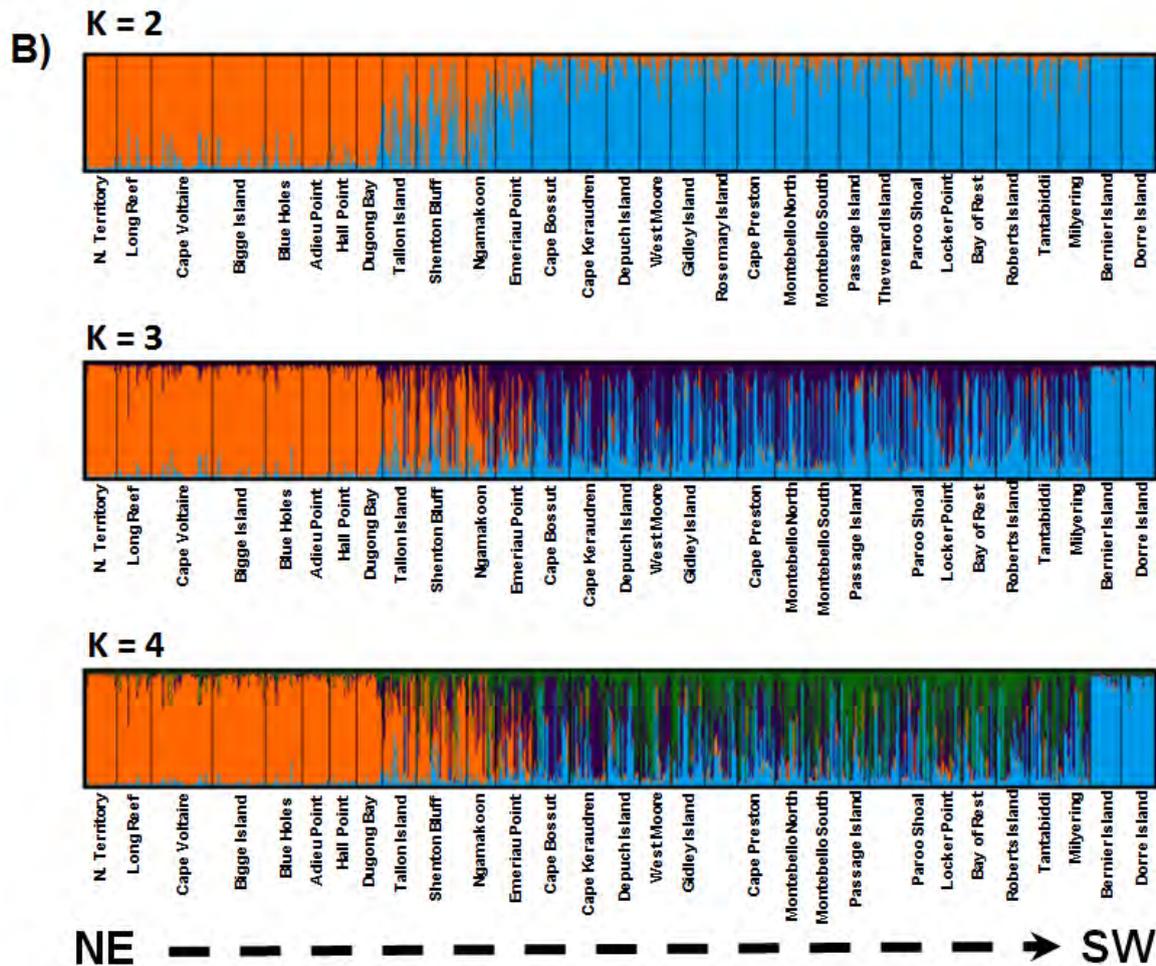
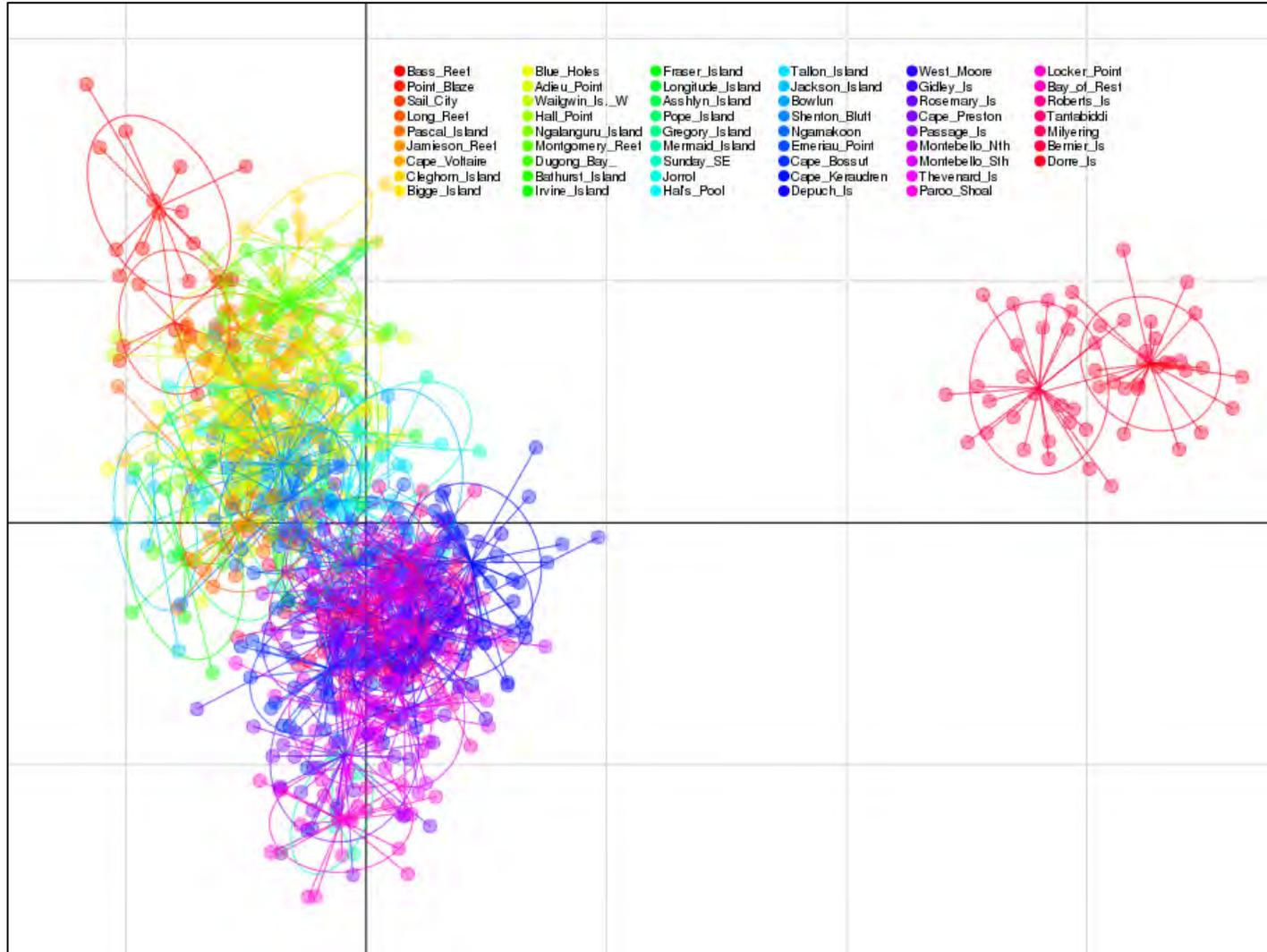


Fig. 3 Results of Bayesian clustering for *L. carponotatus* populations in NWA based on (A) 4,402 SNP loci and (B) 66 outlier loci. Results from $K = 2$, $K = 3$, and $K = 4$ are presented; $K = 2$ was the most likely number of clusters in both cases (see Appendix 3). No prior locations were input as priors in these runs. Individuals are represented by vertical bars, each divided according to their estimated probability of ancestry from each of the genetic clusters (represented by blue and orange). Sites are ordered northeast to southwest and from left to right. Note that the NT sites are pooled for inclusion in the outlier analysis only.

3.4 Discriminant Analysis of Principle Components (DAPC)

The k-means algorithm was optimised at $K = 2$ in the neutral and outlier datasets. Linear discriminant analysis revealed that for the neutral dataset these groups corresponded to the Shark Bay bioregion versus all locations of the North West Shelf (Fig. 4). However, there appeared to be an approximate north to south isolation by distance pattern among the samples from the NT and Kimberley, but little discernible pattern among samples from the Pilbara bioregion (Fig. 4A). The DAPC analysis of the outlier dataset was less discriminating. The points representing samples from Shark Bay formed a distinct group on the right of the plot while those for the Pilbara and Canning exhibited minor overlap but no overlap with those representing Kimberley and NT samples. Pilbara and Kimberley samples were mostly distinct from each other; however sites between the Dampier Peninsula and Buccaneer Archipelago exhibited varying degrees of joint membership and intermediate positions between the majority of the Kimberley and Pilbara clusters (Fig. 4).

(A)



(B)

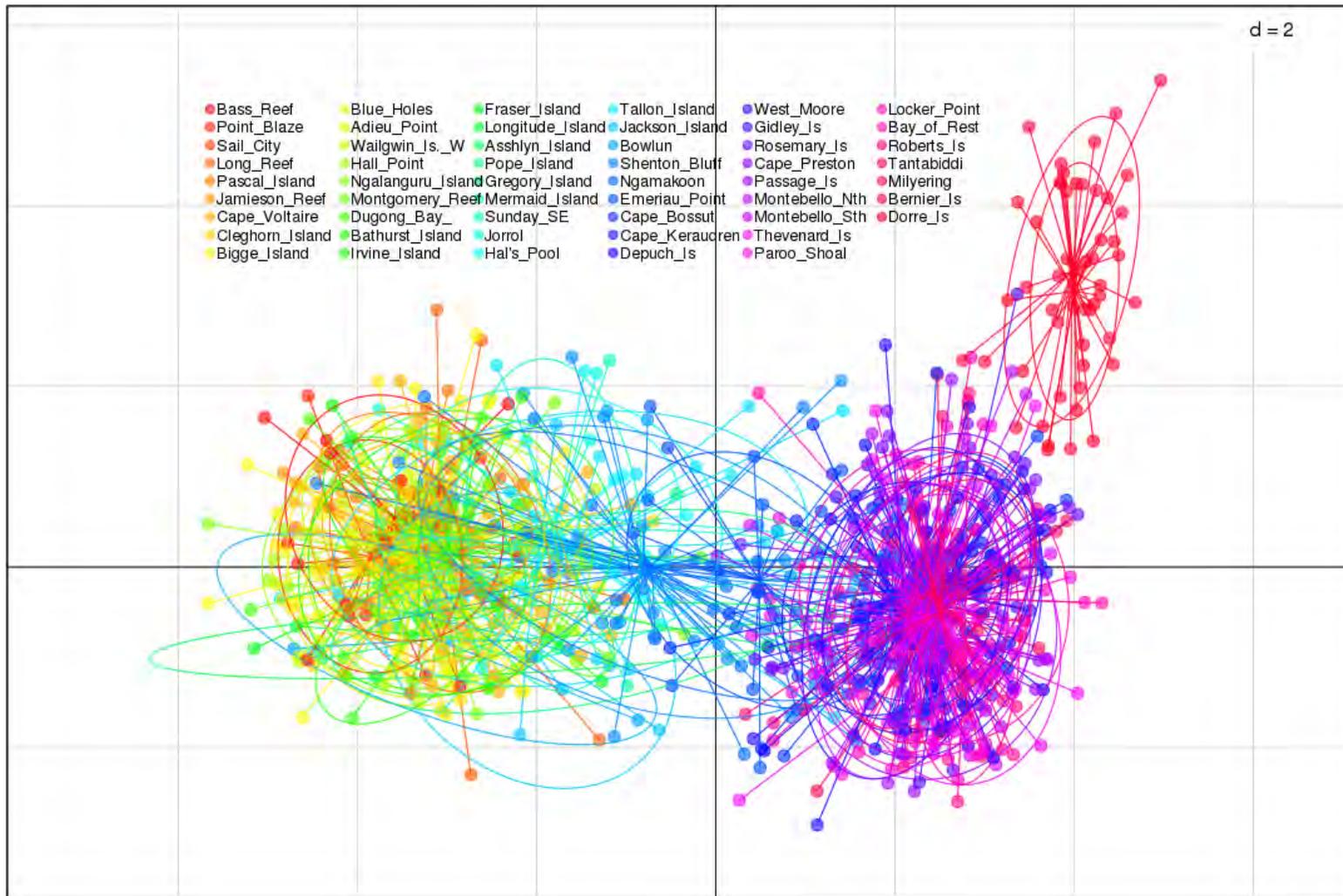


Fig. 4 Scatterplot of DAPC performed on all *L. carponotatus* samples based on (A) 4,402 SNP loci and (B) 66 outlier loci. Populations are coloured in north to south order with 95% inertia ellipses. Dots represent individual genotypes and axes show the first two discriminant functions.

3.5 Determinants of genetic differentiation

One model outperformed all the other models (model likelihood of 1.0 when compared to other models). This model included variables *geo*, *env*, the presence of all three barriers, as well as an interaction between these terms (Appendix 4). We repeated this analysis with outlier loci only and got a different result, only barrier 1 was represented in all four top models (i.e. barrier between the NT and the Kimberley). Thus, modelling the effects of barriers to dispersal, environmental attributes, and geographic distance on genetic differentiation in this species revealed strong effects for all three factors, but in most cases, these effects could not be parsed from each other given the strong correlations among them (see Appendix 5).

3.6 Spatial autocorrelation and IBD

Results from the spatial autocorrelation analysis showed significant local scale genetic structure. The autocorrelation coefficient was modest ($r \sim 0.0025$), but significantly positive as it dropped away from its initial plateau (Fig. 5). The distance where r first crossed the x-axis was roughly 300 km, except in the transition zone where it was only 80 km. Such reference points on the x-axis reveal the distance where the random effects of genetic drift, not gene flow, are the primary determinants of genetic composition in the different regions. A Mantel test revealed that when considering all data, distance was significantly correlated with genetic differentiation between sites ($R = 0.25$, $P < 0.001$; Fig. 6). Distance was not a significant correlate with genetic differentiation when considering sites in the Kimberley only ($R = 0.08$, $P = 0.23$), but was significantly correlated for the sites in the Pilbara ($R = 0.50$, $P = 0.01$).

