Ecological Connectivity of Kimberley Marine Communities

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WAMSI Kimberley Marine Research Program
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WAMSI Kimberley Marine Research Program

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

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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)
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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 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 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.
- Ocean currents are likely to play a significant role in distributing the larvae of Stripey 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 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.
• 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


5.3 Submitted manuscripts


5.4 Presentations


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.


Jim Underwood (2016) Genomics of spawning corals in the Kimberley. AMSA snapchat


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). 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. 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-

2

Kimberley Marine Research Program | Project 1.1.3
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* though 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


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

<table>
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<tr>
<th>Key Questions</th>
<th>Informed Response</th>
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| **How do macro-tidal systems influence ecological connectivity of key taxa?** | 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.  
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.  
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. |
| **What is the extent of fine scale connectivity within and between coastal reefs (up to 100 km)?** | In the Kimberley, with the exception of T. niloticus, 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. |
| **What is the extent of larger scale connectivity within and between coastal and offshore reefs?** | Genetic subdivision (and hence some limitation to dispersal) was observed in all taxa with the exception of T. niloticus within the coastal Kimberley. 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. |
| **What are the dispersal distances of key taxa?** | 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). |
| **Are proposed management areas sufficient for ecological connectivity to support populations of key taxa?** | 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.  
The Montalivet Island group (where a putative genetic diversity hotspot is hypothesised to occur for I. bruggemanni) 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.  
T. niloticus 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.  
In the case of the targeted fish L. carponotatus, 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 L. carponotatus is supported by the observed genetic differentiation |
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

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WAMSI Kimberley Marine Research Program

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

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

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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 re-
circulating 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.](image-url)
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.

<table>
<thead>
<tr>
<th>Species</th>
<th>Sites</th>
<th>Individuals</th>
<th>Markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. aspera_C</td>
<td>14</td>
<td>388</td>
<td>2894</td>
</tr>
<tr>
<td>I. brueggemannii</td>
<td>17</td>
<td>1093</td>
<td>2125</td>
</tr>
<tr>
<td>T. hemprichii</td>
<td>17</td>
<td>749</td>
<td>16</td>
</tr>
<tr>
<td>H. ovalis</td>
<td>11</td>
<td>407</td>
<td>9</td>
</tr>
<tr>
<td>T. niloticus</td>
<td>17</td>
<td>514</td>
<td>5428</td>
</tr>
<tr>
<td>P. milleri</td>
<td>28</td>
<td>842</td>
<td>4472</td>
</tr>
<tr>
<td>L. carponotatus</td>
<td>53</td>
<td>1016</td>
<td>4468</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>157</strong></td>
<td><strong>5009</strong></td>
<td><strong>19412</strong></td>
</tr>
</tbody>
</table>
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:

- **Fine scale: The extent of connectivity differs among species**
- **Fine scale: Population boundaries are shared between some taxa**
- **Fine scale: Some sites act as links between otherwise isolated regions**
- **Fine scale: King Sound and Sunday Strait are barriers to dispersal in some species**
- **Broad scale: the inshore and offshore Kimberley are poorly connected**
- **Broad scale: connectivity between the Kimberley and neighbouring bioregions differs among species**
- **Broad scale: Genetic diversity is distributed differently in each species**
- **Significant cryptic genetic diversity was detected in the broadcast spawning coral**

![Figure 4. Key findings of KMRP Ecological Connectivity Project 1.1.3.](image)

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. bruggemannii* 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. brueggegmanni* 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*. 
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):

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).
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


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

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WAMSI Kimberley Marine Research Program

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

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Front cover images (L-R)

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

Image 2: Pink Seriatopora hystrix nestled into Isopora brueggemannii; 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 brueggemannii, a brooding reef-builder in the intertidal zone of Irvine Island. (Image: Jim Underwood)
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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 Peninsular. 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.
1 Introduction

The coral reef systems of the Kimberley in Northwest Australia (NWA) are diverse, unique, and understudied. They are also among the least impacted coral reefs on the planet (Halpern et al. 2008), and are characterised by extensive reef development, a diversity of reef forms of varied geological origin, and are regarded as a major center of coral biodiversity at the southern margin of the East Indies Coral Triangle (Wilson 2013). Macrotides (up to 12m) combine with complex geomorphology to create complicated hydrodynamics that could either be strong conduits, or barriers, to dispersal of larvae among coral populations. These reefs are also characterised by extreme physical conditions, including large variations in turbidity, nutrient concentrations and temperature. Further, physical evidence suggests that coral reefs of the inshore Kimberley appear to be relatively isolated from neighbouring coral systems; the major currents capable of transporting larvae long distances in this region occur on the continental shelf margin and do not intrude far into the shelf and inshore waters (D’Adamo et al. 2009). Theory predicts reproductive isolation of populations brought about by strong selection pressures from such environmental heterogeneity and physical isolation is conducive to the development of unique patterns of inter- and intra-specific genetic diversity and structure (Felsenstein 1976). A recent study in the Kimberley coast supports such expectations, recording seven new records for coral species in WA and expanding species distributions beyond expected zonation (Richards et al. 2015).

Coral reefs throughout the world are declining rapidly due to increases in sea-water temperatures, ocean acidification, and local anthropogenic disturbances (Hoegh-Guldberg et al. 2007, Hoey et al. 2016), with one third of all reef building corals facing a high risk of extinction (Carpenter et al. 2008). The recent mass bleaching event at Scott Reef (offshore Kimberley) and the inshore reefs of the southern Kimberley in 2016 (Gilmour pers comm), highlights that the worldwide call to conserve coral ecosystems in the face of climate change (see Jones et al. 2016) is especially pertinent in NWA. To manage coral reefs and enhance their resilience, a range of strategies must be employed in tandem, and includes effective spatial planning of Marine Protected Areas (MPAs) and/or Indigenous Protected Areas (IPAs) (McCook et al. 2010, Lamb et al. 2015, Mellin et al. 2016). To provide an empirical basis for a regional network of Kimberley marine parks and reserves, and to inform risk assessments and impact assessments, it is crucial to know where new recruits in a reserve originate from and where larvae from that area disperse to (Kendrick et al. 2016). These patterns of immigration and emigration, or “population connectivity”, are key ecological drivers of population maintenance and recovery after disturbance. Indeed, the current lack of knowledge about the way populations are connected, along with the inability of management agencies to effectively implement the knowledge that is available, is a major impediment to management of marine systems throughout the world (Magris et al. 2014). Because genetic divergence among individuals and populations arises when interbreeding is restricted, a spatial analysis of genetic structure is a pivotal method that provides insights into the degree of connectivity among those individuals and populations over generation by generation time scales.

Patterns of genetic diversity and structure are also required to understand the stability of populations over multigenerational time scales, because adaptation to a changing environment depends on the amount and configuration of standing genetic variation that is available. Further, populations, communities and ecosystems with high genetic diversity are generally resilient to disturbance (Hughes et al. 2008). Therefore, genetic studies that accurately describe evolutionary significant units (reproductively isolated lineages) and how genetic diversity is partitioned among populations, are crucial to management efforts that are intended to protect a representative range of the biodiversity and understand the processes that sustain that diversity (Moritz 2002, Bowen and Roman 2005, Pante et al. 2015). For corals, it is notoriously difficult to resolve species boundaries due to a combination of morphological plasticity and interbreeding (Willis 1990, Miller and Benzie 1997, van Oppen et al. 2001, Willis et al. 2006, Ladner and Palumbi 2012, Ohki et al. 2015, Richards and Hobbs 2015, Rosser 2015, Gilmour et al. 2016b, Richards et al. 2016), and the relationship between morphology and genetics is certainly less well understood for corals of the Kimberley coast than in other reef systems, with concomitant effects for management (Underwood et al. 2013).
Hard corals create the crucial three dimensional structures that provide habitat and protection for many other coral reef species, and research is required to elucidate patterns of genetic diversity and population connectivity within species in these crucial habitat forming organisms. This study utilises single nucleotide polymorphisms (SNPS), isolated across the coral genome to assess the genetic structure and diversity for the brooding hard coral, *Isopora brueggemanni*, and the broadcast spawning coral, *Acropora aspera*, over broad scales (inter-region; tens to hundreds of kilometres), intermediate scales (inter-reef; kilometres to tens of kilometres) and fine scales (within-reef; hundreds of metres) in the Kimberley. The genetic patterns are compared to oceanographic models to elucidate more general biophysical processes influencing patterns of connectivity for coral reef species in this region.

## 2 Materials and Methods

### 2.1 Study species

*Isopora brueggemanni* is one of the most easily identifiable corals within the family Acroporiidae. In the Kimberley, it occurs in shallow (< 20 m) water, especially on exposed upper reef slopes and sand flats, and is abundant and widespread on inshore and offshore reefs. Unlike the majority of species on NWA coral reefs, *I. brueggemanni*’s sexual reproductive mode involves release of sperm into the water column and fertilisation of eggs within the polyp, although self-fertilisation has also been reported (Okubo et al. 2007). The resulting larvae are brooded within the polyp and then released at an advanced developmental stage. Planula release is likely to be extended over several months through spring to autumn in the Kimberley (Gilmour et al. 2016a), but the exact timing is not currently known in this region, as in other parts of the world (Okubo et al. 2007). Similar to other brooders, larvae are probably capable of settling within hours of release (minimum competency period) (Harrison and Wallace 1990). Brooding hard corals are generally characterised by strong levels of genetic subdivision, and self-seeding is well established (Ayre and Dufty 1994, Ayre and Hughes 2000, Underwood et al. 2007, 2009, van Oppen et al. 2011, Starger et al. 2013). However, brooded planulae are relatively large when released into the water column and some contain maternal zooxanthellae, and therefore appear to be provisioned for a long maximum competency period (Harrison and Wallace 1990). Thus, dispersal is likely to be bimodal, with long-distance dispersal (teleplanic) occasionally supplementing more routine, philopatric dispersal or self-seeding. The success of either strategy is likely to depend on environmental and demographic conditions: stable healthy populations are expected to be maintained by locally derived recruits, while recovery after disturbance is expected to be initiated from input of exogenous larvae followed by local recruitment. Both strategies can also be supplemented by asexual reproduction, via vegetative fragmentation in branching corals, which is likely to be more common in exposed, platform habitats. *I. brueggemanni* is listed as “vulnerable” on the IUCN Red List of Threatened Species (Richards et al. 2008).

*Acropora aspera* is widely distributed, and is found in the northern Indian Ocean, the central Indo-Pacific, Australia, Japan and the East China Sea, and the oceanic west Pacific. In Western Australia, *Acropora aspera* occurs from the oceanic shoals in the north to the Abrolhos Islands in the south, but is most abundant at inshore reefs in the Kimberley and Pilbara. *Acropora aspera* is a broadcast spawner, whereby eggs and sperm are released during mass spawning events, after which fertilisation and larval development occur in the plankton. Data on spawning time of *A. aspera* in the Kimberley is sparse, but a primary spawning most likely occurs in the Austral autumn, and there may be a secondary spawning in spring (Gilmour et al. 2016a). In contrast to brooded larvae, broadcast spawned larvae must spend a few days in the plankton before they are competent to settle, and thus have weaker potential for self-seeding compared with brooding corals. However, if suitable substrate is available, the majority of larvae probably settle as soon as they are competent, as they are less well provisioned for teleplanic dispersal than brooded larvae and their probability of settling and surviving drops rapidly the longer they spend in the plankton (Harrison and Wallace 1990, Baird 2004). Like *I. brueggemanni*, asexual reproduction via vegetative fragmentation may well supplement the broadcast spawning reproduction particularly in exposed, platform habitats. *A. aspera* is considered to be relatively easily identified in the field although at some locations it can be confused with *Acropora pulchra* (Richards
2.2 Sampling design

A total of 612 *I. brueggemanni* corals were sampled from 18 sites (between 20 and 60 samples per site) along the Kimberley coast, including the reef systems of the Dampier Peninsula, Buccaneer Archipelago, the central Kimberley, and Ashmore Reef (Fig. 1). A total of 563 corals identified in the field as *A. aspera* were sampled from 16 sites (between 24 and 83 colonies per site) along the Kimberley coast and Ashmore Reef (Fig. 2). Both species were sampled at ten of these sites. Samples were collected by walking on exposed platforms at spring low tides and removing one centimetre fragments from coral colonies. Fragments were placed in 100% ethanol prior to DNA extraction in the laboratory. Photographs were taken of colonies representing common morphologies, along with the collection of voucher specimens for taxonomic identification. Colonies were separated by at least 1.5 metres to reduce the likelihood of collecting clone mates that were the product of fragmentation, and each site spanned no more than 500 metres along the reef flat. In addition to the broad scale collection, we sampled also at an intermediate and fine scale. The intermediate scale sampled multiple reefs separated by kilometres to tens of kilometres through detailed collections from the Buccaneer Archipelago and the Dampier Peninsula in the west of the Kimberley. The fine scale sampling within reefs recorded the location of each colony with GPS for both species, and for the brooding coral, we also sampled a second subsite (separated from the first subsite by ~500 metres) within the Buccaneer Archipelago at three sites (Fig. 1).

![Map of *Isopora brueggemanni* collections from the inshore Kimberley and Ashmore Reef in North West Australia. Insert shows the detailed collections from the Dampier Peninsula (black text) and the Buccaneer Archipelago (blue text).](image-url)
2.3 DNA extractions and DArTseq development of SNPS

Genomic DNA was extracted using a salting out protocol modified from Cawthorn et al. (2011) and purified with Zymo Plate filter plates (Zymo-Spin I-96). Genome-wide single nucleotide polymorphism (SNP) data were generated using a next generation sequencing platform following the DArTseq protocol (Diversity Arrays Technology; Appendix 1), 195 *Isopora* samples and 126 *Acropora* samples were genotyped twice as technical replicates and scoring consistency was used as the main selection criteria for high quality/low error rate markers, and loci with reproducibility less than 0.94 were excluded. The call quality of the initial SNP data set was further assured by setting a cut-off of read depth per locus (coverage) < 7, call rate >0.35, minimum allele frequency >0.00075 for *Isopora* and >0.0017 for *Acropora*. Sequences were blasted on GenBank to check for general contamination and endosymbionts including genomes and transcriptomes of the symbiotic zooxanthellae, *Symbiodinium*, which lives in coral host tissue. No sequences aligned to the *Symbiodinium* genome for *Isopora*, while four sequences from *Acropora* aligned to the *Symbiodinium* genome with E-values between $1.36 \times 10^{-19}$ and $2.73 \times 10^{-27}$ and these were removed from downstream analysis. The primary data set comprised 23,165 SNPS for *I. brueggemanni* and 34,304 SNPS for *A. aspera*.

2.4 Initial quality control, identification of clone and genomic summary statistics

For *I. brueggemanni*, we used adegenet (Jombart 2008) and a custom R script to filter the primary data set at the following levels; call rate > 0.95, coverage > 20, minimum allele frequency >0.05, max heterozygosity <0.75 (graphical summaries in Appendix 2, Fig. A1). In addition, we utilised the reproducibility statistic (calculated from the 195 technical replicates) to filter out all loci with < 0.99 correct calls across individuals. This filtering resulted in 2,946 loci, to which we then filtered out loci that exhibited significant Hardy Weinberg and linkage disequilibrium using custom R scripts and the R packages SNPassoc (Gonzalez et al. 2007), adegenet and pegas (Gonzalez et al. 2007, Paradis 2010). Both Hardy-Weinberg and linkage disequilibrium tests were carried out...
separately for each sampling site (n=21). For Hardy-Weinberg disequilibrium, we removed 133 loci that showed departures from expectations at P < 0.05 in five or more of the 21 sites. For linkage disequilibrium we removed 681 loci with r values > 0.8 among five or more sites. Six individuals with greater than 15% missing data were removed. 2,132 SNPS remained, to which we identified loci under directional selection with OutFLANK v0.1 (Whitlock and Lotterhos 2015) using with 5% left and right trim for the null distribution of Fst, minimum heterozygosity for loci of 0.1, and a 5% false discovery rate (q value). Initial analysis using the entire data set did not detect any outliers, but when OutFLANK v0.1 (Whitlock and Lotterhos 2015) was applied to the inshore data only, seven loci were identified as outliers and were removed from subsequent analyses.

For A. aspera, initial investigations during the SNP development indicated the presence of multiple genetic lineages which were characterised by morphologically cryptic but major genetic divergences among colonies living in sympathy. From the primary data set of 34,304 SNPS, we filtered the data at a highly stringent level (call rate > 0.95, coverage > 20, minimum allele frequency >0.05, max heterozygosity <0.75). In addition, we utilised the reproducibility statistic (calculated from the 126 technical replicates) to filter out all loci with < 0.999 correct calls across individuals (APPENDIX 3, Fig. A1). This extremely stringent filter provided very reliable calls for all samples, and mitigated interference of genotyping (e.g. null alleles) brought about by differences in the target sequences among genetic groups. The result was 585 SNPS that could accurately ascertain the levels of divergence among genetic lineages. We did not filter for Hardy Weinberg or gametic-phase disequilibrium at this stage of the analysis because large (potentially interspecific) divergence would be associated with such disequilibrium, and removal of such markers would likely limit power of the analyses. Seven individuals with greater than 15% missing data were removed.

Four highly divergent lineages were identified in the A. aspera collection that occurred in sympathy and exhibited genetic cohesion among geographically distant populations. Therefore, a targeted re-analysis based on the most common and widespread lineage (Acropora asp-c) was required for population genetic analysis at the intra-specific level. To this end, we re-calculated the descriptive statistics across all SNP loci for those samples identified as Acropora asp-c with the same filters and methods as for the entire A. aspera collection except we relaxed the reproducibility (> 0.98) and call rate (> 0.90) thresholds (Appendix 3, Fig. A2). This filtering resulted in 3,472 loci, to which we then filtered out loci that exhibited significant departures from Hardy Weinberg equilibrium gametic-phase equilibrium as in the I. brueggemanni QC analysis, but because of smaller sample sizes, disequilibrium testing was carried out separately for each sampling site only for those sites with more than 15 samples (N = 5). For Hardy-Weinberg testing, we removed 343 loci that showed departures from expectations at P < 0.05 in three or more (out of five) sites. For gametic-phase disequilibrium, we removed 294 loci with r values > 0.8 among three or more sites. 2,898 SNPS remained, to which we identified loci under directional selection with OutFLANK using the same parameters as for the I. brueggemanni analysis. Four loci were identified as outliers and these were removed from subsequent analyses.

