National Environmental Science Programme

Assisting recovery of seagrass in Shark Bay, Gathaagudu

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Project E6 – Assisting restoration of ecosystem engineers through seed-based and shoot-based programs in the Shark Bay World Heritage Site (WHS)

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Milestone 7 Research Plan version 5 (2019)

Assessing the survivorship of *Amphibolis antarctica* seagrass seedlings recruited onto biodegradable Hessian sandbags at Fowlers Camp, Shark Bay

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Important Disclaimer

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1. EXECUTIVE SUMMARY

The goal of NESP Project E6 is to work alongside the Malgana Traditional Owners to assist recovery of the dominant seagrasses, *Amphibolis antarctica* and *Posidonia australis* in Shark Bay (Gathaagudu) following the 2011 marine heat wave. Therefore, this project has been developed and implemented with consultation and collaboration between University of Western Australia scientists and the Malgana Rangers. Collectively, we have established strong lines of communication and coordinated processes for conducting field work, organising and implementing workshops, engaging in ecological and restoration training exercises as well as brainstorming and organising a successful community event. The community event is the Wirriya Jalyanu (seagrass) Festival which was rescheduled for 7-8 April 2021 in Denham, Shark Bay (out of respect for Sorry Time due to the passing of an Elder).

Our project was divided into three parts, each developed and implemented with consultation and collaboration between UWA scientists and the Malgana people – (1) Collection of baseline genetic/genomic diversity and connectivity estimates across the salinity gradient (within both gulfs) for the two dominant seagrass species – *Amphibolis antarctica* and *Posidonia australis*, (2) Develop methods to assist natural recovery of seagrass meadows, (3) Develop nature-based solutions to climate-change related seagrass loss in Shark Bay. This final report presents results for genomic diversity and connectivity assessments, measuring restoration success through transplant survival and assessment of return to ecosystem function. We have outlined a framework for planning future restoration activities, with step-by step examples. Suggestions are provided for the next steps in assisting people and seagrass ecosystems to heal on sea country. Below we provide outcomes of the three parts to the project:

1. Genomic diversity and connectivity among temperate seagrass meadows in Shark Bay

(i) Ribbon weed (*Posidonia*) meadows contained moderate to high levels of genetic diversity. There was significant differentiation between a single reproductive meadow and all other sampled meadows, which are likely non-reproductive.

(ii) Levels of genomic diversity were similar in all wire weed (*Amphibolis antarctica)* meadows. All meadows were significantly differentiated from each other. More genetic structure was found among meadows in the eastern gulf than in the western.

2. Assessment of success and return of ecosystem function

(i) The early success of using 2.5 m long biodegradable sand-filled hessian sandbags for facilitating *Amphibolis* seedling recruitment means this technique is worth exploring in Shark Bay.

(ii) Monitoring of survival and growth of seedlings and adult transplants at restoration sites continued in collaboration with the Malgana Rangers. There are site specific differences in survival and growth rate. Assessments of 'return to ecosystem function' showed age-related use of transplants by fish and invertebrates. Diversity in five year old *A. antarctica* meadows

was identical to natural meadows. Organic carbon levels in the sediment were absent (sand) to low (older restored meadows) relative to natural meadows.

3. Develop nature-based solutions to climate-change related seagrass loss in Shark Bay

Restoring long-lived large seagrass species takes time, beyond the length of the two year NESP E6 project. However, our results from restoration trials established during training workshops with Rangers, in combination with assessments of more established restoration trials from other research projects, has enabled us to assess the timelines for ecosystem recovery. It provides a guide to how long monitoring should continue (greater than 5 years) to restoration practitioners to have confidence in the restoration success.

Developing restoration programs in remote extreme environments comes with a series of challenges including communication with and coordination of personnel and resources. Next steps are outlined to guide future research and collaboration with Malgana Rangers. Support for 5 to 10 year monitoring programs and scaling up restoration activities are the two main next steps. Embedding restoration activities into a Malgana seasonal calendar would improve planning for timing of activities.

