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

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)

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

Genetic diversity

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

Insights on broad-scale patterns

Population structure and connectivity

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

Genetic diversity

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

Processes influencing population genetic structure, connectivity and diversity

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

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

Implications for management

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

Key residual knowledge gaps

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

1 Introduction

1.1 Seagrasses

Seagrasses are clonal, marine flowering plants that form critical habitat in coastal waters. They are found in all continents except Antarctica, where they provide significant ecosystem services including: primary productivity; a food source for critically endangered fauna such as dugong and green turtles; habitat for many marine flora and fauna including commercially and recreationally important species; sediment stabilization; and carbon storage (Orth et al. 2006). Seagrasses are considered a 'biological group' as they have not evolved from a single lineage, but from four independent evolutionary events between 35 to 65 million years ago (den Hartog 1970, Les et al. 1997, 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 world (Halpern et al. 2008), but one of the most poorly understood. The coast is highly complex with thousands of islands subjected to an extreme tidal range, up to 11m, the world's largest tropical tides (Wilson 2013). Currents around the islands are multidirectional and can exceed 1 ms^{-1}, producing spectacular ocean conditions including whirlpools and extreme standing waves (Cresswell & Badcock 2000, Wilson 2013, Lowe et al. 2015). It is not clear whether these large tidal currents and associated eddies would enhance or limit dispersal between populations. The local currents are heavily influenced by tide, which override the broader scale, outer continental shelf currents (Condie & Andrewartha 2008).

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

1.4 Research questions

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

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

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

2 Materials and Methods

2.1 General approach

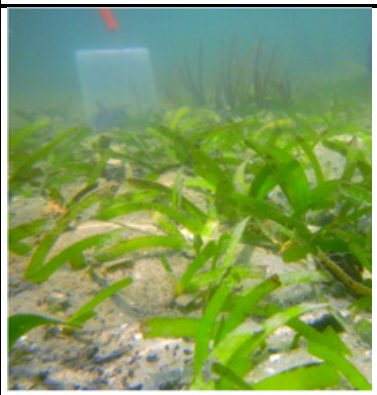
A population genetic approach was used to assess the realized connectivity of seagrass meadows across a range of scales, 5-80 km.

2.2 Species selected

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

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

Table 1. Features of the two focal seagrass species.

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

2.3 Sites sampled

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

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

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

2.4 Sample collection

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

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

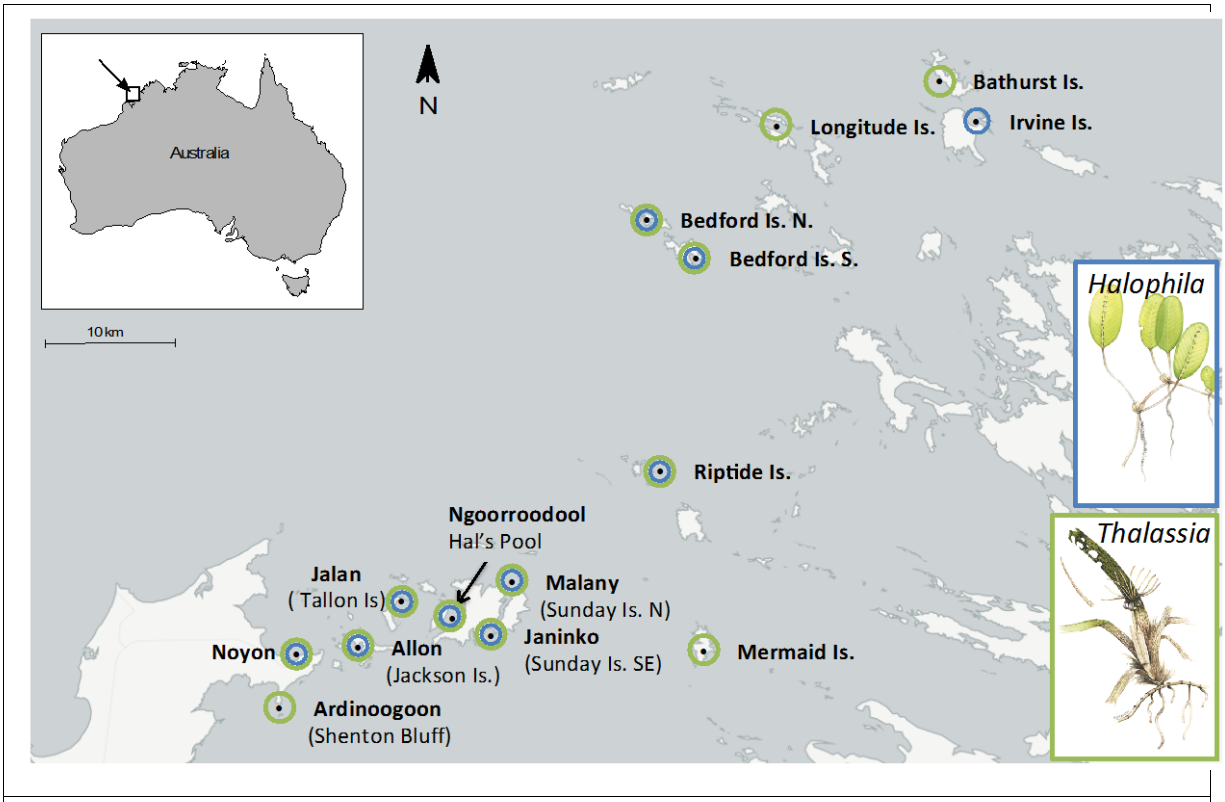


Figure 1: Location of seagrass study sites in the Sunday Islands, Buccaneer Archipelago and Northern King Sound.