To establish whether colonies were clone mates, we used the technical replicates (n = 195 for I. brueggemanni, and n = 126 for A. aspera) to determine a threshold of maximum genetic distance (based on hamming distance) between the two genotypes of each repeat pair, and identified clones (ramets) as samples with genotypic distance below this threshold. For colonies that were identified as ramets, all but one individual was removed from the data, yielding a final data set comprising individual genets. Genotypic richness was calculated as the ratio of number of genets to total number of samples (ramets). Summary statistics of the final data sets were calculated in GenAlEx v6.5 (Peakall and Smouse 2006) and included; number of positive calls (N), number of alleles (Na), observed heterozygosity (H0), unbiased expected heterozygosity (He), and fixation index (Fs) at each site and averaged across sites (± SE).

2.5 Broad and intermediate scale genetic structure

To explore the broad scale of geographic structure in I. brueggemanni, we used the Bayesian software STRUCTURE v2.3 (Pritchard et al. 2000) to estimate membership coefficients (q) of each individual colony to each cluster for a range of populations and identify the optimal number of genetic clusters (K). Initial
exploration of the data used the correlated and independent allele frequency model, both without (NOPRIOR) and with (LOCPRIOR) information on sampling location of colonies. The results of the correlated and independent allele frequency models were extremely congruent in identifying the most appropriate K as well as individual membership to clusters, but correlated allele frequency model and the LOCPRIOR model resolved the data with the most clarity and produced the highest ΔK values, so we only present those results. Mean and variance of log likelihoods and posterior probabilities of the number of clusters from K = 1 to 10 were inferred from 20 independent runs using the admixture models with burn in of 100,000 and then 500,000 MCMC repetitions. All other parameters were default values. Convergence of algorithms was checked by assessing the variability in individual assignment proportions across runs, and the similarity score calculated with the online program CLUMPACK (Kopelman et al. 2015). STRUCTURE runs were performed on the CSIRO Accelerator Cluster "Bragg", which consists of 128 Dual Xeon 8-core E5-2650 computer nodes. CLUMPACK was used to summarise and graphically present the STRUCTURE results as well as to calculate the most appropriate K using the ΔK method of Evanno et al. (2005). When deciding on the most appropriate K, we considered biological interpretations for a range of K values, and chose the K which best addressed our a priori questions and expectations (see Pritchard and Wen 2003, Meirmans 2015). Further, when highly divergent samples were detected, we ran STRUCTURE excluding those samples to ascertain whether they interfered with clustering among the more genetically coherent samples, and thus our ability to describe patterns of genetic structure at intermediate (inter-reef) scales.

To provide an alternative measure of number and membership of major clusters to the Bayesian analyses, we also explored the genetic relationships among all I. brueggemanni individuals with a principal coordinate analysis (PCoA) in GenAlEx. PCoA uses is a simple multi-ordination calculated from a codominant genotypic distance among pairs of samples and does not take into account any a priori information such as sampling location or assumptions of equilibrium, and thus provides a complimentary analysis to the sophisticated Bayesian approach of STRUCTURE. As with the STRUCTURE analysis, to tease out genetic relationships among corals at intermediate and fine scales which were obscured by highly divergent samples, PCoA was repeated excluding those samples. The PCoA was performed using the standardised distance option in GenAlEx.

To estimate the amount of genetic variation that was partitioned among geographic locations in the I. brueggemanni collection at a hierarchy of scales, we conducted an hierarchical AMOVA in GenAlEx with the traditional fixation index of genetic subdivision (FST). This analysis measured variation among the four systems of Ashmore, central Kimberley, Buccaneer Archipelago and Dampier Peninsula (FRT), among sites within systems (FSR), and among all sites (FR). As part of this AMOVA, pairwise FST between all sites was calculated, and tests for statistical significance of all analyses were based on 999 random permutations.

For A. aspera, an initial cluster analysis was performed with STRUCTURE and PCoA (model conditions were the same as for I. brueggemanni but with no a priori information on sampling location in the STRUCTURE runs). This analysis identified four divergent lineages (see results). To gauge the magnitude of divergence among these lineages, we calculated pairwise FST between lineages in GenAlEx, testing for statistical significance with 999 random permutations. Lineages were subsequently deemed reproductively isolated, which meant that a re-analysis of the most common and widespread lineage (Acropora asp-c) was required for inference of intra-specific population connectivity in the broadcast spawning coral.

To this end, we applied the same methods using STRUCTURE, PCoA and AMOVA as for I. brueggemanni to describe genetic structure over multiple geographic scales within the Acropora asp-c lineage. Model runs with and without prior information of sampling location were highly congruent, as were runs using correlated and independent allele frequencies, but correlated allele frequency model and the LOCPRIOR model resolved the data with the most clarity and produced the highest ΔK values so we only present those results. For the AMOVA, some sites had small sample sizes, but because we employed thousands of SNPs, estimation of FST for samples sizes > 4 is likely to be robust (Willing et al. 2012). However, to substantiate this expectation, we also calculated an AMOVA only using those sites where n ≥ 9.
2.6 Fine scale patterns of genetic structure

To explore patterns of fine scale genetic structure and infer routine dispersal distances in *I. brueggemanni* and within the *Acropora* Asp-c lineage, we performed a spatial autocorrelation analysis on the Dampier Peninsula and Buccaneer Archipelago collections that were sampled in most detail in the inshore Kimberley. Spatial autocorrelation utilises the spatial position and genetic identity of each individual coral, and is well suited to establishing the finest scale of genetic structure that is sensitive to recent dispersal processes (Double et al. 2005, Epperson 2005). An autocorrelation was calculated between the genetic distance (codominant genotypic) and geographic (Euclidean) distance of all pairs of individuals that fell within a given distance class, and each autocorrelation coefficient, *r*, was plotted with respect to its given distance class in GenAlEx. Under conditions of restricted gene flow, the autocorrelation coefficient is expected to be positive at short distance classes, and will subsequently decline through zero and become negative at larger distance classes (Sokal and Wartenberg 1983, Epperson and Li 1996, Smouse and Peakall 1999). For *I. brueggemanni*, initial analysis showed that Kooljaman was a clear outlier to the general patterns of spatial genetic structure, and so was excluded from this analysis, which also provided comparability with the study of *A. aspera*. To test for statistical significance of *r* at each distance class, a 95% confidence interval about *r* was generated via 1000 bootstrap trials by drawing (with replacement) from within the set of pairwise comparisons for a specific distance class, and when this interval did not straddle *r* = 0, significant spatial genetic structure was inferred.

In addition to spatial autocorrelation, because we sampled *I. brueggemanni* at level of sub-site at three sites (Bathurst, Pope and Mermaid Islands), we estimated genetic variation among these sites (*F<sub>RT_SITES</sub>), between sub-sites within sites (*F<sub>SR_SUBSITES</sub>), and among all sub-sites (*F<sub>ST_SUBSITES</sub>). As part of this AMOVA, pairwise *F<sub>ST</sub>* between all sub-sites was calculated. Tests for statistical significance were based on 999 random permutations.

2.7 Oceanographic modelling

To estimate the potential for transport of larvae via oceanographic currents among inshore and offshore Kimberley sites, we used a biophysical dispersal model based on Regional Ocean Modelling System with 2 km resolution. The model was nested within the Ocean Forecasting for Australia Model 3 (OFAM3) simulation (Feng et al. 2016) and forced by 3-hourly meteorological measures derived from Kobayashi et al. (2015). The model simulation was based on data from 2011. Hourly sea surface current velocities (0-5 m) were extracted from the model output and used for particle tracking modelling. A 4th-order Runge-Kutta sub-time-stepping scheme was used to update the particle locations every hour (Feng et al. 2010). For *I. brueggemanni*, total of 100 particles were seeded in sampling sites during austral spring-summer-autumn period (September-May), at 3-day intervals. This particle release period represents the season of planulation of *I. brueggemanni* based on field observations that identified gametes and planula larvae in all stages of development during these months (Gilmour et al. 2016a). Using the random walk effect of 1 m2s-1, particles were tracked for two time periods that represented our best estimates of the common competency window for brooding corals (zero to eight days). For *A. aspera*, a total of 100 particles were seeded in sampling sites during predicted time of the main mass spawning in the austral autumn of 2011, which most likely occurred on March 28 (seven to nine nights after the full moon). Because the exact timing of spawning of *Acropora* corals is not known in the Kimberley region, and spawning varies on nearby oceanic atolls by several days (Gilmour et al. 2016a), we also released particles three days before, and three days after the 28<sup>th</sup> of March. Particles were tracked from day three to day eight. These days represented our best estimates of the common competency window of the majority of larvae based on field observations of Acroporans on offshore NWA reefs (Gilmour et al. 2009) and from laboratory studies on the closely related *Acropora pulchra* (Baird 2004). 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 during the competency period. To make this matrix symmetric we summed connectivity between i and j and j and i. Oceanographic connectivity was calculated as the proportion of released particles from i and j that settled at i and j. This value was converted to an oceanic resistance as 1 - oceanographic connectivity. Values were arcsine transformed.
before further analysis. To graphically depict the oceanographic results at the regional scale, we used custom R scripts to produce plots of particle tracks run over eight and 40 days in each austral season of 2011 and incorporated a broader range of sites compared with the genetic collections for corals including several inshore sites and also Scott Reef.

2.8 Oceanographic, geographic and genetic distance

To explore whether genetic structure can be explained by oceanographic and/or geographic distance, we compared the pairwise \( F_{ST} \) (linearised) matrix to the oceanographic resistance matrix and geographic distance (Euclidean) matrix using a simple paired Mantel test in GenAlEx. For \( I. brueggemanni \), initial analysis showed that Kooljaman was a clear outlier to the general patterns, and so was excluded from this analysis, which also provided comparability with the study of \( A. aspera \). Tests for statistical significance of correlation coefficient were based on 999 random permutations.

3 Results: Isopora brueggemanni

3.1 Identification of clones, genotypic richness and gene diversity in \( I. brueggemanni \)

For \( I. brueggemanni \), all technical replicates exhibited a hamming distance of \( \leq 0.02 \), and thus pairs of samples in the complete data set that fell below this threshold were deemed clones (ramets). All ramets of each near-identical genotype were collected at the same site, and the majority were sampled within a few metres of each other reinforcing their clonal origin through fragmentation. Only one genotype of each ramet was retained in the final data set, resulting in the removal of 45 samples.

Genotypic richness (the ratio of number of genets to ramets) was generally high, with an average of 0.93 across all sites (Table 1). Nine sites were comprised entirely of unique genets, although one site (Hedley Island) had a much lower genotypic richness than all other sites (0.39; Table 1). Average observed heterozygosity was 0.176, average expected heterozygosity was 0.173, and average \( F_{IS} \) was -0.069 across all loci (genotypes and summary statistics are given digital data repository; http://catalogue.aodn.org.au/geonetwork/srv/eng/metadata.show?uuid=fb1d80bf-6ef2-4150-9479-22b4240435a7). Gene diversity, as measured by expected heterozygosity, was relatively constant over most of the sampling sites with two exceptions; West Montalivet in the far east had the highest gene diversity and Kooljaman in the far west had the lowest diversity (Fig. 2). These two sites created a general trend of declining gene diversity with increasing latitude (\( r^2 = 0.213 \)).

3.2 Genetic structure among regions, systems and reefs in \( I. brueggemanni \)

The STRUCTURE analysis of the \( I. brueggemanni \) collection revealed distinct genetic clusters among geographically separate (allopatric) populations at a hierarchy of spatial scales and no evidence of cryptic divergence among sympatric corals. The Evanno et al. method indicated highest level of structure at \( K = 2 \) (APPENDIX 2 Fig. A2), with membership coefficients (\( q \)) of 100% of colonies to either the Ashmore cluster, or the inshore Kimberley cluster at all sites, with the exception of the central Kimberley site of West Montalivet which was admixed (with the majority of \( q \leq 50% \) to either cluster; Fig. 3). However, at \( K > 2 \), clusters continued to segregate according to geography with an additional cluster formed by Kooljaman (with \( q = 100% \)) at \( K = 3 \), and a fourth cluster formed by the Dampier Peninsula sites at \( K = 4 \) (with \( q > 70% \); Fig. 3). Furthermore, at \( K = 4 \), the sites of Tide Rip and Mermaid Island\_1 exhibited admixed membership to the Dampier and the Buccaneer clusters either within the individual (at Tide Rip, \( q \sim 50% \) in all individuals), or among individuals (at Mermaid Island\_1, \( q \sim 75% \) to Dampier cluster in 20 individuals, and \( q > 80% \) to the Buccaneer cluster in nine out of the remaining 10 individuals; Fig. 3). At \( K = 5 \), a cluster at Pope Island segregated, and the sites of Irvine and Bathurst\_W segregated at \( K = 6 \), while at \( K > 6 \), the corals from West Montalivet formed their own cluster separate from the Ashmore cluster (APPENDIX 2 Fig. A3).
Table 1 Numbers of samples minus missing data (N), number of genets (Ng) and genotypic richness (Ng:N) in *Isopora brueggemannii* collected from Ashmore reef and sites throughout the inshore Kimberley coast in North West Australia.

<table>
<thead>
<tr>
<th>Region</th>
<th>SITE</th>
<th>N</th>
<th>Ng</th>
<th>Ng:N</th>
</tr>
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<tbody>
<tr>
<td>Ashmore</td>
<td>Ashmore Reef</td>
<td>29</td>
<td>29</td>
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<tr>
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<td>West_Montalivet</td>
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<td>25</td>
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<tr>
<td></td>
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<td>11</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>Irvine_I.</td>
<td>27</td>
<td>27</td>
<td>1.00</td>
</tr>
<tr>
<td>Buccaneer Archipelago</td>
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<td>28</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Bathhurst_W_2</td>
<td>20</td>
<td>20</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Longitude_I.</td>
<td>29</td>
<td>29</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Frazer_I.</td>
<td>31</td>
<td>30</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
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<td>29</td>
<td>27</td>
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</tr>
<tr>
<td></td>
<td>Ashhlyn_I.</td>
<td>31</td>
<td>30</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>Pope_I._1</td>
<td>31</td>
<td>30</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>Pope_I._2</td>
<td>31</td>
<td>30</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
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</tr>
<tr>
<td></td>
<td>Mermaid_I._1</td>
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</tr>
<tr>
<td></td>
<td>Mermaid_I._2</td>
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<td>26</td>
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<tr>
<td>Dampier Peninsula</td>
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<td>26</td>
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<tr>
<td></td>
<td>Ngoorroodoool</td>
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<td>1.00</td>
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<td></td>
<td>Jalan</td>
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<td>1.00</td>
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<td>0.93</td>
</tr>
<tr>
<td></td>
<td>Ardinoogoon</td>
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<tr>
<td></td>
<td>Kooljaman</td>
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<tr>
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<td><strong>606</strong></td>
<td><strong>561</strong></td>
<td></td>
<td><strong>0.93</strong></td>
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</table>
Fig. 2 Estimates of gene diversity based on expected heterozygosity (± standard errors) averaged across loci at all sites for *I. brueggemanni*. Sites are colour coded with Ashmore in orange, central Kimberley in light orange, the Buccaneer Archipelago in blue, and the Dampier Peninsula sites in green. A trend line with $r^2 = 0.213$ is shown.

The results of the PCoA of *I. brueggemanni* were congruent with the STRUCTURE analysis in which the geographic structure was evident over multiple scales. At the broad scale, three separate clusters were associated with samples from Ashmore Reef, a main inshore cluster and Kooljaman, with West Montalivet samples intermediate to Ashmore and main inshore cluster (Fig. 4). Further, the PCoA that focused on the intermediate scale at the southern inshore sites corresponded closely with STRUCTURE results for $K = 4$ (Fig. 3) in which two clusters segregated into the Buccaneer Archipelago and Dampier Peninsula sites, but with most of the samples from Tide-Rip and Mermaid Island intermediate to the Buccaneer and Dampier clusters. Lastly, consistent fine scale patterns were identified in the PCoA and STRUCTURE analysis, with the same nine colonies from Mermaid Island_2 exhibiting strong genetic affinities with the Pope Island corals (Fig. 3 and 5).

The AMOVA also revealed strong geographic structure across broad and intermediate scales. Large and significant variation was attributed to differences among systems ($F_{ST} = 0.151$, $P < 0.001$) and among sites within regions ($F_{SR} = 0.092$, $P < 0.001$), yielding a large overall level subdivision among all sites ($F_{ST} = 0.230$, $P < 0.001$). Average pairwise $F_{ST}$ between Ashmore Reef and West Montalivet was 0.227, but averaged 0.450 (±SE 0.015) with all inshore reefs, and between Kooljaman and the other inshore sites was 0.241 (±SE 0.019) (Appendix 2; Table A1).
Fig. 3 Barplots of membership coefficients of individual corals from *I. brueggemanni* to different clusters calculated in STRUCTURE with the LOCPRIOR model for K = 2 to 4. These are the major mode plot produced by CLUMPAK calculated from 20/20 runs for K = 2 to 5, and 17/20 runs for K = 6. Similarity score = 0.999 and mean (LnProb) = -710322.065 for K = 2, similarity score = 0.991 and mean (LnProb) = -694772.740 for K = 3, similarity score = 0.985 and mean (LnProb) = -682167.990 for K= 4.