The challenges of developing a restoration program in a remote extreme environment included working with highly variable weather conditions and working with higher levels of communication to coordinate effectively among local and University personnel and resources. A shared mission to develop a local industry for people on Country is still in development, but clearly with the right continuous resourcing and oversight this can be accomplished for seagrass restoration in Shark Bay (Gathaagudu).

2. PROJECT BACKGROUND

The Shark Bay World Heritage Site (WHS) is listed under four World Heritage criteria, for outstanding natural **heritage** values. These include the property's lush seagrass beds, as exemplified by the Wooramel Seagrass Bank, which is one of the largest seagrass meadows in the world, with the most seagrass species recorded from one area. The Land and Sea Country of Gathaagudu (Shark Bay) was handed back to the Malgana Peoples through a successful Native Title claim in December 2018. Malgana Country of the Gathaagudu region is approximately 28,800 square kilometres. It is bordered by Yinggarda to the north east, Wajarri to the east and Nanda to the south (see https://aiatsis.gov.au/explore/map-indigenous-australia). The project focuses on two of the NESP Marine Biodiversity Hub listed priorities:

Priority: Trial scientifically-based methods to restore habitat to underpin on-ground management actions.

Restoration is increasingly seen as a management tool in the context of degradation from cumulative impacts, including climate change, but there is limited information to support effective management. This project directly addresses this information gap, with particular emphasis on MNES.

Priority: Research undertaken under all hub priorities should consider the impact of climate change in the research design, delivery and recommendations, as appropriate.

A primary motivation for considering restoration is the loss and decline of extensive temperate seagrass meadows as a result of climate change. These meadows are an important component of the property's World Heritage values.
The project will explicitly incorporate adaptation of seagrasses to decadal climate change through the analysis of genetic provenance in the dominant species prior to selecting source and restoration sites within the Shark Bay WHS.

This program aligns with the social and economic value of the environmental asset/s and research outcomes through engagement with the Malgana Rangers and seed collectors to develop methods to assist natural recovery in preparation for future devastating impacts of climate change as well as showcasing the program at the festival event, Wirriya Jalyanu (seagrass) Festival (Arts meets Restoration Science).

In the context of the research priorities for the NESP Marine Biodiversity Hub provided by the Australian Government Department of the Environment, the proposed work aligns with the following priorities:

- Identify and trial methods to restore degraded habitats such as oyster and mussel beds, seagrass, and intertidal habitats to underpin on-ground management actions.
- Improve the management of marine and coastal biodiversity by evaluating and quantifying the results of management interventions.

- Improve our knowledge of key marine species and ecosystems to underpin their better management and protection.
- Identify key opportunities to collaborate and build Indigenous participation and knowledge into the management and protection of marine species.

2.1 Restoration genomics

Species- and gene-level biodiversity are rarely examined together in the context of restoration, although high genetic diversity of plant populations is a fundamental factor ensuring long-term success of restoration (Aavick & Helm 2018). Genetic diversity is important for the maintenance of the viable populations, as well as the evolutionary or adaptive potential of populations and species (Holderegger et al. 2006). It is an important component of ecosystem resilience (Bernhardt & Leslie 2013), one which should be considered when planning restoration activities, particularly when the climate is changing at unprecedented rates. Several restoration focused studies have shown that genetic diversity can benefit the resistance and recovery potential of seagrasses (e.g. Ehlers et al. 2008; Hughes & Stachowicz 2011; Reynolds et al. 2012; Sinclair et al. 2013).

Genetic or genomic data are often not available when restoration activities are being planned for a number of reasons, although they offer potential for improving restoration outcomes (Williams et al. 2014; Breed et al. 2019),and were outlined as a knowledge gap for many (Australian) seagrass species (York et al. 2017). We have been able to draw on previous microsatellite DNA data from several locations across Shark Bay for *P. australis* (Sinclair et al. 2016, 2020) to make informed decisions on how best to source plant material for the training workshop activities described below. These studies showed there was genetic diversity present within meadows, and that there was some genetic differentiation across the Shark Bay World Heritage Site.