2.5 DNA extraction

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

2.6 Genotyping

2.6.1 *Halophila ovalis*

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

2.6.2 *Thalassia hemprichii*

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

2.7 Genetic analysis

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

2.7.1 *Clonality and diversity*

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

2.7.2 *Genetic differentiation and structure*

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

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

2.7.3 *Spatial autocorrelation*

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

2.7.4 *Genetic connectivity*

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

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

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

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

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

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

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

2.9 Genetic resilience

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

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

3 Results

3.1 Halophila ovalis

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

3.1.1 *Genetic diversity*

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

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

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

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

3.1.2 *Population genetic differentiation and structure*

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

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

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

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

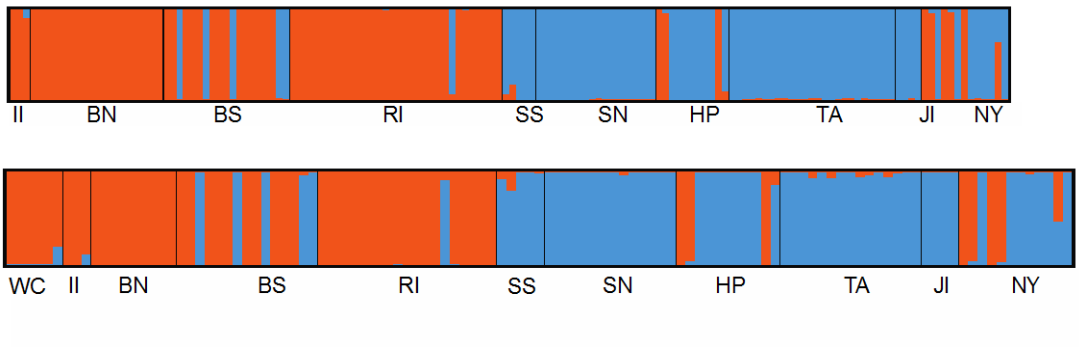


Figure 2: STRUCTURE plots for *Halophila ovalis* showing the spatial arrangement of the two clusters in the western Kimberley (Top) and with the addition of the western Kimberley site of Woobinyeye Creek (Bottom). For site codes refer to Table 3.

3.1.3 *Spatial autocorrelation*

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

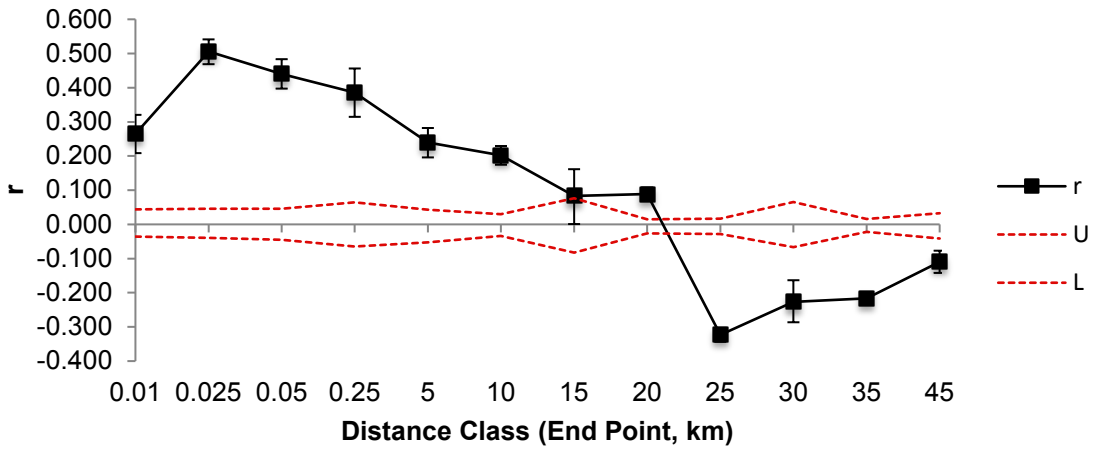


Figure 3: Spatial autocorrelation for *Halophila ovalis* showing significant spatial autocorrelation of individuals over distances up to 20 km, with the most significant spatial autocorrelation at 25 m. r is the autocorrelation value where above the line indicates statistically significant spatial autocorrelation, 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.

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).

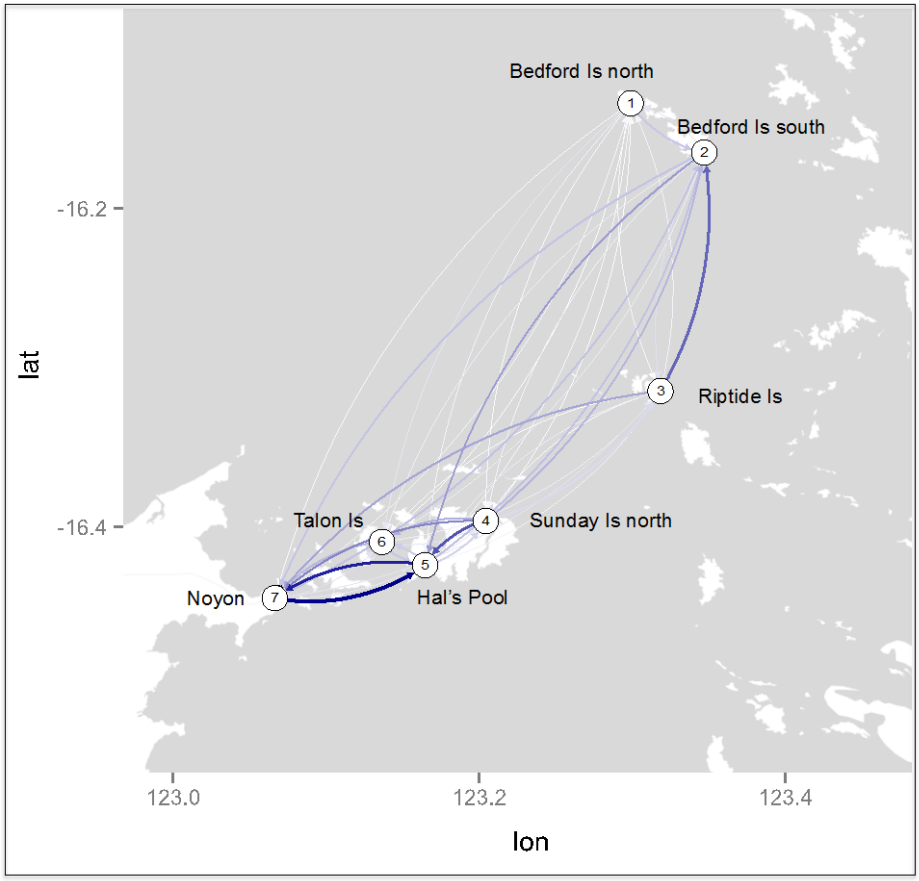


Figure 4: Pattern of gene flow based on $\widehat{Nm}$ (relative number of migrants per generation- Alcala et al. 2014) for *Halophila ovalis*. Sampling sites are identified by name. Levels of $\widehat{Nm}$ among sampling sites are represented by curved lines. The thicker the lines, the higher level 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.