Fig. 4 Principal Coordinates Analysis (PCoA) calculated from individual pairwise genotypic distance of the entire *I. brueggemanni* collection. Individuals are colour coded according to location, with Ashmore Reef represented by diamonds, central Kimberley by squares, the Buccaneer Archipelago by circles and Dampier Peninsula by triangles. Percentage of variation explained by each axis is given in brackets.
3.3 Genetic structure within reefs in *I. brueggemanni*

Results from the analysis that focused on the patterns of genetic structure within the Dampier Peninsula and Buccaneer Archipelago showed significant structure over fine scales. The spatial autocorrelation analysis yielded a large and significant autocorrelation coefficient ($r \sim 0.15$) and relatively constant up to distances of 500 m, and then decreased with distance (Fig. 6). This distance indicates the extent of the genetic patch in which the homogenising influence of localised recruitment on relatedness among individuals first becomes limited. The autocorrelation coefficient crossed the x-axis and becomes negative at just over 20 km, which is the distance at which the random effects of genetic drift, not gene flow, drive genetic relatedness. Results of the AMOVA of the three sites that included replicate subsites that were separated by over 20 km showed that the majority of variation was attributed to significant subdivision among sites ($F_{RT\_SITES} = 0.085$, $P \leq 0.001$), but significant subdivision was also detected between subsites within sites ($F_{SR\_SUBSITES} = 0.010$, $P \leq 0.01$) over distances of about 500m. Pairwise $F_{ST}$ comparisons indicated the significant subdivision between sub-sites was driven by Pope Island ($F_{ST} = 0.013$, $P \leq 0.030$) and Mermaid Island ($F_{ST} = 0.014$, $P \leq 0.020$), but not Bathurst West ($F_{ST} = 0.002$, $P \leq 0.226$).
4 Results: Acropora aspera

4.1 Clonality, gene diversity and major genetic lineages in the entire A. aspera collection

All technical replicates from the entire Acropora aspera collection exhibited a hamming distance of ≤ 0.005, and therefore individuals with a hamming distance of less than this were deemed clones (ramets), and resulted in the removal of about one third (n = 207) of these samples. All ramets of each genotype were collected at the same site, the majority of which were sampled within ten metres of each other. Genotypes and summary statistics of the entire Acropora aspera collection are given digital data repository (http://catalogue.aodn.org.au/geonetwork/srv/eng/metadata.show?uuid=fb1d80bf-6ef2-4150-9479-22b4240435a7).

The results of the cluster analysis of the remaining 349 genets revealed major genetic lineages that were living side by side and only weakly associated with geography. Using the Evanno et al. (2005) method indicated that K = 3 was the most appropriate number of discrete populations for this data, but there was also evidence (i.e. high ΔK) for four clusters at K= 4 (Appendix 3 Fig A3). Moreover, at K = 3, the barplots indicated a fourth group with intermediate ancestry (membership coefficients, q ~ 50% to the blue and green clusters) while at K = 4, this group segregated into a separate cluster with high membership coefficients (q > 0.90; Fig. 7), suggesting this is the most appropriate K. Congruently, the results of the PCoA revealed four distinct genetic clusters within the entire collection of A. aspera (Fig. 8). Some geographic pattern in the distribution of the clusters was apparent with the mainland sites on the Dampier Peninsula of Noyon and Ardinoogoon dominated by Acropora asp-b, the island sites of the Buccaneer Archipelago dominated by Acropora asp-c and the central Kimberley sites of White and Condilac Islands dominated by Acropora asp-d (Fig. 7). However at almost all sites, multiple clusters occur at the same site; for example, all four clusters occur at the central Kimberley site of Condilac Island, while at Ashmore Reef, the three clusters of asp-a, asp-b and asp-c all co-occur (Fig. 7).
Population connectivity and genetic diversity in brooding and broadcast spawning corals in the Kimberley

Fig. 7. Barplot of membership coefficients of individual corals from the entire *Acropora aspera* collection calculated in STRUCTURE v2.3 with no prior information for K = 3 and 4. Orange denotes membership to asp-a, purple to asp-b, blue to *Acropora asp-c* and green to asp-d (four K = 4). CLUMPAK calculated both plots from 20/20 runs and a similarity score = 0.999 and mean (LnProb) = -62402.450 for K = 3, and a similarity score = 0.999 and mean (LnProb) = -71462.630 for K = 4. For comparison at other K’s, barplots for K= 2 to 8 are given in Appendix 3 Fig. A4.

Fig. 8 Principal Coordinates Analysis (PCoA) calculated from individual pairwise genotypic distance of the entire *Acropora aspera* collection. Individuals are colour coded according to the clusters assigned by the STRUCTURE analysis. Percentage of variation explained by each axis is given in brackets.

The magnitude of this differentiation among the four *A. aspera* clusters was very large (overall $F_{ST} = 0.587$), with pairwise $F_{ST}$ ranging from 0.469 to 0.705 (Table 2). Morphological assessments in the field, along with preliminary macro-morphological assessments of skeletal material and photos, indicate that variation in macro-morphology between lineages is similar to variation within lineages (see Fig. 9). We conclude that *A. aspera* in the Kimberley represents a cryptic species complex which comprises four genetic lineages that are reproductively isolated and living in sympatry.
Gene diversity, as measured by unbiased expected heterozygosity, varied considerably among the lineages and was highest in Acropora asp-a, and lowest in Acropora asp-d (Fig. 10). Further, 16 loci were fixed among the different lineages, lending support to species-level, or evolutionary significant, divergence among lineages.
Consequently, heterozygosities were zero and fixation indices could not be calculated at these loci (http://catalogue.aodn.org.au/geonetwork/srv/eng/metadata.show?uuid=fb1d80bf-6ef2-4150-9479-22b4240435a7).

Fig. 10 Estimates of gene diversity of the four Acropora aspera clusters based on expected heterozygosity (± standard errors).

4.2 Clonality and gene diversity in Acropora asp-c

Of the 322 ramets that belonged to the Acropora asp-c lineage, 145 were identified as clones and excluded from further population-level analysis, leaving a total of 177 genets belonging to the Acropora asp-c lineage. Genotypic richness was relatively low, averaging 0.61 across all sites but varied from 0.25 to 1.00 (Table 3). Clones were particularly abundant at White Island, Bathurst N Sat, Bathurst E Sat, Bowles Rock and Pope Island where 60% - 70% of all samples were clones (Table 3). At Bowles Rock and Pope Island, there were lots of clones that represented a few individuals, while the collection at Bathurst N sat was dominated by one common clone. Average observed heterozygosity across loci was 0.205, average expected heterozygosity was 0.249, and average \( F_{IS} \) was 0.115 suggesting a general deficiency in heterozygotes that would be expected under Hardy Weinberg equilibrium (genotypes and summary statistics of the Acropora asp-c collection are given digital data repository (http://catalogue.aodn.org.au/geonetwork/srv/eng/metadata.show?uuid=fb1d80bf-6ef2-4150-9479-22b4240435a7). Gene diversity measured by expected heterozygosity was higher in the center of the sampling area at the Buccaneer Archipelago sites compared with Dampier Peninsula sites (Janinko and Aloon) and the Central Kimberley sites (Condillac and White Islands; Fig. 11), and was very low among the Ashmore Reef samples (\( H_{E} = 0.045 \)). The low diversity at Ashmore Reef created a general trend of increasing gene diversity with decreasing latitude, but the low sample size at Ashmore (n = 5) along with a low correlation coefficient (\( r^2 = 0.126 \)) indicate this trend is well supported.
Table 3 Numbers of samples in entire collection of *Acropora aspera* less those excluded due to missing data [N(all)], total number of samples identified as Acropora asp-c [N(*Acropora asp-c*)], number of genets of *Acropora asp-c* [Ng(*Acropora asp-c*)] and genotypic richness of *Acropora asp-c* [Ng:N(*Acropora asp-c*)] collected from sites in the Kimberley coast and at Ashmore Reef in North West Australia.

<table>
<thead>
<tr>
<th>Region</th>
<th>Site</th>
<th>N (all)</th>
<th>N (asp-c)</th>
<th>Ng (asp-c)</th>
<th>Ng:N(asp-c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ashmore</td>
<td>Ashmore_Reef</td>
<td>34</td>
<td>7</td>
<td>5</td>
<td>0.71</td>
</tr>
<tr>
<td>Central Kimberley</td>
<td>Condillac_I.</td>
<td>32</td>
<td>10</td>
<td>10</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>White_I.</td>
<td>29</td>
<td>20</td>
<td>8</td>
<td>0.40</td>
</tr>
<tr>
<td>Buccaneer Archipelago</td>
<td>Bathurst_N_Sat</td>
<td>27</td>
<td>12</td>
<td>3</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>Bathurst_E_Sat</td>
<td>30</td>
<td>30</td>
<td>12</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>Bowles_Rock</td>
<td>30</td>
<td>30</td>
<td>10</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>Barret_Rock</td>
<td>31</td>
<td>30</td>
<td>19</td>
<td>0.63</td>
</tr>
<tr>
<td></td>
<td>Ashhlyn_I.</td>
<td>61</td>
<td>58</td>
<td>36</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td>Pope_I.</td>
<td>30</td>
<td>30</td>
<td>10</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>Tide_Rip</td>
<td>31</td>
<td>27</td>
<td>20</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td>Mermaid_I.</td>
<td>30</td>
<td>29</td>
<td>15</td>
<td>0.52</td>
</tr>
<tr>
<td>Dampier Peninsula</td>
<td>Janinko</td>
<td>31</td>
<td>28</td>
<td>18</td>
<td>0.64</td>
</tr>
<tr>
<td></td>
<td>Ngoorroodool</td>
<td>32</td>
<td>2</td>
<td>2</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Aloon</td>
<td>24</td>
<td>9</td>
<td>9</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Noyon</td>
<td>28</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Ardinoogoon</td>
<td>83</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td></td>
<td>563</td>
<td>322</td>
<td>177</td>
<td>0.61</td>
</tr>
</tbody>
</table>

Fig. 11 Estimates of gene diversity at sites of the *Acropora asp-c* lineage based on expected heterozygosity (± standard errors). Sites are colour coded with Ashmore in orange, central Kimberley in green, the Buccaneer Archipelago in blue, and the Dampier Peninsula sites in purple. A trend line with $r^2 = 0.126$ is shown.
4.3 Genetic structure among regions, systems and reefs in Acropora asp-c

In contrast to cluster analysis of the entire Acropora aspera collection, results of the STRUCTURE and PCoA analysis within Acropora asp-c lineage identified four genetic clusters that corresponded to geographic location and thus provided evidence that this subsample is one interbreeding metapopulation. The Evanno et al. (2005) indicated that K = 4 is the most appropriate number of discrete populations (Appendix 3, Fig. A5) with membership coefficients greater than 90% to one of the four clusters (Fig. 12, for barplots at K=2 to 8 see Appendix 3, Fig. A6). The four separate groups were associated with Ashmore Reef, the central Kimberley sites (Condillac and White Island), the Buccaneer Archipelago (Bathurst E Sat, Bathurst N Sat, Bowles Reef, Barret Rock, Ashlyn Islands and Pope Island), and the Dampier Peninsula (Janinko, Ngooroodool and Aloon). The sites of Tide-Rip and Mermaid Island were admixed between the Buccaneer and Dampier clusters; half of the colonies from these two sites had strong affinities with Buccaneer cluster (the majority q > 85%), while the other half exhibited evidence of mixed ancestry with q’s between 50 and 65% to either cluster (Fig. 12). This geographic structuring into four major clusters is also obvious in the PCoA, with Tide-Rip and Mermaid Islands formed a fifth group intermediate to the Dampier and Buccaneer clusters (Fig. 13). Analysis of only the inshore Kimberley populations revealed identical patterns to the analysis of the entire collection, showing that the divergent Ashmore Reef samples did not influence clustering resolution in the STRUCTURE analysis (data not shown). However, in the PCoA, the clustering of three genetic groups was more distinct compared with the analysis that included Ashmore Reef, corresponding to the central Kimberley, Buccaneer Archipelago and Dampier Peninsula, with sites of Tide-Rip and Mermaid Island intermediate to Buccaneer Archipelago and Dampier Peninsula (Fig. 14).

The AMOVA showed that the majority of the geographic variation was attributed to differences among systems ($F_{RT} = 0.094$, $P < 0.001$), with small but significant differences among sites within systems ($F_{SR} = 0.008$, $P < 0.05$), revealing a moderate but highly significant level subdivision among all sites ($F_{ST} = 0.101$, $P < 0.001$) (Table 4). Large differences were detected between Ashmore Reef and all the other sites, with average pairwise $F_{ST}$ of 0.380 ($\pm 0.011$) (Appendix 3; Table A1). Therefore, levels of subdivision were weaker when Ashmore and other sites with sample sizes <10 were excluded in the AMOVA, but general patterns and significance were the same (Table 4).

Fig. 12 Barplots of membership coefficients of individual corals from the Acropora asp-c lineage calculated in STRUCTURE v2.3 with the LCOPRIOR model for K = 4. This is the major mode plot produced by CLUMPAK calculated from 12/20 runs and a similarity score = 0.986, and a mean (LnProb) = -423687.042. The minor mode plot was almost identical.
Fig. 13 Principal Coordinates Analysis (PCoA) calculated from individual pairwise genotypic distance of corals from the *Acropora asp-c* lineage. Percentage of variation explained by each axis is given in brackets.

Table 4 Results of AMOVA that partitioned genetic variation among systems, among sites within systems and among all sites. Analysis involved all sites, and only those sites with n ≥ 9. All estimates of differentiation were significant at P < 0.05.

<table>
<thead>
<tr>
<th></th>
<th>all sites</th>
<th>sites (n ≥ 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F_{RT}$: among systems</td>
<td>0.094</td>
<td>0.050</td>
</tr>
<tr>
<td>$F_{SR}$: within systems</td>
<td>0.008</td>
<td>0.006</td>
</tr>
<tr>
<td>$F_{ST}$: among all sites</td>
<td>0.101</td>
<td>0.056</td>
</tr>
</tbody>
</table>

Fig. 14 Principal Coordinates Analysis (PCoA) calculated from individual pairwise genotypic distance of corals from the *Acropora asp-c* lineage from the inshore Kimberley only (i.e. Ashmore Reef samples are excluded). Percentage of variation explained by each axis is given in brackets.
4.4 Genetic structure within-reefs in Acropora asp-c

Within *Acropora* asp-c, there was evidence of spatial autocorrelation, with small but significant positive correlation coefficient that remained relatively constant up to 500 m, and then dropped away after this initial plateau (Fig. 15). This distance indicates the extent of the genetic patch and the spatial limit of completely mixed genotypes. Additionally, r first crossed the x-intercept at 35 km, revealing the distance at which the random effects of genetic drift, not the homogenising influence of gene flow, drive genetic relatedness.

![Fig. 15 Spatial autocorrelation analyses of the genetic correlation coefficient (r) as a function of distance for the *Acropora* asp-c lineage at the Dampier Peninsula and the Buccaneer Archipelago. The bootstrapped 95% confidence error bars generated via 1000 bootstrap trials are shown.](image)

5 Results: Oceanographic modelling and tests for isolation by distance and oceanographic resistance

The mantel tests for correlation between genetic distance, geographic distance, and oceanographic resistance showed that geographic distance explained more (for *I. brueggemanni* R = 0.634, for *Acropora* asp-c R = 0.681) of the genetic structure among sites from the Buccaneer Archipelago and Dampier Peninsula than the oceanographic model (for *I. brueggemanni* R = 0.460, for *Acropora* asp-c R = 0.477; Table 5). However, there was also a strong correlation between oceanic resistance and geographic distance (for *I. brueggemanni* R = 0.720, for *Acropora* asp-c R = 0.517). The outputs of the oceanographic model that was run for eight or 40 days in the different austral seasons showed a lack of cross shelf connectivity of passive particles between the offshore and inshore reefs in any season (Appendix 4 Fig. A1 and Fig. A2).

Table 5 Results of mantel test for correlation between genetic distance (pairwise F_{ST}) between sites in the Buccaneer Archipelago and Dampier Peninsula, geographic distance (GGD) and oceanographic resistance (OR) in *I. brueggemanni* and *Acropora* asp-c. The outlying site of Kooljaman was not included in the analysis.

<table>
<thead>
<tr>
<th></th>
<th>OR vs FST</th>
<th>GGD vs FST</th>
<th>OR VS GGD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>P</td>
<td>R</td>
</tr>
<tr>
<td><em>I. brueggemanni</em></td>
<td>0.460</td>
<td>0.001</td>
<td>0.634</td>
</tr>
<tr>
<td><em>Acropora asp-c</em></td>
<td>0.477</td>
<td>0.001</td>
<td>0.681</td>
</tr>
</tbody>
</table>
6 Discussion

This first-ever population genomic study of connectivity in hard corals of the inshore Kimberley shows that most recruits to populations of both the brooding coral (Isopora brueggemanni) and broadcast spawning coral (Acropora aspera) originate from their natal reef or reef patch, with few larvae dispersing and recruiting successfully between reefs over distances of more than 35 kilometres. Further, cross-shelf connectivity between the offshore and inshore reefs appears to be negligible, even over multiple generations. Finally, in the A. aspera collection, we identified four distinct genetic lineages that are morphologically cryptic, but reproductively isolated despite coexisting on the same reefs.

6.1 Patterns of ecological connectivity

Over the broadest, inter-regional, scale of the study (10s – 100s of kilometres), the greatest level of divergence among populations of I. brueggemanni and the major A. aspera lineage (Acropora asp-c) was detected between offshore reef at Ashmore Reef and the inshore reefs of the Kimberley. These results indicate that cross shelf connectivity for both the brooding and spawning coral occurs rarely, even over evolutionary time scales. This conclusion is well supported by the oceanographic results which exhibited an absence of cross shelf connectivity even when the model was run over the upper competency of corals of 40 days.

There was only one exception to this regional scale divergence; the cluster analysis revealed that I. brueggemanni corals from the most northern of our inshore Kimberley sites - West Montalivet Island in the Bonaparte Archipelago, exhibited genetic affinities with Ashmore Reef. However, several lines of indirect evidence indicate that this relationship most likely reflects the limited sampling in the northern and central Kimberley in which unsampled “ghost” populations may have created the appearance of cross shelf migration between Ashmore and West Montalivet but they may not actually exchange migrants (Slatkin 2005). First, genetic differentiation between Ashmore Reef and West Montalivet as measured by FST was large (FST = 0.227). Second, gene diversity was very high at West Montalivet, suggesting that this site is part of a large population that has been well connected to exogenous sources of genetic variation. Third, in the STRUCTURE analysis for K > 6, I. brueggemanni corals from West Montalivet formed a coherent cluster with high membership coefficients that was separate from corals at Ashmore Reef. Thus, we conclude that I. brueggemanni corals on the offshore and inshore reefs are separate evolutionary significant units (sensu Moritz 2002), while also recognising the need for future research that targets sites in the central Kimberley to clarify origins of the striking genetic diversity at West Montalivet.