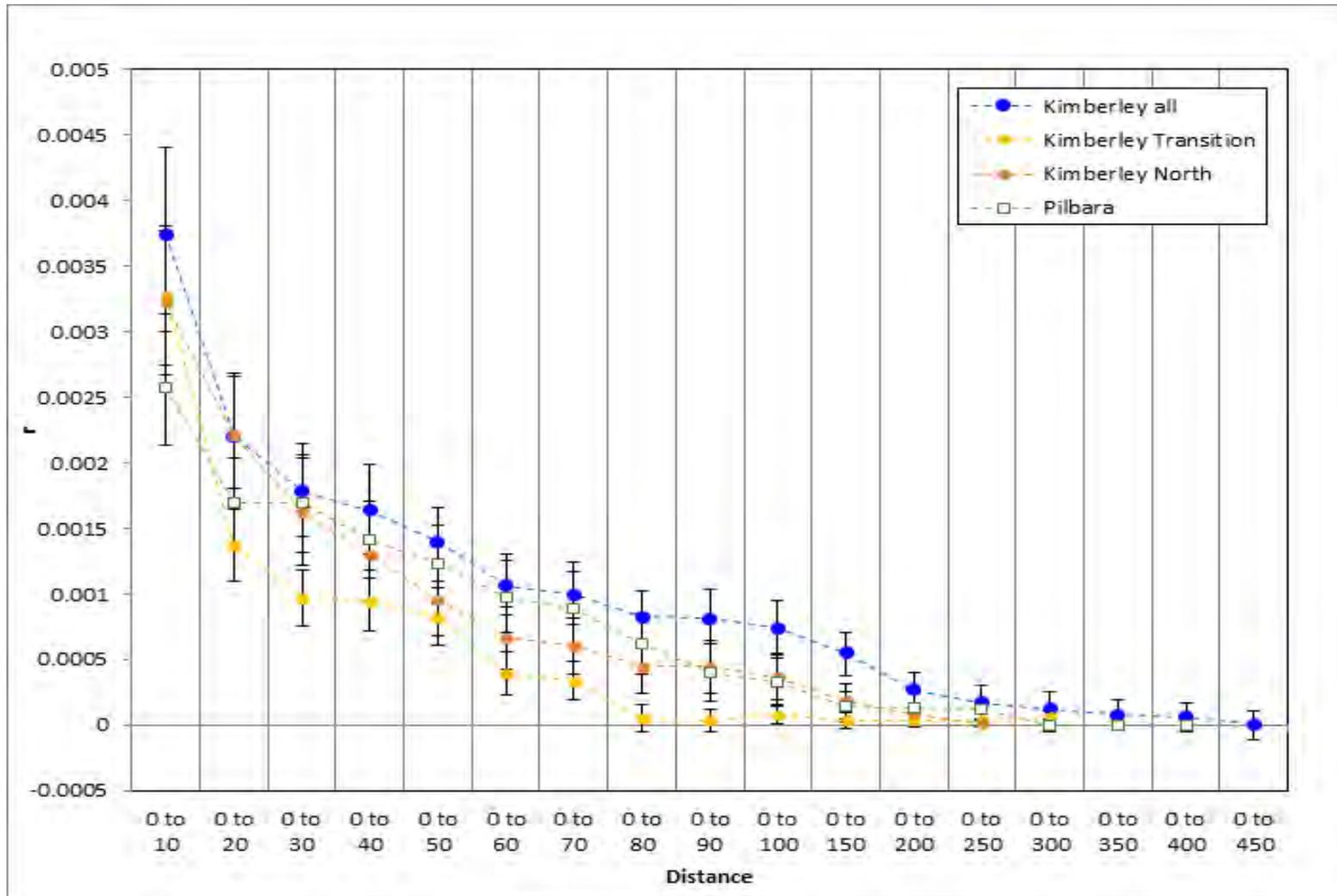


Fig. 5 Spatial autocorrelation as a function of cumulative geographic distance (in kilometres) for *L. carponotatus* populations with 20 or more individuals in NWA based on 4,402 SNP loci in all of the Kimberley, the Kimberley transition zone only, the Northern Kimberley only, or the Pilbara.

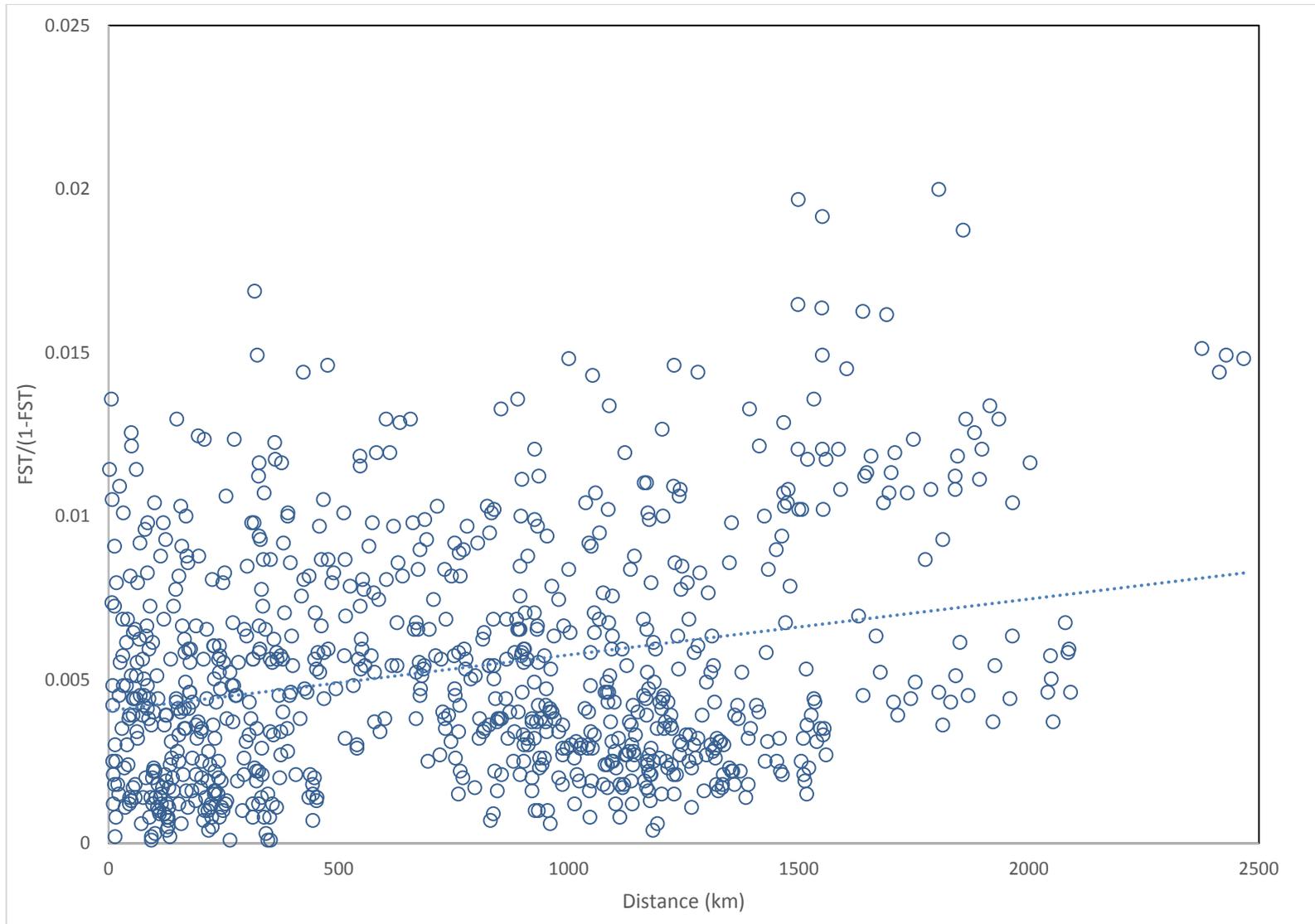


Fig. 6 Isolation by distance for all *L. carponotatus* samples illustrating the relationship between geographic distance and linearised F_{ST} . Dashed line indicate best linear fit.

4 Discussion and Conclusions

This study, along with a companion study on the Miller's Damselfish (*Pomacentrus milleri*; sub-report 1.1.3.4a), is the first to investigate genetic connectivity within the coastal Kimberley Bioregion as well as other parts of WA and NT. The Stripey Snapper is probably representative of many widespread and abundant, pelagic spawning reef-associated fish species with relatively long pelagic larval durations in NWA. Many of these species are also recreationally and/or commercially targeted and therefore understanding the processes driving connectivity between populations can support more appropriate management decisions. Although larvae from these species are able to actively exercise some control over their dispersal and settlement, the powerful hydrodynamic forces in this region appear to play a significant role in distributing the larvae of *L. carponotatus*.

Primarily, the distribution of genetic subdivision in *L. carponotatus* across NWA follows an isolation-by-distance model of connectivity. This is probably facilitated by the prolonged duration larvae spend in the plankton where, on average, dispersal potential is in the order of up to 450km. However, it is also clear that within this, significant genetic breaks exist at well-recognised biogeographic boundaries. These results support previously hypothesised restrictions to connectivity between the Pilbara, northern Ningaloo and the Shark Bay bioregions based on allozyme electrophoresis for four other commercial fish species (Johnson *et al.* 1993) and allozyme and SNP data for the coral, *Pocillopora damicornis* (Whitaker 2006; Thomas *et al.* in review). Our study also provides evidence of restricted connectivity between geographically distant sites and, in some cases, neighbouring sites *within* bioregions separated by a few kilometres. The increased genetic resolution in the present study provided by thousands of SNP loci, with some under natural selection, also revealed a genetic transition zone in the macro-tidal region at the mouth of King Sound that has not been shown using other markers (Veilleux *et al.* 2011). This corresponds to a well-defined IMCRA biogeographic boundary between the Kimberley and Canning marine bioregions, based on shifts in faunal composition in a number of taxa, including fishes (Hutchins 2001; Travers *et al.* 2010) and molluscs (Wilson 2013), but now genetic differentiation is also confirmed at this location.

4.1 Genetic diversity is highest at range limits

Reduced levels of neutral genetic diversity are characteristic of populations at the edge of their range (Messmer *et al.* 2012), and can be attributed to isolation, small population size, and associated increases in genetic drift, as well as potentially strong selection (Kawecki 2008; Cahill & Levinton 2016). The range of *L. carponotatus* extends from Taiwan in the north to Shark Bay in the south and into eastern Australia. Although our sampling efforts did not include the full distributional range of this species, it was extensive (13° of latitude). Considering this, one might expect shifts in genetic connectivity and diversity to be present over this large spatial scale. Surprisingly, the reverse was observed, with levels of genetic diversity being similar throughout the sampling range. This homogeneity may be due to the relatively long PLD of this species and large genetic neighbourhoods that we observed, as evidenced in positive spatial autocorrelation up to 450 km. Despite such forces acting to homogenise genetic diversity metrics, many populations, particularly in the Kimberley bioregion, exhibited low but significant differentiation. The reasons for this pattern are unclear. However, its predominance among samples from the Kimberley and not the Pilbara indicates that patterns of dispersal are likely to differ between these bioregions, perhaps owing to their markedly different hydrodynamic conditions.

4.2 Broad-scale subdivision across NWA

Currently harvest of *L. carponotatus* from the Gascoyne and Ningaloo regions is considered separately from harvest throughout the remainder of NWA. Our results do not support this division, but instead show that *L. carponotatus* from Ningaloo has much higher levels of connectivity with samples from the Pilbara than with those from the Gascoyne. Two dominant patterns of genetic subdivision were evident from the SNP genotyping

of *L. carponotatus*. The first was a clear overall isolation by distance (IBD) effect, where on average sampling sites were genetically most similar to their closest neighbours and least similar to distant sites (Fig. 6). By implication, dispersal is limited on the scale of this investigation (~ 3000 km), and proceeds in a stepping-stone manner. Comparison with the demersal nesting and reef-obligate fish *P. milleri*, indicates that the IBD effect is much weaker in *L. carponotatus*, implying that, as expected considering its longer PLD and less reliance on patchy coral reefs to spawn, it has a higher level of connectivity throughout NWA. This pattern is likely also true for other lutjanid and lethrind species with similar life histories in the region.

In addition to the isolation by distance effect, several pronounced genetic discontinuities were evident among samples of *L. carponotatus* from across NWA. The two most obvious genetic breaks were firstly, a significant genetic subdivision between the Shark Bay Bioregion and all locations of the North West Shelf (Ningaloo, Pilbara, Canning and Kimberley) including the NT. This coincides with well-recognised biogeographic boundaries and oceanographic features south of the North West Cape (Commonwealth of Australia 2006; Woo *et al.* 2006; Spalding *et al.* 2007). Wilson (2013) suggested that the effect of the Leeuwin Current across this region results in a barrier that is probably ineffective in preventing exchange for species with planktotrophic larvae (such as *L. carponotatus*). However, these results (and those for *P. milleri*, subproject 1.1.3.4a), as well as for the coral *Cyphastrea microphthalmia* (Evans *et al.* in prep) and mangrove *Avicennia marina* (Binks *et al.* in prep) indicate the presence of some form of a barrier to genetic exchange even for planktotrophic species. Some studies have suggested that this barrier is probably gradual rather than abrupt (e.g. Johnson *et al.* 1993; Whitaker 2006; Thomas *et al.* 2014; Thomas *et al.* in review; R. Evans unpublished data), and potentially results from a mesoscale eddy at Point Cloates that advects larvae offshore (Woo *et al.* 2006). Our sampling was sparse in this region, however, and therefore we are unable to comment further on this hypothesis. Additional sampling south of Point Cloates would enable us to determine whether it represents an abrupt barrier, a similar isolation by distance pattern observed elsewhere in the range of *L. carponotatus*, or a more pronounced isolation by distance effect indicative of reduced connectivity compared to elsewhere on the NWA coastline.

A second apparent boundary was observed between the Kimberley and Canning marine bioregions (Fig. 4). This pattern was most evident in the STRUCTURE analysis of outlier SNPs as a region of progressive admixture between two apparently homogenous genetic clusters representing the Kimberley/NT, and the combined Pilbara and Canning bioregions. The result was also reflected in the DAPC analysis of both neutral and outlier SNPs, but again less clearly for the neutral dataset. These results, supported by a distinctive pattern of low spatial autocorrelation in this region (Fig. 5) indicate that it likely represents a region of restricted dispersal over a distance of ~ 80km near the tip of the Dampier Peninsula and the entrance to King Sound. Wilson (2013) has previously identified the tip of the Dampier Peninsula as an important biogeographic break in marine species that also reflects a change in the underlying geology and benthic habitat. It also represents an abrupt genetic break in *P. milleri* and the coral *Isopora breuggemanni* (see chapters 1.1.3.4a and 1.1.3.1). The uniquely powerful tidal regime in this region is a likely driver of this pattern. Hydrodynamic modelling conducted in WAMSI Kimberley project 2.2.7 (M. Feng, CSIRO, pers. comm.) show few opportunities for the movement of larvae westwards across Sunday Strait (see figure in sub-report 1.1.3 Synthesis).