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

3.1.5 *Drivers of genetic differentiation*

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

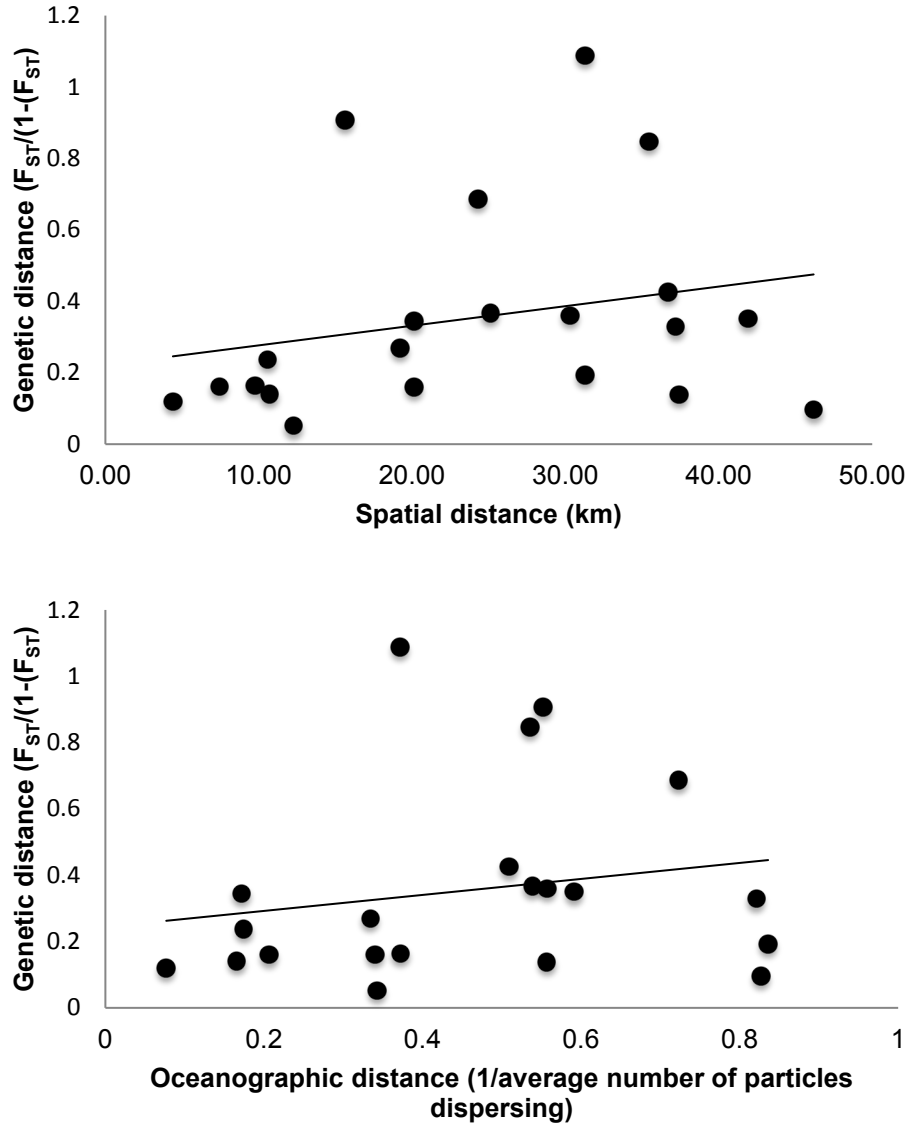


Figure 5: Isolation by spatial distance (Top) and by oceanographic distance (Bottom) between seven sites in the Sunday Islands Group and the Buccaneer Archipelago. There was no significant isolation by distance (p=0.06) but a significant isolation by oceanographic distance (p<0.05, r^2=0.143), although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