At the intermediate, inter-reef scale of the study (kilometres – 10s of kilometres), there was a clear association between genotype and geography for both corals, with three distinct genetic groups identified in the inshore systems of the Central Kimberley, the Buccaneer Archipelago, and the Dampier Peninsula. The pattern of divergence of these two latter systems in the southern Kimberley identified the Sunday Strait as a semi-permeable barrier to gene flow between the Buccaneer and Dampier reefs, a pattern that was common to both species. The Sunday Strait is a relatively deep water channel, through which majority of water that fills and drains King Sound funnels at extreme velocities. Putative restrictions to dispersal of brooded and broadcast spawned larvae between of the Buccaneer and Dampier systems across this barrier explains the genetic divergence between the two systems, with occasional dispersal across the barrier via the stepping stones of Tide Rip and Mermaid Islands likely facilitating the evolutionary important exchange of genes. In addition, within the Dampier peninsula, I. brueggemanni corals from the mainland site of Kooljaman in the far west of the sample area were very divergent from the other inshore Kimberley corals and was characterised by the lowest gene diversity of all sites, suggesting that it is a small and isolated population that may well be end of its range at least within the Kimberley region. This explanation is consistent with observations in the field; we did not find populations of I. brueggemanni along the west coast of the Dampier Peninsula further south than Kooljaman, which is the southernmost extent of the Kimberley. These results indicate that the population of Kooljaman may be reliant on its own genetic variation to adapt to environmental change in coming decades.
At the fine, within-reef scale of the study (100s metres), colonies of both species within 500 m of each other are genetically more related than colonies further apart, indicating the general size of the genetic patch. Congruently, significant differentiation was detected in *I. brueggemanni* between subsites separated by 500 metres, although the magnitude of differentiation was relatively small compared with that among sites. More importantly, colonies separated by more than 20 km for *I. brueggemanni* and 35 km for *A. aspera* were not positively related, revealing the distance where the random effects of genetic drift, not homogenising influence of gene flow mediated by dispersal, are the primary determinants of genetic composition and thus providing inference of the general scale of demographic independence.

Finally, geographic distance was a better predictor of genetic structure for both corals than the oceanographic model, indicating the simple pattern of isolation by distance and a need for more biologically relevant and finer scale modelling. Outputs of the model that was run over eight days also show that there is no distinct directional current in operation along the inshore Kimberley, but large complex tidal flows and wind driven currents prone to reversals and multidirectionality are the primary drivers of connectivity for species with pelagic larvae in this region.

### 6.2 Patterns of clonality and genetic diversity

The results presented here indicate that in the branching coral *Acropora asp-c*, clonal propagation is an important mode of reproduction at some sites in the Kimberley. In particular, genotypic richness was particularly low at the four sites in the Buccaneer Archipelago (Bathurst_N_Sat, Bathurst_E_Sat, Bowles_Rock and Pope_Is) as well as at White Island. This conclusion is congruent with other work in NWA, that indicates the establishment of vegetative fragments that aid recovery after tropical storms on the inshore reefs with shallow depth gradients is common (Underwood 2009). The relatively high levels of clonality were not associated with reduced gene diversity, with high expected heterozygosity detected at all these sites (Fig. 11). Therefore, even for those reefs where vegetative fragmentation dominates, sexual reproduction continues to be important for maintenance of genetic variation. In contrast, vegetative fragmentation appears to be much less common in the more robust branching growth form of *I. brueggemanni*, with high genotypic richness at all the sites except one, suggesting that sexually produced larvae dominate reproduction in these populations.

The presence of a either inter or intra-specific declining diversity gradient from north to south along the North West Shelf is often alluded to, but empirical supporting data has been lacking (Wilson 2013). Our results suggest such a pattern does exist for the brooding coral, but sampling further south in the Pilbara is required to verify whether the regional scale pattern holds over broader scales. In contrast, for the spawner, there was little evidence of a diversity gradient in the inshore Kimberley, suggesting that the diversification of the *Acropora aspera* species in the Kimberley has had a dominant influence on distribution of genetic diversity of the *Acropora asp-c* lineage. In particular, our results indicate that at the regional scale, the centre of genetic diversity within *Acropora asp-c* is in Buccaneer Archipelago in the Kimberley, with expected heterozygosity attenuating to the west and east from this centre. Furthermore, *Acropora asp-c* was rare or non-existent in our collections from the Dampier Peninsula, and much less abundant in the central Kimberley and Ashmore Reef collections. Therefore, the largest and most well connected populations of this lineage seem to occur in the central Buccaneer Archipelago. However, the sampling scale of this study needs to be extended to the central and northern Kimberley, the offshore atolls and the Pilbara to test these hypotheses, along with a consideration of distribution of diversity of the other *Acropora aspera* lineages. Nevertheless, results from our oceanographic model support the prediction that, in contrast to the offshore reefs on the shelf margin, there is no distinct north south current along the inner shelf, but complex tidal flows and wind driven currents prone to reversals are the primary drivers of connectivity for species with pelagic larvae.
6.3 Cryptic diversity in Acropora aspera

In the marine realm, it is estimated that tens of thousands of cryptic species are undescribed (Appeltans et al. 2012), and such cryptic diversity appears to be particularly prevalent among coral reef taxa (Rocha et al. 2007). Here, the detection of four distinct genetic lineages that are morphologically cryptic is consistent with numerous genetic studies in hard corals throughout the world (Wallace and Willis 1994, Miller and Benzie 1997, van Oppen et al. 2001, Willis et al. 2006, Ladner and Palumbi 2012, Pinzon et al. 2013, Prada and Hellberg 2013, Schmidt-Roach et al. 2014, Combosch and Vollmer 2015, Ohki et al. 2015, Warner et al. 2015), and particularly in NWA (Richards et al. 2013, Rosser 2015, Gilmour et al. 2016b, Richards et al. 2016, Rosser 2016). The delimitation of four clusters by 100% agreement with two alternative methods (PCoA and Bayesian clustering) with large fixation indices greater than 0.469 show that these corals living in sympatry represent unique evolutionary significant units. However, morphological assessments in the field, along with preliminary macro-morphological assessments of skeletal material and photos, indicate that no clear macro-morphological differences exist among the lineages (see Fig. 9). This pattern contrasts to that observed on the Great Barrier Reef in Eastern Australia of where A. aspera was the only morphospecies among five sister species which was genetically distinct (Van Oppen et al. 2002). However, Van Oppen et al. (2002) also showed evidence of successful hybridization between A. aspera and one of these sister species and it thus appears that reproductive barriers in this species group are semi-permeable, the strength of which varies between species and over time. A major revision of the taxonomic status of Acropora aspera is thus warranted.

Coral reefs of the Kimberley are recent phenomena: during the low sea levels of the last interglacial period the coastline occurred along the continental shelf margin many hundreds of kilometres to the north and west of today's coast line (Wilson 2013), with the formation of coral reefs in the inshore Kimberley commencing only 8,000 years ago (Solihuddin et al. 2016). Indeed, such transgressions and regressions have occurred many times during the late quaternary due to eustatic sea level change, and these processes likely underlie the current divergence of Acropora aspera lineages. Further, the patchy distribution and habitat heterogeneity of present day reefs provide fertile conditions for strong local selection, and thus processes of ecological speciation appear to have played an important role along with the vicariant processes and founder effects in the evolution of corals in this region. One documented mechanism that appears to be central to the diversification of sympatric broadcast spawners in NWA involves differences in timing of spawning, with direct evidence that distinct genetic lineages within morphologically cryptic Acropora "species" exist between corals that spawn in Spring and Autumn (Rosser 2015, Gilmour et al. 2016b, Rosser 2016). Such temporal reproductive barriers are the most likely explanation for diversification of the A. aspera lineages detected here.

The discovery of cryptic diversity has important implications for management of coral reefs in the region that concern estimates of biodiversity and effective population size. The most concerted biodiversity study based on morphological identifications of museum-registered specimens published so far for the Kimberley coast reported seven new coral records for WA (Richards et al. 2015). Additionally, many coral species that had previously been recorded in clear offshore habitats were found to occur on inshore reefs, and 34 species were found in the intertidal zone that have only been recorded to occur in the subtidal zone. Richards et al. (2015) undertook their study in a relatively small sample area in the Bonaparte Archipelago, central Kimberley, in tandem with an analysis of historical specimen-based records in Australian Museums (Richards et al. 2014), and show a total of 338 species of coral have been recorded from the Kimberley. If cryptic speciation is common throughout the Kimberley, then this estimate of coral biodiversity may be a substantial underestimate. Additionally, given these A. aspera lineages are likely reproductively isolated, the effective population size of each is much smaller than expected, which has implications for their ecological and evolutionary capacity to recover after disturbance. In particular, lineages may have different susceptibility to environmental change, such as fluctuations in water temperature, acidity, or incidence of disease (Hoegh-Guldberg and Bruno 2010, Hughes et al. 2010), and with a smaller effective population likely have less standing genetic variation to draw on for adaptation. Further, the demographic consequences of a higher susceptibility to disturbances, whether lethal or sublethal, are likely to be reduced reproductive output and recruitment (Oliver and Babcock 1992,
Population connectivity and genetic diversity in brooding and broadcast spawning corals in the Kimberley

Hughes et al. 2000, Levitan et al. 2004) and therefore, a slower rate of recovery from disturbance. In the worst instances, recovery from severe disturbances would be severely compromised if reproductive isolation was further compounded by Allee effects (Knowlton 2001). 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.

6.4 Conclusions

This study utilised thousands of genome-wide SNPS to reveal that populations of the brooding coral I. brueggemannii and the broadcast spawning coral A. aspera are characterised by strong geographic structure over multiple scales in NWA. For the brooder, collections within the inshore Kimberley comprised one species, with no evidence of cryptic diversity. Thus, the brooding mode of reproduction in this species appears to maintain abundant populations by local recruitment over scales of a few hundred metres, with occasional longer distance dispersal over scales of few tens of kilometres that prevents inter-specific genetic divergence.

In contrast, the morphospecies A. aspera comprises several discrete lineages in NWA that not only occur in sympathy but also exhibit genetic affinities across geographically distant sites. The implication is that reproductive barriers exist between lineages in the broadcast spawner, most likely through a combination of allopatric speciation followed by reconnection during sea level change, in conjunction with ecological divergence through localised adaptation to heterogeneous environments and reproductive isolation through difference in the timing of spawning. Consistent with a greater propensity for widespread dispersal in spawning corals, the level of genetic subdivision within the Acropora asp-c lineage (\(F_{ST} = 0.101\)) was half that of the brooder I. brueggemannii (\(F_{ST} = 0.230\)). However, the general patterns of ecological connectivity were remarkably similar between the two corals; migration is rare among reefs that are separated by more than a few tens of kilometres, the Sunday Strait appears to be a semi-permeable barrier in which Tide Rip and Mermaid Islands provide stepping stone connections between the Dampier Peninsula and Buccaneer Archipelago, and the offshore populations of Ashmore Reef are separate evolutionary significant units from those of the inshore reefs of the Kimberley. These common findings among two species with different reproductive modes suggest our conclusions maybe applicable to many hard corals in the region, and have important implications for spatial management strategies aimed at maximising the resilience of these ecosystems to climate change and other human induced disturbances.
References


Ladner, J. T., and S. R. Palumbi. 2012. Extensive sympatry, cryptic diversity and introgression throughout the geographic distribution of two


8 Acknowledgements

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9 Data Availability

Data associated with this research is available on the AIMS Data Access Portal at http://catalogue.aodn.org.au/geonetwork/srv/eng/metadata.show?uuid=fb1d80bf-6eF2-4150-9479-22b4240435a7
Appendices

Appendix 1 General Diversity Array Technologies SNP development protocol.

Genome-wide single nucleotide polymorphism (SNP) data were generated at Diversity Arrays Technology (DArT) with DArTseq methodology using the next generation sequencing platform. DArTseq represents a new implementation of sequencing of complexity reduced representations (Altshuler et al. 2000) and recent applications of this concept using the next generation sequencing platforms (Elshire et al. 2011). Detailed protocols are provided in Kilian et al. (2012), and examples of recent applications are Cruz et al. (2013) and Raman et al. (2014). The method is conceptually similar to RAD-seq methods (Baird et al. 2008), but in comparison, because generation of restriction fragments with appropriate adapters is more straightforward during the complexity reduction stage, there is a high degree of qualitative and quantitative reproducibility in sampling genomic fragments. A subsample of six to eight individuals from 12 sites (n = 94) spread across the entire sample area (to avoid ascertainment bias) was used to optimise the DArTseq methodology. Four methods of complexity reduction were tested in corals (data not presented) to select the most appropriate method based on both the size of the representation and the fraction of a genome selected for assays, and the PstI-HpaII method was selected. DNA samples were processed in digestion/ligation reactions principally as per Kilian et al (2012) but replacing a single PstI-compatible adaptor with two different adaptors corresponding to two different Restriction Enzyme (RE) overhangs. The PstI-compatible adapter was designed to include Illumina flowcell attachment sequence, sequencing primer sequence and “staggered”, varying length barcode region, similar to that reported by Elshire et al. (2011). Reverse adapter contained flowcell attachment region and HpaII-compatible overhang sequence.

Only “mixed fragments” (PstI-HpaII) are effectively amplified in 30 rounds of PCR using the following reaction conditions: PCR conditions consisted of an initial denaturation 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 amounts of amplification products from each sample of the 96-well microtiter plate were bulked and applied to c-Bot (Illumina) bridge PCR followed by sequencing on Illumina Hiseq2500. The sequencing (single read) was run for 77 cycles.

Once optimised, the above method was used to generate sequences from the entire collection, and then processed using proprietary DArT analytical pipelines. In the primary pipeline the FASTQ files are first processed to filter away poor quality sequences, applying more stringent selection criteria to the barcode region compared to the rest of the sequence. In that way the assignments of the sequences to specific samples carried in the “barcode split” step are very reliable. Approximately 2,500,000 (+/- 7%) sequences per barcode/sample were used in marker calling. Finally, identical sequences were collapsed into “fastqcall files”, which were groomed using DArT’s proprietary algorithm that corrects low quality bases from singleton tags using collapsed tags with multiple members as a template. These files are used in the secondary pipeline for DArT PL’s proprietary SNP and SilicoDArT (presence/absence of restriction fragments in representation) calling algorithms (DArTsoft14). All tags from all libraries were clustered using DArT PL’s C++ algorithm at the threshold distance of 3, followed by parsing of the clusters into separate SNP loci using a range of technical parameters, especially the balance of read counts for the allelic pairs. Additional selection criteria were added to the algorithm based on analysis of approximately 1,000 controlled cross populations. Testing for Mendelian distribution of alleles in these populations facilitated selection of technical parameters discriminating true allelic variants from paralogous sequences.
Appendix 2 Additional results of quality control statistics, descriptive statistics and STRUCTURE analysis for *Isopora brueggemannii*.

**APPENDIX 2 Fig. A1 Histograms of descriptive statistics of 23, 165 SNPS for the entire *Isopora brueggemannii* data set showing reproducibility (A), call rate (B), coverage (C), SNP allele frequency (D) and heterozygosity (E), and summary of numbers of loci remaining after each filter was applied.**

\[
\Delta K = \text{mean}(L^*(K)) / \text{stdev}(L(K))
\]

**APPENDIX 2 Fig. A2 Plot of \(\Delta K\) for increasing K from STRUCTURE analyses of the entire *Isopora brueggemannii* collection without any prior information and run with correlated allele frequency model.**
Appendix 2 Fig. A3 Barplots from STRUCTURE analysis using the LOCPRIOR model showing membership coefficients for K = 2 to 10 of the entire *Isopora brueggemanni* collection. Major modes calculated in CLUMPAK are presented.
### Appendix 2 Table A1 Pairwise FST estimates between sites for *I. brueggemannii* in the Kimberley below diagonal, and P-values significance based on 999 permutations are shown above diagonal.

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Appendix 3 Additional results of quality control statistics, descriptive statistics and STRUCTURE analysis for *Acropora aspera*.

APPENDIX 3 Fig. A1 Histograms of descriptive statistics of 34,304 SNPS for the entire *Acropora aspera* data set showing reproducibility (A), call rate (B), coverage (C), SNP allele frequency (D) and heterozygosity (E), and summary of numbers of loci remaining after each filter was applied.
APPENDIX 3 Fig. A2 Histograms of descriptive statistics of 27,304 SNPS for the Acropora asp-c data set showing reproducibility (A), call rate (B), coverage (C), SNP allele frequency (D) and heterozygosity (E), and summary of numbers of loci remaining after each filter was applied.

\[ \Delta K = \frac{\text{mean}(\Delta L(K))}{\text{std}(L(K))} \]

APPENDIX 3 Fig. A3 Plot of $\Delta K$ for increasing $K$ from STRUCTURE analyses of the entire Acropora aspera collection without any prior information and run with correlated allele frequency model.
Appendix 3 Fig. A4 Barplots from STRUCTURE analysis showing membership coefficients for $K = 2$ to $8$ of the entire Acropora aspera collection. Major modes calculated in CLUMPAK are presented.
APPENDIX 3 Fig. A5 Plot of ΔK for increasing K from STRUCTURE analyses of the Acropora asp-c lineage with prior information on sampling location and run with correlated allele frequency model.
APPENDIX 3 Fig. A6 Barplots from LOCPRIOR runs in STRUCTURE showing membership coefficients for $K = 2$ to $8$ of colonies in the Acropora asp-c lineage. Major modes calculated in CLUMPAK are presented.
Appendix 3 Table A1 Pairwise $F_{ST}$ estimates between sites for *Acropora* asp-c in the Kimberley below diagonal, and P-values based on 999 permutations are shown above diagonal. Sites with sample size <4 were excluded.

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Appendix 4 Results of oceanographic model

Appendix 4 Fig. A1 Particle tracks from oceanographic model run over 8 days in different austral seasons. Orange circles represent release sites, with particles released 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).
Appendix 4 Fig. A2 Particle tracks from oceanographic model run over 40 days in different austral seasons. Orange circles represent release sites, with particles released 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).
Population genetic diversity, structure and connectivity of two seagrass species, *Thalassia hemprichii* and *Halophila ovalis* in the Kimberley

Kathryn McMahon¹,³, Udhi Hernawan¹,³, Kathryn Dawkins¹,³, Kor-Jent van Dijk²,³, Michelle Waycott²,³

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**

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

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**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)

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

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4 DISCUSSION ........................................................................................................................................... 27
  4.1 GENETIC CONNECTIVITY .................................................................................................................... 27
    4.1.1 Fine-scale ..................................................................................................................................... 27
    4.1.2 Broad-scale ................................................................................................................................. 27
  4.2 GENETIC DIVERSITY .......................................................................................................................... 28
    4.2.1 Fine-scale ..................................................................................................................................... 28
    4.2.2 Broad scale .................................................................................................................................. 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
Population genetic diversity, structure and connectivity of two seagrass species, Thalassia hemprichii and Halophila ovalis in the Kimberley

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. It’s 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 FST), 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.
Population genetic diversity, structure and connectivity of two seagrass species, Thalassia hemprichii and Halophila ovalis in the Kimberley

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).
Population genetic diversity, structure and connectivity of two seagrass species, *Thalassia hemprichii* and *Halophila ovalis* in the Kimberley
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, Jannsen & 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 the 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). Its 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.