Management of *L. carponotatus* in NWA is based in part on recognising three stocks corresponding to: 1) the Gascoyne (which includes both Shark Bay and Ningaloo in fisheries management arrangements); 2) combined Pilbara, Canning, and Kimberley; and 3) the NT. The distinctiveness of the Shark Bay samples from all other bioregions indicates that the Gascoyne management boundary is not supported. In addition, support for separate management of *L. carponotatus* from the NT is equivocal. NT samples were significantly, albeit weakly, genetically differentiated from all other samples (Fig. 2), and appeared weakly divergent in both STRUCTURE and DAPC analyses. However, a large sampling gap exists between the Kimberley and NT sites, and it is unclear whether the genetic differentiation of the NT samples reflects a genuine discontinuity, or a continuation of the isolation by distance effect observed elsewhere in the range of *L. carponotatus*. Unlike the region between Ningaloo and Shark Bay, *L. carponotatus* is abundant between the Kimberley and NT (Travers *et al.* 2010), and further sampling in this region is required to reveal the true nature of the relationship between these recognised stocks.

The integration of oceanographic and environmental variables to explain genetic signals of differentiation, often referred to as seascape genetics, is a growing field (Selkoe *et al.* 2016). Although we explored

environmental variables across the geographic range of *L. carponotatus*, linear distance provided a better explanation for the observed patterns of genetic structure. This reflects that the environmental data almost exactly tracked linear distance (i.e. collinearity) due to the large spatial scale of the study. That is, large distances between sampling sites (up to hundreds of kilometres) over a gradual latitudinal gradient lends itself to environmental change relative to that particular gradient. The long distances, therefore, drive the collinearity of the environmental and the geographical distance. In addition, the environmental variables available had some limitations based on the nature of the data used in the PCA (i.e. remote sensing). That is, many of the sites are on shallow coral reefs or very close to islands or the mainland, and so this proximity reduces data confidence and results in the shifting of focal pixels to slightly deeper water. Pixel shifting creates a deviation from the modelled data to the actual environmental influence on the survival of individuals and their genetic expression. Therefore more confidence is placed in the outcomes of the geographical distance as a predictor in our models.

4.3 Fine-scale connectivity across NWA

The broad-scale genetic discontinuities between bioregions were overlaid by subtle genetic differentiation within each bioregion. Patterns of genetic differentiation also differed between the bioregions, indicating that *L. carponotatus* dispersal behaviour also differs between the bioregions. On average, genetic differentiation between sites was higher in the Kimberley than the Pilbara (Fig. 2), implying that on average dispersal is more restricted in the Kimberley. A moderately pronounced isolation by distance effect was evident among Pilbara samples, yet not in the Kimberley. This also suggests greater restriction to gene flow in the Kimberley than the Pilbara, and its more idiosyncratic patterning likely reflects the more powerful tidal regime and complex coastal topography present in the Kimberley. Larval *L. carponotatus* on the Great Barrier Reef have an effective swimming ability and are capable of actively influencing their dispersal and settlement (Quere & Leis 2010). However, the maximum reported swimming speed recorded is $\sim 33\text{cm/s}^{-1}$, which is considerably less than the maximum tidal velocity in the vicinity of the transition zone (100cm/s^{-1} ; Wolanski & Spagnol, 2003; Lowe et al. 2015). Although spawning probably occurs during neap tides (Quere & Leis 2010), *L. carponotatus* have a relatively long PLD (33-38 days; Quere & Leis 2010), which would expose them to the full spectrum of tidal action in this region. This may limit opportunities for active dispersal to short windows of time around the change of tides and during neap tides. These results also closely reflect that observed for *P. milleri* and the seagrass *Thalassia hemprichii* (subchapter 1.1.3.2), indicating a consistent imprint of environment on the spatial ecology of a diverse range of marine taxa. Although the larger tidal flows in the Kimberley might be expected to promote greater dispersal and genetic homogenisation, the results for *L. carponotatus* and other taxa investigated in this project consistently exhibit the opposite.

Management of *L. carponotatus* north of Sunday Strait within the Kimberley Bioregion could treat it as being effectively a single stock over the ecological timeframes relevant to harvest management. Significant spatial autocorrelation indicates that dispersal is limited on average to distances of several hundred kilometres and less. However, local hydrodynamics probably also promote idiosyncratic spatial relationships among sites, so that a model of stepping stone connectivity doesn't apply like it does in the Pilbara. The transition zone identified around the tip of the Dampier Peninsula represents a region of limited connectivity and mixing between *L. carponotatus* from the Kimberley and the Pilbara/Canning populations. This region should be recognised by managers of coastal resources along these coasts.

5 References

- Anderson DR (2008) Information Theory and Entropy (pp. 51-82). Springer, New York.
- Andrews KR, Good JM, Miller MR, Luikart G, Hohenlohe PA (2016) Harnessing the power of RADseq for ecological and evolutionary genomics. *Nature Reviews Genetics*, 17, 81-92.
- Balkenhol N, Waits LP, Dezzani RJ (2009) Statistical approaches in landscape genetics: an evaluation of methods for linking landscape and genetic data. *Ecography*, 32, 818-830.
- Burgess SC, Nickols KJ, Griesemer CD, Barnett LA, Dedrick AG, Satterthwaite EV, Yamane L, Morgan SG, White JW, Botsford LW (2014) Beyond connectivity: how empirical methods can quantify population persistence to improve marine protected-area design. *Ecological Applications*, 24, 257-270.
- Cahill AE, Levinton JS (2016) Genetic differentiation and reduced genetic diversity at the northern range edge of two species with different dispersal modes. *Molecular Ecology*, 25, 515-526.
- Calaway R, Weston S, Tenenbaum D (2014) doParallele: Foreach Parallel Adaptor for the 'parallel' Package. Available from
- Cawthorn D-M, Steinman HA, Witthuhn RC (2011) Comparative study of different methods for the extraction of DNA from fish species commercially available in South Africa. *Food Control* 22:231-244.
- Commonwealth of Australia (2006) A Guide to the Integrated Marine and Coastal Regionalisation of Australia Version 4.0., Department of the Environment and Heritage, Canberra, Australia.
- Commonwealth of Australia (2006) IMCRA.
- Condie S, Andrewartha J (2008) Circulation and connectivity on the Australian North West shelf. *Continental Shelf Research*, 28, 1724-1739.
- Correa C, Hendry AP (2012) Invasive salmonids and lake order interact in the decline of puye grande *Galaxias platei* in western Patagonia lakes. *Ecological Applications*, 22, 828-842.
- Cowen RK, Sponaugle S (2009) Larval dispersal and marine population connectivity. *Marine Science*, 1.
- Cruz VM, Kilian A, Dierig DA (2013) Development of DArT marker platforms and genetic diversity assessment of the U.S. collection of the new oilseed crop *lesquerella* and related species. *PLoS One*, 8, e64062.
- D'Adamo ND, Fandry C, Buchan S, Domingues C (2009) Northern sources of the Leeuwin Current and the 'Holloway Current' on the North West Shelf. *Journal of the Royal Society of Western Australia*, 92, 53-66.
- Domingues CM, Maltrud ME, Wijffels SE, Church JA, Tomczak M (2007) Simulated Lagrangian pathways between the Leeuwin Current System and the upper-ocean circulation of the southeast Indian Ocean. *Deep-Sea Research Part II -Topical Studies in Oceanography*, 54, 797-817.
- Duke NC (2006) Australia's mangroves: the authoritative guide to Australia's mangrove plants. MER.
- Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology*, 14, 2611-2620.
- Evans RD, Wilson SK, Field SN, Moore JAY (2014) Importance of macroalgal fields as coral reef fish nursery habitat in north-west Australia. *Marine Biology*, 161, 599-607.
- Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics*, 164, 1567-1587.
- Fox NJ, Beckley LE (2005) Priority areas for conservation of Western Australian coastal fishes: a comparison of hotspot, biogeographical and complementarity approaches. *Biological Conservation*, 125, 399-410.
- González JR, Armengol L, Solé X, Guinó E, Mercader JM, Estivill X, Moreno V (2007) SNPAssoc: an R package to perform whole genome association studies. *Bioinformatics* 23:654-655.
- González JR, Armengol L, Solé X, Guinó E, Mercader JM, Estivill X, Moreno V (2007) SNPAssoc: an R package to perform whole genome association studies. *Bioinformatics*, 23, 654-655.
- Grewe PM, Feutry P, Hill PL, Gunasekera RM, Schaefer KM, Itano DG, Fuller DW, Foster SD, Davies CR (2015) Evidence of discrete yellowfin tuna (*Thunnus albacares*) populations demands rethink of management for this globally important resource. *Scientific Reports*, 5.
- Harrison HB, Williamson DH, Evans RD, Almany GR, Thorrold SR, Russ GR, Feldheim KA, van Herwerden L, Planes S, Srinivasan M, Berumen ML (2012) Larval export from marine reserves and the recruitment benefit for fish and fisheries. *Current Biology*, 22, 1023-1028.
- Horne JB, Momigliano P, van Herwerden L, Newman SJ (2013) Murky waters: Searching for structure in genetically depauperate blue threadfin populations of Western Australia. *Fisheries Research*, 146, 1-6.
- Horne JB, Momigliano P, Welch DJ, Newman SJ, van Herwerden L (2011) Limited ecological population connectivity suggests low demands on self-recruitment in a tropical inshore marine fish (*Eleutheronema tetradactylum*: Polynemidae). *Molecular Ecology*, 20, 2291-2306.
- Horne JB, Momigliano P, Welch DJ, Newman SJ, van Herwerden L (2012) Searching for common threads in threadfins: phylogeography of Australian polynemids in space and time. *Marine Ecology Progress Series*, 449, 263-276.

- Hutchins JB (2001) Biodiversity of shallow reef fish assemblages in Western Australia using a rapid censusing technique. *Records of the Western Australian Museum*, 20, 247-270.
- Johnson MS, Hebbert DR, Moran MJ (1993) Genetic analysis of populations of north-western Australian fish species. *Marine and Freshwater Research*, 44, 673-685.
- Johnson MS, Joll LM (1993) Genetic subdivision of the pearl oyster *Pinctata maxima* (Jameson, 1901)(Mollusca: Pteriidae) in northern Australia. *Marine and Freshwater Research*, 44, 519-526.
- Jombart T (2008) adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics* 24:1403-1405.
- Kawecki TJ (2008) Adaptation to marginal habitats. *Annual Review of Ecology, Evolution, and Systematics*, 39, 321-342.
- Kilian A, Wenzl P, Huttner E, Carling J, Xia L, Blois H, Caig V, Heller-Uszynska K, Jaccoud D, Hopper C, Aschenbrenner-Kilian M, Evers M, Peng K, Cayla C, Hok P, Uszynski G (2012) Diversity Arrays Technology: A Generic Genome Profiling Technology on Open Platforms. In: Pompanon F, Bonin A (eds) *Data Production and Analysis in Population Genomics: Methods and Protocols*. Humana Press, Totowa, NJ.
- Kritzer JP (2004) Sex-specific growth and mortality, spawning season, and female maturation of the stripey bass (*Lutjanus carponotatus*) on the Great Barrier Reef. *Fisheries Bulletin*, 102, 94-107.
- Lischer HEL, Excoffier L (2012) PGDSpider: an automated data conversion tool for connecting population genetics and genomics programs. *Bioinformatics*, 28, 298-299.
- Lotterhos KE, Whitlock MC (2015) The relative power of genome scans to detect local adaptation depends on sampling design and statistical method. *Molecular Ecology* 24:1031-1046.
- Lowe RJ, Leon AS, Symonds G, Falter JL, Gruber R (2015) The intertidal hydraulics of tide-dominated reef platforms. *Journal of Geophysical Research: Oceans*, 120, doi:10.1002/2015JC010701.
- McLean DL, Langlois TJ, Newman SJ, Holmes TH, Birt MJ, Bornt KR, Bond T, Collins DL, Evans SN, Travers MJ, Wakefield CB, Babcock RC, Fisher R (2016) Distribution, abundance, diversity and habitat associations of fishes across a bioregion experiencing rapid coastal development. *Estuarine and Coastal Shelf Science*, 178, 36-47.
- Meirmans PG, Van Tienderen PH (2004) GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. *Molecular Ecology Notes*, 4, 792-794.
- Messmer V, Jones GP, Munday PL, Planes S (2012) Concordance between genetic and species diversity in coral reef fishes across the Pacific ocean biodiversity gradient: parallel patterns in species and genetic diversity. *Evolution*, 66, 3902-3917.
- Moore GI, Morrison SM, Hutchins JB, Allen GR and Sampey A (2014) Kimberley marine biota. Historical data: fishes. *Records of the Western Australian Museum Supplement* 84: 161-206.
- Nosil P, Funk DJ, Ortiz-Barrientos, D (2009) Divergent selection and heterogeneous genomic divergence. *Molecular Ecology*, 18, 375-402.
- Oksanen J, Kindt R, Legendre P, et al. (2007) The vegan package. *Community ecology package*, 10.
- Pante E, Simon-Bouhet B (2013) marmap: A Package for Importing, Plotting and Analyzing Bathymetric and Topographic Data in R. *PLoS ONE* 8:e73051.
- Paradis E (2010) pegas: an R package for population genetics with an integrated-modular approach. *Bioinformatics*, 26, 419-420.
- Peakall R, Ruibal M, Lindenmayer DB (2003) Spatial autocorrelation analysis offers new insights into gene flow in the Australian bush rat, *Rattus fuscipes*. *Evolution* 57:1182-1195.
- Peakall ROD, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. *Population genetic software for teaching and research*. *Molecular Ecology Notes*, 6, 288-295.
- Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, 155, 945-959.
- Pusack TJ, Christie MR, Johnson DW, Stallings CD, Hixon MA (2014) Spatial and temporal patterns of larval dispersal in a coral-reef fish metapopulation: evidence of variable reproductive success. *Molecular Ecology*, 23, 3396-3408.
- Quére G, Leis JM (2010) Settlement behaviour of larvae of the Stripey Snapper, *Lutjanus carponotatus* (Teleostei: Lutjanidae). *Environmental Biology of Fishes*, 88, 227-238.
- Rellstab C, Gugerli F, Eckert AJ, Hancock AM, Holderegger R (2015) A practical guide to environmental association analysis in landscape genomics. *Molecular Ecology*, 24, 4348-4370.
- Richards ZT, Garcia RA, Wallace CC, Rosser NL, Muir PR (2015) A diverse assemblage of reef corals thriving in a dynamic intertidal reef setting (Bonaparte Archipelago, Kimberley, Australia). *PLoS One*, 10, e0117791.
- Riginos C, Liggins L (2013) Seascape genetics: populations, individuals, and genes marooned and adrift. *Geography Compass*, 7, 197-216.
- Sbrocco EJ, Barber PH (2013) MARSPEC: ocean climate layers for marine spatial ecology. *Ecology*, 94, 979-979.
- Schiller A (2011) Ocean circulation on the North Australian Shelf. *Continental Shelf Research*, 31, 1087-1095.
- Selkoe K, D'Aloia C, Crandall E et al. (2016) A decade of seascape genetics: contributions to basic and applied marine connectivity. *Marine Ecology Progress Series*, 554, 1-19.
- Spalding MD, Fox HE, Allen GR, Davidson N, Ferdaña ZA, Finlayson M, Halpern BS, Jorge MA, Lombana