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

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

3.2.2 *Population genetic differentiation and structure*

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

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

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

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

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

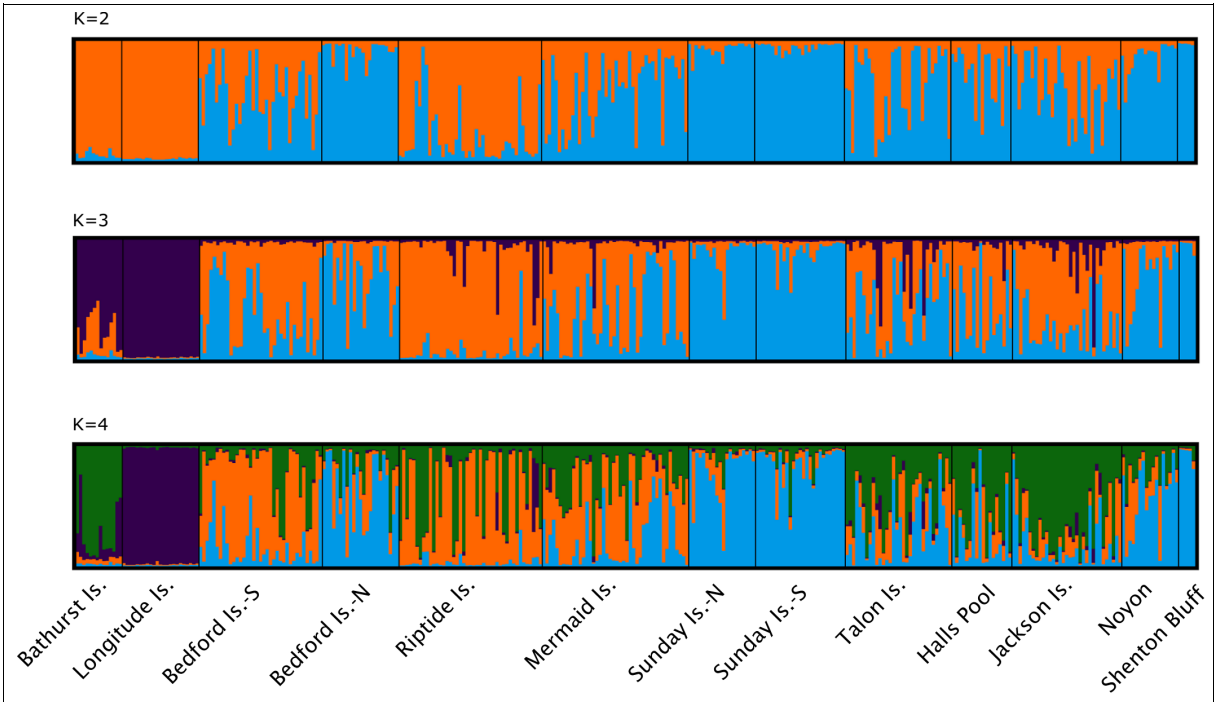


Figure 6: Cluster of populations resulted STRUCTURE analysis. Each individual is represented by a thin vertical line, which is partitioned into K segments that represent the estimated population group membership fractions. Each colour represents a distinct population. Black lines separate individuals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

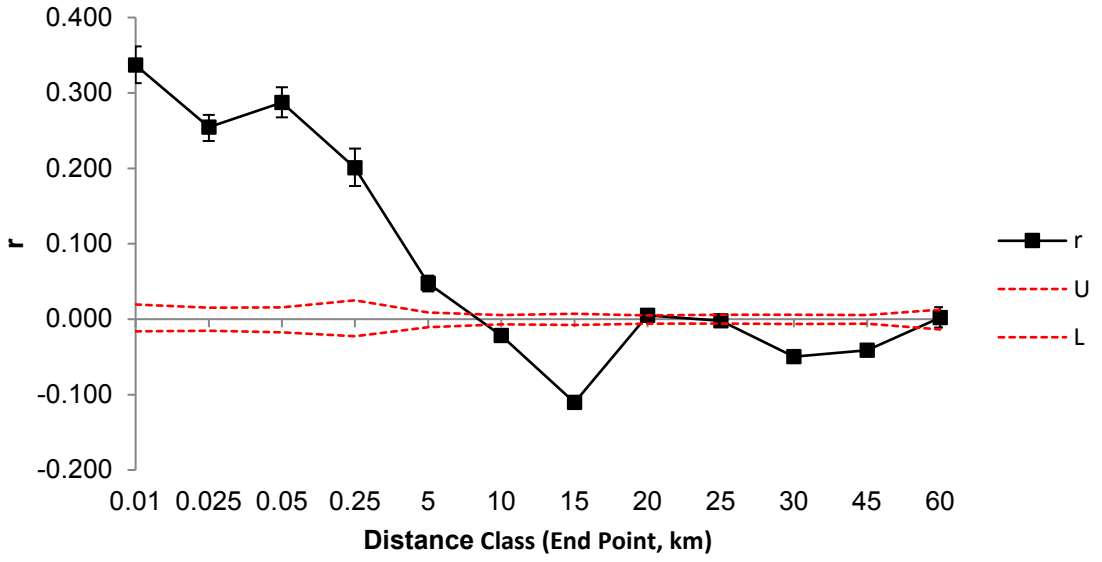


Figure 7: Spatial autocorrelation of *T. hemprichii* individuals among sites with significant autocorrelation over distances up to 5 km, with the most significant spatial autocorrelation at 10 m. r is the autocorrelation value where above the line indicates statistically significant spatial autocorrelation,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