Early population genetic studies using allozymes and Restriction Fragment Length Polymorphisms (RFLP) failed to detect any genetic variation in *Amphibolis antarctica* (Waycott et al. 1996). A recently published study developed 14 microsatellite DNA markers and published diversity data from four meadows (van Dijk et al. 2018). This study showed there was considerably more genetic diversity in meadows of this dioecious species (Clonal diversity = 0.23 - 0.98; Ho = 35 - 45%). However, no such data are currently available for *A. antarctica* meadows in Shark Bay.

The sourcing of genetically diverse plants from a local provenance is generally regarded as ‘best-practice’ in restoration (Vander Mijnsbrugge et al. 2010). ‘Home-site’ advantage suggests a fitness advantage to local plants, although this may not always be the case (Jones 2013). For example, when sites have been heavily degraded, and landscapes (or seascapes) have been modified. Alternative approaches, such as mixing seed sources (Breed et al. 2013), or predictive sourcing to match future conditions are being considered under climate change scenarios (Breed et al. 2013; Prober et al. 2015; Bucharova et al. 2019). Most of these conversations have been initiated in the context of restoration of terrestrial ecosystems, however, these concepts are also directly relevant to marine ecosystem restoration where oceans are subject to warming and extreme events. A conservative *'local is best'* approach was taken - using plant material sourced from the nearest meadows (within the same salinity range). This '*local is best*' strategy will be revised as required following our new genomic assessments.

Most genetic studies collect data for neutral genetic markers, which have great potential for investigating processes such as gene flow, and migration, but have no direct effect on fitness or adaptive potential. (Putative) adaptive markers (under natural selection) are markers that may be associated with local adaptation. Population genomics approaches offer an opportunity to identify adaptive (or outlier markers), so are of interest to identify whether local adaptation is associated with environmental gradients, such as the steep salinity gradient in Shark Bay. So, the underlying driver for conducting genomic diversity assessment in conjunction with restoration training activities was to advise on what the most suitable genetic provenance is for sourcing plant material (adult plants, seedlings, or seeds) for future scaled-up restoration activities of degraded *Amphibolis antarctica* (wire weed) and *Posidonia australis* (ribbon weed) meadows across Shark Bay.

2.2 Seagrass restoration in Shark Bay (Gathaagudu)

Our main aims from this section is to: 1.) Develop methods to assist natural recovery of seagrass meadows, and 2.) Develop nature-based solutions to climate-change related seagrass loss in Shark Bay.

Our goal is to assist natural recovery of seagrass meadows post the 2011 extreme marine heat wave (see Kendrick et al. 2019) through a suite of on-ground restoration activities. A recent review of restoration methods (Tan et al. 2020) and three successful approaches to seagrass restoration in Australia (Sinclair et al. 2021) provide a wealth of background to refining restoration methods with Malgana Rangers for use in Shark Bay.

Relatively little is known about the restoration potential of seagrass meadows in the Shark Bay WHS, but such knowledge is needed when designing and implementing adaptive management strategies after a large scale loss of seagrasses. Shark Bay seagrasses were devastated by the marine heatwave of 2010-2011 and these events are predicted to increase in frequency and intensity with global warming. The loss and or damage to 36% of seagrass cover in the bay (approx. 1,300 km^2; Strydom et al. 2020) had a flow on effect to biodiversity, including culturally significant species, tourism and the recreational and commercial aquaculture and fisheries industries (Kendrick et al. 2019). There is a critical need to develop management actions to respond to such events and to prepare for predicted future events. Seagrass restoration methods have been trialled at Useless Loop, with some successes (Statton et al. 2015). Experimental transplant sites on both sides of the Peron Peninsula are exploring the response of *Posidonia* plants to salinity will lead to an increased understanding how local adaptation and plasticity contribute to stress response. This kind of information will guide how plants can be sourced for restoration (whether plants can cope with different changes across the salinity gradient).