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

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.

<table>
<thead>
<tr>
<th>Site No</th>
<th>Sub-region</th>
<th>Population</th>
<th>Thalassia hemprichii</th>
<th>Halophila ovalis</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Buccaneer Archipelago</td>
<td>Bathurst Is.</td>
<td>-16.04164</td>
<td>-16.06437</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>123.52317</td>
<td>123.55263</td>
</tr>
<tr>
<td>4</td>
<td>Buccaneer Archipelago</td>
<td>Irvine Is.</td>
<td>-16.13672</td>
<td>-16.13460</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>123.32988</td>
<td>123.30029</td>
</tr>
<tr>
<td>7</td>
<td>Buccaneer Archipelago</td>
<td>Longitude Is.</td>
<td>-16.06936</td>
<td>-16.16476</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>123.39378</td>
<td>123.34789</td>
</tr>
<tr>
<td>8</td>
<td>Buccaneer Archipelago</td>
<td>Bedford Is. North</td>
<td>-16.16476</td>
<td>-16.16484</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>123.34789</td>
<td>123.34796</td>
</tr>
<tr>
<td>9</td>
<td>Buccaneer Archipelago</td>
<td>Bedford Is. South</td>
<td>-16.16476</td>
<td>-16.16484</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<td>123.34796</td>
</tr>
<tr>
<td>10</td>
<td>North-eastern King Sound</td>
<td>Riptide Is./Gregory Is.</td>
<td>-16.31016</td>
<td>-16.31123</td>
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<td></td>
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<td>123.31913</td>
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<tr>
<td>11</td>
<td>North-eastern King Sound</td>
<td>Mermaid Is.</td>
<td>-16.44516</td>
<td>-16.39106</td>
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<td></td>
<td></td>
<td></td>
<td>123.35142</td>
<td>123.20641</td>
</tr>
<tr>
<td>12</td>
<td>Sunday Island Group</td>
<td>Sunday Is. –north, Maleny</td>
<td>-16.39642</td>
<td>-16.42949</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>123.21020</td>
<td>123.19780</td>
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<tr>
<td>13</td>
<td>Sunday Island Group</td>
<td>Sunday Is. –south east, Janinko</td>
<td>-16.42537</td>
<td>-16.41819</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>123.19805</td>
<td>123.16698</td>
</tr>
<tr>
<td>14</td>
<td>Sunday Island Group</td>
<td>Hal’s Pool, Ngoorroodool</td>
<td>16.41813</td>
<td>-16.41819</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>123.16699</td>
<td>123.16698</td>
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<tr>
<td>15</td>
<td>Sunday Island Group</td>
<td>Tallon Is., Jalan</td>
<td>-16.40182</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>123.3517</td>
<td>123.13906</td>
</tr>
<tr>
<td>16</td>
<td>Sunday Island Group</td>
<td>Jackson Is., Aloon</td>
<td>-16.44053</td>
<td>-16.44052</td>
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<tr>
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<td></td>
<td>123.10225</td>
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<td>17</td>
<td>Sunday Island Group</td>
<td>Noyon</td>
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<td></td>
<td></td>
<td></td>
<td>123.06940</td>
<td>123.06958</td>
</tr>
<tr>
<td>19</td>
<td>Sunday Island Group</td>
<td>Shenton Bluff, Ardinoogoon</td>
<td>16.48246</td>
<td>-16.15289</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>123.04702</td>
<td>126.53329</td>
</tr>
<tr>
<td>20</td>
<td>Woobinbeye Creek</td>
<td></td>
<td>-12.19832</td>
<td>96.84287</td>
</tr>
</tbody>
</table>

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.
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, TH37-5 alleles, TH43-6 alleles, Thh8-5 alleles, TH34-Balleles, 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 (rD) 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 (FIS) 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 = 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 FST and GST. Since mutation rates can affect FST, particularly with highly polymorphic markers, such as microsatellites, then using FST can lead to bias in estimating genetic differentiation, usually resulting in an underestimation of genetic differentiation. Wang (2015) showed that the mutational effects on FST can be examined by correlating GST and Hs across loci. If the correlation (rGH) is highly negative and significant then the FST is likely to be biased and should not be used (Wang 2015). We examined the rGH using the program CoDiDi and for *Halophila* found a significant and highly negative relationship, raising concern on the use of FST. However, for *Thalassia* we found a positive relationship (rGH = 0.554) suggesting that in our dataset the FST 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. DISTUCTv1.1 (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 $\theta$ (Alcala et al. 2014). This measure is based on the complementary function of both $F_{ST}$ and $D$. To calculate $\theta$, 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 $\theta$ 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
Population genetic diversity, structure and connectivity of two seagrass species, *Thalassia hemprichii* and *Halophila ovalis* in the Kimberley

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 collinearity 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 ~ 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.

### 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 ($r^2d = 0.404, p=0.001$), 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.

<table>
<thead>
<tr>
<th>No</th>
<th>Code</th>
<th>Population</th>
<th>N</th>
<th>G</th>
<th>G Max</th>
<th>R</th>
<th>nA</th>
<th>Ar</th>
<th>pA</th>
<th>Re</th>
<th>Ho</th>
<th>Hexp</th>
<th>F IS</th>
<th>LD</th>
</tr>
</thead>
<tbody>
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<td>0.03</td>
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<td>0.048</td>
<td>0.124</td>
<td>0.667</td>
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<tr>
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<td>5</td>
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<td>14</td>
<td>1.56</td>
<td>0.26</td>
<td>0.72</td>
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<td>0.215</td>
<td>0.103</td>
<td>0.01</td>
</tr>
<tr>
<td>9</td>
<td>BS</td>
<td>Bedford Is. – south</td>
<td>41</td>
<td>19</td>
<td>12</td>
<td>0.45</td>
<td>27</td>
<td>2.62</td>
<td>0.18</td>
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<td>0.353</td>
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<td>3</td>
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<tr>
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<tr>
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<td>5</td>
<td>31</td>
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<td>19</td>
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<td>0.11</td>
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<td>0.543</td>
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<td>11</td>
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<td>0.10</td>
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<td>0.695</td>
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<td>0.83*</td>
</tr>
<tr>
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<td>Woobinbeye Creek</td>
<td>28</td>
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<td>17</td>
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<td>1.5*</td>
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<td>149</td>
<td>24</td>
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<td>0.32</td>
<td>0.338</td>
<td>0.722</td>
<td>0.312*</td>
<td>0.40*</td>
</tr>
</tbody>
</table>

3.1.2 Population genetic differentiation and structure

Overall we detected significant genetic differentiation, global FST 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 (FST 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 (FST = 0.521), Bedford Island North and Talon Island (FST = 0.459) and Riptide Island with Sunday Island North (FST = 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.
Population genetic diversity, structure and connectivity of two seagrass species, Thalassia hemprichii and Halophila ovalis in the Kimberley

Table 4. Genetic differentiation statistics for *Halophila ovalis*. Top matrix is $G_{ST}^{\text{NEI}}$ 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.

<table>
<thead>
<tr>
<th></th>
<th>II</th>
<th>BN</th>
<th>BS</th>
<th>RI</th>
<th>SN</th>
<th>SS</th>
<th>HP</th>
<th>TI</th>
<th>JI</th>
<th>NY</th>
<th>WC</th>
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<td>0.396</td>
<td>0.570</td>
<td>0.632</td>
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</tr>
<tr>
<td>SN</td>
<td>0.601</td>
<td>0.521</td>
<td>0.265</td>
<td>0.476</td>
<td>-</td>
<td>0.005</td>
<td>0.176</td>
<td>0.303</td>
<td>0.070</td>
<td>0.319</td>
<td>0.655</td>
</tr>
<tr>
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<td>-</td>
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<td>0.221</td>
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</tr>
<tr>
<td>HP</td>
<td>0.361</td>
<td>0.299</td>
<td>0.122</td>
<td>0.269</td>
<td>0.124</td>
<td>0.081</td>
<td>-</td>
<td>0.151</td>
<td>0.183</td>
<td>0.029</td>
<td>0.475</td>
</tr>
<tr>
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<td>0.192</td>
<td>0.097</td>
<td>0.107</td>
<td>-</td>
<td>0.252</td>
<td>0.215</td>
<td>0.648</td>
</tr>
<tr>
<td>JI</td>
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<td>0.079</td>
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<td>0.279</td>
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<td>0.361</td>
<td>0.508</td>
<td>0.575</td>
<td>0.331</td>
<td>-</td>
</tr>
</tbody>
</table>

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.

![Figure 3: Spatial autocorrelation for Halophila ovalis showing significant spatial autocorrelation of individuals over distances up to 20 km, with the most significant spatial autocorrelation at 25 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.](image)

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).
Figure 4: Pattern of gene flow based on $\hat{N_m}$ (relative number of migrants per generation- Alcala et al. 2014) for Halophila ovalis. Sampling sites are identified by name. Levels of $\hat{N_m}$ among sampling sites are represented by curved lines. The thicker the lines, the higher level of gene flow between populations. Only those sites with more than 10 individuals have been included.

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

<table>
<thead>
<tr>
<th></th>
<th>Total strength</th>
<th>Closeness</th>
<th>Transitivity</th>
<th>Betweenness</th>
</tr>
</thead>
<tbody>
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<td>0.78</td>
<td>21</td>
</tr>
<tr>
<td>BS</td>
<td>2.62</td>
<td>2.46</td>
<td>1.03</td>
<td>0</td>
</tr>
<tr>
<td>RI</td>
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<td>0.74</td>
<td>8</td>
</tr>
<tr>
<td>SN</td>
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<td>3.25</td>
<td>0.62</td>
<td>7</td>
</tr>
<tr>
<td>HP</td>
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<td>2.87</td>
<td>1.00</td>
<td>0</td>
</tr>
<tr>
<td>TI</td>
<td>1.54</td>
<td>4.46</td>
<td>0.92</td>
<td>5</td>
</tr>
<tr>
<td>NY</td>
<td>3.83</td>
<td>2.22</td>
<td>0.99</td>
<td>0</td>
</tr>
</tbody>
</table>
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).

![Figure 5: Isolation by spatial distance (Top) and by oceanographic distance (Bottom) between seven sites in the Sunday Islands Group and the Buccaneer Archipelago. There was no significant isolation by distance ($p=0.06$) but a significant isolation by oceanographic distance ($p<0.05$, $r^2=0.143$), although it only explained 14% of the variation.](image)

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 Whalund 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 ($nA$) 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, \( G_{\text{Max}} \)-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, \( F_S \)-Inbreeding coefficient (*=significant, \( p<0.05 \)), LD-linkage disequilibrium. Grey shading indicates a low number of individuals and less certainty in genetic statistics.

<table>
<thead>
<tr>
<th>No</th>
<th>Population</th>
<th>Abb.</th>
<th>N</th>
<th>G</th>
<th>( G_{\text{Max}} )</th>
<th>R</th>
<th>nA</th>
<th>Ar</th>
<th>pA</th>
<th>Re</th>
<th>Ho</th>
<th>Hexp</th>
<th>( F_S )</th>
<th>LD</th>
</tr>
</thead>
<tbody>
<tr>
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<td>24</td>
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</tr>
<tr>
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<td>30</td>
<td>1.83</td>
<td>0.06</td>
<td>0.56</td>
<td>0.291</td>
<td>0.216</td>
<td>-</td>
<td>0.357*</td>
</tr>
<tr>
<td>8</td>
<td>Bedford Is. – north</td>
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<td>7</td>
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<td>24</td>
<td>1.47</td>
<td>0</td>
<td>0.59</td>
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<td>0.133</td>
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</tr>
<tr>
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<td>Bedford Is. – south</td>
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<td>37</td>
<td>4</td>
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<td>1.66</td>
<td>0.01</td>
<td>0.68</td>
<td>0.120</td>
<td>0.139</td>
<td>0.138*</td>
<td>-0.02</td>
</tr>
<tr>
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<td>Riptide Is.</td>
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<td>43</td>
<td>2</td>
<td>0.94</td>
<td>31</td>
<td>1.82</td>
<td>0.03</td>
<td>0.40</td>
<td>0.199</td>
<td>0.211</td>
<td>0.059*</td>
<td>-0.01</td>
</tr>
<tr>
<td>11</td>
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<td>36</td>
<td>1.84</td>
<td>0.09</td>
<td>0.44</td>
<td>0.215</td>
<td>0.196</td>
<td>-</td>
<td>0.097*</td>
</tr>
<tr>
<td>12</td>
<td>Sunday Is. – north</td>
<td>SN</td>
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<td>27</td>
<td>5</td>
<td>0.57</td>
<td>27</td>
<td>1.56</td>
<td>0.12</td>
<td>0.65</td>
<td>0.130</td>
<td>0.131</td>
<td>0.009</td>
<td>-0.01</td>
</tr>
<tr>
<td>13</td>
<td>Sunday Is. – south</td>
<td>SI</td>
<td>47</td>
<td>20</td>
<td>6</td>
<td>0.43</td>
<td>27</td>
<td>1.58</td>
<td>0.05</td>
<td>0.52</td>
<td>0.119</td>
<td>0.132</td>
<td>0.105*</td>
<td>0.01</td>
</tr>
<tr>
<td>14</td>
<td>Halls Pool</td>
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<td>6</td>
<td>0.66</td>
<td>27</td>
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<td>0.07</td>
<td>-0.01</td>
<td>0.104</td>
<td>0.171</td>
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<td>0.00</td>
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<td>16</td>
<td>0.36</td>
<td>31</td>
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<td>0.141</td>
<td>0.047</td>
<td>0.00</td>
</tr>
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<td>0</td>
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</tr>
<tr>
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<td>32</td>
<td>1.76</td>
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<td>0.01</td>
<td>0.240</td>
<td>0.218</td>
<td>-</td>
<td>0.102*</td>
</tr>
</tbody>
</table>

**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.

<table>
<thead>
<tr>
<th></th>
<th>BAT</th>
<th>LI</th>
<th>BN</th>
<th>BS</th>
<th>RI</th>
<th>MI</th>
<th>SN</th>
<th>SS</th>
<th>HP</th>
<th>TI</th>
<th>JI</th>
<th>NY</th>
<th>SB</th>
<th>CK</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>-</td>
<td>0.22</td>
<td>0.237</td>
<td>0.132</td>
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<td>0.091</td>
<td>0.102</td>
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<td>0.054</td>
<td>0.057</td>
<td>0.058</td>
<td>0.08</td>
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<td>0.055</td>
<td>0.038</td>
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<td>0.13</td>
<td>0.145</td>
<td>0.044</td>
<td>0.055</td>
<td>0.054</td>
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<td>-</td>
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<td>0.08</td>
<td>0.065</td>
<td>0.077</td>
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</tr>
<tr>
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<td>0.152</td>
<td>0.186</td>
<td>-</td>
<td>0.03</td>
<td>0.029</td>
<td>0.079</td>
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<td>0.199</td>
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<td>0.160</td>
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<td>0.135</td>
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<td>0.085</td>
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<td>0.13</td>
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<td>0.429</td>
<td>0.125</td>
<td>0.224</td>
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<td>0.176</td>
<td>0.092</td>
<td>0.099</td>
<td>0.197</td>
<td>0.189</td>
<td>0.265</td>
<td>0.237</td>
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<td>C</td>
<td>0.499</td>
<td>0.491</td>
<td>0.561</td>
<td>0.533</td>
<td>0.456</td>
<td>0.489</td>
<td>0.558</td>
<td>0.561</td>
<td>0.512</td>
<td>0.517</td>
<td>0.530</td>
<td>0.569</td>
<td>-</td>
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</tr>
</tbody>
</table>
Population genetic diversity, structure and connectivity of two seagrass species, Thalassia hemprichii and Halophila ovalis in the Kimberley

Figure 6: Cluster of populations resulted STRUCTURE analysis. Each individual is represented by a thin vertical line, which is partitioned into K segments that represent the estimated population group membership fractions. Each colour represents a distinct population. Black lines separate individuals from geographical site locations.
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.

![Spatial autocorrelation graph](image)

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 (*N*<sub>mt</sub>) 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 (*N*<sub>mt</sub> = 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).
Population genetic diversity, structure and connectivity of two seagrass species, Thalassia hemprichii and Halophila ovalis in the Kimberley

Figure 8: Map of the sampling sites and the pattern of gene flow based on $\hat{N}_m$ (relative number of migrants per generation). Sampling sites (populations) were represented by numbers within circles (referred to Table 1). Levels of $\hat{N}_m$ among sampling sites were represented by curved lines. The thicker the lines, the higher levels of gene flow between populations. The base map was obtained from OPENSTREETMAP contributors (https://www.openstreetmap.org/copyright).

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

<table>
<thead>
<tr>
<th></th>
<th>Total strength</th>
<th>Closeness</th>
<th>Transitivity</th>
<th>Betweenness</th>
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<tr>
<td>BAT</td>
<td>4.70</td>
<td>0.74</td>
<td>1.31</td>
<td>50</td>
</tr>
<tr>
<td>LI</td>
<td>10.26</td>
<td>0.71</td>
<td>0.70</td>
<td>33</td>
</tr>
<tr>
<td>BN</td>
<td>19.68</td>
<td>0.35</td>
<td>1.12</td>
<td>0</td>
</tr>
<tr>
<td>BS</td>
<td>15.96</td>
<td>0.78</td>
<td>0.95</td>
<td>1</td>
</tr>
<tr>
<td>RI</td>
<td>20.19</td>
<td>0.70</td>
<td>1.06</td>
<td>15</td>
</tr>
<tr>
<td>MI</td>
<td>16.53</td>
<td>0.66</td>
<td>0.96</td>
<td>0</td>
</tr>
<tr>
<td>SN</td>
<td>18.46</td>
<td>0.34</td>
<td>0.83</td>
<td>11</td>
</tr>
<tr>
<td>SS</td>
<td>20.32</td>
<td>0.37</td>
<td>1.03</td>
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<tr>
<td>HP</td>
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<td>0.59</td>
<td>0.93</td>
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</tr>
<tr>
<td>TI</td>
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<tr>
<td>JI</td>
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<td>0.42</td>
<td>0.80</td>
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<tr>
<td>NY</td>
<td>17.13</td>
<td>0.36</td>
<td>1.07</td>
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</tr>
</tbody>
</table>
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).