3.2.4 *Genetic connectivity*

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

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

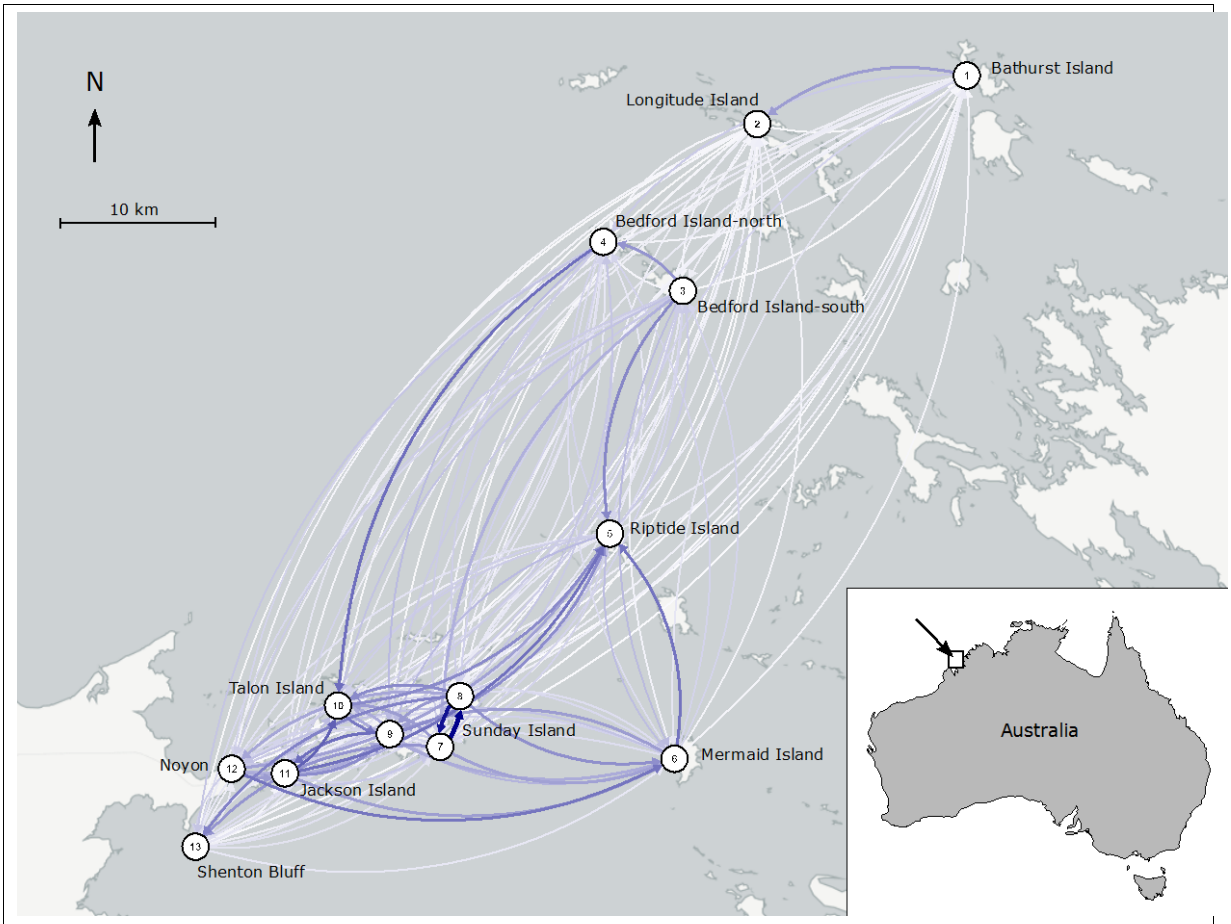


Figure 8: Map of the sampling sites and the pattern of gene flow based on $\widehat{Nm}$ (relative number of migrants per generation). Sampling sites (populations) were represented by numbers within circles (referred to Table 1). Levels of $\widehat{Nm}$ among sampling sites were represented by curved 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.

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

3.2.5 *Drivers of genetic differentiation*

Isolation by distance

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

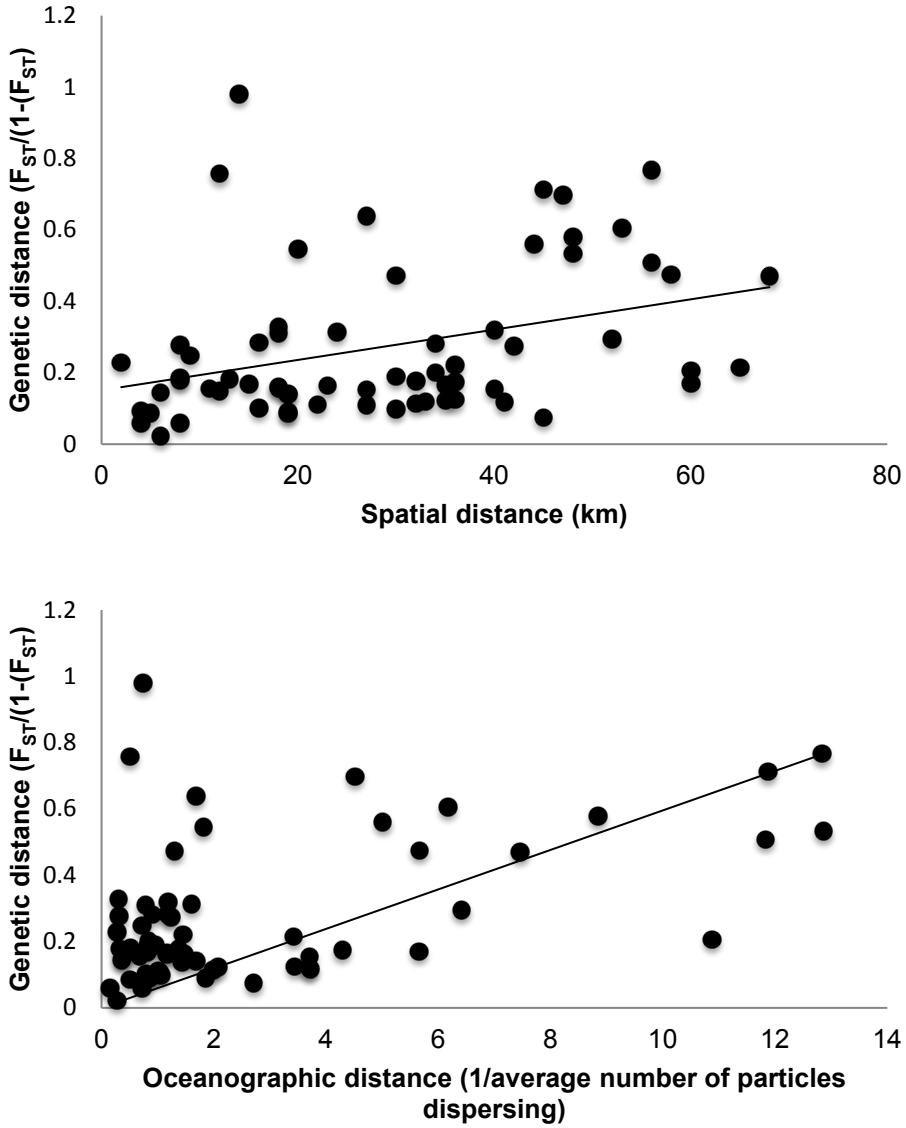


Figure 9: Isolation by spatial distance (Top) and oceanographic distance (Bottom) of *Thalassia hemprichii* between twelve sites in the Sunday Islands Group and the Buccaneer Archipelago. There was significant but weak isolation by spatial distance (r^2=0.18, p<0.001) and significant isolation by oceanographic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

