Project 5.2 Genetic variability of seagrass in NW Australia

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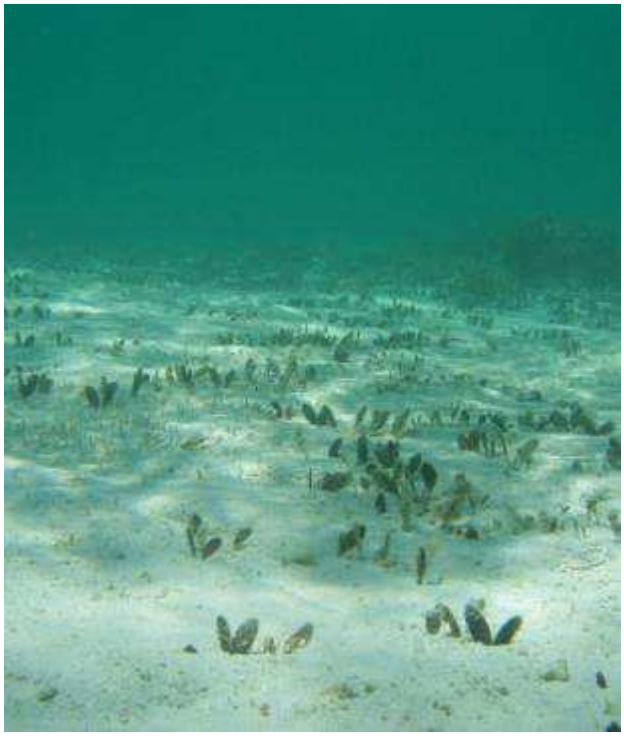
Competing Interests

The authors have declared that no competing interests exist.

Collection permits

A permit for entering marine parks and collecting plants was granted to Dr Kathryn McMahon from Department of Parks and Wildlife.



Front cover image

Halophila ovalis meadow at the Muiron Islands (Kathryn McMahon)

Project 5.2. Genetic variability of seagrass in NW Australia

Table of Contents

1 Executive summary

The response of seagrass species to on-going pressures such as dredging can be strongly influenced by their ability to adapt to, resist or recover from these pressures. The ability of species to adapt to a pressure, over generations, is influenced by the amount of genetic variation in a population: greater genetic diversity can enhance resistance and higher levels of gene flow between populations can enhance the rate of recovery following complete habitat loss. As seagrass are clonal plants, genetic diversity in a meadow is dependent on both the number of unique clones within the meadow, and distribution of this variation within and among meadows. Understanding the genetic diversity of seagrass meadows can provide important fundamental knowledge for the prediction of dredging impacts, by providing insights into the likelihood of recovery and the processes that may drive that recovery (vegetative regrowth, seed bank recruitment or immigration of recruits). It can also inform management, for example by providing insights into relative vulnerability to pressures, sources of recruitment populations and the importance of maintaining seed banks. However, for most seagrasses and in most parts of the world, extremely little is known about the genetic diversity and connectivity of populations.

The aims of this study for the WAMSI Dredging Science Program were:

1. To establish fundamental knowledge on the genetic diversity of seagrass meadows; and if this varies among sites and with different environmental conditions, particularly clear and turbid water;
2. To understand the gene flow among populations; and
3. To inform the design of mesocosm and laboratory experiments on seagrass resilience.

This study was the first of its kind to examine the patterns of genetic diversity in seagrasses in the Pilbara region of WA. Three species were assessed: *Halophila* ovalis (6 populations), *Halodule uninervis* (8 populations) and *Thalassia hemprichii* (3 populations) at a range of spatial scales, within a meadow (centimetres–metres), among meadows at a local scale (2–60 km) and among meadows at a regional scale (up to 500 km). Due to the varied distribution of species we could not sample all species across the same spatial scale and range of environments, so we designed a nested approach, with sites replicated at a distance of 2–5 km, and then different species at varied larger spatial scales.

Genetic diversity and connectivity was assessed using microsatellite markers for *H. ovalis* and *T. hemprichii*, however this was not possible for *H. uninervis* and we used an alternative approach, genotype by sequencing using single nucleotide polymorphism markers. For all species, population level genetic diversity was characterised in a number of ways: clonal richness; genotypic diversity, estimated from allelic richness; and heterozygosity. Genetic connectivity among populations was assessed in three ways: first, by examining the genetic differentiation between pairs of populations; second, through an analysis of the population structure; and third, through migration network analysis. We also undertook a preliminary examination of the relationship of genetic diversity with environmental conditions, using AMOVA to assess if there was significant partitioning of variation within habitats, locations and sites and a Principal Components Analysis was performed to visually inspect the partitioning of the variation.

For *Halophila ovalis* analysed over a small spatial scale (~60 km) in the Exmouth region, genetic diversity was moderate to high. Both sexual reproduction and vegetative growth appear to be important for maintaining population viability. All sites were identified as one genetic cluster, meta-population or management unit. High levels of migration were detected among sites within 2–5 km of each other, and low to moderate levels over larger distances. There was no strong association of genotypes with habitat. Overall these populations appear to be resilient from a genetic perspective.

For *Halodule uninervis*, analysed over a regional scale (~500 km) in the Pilbara, two types of meadow were identified. Some meadows were similar to *H. ovalis* sites, in that genetic diversity was moderate to high and high levels of connectivity were detected among meadows 2–5 km apart. The second type of meadow had very low genetic diversity, driven by particularly low numbers of individuals; a few clones dominated these meadows. At these low diversity meadows, vegetative growth appears more important for maintaining population growth than sexual reproduction, hence these meadows may take longer to recover if there is complete seagrass loss.

Based on genetic differentiation, two to three management units were supported, Exmouth Gulf was distinct from Thevenard Island, Rosemary Island and Balla Balla, and Balla Balla was distinct from the island sites. Long distance migration was detected but this is likely to be rare based on the genetic differentiation among sites. This long distance dispersal was occurring both in a northward direction, against the direction of the dominant oceanographic current, and southwards, with the direction of the dominant current. There is the possibility for rare long distance dispersal by dugongs, therefore maintaining dugongs may be important for seagrass habitat connectivity and resilience. There was no association of genotypes with habitat. Some meadows appear to be resilient from a genetic perspective, but others not.

Three populations of *Thalassia hemprichii* were assessed in the Pilbara (~200 km) and compared to populations in the Kimberley and Indonesia. The Pilbara populations were genetically diverse and showed moderate to high levels of connectivity, predominantly in a northerly direction. This northerly movement could be facilitated by the continental counter-current, which is present in the austral summer.

Based on genetic theory we developed a genetic resilience assessment for seagrass meadows in the Pilbara, compiling data from all three species. This assessment consisted of three main assumptions:

- populations with a high allelic richness have greater potential to adapt to pressures over generations;
- high levels of heterozygosity in a population provide a better capacity to recover from a disturbance immediately following the event;
- if a meadow is completely lost, recovery is more likely or will occur at a faster rate in meadows where both sexual reproduction and vegetative growth contribute to maintenance of the population. Therefore where clonal richness is moderate to high, there is a greater likelihood of recovery from sexual reproduction, but if clonal richness is low, the likelihood of recovery from a seed bank is low.

Following this assessment, the least genetically resilient meadows were at Rosemary Island and the most genetically resilient meadows were at Exmouth Gulf, Barrow Island and Thevenard Island.

2 Considerations for predicting and managing the impacts of dredging

The ability of seagrass species to adapt to, resist, or recover from pressures can be strongly influenced by the amount of genetic variation in a population. Typically, greater genetic diversity can enhance resistance, and higher levels of gene flow between populations can enhance the rate of recovery following habitat loss. It follows that knowledge of the genetic diversity of seagrass meadows likely to be influenced by dredging, could provide information on which are potentially more resilient (hence better able to cope with or recover from dredging related pressures), or which are less resistant and need more careful management attention. In addition, source and sink populations can potentially be identified which would help in the identification of potential recovery pathways for seagrass meadows.

This study identified that (1) most meadows examined had quite a high clonal diversity (i.e. many unique individuals in the meadow), so both sexual reproduction and vegetative growth are important for maintaining populations, and (2) that there was a reasonably high level of migration of genes over distances of 2–5 km, but lower levels over greater distances. The study also showed that not all seagrass meadows and species in NW Australia have a similar level of genetic diversity therefore from a management perspective, they should be considered differently.

According to the policy framework and zonation schemes used to assess and manage the impacts of dredging projects in Western Australia, temporary loss of seagrass meadows (through poor water quality) although not desirable is still permissible in some zones, as long as the meadow recovers to pre-dredging levels within five years. To manage to that framework, this study shows that it is important to establish: (1) which species of seagrass are present and likely to be influenced by plumes, (2) the genetic diversity of the seagrass meadows (i.e. clonal richness, allelic diversity, heterozygosity), (3) the life-history traits of the species (with respect to recovery mechanisms and sexual reproduction [seed banks] and vegetative growth), and (4) the magnitude of gene flow and proximity of local communities (outside of the zone of impact) for repopulation. Better knowledge of these areas could increase confidence in predictions of likely effects and recovery potential, and also allow the identification of higher risk situations should impacts occur - i.e. highly isolated populations of

genetically poor meadows (dominated by a few clones and indicating that sexual reproduction is not a large contributor to population maintenance).

3 Introduction

Seagrasses are clonal, marine flowering plants that form critical habitat in coastal waters. They are found in coastal waters of 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 a saline water medium. 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 in other plants can significantly affect their resilience (Hughes & Stachowicz 2004, Engelhardt et al. 2014, Salo et al. 2015, see Appendix 1 for a gloassary of all genetic terms).

Genetic diversity within a species is explained by the amount of genetic variation within individuals and populations and the distribution of this variation within and among populations (differentiation). This variation is created by mutations and eroded by the evolutionary processes of selection and drift. Four main factors influence genetic diversity and differentiation: the population size, gene flow within and among populations, the reproductive system in a species and natural selection (Lowe et al. 2004). As seagrasses are clonal plants, the genetic make-up of a population is dependent not only on the variation within and among individuals, but also on the number of clones or unique genotypes (multi-locus genotypes, MLGs) within that population, measured as clonal richness (See Appendix 1 for a glossary of genetic terms).

The degree to which we understand genetic diversity and connectivity within and between seagrass populations can significantly influence our ability to predict and manage dredging-related pressures on seagrasses. The ability of a population to resist and recover from a pressure, such as that imposed by dredging can be strongly influenced by the inherent genetic diversity of that population. Increased genetic diversity has been shown within some seagrass species to increase the resistance to disturbance (Hughes & Stachowicz 2004). This could be due to a higher clonal richness or higher diversity measured as heterozygosity. Heterozygosity is a measure of the variability of alleles of a gene. For example, at a gene the alleles could be homozygous (e.g. AA or aa) or heterozygous (e.g. Aa). In a population, heterozygosity measures the proportion of individuals heterozygous over a range of genes or loci. High heterozygosity indicates a better ability for a population to respond immediately after a disturbance (Lowe et al. 2004). Greater genetic diversity will also increase the probability of a species or population adapting to stressful or changed conditions, and a greater number of alleles indicate a greater capacity for a population to respond over generations to pressures (Lowe et al. 2004). Clonal richness can also indicate the relative contributions of sexual reproduction and clonal growth to maintenance of the population. High clonal richness indicates that sexual reproduction is important for maintaining populations, so

any impacts to reproductive processes such as flowering, pollination, seed set, seed germination and survival are likely to reduce the population's resilience to pressures. But if a population with high clonal richness is impacted, the likelihood of recovering is greater as both recruitment from seed and regrowth of remaining fragments is possible. Conversely, if clonal richness is very low and only a few genotypes dominate (e.g. Evans et al. 2014) then there is little evidence that sexual reproduction is important for maintaining population growth, the likelihood of recovery from seed is lower, so management focus should be on preventing complete loss and maintaining the meadow and the clones present.

The ability of a meadow to recover from complete loss 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). Over smaller scales, gene flow and connectivity can also be achieved through movement of pollen (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).

As well as understanding the genetic diversity and connectivity among populations, it is also useful to understand the partitioning of the variation and its relationship to the environment. Because a species of seagrass may grow in a variety of habitats or under different environmental conditions, it is possible that certain genotypes are adapted to particular conditions. From a management perspective it is useful to know if particular genotypes are linked to particular environmental conditions. For example, if seagrass were lost from a turbid, muddy site, would recruits from a clear water coral reef site be able to survive in this habitat. While definitive studies on this aspect of seagrass diversity are rare, it can be investigated at a coarse scale by correlating similarity in genetic diversity across sites with similarity in environmental conditions.

Many experimental studies are carried out to determine thresholds and response to different environmental pressures (e.g. Collier et al. 2009, Lavery et al. 2009). Few of these studies take into account the clonality and diversity of the seagrasses used when establishing experimental treatments. A few seagrass studies have shown significant effects of genetic diversity on the response to environmental pressures (Hughes & Stachowicz 2004). Most genetic diversity studies are carried out at a 'meadow', over tens to hundreds of metres. On the other hand, most experiments work on the smaller 'patch' scale, over a metre or less. Consequently, the data available from most genetic diversity studies may not be appropriate for guiding the design of experimental studies, which requires an understanding of genetic diversity at a patch scale.