![Graph showing isolation by spatial and oceanographic distance](image_url)

Figure 9: Isolation by spatial distance (Top) and oceanographic distance (Bottom) of *Thalassia hemprichii* between twelve sites in the Sunday Islands Group and the Buccaneer Archipelago. There was significant but weak isolation by spatial distance ($r^2=0.18$, $p<0.001$) and significant isolation by oceanographic distance ($r^2=0.24$, $p<0.001$).

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

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.

<table>
<thead>
<tr>
<th></th>
<th>$R^2_{adj}$ (%)</th>
<th>$df_{mod}$</th>
<th>$df_{res}$</th>
<th>$F$</th>
<th>p-value</th>
</tr>
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<tbody>
<tr>
<td>Marginal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>EN</td>
<td>54.51</td>
<td>3</td>
<td>9</td>
<td>5.793</td>
<td>0.025</td>
</tr>
<tr>
<td>OC</td>
<td>62.46</td>
<td>3</td>
<td>9</td>
<td>7.655</td>
<td>0.002</td>
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<td>GD</td>
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<td>8</td>
<td>1.334</td>
<td>0.292</td>
</tr>
<tr>
<td>Residual</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conditional</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EN</td>
<td>(OC + GD)</td>
<td>17.49</td>
<td>3</td>
<td>2</td>
<td>2.034</td>
</tr>
<tr>
<td>OC</td>
<td>(EN + GD)</td>
<td>23.00</td>
<td>3</td>
<td>2</td>
<td>2.359</td>
</tr>
<tr>
<td>GD</td>
<td>(OC + GD)</td>
<td>13.11</td>
<td>4</td>
<td>2</td>
<td>1.697</td>
</tr>
</tbody>
</table>

Figure 11. Decomposition of among-population variation (expressed as percentage) on genetic differentiation into spatial (GD), environmental (EN) and oceanographic (OC) components.
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).
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.
Population genetic diversity, structure and connectivity of two seagrass species, *Thalassia hemprichii* and *Halophila ovalis* in the Kimberley


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).](image)

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.

<table>
<thead>
<tr>
<th>Population</th>
<th>Thalassia hemprichii</th>
<th></th>
<th>Halophila ovalis</th>
<th></th>
<th>TOTAL BOTH SPECIES</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Connection</td>
<td>Stepping stone</td>
<td>Genetic diversity</td>
<td>TOTAL</td>
<td>Connection</td>
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<td>0</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Irvine Is.</td>
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<td>nd</td>
<td>0</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Longitude Is.</td>
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<td>nd</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Bedford Is. North</td>
<td>x</td>
<td>1</td>
<td>0</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Bedford Is. South</td>
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<td>x</td>
<td>2</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Riptide Is./Gregory Is.</td>
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<td>x</td>
<td>2</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Mermaid Is.</td>
<td>x</td>
<td>1</td>
<td>nd</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Sunday Is. --north</td>
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<td>0</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Sunday Is. --south east, Janinko</td>
<td>x</td>
<td>1</td>
<td>x</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Hal’s Pool, Ngoorroodool</td>
<td>x</td>
<td>1</td>
<td>x</td>
<td></td>
<td>x</td>
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Meirmans P (2015) Seven common mistakes in population genetics and how to avoid them. Molecular Ecology 24:3223–3231


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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.


- 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


- 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 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 though 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

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WAMSI Kimberley Marine Research Program

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

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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)
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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 ≤ 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.
1 Introduction

Australia’s North-West Shelf is a large ecologically and economically significant marine bioregion encompassing coastal and oceanic waters to the edge of the Australia’s north-west continental shelf. The region is a marine biodiversity hotspot, but in comparison to other parts of the world, it is little studied and poorly understood, particularly the northernmost Kimberley region (Wilson 2013; Figure 1). The Kimberley is the subject of growing scientific interest because of its pristine state and unique biota (Anon 2011). It is also subject to increasing interest from industry (Simpson 2011), which has motivated the establishment of strategically placed marine reserves for the management of regional biodiversity (Anon 2011).

Two faunal assemblages dominate coral reefs of the North-West Shelf bioregion: those inhabiting offshore reefs and atolls along the continental shelf margin (“oceanic”), and those inhabiting shallow coastal waters (“coastal”; Wilson 2013). The existence of these characteristic assemblages has been attributed to the distinctive hydrodynamic, geological, and water column properties of the coastal and oceanic regions (Wilson 2013). The hydrodynamics of the oceanic reefs are dominated by a southwest flowing extension of the Indonesian Through Flow (ITF). This current carries warm clear oligotrophic waters from the west Pacific Ocean southwards through passages in the eastern Indonesian archipelago and along the western Australian continental margin (Figure 1). The biogeographic affinities of offshore biota to Indonesian marine communities, and the prevalence of species with planktotrophic larvae indicates that the ITF provides significant biogeographic linkage among oceanic reefs (Wilson 2013).

The ITF does not typically reach coastal Kimberley waters (Cresswell et al. 1993, Condie & Andrewartha 2008). In contrast to oceanic reefs, the hydrodynamics of the coast are dominated by extremely large semi-diurnal tides (up to 11 metres), which, although they typically act cross-shelf, interact with complex bathymetries and island archipelagos to produce powerful multidirectional and highly idiosyncratic currents (Holloway 1983). Owing to this turbulent environment, higher levels of nutrients, as well as freshwater and sediment inputs from large rivers, coastal Kimberley environments are highly turbid and hostile for many organisms (Richards et al. 2015).

Whether the distinctive oceanic and coastal faunas can be attributed to these pronounced environmental differences or to hydrodynamic isolation has been a point of discussion among biogeographers (Wilson 2013). Levels of genetic connectivity between corals and fishes inhabiting oceanic reefs has been investigated recently, and confirmed that occasional gene flow occurs between widely separated reefs (c. 500km apart; Underwood et al. 2009, Underwood et al. 2012). This is consistent with hydrodynamic modelling of the ITF and its derivative currents, which predict organisms could be transported between oceanic reefs if larval durations exceed a month (Condie & Andrewartha 2008, Kool & Nichol 2015).

Hydrodynamic models also indicate that connectivity between the oceanic reefs and coastal Kimberley environments is significantly weaker than among oceanic reefs (Condie & Andrewartha 2008, Kool & Nichol 2015). However, the scarcity of species common to both environments has meant that this hypothesis is untested. This is significant because of interest in understanding the origins and affinities of the coastal Kimberley faunas (Wilson 2013). In addition, both coastal and oceanic faunas are harvested by commercial and artisanal fishers (Berry 1993, Fletcher & Santoro 2013), and there is a need to understand the likely demographic and genetic dependencies among these harvested reefs (Rees et al. 2003).

The top shell, *Tectus niloticus* (“trochus”) provides an excellent model to test notions of connectivity within and between the oceanic and coastal environments of north-western Australia. Unusually, this large herbivorous gastropod (up to 12cm diameter) occurs in both oceanic and coastal environments of Australia’s north-west, and on intertidal and subtidal reefs throughout the Indo-Pacific. Understanding levels of connectivity among *T. niloticus* populations has practical significance since the species is harvested for food and mother-of-pearl shell in coastal Australian waters by indigenous traditional owners and on oceanic reefs by traditional Indonesian trepang fisherman (Purcell & Cheng 2010).
Over-harvest of *T. niloticus* has been documented on numerous occasions throughout its range (Foale 1998, Bartlett et al. 2009), and notably on Australia’s north-western offshore reefs (Skewes et al. 1999). Local depletions have also been documented in coastal Kimberley regions where indigenous harvest has been strongest (Purcell & Cheng 2010). These observations are consistent with the short non-feeding larval phase documented for *T. niloticus* (c. 4 days; Heslinga 1981, Heslinga & Hillmann 1981). In marine species this trait means capacities for dispersal are limited and local environmental and hydrodynamic processes strongly dictate the dynamics of recruitment (Sammarco & Andrews 1989). For this reason there has been concern that recruitment in *T. niloticus* may primarily be local, making the species prone to over-harvest (Heslinga 1981, Bartlett et al. 2009). Likewise, it’s been argued that placement of reserves adjacent to harvested regions would be an effective way to sustain populations of *T. niloticus* (Heslinga et al. 1984).

Yet, little is understood about realised dispersal in *T. niloticus*, and this remains a major limitation to its effective management (Foale 1998, Stutterd & Williams 2003). An investigation of genetic sub-division in *T. niloticus* on the Australian Great Barrier Reef based on 3 polymorphic allozyme loci indicated that gene flow was extensive over almost 1000 km (Borsa & Benzie 1996). However, these conclusions were weakly supported because the markers exhibited little variation. Nevertheless, they point to a potential paradox between the observation of short larval duration and susceptibility to local overharvest, and widespread realised dispersal. One explanation for this may be that the capacity for oceanographic processes to distribute *T. niloticus* larvae is not properly appreciated, even though they have an apparently short larval duration (Heslinga 1981, Foale 1998). Indeed, the species has a reasonably widespread native distribution across Indo-Malaysian and west Pacific island archipelagos, indicating capacity for broad dispersal over long time frames.

Here, we employ a genotype-by-sequencing approach to reveal the extent of genetic structure within harvested coastal and oceanic *T. niloticus* populations in north-western Australia, testing the notion that connectivity is greater within these regions than between them. We also employ particle tracking simulations to evaluate, for the first time, the role of coastal currents and tides in dictating the observed genetic structure in this prominent species from the coastal Kimberley environment.
2 Materials and Methods

2.1 Sampling

Field trips were conducted to the Dampier Peninsular and Buccaneer Archipelago in the coastal Kimberley in August and October 2014 respectively (Figure 1). This region is the centre of trochus harvest by indigenous fishers (Ostle 1996), and falls within the only gazetted commercial trochus fishery in Western Australia (Anon 2010). In addition, samples were collected from the offshore sites the Rowley Shoals and Scott Reef in October 2014 and April 2015 respectively. Trochus from both Scott Reef and the Rowley Shoals were historically targeted by Indonesian fishers, but only Scott Reef remains accessible to them (Fox 2009). Sites were selected based on past records of occurrence, and suitability of rocky reef habitat (Lee & Lynch 1997). *T. niloticus* individuals were collected by hand from reef platforms at low tide, or at Scott Reef and the Rowley Shoals by scuba in < 10 metres water depth. GPS coordinates were obtained for each individual. Samples were collected from a total of 16 sites on the Dampier Peninsular and the Buccaneer Archipelago, 6 sites at the Rowley Shoals, and 3 sites at Scott Reef. Individuals were returned to a central processing area where a small biopsy (c. 3mm x 8mm) was taken with sharp dissecting scissors from the edge of the extended foot. Scissors were sterilised between samples. The maximal basal width of each individual was measured to within 1mm. After processing the *T. niloticus* individuals were returned to their approximate point of capture. A total of 524 individuals were sampled. All activities took place under exemption permits SF009910 from the Western Australian Department of Parks and Wildlife, and 2485 and 2295 from the Western Australian Department of Fisheries.

2.2 DNA Extraction

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

2.3 Reduced Representation SNP Genotyping

SNP genotypes were obtained with DArTSeq, a combination of the DArT™ complexity reduction methods and next generation sequencing (Sansaloni et al. 2011, Killian et al. 2012, Cruz et al. 2013). The method is conceptually similar to Rad-Seq methods but offers a number of advantages including: 1) use of lower DNA input; 2) greater tolerance to lower quality DNA; and 3) higher call rate/frequency of markers shared among the samples in the experiment (Sansaloni et al. 2011). Four enzyme systems for complexity reduction were tested in *P. milleri* (data not presented) and the PstI- HpaII method selected. DNA samples were processed in digestion/ligation reactions principally (as per Kilian et al. 2012) but replacing a single PstI-compatible adaptor with PstI and HpaII adaptors. The PstI-compatible adapter was designed to include an Illumina flow cell attachment sequence, sequencing primer and a “staggered” barcode region of varying lengths (see Elshire et al. 2011). The reverse adapter contained a flow cell attachment region and a HpaII-compatible overhang sequence. Only “mixed fragments” (PstI-Hpall) were effectively amplified by PCR. PCR conditions consisted of an initial denaturation 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.
Isolation of oceanic and coastal populations of the harvested mother-of-pearl shell *Tectus niloticus* in the Kimberley

**Figure 1.** Map showing the entire study region, bathymetry and sampling sites for *T. niloticus* (yellow circles). Dashed area indicates coastal locations (see Figure 2).

**Figure 2.** Map showing the sampling sites within the coastal Kimberley region (yellow circles).
2.4 **SNP Calling**

Sequences were processed using proprietary DArTseq analytical pipelines. In the primary pipeline, poor quality sequences were initially filtered from FASTQ files, applying higher stringency to the barcode region than to the rest of the sequence (barcode min. Phred score 30, min. pass % 75; whole read min. Phred score 10, min. pass % 50). Approximately 2,000,000 sequences per barcode/sample were identified and used for marker calling. Identical sequences were collapsed into “fastqcoll files”, which were groomed using DArT’s proprietary algorithm that corrects low quality bases from singleton reads using collapsed reads with multiple members as a template. The groomed fastqcoll files were used in the secondary pipeline for DArT’s proprietary SNP calling algorithms (DArTsoft14). All reads from all libraries were clustered using DArT PL’s C++ algorithm at the threshold distance of 3 (number of difference in bases occupying specific position in the sequence), followed by parsing of the clusters into separate SNP loci using a range of technical parameters, especially the balance of read counts for the allelic pairs. Additional selection criteria were added to the algorithm based on analysis of approximately 1,000 controlled cross populations. These crosses permitted testing for Mendelian distribution of alleles in these populations and facilitated selection of technical parameters discriminating true allelic variants from paralogous sequences. In addition, approximately one third of samples were genotyped twice as technical replicates and scoring consistency was used as the main selection criteria for high quality/low error rate markers. A total of 29,552 SNPs were identified.

2.5 **SNP Quality Control Filtering**

SNPs identified by the DArTsoft14 pipeline were subject to a further series of quality control filters based on descriptive statistics from the DArTseq pipeline. These settings were as follows: minimum allele frequency ≥ 0.05, heterozygosity ≤ 0.75, number of reads ≥ 20 and ≤ 200, repeatability of technical replicates ≥ 0.98, call rate/locus ≥ 0.95, missing data/individual ≤ 0.01, and missing data per individual < 5%. After filtering, a total of 6398 loci and 516 (of 524) individuals remained (Appendix 1).

2.6 **Locus Selection**

Filtered SNPs were subject to further checks for departure from Hardy-Weinberg equilibrium and gametic-phase disequilibrium expectations. Testing for Hardy-Weinberg equilibrium made use of custom R scripts and the R packages SNPassoc (González et al. 2007) and pegas (González et al. 2007, Paradis 2010, R Core Team 2014). Testing for gametic-phase disequilibrium made use of custom R scripts (supplementary material) and the R packages doParallel (Calaway et al. 2014) and Adegenet (Jombart 2008). Both Hardy-Weinberg and gametic-phase disequilibrium testing was carried out separately for each sampling site, and only applied to sites where the sample size was greater than 20. For Hardy-Weinberg testing we removed loci that showed departures from expectations at P < 0.05 in 5 or more of the 13 sample sites. For gametic-phase disequilibrium we removed loci with $R^2$ values > 0.8 in 5 or more of the 13 sampling sites. After these filters 5584 loci remained. Further analyses completed with different thresholds (3-7 populations) for numbers of populations in LD and HWE testing returned near-identical results (data not shown).

2.7 **Testing for Markers Under Selection**

We used the R package OutFlank (Whitlock & Lotterhos 2015) to identify outlier loci putatively under the influence of directional selection. 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 are more 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). 156 putatively SNPs under putative directional selection were identified. These loci were removed from further analyses unless noted. The outlier analysis was repeated separately on a dataset consisting of samples collected from the coastal Kimberley only (N = 484; i.e. excluding the Rowley Shoals and Scott Reef).
Outlier loci were Blasted against the Ensemble database to search for significant homology to genes of known function. Search criteria were maximum E-value of 1.00E-05 and identity ≥ 85%.

2.8 Descriptive Statistics

Levels of genetic diversity (observed and expected heterozygosity, allelic richness) and the inbreeding coefficient (Fst) were calculated for each sampling site with the R package Hierfstat (Goudet 2005).

2.9 Genetic Sub-Division

The fixation index of genetic sub-division (Fst) was estimated overall and pairwise between each sampling site according to the (Weir & Cockerham 1984) method with the R package STAMPP (Pembleton et al. 2013). The significance of the observed subdivision between all pairs of sampling sites was tested with 9999 bootstraps over loci. Tests of overall genic differentiation among sampling sites and between coastal Kimberley sampling sites were conducted with GenePop (Rousset 2008), based on genotypic differentiation and exact G tests. MCMC settings were as follows: dememorization 1000, batches 100, iterations per batch 1000.

2.10 Isolation by Distance

We applied Mantel tests to evaluate correlations between linearised FST (FST/(1-FST)) and distance as well as oceanographic distance. Mantel tests were conducted on the whole dataset, and on the coastal Kimberley dataset. Geographic distances between sites were calculated based on the shortest across-water distance with a minimum water depth of 1m as this was the minimum depth specifiable. 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. Oceanographic resistance was calculated from particle tracking simulations (see below). We employed partial Mantel tests to test for a correlation between linearised FST and oceanographic resistance while controlling for log(geographic distance). This analysis was conducted with the Vegan package in R (Oksanen et al. 2007).

2.11 Oceanographic Connectivity

Hydrodynamic connectivity between sampling sites in the coastal Kimberley was calculated through particle tracking simulations nested within a Regional Ocean Modelling System with 2 km resolution to construct a site-pairwise matrix of oceanographic connectivity. The model was nested within the Ocean Forecasting for Australia Model 3 (OFAM3) simulation (Feng et al. 2016) and forced by 3-hourly meteorological measures derived from Kobayashi et al (2015). The model simulation was based on data from 2011. Hourly sea surface current velocities (0-5 m) were extracted from the model output and used for particle tracking modelling. A total of 100 particles were seeded at each sampling site for the entire year at 3-day intervals. *T. niloticus* in King Sound is reproductively active all year, whereas elsewhere its reproduction is seasonal (Gimin & Lee 1996). A 4th-order Runga-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 m2s-1, particles were tracked for 8 days. The locations of particles were recorded during the period 4-8 days. *T. niloticus* larvae are non-feeding and become competent to settle after 3-4 days (Heslinga & Hillmann 1981), but because larvae can remain swimming for up to 10 days in the absence of settlement cues (Heslinga 1981) we allowed a further competency period of 4 days for settlement. The grid size for tracking the particles from each sampling site was set to 500m x 500m. Connectivity among sampling sites was estimated as one minus the proportion of particles released from site i that were tracked to be in site j during the competency period. To make this matrix symmetrical we summed connectivity between i and j and j and i. Oceanographic connectivity was calculated as the proportion of released particles from i and j that settled at i and j. This value was converted to an oceanic resistance as 1 - oceanographic connectivity. Values were arcsine transformed.
before further analysis.