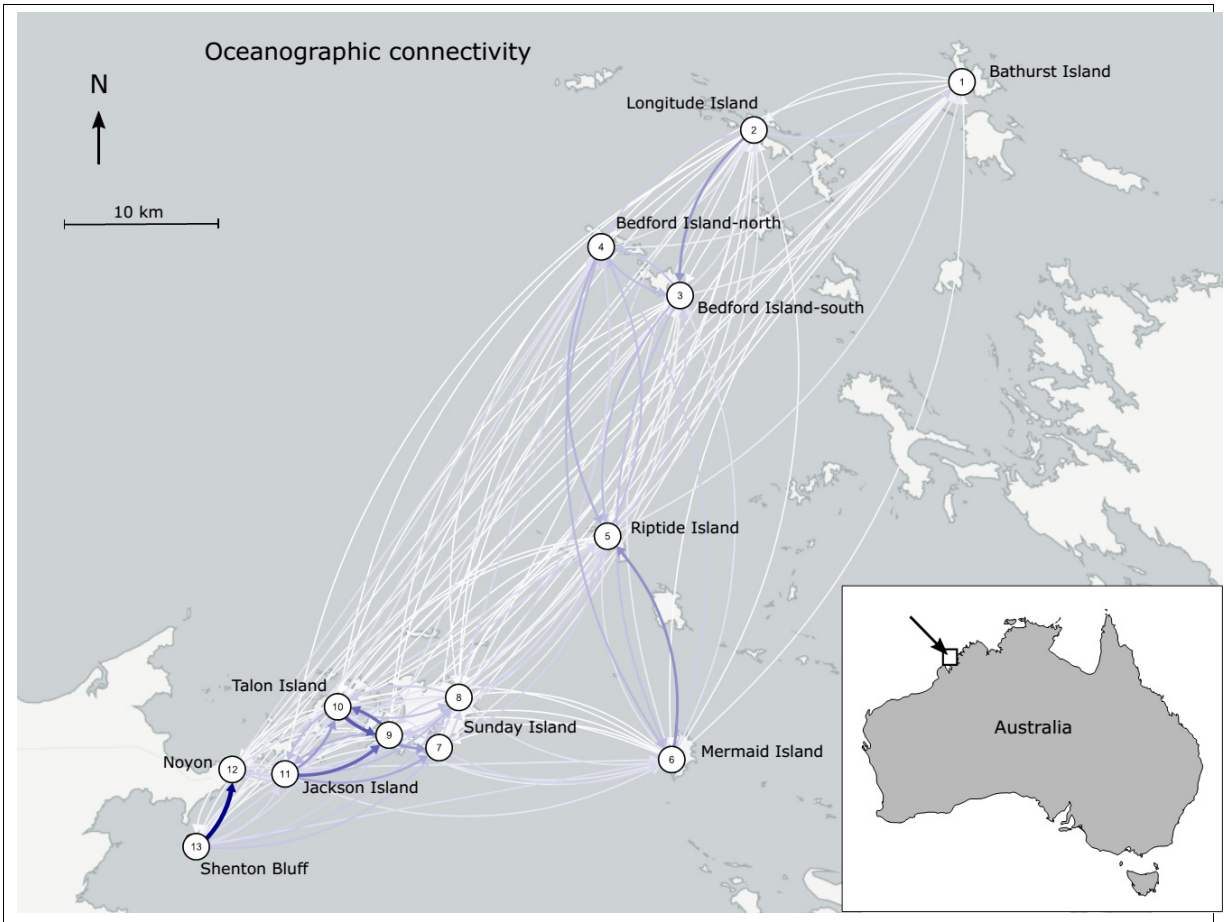


Figure 10: Oceanographic connectivity of *Thalassia hemprichii* particles as the average number of particles released from site *i* that were tracked to be in site *j,* ranging from 0 to 7.49 particles/release period. Sampling sites (populations) are named on the map. Curved lines represent number of particles. The thicker the lines, the higher level of connectivity between populations. The base map was obtained from OpenStreetMap contributors (https://www.openstreetmap.org/copyright).

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^2adj values. df_{mod}: degrees of freedom of model; df_{res}: degrees of freedom of residuals

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

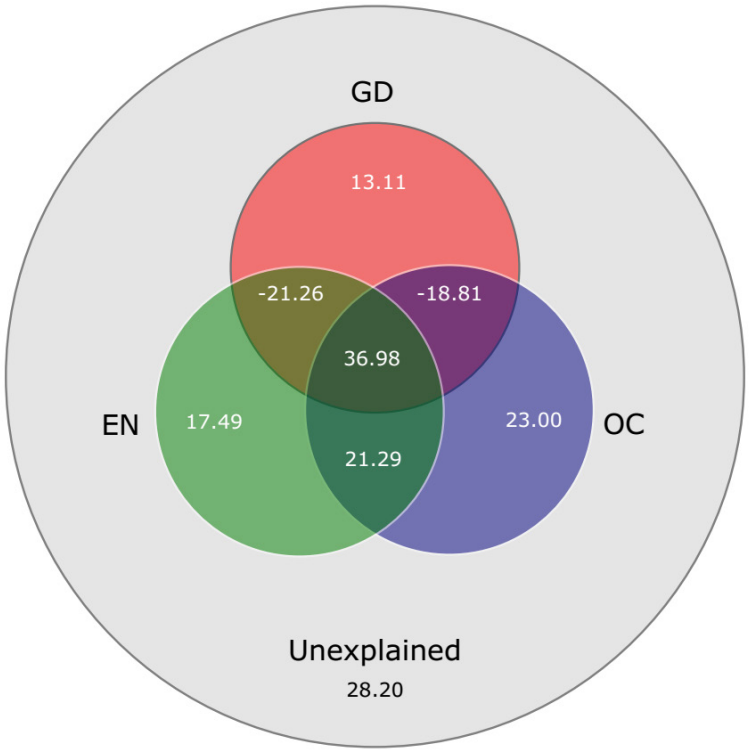


Figure 11. Decomposition of among-population variation (expressed as percentage) on genetic differentiation into spatial (GD), environmental (EN) and oceanographic (OC) components.