The Malgana people have cultural responsibilities and obligations to look after sea country in Shark Bay (Gathaagudu), with a strong connection to the land and inshore seas that make up the Shark Bay WHS. This project is developing seagrass restoration methods using plants, seedlings and seeds. Our collective vision is to scale up the existing restoration research to practice and assist recovery of the dominant seagrasses, wire weed (*Amphibolis antarctica*) and ribbon weed (*Posidonia australis*) following the 2011 marine heat wave. In this project, we work alongside the Malgana Rangers. This final report provides the outcomes of our genomic diversity and connectivity studies of *P. australis* and *A. antarctica* (milestone 2), describes the final training workshop (milestone 3), and provides results on assisted seagrass recovery (milestones 4 and 5). These results are incorporated into an assessment of return to ecosystem function and development of tools for restoration (milestones 6 and 7). Collaborative Research and training with traditional owners

Jointly designed research into optimizing restoration practices with Traditional Owners has been on-going. Communication is key to the success of any research project. The remoteness of Shark Bay and its seagrass meadows present very real challenges for researchers (Perth-based) and Rangers (based in Albany, Perth, Geraldton, and Denham). This has been highlighted by travel bans between regions within Western Australia as a result of COVID-19 in 2020.

Phone, email and video conferencing have played an important role in communication during 2020. It has led to the use of different knowledge sharing methods, but some individuals being in better communication than others:

1. Regular video conference calls around the planning of Wirrya Jalyanu (seagrass) Festival have enabled the development of long-term relationships and mutual respect.

2. Video link up for events between Perth and Denham for an official sharing of 70 years of research on Shark Bay.

3. Email communications were necessary, but not terribly effective for many.

4. ‘Open house’ policy to meet, socialise, and participate in sample processing during field trips to Shark Bay.

2.3 Overcoming challenges of remoteness and cross-culture

Indigenous communities and researchers have important roles to play in identifying challenges, seeking opportunities and developing productive relationships (Hedge et al. 2020). They should also be developing solutions to these challenges. One significant barrier is the recognition of Indigenous knowledge as a science, and this will take time to be overcome through education, ‘two-ways’. Collaborative projects, such as this one being described here, provide a positive way for traditional knowledge, culture, and science to come together for a mutual understanding and recognition - that we have a shared vision for restoring and looking after the environment.

We have taken advice from a recent success in shellfish ecosystem restoration - ‘*Seven pearls of wisdom generated by Traditional Owners*’ (McLeod et al. 2018). A number of key elements have been used:

1. **Recognition.** Malgana People have inhabited Shark Bay for more than 30,000 years and now have formal access and determination of their land through the Native Title Act since 2018. The Malgana Aboriginal Corporation (MAC) and a new Traditional Owner group. They were officially recognised as Traditional Owners of their country in December 2018. Changes to the Indigenous engagement partners due to native title being awarded occurred between submission of the original NESP project proposal and awarding of the project in late December 2018. The contract was revised to acknowledge the Malgana Aboriginal Corporation (MAC) as the official collaborators. However, relationships had to be built with the new Board. There have been several changes to key board members, including the Chair. The young board is finding its ways, and two years on, it is starting to establish itself.

2. **Working together from the beginning.** This seagrass project was designed by Bianca McNeair and Gary Kendrick under the stars in Shark Bay and represents the first joint collaboration with the MAC and the University of Western Australia. We are developing long term friendships to enable long term working partnerships between the Malgana and researchers, and to set a new standard for joint collaborative research and on ground management. This has been a very rewarding part of the NESP collaboration.

3. **Local employment.** Significant resources were available to appropriately reward time invested, rather than creating a sense of burden through additional work. Six Malgana rangers in training (3 male and 3 female) are now fully certified Rangers. Our budget included payment for employment during training and support for continued restoration practice.