From the above, it is apparent that genetic diversity and population connectivity are likely to influence the ability of seagrass populations to resist or to recover from dredging-related pressures and may also have implications for the laboratory or mesocosm-based experimental studies on which much of our current management of seagrasses is based. With this in mind, the objective of this project is to examine patterns in the genetic diversity and connectivity of populations of three seagrass species across environmental gradients and habitats, at a range of spatial scales. This information will provide an overall picture of the genetic diversity of seagrasses in NW WA and will be used for three purposes:

1. To establish fundamental knowledge on the genetic diversity of natural seagrass meadows and if this varies among sites with different environmental conditions, particularly turbid and clear water. We seek to determine whether seagrass patches are highly clonal or whether they have multiple genotypes, and within these genotypes is there a high genetic diversity that implies greater resilience? This will be relevant to predicting the impact of dredging activities and potential seagrass resilience in different habitats;

2. To understand the gene flow among and between populations. This information can improve our understanding of the distances over which populations are connected and over which recruits can move to colonise new habitats or aid in the recovery of impacted areas; and

3. To inform the design of mesocosm and laboratory experiments on seagrass resilience. The WAMSI DSN Project Theme 5 aims to determine the thresholds and indicators of response to dredging-related pressures for three species of seagrasses, *Halophila ovalis* (R Br.) Hook.f., *Halodule uninervis* (Forssk.) Asch. and *Cymodocea serrulata* (R.Br.) Asch. & Magnus. The project aims to achieve this through a series of laboratory experiments conducted on appropriate units of seagrass. One of the determinants of 'appropriateness' of those seagrass units is the amount of genetic diversity that should, ideally, be incorporated into them. Surveys of natural seagrass populations of two of these species, *H. ovalis* and *H. uninervis* will provide insights into the degree of variation that may need to be incorporated into laboratory seagrass 'populations'.

4 Materials and Methods

4.1 General approach

Three species of seagrass, *H. ovalis*, *H. uninervis* and *Thalassia hemprichii* (Ehrenberg) Ascherson (year) were assessed across a range of spatial scales (Table 1) to determine:

- the variation in genetic diversity within and among sites;
- the patterns in connectivity among sites; and
- the relationship between genetic diversity and relevant environmental conditions.

These species were selected as they are being examined in other WAMSI projects so this genetic data can be linked to our broader understanding of their ecology, natural variation and response to dredging related pressures. In addition, *H. ovalis* and *H. uninervis* are the most widespread seagrass species throughout the Pilbara and they are an important food source for dugong and turtle and provide habitat for commercially important fisheries. *T. hemprichii* is less widespread, and is found more on islands throughout the Pilbara, and is an important food source for green turtles (McMahon et al. 2015), although, unlike dugongs, green turtles have a more varied diet including algae and invertebrates.

Table 1. The spatial scale of sampling and the associated information it provides along with the species linked to each spatial scale.

		Provides information on			Seagrass species		
Sampling scale	Sampling unit	Clonal structure	Genetic diversity	Connectivity	*Halophila ovalis*	*Halodule uninervis*	*Thalassia hemprichii*
Fine (m's)	Cores in a site	✓			✓		
Small (10–100s km)	Sites in Locations		✓	✓	✓	✓	
Regional (100–500 km)	Sites in Locations		✓	✓		✓	
Large(>500 km)	Sites in Locations		✓	✓			✓

Ideally, each species would be assessed over the same spatial scales, however, this was not possible, as not all species were found in the same locations. To find locations with the same suite of species would have required a large amount of additional sampling, well beyond the financial scope of this study. The design outlined above uses species relevant to other aspects of the WAMSI Dredging Science studies (Project 5.5), where *H. ovalis* and *H. uninervis* were selected as target species, maximised our understanding on the spatial scale of connectivity, and was logistically feasible. Each species was assessed for the three main questions but over a different spatial scale (Table 1).

Four spatial scales were assessed: fine scale (metres); small scale (10-100s km); regional spatial scale (100-500 km); and large scale (>500 km), from Indonesia to the Pilbara (Table 1). The large-scale sampling was part of a different project being undertaken in Indonesia and the Kimberley by ECU, but which was extended to include the Pilbara in order to provide insights into the genetic diversity of the species in this region. For each spatial scale, a number of locations were sampled, with two sites sampled within each location (Table 2). *H. ovalis* was

sampled over the 'small' scale in the Exmouth region at three locations (Figure 1). Two sites were sampled from a clear-water island location (Muiron Islands), a clear-water coastal location (Bundegi and Mangrove Bay) and a turbid-water coastal location (Exmouth Gulf). For each habitat type, the sites were within 5 km of each other. The single exception was the clear-water coastal habitat where sampling restriction due to Marine Park Sanctuary Zone regulations prevented sampling sites located close to each other. In this case, we sampled Mangrove Bay and Bundegi sites around 47 km apart. *Halodule uninervis* was sampled over the Pilbara on a regional scale from four locations (Figure 2). Two sites were sampled from each of two turbid coastal locations (Exmouth Gulf and Balla Balla), and each of two turbid island locations (Rosemary Island and Thevenard Island). *Thalassia hemprichii* was sampled in the Pilbara (Muiron Islands [North and South], Barrow Island) and the locations incorporated into a larger study across the Indo-Australian Archipelago, which included sites from the Sunday Island and Buccaneer Archipelago in the south western Kimberley (PhD project and WAMSI Kimberley research project). The 'fine' scale was assessed with a pilot study at Thevenard Island Site 2 (Table 1) using *H. ovalis*.

4.2 Sample collection

A site was defined as a circular area of 50 m diameter. At each site, 50 samples were randomly collected based on randomly generated bearings and distances along the bearing which were located using compasses and transect tapes to identify positions. 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, and the position 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 *H. uninervis and 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.

For the 'fine' scale, all seagrass was harvested from 3 replicate 50 cm diameter cores. Up to 12 independent ramets were identified, and preserved for DNA extraction as described below. Only *H. ovalis* was analysed at this scale.

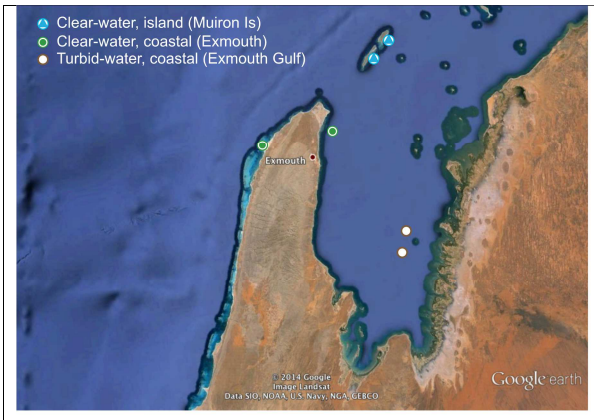


Figure 1. *Halophila ovalis* sites used to assess genetic diversity at a 'small' spatial scale in the Exmouth region. Three habitats were sampled (Clear water island: Muiron Islands, Clear water coastal: Bundegi and Mangrove Bay, and Turbid water coastal: Exmouth Gulf) with two sites in each habitat.

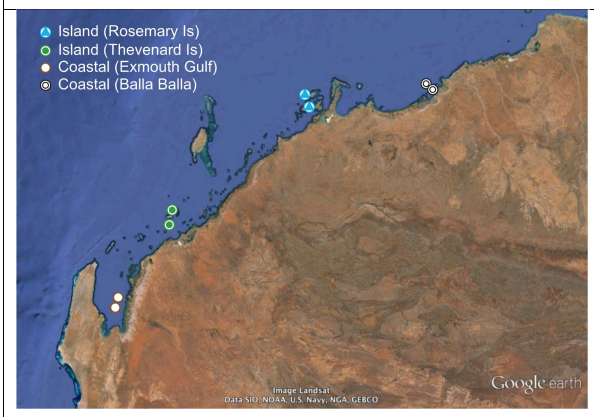


Figure 2. *Halodule uninervis* sites used to assess genetic diversity at a 'regional' spatial scale, in the Pilbara. Four locations were sampled, two in coastal (Exmouth Gulf, Balla Balla) and two in island habitats (Rosemary Island Thevenard Island). Within each location, two sites were sampled.

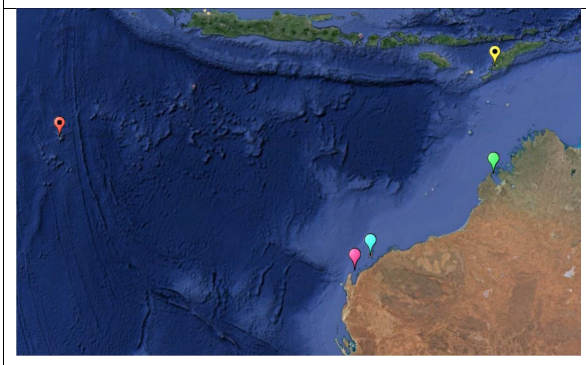


Figure 3. *Thalassia hemprichii* sites used to assess genetic diversity at a 'large' spatial scale, across the Pilbara: Muiron Islands (pink), Barrow Island (blue); Kimberley (green); Cocos (Keeling) Islands (red); and Indonesia (yellow). Two locations were sampled in the Pilbara, Muiron Islands (two sites) Barrow Island (one site).

4.3 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).

4.4 Genotyping

4.4.1 Halophila

Forty–eight samples from each site were analysed. Genotyping was conducted using 12 species-specific microsatellite markers (Xu et al. 2010), of which 8 (Ho5-4 alleles, Hpo34-12 alleles, Ho31-2 alleles, Hpo55-15 alleles, Ho20-8 alleles, Ho2-8 alleles, Ho51-4 alleles, Ho8-8 alleles) amplified consistently and were informative. The number of alleles per locus ranged from 2–15. Fluorescently labelled primers were run in multiplex reactions using *QIAGEN Type-it microsatellite PCR Kit* and 0.1–1 µg of DNA template. Fragment analysis by capillary separation was run at the GGF (Georgia Genomic Facility, USA, http://dna.uga.edu) with GGF's size standard 500 ROX. Microsatellite alleles were scored using Geneious R7 version 7.1.7 (Biomatters, Auckland, New Zealand). Following genetic analysis (described below), one site, Bundegi, and five samples from the remaining sites were grouped as unique after assignment of individuals to populations using the Bayesian

clustering algorithm of Structure 2.3.4 (Pritchard et al. 2000). In addition, no migration was detected between Bundegi and the remaining sites. This indicates that these samples may be from a different lineage, and it may not be appropriate to include them in a population genetic analysis. Therefore, due to this uncertainty, the Bundegi site, as well as samples that grouped into the same cluster as determined by the Structure analysis were removed and the data re-analysed without this site and samples.

Table 2. Location of sites where population genetic diversity samples have been collected, including the seagrass species collected. All sites were subtidal with the exception of Barrow Island, which was intertidal.

Habitat	**Location**	**Sites**	**GPS**	***Halophila ovalis***	***Halodule uninervis***	***Thalassia hemprichii***
Clear-water island	Muiron Islands	Muiron Islands North	S 21.65018 E 114.3724	✓		✓
Clear-water island	Muiron Islands	Muiron Islands South	S 21.68615 E 114.33107	✓		✓
Clear-water coastal	Exmouth	Mangrove Bay Lagoon	S 21.9798 E113.91946	✓		
Clear-water coastal	Exmouth	Bundegi	S 21.85084 E 114.16379	✓		
Turbid-water coastal	Exmouth Gulf	Exmouth Gulf Site 1	S 22.31487 E 114.35371	✓	✓	
Turbid-water coastal	Exmouth Gulf	Exmouth Gulf Site 2	S22.33603 E114.34951	✓	✓	
Clear offshore	Barrow Island	Barrow Island Bandicoot Bay*	S 20.87835 E 115.36259			✓
Turbid nearshore	Balla Balla	Balla Balla Creek Site 2	S 20.65035 E 117.042516		✓	
Turbid nearshore	Balla Balla	Balla Balla Creek Site 1	S 20.64493 E 117.686067		✓	
Turbid offshore	Rosemary Island	Rosemary Island Site 1	S 20.457762 E 116.610594		✓	
Turbid offshore	Rosemary Island	Rosemary Island Site 2	S 20.462 E 116.6091		✓	
Turbid offshore	Thevenard Island	Thevenard Island Site 1	S 21.44983 E 114.988895		✓	
Turbid Offshore	Thevenard Island	Thevenard Island Site 2	S 21.46543 E 115.016494	✓	✓	

* collected by Wes Manson at the Department of Parks and Wildlife (WA), (DPaW)

4.4.2 Halodule

Microsatellite markers (van Dijk unpublished) were trialled for *H. uninervis* but did not amplify consistently and were not informative. Of the 12 microsatellite markers trialled 6 had fixed alleles, 4 had multiple alleles but only a few samples showed variation and 2 loci did not amplify. These markers were developed on eastern Australian samples, so new screening is required on a more local basis to develop a suite of markers appropriate to NW WA. An alternative approach, Complexity reduction of polymorphic sequences (CRoPS), was used for genotyping with single nucleotide polymorphisms (SNP's), trialling a methodology following the approach of Jardine et al (2015), which incorporates next generation sequencing technology. A representative library was produced using a double restriction enzyme digest method (Vos et al. 1995, van Orsouw et al. 2007). This involved a restriction digest of DNA (~ 1 ng μL^{-1}) using Eco R1 HF® and Mse1 (New England Biolabs, Beverly, MA), then ligation by adding double stranded adapters with restriction site specific sticky ends (Eco RI and MseI) using T4 ligase (New England Biolabs) which were incubated overnight at 16°C. The next stage was a pre-selective amplification using PCR (25 cycles) with DyNAzymeTM polymerase (Finnzyme, Thermo Fisher Scientific, Waltham, MA) using adapter sites for primer annealing (Eco RI + A primer and Mse I + C primer). The additional 'selective' base (A or C) was added to reduce the amplicon pool to $1/16^{th}$, basically following an AFLP approach. This product was then diluted (1/20) and a second PCR (5 + 12 cycles) used to add Ion Torrent sequencing keys with barcodes for use in the Ion Torrent Next Generation Sequencing machine using Amplitaq Gold (Life Technologies, Carlsbad, CA) polymerase. PCR products were pooled and purified using AMPureTM XP (Agencourt, Beckman Coulter, Inc., Brea, CA) to remove leftover primer and

primer dimers. This library was then size selected using an E-Gel cartridge (Life Technologies) into 200, 250 and 300 bp lengths. The size-selected amplification pool was quantified using a 2200 TapeStationTM (Agilent, Santa Clara, CA) with the High-Sensitivity D1000 ScreenTape. Sequencing followed using the 250 bp lengths fragments on the Ion Torrent Protom™ (Life Technologies) at the Australian Cancer Research Foundation (ACRF) Cancer Genomics Facility in Adelaide. Sequencing reads were analysed using CLC-Genomic Workbench (Qiagen, Venlo, The Netherlands). Reads were de-multiplexed, trimmed and assembled to generate a 'Provisional Reference Genome' (Hird et al. 2011). Each sample's individual reads were mapped onto this reference and the consensus sequences extracted. These results were exported into the genetic analysis program Geneious version R6 for manual selection of SNP loci and 130 were identified and used for further genetic analysis. Due to the nature of SNP data, each locus had 2 alleles.