- A, Lourie SA (2007) Marine ecoregions of the world: a bioregionalization of coastal and shelf areas. *BioScience*, 57, 573-583.
- Sprintall J, Wijffels S, Chereskin T, Bray N (2002) The JADE and WOCE I10/IR6 throughflow sections in the southeast Indian Ocean. Part 2: velocity and transports. *Deep-Sea Research Part II - Topical Studies in Oceanography*, 49, 1363-1389.
- Thackway R, Cresswell ID (1998) Interim Marine and Coastal Regionalisation for Australia: an ecosystem-based classification for marine and coastal environments. Version 3.3. In: *Environment Australia, Commonwealth Department of the Environment, Canberra*, p 102.
- Thomas L, Kendrick G, Stat M et al. (2014) Population genetic structure of the *Pocillopora damicornis* morphospecies along Ningaloo Reef, Western Australia. *Marine Ecology Progress Series*, 513, 111-119.
- Thomas L, Kennington WJ, Evans RD, Kendrick GA, Stat M (In review) Restricted gene flow and local adaptation highlight the vulnerability of high latitude reefs to rapid environmental change. *Global Change Biology*.
- Travers MJ, Potter IC, Clarke KR, Newman SJ, Hutchins JB (2010) The inshore fish faunas over soft substrates and reefs on the tropical west coast of Australia differ and change with latitude and bioregion. *J Biogeogr* 37:148-169.
- Trembl EA, Ford JR, Black KP, Swearer SE (2015) Identifying the key biophysical drivers, connectivity outcomes, and metapopulation consequences of larval dispersal in the sea. *Movement Ecology*, 3, 1.
- Tyberghein L, Verbrugge H, Pauly K, Troupin C, Mineur F, De Clerck O (2012) Bio-ORACLE: a global environmental dataset for marine species distribution modelling. *Global Ecology and Biogeography*, 21, 272-281.
- Veilleux HD, van Herwerden L, Evans RD, Travers MJ, Newman SJ (2011) Strong genetic subdivision generates high genetic variability among eastern and western Australian populations of *Lutjanus carponotatus* (Richardson). *Fisheries Research*, 108, 74-80.
- Waayers D, Fitzpatrick J (2013) Genetic affiliations and key habitats of marine turtles in the Kimberly region, western Australia. In *Proceedings of the First Western Australian Marine Turtle Symposium* (pp. 34-36).
- Walker DI (1990) Seagrass in Shark Bay. In: Berry PF, Bradshaw SD, Wilson BR (eds) *Research in Shark Bay: Report of the France-Australe Bicentenary Expedition Committee*. Western Australian Museum, Perth, p 101-106.
- Walker DI (1990) Seagrass in Shark Bay. In: Berry PF, Bradshaw SD, Wilson BR (eds) *Research in Shark Bay: Report of the France-Australe Bicentenary Expedition Committee*. Western Australian Museum, Perth.
- Wang JJ, Bradburd GS (2014) Isolation by environment. *Molecular Ecology*, 23, 5649-5662.
- Whitaker K (2006) Genetic evidence for mixed modes of reproduction in the coral *Pocillopora damicornis* and its effect on population structure. *Marine Ecology-Progress Series*, 306, 115-124.
- Whitlock MC, Lotterhos KE (2015) Reliable detection of loci responsible for local adaptation: inference of a null model through trimming the distribution of *F_{ST}*. *The American Naturalist*, 186, S24-36.
- Willing EM, Dreyer C, Van Oosterhout C (2012) Estimates of genetic differentiation measured by *F_{ST}* do not necessarily require large sample sizes when using many SNP markers. *PLoS One*, 7, e42649.
- Wilson B (2014) Kimberley marine biota. History and environment. *Records of the Western Australian Museum Supplement*, 84, 1-18.
- Wilson BR (2013) *The biogeography of the Australian North West Shelf: environmental change and life's response*, Vol. Elsevier Science Publishing Co., Inc., Philadelphia, PA.
- Wilson SK, Depczynski M, Fisher R et al. (2010) Habitat associations of juvenile fish at Ningaloo Reef, Western Australia: the importance of coral and algae. *PLoS ONE*, 5, e15185.
- Wolanski E, Spagnol S (2003) Dynamics of the turbidity maximum in King Sound, tropical Western Australia. *Estuarine Coastal and Shelf Science* 56, 877-890.
- Woo M, Pattiaratchi C, Schroeder W (2006) Dynamics of the Ningaloo Current off Point Cloates, Western Australia. *Marine and Freshwater Research*, 57, 291.
- Zhang Z, Lowe R, Falter J, Ivey G (2011) A numerical model of wave- and current-driven nutrient uptake by coral reef communities. *Ecological Modelling*, 222, 1456-1470.

6 Acknowledgements

The development and then implementation of this project benefited greatly from our collaborating scientists on the WAMSI 1.1.3 project: Kathryn McMahon, Jim Underwood, Zoe Richards, and James Gilmour. For funding and logistic support as well as stimulation and encouragement we sincerely thank The Western Australian Marine Science Institution, especially Kelly Waples, Stuart Field and Kim Friedman.

Many other individuals and organisations assisted us. For field assistance, Fiona Webster, Sam Moyle, Nicole Ryan. For field assistance, cultural advice, and permissions, Kimberley Land Council, One Arm Point PBC, Damon Pyke, Danial Oades, Kevin George, Kevin Ejai, Azden Howard, Kevin Dougal, Tesha Stumpagee, Phillip McCarthy, Peter Hunter, Zac Ejai, Paul Davey, Trevor Sampey, Chris Sampey, Mayala traditional owners, Sandy Isaac, Alec Isaac. The Western Australian Marine Science Institute, Kelly Waples, Kim Friedman, Stuart Field. For logistics and advice Cygnet Bay Pearls, James Brown, Erin McGinty, Ali McCarthy, Scott Whitlam, Duncan Smith. For molecular guidance and genotyping service, DART and Andrzej Killian. For computing assistance Ian LeCoultré, Tim Ho, CSIRO High Performance Computing Centre, Philippe Moncuquet, Annette McGrath and the CSIRO Bioinformatics Core. Bernd Gruber for R scripting lessons. Pilbara and Gascoyne sample collections were funded by the Chevron-operated Wheatstone Project's State Environmental Offsets Program and administered by the Department of Parks and Wildlife. The Wheatstone Project is a joint venture between Australian subsidiaries of Chevron, Kuwait Foreign Petroleum Exploration Company (KUFPEC), Woodside Petroleum Limited and Kyushu Electric Power Company, together with PE Wheatstone Pty Ltd (part owned by TEPCO).

7 Data Availability

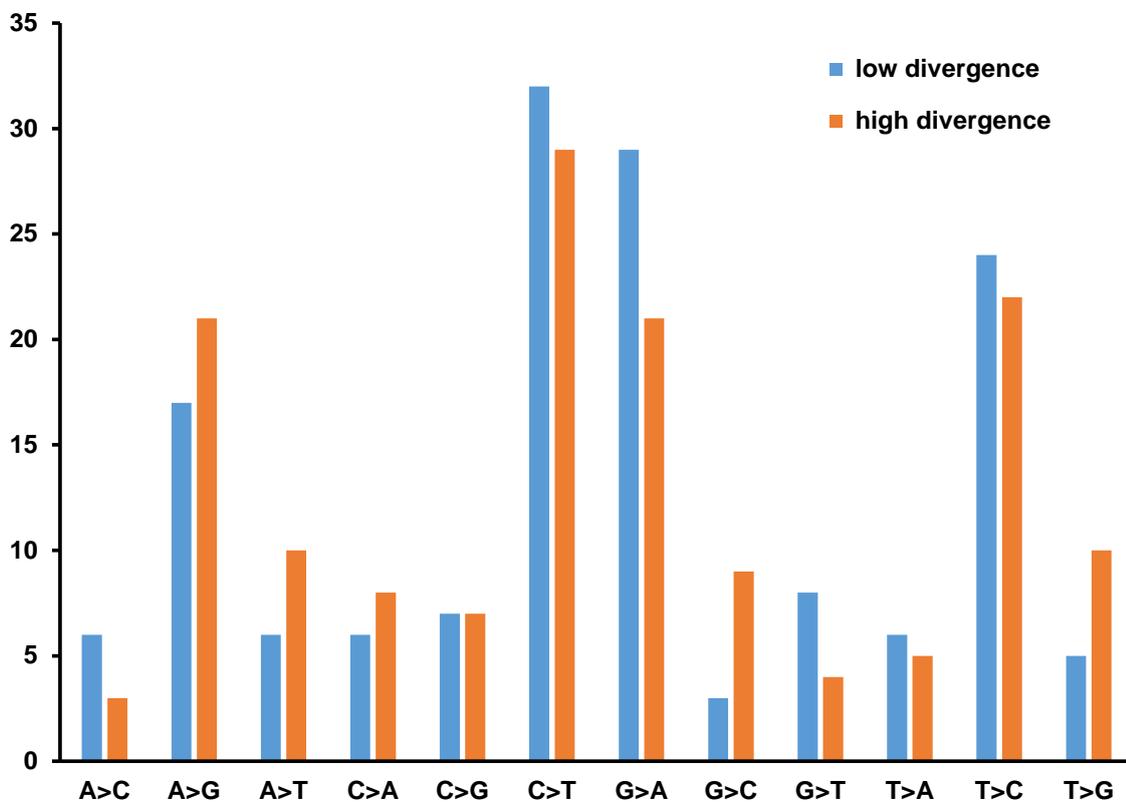
Data associated with this research is available on the CSIRO Data Access Portal at:

<https://data.csiro.au/dap/landingpage?pid=csiro:20195>.

8 Appendices

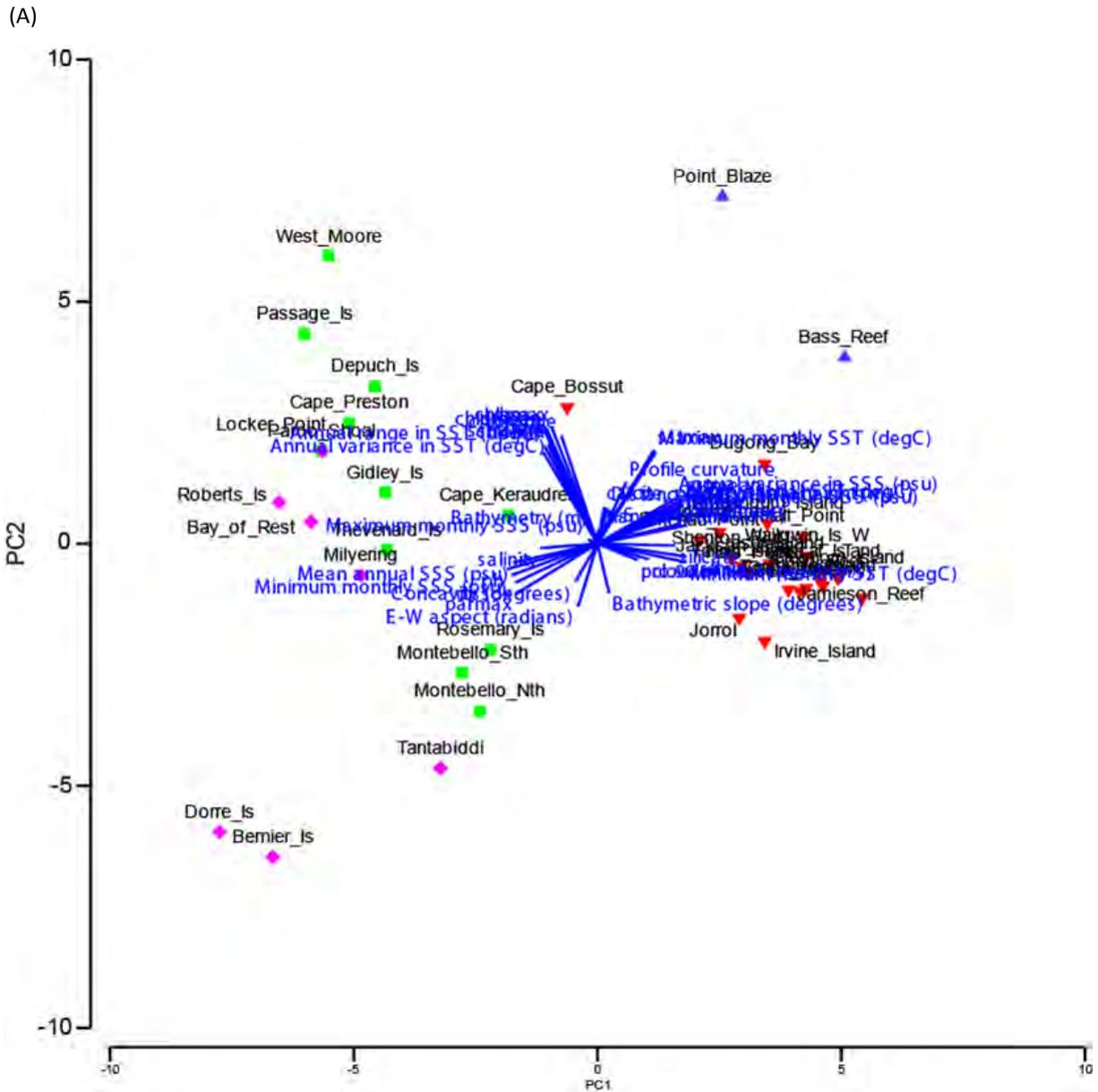
Appendix 1. A comparison of SNP type

A comparison of SNP type (e.g. transition versus transversion) for 30 individual *L. carponotatus* sampled in 2002 and 2015, respectively, from the Pilbara bioregion. The comparison is based on the 150 SNPs that were the most different in frequency between the two groups based on F_{ST} (high divergence) and the 150 SNPs that were the least different (but still variable) in frequency between the two groups (low divergence). These two categories were not significantly different from each other based on a one-way ANOVA of the logarithmically transformed values ($P = 0.846$). This result indicates deamination (C/T transitions) is not a likely cause of the genetic divergence between samples collected in 2002 versus 2015, although we cannot rule out other forms of DNA damage.