2.12 Principal Components Analysis and Discriminant Analysis of Principal Components

Initially we employed the k-means algorithm to evaluate support for between one and fifteen potential clusters (K) in the data. This was implemented in the R package adegenet 2.0. Support for alternative values of K was evaluated with the Bayesian information content (BIC) based on retaining all principal components. A discriminant analysis of principal components (DAPC) was then conducted on the data without specifying K and based on 100 retained principal components. In addition, a principal components analysis was conducted on the data with the gIPCA function in adegenet 2.0. All analysis was repeated on the coastal sites only (excluding the Rowley Shoals and Scott Reef).

2.13 Model-based Clustering Analysis

We used a model-based clustering approach to evaluate whether genetic variation was partitioned geographically, and at what scale. This was implemented in the software Structure 2.3.4 (Pritchard et al. 2000) run on the CSIRO Accelerator Cluster “Bragg”. Structure seeks to group individuals in such a way that the groups maximise conformity to Hardy-Weinberg and linkage equilibrium. We ran Structure across multiple pre-defined values for K (number of clusters), and evaluated the fit of the data to different values of K. We conducted an overall analysis incorporating all 13 sampling sites and varied K between 2 and 16, we conducted the same analysis on the coastal sites only. The fits of alternative models were evaluated with the Delta K method (Evanno et al., 2005) implemented in Clumpak (Kopelman et al., 2015) and based on 20 independent runs for each value of K. For all runs we incorporated a 200,000 iteration burnin followed by 500,000 clustering iterations. We ensured the adequacy of the run length by checking the runtime likelihood and alpha for stability. For all runs we assumed that allele frequencies were correlated between sampling sites and allowed for admixture. We repeated runs with and without location information incorporated as a Bayesian prior.

3 Results

3.1 Descriptive Statistics

5,428 SNP loci remained after QC filtering, Hardy-Weinberg and Linkage disequilibrium filtering and outlier analyses. Table 1 reports estimates of genetic diversity at each sampling site and overall. The two offshore sites exhibited lower observed and expected heterozygosity than coastal sites.

3.2 Loci under selection

In an analysis of the full dataset, 156 loci were identified as being positive outliers, consistent with being under directional selection. No loci showed evidence of stabilising selection. In the coastal Kimberley dataset (excluding the Rowley Shoals and Scott Reef) no outlier loci were identified. None of the outlier loci showed significant Blast homology to genes against the Ensembl genome database.

3.3 Genetic Sub-division

The overall level of genetic subdivision based on the putatively neutral markers as estimated by $F_{ST}$ was 0.0053 (±0.0001SE), which corresponded to a significant genotypic differentiation among sampling sites ($P < 0.001$). $F_{ST}$ based on outlier loci was 0.0998 (±0.0051SE). $F_{ST}$ among the coastal Kimberley sites was 5.44 x 10-5 (± 8.89 x 10-5SE), which corresponded to a non-significant difference in genotypic differentiation ($P = 0.19$). Pairwise $F_{ST}$ between Kimberley sites was typically low (Table 2), and only 11 of 105 pairs were significantly different from zero at $P < 0.05$. $F_{ST}$ between Scott Reef and the Rowley Shoals was 0.0163 (±0.00015E), which was significantly different from zero ($P < 0.001$). $F_{ST}$ between combined Kimberley sites and combined offshore sites was 0.0348 (± 0.0008), which was significantly different from zero ($P < 0.0001$), but $F_{ST}$ based on outlier loci was 0.3518 (±0.0022).
Table 1. Descriptive statistics for 5428 neutral SNP markers genotyped in 514 individual *T. niloticus* from Western Australia. N mean number of samples; *He* expected heterozygosity; *Ho* observed heterozygosity; *Fis* inbreeding coefficient; *Ar* allelic richness. All values are ± standard error in parentheses.

<table>
<thead>
<tr>
<th>Site (north-south)</th>
<th>Region</th>
<th>N</th>
<th><em>He</em></th>
<th><em>Ho</em></th>
<th><em>Fis</em></th>
<th><em>Ar</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Bathurst Is. North</td>
<td>Coastal</td>
<td>27.836</td>
<td>0.304</td>
<td>0.280</td>
<td>0.073</td>
<td>1.550</td>
</tr>
<tr>
<td>Bowles Reef</td>
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<td>0.307</td>
<td>0.282</td>
<td>0.079</td>
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</tr>
<tr>
<td>Irvine Island</td>
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<td>0.306</td>
<td>0.278</td>
<td>0.064</td>
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<tr>
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<td>0.068</td>
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</tr>
<tr>
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<td>0.078</td>
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</tr>
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<td>0.286</td>
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<tr>
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<td>0.264</td>
<td>0.084</td>
<td>1.551</td>
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</table>
Table 2. $F_{ST}$ values for pairwise comparisons (below diagonal). Calculated in R using package STAMPP based on the Weir & Cockerham (1984) method. P values ≤ 0.5 indicated with * calculated based on 1000 bootstraps over loci. Grey cells indicate $F_{ST}$ of zero.
3.4 Isolation by distance and oceanic resistance

Within the coastal Kimberley there was no significant relationship between linearised $F_{ST}$ and shortest cross-water distance between sampling sites ($R = 0.042, P = 0.36$). Neither was there a significant relationship between linearised $F_{ST}$ and oceanographic resistance ($R = -0.04, P = 0.62$). Geographic distance and oceanographic resistance were strongly correlated based on both the 8 day particle tracking run ($R = 0.76, P < 0.01$), and the 40 day particle tracking run ($R = 0.77, P < 0.01$). A partial Mantel test that controlled for geographic distance did not reveal a significant relationship between linearised $F_{ST}$ and oceanographic resistance based on 8 day ($R = -0.118, P = 0.809$), or 40 day runs ($R = -0.169, P = 0.902$).

3.5 Model-based clustering

The data best supported two genetically discrete groups of $T$. niloticus individuals; maximum $\Delta K$ was obtained for $K$ of two whether location prior was included or not ($\Delta K = 321.4$ and $34.8$ respectively; Appendix 2).

3.6 Principal Components Analysis and Discriminant Analysis of Principal Components

The k-means algorithm was optimised at $K = 2$ (Appendix 2). Both principal components and DAPC analyses revealed that these groups corresponded exactly to offshore and coastal sites (Figure 4), and that all individuals were assigned to their correct group at $\geq 0.99$ probability of membership in the DAPC analysis. No grouping with a geographical basis was observed in the analysis of the coastal sites only.

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**Figure 3.** Bar plots indicating probability of ancestry (q) for 514 individual $T$. niloticus individuals from the coastal Kimberley and from the Rowley Shoals and Scott Reef. Plots are based on $K = 2$ (2 genetic clusters assumed). Individuals are represented by vertical bars, each divided according to their estimated probability of ancestry from each of 2 genetic clusters (represented by blue and orange). Coastal sites are ordered north-south from left-right. Upper panel indicates analysis completed with locations set as Bayesian priors in the analysis. Lower panel shows results without prior location assumptions.
Figure 4. Principal components analysis for 514 *T. niloticus* individuals. Inertia ellipses are centred on the mean coordinates of points from each sampling site and width and height correspond to variances on each axis. Here they are scaled to incorporate c. 77% of the points from each sampling site (1.5 * variance). In the legend samples are ordered north to south, but with the two offshore sites listed last.
4 Discussion and Conclusions

Harvested trochus populations from the west Kimberley and adjacent offshore reefs represent two genetically and demographically independent units, with the oceanic trochus populations being further subdivided into two largely independent units corresponding to the Rowley Shoals and Scott Reef. The very high levels of genetic subdivision between oceanic and coastal populations exhibited in a subset of loci, also indicate that divergent selection is driving evolutionary adaptation to these distinctive environments.

These conclusions are based on a large dataset of both individuals, and particularly, SNP markers, meaning that unlike previous genetic studies of *T. niloticus* (Borsa & Benzie 1996), the power to detect population structure was high (Willing et al. 2012). Accordingly, the lack of genetic structure observed within the most heavily harvested region between the Dampier Peninsular and Buccaneer Archipelago strongly indicates that it represents a single genetic unit.

The small sample size for Scott Reef reflects the difficulty of collecting *T. niloticus* at this heavily harvested location (Skewes et al. 1999). While it would be ideal to base analyses on larger samples from this location, the principal components and model-based clustering analyses based on individual genotypes rather than population-based allele frequencies indicate that samples from Scott Reef are readily distinguished from coastal populations, and are also distinct from the Rowley Shoals. Furthermore, the large number of SNP markers employed here means that the estimate of $F_{ST}$ are likely highly accurate (Willing et al. 2012).

4.1 Connectivity between coastal and oceanic reefs

This is the first time that levels of realised connectivity between coastal Kimberley and offshore reefs has been characterised. It confirms modelling results that the two regions have very limited hydrodynamic connectivity (Condie & Andrewartha 2008, Kool & Nichol 2015), which in part explains the widely divergent reef biotas. It is also consistent with recent observations in a species of coral that exhibit strong genetic subdivision between oceanic and coastal regions (sub-report 1.1.3.1). The magnitude of the subdivision in the corals was up to an order of magnitude greater than in *T. niloticus*, however, likely indicative of a much smaller effective population size, or less dispersive ability in the corals.

Hydrodynamic modelling predicts that if larvae can remain suspended for longer than a month they may be transported between the isolated oceanic atoll systems such as Scott Reef and the Rowley Shoals (Kool & Nichol 2015). This has now been tested in a number of species including fishes and corals, which confirm that transport is uncommon enough that the systems are demographically independent (Underwood et al. 2009, Underwood et al. 2012). *T. niloticus* adds a further example but the magnitude of the differentiation between Scott Reef and the Rowley Shoals is an order of magnitude larger than that observed in the Pomacentrid fish *Chromis margaritifer* (Underwood et al. 2012), and an order of magnitude lower than that observed between corals (Underwood in review), likely reflecting different capacities for dispersal and/ or population sizes in these organisms.

These oceanic atolls systems therefore can be considered to operate independently on ecological timescales relevant to management since recovery after disturbances is unlikely to be driven by recruitment from outside in the short term. This is relevant to the ongoing harvest of *T. niloticus* from Scott Reef by artisanal Indonesian fishers, where the stock is believed to be significantly depleted (Skewes et al. 1999). This stock is unlikely to recover unless local recruitment is increased because recruitment from other sources is demographically insignificant. Similar conclusions have been drawn for corals on these atolls, which are vulnerable to bleaching (Gilmour et al. 2013).

Translocations have been extensively used throughout the range of *T. niloticus* to supplement over-harvested stocks, with mixed success (Stutterd & Williams 2003). Our finding that oceanic populations exhibit signs of adaptive differentiation from coastal populations indicate that any supplementation of oceanic populations like
Scott Reef would best be sourced from populations sharing similar oceanic environmental conditions. The same principle should be considered elsewhere in the Indo-Pacific for translocations, de novo establishment, or aquaculture in this species. Whether the low success rate of re-seeding of *T. niloticus* (Stutterd & Williams 2003) can be attributed in part to mal-adaptation is unclear.

4.2 Connectivity among coastal reefs in the Kimberley

The lack of any observable genetic structure within the coastal Kimberley region indicates that this region functions effectively as a single demographic unit, and should be considered as such for management purposes. This result is consistent with those for *T. niloticus* on the Great Barrier Reef (Borsa & Benzie 1996), yet is somewhat paradoxical considering the apparently brief non-feeding larval period, and belief that larval dispersal and gene flow is limited in the species (Foale & Day 1997), as it is in related marine gastropods with similar larval life-histories (Prince et al. 1987, Temby et al. 2007). The reasons for this observation are unclear, however two unique properties of the Dampier peninsular and Buccaneer archipelagos may be relevant. First, the region experiences extreme tidal velocities up to 2 m/s (Cresswell & Badcock 2000), which in principle are capable of transporting buoyant objects almost the length of the region in a single 6 hour tidal cycle. The second is that *T. niloticus* in the region, unlike most other parts of their range, reproduce continuously throughout the year. Larvae are therefore exposed to the complete spectrum of hydrodynamic conditions experienced in the region.

It’s been argued that close placement of reserves for *T. niloticus* to harvested areas is a way to account for local recruitment patterns in the species (Heslinga et al. 1984). In the case of King Sound, however, the hydrodynamic mixing appears to be strong enough that reserves within the region would be well connected to harvested areas. It’s unclear why other species investigated in this project, specifically the seagrasses *Thalassia hemprichii* and *Halophila ovalis* (sub-report 1.1.3.2) and the corals *Acropora aspera* and *Isopora brueggmanni* (sub-report 1.1.3.1) do not also exhibit the same level of mixing in this region. It points to differences in larval behaviour in relation to the hydrodynamics, the more seasonal reproduction, or potentially differences in settling behaviour as being key differences.

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 (Stutterd & Williams 2003). 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 (Amos 1997), a broader investigation of population structure in *T. niloticus* and its biophysical drivers deserves consideration.

One aspect of interest is the relationship between *T. niloticus* populations in Indonesia to those on the Australian coast and oceanic atolls. Based on the path of the ITF, populations inhabiting oceanic atolls may have a greater affinity to those in Indonesia than coastal Australia, with the Kimberley populations being relatively isolated from the “coral triangle” centre of marine tropical biodiversity (Wilson 2013). This was an observation made in the seagrass *Thalassia hemprichii* (sub-report 1.1.3.2), for which Kimberley populations exhibit lower genetic diversity, and less genetic affinity to those in Indonesia than Australian populations further south and with more direct exposure to ITF derivative currents. The Kimberley maintains a biodiverse reef fauna (Wilson 2013), and if insularity is common among these species, it indicates that the origins of this biodiversity may to some extent be independent of the mega-biodiverse coral triangle.

Conclusions

Both hydrodynamic isolation and environmental differences have been hypothesised to explain the starkly different coral reef faunas on oceanic and coastal regions of north-western Australia (Wilson 2013). Our results indicate that both of these hypotheses are likely true. Not only is there little genetic exchange in *T. niloticus*
between the coast and oceanic reefs, but a significant number of genomic regions exhibit very strong differentiation that is consistent with evolutionary adaptation to local conditions. We anticipate similar results would be evident for other species with equal or lower larval dispersal capabilities to *T. niloticus*.

*T. niloticus* is harvested from both coastal and oceanic reefs, each under separate management (Fletcher & Santoro 2013). Populations in both environments exhibit signs of over-harvest, particularly the oceanic reefs (Skewes et al. 1999). The separate management of these regions is consistent with the evidence for their isolation presented here. There are two additional implications for management of *T. niloticus*. First, the isolation of the offshore reefs from any other source of recruits indicates that they are almost entirely reliant on local recruitment to offset harvest. The heavy harvest and resulting low density of *T. niloticus* at Scott Reef, coupled with a broadcast-spawning reproduction, indicates the capacity for local recruitment is likely heavily reduced. Second, *T. niloticus* on the Dampier Peninsular and Buccaneer Archipelago can be considered 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, so long as management ensures that such sites exist, and allowing for the slow growth of the species.
5 References


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

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7 Data Availability

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

8 Communication

8.1 Presentations
- WAMSI Research Conference, March 30th – 1st April, 2015
- Society of Australian Systematic Biologists (SASB) and Invertebrate Conservation conference, Fremantle, December 6-9, 2015.
- WAMSI Lunch and Learn Seminar, August 19, 2016.

8.2 Other communications achievements
- Field trip report in the Bardi-Jawi Newsletter
- Presentation to the Kimberley Indigenous Saltwater Science Project, 10th March 2017.
9 Appendices

Appendix 1. Frequency histograms of descriptive statistics used to filter 28,076 single nucleotide polymorphisms in *Tectus niloticus.*
Appendix 2. Identification of the number of genetic clusters in *Tectus niloticus*

$$
\text{Delta } K = \text{mean}(\text{L}''(K)) / \text{stdev}[\text{L}(K)]
$$

Figure A.1. Change in $\Delta K$ for different numbers of clusters in the data. Maximal $\Delta K$ indicates the best supported solution (Evanno et al. 2005).

Figure A.2. Change in Bayesian Information Content (BIC) for increasing numbers of clusters in the data generated by the find.clusters function in the R package adegenet 2.0. The best supported number of clusters is indicated by the inflection point (Jombart et al. 2010).
Genomic Connectivity in a Tropical Reef Fish from the Kimberley, Pilbara and Gascoyne Bioregions of Western Australia

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WAMSI Kimberley Marine Research Program

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

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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 *Pomacentus milleri* (Image: Western Australian Museum)

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

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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.

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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 that 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

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WAMSI Kimberley Marine Research Program

Final Report

Subproject 1.1.3.4b

August 2017
WAMSI Kimberley Marine Research Program

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

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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)


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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 EXTRACTION ........................................................................................................................................ 8
   2.3 REDUCED REPRESENTATION SNP GENOTYPING ............................................................................................... 8
   2.4 SNP CALLING ............................................................................................................................................ 8
   2.5 SNP QUALITY CONTROL FILTERING ................................................................................................................ 8
   2.6 POPULATION GENETIC STATISTICS................................................................................................................. 8
   2.7 MODEL-BASED CLUSTERING ANALYSIS ........................................................................................................... 9
   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 DIVERSITY .................................................................................................................................... 10
   3.2 GENETIC SUBDIVISION................................................................................................................................. 11
   3.3 MODEL-BASED CLUSTERING ANALYSIS ......................................................................................................... 13
   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 LIMITS .......................................................................................... 20
   4.2 BROAD-SCALE SUBDIVISION ACROSS NWA ................................................................................................. 20
   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 ANALYSIS .............................................................................................. 28
   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 Regionisation 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* ≥ 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 Fst, 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 Striped 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 Striped 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 Striped 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 Striped 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 Striped 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 faunaal communities in the Kimberley display heterogeneous compositions 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; F_{IS} = Inbreeding coefficient) for *L. carponotatus* based on 4,402 SNP loci.