4.4.3 Thalassia

Forty-eight samples from each site were analysed. These sites were compared to thirteen sites in the Kimberley (in the Buccaneer Archipelago and the Sunday Island Group), as well as Cocos (Keeling) Islands and Kupang in Indonesia. The Australian samples were collected through the WAMSI Kimberley Science Program and the Indonesian sample through the ECU-UWA CRN project by PhD student Udhi Hernawan. Genotyping was conducted on 16 microsatellite markers developed by van Dijk et al. (2014): 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. Fluorescently labelled primers were used to amplify the DNA in multiplex reactions using the QIAGEN Type-it microsatellite PCR Kit. DNA template (0.1−1 µg) was added to the PCR reaction mix. Fragment analysis and capillary separation were run at the GGF with GGF's size standard 500 ROX. Alleles were scored using Geneious R7 version 7.1.7

4.5 Genetic analysis

4.5.1 Clonality and Diversity

For *H. ovalis* and *T. hemprichii* where microsatellite markers were used, MLGs were determined using the *poppr* package in R (Kamvar et al. 2014) and expressed as clonal richness (R = MLG-1/N-1). Clone mates were removed from further analyses, so that only one representative of each MLG were 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*: 28 *T. hemprichii*: 14) using rarefaction in HP-Rare (Kalinowski 2005). The genetic diversity including unbiased expected heterozygosity (H'_{exp}) and observed heterozygosity (H_{obs}) was calculated using GENETIX 4.05.2 (Nei 1978).

For *H. uninervis* where SNP markers were used, the identification of clones was performed in the genetic analysis program GenoDive version 2.0b27 using the Infinite Allele Model, and missing data was not counted. The threshold for the number of SNP pairs that can vary within a clone was set at 5. There is not a good understanding of the number of SNP pairs that vary within clones of seagrasses, but the proposed method of Douhovnikoff and Dodd (2003) provides an objective approach to choose the appropriate distance threshold allowed between two samples, based on the means and standard deviations. Due to the clonal and long-lived nature of seagrasses somatic mutations can develop over time, hence the DNA sequences within ramets of the same individual can vary. It is also very likely that some null alleles (alleles being present but not amplifying) might be present or that scoring errors were committed. Therefore there is less certainty in identification of MLGs with this approach, and they should be considered putative MLGs. Due to the different nature of the SNP data not all of the same genetic statistics could be calculated. Genotypic diversity was estimated in GenoDive by the effective number of alleles (average number of alleles per locus weighted for their frequencies) and the observed and expected heterozygosity per population.

4.5.2 Genetic Connectivity

Connectivity among sites was assessed in three ways; first, by examining the genetic differentiation between pairs of populations; secondly, through an analysis of the population structure; and third through a migration network analysis, which identifies the direction and relative magnitude of migration among all sites. For *H. ovalis* and *T. hemprichii* both F_{ST} (Wright 1969) and G'_{ST} (Meirmans & Hedrick 2011) were estimated in the genetics

analysis program Genalax version 6.501. F_{ST} is the most common index used and hence for comparisons to other studies is included here, but it is only suitable for bi-allelic data generated with molecular techniques such as SNPs. When there are more than two alleles per locus such as occurs with microsatellite data, a different calculation is required, and this is termed either F_{ST} or G_{ST} (Nei 1973). Here we use the term F_{ST} to cover both measures. However, there are some issues with G_{ST} and a standardised measure, G'_{ST} has been developed to deal with this (Hedrick 2005). We present both F_{ST} and G'_{ST} for the species analysed with microsatellites. For *H. uninervis*, only F_{ST} (Wright 1969) was calculated in GenoDive. Population structure was examined using a Bayesian assignment test of Structure v2.3.4 (Pritchard et al. 2000). This identifies 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 = 6 for *Halophila*, K = 1 to K = 8 for *Halodule* and K = 1 to K = 16 for *Thalassia* with burnin = 200,000 and replications after burn-in = 500,0000. 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). Clump v1.1.2 was then employed to align the multiple replicate analysis of the appropriate K (Jakobsson & Rosenberg 2007). Distruct v1.1 (Rosenberg 2004) was then used to visualize the population structure.

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) for *H. ovalis* and *T. hemprichii*. This measure is based on the complementary function of both G_{ST} and D calculated in Genalex 6.501. For *H. uninervis* Nm was estimated in GenoDive. To calculate the site pair-wise $\widehat{Nm}$ or Nm, the function *divMigrate* of *diveRsity* package in R (Keenan et al. 2013) was used. Visualization of the gene flow was built on the qgraph package (Epskamp et al. 2012).

4.5.3 The relationship between genetic diversity and environmental conditions

For a preliminary examination of the relationship of genetic diversity with environmental conditions, an analysis of molecular variance (AMOVA) was carried out to assess if there was significant partitioning of variation within habitats, locations and sites and a principal component analysis (PCA) was performed to visually inspect the partitioning of the variation. For *H. ovalis*, the habitats were coastal turbid water and island clear water with two sites in each habitat. The coastal clear habitat was not incorporated due to the removal of the Bundegi site. For *H. uninervis*, two AMOVAs were carried out: the first by habitat, with the coastal turbid sites (Balla Balla and Exmouth Gulf) grouped and the island turbid sites (Rosemary Island and Thevenard Island.) grouped, with two sites in each location; and the second grouped the sites by the four regions. These analyses were not carried out on the *T. hemprichii* populations due to the low number of populations sampled in the Pilbara region.

5 Results

5.1 *Halophila ovalis* – local scale

5.1.1 Clonal structure and genetic diversity

The number of samples successfully amplified among sites ranged from 38–48, with 14 to 46 MLGs identified (Table 3). Clonal richness was greatest in Exmouth Gulf (R = 0.96), least at the Muiron Islands (R = 0.35–0.36) and intermediate at Mangrove Bay (R = 0.63). The allelic diversity also varied among sites, from a minimum of 30 alleles at the Muiron Islands to 47 at Exmouth Gulf 1. The average allelic richness (averaged over all loci) was lowest at Muiron Island North (3.68–3.75) and from 4.1 to 4.62 at all other sites. Private alleles were, on average, most common at Mangrove Bay (5 observed, pA = 0.59) and least common at the Muiron Islands (3 observed, pA = 0.13–0.29). The observed heterozygosity was generally high from 0.56–0.67 (Table 3).

Table 3. Allelic richness and genetic diversity of *Halophila ovalis* obtained from 9 microsatellite loci with standardized genets of 28. N: total number of individuals examined; MLG: number of multi-locus genotypes; R: clonal richness (MLG-1/N-1); nA: observed alleles; Ar: allelic richness and pA: private allele richness, standardized at 30 genets (mean of alleles per loci); *Hobs:* observed heterozygosity*; Hexp*: non-biased expected heterozygosity (Nei 1978).

Habitat	Site	Number	N	MLG	R	nA	Ar	pA	H_{obs}	H_{exp}
Coastal Turbid	Exmouth Gulf	1	48	46	0.96	47	4.62	0.57	0.60	0.59
	Exmouth Gulf 2	2	46	44	0.96	43	4.29	0.30	0.56	0.52
Coastal Clear	Mangrove Bay	3	41	26	0.63	36	4.06	0.59	0.57	0.50
Offshore Clear	Muiron Is. North	4	46	17	0.36	30	3.68	0.13	0.66	0.54
	Muiron Is. South	5	38	14	0.35	30	3.75	0.29	0.67	0.53
TOTAL			220	182	0.82					

5.1.2 Genetic connectivity and structure

There was significant, although low, genetic differentiation among sites, the overall F_{ST} = 0.040 ± 0.012. All site pair-wise comparisons were significant, with the greatest genetic differentiation being between Mangrove Bay with Muiron Islands North, and Mangrove Bay with the Exmouth Gulf sites (G'_{ST} ranged from 0.065–0.096, compared to 0.009 between the Exmouth Gulf sites, Table 4). Although the Muiron Islands sites are a similar distance apart to the Exmouth Gulf sites, they have significantly more genetic differentiation. Two population clusters were most supported with Structure (k = 2) across the five sites; however, there was not a consistent spatial pattern associated with these clusters, the common blue cluster was spread across all sites, and the orange cluster was present in all sites except Muiron Islands North, where there was an intermediate of the orange and blue cluster (Figure 4). Generally Exmouth Gulf sites were most similar, and the remaining three sites were characterised with intermediates of the blue and orange cluster. The greatest level of migration was between sites separated by ~5 km, the Exmouth Gulf sites, followed by the Muiron Islands sites (Figure 5). The next most significant migration was from Exmouth Gulf site 2 to Muiron Islands South (62 km), Muiron Islands South to Exmouth Gulf 1 (59 km) and from Muiron Islands South to Mangrove Bay in both directions (53 km). Although there are differences in the levels of connectivity among populations, *H. ovalis* appears to be acting as a well-mixed meta-population across these sites, at this scale of tens of kilometres.

Table 4. Population pair-wise F_{ST} (bottom triangular matrix) and G'_{ST} (top triangular matrix) among sites. All population pair-wise comparisons are significant at $p<0.05$.

	Population	EG1	EG2	MB	MIN	MIS
1	Exmouth Gulf Site 1	-	0.009	0.065	0.040	0.029
2	Exmouth Gulf Site 2	0.010	-	0.088	0.056	0.044
3	Mangrove Bay	0.041	0.053	-	0.096	0.040
4	Muiron Islands North	0.030	0.038	0.062	-	0.040
5	Muiron Islands South	0.024	0.031	0.030	0.033	-

Figure 4. The Structure analysis for *H. ovalis* across five sites over a local scale in the Exmouth region had the strongest support for two clusters (K = 2, depicted by the orange and blue colours). Sites are presented in blocks, with lines separating them and individuals are vertical bars, either one-colour or cluster or a combination of clusters. There is not a distinct spatial patterning associated with the clusters, individuals belonging to the blue and orange clusters are found at all sites, indicating these sites are a single management unit. 1 = Exmouth Gulf 1, 2 = Exmouth Gulf site 2, 3 = Mangrove Bay, 4 = Muiron Islands North, 5 = Muiron Islands South.

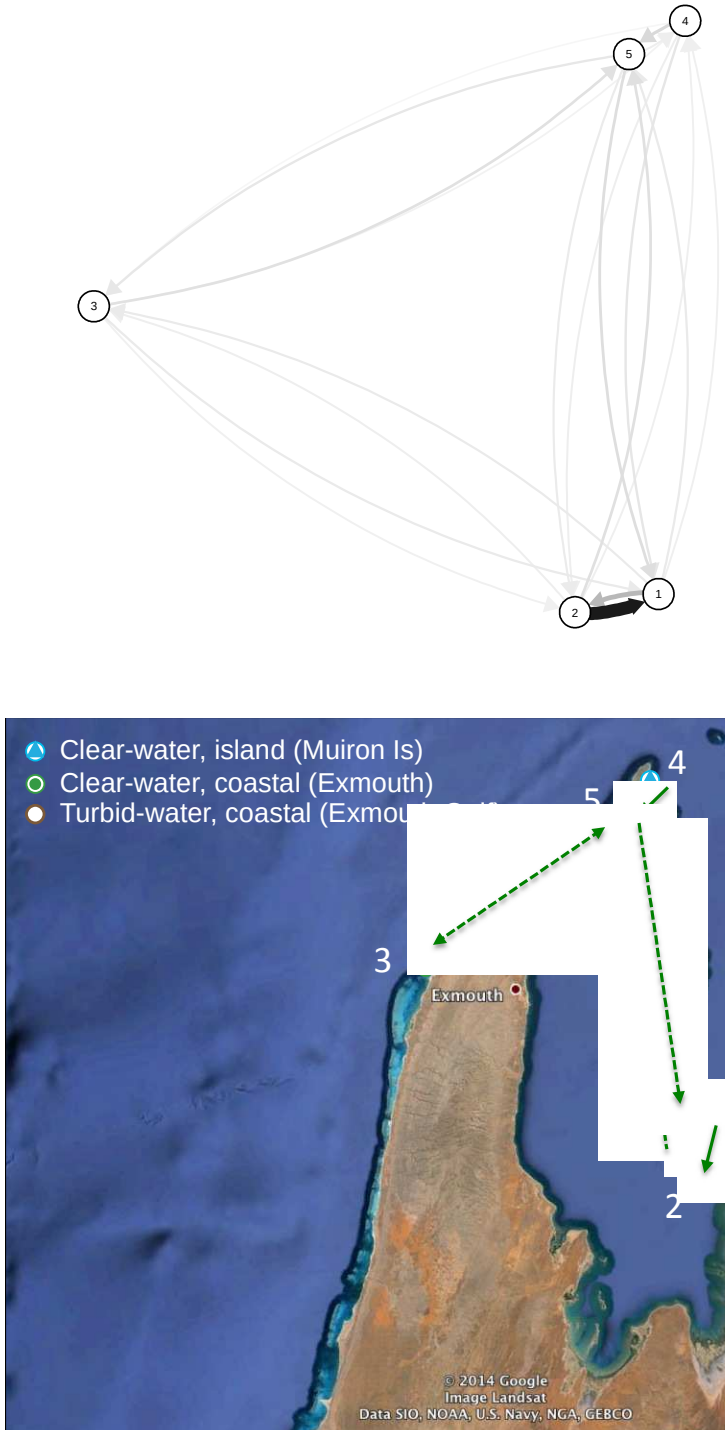


Figure 5. *Halophila ovalis* genetic connectivity among sites over a local scale in the Exmouth region. Above: the migration network shows the relative rates of migration per generation, thicker lines indicate more migration and the arrows indicate the direction. Below: This network analysis is stylised on the site map to show the major migration pathways (relative migration rates >0.8). 1 = Exmouth Gulf site 1 2 = Exmouth Gulf site 2, 3 = Mangrove Bay, 4 = Muiron Islands (North), 5 = Muiron Islands (South).