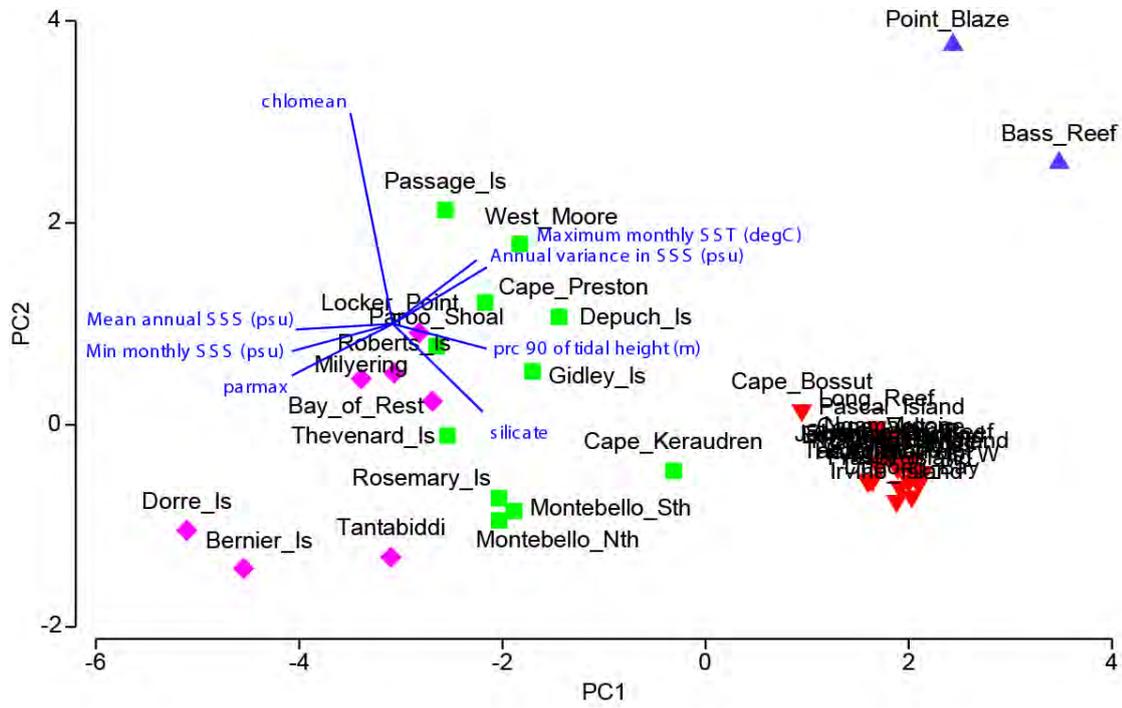


Appendix 2. Principal Component Analysis

Principal Component Analysis (PCA) of 43 environmental variables extracted from freely available ocean climate layers MARSPEC (Sbrocco and Barber 2013) and Bio-ORACLE (Tyberghein et al. 2012). We here present PCA plots with: (A) all environmental variables included, and (B) the eight most influential variables (using Draughtsmans plots and inspecting the pairwise correlation matrix in all cases; data not shown).



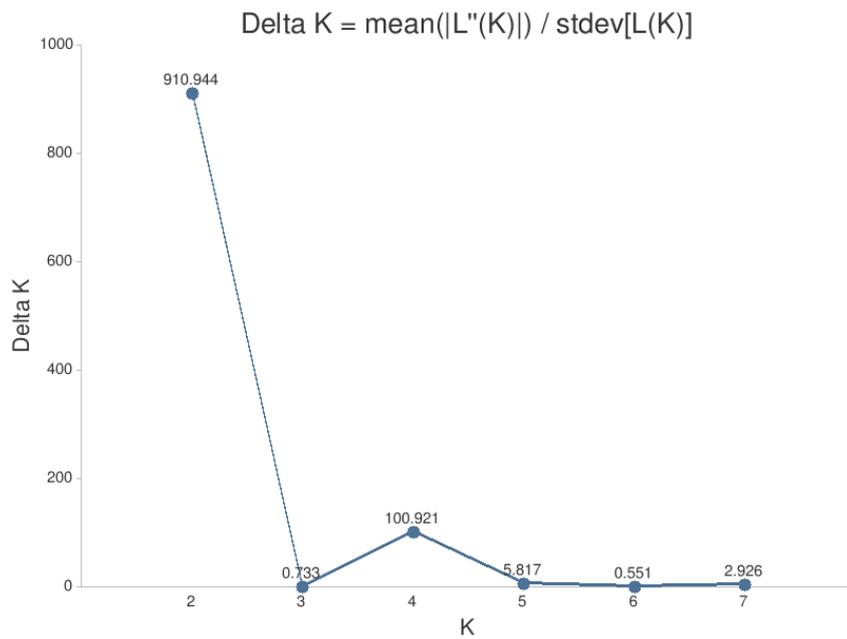
(B)



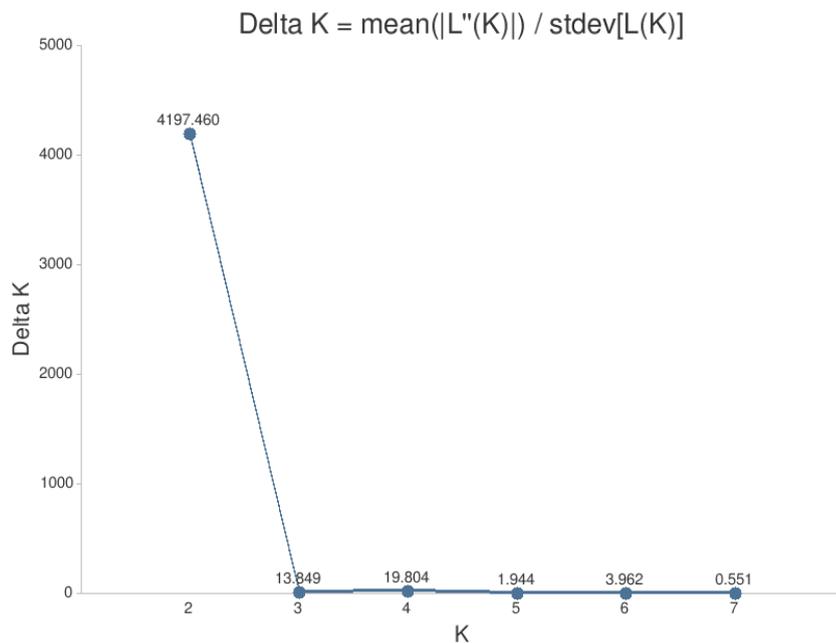
Appendix 3. Structure Harvester analyses

Structure Harvester analyses used to determine that the most likely value of K was 2 for *L. carponotatus* populations with 20 or more individuals in NWA based on (A) 4,402 SNP loci and (B) 66 outlier loci.

(A)



(B)



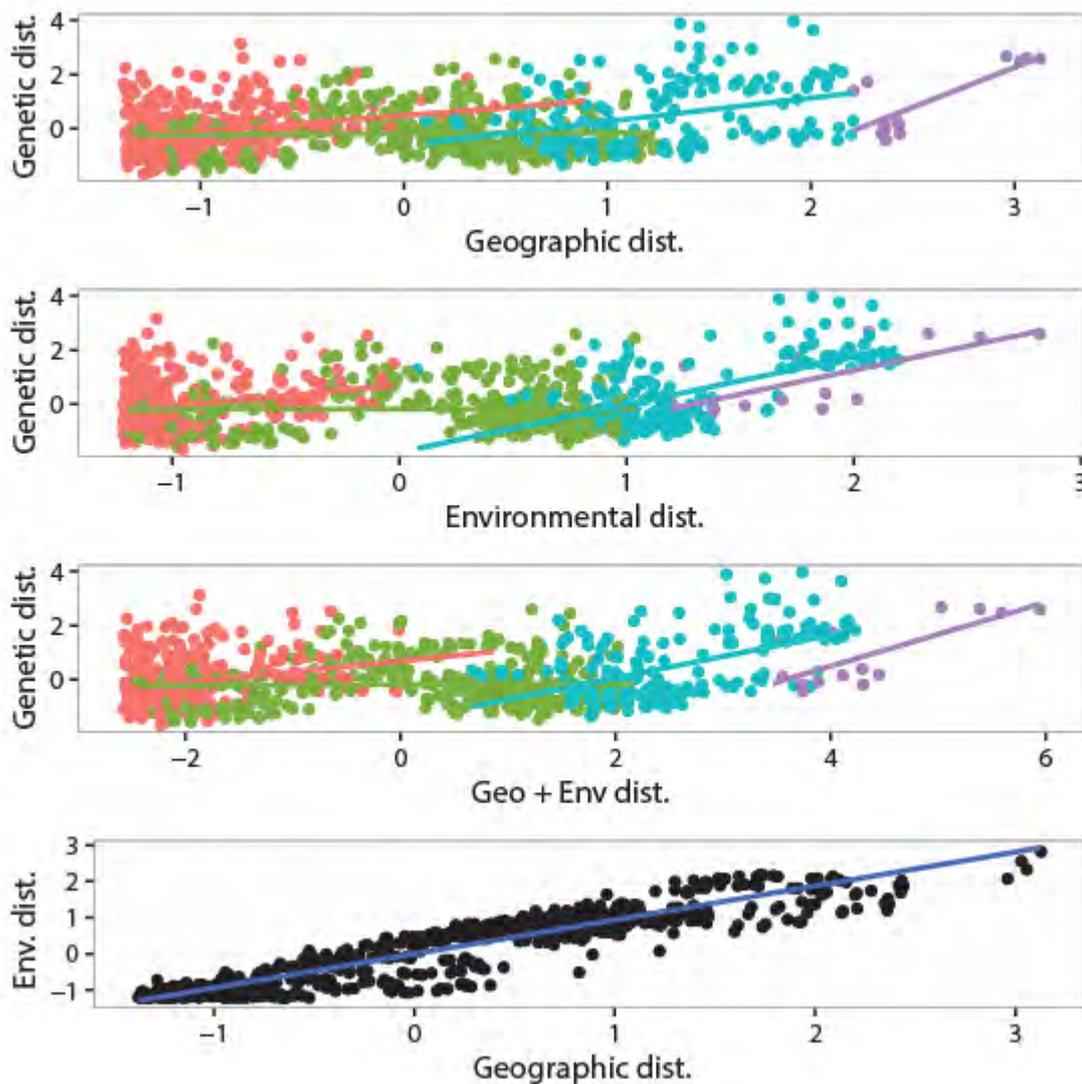
Appendix 4. Linear model ranking

Linear model ranking for the effects of geographic distance, environmental distances, or a priori barriers to dispersal for *L. carponotatus* populations with 20 or more individuals in NWA based on (a) 4,402 SNP loci or (b) 66 outlier loci. Only models with a likelihood > 0 are presented here.

	Formula	K	AIC	AICc	RSS	R ²	Adjusted R ²	Delta AICc	Model likelihood	Model probability	Evidence ratio
(a) 4,402 SNP loci											
m65	b1_2_3 + env + geo + b1_2_3 * env + b1_2_3 *	13	2249.909	2250.339	667.2432	0.2241	0.2141	0	1	0.4918	1
m66	b1_2_3 * env + geo	10	2251.093	2251.352	672.8341	0.2176	0.2103	1.0134	0.6025	0.2963	1.6598
m64	b1_2_3 * env	9	2251.921	2252.133	675.0476	0.2151	0.2086	1.794	0.4078	0.2006	2.4522
m38	b3 + env + geo + b3 * env + b3 * geo	7	2257.751	2257.882	682.7983	0.206	0.2014	7.5431	0.023	0.0113	43.4477
(b) 66 outlier SNP loci											
m22	b1 * geo + env	6	1091.7667	1091.8651	176.6732	0.7946	0.7936	0	1	0.4107	1
m21	b1 * env + geo	6	1091.7694	1091.8678	176.6738	0.7946	0.7936	0.0027	0.9986	0.4102	1.0014
m20	b1 + env + geo + b1 * env + b1 * geo	7	1093.6166	1093.7479	176.6424	0.7946	0.7934	1.8828	0.3901	0.1602	2.5636
m14	b1 + env + geo	5	1097.9555	1098.0257	178.3616	0.7926	0.7919	6.1607	0.0459	0.0189	21.7656

Appendix 5. Correlation between pairwise genetic distance, geographic, and environmental distances

Correlation between pairwise genetic distance, geographic, and environmental distances for *L. carponotatus* in NWA based on 4,402 SNP loci. In each case, genetic distance (F_{ST}) was compared to geographic distance, environmental distance, and the combined geographic-environmental distance, with red dots corresponding to the pairwise comparison of sites with no modelled barriers between them, green dots corresponding to the pairwise comparison of sites with one barrier between them, blue dots corresponding to the pairwise comparison of sites with two barrier between them, and purple dots corresponding to the pairwise comparison of sites with all three barriers between them.





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

Sarah Hearne^{1,5}, Mike Travers^{2,5}, Richard Evans^{3,5}, Alison Blythe^{4,5}, Kate Trinajstić^{1,5},
Jennifer McIlwain^{1,5}, Stephen Newman^{2,5}

¹Department of Environment and Agriculture, Curtin University, Bentley, Western Australia

²Fisheries Division, Department for Primary Industries and Regional Development, Hillarys, Western Australia

³Department of Biodiversity, Conservation and Attractions, Kensington, Western Australia

⁴Department of Environment and Agriculture, Curtin University, Bentley, Western Australia

⁵Western Australian Marine Science Institution, Perth, Western Australia

WAMSI Kimberley Marine Research Program

Final Report

Subproject 1.1.3.4c

September 2017



western australian
marine science institution



Department of Biodiversity,
Conservation and Attractions



Department of
Primary Industries and
Regional Development



WAM WESTERN
AUSTRALIAN
MUSEUM



Mayala
Community



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.

Ownership of Intellectual property rights

Unless otherwise noted, copyright (and any other intellectual property rights, if any) in this publication is owned by the Western Australian Marine Science Institution, CSIRO, Australian Institute of Marine Science, Edith Cowan University, The University of Western Australia, Western Australia Department of Primary Industries and Regional Development, Western Australian Museum, Curtin University and the Western Australian Department of Biodiversity, Conservation and Attractions.

Copyright

© Western Australian Marine Science Institution

All rights reserved.

Unless otherwise noted, all material in this publication is provided under a Creative Commons Attribution 3.0 Australia License. (<http://creativecommons.org/licenses/by/3.0/au/deed.en>)



Legal Notice

The Western Australian Marine Science Institution advises that the information contained in this publication comprises general statements based on scientific research. The reader is advised and needs to be aware that such information may be incomplete or unable to be used in any specific situation. This information should therefore not solely be relied on when making commercial or other decision. WAMSI and its partner organisations take no responsibility for the outcome of decisions based on information contained in this, or related, publications.