4. **Knowledge sharing.** Two-way knowledge sharing occurred through informal on Country (field-based) activities. It is best shared in person. Rangers and key researchers were not always able to participate in all seagrass restoration workshops.

5. **Creating a shared vision.** Discussions around the role of seagrass meadows in shaping the marine environment on Shark Bay generated a shared desire to look after Country and restore seagrass meadows.

6. **Early engagement.** This seagrass restoration project was conceived through informal discussions between Malgana People and researchers on Country and a grant application submitted before Native Title was awarded.

7. **Connections.** Discussions around connectivity between terrestrial and marine ecosystems and species (including humans) and the role of seagrass meadows in shaping the marine environment on Shark Bay. Developing (human) connections with Malgana people requires spending time on Country. The short time frame of NESP projects (two years), combined with remote working locations, significantly increases challenges associated with Indigenous collaboration projects. Several of the Malgana Rangers embraced electronic communications during COVID-19 travel restrictions in 2020, which greatly assisted the success of the project.

8. This seagrass restoration project was the **first jointly conceived and actioned research collaboration between the Malgana Aboriginal Corporation** (MAC) and the University of Western Australia. We acknowledge that we are all establishing the new way to conduct research on Country and that MAC was establishing itself and protocols.

9. The use of more **informal work spaces** removes potential learning and sharing barriers. This is more easily achieved in the field, but more challenging when locations are remote. Regional travel restrictions associated with COVID-19 in Western Australia prevented face to face activities during 2020. The use of digital communications became important to maintain contact, but varied with individuals.

10. It is not appropriate for Indigenous people to speak about Country other than their own. However, this goes deeper into family groups and roles within culture – finding the **'*right person with authority to speak*'**. The focus has therefore been on building relationships and trust with many people, rather than just through a single person.

11. All activities were conducted in groups that were **balanced by culture and gender**

12. Cultural practice often takes priority over work milestones. This can result in significant delays to activities and/or limit participation by individuals/family members. Additional flexibility by researchers and funding agencies is required to accommodate.

13. Many Indigenous people have been separated from Country due to colonisation. Ranger programs enable them to rediscover their identity through **reconnecting with Country**. These steps can assist in a joint recovery program for ecosystems across Australia – allowing people and Country to heal together.

2.4 Advice for future collaborations

Indigenous knowledge and languages are not lost, they are 'just sleeping'. They will continue to be revived through ongoing conversations, sharing knowledge, and recording information through written and spoken words and visual or artistic means. The First Malgana Dictionary 'Malgana Wangganyina' (= Talking Malgana) was an Illustrated Wordlist of the Malgana Language of Western Australia (2003) edited by Doreen Mackman. We have been learning Malgana words and listening to stories during this two plus years collaboration with Malgana Peoples. Some of these are being shared in Appendix A. Our advice for future collaborations include:

- Amplify the voice of Indigenous peoples
- Share in and respect their knowledge
- Interpret western science results with the aid of Indigenous knowledge
- Level people and knowledge to an equal playing field
- Get involved to build cultural confidence in local TOs within their communities

3. INCORPORATING GENOMIC DIVERSITY AND CONNECTIVITY ASSESSMENTS INTO RESTORATION (MILESTONE 2)

3.1 Sampling seagrass meadows for genomic assessment

A sampling strategy was developed in order to cover the full geographic range of each species across the salinity gradient in the eastern and western gulfs of Shark Bay (Gathaagudu). We focused on selecting locations in which both *P. australis* and *A. antarctica* meadows were in close proximity (~35 – 52 PSU), accessible, and healthy (i.e. not showing signs of degradation as a result of the 2010/2011 heatwave). Ten meadows were sampled for 30 samples each of these persistent seagrasses between March and August 2019 (Figure 1, Table 1).