<table>
<thead>
<tr>
<th>Site</th>
<th>Fisheries Management Area</th>
<th>MEOW Province</th>
<th>MEOW Ecoregion</th>
<th>IMCRA Bioregion</th>
<th>N</th>
<th>Na</th>
<th>Ho</th>
<th>He</th>
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Population connectivity of the Stripey Snapper *Lutjanus carponotatus* along the ecologically significant coast of northwestern Australia

<table>
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<th>Region</th>
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<th>Marine Ecoregion</th>
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<th>Connectivity</th>
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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).
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).

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.

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.

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 \( N > 6 \) or \( N > 20 \) individuals collected (reduced dataset) to mitigate the effects of low sample size, where appropriate.

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
We ran Outflank with 5% left and right trim for the null distribution of F\textsubscript{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 \textit{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 \text{ln Pr} (X|K) among different values of K. The value of K for which \text{ln Pr} (X|K) was highest or reached a plateau was selected as the most parsimonious number of populations in our sample. The \textit{ad hoc} statistic \Delta K (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 \text{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 \text{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\textsubscript{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 (\textit{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 (\textit{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 ($AIC_c$) was computed as $AIC_c = AIC + 2K(K+1)/(n - K -1)$, where $AIC = -2\log\text{-likelihood} + 2K$ ($K$= number of parameters in model, $n$= number of observations). Models were then ranked based on increasing $AIC_c$ 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).
Population connectivity of the Stripey Snapper *Lutjanus carponotatus* along the ecologically significant coast of northwestern Australia

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 $\Delta K$ (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.
Population connectivity of the Stripey Snapper *Lutjanus carponotatus* along the ecologically significant coast of northwestern Australia

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).
Population connectivity of the Stripey Snapper Lutjanus carponotatus along the ecologically significant coast of northwestern Australia
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 lethrinid 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 microphthalma* (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 breuggemannii* (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 ~33cm/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


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


Spalding MD, Fox HE, Allen GR, Davidson N, Ferdaña ZA, Finlayson M, Halpern BS, Jorge MA, Lombana


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7 Data Availability

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

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 FST (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).
Population connectivity of the Stripey Snapper Lutjanus carponotatus along the ecologically significant coast of northwestern Australia
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)

\[
\Delta K = \text{mean}(L''(K)) / \text{stdev}[L(K)]
\]

(B)

\[
\Delta K = \text{mean}(L''(K)) / \text{stdev}[L(K)]
\]
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.

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<th>AICc</th>
<th>RSS</th>
<th>R²</th>
<th>Adjusted R²</th>
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<th>Model likelihood</th>
<th>Model probability</th>
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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 (FST) 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 barriers 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

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WAMSI Kimberley Marine Research Program
Final Report
Subproject 1.1.3.4c
September 2017
WAMSI Kimberley Marine Research Program

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

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Front cover images (L-R)

Image 1: Satellite image of the Kimberley coastline (Image: Landgate)
Image 3: Humpback whale breaching (Image: Pam Osborn)
Image 3: Damselfish (Pomacentus milleri) (Image: Gerry Allen)
# 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* (23Na, 24Mg, 60Ni, 63Cu, 88Sr and 137Ba), while seven trace elements were significant and above the LOD for *P. milleri* (23Na, 29Si, 31P, 60Ni, 63Cu, 88Sr and 137Ba). A PCA on the *L. carponotatus* data showed that 137Ba and 63Cu were the cause of most of the variation in the core while 24Mg and 137Ba were most significant for the margin. The PCA results for *P. milleri* showed that for both the core and the margin 31P was the main influence on the variation. The pelagic larval duration (PLD) of each fish species was determined and values were input into associated hydrodynamic models to inform the genetic results for these species.

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

A key finding of this study was that the trace element data from the margins of the otoliths (older fish) in both *L. carponotatus* and *P. milleri* show population separation between the major bioregions examined. The results agree with the findings of the genetic companion studies of these two fish species (Chapters 1.1.3.4a,b), and add support...
to their conclusions that there are genetically distinguishable populations of both species in the Kimberley, Pilbara and Gascoyne management bioregions. However, this result should be considered cautiously as the margin otolith microchemistry only tells part of the story and additional core samples will need to be analysed to allow interpretation of population connectivity. Differences in otolith chemistry between the Kimberley and other bioregions are likely to reflect variation in geology and climate. High summer rainfall brings terrigenous muds and gravels into the coastal waters of the Kimberley, while regions further south have far fewer rivers and estuaries, limiting terrestrial input. These differences will then be reflected in the types and abundances of trace elements input to the local marine waters. While data from the otolith cores (juvenile life stage) was much more equivocal, this disparity is most likely due to the margins all representing a single temporal period of otolith formation (the time immediately prior to capture), while the cores represent multiple periods, depending on the age of the fish. Furthermore, the varied core signatures (larval phase) may reflect a pre-settlement environment that differs from the area where it was collected as an adult. Additional analysis of the extent of differences between core, near core and marginal signatures within individual fish will be undertaken to elucidate the underlying importance of any differences in relation to connectivity.

Implications for management

Broad scale

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

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

Fine scale

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

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

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

- Each analysed otolith in this study will be individually aged to fully understand the juvenile phase trace element data, and patterns of individual change through time. ~20% of otoliths have been aged so far.

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

- Additional geochemical analyses of *P. milleri* otoliths at sites in the Kimberley bioregion would enable an improved understanding of both broad and fine-scale connectivity.

- Each individual geochemical proxy will be subject to a number of complicating factors in its interpretation, due to the complexity of the natural environment. To obtain the most robust interpretation, it is therefore advisable to conduct multi-proxy investigations, combining two or more approaches. To further understand the ontogenetic movement of these fish, we suggest that $\delta^{18}O$ and trace element analyses should be more extensively paired, and that additional consideration is given to including other proxies, such as compound specific stable isotope analysis of carbon, which can reflect the organic and trophic system in which a fish exists. By combining multiple parameters in this way, distinct geochemical fingerprints for each population and sub-population can be developed.
Population connectivity of two reef fish species in northwestern Australia using otolith geochemistry: A pilot study
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. 2017).
Population connectivity of two reef fish species in northwestern Australia using otolith geochemistry: A pilot study

Strontium isotopes are used as a proxy for measuring salinity. $^{87}\text{Sr}$ is the product of the radioactive decay of $^{87}\text{Rb}$ while $^{86}\text{Sr}$ 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 multicolonlector 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. $^{18}\text{O}$ is the most common isotope of oxygen, accounting for 99.76% of all naturally occurring oxygen isotopes. However, oxygen also occurs naturally as $^{17}\text{O}$ and $^{18}\text{O}$. $^{17}\text{O}$ occurs very rarely (natural abundance is 0.04%) while $^{18}\text{O}$ is slightly more common, accounting for 0.2% of all naturally occurring oxygen isotopes. $^{16}\text{O}$ is lighter than $^{18}\text{O}$ which means that it evaporates more readily, so that seawater has more $^{18}\text{O}$ than freshwater. The ratio of $^{18}\text{O}$ to $^{16}\text{O}$ ($\delta^{18}\text{O}$) 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 Rottnest Island in the southwest (Allen 1991). The contrasting reproductive strategies combined with the recent genetic data made these two species highly suitable for use in the current study.
Figure 2: Map of northern Western Australia showing the sample sites for *L. carponotatus* (top) and *P. milleri* (bottom) and the extent of the four IMCRA Bioregions. Illustration of *L. carponotatus* © R.Swainston/www.anima.net.au.
Table 1: Sample sites classified by biogeographic regime with the number of fish sampled (N) from each.

<table>
<thead>
<tr>
<th>Site</th>
<th>MEOW Province</th>
<th>MEOW Ecoregion</th>
<th>IMCRA Bioregion</th>
<th>Fisheries Management Zone</th>
<th>Lutjanus carponotatus</th>
<th>Pomacentrus milleri</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adieu Point</td>
<td>Sahul</td>
<td>Bonaparte</td>
<td>Kimberley</td>
<td>Kimberley</td>
<td>1</td>
<td>N</td>
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<tr>
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<td>Sahul</td>
<td>Bonaparte</td>
<td>Kimberley</td>
<td>Kimberley</td>
<td>11</td>
<td>N</td>
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<td>Sahul</td>
<td>Bonaparte</td>
<td>Kimberley</td>
<td>Kimberley</td>
<td>2</td>
<td>N</td>
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<tr>
<td>Hall Point</td>
<td>Sahul</td>
<td>Bonaparte</td>
<td>Kimberley</td>
<td>Kimberley</td>
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<td>N</td>
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<td>N</td>
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<td>Kimberley</td>
<td>Kimberley</td>
<td>4</td>
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<td>Kimberley</td>
<td>Kimberley</td>
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<td>N</td>
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<td>EX to BRM</td>
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<td>EX to BRM</td>
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<td>EX to BRM</td>
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<td>EX to BRM</td>
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<td>Pilbara (Nearshore)</td>
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<td>Pilbara (Nearshore)</td>
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<td>Paroo Shoal</td>
<td>NWA</td>
<td>EX to BRM</td>
<td>Pilbara (Nearshore)</td>
<td>Pilbara</td>
<td>4</td>
<td>N</td>
</tr>
<tr>
<td>Passage Island</td>
<td>NWA</td>
<td>EX to BRM</td>
<td>Pilbara (Nearshore)</td>
<td>Pilbara</td>
<td>4</td>
<td>N</td>
</tr>
<tr>
<td>Rosemary Island</td>
<td>NWA</td>
<td>EX to BRM</td>
<td>Pilbara (Nearshore)</td>
<td>Pilbara</td>
<td>4</td>
<td>N</td>
</tr>
</tbody>
</table>
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 Kimberley 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, 23Na, 24Mg, 55Mn, 60Ni, 65Cu, 66Zn, 85Rb, 86Sr, 87Sr, 88Sr, 137Ba and 208Pb using NIST612 as a standard. Data were processed relative to the standard using Iolite v3.32 (School of Earth Sciences, University of Melbourne; www.iolite.org.au) running on IgorPro v6.37 (WaveMetrics, Inc; www.wavemetrics.com) (Paton et al. 2011).

2.9 Strontium Isotope Analysis

Strontium isotope analysis was performed on all the sampled (39) *P. milleri* otoliths and 61 of the *L. carponotatus* otoliths at the University of Melbourne using multi-collector inductively coupled plasma mass spectrometry (MC-ICP-MS). Transects from the core to the margin were made using a laser spot size of 72 μm, a translation rate of 5 μm/second and a laser fluence of 2J cm².

2.10 Oxygen Isotope Analysis

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

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

3 Results

3.1 Pelagic Larval Duration (PLD)

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

3.2 Trace Element Analysis

Of the trace elements that were measured nine were found to have sufficiently small error to be included in analyses: $^{23}$Na, $^{29}$Si, $^{24}$Mg, $^{31}$P, $^{60}$Ni, $^{63}$Cu, $^{88}$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.

<table>
<thead>
<tr>
<th>Bioregion (Fisheries Management)</th>
<th>Core</th>
<th></th>
<th></th>
<th>Margin</th>
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</thead>
<tbody>
<tr>
<td>df</td>
<td>MS</td>
<td>$F$</td>
<td>$P$</td>
<td>MS</td>
<td>$F$</td>
<td>$P$</td>
</tr>
<tr>
<td>Bioregion (Fisheries Management)</td>
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<td>1.16</td>
<td>1.8</td>
<td>0.110</td>
<td>2.746</td>
<td>3.8</td>
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</table>
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.

<table>
<thead>
<tr>
<th></th>
<th>Core</th>
<th>Margin</th>
<th>Bioregion (Fisheries Management)</th>
<th>Kimb. vs Gasc. (0.007), Kimb. vs Pilb. (0.014)</th>
<th>Bioregion (IMCRA)</th>
<th>Kimb. vs Pilb-Nearshore (0.002)</th>
<th>Ecoregion (MEOW)</th>
<th>Bonaparte vs Ex to BRM (&lt;0.001)</th>
<th>Province (MEOW)</th>
<th>Sahul vs NW Shelf (0.002)</th>
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</thead>
<tbody>
<tr>
<td>Bioregion (IMCRA)</td>
<td>ns</td>
<td></td>
<td>Kimb. vs SB</td>
<td>(0.027)</td>
<td></td>
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<tr>
<td>Ecoregion (MEOW)</td>
<td>ns</td>
<td></td>
<td>Kimb. vs Pilb-Nearshore</td>
<td>(0.002)</td>
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<td>Province (MEOW)</td>
<td>ns</td>
<td></td>
<td>Saul vs NW Shelf</td>
<td>(0.002)</td>
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The geochemistry at the core region of \( P. milleri \) differed significantly between sites but not for any of the four bioregional classifications (Table 4). In stark contrast, the margin showed significant differences for each of the bioregional classifications tested as well as between sites (Table 4). The PCA results for \( P. milleri \) showed that for both the core and the margin \( ^{31}P \) was the main influence on the variation and the first two principal components represented over 90% of the variation overall. The PCA plots for the otolith margins (using averaged data) again illustrate the significant regional differences demonstrated in the pairwise PERMANOVA tests (Table 5). For example, in the case of fisheries management bioregions, all of the points representing the Gascoyne lay above all those from the Pilbara and in the case of IMCRA bioregions, all of the points representing the Pilbara nearshore lay below all those from the Shark Bay and to the left of all those from the Pilbara offshore bioregion (Figure 4 b,d). There are visible differences between the core and the margin in the PCA plots, even though the same isotopes are driving that variation.

### 3.3 Strontium Analysis

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

### 3.4 Oxygen Isotope Analysis

\( \delta^{18}O \) shows variation outside the margin of error in all six fish. In fish 5 and 6, and to a lesser extent in fish 4, there is a sequential increase in \( \delta^{18}O \) towards the margin, while in fish 1, there is a spike at the fourth analytical spot, and then a relatively stable signal from the sixth spot onwards.
Figure 3: PCA analysis on the core (a,c,e,g) and margin (b,d,f,h) geochemistry (\(^{23}\)Na, \(^{24}\)Mg, \(^{60}\)Ni, \(^{63}\)Cu, \(^{88}\)Sr and \(^{137}\)Ba) of *Lutjanus carponotatus* otoliths.
Table 4: Mean squares (MS), F values and significance levels (P) for PERMANOVAs of the isotopic values derived from LA-ICPMS analysis of *Pomacentrus milleri* otoliths (core and margin) along the north-western Australian coast. df, degrees of freedom. Significant P values in bold.

<table>
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<tr>
<th></th>
<th>Core</th>
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<td>MS</td>
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<tr>
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<td>0.6</td>
<td>0.486</td>
<td>5.238</td>
<td>4.5</td>
<td><strong>0.018</strong></td>
<td></td>
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<tr>
<td>Bioregion (IMCRA)</td>
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<td>0.94</td>
<td>0.9</td>
<td>0.382</td>
<td>4.802</td>
<td>4.5</td>
<td><strong>0.004</strong></td>
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<tr>
<td>Ecoregion (MEOW)</td>
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<td>&lt;0.01</td>
<td>0.8</td>
<td>0.435</td>
<td>4.386</td>
<td>3.7</td>
<td><strong>0.029</strong></td>
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<tr>
<td>Province (MEOW)</td>
<td>1</td>
<td>1.34</td>
<td>1.4</td>
<td>0.232</td>
<td>4.386</td>
<td>3.7</td>
<td><strong>0.031</strong></td>
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<tr>
<td>Site</td>
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<td>&lt;0.01</td>
<td>1.9</td>
<td><strong>0.038</strong></td>
<td>2.090</td>
<td>2.1</td>
<td><strong>0.026</strong></td>
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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.

<table>
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<tr>
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<th>Core</th>
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<tr>
<td>Bioregion (Fisheries Management)</td>
<td>ns</td>
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<td></td>
<td></td>
<td>Pilb. vs Gascoyne (<strong>0.016</strong>)</td>
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<tr>
<td>Bioregion (IMCRA)</td>
<td>ns</td>
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<td></td>
<td></td>
<td>SB vs Pilb(Off) (<strong>0.007</strong>), SB vs Pilb. (<strong>0.078</strong>), Pilb(Off) vs Pilb (<strong>0.010</strong>)</td>
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<td>Ecoregion (MEOW)</td>
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<td></td>
<td>SB vs Ex to BRM (<strong>0.03</strong>)</td>
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<tr>
<td>Province (MEOW)</td>
<td>ns</td>
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<td></td>
<td></td>
<td></td>
<td>WCAS vs NWA Shelf (<strong>0.029</strong>)</td>
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</table>
Figure 4: PCA analysis on the core (a,c,e,g) and margin (b,d,f,h) geochemistry ($^{23}$Na, $^{29}$Si, $^{31}$P, $^{60}$Ni, $^{63}$Cu, $^{88}$Sr and $^{137}$Ba) of *Pomacentrus millerii* otoliths.
Figure 5: Mean Sr87/86 results for each sub-region for *L. carponotatus* (top) and *P. milleri* (bottom).

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

4.1 Population Structure

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

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

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

4.2 Individual Movement

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

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

The six fish analysed for $\delta^{18}$O via SIMS show variation through time which is outside the margin of error (Figure
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 δ¹⁸O 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 δ¹⁸O behaviour in the Kimberley and Pilbara waters is required in order to establish where the variations occur, and to what extent factors such as geography, proximity to river mouths, temperature, salinity and depth are influencing the signal in these areas.

4.3 Comparisons with Genetics

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

5 Conclusions

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

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

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

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

- Each analysed otolith in this study will be individually aged to fully understand the juvenile phase trace element data, and patterns of individual change through time.

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

- Additional geochemical analyses of *P. milleri* otoliths at sites in the Kimberley bioregion would enable an improved understanding of both broad and fine-scale connectivity.

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


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8 Communication

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

8.2 Submitted manuscripts
Manuscripts relating to this work will be produced as part of a PhD thesis
A manuscript with the proposed title “Comparison of otolith geochemistry from two reef fish from northwestern Australia” is currently being drafted.

8.3 Presentations
WAMSI Lunch & Learn 28 February 2017
A presentation titled “Connectivity of fishes from the Kimberley region, Western Australia, using otolith geochemistry” was given by Sarah Hearne at the Australian Society of Fish Biology annual conference to be held in Albany, WA, 21-23 July 2017.

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