Front cover images (L-R)

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

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

Image 3: Humpback whale breaching (Image: Pam Osborn)

Image 3: Damsel fish (*Pomacentus milleri*) (Image: Gerry Allen)

Year of publication: September 2017

Metadata: <http://catalogue.aodn.org.au/geonetwork/srv/eng/metadata.show?uuid=fb1d80bf-6ef2-4150-9479-22b4240435a7>

Citation: Hearne S, Travers M, Evans R, Blyth A, Trinajstic K, McIlwain J, Newman S (2017) Population connectivity of two reef fish species in northwestern Australia using otolith geochemistry: A pilot study. Report of Project 1.1.3 - Project 1.1.3.4c prepared for the Kimberley Marine Research Program, Western Australian Marine Science Institution, Perth, Western Australia, 31pp.

Author Contributions: All authors contributed to the drafting of this text.

Corresponding author and Institution: Oliver Berry, CSIRO Oceans and Atmosphere. oliver.berry@csiro.au

Funding Sources: This project was funded (commissioned) by the Western Australian Marine Science Institution as part of the WAMSI Kimberley Marine Research Program, a \$30M program with seed funding of \$12M provided by State government as part of the Kimberley Science and Conservation Strategy. The Program has been made possible through co-investment from the WAMSI Joint Venture partners and further enabled by data and information provided by Woodside Energy Ltd.

Competing Interests: The commercial investors and data providers had no role in the data analysis, data interpretation, the decision to publish or in the preparation of the manuscript. The authors have declared that no competing interests exists.

Kimberley Traditional Owner agreement: This research was enabled by the Traditional Owners through their advice, participation and consent to access their traditional lands.

Acknowledgements: We are grateful to all WAMSI staff for their assistance, and in particular Kelly Waples, Stuart Field and Kim Friedman for providing advice and constructive criticism throughout the project. Many other people and organisations contributed to the success of this project, and we thank them sincerely. We especially thank the following Indigenous communities. The Bardi and Jawi Niimidiman Aboriginal Corporation, the Bardi Jawi Rangers and the Bardi Jawi Traditional Owners and specifically Daniel Oades, Damon Pyke, Azton Howard, Chris Sampi, Kevin George, Kevin Ejai, Kevin Dougal, Tasha Stumpagee, Phillip McCarthy, Peter Hunter, Zac Ejai, Paul Davey and Trevor Sampi. The Mayala people, specifically Sandy, Alec and Janella Isaacs. The Wunambal Gaambera Aboriginal Corporation, Traditional Owners, Unguu Rangers and Tom Vigilante. The Dambimangari Aboriginal Corporation, Dambimangari Traditional Owners and the Dambimangari Rangers. Thanks also to the Bioinformatic pipeline development: Bernd Gruber (University of Canberra); Oceanographic modelling: Ming Feng, Dirk Slawinski (CSIRO); SNP marker development and genotyping: Andrzej Kilian (Diversity Arrays Technology); Field assistance: Sam Moyle and Fiona Webster; Kimberley Marine Research Station for facilities and logistical support: James Brown, Michael Flynn, Scott Whitlam, Duncan Smith and Erin McGinty; and advice on project development: Karen Miller (AIMS).

Collection permits/ethics approval: SF008440, SF009910, SC001362 (Western Australian Department of Parks and Wildlife); 2485 2085, 2344 (Western Australian Department of Fisheries)

Contents

EXECUTIVE SUMMARY	I
IMPLICATIONS FOR MANAGEMENT	II
RESIDUAL KNOWLEDGE GAPS	III
1 INTRODUCTION	1
1.1 MARINE ENVIRONMENT OF NORTHWESTERN AUSTRALIA.....	1
1.2 POPULATION CONNECTIVITY	1
1.3 OTOLITH GEOCHEMISTRY	2
1.4 RESEARCH OBJECTIVES	3
2 MATERIALS AND METHODS	4
2.1 GENERAL APPROACH	4
2.2 SITE SELECTION	4
2.3 SPECIES SELECTION.....	4
2.4 SAMPLE COLLECTION.....	7
2.5 SAMPLING STRATEGY	7
2.6 OTOLITH PREPARATION	8
2.7 AGE DETERMINATION	8
2.8 TRACE ELEMENT ANALYSIS	8
2.9 STRONTIUM ISOTOPE ANALYSIS.....	8
2.10 OXYGEN ISOTOPE ANALYSIS.....	8
2.11 STATISTICAL ANALYSES	9
3 RESULTS.....	9
3.1 PELAGIC LARVAL DURATION (PLD)	9
3.2 TRACE ELEMENT ANALYSIS	9
3.3 STRONTIUM ANALYSIS	10
3.4 OXYGEN ISOTOPE ANALYSIS.....	10
4 DISCUSSION AND CONCLUSIONS	15
4.1 POPULATION STRUCTURE	15
4.2 INDIVIDUAL MOVEMENT.....	15
4.3 COMPARISONS WITH GENETICS	16
5 CONCLUSIONS.....	16
5.1 FUTURE WORK	17
6 REFERENCES.....	18
7 ACKNOWLEDGEMENTS	21
8 COMMUNICATION	21
8.1 STUDENTS SUPPORTED.....	21
8.2 SUBMITTED MANUSCRIPTS.....	21
8.3 PRESENTATIONS	21
8.4 OPPORTUNITIES CREATED AS A RESULT OF THIS PROJECT	21

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 $\delta^{18}\text{O}$ 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 sagitta, 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.

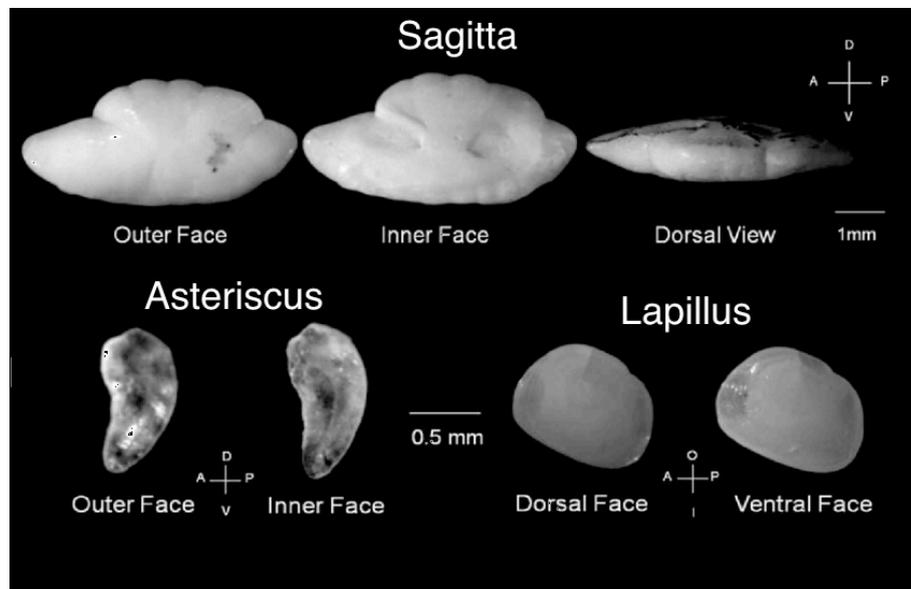


Figure 1: An example of sagitta, asteriscus and lapillus of *Lepidonotothen larseni* (modified from(Curcio et al. 2014)).

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 $^{87}\text{Sr}/^{86}\text{Sr}$ 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 ($\delta^{18}\text{O}$) 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 $\delta^{18}\text{O}$ 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 $\delta^{18}\text{O}$ 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* Commonwealth 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 Rottneest 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.

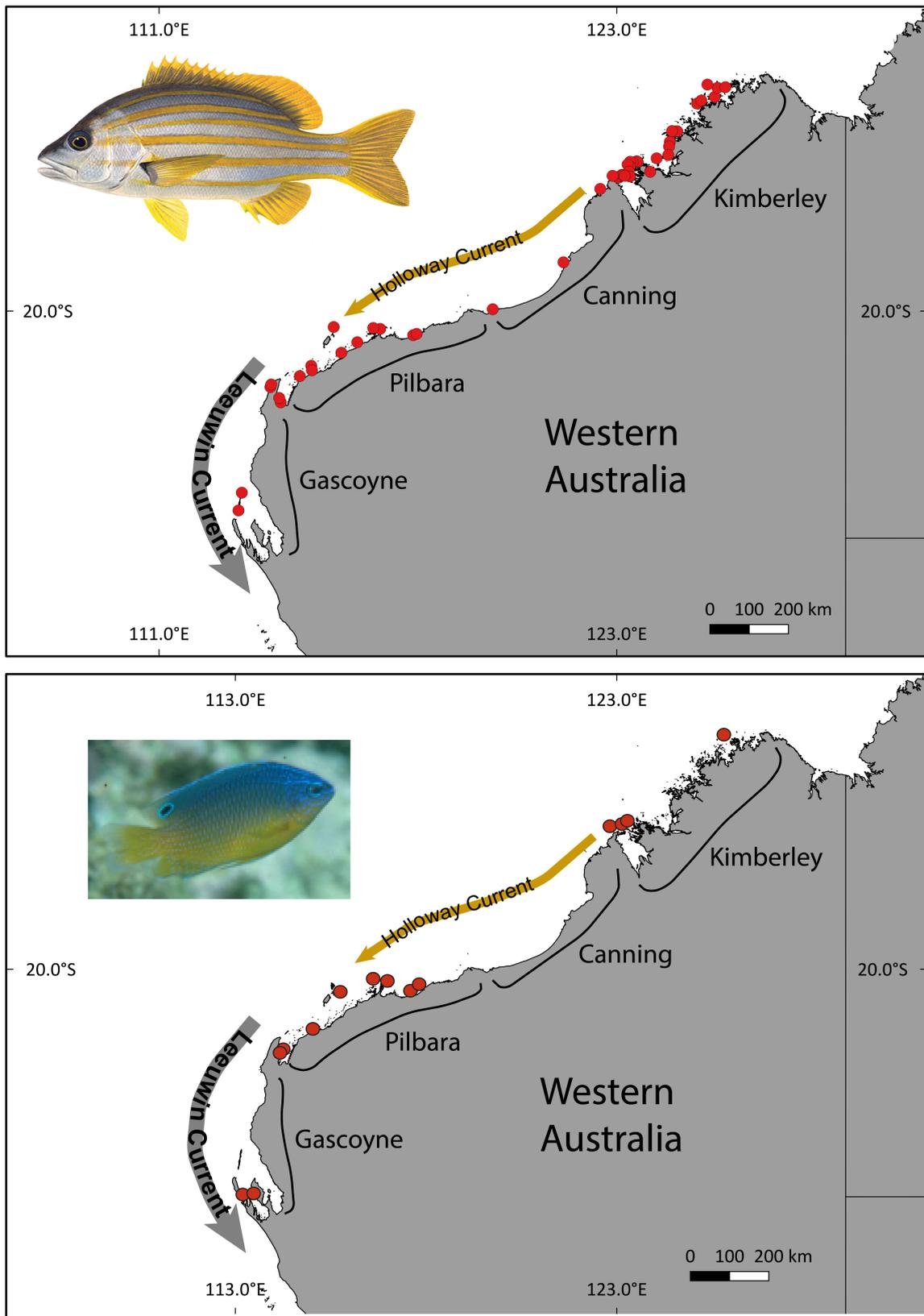


Figure 2: Map of northern Western Australia showing the sample sites for *L. carponotatus* (top) and *P. milleri* (bottom) and the extent of the four IMCRA Bioregions. Illustration of *L. carponotatus* © R.Swainston/www.anima.net.au.

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	<i>Lutjanus carponotatus</i> N	<i>Pomacentrus milleri</i> 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: ^7Li , ^{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 $\mu\text{m}/\text{second}$ 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 15 μm 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 $^{87}\text{Sr}/^{86}\text{Sr}$ analysis. A qualitative analysis of $\delta^{18}\text{O}$ 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 3b,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 3b**).

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.

	df	Core			Margin		
		MS	<i>F</i>	<i>P</i>	MS	<i>F</i>	<i>P</i>
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

$\delta^{18}\text{O}$ 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 $\delta^{18}\text{O}$ 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.

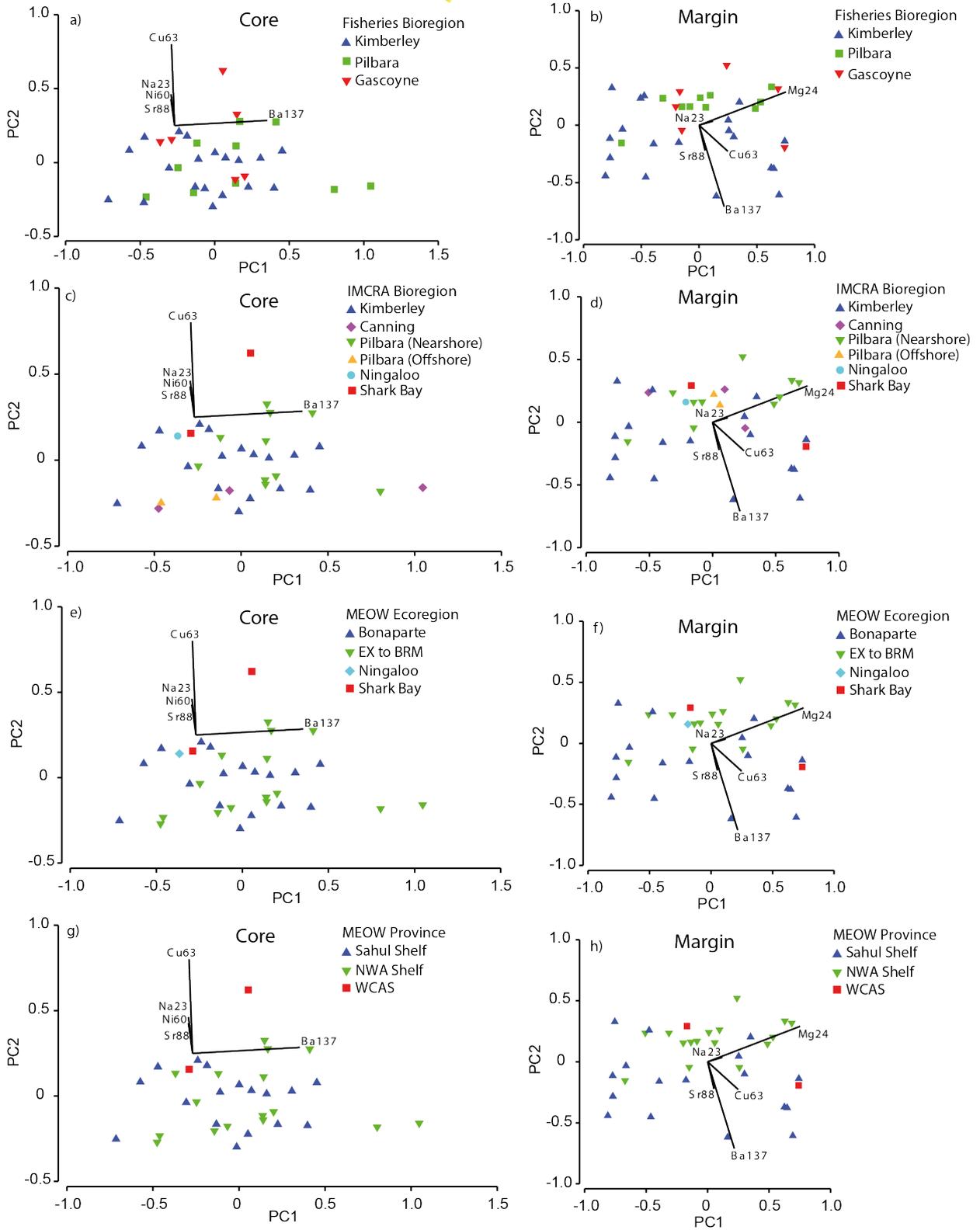


Figure 3: PCA analysis on the core (a,c,e,g) and margin (b,d,f,h) geochemistry (^{23}Na , ^{24}Mg , ^{60}Ni , ^{63}Cu , ^{88}Sr and ^{137}Ba) of *Lutjanus carponotatus* otoliths.