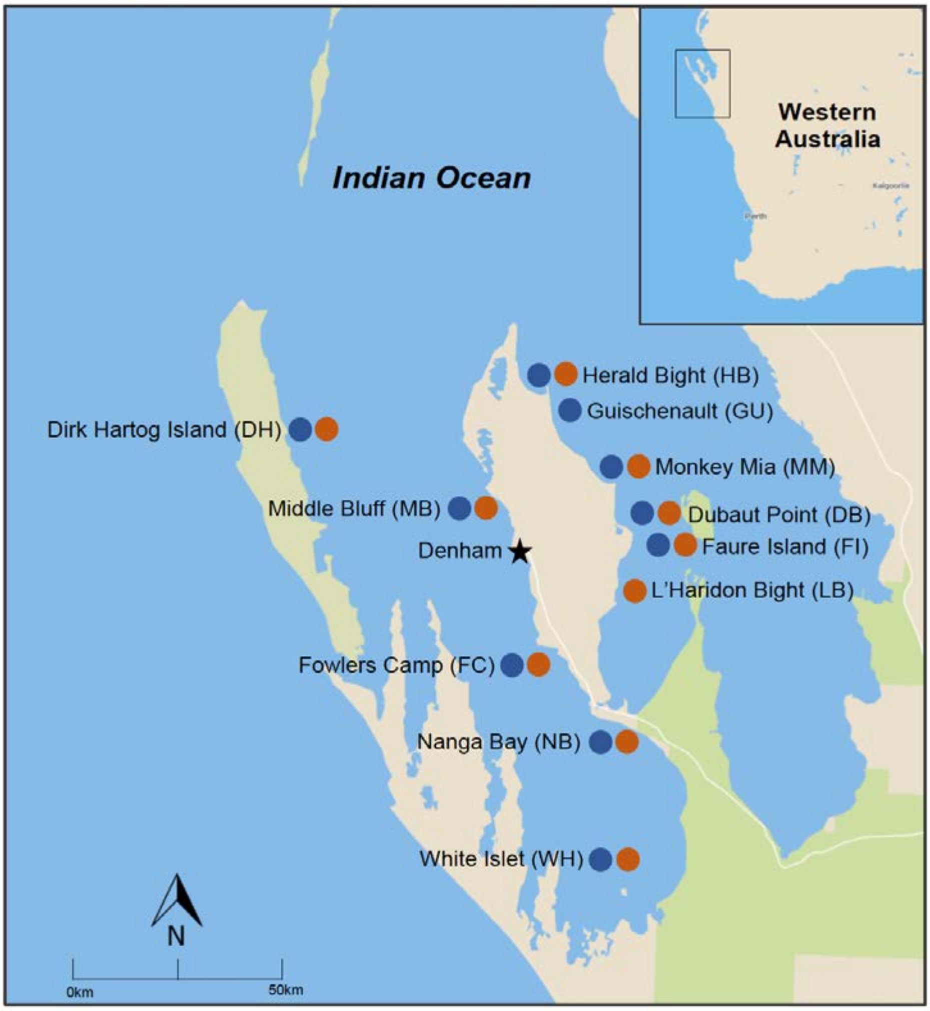

Figure 1 Seagrass meadows sampled for genomic diversity and connectivity estimates by species: *Posidonia australis* (blue circles) and *Amphibolis antarctica* (orange circles) in the eastern and western gulfs of Shark Bay, Western Australia. This sampling effort covers the geographic range for each species across the salinity gradient from ~ 35 salinity units (psu) in the north to >50 psu in the south.

Table 1 Locations for seagrass meadow sampling for genomic assessment by species and conditions at the time of sampling.