5.1.3 Genetic diversity by environment

There was significant partitioning of variation among all sources of variation but habitat and among population variation explained only 4% and 3% respectively, while the variation within populations accounted for 93% of

the variation (Table 5). This does not support the hypothesis that particular genotypes are associated with different habitats. The partitioning of the variation is illustrated in the Principal Coordinates Analysis (PCoA, Figure 6), where there are two main clusters of samples. The cluster on the right contains the majority of samples from all sites, whereas the cluster of the left has fewer samples, and no individuals from Muiron North are represented.

Table 5. Analysis of molecular variance (AMOVA) for the Muiron Islands clear-water sites and the Exmouth Gulf turbid-water sites, and the sites within these habitats.

Source	Df	MS	p	% var
Among habitats	1	16.7	<0.001	4
Among populations	2	8.57	<0.001	3
Within populations	117	4.24	<0.001	93
Total	120			

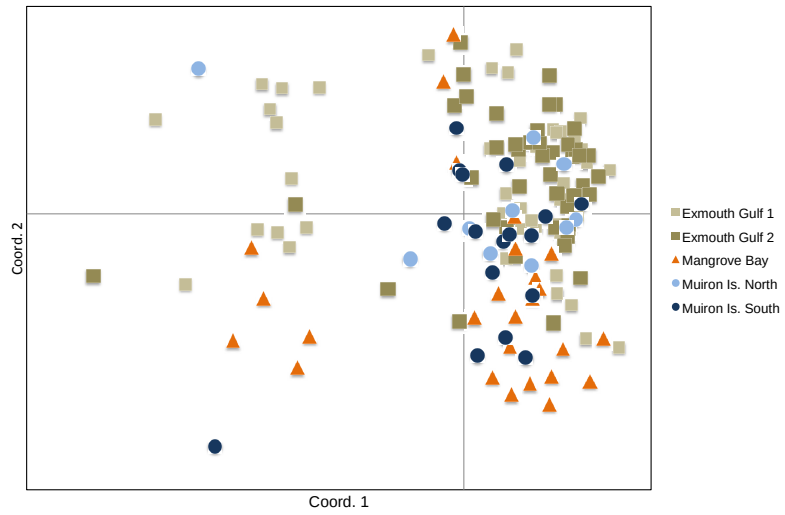


Figure 6. A principal coordinate analysis showing the association of similar genotypes of *H. ovalis* from five sites in the Exmouth region. The two axes account for 35% of the variation.

5.2 *Halophila ovalis* – patch scale

The pilot study to assess genetic diversity at a patch scale (50 cm diameter plot) identified from 4–9 genotypes out of 12 samples genotyped from each plot (Table 6). This clonal richness was similar to that observed at the 50 m diameter scale, R = 0.27–0.73, compared to 0.37–0.96 at the meadow scale (Table 3).

Table 6. The number of samples, MLGs and clonal richness at a small patch scale, from three replicate cores in a shallow water *H.* ovalis meadow from Thevenard Island This was obtained from 9 microsatellite loci. N: total number of individuals examined; G: number of multi-locus genotype; R: clonal richness (MLG-1/N-1).

Population	N	G	R
1	12	5	0.36
2	12	4	0.27
3	12	9	0.73

5.3 *Halodule uninervis* – regional scale

5.3.1 Clonal structure and genetic diversity

All samples were successfully genotyped at most sites (n = 16), except for Exmouth Gulf (site 1) and Balla Balla (site 1) where only 15 samples were successfully amplified (Table 7). The number of MLGs detected varied greatly, from 3 at the Rosemary Island sites and Thevenard Island (site 2) to 15 at Exmouth Gulf (site 1). Clonal richness was highest at the Exmouth Gulf sites (R = 1 and 0.87), followed by Balla Balla (R = 0.92 and 0.73), generally low at Thevenard Island (R = 0.47 and 0.13) and low at Rosemary Island (R = 0.13). Due to the low number of MLGs detected at Rosemary Island and Thevenard Island (site 2), three individuals; the remaining genetic data needs to be interpreted cautiously.

Unlike the microsatellite data, the maximum average allelic richness for the SNP data was two per loci, and allelic richness was lowest at the sites where clonal richness was lowest (R = 0.13). However, at Thevenard Island site 1, with low to moderate clonal richness, the allelic richness was one of the highest observed (Table 7). In general, allelic richness was highest at Exmouth Gulf, followed by Thevenard Island, then Balla Balla, and lowest at Rosemary Island. The greatest observed heterozygosity followed a slightly different pattern; it was highest at Thevenard Island, followed by Exmouth Gulf, then Rosemary Island and lowest at Balla Balla (Table 7).

5.3.2 Genetic connectivity and structure

In comparison to *H. ovalis*, there was greater genetic differentiation among the *H. uninervis* sites (overall F_{ST} = 0.097 ± 0.011) and this was significant among most pair-wise comparisons of sites (Table 8). The greatest differentiations were between Balla Balla site 1 and three other sites, the Exmouth Gulf sites and Thevenard

Island site 2 (F_{ST} = 0.165–0.183). However, there was no significant differentiation among the paired sites in the Exmouth Gulf, Thevenard Island and Rosemary Island. In addition, Thevenard Island site 1 was not significantly different to Rosemary Island site 1, and Thevenard Island site 2 was not significantly different to Rosemary Island site 2. These relationships were further supported in the Structure analysis, where K = 2 or K = 3 population clusters were strongly supported (Figure 7). At K = 3, the Balla Balla sites formed one group, the Thevenard and Rosemary Island sites another group, and the Exmouth Gulf sites the final group. At K = 2 Exmouth Gulf was distinct from all other sites (Figure 7).

Table 7. Genetic statistics for *Halodule uninervis* obtained from 130 SNP loci. N: total number of individuals examined; MLG: number of multi-locus genotypes; R: clonal richness (MLG-1/N-1); Ae: effective allelic richness; *Hobs:* observed heterozygosity; and *Hexp*: expected heterozygosity).

Habitat	Population	N	MLG	R	Ae	H_{obs}	H_{exp}
Coast	Exmouth Gulf 1	15	15	1.00	1.43	0.266	0.266
Turbid	Exmouth Gulf 2	16	14	0.87	1.44	0.269	0.269
Island	Thevenard Island 1	16	8	0.47	1.44	0.295	0.280
Turbid	Thevenard Island 2	16	3	0.13	1.39	0.300	0.253
Island	Rosemary Island 1	16	3	0.13	1.37	0.270	0.285
Turbid	Rosemary Island 2	16	3	0.13	1.39	0.249	0.283
Coast	Balla Balla 1	15	15	1.00	1.40	0.242	0.250
Turbid	Balla Balla 2	16	12	0.73	1.39	0.243	0.251
Total		126	72	0.57	1.35	0.262	0.291

The genetic connectivity, as estimated by relative migration rates, showed that the main pathways of migration are between closely located sites within a region (Figure 8). The highest migration rates were between the Exmouth Gulf sites in both directions. Moderate migration rates were detected from Thevenard Island site 2 (# 6 in Figure 8) to Thevenard Island site 1 (#5 in Figure 8), with low migration rates in the opposite direction. Low migration rates were detected from Rosemary Island site 2 (# 4 in Figure 8) to Rosemary Island site 1, and in both directions between the Balla Balla sites. Low, but still detectable levels of long distance migration were detected between Thevenard Island and the Exmouth Gulf sites in both directions, a distance of ~115 km, and from Balla Balla to Exmouth Gulf, a distance of 450 km.

Table 8. Population pair-wise F_{ST} (bottom matrix) comparisons among sites. Bold population pair-wise comparison are significant at p<0.05, all others are not significant.

Population	EG1	EG2	T1	T2	R1	R2	BB1	BB2
Exmouth Gulf site 1	-							
Exmouth Gulf site 2	0.002	-						
Thevenard Island site 1	**0.084**	**0.084**	-					
Thevenard Island site 2	**0.127**	**0.137**	0.002	-				
Rosemary Island site 1	**0.095**	**0.123**	0.044	0.094	-			
Rosemary Island site 2	**0.116**	**0.124**	**0.052**	0.097	0.000	-		
Balla Balla site 1	**0.165**	**0.183**	**0.136**	**0.181**	**0.093**	**0.094**	-	
Balla Balla site 2	**0.131**	**0.137**	**0.099**	**0.161**	**0.092**	**0.090**	**0.089**	-

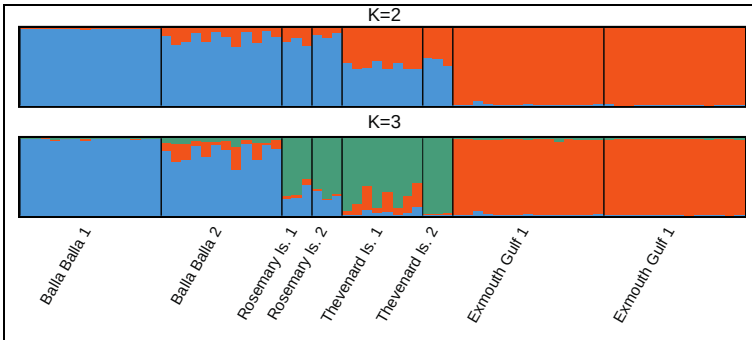


Figure 7. *Halodule uninervis* genetic structure over a regional scale in the Pilbara. Both K = 2 and K = 3 were strongly supported. At K = 3, Exmouth Gulf clearly separates from Rosemary and Thevenard Island, which separates again from Balla Balla further north based on the structure assignment.

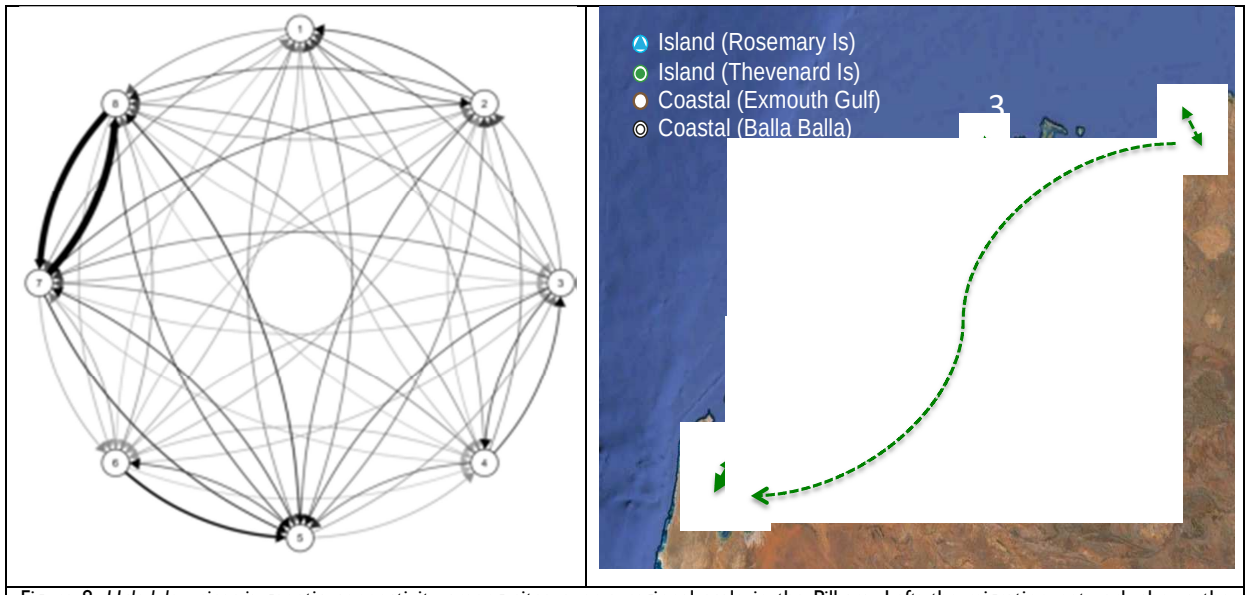


Figure 8. *Halodule uninervis* genetic connectivity among sites over a regional scale in the Pilbara. Left: the migration network shows the relative rates of migration per generation between sites, with the thicker lines indicating more migration and the arrows indicating the direction. Right: A stylised version showing the major migration pathways between sites. Site numbers in the network analysis correspond to 1= Balla Balla site 1, 2 = Balla Balla site 2, 3 = Rosemary Island site 1, 4 = Rosemary island site 2, 5 = Thevenard Island site 1, 6 = Thevenard Island site 2, 7 = Exmouth Gulf site 1, 8 = Exmouth Gulf site 2.

5.3.3 Genetic diversity by environment

There was not a significant partitioning of variation by habitat, but there was among (15%) and within (82%) populations (Table 9, left). When examining the partitioning of variation by region, there was a significant effect of region (14%), among populations (5%) and within populations (81%). In contrast to habitat, there is a significant effect of region on the partitioning of genetic variation (Table 9, right). The principal components analysis visually displays the genetic distance among individuals, populations and regions, highlighting the differences among regions and populations. Interestingly the Exmouth Gulf sites are more closely related than the Balla Balla sites.

Table 9. Analysis of molecular variance (AMOVA) for coastal habitat (Exmouth Gulf and Balla Balla) and island habitats (Rosemary and Thevenard) (left); and for regions (right) within the Pilbara.