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.

	df	Core			Margin		
		MS	<i>F</i>	<i>P</i>	MS	<i>F</i>	<i>P</i>
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)

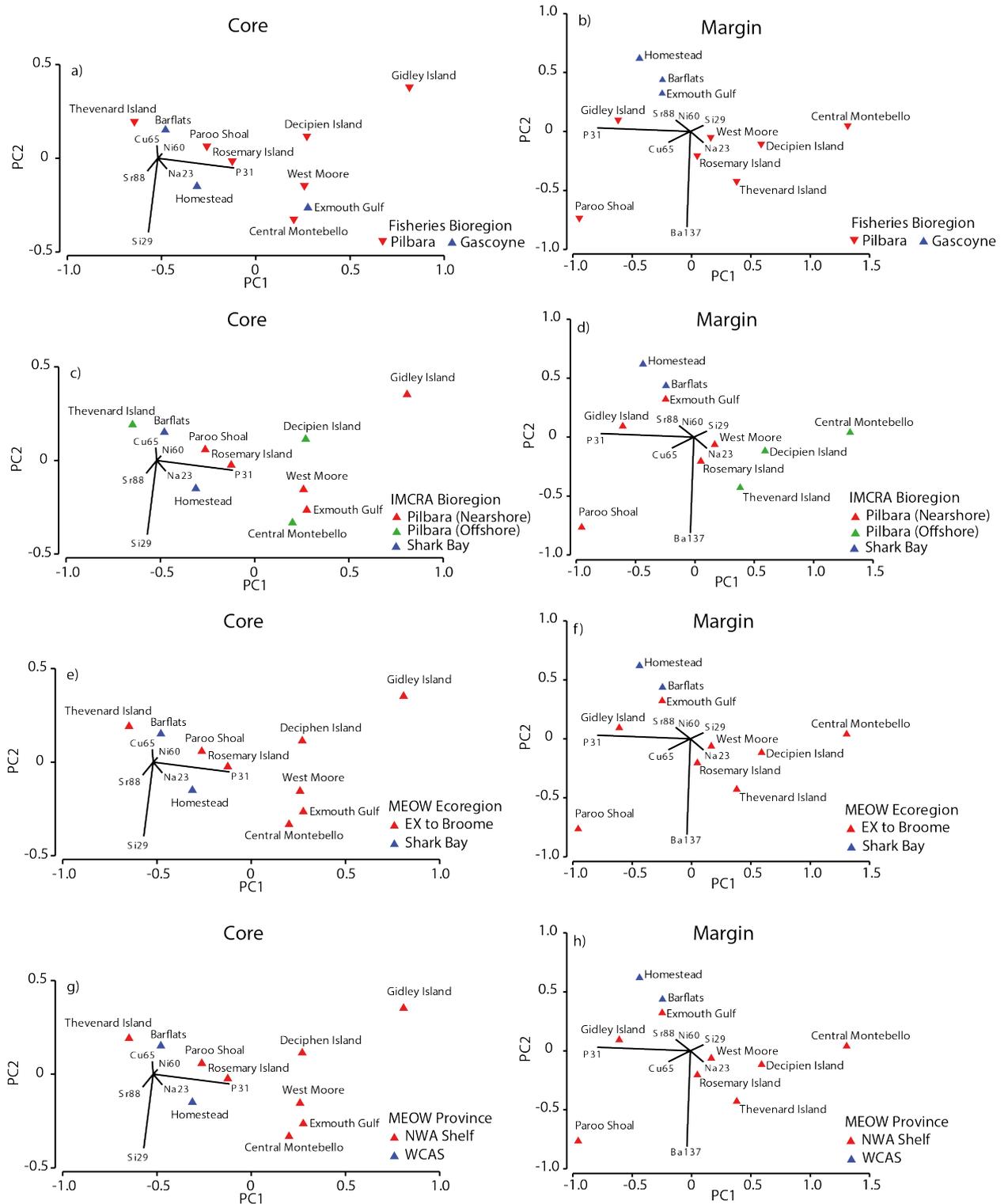


Figure 4: PCA analysis on the core (a,c,e,g) and margin (b,d,f,h) geochemistry (^{23}Na , ^{29}Si , ^{31}P , ^{60}Ni , ^{63}Cu , ^{88}Sr and ^{137}Ba) of *Pomacentrus millerii* otoliths.

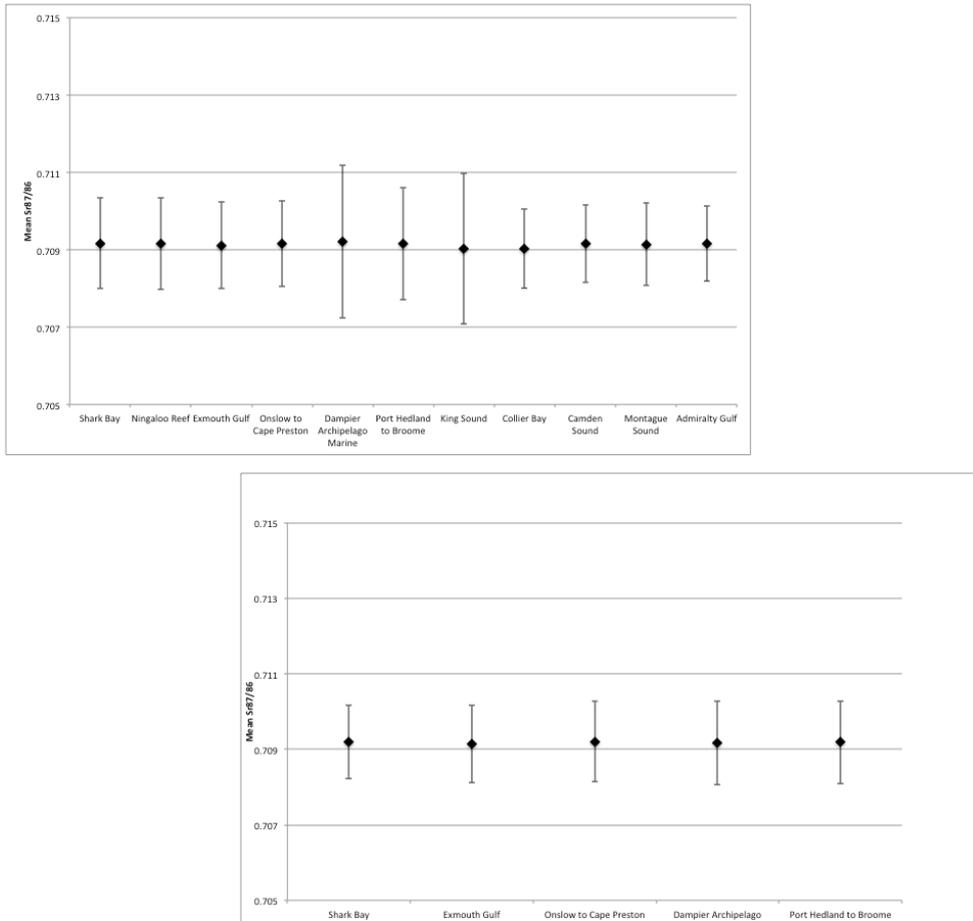


Figure 5: Mean Sr87/86 results for each sub-region for *L. carponotatus* (top) and *P. milleri* (bottom).

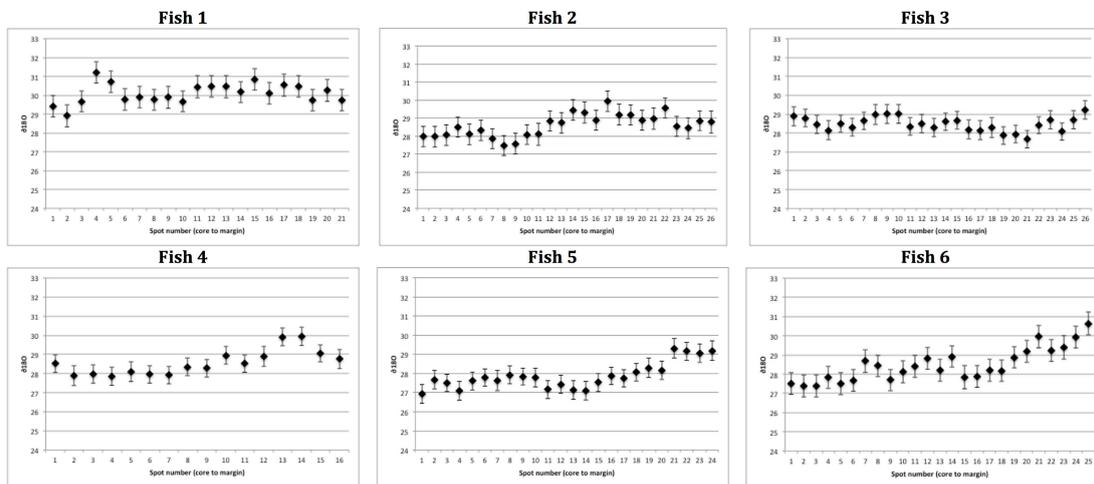


Figure 6: Oxygen isotope analysis results for *P. milleri* from the Pilbara region.

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, $\delta^{18}\text{O}$ 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 $\delta^{18}\text{O}$ 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 $\delta^{18}\text{O}$ 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 $\delta^{18}\text{O}$ 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 $\delta^{18}\text{O}$ 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.

6 References

- Allen, G.R., 1991. Damsel fishes of the world, Melle, Germany: Mergus Publishers.
- Allen, G.R., 1985. Vol.6. Snappers of the world. An annotated and illustrated catalogue of lutjanid species known to date, Rome, Italy: Food and Agriculture Organisation of the United Nations.
- Amakawa, H. et al., 2012. Sr isotopic compositions of ayu otolith and its ambient water. *Fisheries Science*, 78(5), pp.1023–1029.
- Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecology*, 26(1), pp.32–46.
- Anderson, M.J., Gorley, R.N. & Clarke, K.R., 2008. PERMANOVA+ for PRIMER. Guide to software and statistical methods.
- Arai, T. & Hirata, T., 2006. Differences in the trace element deposition in otoliths between marine- and freshwater-resident Japanese eels, *Anguilla japonica*, as determined by laser ablation ICPMS. *Environmental Biology of Fishes*, 75(2), pp.173–182.
- Ashford, J.R. et al., 2005. Can otolith elemental signatures record the capture site of Patagonian toothfish (*Dissostichus eleginoides*), a fully marine fish in the Southern Ocean? *Canadian Journal of Fisheries and Aquatic Sciences*, 62, pp.2832–2840.
- Ashford, J.R., Arkhipkin, A.I. & Jones, C.M., 2006. Can the chemistry of otolith nuclei determine population structure of Patagonian toothfish *Dissostichus eleginoides*? *Journal of Fish Biology*, 69(3), pp.708–721.
- Ashford, J.R. & Jones, C.M., 2007. Oxygen and carbon stable isotopes in otoliths record spatial isolation of Patagonian toothfish (*Dissostichus eleginoides*). *Geochimica et Cosmochimica Acta*, 71(1), pp.87–94.
- Bailey, D.S., Fairchild, E.A. & Kalnejais, L.H., 2015. Microchemical signatures in juvenile winter flounder otoliths provide identification of natal nurseries. *Transactions of the American Fisheries Society*, 144(1), pp.173–183.
- Begg, G.A., Friedland, K.D. & Pearce, J.B., 1999. Stock identification and its role in stock assessment and fisheries management: An overview. *Fisheries Research*, 43, pp.1–8.
- Ben-Tzvi, O. et al., 2008. Tracking recruitment pathways of *Chromis viridis* in the Gulf of Aqaba using otolith chemistry. *Marine Ecology Progress Series*, 359, pp.229–238.
- Berry, O. et al., 2017. WAMSI Ecological Connectivity 1.1.3.4a Genomic Connectivity in a Tropical Reef Fish from the Kimberley, Pilbara and Gascoyne Bioregions of Western Australia,
- Brazner, J.C., Campana, S.E. & Tanner, D.K., 2004. Habitat fingerprints for Lake Superior coastal wetlands derived from elemental analysis of yellow perch otoliths. *Transactions of the American Fisheries Society*, 133(3), pp.692–704.
- Breder, C.M. & Rosen, D.E., 1966. Modes of reproduction in fishes, New York: The American Museum of Natural History.
- Brennan, S.R. et al., 2015. Strontium isotopes in otoliths of a non-migratory fish (slimy sculpin): Implications for provenance studies. *Geochimica et Cosmochimica Acta*, 149, pp.32–45.
- Bruland, K.W., 1983. Trace elements in sea-water. In J. P. Riley & R. Chester, eds. *Chemical Oceanography*, Volume 8. London, UK: Academic Press, pp. 157–220.
- Campana, S.E., 1999. Chemistry and composition of fish otoliths: pathways, mechanisms and applications. *Marine Ecology Progress Series*, 188, pp.263–297.
- Chittaro, P.M. et al., 2006. Spatial variation in otolith chemistry of *Lutjanus apodus* at Turneffe Atoll, Belize. *Estuarine, Coastal and Shelf Science*, 67(4), pp.673–680.
- Clarke, K.R. & Gorley, R.N., 2015. PRIMER v7: User Manual/Tutorial. , p.296.
- Collins, J.H., 2008. Marine tourism in the Kimberley region of Western Australia. *Geographical Research*, 46(1), pp.111–123.
- Commonwealth of Australia, 2006. A guide to the integrated marine and coastal regionalisation of Australia version 4.0, Canberra, Australia.
- Correia, A.T. et al., 2012. Population structure of the black seabream *Spondyliosoma cantharus* along the south-west Portuguese coast inferred from otolith chemistry. *Journal of Fish Biology*, 80(2), pp.427–443.
- Cowen, R.K. & Sponaugle, S., 2009. Larval dispersal and marine population connectivity. *Annual Review of Marine Science*, 1, pp.443–466.
- Cuif, M. et al., 2015. Monthly variability of self-recruitment for a coral reef damselfish. *Coral Reefs*, 34(3), pp.759–770.
- Curcio, N., Tombari, A. & Capitanio, F., 2014. Otolith morphology and feeding ecology of an Antarctic nototheniid, *Lepidonotothen larseni*. *Antarctic Science*, 26(2), pp.124–132.
- DiBattista, J. et al., 2017. WAMSI Ecological Connectivity 1.1.3.4b Population Population connectivity of the Stripey snapper *Lutjanus carponotatus* along the ecologically significant coast of northwestern Australia,
- Edmonds, J.S., Moran, M.J. & Caputi, N., 1989. Trace element analysis of fish sagittae as an aid to stock identification: pink snapper (*Chrysophrys auratus*) in Western Australian waters. *Canadian Journal of Fisheries and Aquatic Sciences*, 46, pp.50–54.