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).

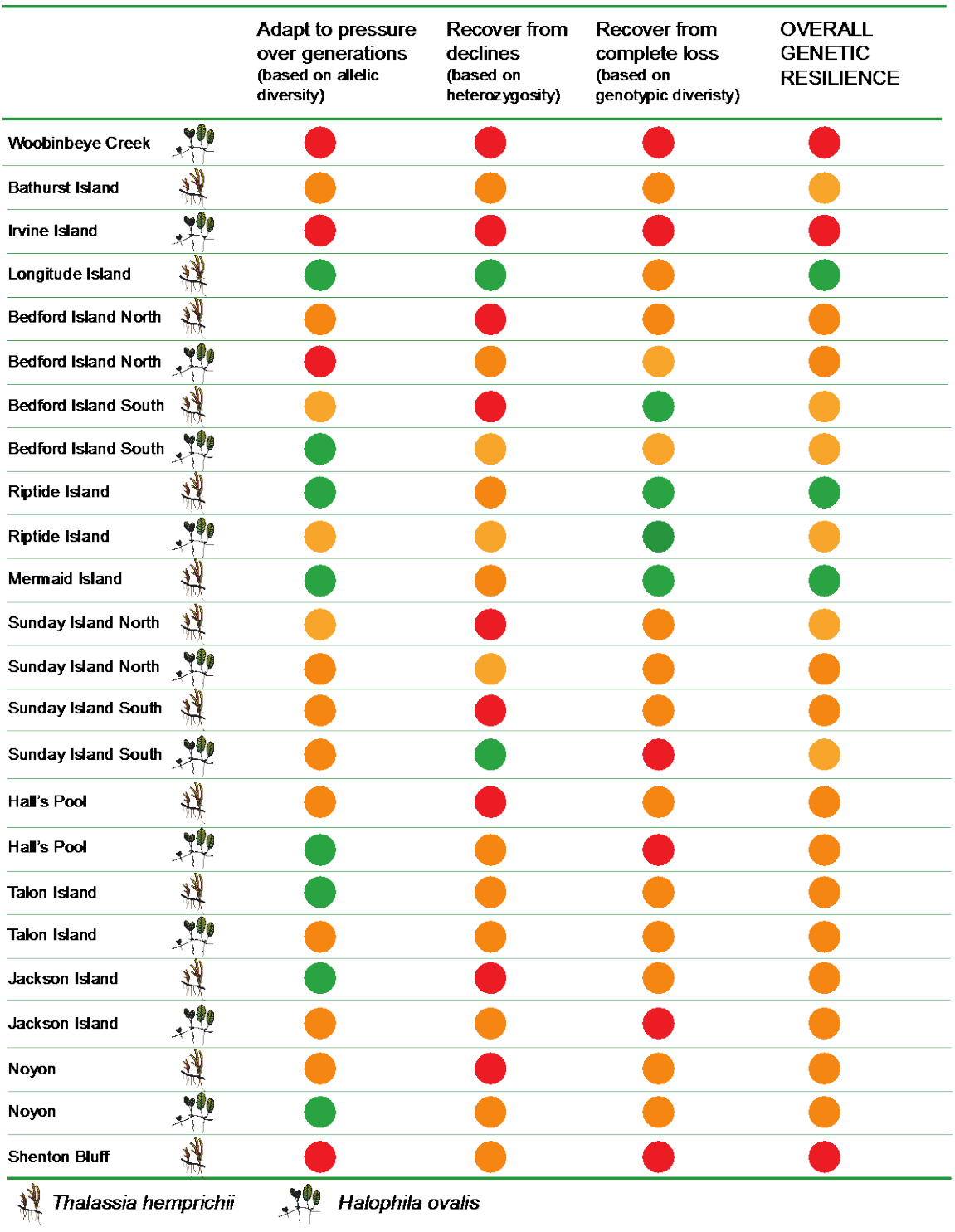


Figure 12: A summary of genetic resilience of both species across sites in the Kimberley.

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.

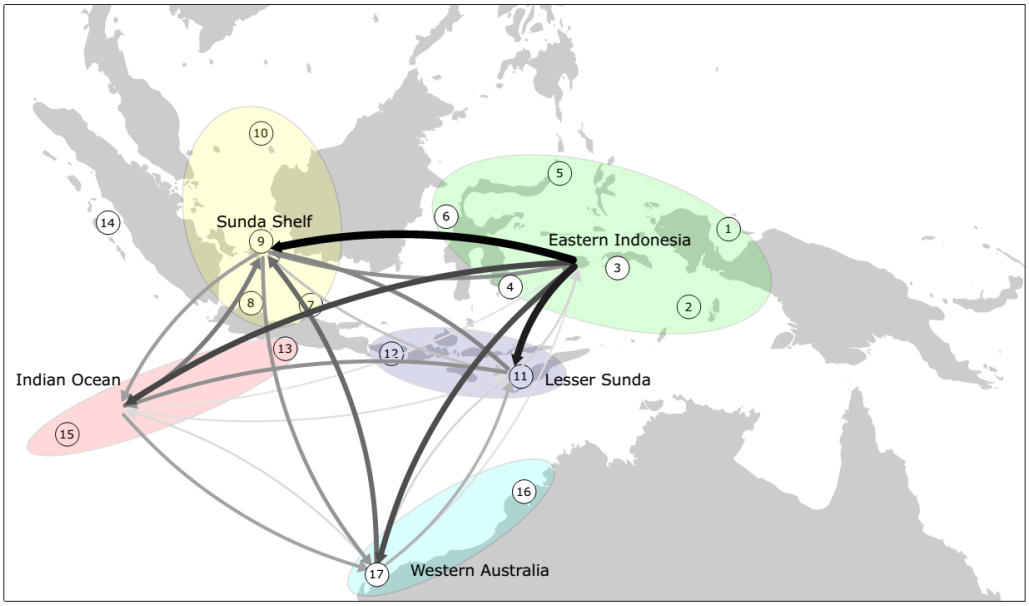


Figure 13. Samples were pooled based on five spatial clusters of populations determined from Bayesian assignment at K=5 (Pritchard et al. 2000). Lines between population clusters indicate migration estimates based on microsatellite data calculated in Migrate (Beerli & Felsenstein 2001) where thicker lines indicate greater levels of migration. Sampling site: 1. Biak; 2. Tual; 3. Ambon; 4. Kendari; 5. Bitung; 6. Palu; 7. Jepara; 8. Pari Is.; 9. Bangka; 10. Natuna, 11. Kupang; 12. Lombok; 13. Drini; 14. Padang; 15. Cocos Keeling, 16. Kimberley; and 17. Exmouth. From Hernawan et al. (in review).