Source	df	MS	P	% var	Source	Df	MS	P	% var
Among habitats	1	227	ns	3	Among regions	3	305	<0.05	14
Among populations	6	186	<0.01	15	Among populations	4	108	<0.01	5
Within populations	64	72	<0.05	82	Within populations	64	72	<0.01	81
Total	72				Total	72			

5.4 Thalassia hemprichii – large scale

5.4.1 Clonal structure and genetic diversity

Within the three sites in the Pilbara, from the 47–48 samples collected at each site, 20–39 unique genotypes (MLGs) were detected. The greatest number of MLGs was detected at Muiron Islands (North), and the least at Muiron Islands (South) with corresponding clonal richness ranging from 0.41 at Muiron Island (South) up to 0.81 at Muiron Island (North).

The Pilbara populations were genotypically more diverse than the Kimberley populations (Table 10). This is unusual as the Pilbara populations are at the edge of the species distribution range. In the Pilbara, the allelic richness (A_r) ranged from 2.05–2.52 alleles per locus, with the greatest at Barrow Island, compared to a range of 1.51–1.87 alleles per locus in the Kimberley. The highest allelic richness was in Indonesia, which is

understandable as it is at the centre of the species' range. The greatest number of private alleles was observed at Barrow Island. Genetic diversity, expressed as the observed heterozygosity ranged from 0.188–0.213 in the Pilbara, compared to the Kimberley, 0.104–0.291 (site range). As was observed with genotypic diversity, genetic diversity expressed as heterozygosity was also higher in Indonesia (Table 10).

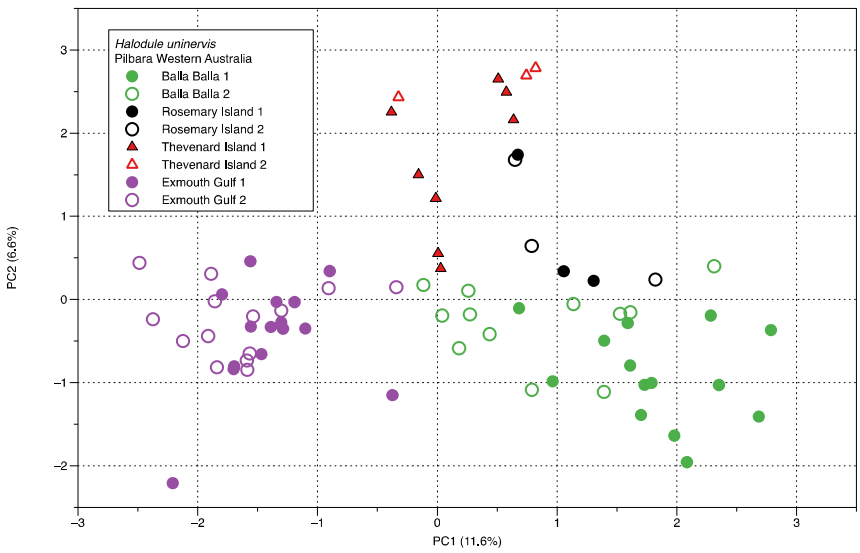


Figure 9. *Halodule uninervis* PCA of genetic distance among sites over a regional scale in the Pilbara.

Table 10. Allelic richness and genetic diversity of *Thalassia hemprichii* obtained from 16 microsatellite loci with standardized genets of 30. N: total number of individuals examined; G: number of multi-locus genotype; clonal size (number of samples with same MLG); R: clonal richness (MLG-1/N-1); nA: observed alleles; Ar: allelic richness and pA: private allele richness, standardized at 14 genets (mean of alleles per loci); *Hexp*: genetic diversity (non-biased expected heterozygosity, Nei 1978)). Shaded rows highlight the Pilbara samples which are the focus of this study.

No	Region	Population	Abb.	N	MLG	R	nA	Ar	pA	H_{obs}	H_{exp}
16	Pilbara	Barrow Island	BW	48	29	0.60	45	2.55	0.52	0.211	0.272
17		Muiron Island South	MS	47	20	0.41	33	1.98	0.02	0.213	0.202
18		Muiron Island North	MN	48	39	0.81	38	2.07	0.08	0.188	0.184
3	Kimberley	Bathurst Island	BAT	30	14	0.45	24	1.50	0.00	0.232	0.167
4		Irvine Island	LI	48	23	0.47	30	1.83	0.06	0.291	0.216
5		Bedford Island–south	BF	48	37	0.77	28	1.65	0.01	0.120	0.139
6		Bedford Island–north	BN	48	23	0.47	24	1.47	0.00	0.133	0.133
7		Riptide Island	GI	48	43	0.89	31	1.82	0.03	0.199	0.211
8		Mermaid Island	MI	48	44	0.91	36	1.84	0.09	0.215	0.196
9		Sunday Island–south	SI	47	20	0.41	27	1.58	0.05	0.119	0.132
10		Sunday Island–north	SN	48	27	0.55	27	1.56	0.12	0.130	0.131
11		Halls Pool	HP	48	32	0.66	27	1.61	0.07	0.104	0.171
12		Talon Island	TI	48	18	0.36	31	1.84	0.12	0.208	0.180
13		Jackson Island	JI	48	33	0.68	31	1.73	0.08	0.135	0.141
14		Noyon	NY	48	17	0.34	26	1.59	0.00	0.092	0.107
1	Indonesia	Kupang – Indonesia	KUP	48	43	0.89	56	3.32	0.13	0.430	0.415
2	Cocos	Cocos Keeling Island	CK	48	43	0.89	32	1.85	0.00	0.240	0.218

5.4.2 Population structure and patterns of connectivity

In the Pilbara, genetic differentiation was low but significant, with pair-wise G'_{ST} ranging from 0.015–0.037. Barrow Island and Muiron Island south were more similar than the two closely paired sites, Muiron Island

North and South. Genetic differentiation was much higher between the Pilbara and Kimberley sites, ranging from a pair-wise G_{ST} of 0.226–0.416. This was supported in the Structure analysis, where three distinct populations were detected (K = 3). All Pilbara sites grouped in one cluster (blue), the Kimberley sites in another cluster (red) and the Cocos (Keeling) and Kupang sites in another cluster (yellow)(Figure 10).

Within the Pilbara region, significant levels of migration were detected between sites (Figure 11). The highest levels of migration were from Muiron Islands North to South, Muiron Islands North to Barrow Island, and Muiron Island South to Barrow Island. Moderate levels of gene flow have occurred over distances of 5–145 km within this region, with the flows over the longer distances in a south to north direction. There appears to be little migration between the Pilbara and the Kimberley, and as supported by Structure they are operating as distinct populations.

Table 11. Population pair-wise F_{ST} (bottom triangular matrix) and G'_{ST} (top triangular matrix) comparisons among sites. All population pair-wise comparisons are significant at $p<0.05$. Dark grey shading highlights the comparison within Pilbara sites, and the light grey shading highlights the comparisons of the Pilbara populations with sites outside of the Pilbara.

	BW	**MS**	**MN**	**BAT**	**LI**	**BF**	**BN**	**GI**	**MI**	**SI**	**SN**	**HP**	**TI**	**JI**	**NY**	**KUP**	**CK**
BW	-	0.015	0.036	0.290	0.251	0.334	0.343	0.226	0.293	0.368	0.359	0.248	0.277	0.295	0.354	0.247	0.455
MS	0.027	-	0.037	0.323	0.263	0.369	0.379	0.252	0.315	0.400	0.393	0.269	0.307	0.329	0.394	0.270	0.512
MN	0.045	0.046	-	0.332	0.268	0.376	0.395	0.250	0.330	0.416	0.406	0.283	0.315	0.336	0.404	0.277	0.514
BAT	0.302	0.334	0.341	-	0.220	0.132	0.237	0.078	0.135	0.189	0.197	0.096	0.079	0.095	0.187	0.283	0.492
LI	0.260	0.271	0.275	0.228	-	0.265	0.329	0.191	0.216	0.262	0.259	0.208	0.227	0.226	0.287	0.231	0.486
BF	0.342	0.378	0.382	0.142	0.271	-	0.122	0.074	0.054	0.058	0.057	0.080	0.056	0.055	0.038	0.299	0.529
BN	0.353	0.389	0.403	0.248	0.336	0.132	-	0.149	0.130	0.091	0.082	0.102	0.075	0.139	0.122	0.301	0.556
GI	0.234	0.260	0.256	0.088	0.198	0.081	0.158	-	0.048	0.145	0.130	0.044	0.055	0.054	0.099	0.244	0.451
MI	0.300	0.323	0.336	0.143	0.223	0.060	0.138	0.054	-	0.080	0.070	0.065	0.077	0.073	0.054	0.250	0.485
SI	0.379	0.411	0.425	0.201	0.271	0.069	0.103	0.155	0.089	-	0.011	0.106	0.066	0.111	0.084	0.287	0.554
SN	0.368	0.402	0.412	0.207	0.266	0.066	0.092	0.138	0.077	0.022	-	0.083	0.067	0.078	0.042	0.295	0.553
HP	0.258	0.280	0.291	0.109	0.217	0.089	0.113	0.052	0.073	0.119	0.094	-	0.030	0.029	0.079	0.276	0.506
TI	0.289	0.318	0.323	0.090	0.235	0.066	0.086	0.064	0.086	0.079	0.078	0.042	-	0.041	0.086	0.273	0.510
JI	0.304	0.338	0.343	0.105	0.233	0.063	0.148	0.061	0.079	0.122	0.086	0.038	0.051	-	0.047	0.300	0.525
NY	0.367	0.406	0.414	0.200	0.296	0.051	0.136	0.110	0.064	0.100	0.055	0.093	0.100	0.059	-	0.322	0.562
KUP	0.254	0.279	0.282	0.292	0.238	0.305	0.308	0.250	0.255	0.295	0.302	0.283	0.282	0.306	0.331	-	0.276
CK	0.462	0.518	0.518	0.499	0.491	0.533	0.561	0.456	0.489	0.561	0.558	0.512	0.517	0.530	0.569	0.281	-

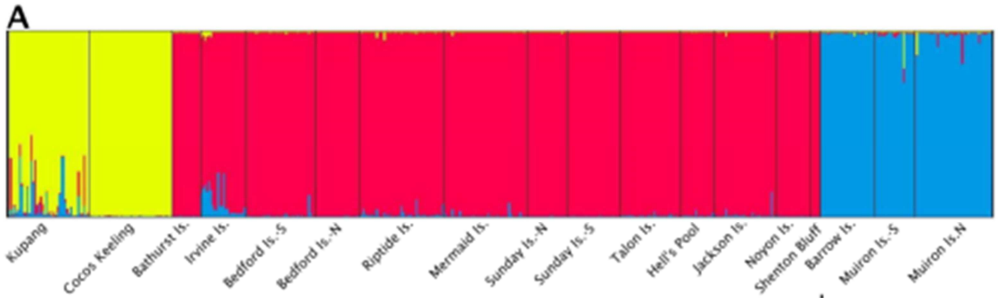


Figure 10. Estimated population structure of *Thalassia hemprichii* using Structure.

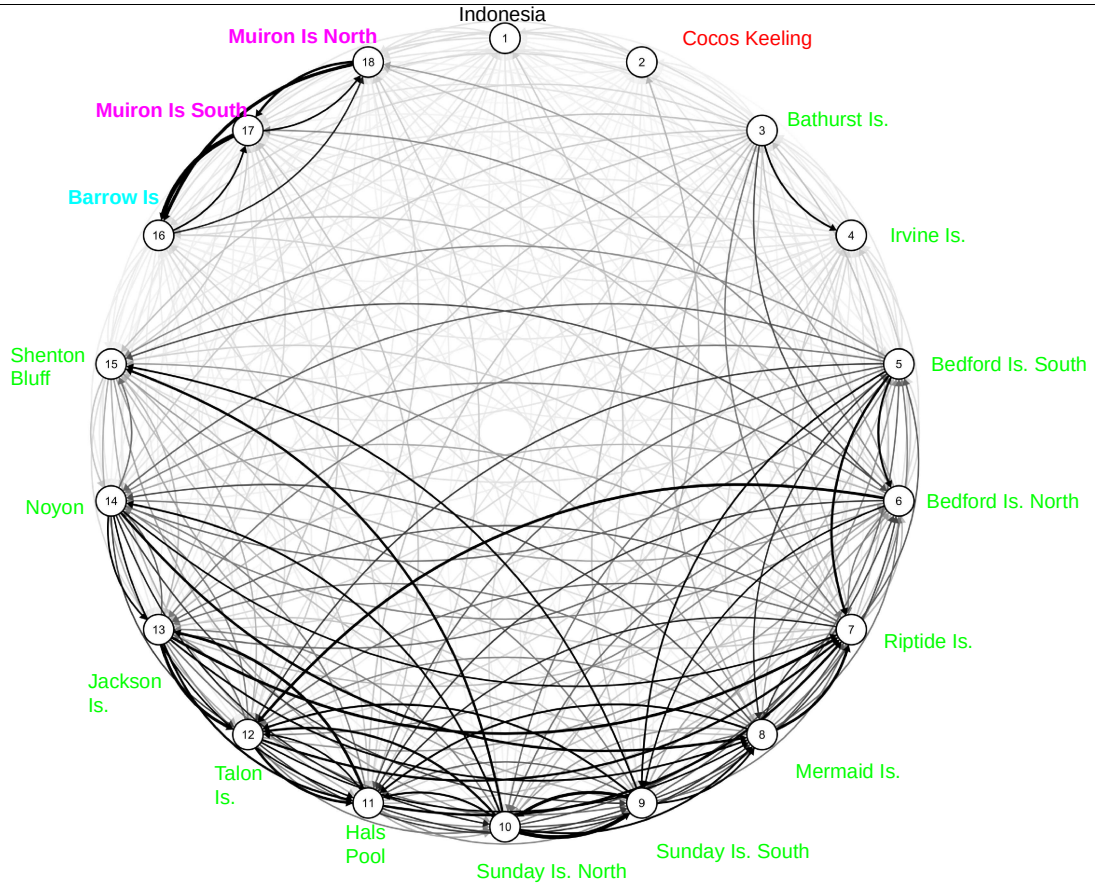


Figure 11. Pattern of gene flow based among Pilbara, Kimberley and Indonesian populations of *T. hemprichii*, based on $\widehat{Nm}$ (number of migrants per generation- Alcala et al. 2014). Sampling sites are represented by numbers within circles (See Table 9). Levels of $\widehat{Nm}$ among sampling sites are represented by curved lines. The thicker the lines, the higher levels of gene flow between populations. Blue and pink site names are from the Pilbara and green site names are from the Kimberley.