- Eldson, T.S. et al., 2008. Otolith chemistry to describe movements and life-history parameters of fishes: Hypotheses, assumptions, limitations and inferences. *Oceanography and Marine Biology: An Annual Review*, 46, pp.297–330.
- Epstein, S. & Mayeda, T., 1953. Variation of O18 content of waters from natural sources. *Geochimica et Cosmochimica Acta*, 4, pp.213–224.
- Fairclough, D. V. et al., 2011. Rapid and cost-effective assessment of connectivity among assemblages of *Choerodon rubescens* (Labridae), using laser ablation ICP-MS of sagittal otoliths. *Journal of Experimental Marine Biology and Ecology*, 403(1–2), pp.46–53.
- Fletcher, W.J., Mumme, M.D. & Webster, F.J. eds., 2017. Status Reports of the Fisheries and Aquatic Resources of Western Australia 2014/15: The State of the Fisheries. In Department of Fisheries, Western Australia.
- Fletcher, W.J. & Santoro, K., 2009. State of the fisheries report 2008/09, Perth, Australia.
- Fraille, I. et al., 2016. Otolith chemistry as an indicator of movements of albacore (*Thunnus alalunga*) in the North Atlantic Ocean. *Marine and Freshwater Research*, 67(7), pp.1002–1013.
- Di Franco, A. et al., 2012. Dispersal patterns of coastal fish: Implications for designing networks of marine protected areas. *PLoS ONE*, 7(2), p.e31681.
- Halpern, B.S. et al., 2008. A global map of human impact on marine ecosystems. *Science*, 319(5865), pp.948–952.
- Hobbs, J.A. et al., 2010. The use of otolith strontium isotopes ($^{87}\text{Sr}/^{86}\text{Sr}$) to identify nursery habitat for a threatened estuarine fish. *Environmental Biology of Fishes*, 89(3), pp.557–569.
- Humphreys Jr, R.L., Campana, S.E. & DeMartini, E.E., 2005. Otolith elemental fingerprints of juvenile Pacific swordfish *Xiphias gladius*. *Journal of Fish Biology*, 66(6), pp.1660–1670.
- Izzo, C. et al., 2017. Integrated approach to determining stock structure: implications for fisheries management of sardine, *Sardinops sagax*, in Australian waters. *Reviews in Fish Biology and Fisheries*, 27, pp.267–284.
- Kalish, J.M., 1991. Oxygen and carbon stable isotopes in the otoliths of wild and laboratory-reared Australian salmon (*Arripis trutta*). *Marine Biology*, 110, pp.37–47.
- Kennedy, B.P. et al., 2000. Using natural strontium isotopic signatures as fish markers: methodology and application. *Canadian Journal of Fisheries and Aquatic Sciences*, 57(11), pp.2280–2292.
- King, B. et al., 2001. River plume dynamics in the central Great Barrier Reef. *Oceanographic Processes of Coral Reefs: Physical and Biological Links in the Great Barrier Reef*, pp.145–159.
- Kritzer, J.P. & Liu, O.R., 2014. Fishery management strategies for addressing complex spatial structure in marine fish stocks. In S. X. Cadarin, L. A. Kerr, & S. Mariani, eds. *Stock Identification Methods: Applications in Fishery Science: Second Edition*. Elsevier, pp. 29–57.
- Kritzer, J.P. & Sale, P.F., 2004. Metapopulation ecology in the sea: from Levins' model to marine ecology and fisheries science. *Fish and Fisheries*, 5(2), pp.131–140.
- Labonne, M. et al., 2008. Otolith chemical signature and growth of *Chaetodon speculum* in coastal areas of New Caledonia. *Estuarine, Coastal and Shelf Science*, 78(3), pp.493–504.
- Matta, M.E. et al., 2013. Otolith oxygen isotopes measured by high-precision secondary ion mass spectrometry reflect life history of a yellowfin sole (*Limanda aspera*). *Rapid Communications in Mass Spectrometry*, 27(6), pp.691–699.
- McMahon, K.W., Hamady, L.L. & Thorrold, S.R., 2013. Ocean ecogeochemistry: a review. *Oceanography and Marine Biology: An Annual Review*, 51, pp.327–374.
- Meredith, M.P. et al., 1999. Distribution of oxygen isotopes in the water masses of Drake Passage and the South Atlantic. *Journal of Geophysical Research-Oceans*, 104(C9), pp.20949–20962.
- Miller, J.A. et al., 2011. Life history variation in upper Columbia River Chinook salmon (*Oncorhynchus tshawytscha*): a comparison using modern and ~500-year-old archaeological otoliths. *Canadian Journal of Fisheries and Aquatic Sciences*, 68, pp.603–617.
- Pais, I. & Jones Jr, J.B., 1997. *The Handbook of Trace Elements*, Boca Raton, Florida, USA: St Lucie Press.
- Palmer, M.R. & Edmond, J.M., 1989. The strontium isotope budget of the modern ocean. *Earth and Planetary Science Letters*, 92(1), pp.11–26.
- Paton, C. et al., 2011. Iolite: Freeware for the visualisation and processing of mass spectrometric data. *Journal of Analytical Atomic Spectrometry*, 26, p.2508.
- Petroleum Division & Geological Survey of Western Australia, 2014. *Western Australia's Petroleum and Geothermal Explorer's Guide 2014 Edition*,
- Popper, A.N. & Lu, Z., 2000. Structure-function relationships in fish otolith organs. *Fisheries Research*, 46, pp.15–25.
- QGIS Development Team, 2016. *QGIS Geographic Information System*.
- Reis-Santos, P., Tanner, S.E., Vasconcelos, R.P., et al., 2013. Connectivity between estuarine and coastal fish populations: Contributions of estuaries are not consistent over time. *Marine Ecology Progress Series*, 491, pp.177–186.

- Reis-Santos, P., Tanner, S.E., Elsdon, T.S., et al., 2013. Effects of temperature, salinity and water composition on otolith elemental incorporation of *Dicentrarchus labrax*. *Journal of Experimental Marine Biology and Ecology*, 446, pp.245–252.
- Reis-Santos, P. et al., 2012. Temporal variability in estuarine fish otolith elemental fingerprints: Implications for connectivity assessments. *Estuarine, Coastal and Shelf Science*, 112, pp.216–224.
- Rohling, E.J., 2013. Oxygen Isotope Composition of Seawater. In S. A. Elias & C. J. Mock, eds. *Encyclopedia of Quaternary Science*. Amsterdam: Elsevier Inc., pp. 915–922.
- Ryan, K.L. et al., 2015. State-wide survey of boat-based recreational fishing in Western Australia 2013/14.
- Saenz-Agudelo, P. et al., 2009. Estimating connectivity in marine populations: An empirical evaluation of assignment tests and parentage analysis under different gene flow scenarios. *Molecular Ecology*, 18(8), pp.1765–1776.
- Secor, D.H., Dean, J.M. & Laban, E.H., 1992. Otolith removal and preparation for microstructural examination. In D. K. Stevenson & S. E. Campana, eds. *Otolith Microstructure and Analysis*. Ottawa, Canada, pp. 19–57.
- Shiao, J.C. et al., 2014. Oxygen isotopic distribution along the otolith growth axis by secondary ion mass spectrometry: Applications for studying ontogenetic change in the depth inhabited by deep-sea fishes. *Deep-Sea Research Part I: Oceanographic Research Papers*, 84, pp.50–58.
- Spalding, M.D. et al., 2007. Marine ecoregions of the world: A bioregionalization of coastal and shelf areas. *BioScience*, 57(7), pp.573–583.
- Standish, J.D., Sheehy, M.S. & Warner, R.R., 2008. Use of otolith natal elemental signatures as natural tags to evaluate connectivity among open-coast fish populations. *Marine Ecology Progress Series*, 356, pp.259–268.
- Stanley, R.R.E. et al., 2016. Regional variation in otolith geochemistry of juvenile Atlantic cod (*Gadus morhua*) in coastal Newfoundland. *Canadian Journal of Fisheries and Aquatic Sciences*, 73, pp.1507–1519.
- Steer, M.A., Fowler, A.J. & Gillanders, B.M., 2009. Age-related movement patterns and population structuring in southern garfish, *Hyporhamphus melanochir*, inferred from otolith chemistry. *Fisheries Management and Ecology*, 16(4), pp.265–278.
- Sturrock, A.M. et al., 2012. Can otolith elemental chemistry retrospectively track migrations in fully marine fishes? *Journal of Fish Biology*, 81(2), pp.766–795.
- Swan, S.C. et al., 2003. Otolith microchemistry of *Nezumia aequalis* (Pisces: Macrouridae) from widely different habitats in the Atlantic and Mediterranean. *Journal of the Marine Biological Association of the UK*, 83(4), pp.883–886.
- Tanner, S.E. et al., 2012. Otolith geochemistry discriminates among estuarine nursery areas of *Solea solea* and *S. senegalensis* over time. *Marine Ecology Progress Series*, 452, pp.193–203.
- Thorrold, S.R. et al., 1997. Factors determining $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ fractionation in aragonitic otoliths of marine fish. *Geochimica et Cosmochimica Acta*, 61(14), pp.2909–2919.
- Thorrold, S.R. et al., 2001. Natal homing in a marine fish metapopulation. *Science*, 291(5502), pp.297–299.
- Thorrold, S.R. & Shuttleworth, S., 2000. In situ analysis of trace elements and isotope ratios in fish otoliths using laser ablation sector field inductively coupled plasma mass spectrometry. *Canadian Journal of Fisheries and Aquatic Sciences*, 57(6), pp.1232–1242.
- Vasconcelos, R.P. et al., 2008. Evidence of estuarine nursery origin of five coastal fish species along the Portuguese coast through otolith elemental fingerprints. *Estuarine, Coastal and Shelf Science*, 79(2), pp.317–327.
- Warner, R.R. et al., 2005. Natal trace-elemental signatures in the otoliths of an open-coast fish. *Limnology and Oceanography*, 50(5), pp.1529–1542.
- Welch, D.J. et al., 2009. Determination of management units for grey mackerel fisheries in northern Australia. Final Report, Fisheries Research and Development Corporation Project 2005/010, Fishing & Fisheries Research Centre Technical report No. 4, Townsville, Australia.
- Welch, D.J. et al., 2015. Integrating different approaches in the definition of biological stocks: A northern Australian multi-jurisdictional fisheries example using grey mackerel, *Scomberomorus semifasciatus*. *Marine Policy*, 55, pp.73–80.
- Wilson, B., 2014. Kimberley marine biota. History and environment. Records of the Western Australian Museum, Supplement, pp.001–018.
- Wilson, B., 2013. The Contemporary Physical Environment. In *The Biogeography of the Australian North West Shelf*. Elsevier, pp. 13–57.
- Zimmerman, C.E. et al., 2013. Species and life history affect the utility of otolith chemical composition for determining natal stream of origin for Pacific salmon. *Transactions of the American Fisheries Society*, 142(5), pp.1370–1380.

7 Acknowledgements

This work could not have been completed without the help of the laboratory staff at Fisheries WA who gave invaluable assistance with the preparation and sectioning of the otoliths; the staff of the Laser Ablation Facility, John de Laeter Centre, Curtin University who provided help and guidance with the trace element analysis and data processing, the staff at the Department of Earth Sciences who provided the strontium isotope analysis and the staff at the University of Adelaide who provided additional trace element analysis and data processing. Thanks must also go to the staff of the Centre for Microscopy, Characterisation and Analysis, UWA, for their help with the oxygen isotope analysis and the staff in the Electron Microscope Facility, John de Laeter Centre, Curtin University for their help with the preparation of otolith mounts. For cultural advice and permissions we thank the Kimberley Land Council and the Wunambal Gaambera, Dambimangari, Mayala, Bardi-Jawi, and Karajarri people. For field assistance, we thank Francis Woolagoodja, Danial Oades, Kevin George, Damon Pyke, Kevin Ejai, Azden Howard, Kevin Dougal, Tesha Stumpagee, Phillip McCarthy, Peter Hunter, Zac Ejai, Paul Davey, Trevor Sampey, Chris Sampey, Sandy Isaac, Alec Isaac, Craig Skepper, Grace Davis, Gabby Mitsopoulos, Fiona Webster, Todd Bond, Matt Birt, Tyrone Ridgway, and Nicole Ryan. Funding for Kimberley sampling was administered through the Western Australian Marine Science Institution (WAMSI) Kimberley Marine Research Program and the Department of Primary Industries and Regional Development (Fisheries; Government of Western Australia) Lalang-garram Camden Sound Marine Park Project. We thank Kelly Waples, Kim Friedman, and Stuart Field from WAMSI. Pilbara and Gascoyne sample collections were funded by the Chevron-operated Wheatstone Project's State Environmental Offsets Program and administered by the Department of Biodiversity, Conservation and Attractions. The Wheatstone Project is a joint venture between Australian subsidiaries of Chevron, Kuwait Foreign Petroleum Exploration Company (KUFPEC), Woodside Petroleum Limited and Kyushu Electric Power Company, together with PE Wheatstone Pty Ltd (part owned by TEPCO). The authors would like to acknowledge the contribution of an Australian Government Research Training Program Scholarship in supporting this research.

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.