4.2 Genetic diversity

4.2.1 *Fine-scale*

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

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

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

management.

4.2.2 *Broad scale*

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

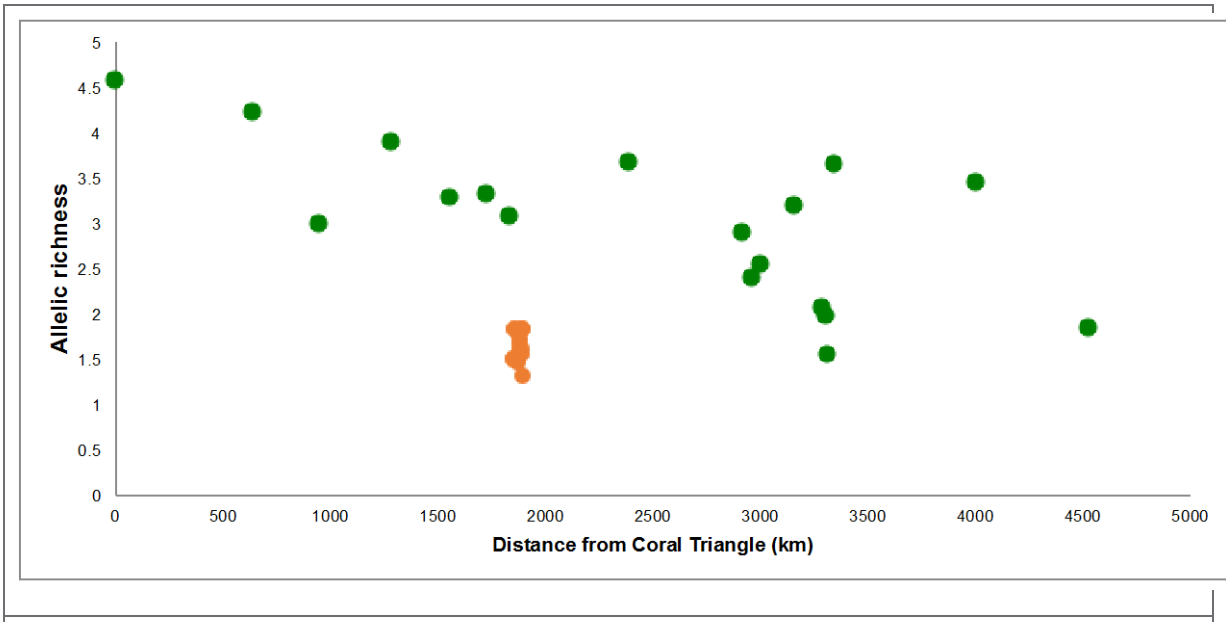


Figure 14: Patterns in genetic diversity expressed as allelic richness with increasing distance from the Coral Triangle. Orange dots indicate the lower diversity in the Kimberley. Data from Hernawan et al (in review) and McMahon et al (2016).

4.3 Drivers of genetic connectivity

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

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

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

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

4.4 Recommendations for management

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

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

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

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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.

- One specifically on the patterns of genetic connectivity for *T. hemprichii* (Hernanwan, U, van Dijk, KJ, Kendrick, GA, Feng, M, Berry, O, Kavazos, C, McMahon, K. Extreme ocean currents and habitat characteristics drive genetic divergence in a tropical seagrass. (In review Molecular Ecology),
- and one on the broader patterns from Indonesia to the Pilbara, in WA, including the Kimberley (Hernawan U, van Dijk K, Kendrick G, Feng M, Biffin E, Lavery P, McMahon K. Historical processes and contemporary ocean currents drive genetic structure in the seagrass *Thalassia hemprichii* in the Indo-Australian Archipelago. (Accepted, Molecular Ecology).

8.5 The following conference presentations were made during this project

- Australian Marine Sciences Association, Geelong, Australia. 2015. Genetic connectivity of the seagrass *Thalassia hemprichii* in the Kimberley and Pilbara. **Kathryn McMahon**, Udhi Hernawan, Gary Kendrick, Korjent van Dijk, Paul Lavery, Oliver Berry, Mike Travers, Jim Underwood.
- Coastal and Estuarine Research Federation, Oregon, Portland, USA. 2015. So near, yet so far: Genetic connectivity of the seagrass *Thalassia hemprichii* in tropical Australia. **Udhi Hernawan**, Kathryn McMahon, Gary Kendrick, Korjent van Dijk, Paul Lavery.
- University of Jogjakarta, Natural resources from local to global conference. 2015. Invited speaker. Molecular ecology of seagrasses: tools for conservation and management. **Kathryn McMahon**
- Indonesian Institute of Sciences, 2015. Management and conservation of valuable seagrass ecosystems. **Kathryn McMahon**
- ECU Research Week 2015. What we know about connections in seagrasses: Long-distance dispersal, millennial movements and emerging patterns in NW WA. **Kathryn McMahon**

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

8.6 The following poster presentations were made during this project

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

8.7 Other communications achievements

8.8 Through this project additional genetic connectivity work has been funded as part of a collaboration between ECU and Parks and Wildlife, to investigate further the genetic connectivity of the seagrass H. ovalis 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.