6 Discussion

6.1 Halophila ovalis

H. ovalis is widespread throughout the Pilbara and has a broad Indo-Pacific distribution in temperate and tropical waters (Waycott et al. 2004). It is a considered to have a colonising life-history strategy with fast shoot turn-over, significant investment in reproduction, a low physiological resistance to disturbance but a rapid ability to recover from disturbance due to its dormant seed bank (Kilminster et al. 2015). Due to this life-history strategy it is likely that sexual reproduction is important for maintaining *Halophila* populations and the results from this study support this. The meadows sampled are genotypically diverse, with moderate to high

levels of clonal richness and relatively high levels of allelic richness and high levels of genetic diversity, measured as heterozygosity. Clonal and allelic richness, as well as heterozygosity are in a similar range to that observed by van Dijk et al. (in review) for *H. ovalis* on the east coast of Australia over a slightly smaller spatial scale. These genetic measures indicate the meadows are likely to be relatively resilient to disturbance. High levels of heterozygosity indicate that they have the ability to recover from a disturbance or a reduction in the effective population size within a generation and the high allelic diversity indicates that they have the potential to adapt to pressures over a number of generations, subject to the level of pressure.

The genetic differentiation among meadows is quite low considering the spatial scale sampled in this study. van Dijk et al. (in review) noted slightly higher levels of genetic differentiation (based on F_{ST}) but this was over a smaller spatial scale, < 15 km and Ngugen et al. (2014) estimated higher levels of genetic differentiation but this was expected as the spatial scale was an order of magnitude higher, over 1000s km. Within this study, genetic connectivity among meadows is moderate to high over distances of 5 km, and moderate over distances up to 60 km. These are significant distances for a species with non-buoyant seeds that are generally released into the sediment when mature. Vegetative fragments could disperse with currents or fragments containing seeds, but little is known about the longevity of fragments (McMahon et al. 2014). Another mechanism for genetic connectivity is the biotic dispersal of seeds by grazers such as dugongs (McMahon et al. 2014). A number of seagrass seeds, including *H. ovalis* are viable after passing through a dugong gut (Tol et al. 2015) and dugongs are present in the region, grazing in the Exmouth Gulf, around Bundegi and the Ningaloo Reef lagoon near Mangrove Bay but are less common around the Muiron Islands, although fewer surveys have covered that region (Hodgson 2007). The low differentiation of sites over these distances, as well as the relative migration estimates indicate that movement of vegetative fragments with fruits or biotic dispersal may be important mechanisms for connectivity of *H. ovalis* populations in this region.

The exception to the generally low degree of genetic differentiation was the site at Bundegi. When Bundegi was included in the large dataset, it had high levels of genetic differentiation from all other sites, the greatest number of private alleles and formed a distinct population with no evidence of migration between all other populations. This is surprising as gene flow was detected between other sites up to 60 km apart, and the closest site to Bundegi is only 25 km away. There are a number of hypotheses we can propose to explain this. Firstly, as stated in the methods, the sample design may have influenced the results. The initial design called for a second Bundegi site to be sampled within 5 km of Bundegi, however, the only site containing *H. ovalis* within that range was in Marine Park Sanctuary Zone, where removal of any material is prohibited, and we were forced to sample outside the Exmouth Gulf. Therefore, the genetic isolation measured at Bundegi could be a result of not capturing the representative genetic variation in the region. For example, the population may have been recently recruited from a genetic source not captured in this study. Secondly, if genetic connectivity across 40–60 km is facilitated by dispersal of vegetative fragments or by dugongs then oceanographic currents or limited dugong movement from the Exmouth Gulf to Bundegi, or Bundegi to the Ningaloo site may isolate Bundegi from the other sites, resulting in higher levels of differentiation. This seems unlikely; as dugongs are regularly observed at Bundegi and they are known to move between sites on a regular basis (Gales et al. 2004, Sheppard et al. 2006, Hodgson 2007, Sheppard et al. 2010). Finally, the samples collected at Bundegi may be a different species of *Halophila*, and the isolation is due to a different evolutionary history. There is confusion regarding the taxonomy of the *H. ovalis* group (Waycott et al. 2014) with some evidence emerging of cryptic species and hybridisation among lineages (McMahon et al unpublished data). As this is a possible explanation, which if this is the case, would confound the population genetic analysis, we decided to be conservative and remove this site from the overall analysis. Work is underway at present to assess whether this is a different lineage, species or hybrid of the *Halophila ovalis* 'complex' using next generation sequencing.

Overall, this work has shown that the *H. ovalis* populations around Exmouth are genotypically and genetically diverse, indicating that they are likely to be resilient to disturbance. Some sites (Bundegi and Muiron Islands) had lower levels of genetic diversity suggesting they may be less resilient than others. At Bundegi, its apparent isolation from other sites, may result in slower recovery from impacts and its lower heterozygosity indicates that it may have a lower potential to respond to a disturbance within a generation. The Muiron Islands sites had the lowest clonal richness, making them potentially the least resistant to disturbance and Muiron Island North had the lowest allelic diversity so the least ability to adapt over generations to sustained pressure. These

sites are likely representative of other low diversity sites throughout the region, and because of this possibility, it may be useful to incorporate genetic diversity assessments in pre-dredging surveys and for management to focus on minimising threats at low diversity sites.

6.2 Halodule uninervis

H. uninervis is also widespread throughout the Pilbara and has a broad Indo-Pacific distribution (Waycott et al. 2004). It also has a colonising life-history strategy with fast shoot turn-over, significant investment in reproduction, a low physiological resistance to disturbance and a rapid ability to recover from disturbance due to its dormant seed bank (Kilminster et al. 2015). Due to this life-history strategy it is likely that sexual reproduction is important for maintaining *Halodule* populations. The results from this study support the important role of sexual reproduction in maintaining populations at five of the eight sites. At these sites, recruitment via seed banks is likely to occur and contribute to meadow recovery following impacts. However, at the remaining three sites, very low numbers of individuals were detected (clonal richness = 0.13), and vegetative growth is likely the main mechanism for maintaining populations, not sexual reproduction. Low clonal diversity has implications for the management of these populations. If they are completely lost due to human activities or natural events, such as cyclones, recovery will potentially be slow and will rely not on recruitment from seed banks, but from immigration of recruits from other meadows. The lack of seed banks and the inability to recover following complete meadow loss has been documented for some *H. uninervis* dominated meadows in Queensland. These Queensland meadows were in shallow-water sites where *H. uninervis* only used vegetative recovery, whereas deeper water meadows dominated by *Halophila* spp. used a combination of recruitment from seed and vegetative growth (Taylor et al. 2013, Rasheed et al. 2014). Our results for the Pilbara indicate that in some sites, recovery is likely to be from vegetative growth rather than seed banks, due to the high clonality of these meadows. At the other sites, the relatively high clonal richness indicates that recovery from seed banks may play an important role. There are no other published studies on the population genetics of *H. uninervis*, however, there are a few on its sister species (*H. wrightii*) in the Atlantic. The low clonal richness we observed at some *H. uninervis* sites in the Pilbara has not been observed in Atlantic *H. wrightii* (Angel 2002, Travis & Sheridan 2006), though it is difficult to make a direct comparison as different markers and methods of determining clonal richness were used.

Genetic diversity measured as allelic richness and heterozygosity, was as expected, lowest at the sites where only three individuals were detected. However, at the sites with moderate to high clonal richness, a higher clonal richness did not necessarily reflect a high allelic or genetic diversity. Due to the low number of genotypes identified at some of these sites, there are limited conclusions we can draw on the significance of the levels of heterozygosity and allelic diversity in these populations. The allelic diversity was greatest at Exmouth Gulf and Thevenard Island, indicating the greatest potential to adapt to change over generations, and the heterozygosity was greatest at Thevenard Island, indicating that these populations have the highest potential to recover from a disturbance or a reduction in the effective population size within a generation.

Unlike *H. ovalis*, over the larger, regional scale there was significant population structure in *H. uninervis*. The data strongly support the presence of two or three clusters of genotypes, with Exmouth Gulf clearly separating from the other populations and, at a higher level of structure, Balla Balla separating from the central islands sites. As was observed with *H. ovalis*, the greatest levels of connectivity were between sites separated by 2–5 km. Exmouth Gulf is ~100 km from the next closest sites we sampled, but the other sites that cluster together spanned distances of 200–340 km, depending on the Structure model accepted. Despite this, only very low levels of migration were detected between these regions that grouped together, the highest levels of migration were among sites in the same region, separated by 2–5 km. The low levels of long-distance migration between Exmouth Gulf and Thevenard (~100 km) and Exmouth Gulf and Balla Balla (~450 km) are significant for a species with non-buoyant seeds that are generally released into the sediment when mature. Vegetative fragments could disperse with currents or fragments containing seeds, but little is known on the longevity of fragments (McMahon et al. 2014), particularly over these distances. Biotic dispersal by dugongs (McMahon et al. 2014) is a possible mechanism. It is known that *H. uninervis* seeds found in dugong faeces are viable (Tol et al. 2015) and these three regions are dugong population hotspots in the Pilbara (Hodgson 2007). Although low levels of migration were detected, there is significant differentiation between the Balla Balla sites and Exmouth

Gulf, indicating that if biotic dispersal does occur, it is a rare event. These initial findings show that further research into the mechanisms of connectivity in the region are warranted.

Over the regional scale we identified two to three discrete management units, based on spatial distribution of the genetic clusters. Due to the low number of individuals identified at Rosemary and Thevenard Island, further analysis is required with a larger number of samples to guide decisions as to whether they should be designated as a discrete management unit. The low and often insignificant genetic differentiation over short distances (up to 5 km) and the high connectivity over these distances, implies that if large areas of seagrass are lost they could recover from immigration at this scale. However, recovery over larger distances, although there was evidence that it occurs, it is most likely rare, so it should not be considered a possible recovery pathway in the management of habitat loss.

6.3 Thalassia hemprichii

T. hemprichii has a broad distribution in the Indo-Pacific but is restricted to tropical waters. The southern most limit of its distribution on the west coast of Australia is around Exmouth, hence the Muiron Islands are at the edge of its range. It is considered to have a persistent life-history habitat with relatively slow turnover, long-lived, no seed dormancy, a high physiological resistance to disturbance and a slow ability to recover (Kilminster et al. 2015). Most studies of *T. hemprichii* have shown that it reproduces seasonally, although no detailed studies have been carried out in WA, so flowering and fruiting times are unknown. The fruits are buoyant, so are an excellent dispersal unit, remaining afloat up to 2–7 days. Once the seeds are released from the buoyant fruit they immediately sink (Lacap et al. 2002). Dispersal distances from 23–74 km have been recorded in the Bolinao Reef system, Philippines (Lacap et al. 2002), and up to 300 km in the Atlantic sister species *T. testudinum* (van Dijk et al. 2009). Therefore, compared to other species such as *H. ovalis* and *H. uninervis,* we predict *T. hemprichii* meadows would experience genetic connectivity over greater spatial scales.

The meadows sampled in the Pilbara were genotypically diverse, with moderate to high levels of clonal richness, moderate levels of allelic diversity and heterozygosity. The fact that in general more than 50% of samples collected within a site were unique individuals (MLGs), shows that both sexual reproduction and vegetative growth are important for maintaining population growth. Overall, these features indicate they are resilient to disturbance and pressures over generational and multi-generational time scales. For *T. hemprichii*, the Pilbara is at the edge of the range, where lower genetic diversity is predicted (sensu Eckert et al. 2008). A number of recent seagrass papers have supported this pattern (Evans et al. 2014, Nakajima et al. 2014). Relative to the Indonesian populations, which are at the centre of the range, the genetic diversity at the edge of the range was less than Indonesia (Kupang). But the allelic diversity in the Pilbara, based on three sites is greater than in the Kimberley, based on 12 sites, which does not hold with the predictions of lower diversity at the range edge. This implies that the Pilbara meadows have a greater capacity to respond and adapt to multi-generational pressures compared to the Kimberley. The Pilbara and Kimberley populations should also be considered discrete management units due to the very limited gene flow among them and the support of separate populations clusters.

Within the Pilbara, there is evidence of moderate levels of gene flow over distances of 200 km. This is congruent with previously published data on the potential dispersal distances of this species and its sister species, *T. testudinum* (Lacap et al. 2002, van Dijk et al. 2009). Populations from the Muiron Islands are connected with populations at Barrow Island. Interestingly, there is directionality in the gene flow, with a predominant south to north movement. This movement is in the opposite direction of the dominant oceanographic currents in the region, the Holloway and Leeuwin Currents flowing along the continental shelf edge, which moves in a southward direction. However, this pattern of movement is congruent with a northward counter-current on the continental shelf, which is present in the region during austral summer (Feng et al. in prep). As *Thalassia* fruits are buoyant, a combination of water currents and windage could facilitate dispersal. The timing of fruit release is not known in this region but if it does occur at this time, it would facilitate this dispersal. This directionality also implies that southern populations are a potential source for more northern populations.

6.4 Species comparison and insights for resilience of seagrasses in the Pilbara

We performed genetic diversity and connectivity analysis on three NW seagrass species, each at a different spatial scale. Ideally, we would have performed all species at the same scale, but this is challenging due to the distribution of species in this area. However, we can compare the genetic differentiation among species at the same sites for a number of cases. Firstly *H. ovalis* and *T. hemprichii* were both collected at the Muiron Islands North and South, using the same type of markers, microsatellites. The genetic differentiation measured as F_{ST} was greater in *T. hemprichii* (0.037 vs. 0.016), a surprising outcome since you would predict this species to have a better ability to disperse between meadows due to the buoyant fruits, thereby reducing differentiation. The other case was in Exmouth Gulf site 1 and site 2 where both *H. ovalis* and *H. uninervis* were found. In this case, very low and similar levels of differentiation were found for both species (Table 12). In this case, both species have similar reproductive biology with non-buoyant and dormant seeds that are often released into the sediments, therefore, based on the dispersal biology, we would predict similar levels of genetic differentiation between sites. Further work is needed to develop a comparative understanding of the genetic structure and connectivity of seagrass species in this region.

Table 12. Population pair-wise F_{ST} between sites where multiple species were measured, note that the pair-wise comparison in bold are not significant at $p<0.05$.

Site	Site	*H. ovalis*	*T. hemprichii*	*H. uninervis*
MS	**MN**	0.016	0.037	-
EG1	**EG2**	0.004	-	**0.002**

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 genotype. 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 (Figure 12). We used a relative scale of clonal richness, allelic richness and heterozygosity within species, ranking the higher values as relatively more resilient. Overall, based on the genetic measures, the most resilient meadows were in Exmouth Gulf (*H. ovalis* and *H. uninervis*), Barrow Island (*T. hemprichii*) and Thevenard Island (*H. uninervis*). The least resilient meadows were at Rosemary Island (*H. uninervis*).

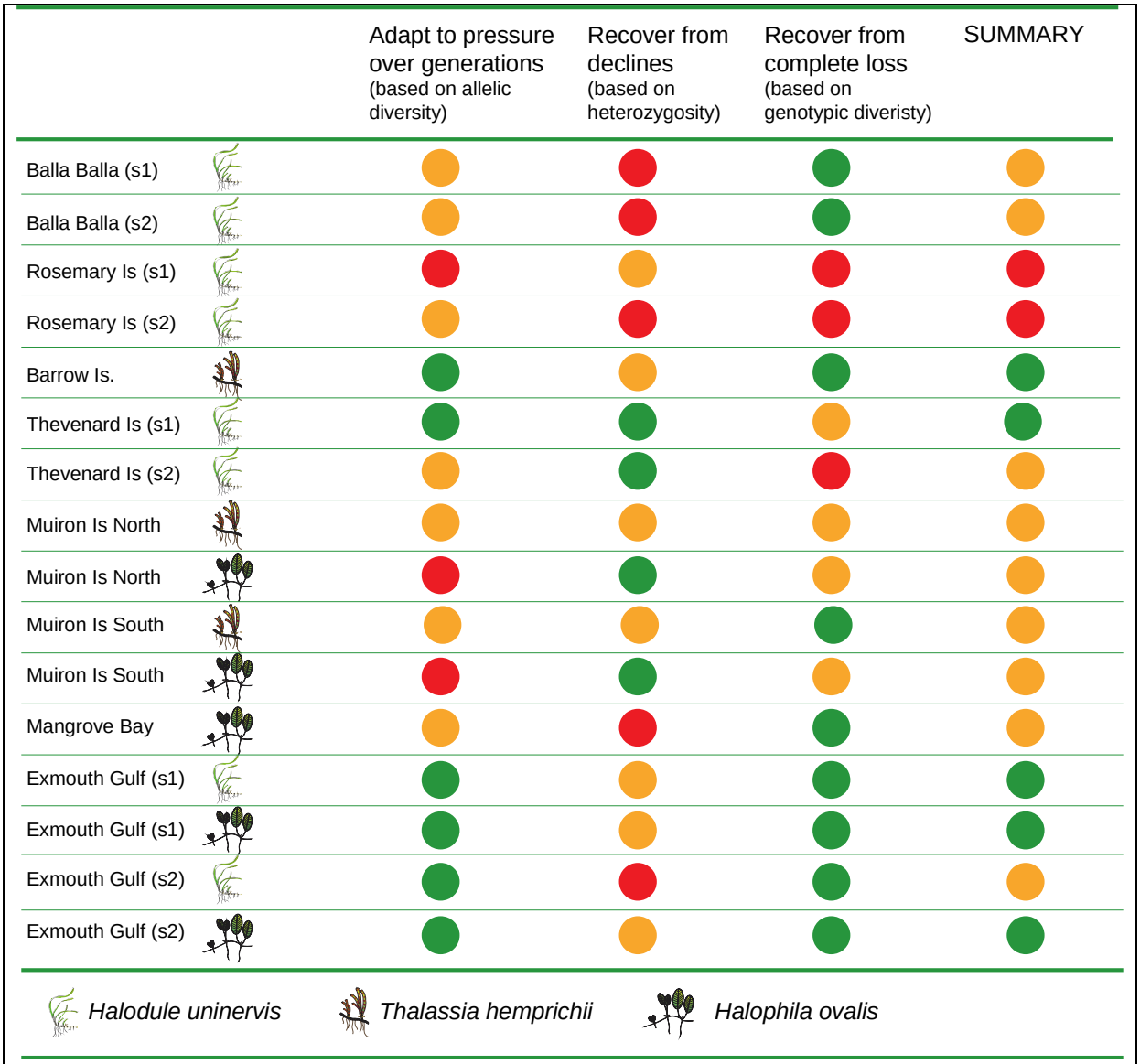


Figure 12. A summary of the genetic resilience of seagrass meadows in the Pilbara. The potential to adapt to pressures over generations was based on allelic richness, to recover from declines in a generation was based on heterozygosity and to recover from complete loss using seed banks was based on genotypic diversity (clonal richness). Red = least resilient ; orange = moderate resilient; Green most resilient????

6.5 Insights for other Theme 5 projects

Across three seagrass species in the Pilbara from 12 sites, we found a range of diversity based on the clonal richness. Generally, across all meadows and species in this study, clonal richness was moderate to high. So within meadows, over an area of ~8,000 m^2 multiple unique individuals were detected, in fact the chance of selecting a unique genotype in the sites with moderate to high clonal richness ranged from 36–96%. At a smaller scale, 0.2 m^2 for *H. ovalis* the probability was similar, ranging from 33–75%. However, there was a number of sites where this was not the case, and these were within *H. uninervis* meadows where only 3/16 unique genotypes were selected, a 16% chance of selecting a unique genotype. These sites did have a smaller sample size, compared to the other species, due to the challenges with developing the genotyping approach. However, work is underway, funded by Department of Parks and Wildlife and ECU in collaboration with University of Adelaide, to improve these sample numbers and develop a better understanding of clonality in these and other meadows in the Pilbara (K. McMahon pers. comm.).

This understanding of the levels of clonality can help to inform further experimental work. Firstly, if the aim is to replicate natural conditions in mesocosms and impose stressors, then in general multiple genotypes should be used, as this is to date, the most common situation encountered in the field. However, if the question is very site specific, then the genetic diversity of the conditions at that site should be incorporated, either by assessing the genetic diversity before experimentation, or collecting from the particular sites of interest. If experiments are to be carried out in the field, the preliminary results suggest that clonal diversity, at least for *H. ovalis* is generally high at a small scale, at similar levels to the meadow. So replicated, small-scale experiments are likely to encompass the genetic diversity in the meadow.

7 References

Alcala N, Goudet J, Vuilleumier S (2014) On the transition of genetic differentiation from isolation to panmixia: What we can learn from G(ST) and D. Theoretical Population Biology 93:75-84

Angel R (2002) Genetic diversity of Halodule wrightii using random amplified polymorphic DNA. Aquatic Botany 74:165-174

Collier CJ, Lavery PS, Ralph PJ, Masini RJ (2009) Shade-induced response and recovery of the seagrass *Posidonia sinuosa*. Journal of Experimental Marine Biology And Ecology 370:89-103

den Hartog C (1970) Sea-grasses of the world. North Holland Publishing Company, Amsterdam

Douhovnikoff V, Dodd R (2003) Intra-clonal variation and a similarity threshold for identification of clones: application to *Salix exigua* using AFLP molecular markers. Theoretical and Applied Genetics 106:1307-1315

Earl DA, vonHoldt BM (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources 4:359-361

Eckert CG, Samis KE, Lougheed SC (2008) Genetic variation across species' geographical ranges: the central-marginal hypothesis and beyond. Molecular Ecology 17:1170-1188

Engelhardt KAM, Lloyd MW, Neel MC (2014) Effects of genetic diversity on conservation and restoration potential. at individual, population, and regional scales. Biological Conservation 179:6-16

Epskamp S, Cramer AOJ, Waldorp LJ, Schmittmann VD, Borsboom D (2012) qgraph: Network Visualizations of Relationships in Psychometric Data. Journal of Statistical Software 48:1-18

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology 14:2611-2620

Evans SM, Sinclair EA, Poore AGB, Steinberg PD, Kendrick GA, Verges A (2014) Genetic diversity in threatened *Posidonia australis* seagrass meadows. Conservation Genetics 15:717-728

Feng M, Colberg F, Slawinski D, Berry O, Babcock R (in prep) Ocean circulation, larval retention, and connectivity among coral populations on the North West Shelf of Australia. Coral Reefs

Gales N, McCauley RD, Lanyon J, Holley D (2004) Change in abundance of dugongs in Shark Bay, Ningaloo and Exmouth Gulf, Western Australia: evidence for large-scale migration. Wildlife Research 31:283-290

Grech A, Chartrand-Miller K, Erftemeijer P, Fonseca M, McKenzie L, Rasheed M, Taylor H, Coles R (2012) A comparison of threats, vulnerabilities and management approaches in global seagrass bioregions. Environmental Research Letters 7:8

Hedrick P (2005) A standardized genetic differentiation measure. Evolution 59:1633–1638

Hird SM, Brumfield RT, Carstens BC (2011) RGmatic: an efficient pipeline for collating genome-enriched second-generation sequencing data using a 'provisional-reference genome'. Molecular Ecology Resources 11:743–748

Hodgson A (2007) The distribution, abundance and conservation of dugongs and other marine megafauna in Shark Bay Marine Park, Ningaloo Reef Marine Parka nd Exmouth Gulf. Unpublished report to WA Department of Environment and Conservation

Hughes AR, Stachowicz JJ (2004) Genetic diversity enhances the resistance of a seagrass ecosystem to disturbance. PNAS 101:8998-9002

Jakobsson M, Rosenberg NA (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics 23:1801-1806

Jannsen T, Bremer K (2004) The age of major monocot groups inferred from 800+ rbcL sequences. Botanical Journal of the Linnean Society 146:385–398

Jardine DI, Dormontt EE, van Dijk KJ, Dixon RRM, Dunker B, Lowe AJ (2015) A set of 204 SNP and INDEL markers for Bigleaf maple (*Acer macrophyllum* Pursch). Conservation Genetics Resources 7:797-801

Kalinowski ST (2005) HP-RARE 1.0: a computer program for performing rarefaction on measures of allelic richness. Molecular Ecology Notes 5:187-189

Kamvar ZN, Tabima JF, Gruenwald NJ (2014) Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. Peerj 2:e281

Keenan K, McGinnity P, Cross TF, Crozier WW, Prodoehl PA (2013) diveRsity: An R package for the estimation and exploration of population genetics parameters and their associated errors. Methods in Ecology and Evolution 4:782-788

Kilminster K, McMahon K, Waycott M, Kendrick GA, Scanes P, McKenzie L, O'Brien KR, Lyons M, Ferguson A, Maxwell P, Glasby T, Udy J (2015) Unravelling complexity in seagrass systems for management: Australia as a microcosm. Science of the Total Environment 534:97-109

Lacap CDA, Vermaat JE, Rollon RN, Nacorda HM (2002) Propagule dispersal of the SE Asian seagrasses *Enhalus acoroides* and *Thalassia hemprichii*. Marine Ecology Progress Series 235:75-80

Lavery P, McMahon K, Mulligan M, Tennyson A (2009) Interactive effects of timing, intensity and duration of light reduction on *Amphibolis griffithii* Marine Ecology Progress Series 394:21-33

Les DH, Cleland MA, Waycott M (1997) Phylogenetic studies in Alismatidae, II: Evolution of marine angiosperms (seagrasses) and hydrophily. Syst Bot 22:443-463

Lowe AJ, Harris SA, Ashton SA (2004) Ecological Genetics; Design, Analysis and Application. Blackwells

McMahon K, Statton J, Lavery PS (2015) Identify the primary producers for research into thresholds and indicators of response to dredging-related pressures WAMSI Dredging Science Node Primary Producer Theme 51b. Western Australian Marine Science Institution, Perth

McMahon K, van Dijk K-j, Ruiz-Montoya L, Kendrick GA, Krauss SL, Waycott M, Verduin J, Lowe R, Statton J, Brown E, Duarte C (2014) The movement ecology of seagrasses. Proceedings of the Royal Society B-Biological Sciences 281:20140878

Meirmans PG, Hedrick PW (2011) Assessing population structure: F-ST and related measures. Molecular Ecology Resources 11:5-18

Nakajima Y, Matsuki Y, Lian C, Fortes MD, Uy WH, Campos WL, Nakaoka M, Nadaoka K (2014) The Kuroshio Current influences genetic diversity and population genetic structure of a tropical seagrass, *Enhalus acoroides*. Molecular Ecology 23:6029–6044

Nei M (1973) Analysis of gene diversity in subdivided populations. Proceedings of the National Academy of Sciences 70:3321–3323

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583-590

Nguyen VX, Detcharoen M, Tuntiprapas P, Soe-Htun U, Sidik JB, Harah MZ, Prathep A, Papenbrock J (2014) Genetic species identification and population structure of Halophila (Hydrocharitaceae) from the Western Pacific to the Eastern Indian Ocean. BMC Evolutionary Biology 14:1-18

Orth RJ, Carruthers TJB, Dennison WC, Duarte CM, Fourqurean JW, Heck KLJ, Hughes AR, Kendrick GA, Kenworthy WJ, Olyarnik S, Short FT, Waycott M, Williams SL (2006) A global crisis for seagrass ecosystems. Bioscience 56:987-996

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945-959

Rasheed MA, McKenna SA, Carter AB, Coles RG (2014) Contrasting recovery of shallow and deep water seagrass communities following climate associated losses in tropical north Queensland, Australia. Marine Pollution Bulletin 83:491-499

Rosenberg NA (2004) DISTRUCT: a program for the graphical display of population structure. Molecular Ecology Notes 4:137-138

Salo T, Reusch TBH, Bostrom C (2015) Genotype-specific responses to light stress in eelgrass *Zostera marina*, a marine foundation plant. Marine Ecology Progress Series 519:129-140

Sheppard JK, Marsh H, Jones RE, Lawler IR (2010) Dugong habitat use in relation to seagrass nutrients, tides, and diel cycles. Marine Mammal Science 26:855-879

Sheppard JK, Preen AR, Marsh H, Lawler IR, Whiting SD, Jones RE (2006) Movement heterogeneity of dugongs, *Dugong dugon* (Muller), over large spatial scales. Journal of Experimental Marine Biology and Ecology 334:64-83

Short FT, Polidoro B, Livingstone SR, Carpenter KE, Bandeira S, Bujang JS, Calumpong HP, Carruthers TJB, Coles RG, Dennison WC, Erftemeijer PLA, Fortes MD, Freeman AS, Jagtap TG, Kamal AHM, Kendrick GA, Kenworthy WJ, La Nafie YA, Nasution IM, Orth RJ, Prathep A, Sanciangco JC, van Tussenbroek B, Vergara SG, Waycott M, Zieman JC (2011) Extinction risk assessment of the world's seagrass species. Biological Conservation 144:1961-1971

Taylor H, Rasheed M, McKenna SA, Carter AB, Reason CL, Loban F (2013) Seagrass productivity, resilinece to climate change and capacity for recovery in the Torres Strait 2011-2012. JCU Publication 13/26, Centre for Tropical Water and Aquatic Ecosystem Research, Cairns

Tol SJ, Jarvis JC, Coles RC, York PY, Congdon BC (2015) Tropical seagrass seed dispersal by marinemega-herbivores. Poster presentation from Australian Marine Sciences Association. Deakin University, Geelong

Travis SE, Sheridan P (2006) Genetic structure of natural and restored shoalgrass Halodule wrightii populations in the NW Gulf of Mexico. Marine Ecology Progress Series 322:117-127

van Dijk JK, van Tussenbroek BI, Jimenez-Duran K, Judith Marquez-Guzman G, Ouborg J (2009) High levels of gene flow and low population genetic structure related to high dispersal potential of a tropical marine angiosperm. Marine Ecology Progress Series 390:67-77

van Dijk K-J, Mellors J, Waycott M (2014) Development of multiplex microsatellite PCR panels for the seagrass *Thalassia hemprichii* (Hydrocharitaceae). Applications in Plant Sciences 2:1400078

van Dijk KJ, McMahon K, Waycott M (in review) Genetic diversity, structure and connectivity of the seagrass *Halophila ovalis* in a highly grazed marine embayment, Moreton Bay, Australia. PLOS ONE

van Orsouw NJ, Hogers R, Janssen A, Yalcin F, Snoeijers S, Verstege E, Schneiders H, van der Poe H, van Oeveren J, Verstegen H, van Eijk MJT (2007) Complexity reduction of polymorphic

sequences (CRoPS): a novel approach for large-scale polymorphism discovery in complex genomes. PLoS ONE 2:e1172

Vos P, R H, Bleeker M, Reijans M, Van de Lee T, Hornes M, Friters A, Pot J, Paleman J, Kuiper M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research 23:4407–4414

Waycott M, Duarte CM, Carruthers TJB, Orth RJ, Dennison WC, Olyarnik S, Kenworthy WJ, Short FT, Williams SL (2009) Accelerating loss of seagrasses across the globe threatens coastal ecosystems. Proceedings of the National Academy of Sciences in press:12377-12381

Waycott M, McMahon KM, Lavery PS (2014) A Guide to Temperate Southern Seagrasses. CSIRO, Melbourne, p108, ISBN13 9780864437266

Waycott M, McMahon KM, Mellors JE, Calladine A, Kleine D (2004) A Guide to Tropical Seagrasses of the Indo-West Pacific. James Cook University, Townsville, p 72, ISBN10 0864437269

Wright S (1969) Evolution and the Genetics of Populations, Vol 2. University of Chicago Press, Chicago

Xu N-N, Yu S, Zhang J-G, Tsang PKE, Chen X-Y (2010) Microsatellite primers for *Halophila ovalis* and cross-amplification in *H. minor* (Hydrocharitaceae). American Journal of Botany 97:E56-E57

8 Appendices

8.1 Glossary of genetic terms

adaptation	the evolution of a particular character or characters in response to a specific selective pressure
allele	one or two o rmore alternative forms of a gene, locus or DNA sequence
allele frequency	the abundance of a particular allele in a population or species (expressed as a proportion of one)
allelic richness	the number of alleles in a given locus or the mean number of alleles per locus
average gene diversity	a measure of the expected level of heterozygosity calculated from allele frequencies revealed by assay, averaged across all loci
clonal richness	number of unique genotypes in a population and expressed as MLG-1/N-1
clone mate	different samples but with the same identity or multilocus genotype (MLG)
cluster	a group of genetically similar samples positioned together in a pictorial representation such as a dendrogram or a multivariate plot
co-dominant marker	a genetic marker in which both alleles are expressed, thus heterozygous individuals can be distinguished from either homozygous state
CRoP	a molecular technique - complexity reduction of polymorphic sequences
demographic connectivity	relative contribution dispersal vs. local recruitment to population growth
dispersal	the movement of individuals from one location to another
dominant marker	a marker that shows dominat inheritance with homozygous dominant individuals indistinguishable from heterozygous individuals
effective population size	the number of individuals in a population reproducing and contributing to alleles present in the next generation
effective number of alleles	the number of alleles in a population, weighted for their frequencies, very dependent on sample size
expected heterozygosity	the probability that two gametes, randomly chosen from the gene pool, are of different alleles, are by far the measures most commonly used by the majority of papers that present a genetic summary of populations
fitness	the ability of an individual, relative to other members of the same species, to produce viable offspring which themsleves survive to reproduce and leave viable offspring
fixation	the loss of alleles from a polymorphic population until only one remains, i.e. becomes monomorphic
Fst	fixatoin index, a measure of population differentiation
F'st	a standardised measure of Fst where Fst is expressed relative to the maximum value possible giventhe observed amount of within-population diversity
gene	specific nucelotide sequence of DNA that codes for a particular protein, tRNA, or rRNA
gene flow	movement of genes from one population to another that then interbreed with the recipient population
gene pool	the genes of all the breeding individuals in a discrete population

genet	a genetically distinct individual
genetic connectivity	dispersal of indiviudals or gene from one population to another
genetic differentation	a measure of the allocation of genetic variaiton within a species and among populations. Species with a high level of genetic differentiation show high variation between populations
genetic distance	the degree of genetic similarity between a pair of individuals, populations or species. Values typically vary between 0 (identical) and 1 (completely different)
gene/genetic diversity	a measure of the genetic variation found in a population or species based on the mean expected heterozygosity
genetic drift	change in allele frequencies within a population over time due to the sampleing effects of small populations
genetic marker	a sequence of DNA or protein that can be screened to reveal key attricutes of its state or composition and thus used to reveal genetic variation
genome	the full complement of genes present in a haplod set of chromosomes in an organism
genotype	state (e.g. allelic composition) for a particular genetic locus of an organism
genotypic diversity	a measure of the number of genotypes in a population sample
G'st (Nei)	a measure of population differentiation which compares the heterozygosity within and between populations, with a correction for sampling a limited number of populations
G"st	a standardised measure of population differentation based on Nei's Gst
heterozygosity	a measure of frequency of heterozygotes in a subpopulation
heterozygote	an individual with two different alleles at a locus
homozygote	an individual with two copies of the same allele at a locus
inbreeding	reproduction between closely realted individuals, includes self-fertilisation
infinite allele model	mutation pattern where an allele can spontaneously mutate into an allele of any size or character
Jost's D	index of population differentiation that is independent of the amount of within population diversity (Hs)
lineage	a particular monoplyletic group of genotypes (taxa, populations or individuals) that are closely related by descent more closely than other such groups
locus	a specific region or position on the genome of chromosome
marker	an observable, typically discrete, genetically controlled trait
metapopulation	a population consisting of a network of partially isolated smaller populations
microsatellites	short tandem repeats of a short sequence of typically two to four nucleotides randomly distirbuted throughout the genome
migration	the movement of organisms form one location to another, in population genetics this usually refers to the movement of an individual form one population to another which then breeds with individuals from the recipient population
MLG	multi-locus genotype, a terminology used when identifying individuals with a clonal species where the genetic signature is unique
mutation	change in nucleotide sequence of an organism

Nm	the number of migrants successfully entering a population per generation
null alleles	an allele that is not expressed and therefore does not produce a band on a gel
observed heterozygosity	the frequency of heterozygous individuals in a subpopulation ranging from 0 (all individuals homozygpous) to 1 (all individuals heterozygous)
panmixia	mating between individuals in a population which is random with respect to phenotype/genotype
polymerase chain reaction (PCR)	a techinique for increasing the number of target DNA sequences by several orders of magnitude by repeated cycles of denaturations, primer annealing, and fragment extension using natural properties of a thermostable DNA polymerase
polymorphic	loci with more than one allele leading to different phenotypes in the population
population	ecological population; a group of individuals of the same species within the same habitat at the same time. Statisitcal population: all the items under study. Genetical population: all the individuals connected by the gene flow, i.e. gene pool
private alleles	the possession of unique alleles within a sampling unit (e.g. population)
ramet	a connected piece of rhizome containing roots and shoots of a clonal plant
rarefaction	method od addressing unequal sample sizes by recording the frequency of alleles in a large sample and estimating the number of each allele that would occur at these frequencies in smaller samples
relatedness	a value ® that records the degree of shared genetic material between indivudals
richness	a measure of the absolute number of variants in a sample
selection	the influence of the environment (in its broadest sense) in determining which individuals will breed and pass their genes on to the next generation and those who will not breed
SNP	single nucelotide polymorphism - a DNA sequence variation occuring when a single nucelotide, A, T, C or G in the genome of other shared sequence varies
somatic mutation	a change in the genetic signature of a somatic cell in an individual , which in long-lived clonal plants can add to the genetic variation
speciation	the process of generating new species due to reproductive isolation, ecological differentiation, and/or character change
subpopulation	spatially distinct unit of a statistical population
tandem repeats	a form of repetitive DNA with units of usually short sequences repeated hundreds or thousands of times

8.2 Genetic data for *Halophila* from Bundegi site including structure and migration outputs when included in the complete data set.

Table 3. Allelic richness and genetic diversity of *Halophila ovalis* obtained from 9 microsatellite loci with standardized genets of 28. N: total number of individuals examined; MLG: number of multi-locus genotypes; R: clonal richness (MLG-1/N-1); nA: observed alleles; Ar: allelic richness and pA: private allele richness, standardized at 30 genets (mean of alleles per loci); *Hobs:* observed heterozygosity*; Hexp*: non-biased expected heterozygosity;(Nei 1978)).

Habitat	**Population**	**Site**	**N**	**MLG**	**R**	**nA**	**Ar**	**pA**	**H_{obs}**	**H_{exp}**
Coastal Clear	Bundegi	5	47	35	0.74	39	4.10	0.64	0.34	0.49

Figure A. *Halophila ovalis* genetic structure (K=2) over a local scale in the Exmouth region. Bundegi clearly separates from the remaining sites based on the structure assignment.

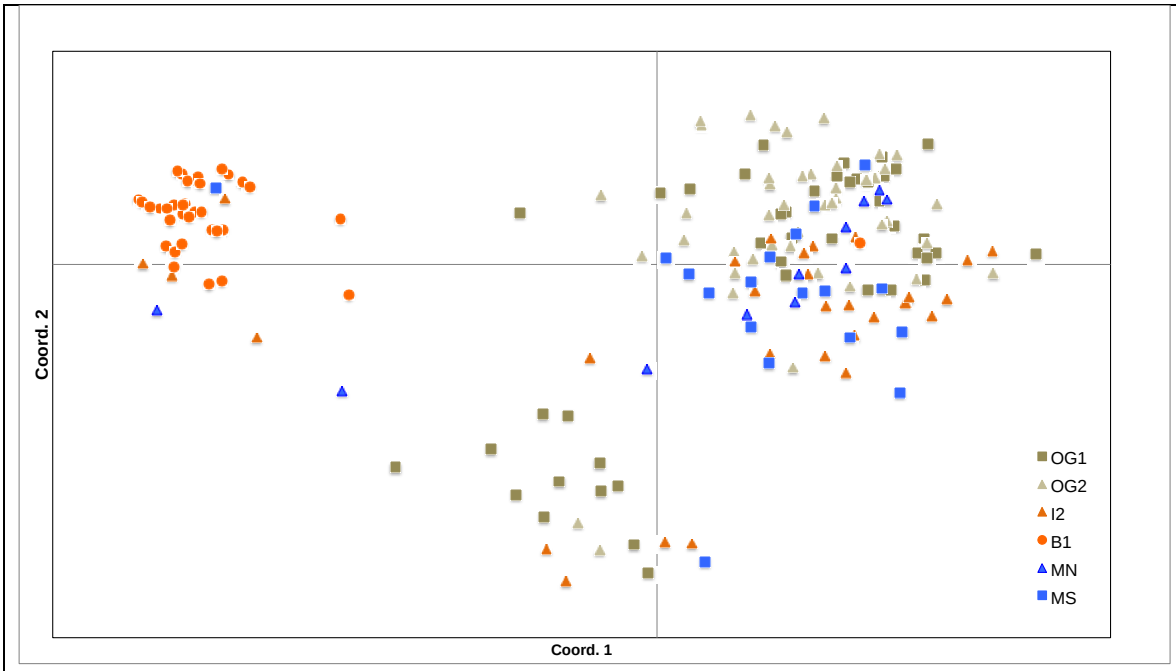


Figure B. A principal coordinate analysis showing the association of similar genotypes. Bundegi is different to the other sites. These two axes account for 35% of the variation.