Location	Abbrev.	Gulf	Date	Water temp (˚C)	Depth (m)	Salinity (PSU)
Posidonia australis (ribbon weed)						
Dirk Hartog Island	DH	west	15/3/19	25.4	4.2	35.7
Middle Bluff	MB	west	12/3/19	25.3	1.1	39.6
Fowlers Camp	FC	west	10/3/19	27.9	0.6	40.5
Nanga Bay	NB	west	18/3/19	23.7	0.5	49.1
White Islet	WH	west	12/8/19	18.0	2.9	35.1
Herald Bight	HB	east	13/3/19	25.8	0.8	38.7
Guischenault Point	GU	east	14/11/12	-	0.5	-
Monkey Mia	MM	east	15/11/12	-	<3.0	-
Dubaut Point	DB	east	17/3/19	25.3	1.2	44.7
Faure Island	FI	east	14/3/19	25.6	2.8	45.4
Amphibolis antarctica (wire weed)						
Dirk Hartog Island	DH	west	15/3/19	26.9	2.3	35.0
Middle Bluff	MB	west	12/3/19	25.3	1.1	39.6
Fowlers Camp	FC	west	10/3/19	26.9	1.1	40.6
Nanga Bay	NB	west	16/3/19	23.6	0.8	48.6
White Islet	WH	west	12/8/19	18.0	2.4	35.1
Herald Bight	HB	east	13/3/19	25.8	0.8	38.7
Monkey Mia	MM	east	17/3/19	25.1	2.2	42.1
Dubaut Point	DB	east	17/3/19	25.3	1.2	44.7
Faure Island	FI	east	14/3/19	24.7	1.2	47.4
L'Haridon Bight	LH	east	14/3/19	24.4	1.6	51.9

3.2 Genomic laboratory methods

Genomic DNA was extracted from a subset samples (*P. australis* n = 12-14/meadow; *A. antarctica*, n = 19-20/meadow) using a Qiagen DNeasy® Plant Pro Kit (Qiagen, Germany). Extraction protocols were modified as required from the suppliers' instructions to improve the DNA quality and yield. Double digest restriction-associated DNA sequencing (ddRAD-seq; Peterson et al. 2012) was used to generate reduced representation Single Nucleotide Polymorphic (SNP) markers for each species. DNA libraries were prepared in the Batley genomics laboratory at the University of Western Australia, following the protocols outlined in Severn-Ellis et al. (2020). Pooled libraries were then sequenced on a HighSeqX10 sequencing machine as 2 x 150 bp paired-end reads (KCCG Sequencing Laboratory, Garvan Institute of Medical Research, New South Wales).

3.3 Bioinformatics and analyses

Raw sequence reads were processed following the pipeline detailed in Severn-Ellis et al. (2020) (available at: (https://github.com/ascheben/RAD_analysis_workflow#Diversity-analysis-protocol) and performed in close collaboration with the Batley Lab at the University of Western Australia. A *de novo* ddRAD loci assembly and SNP identification was performed using the denovo_map pipeline in STACKS v2.52 (Rochette et al. 2019). Key parameters in Ustacks and Cstacks were selected based on maximising the number of individual samples remaining in the dataset and the number of high-quality SNPs (Paris et al. 2017; O'Leary et al. 2018). Assemblies for both seagrass species were conducted *de novo* as annotated genomes were not available at the time of sequence assembly. SNP profiles for a total of 133 out of 144 individuals remained for *P. australis* and 184 out of 192 for *A. antarctica*.

3.3.1 Identification of outlier loci

Three methods were used to identify outlier or putative adaptive SNP loci: OutFLANK (Whitlock & Lotterhos 2015), BayeScan v.2.1 (Foll & Gaggiotti 2008) and PCadapt (Luu et al. 2016). All methods were run with a false discovery rate (FDR), or q-value, of 0.05. A SNP locus was regarded as an outlier if it was identified by two or more methods using the R package *eulerr* (Larsson 2020). Outlier loci were removed from the main data sets for both species.

A total of 18, 021 SNP markers were identified in *P. australis*, of which 67 were identified as outliers (0.37 %; Figure 2 left). Interestingly, 43 out of the 67 outlier markers were unique to the Guischenault Point meadow. A total of 6,534 SNP markers were identified in *A. antarctica,* with only 7 outlier loci identified (0.11%; Figure 2 right). The BayeScan and OutFLANK methods appear to be much more conservative estimators of outlier loci than PCadapt for both species.

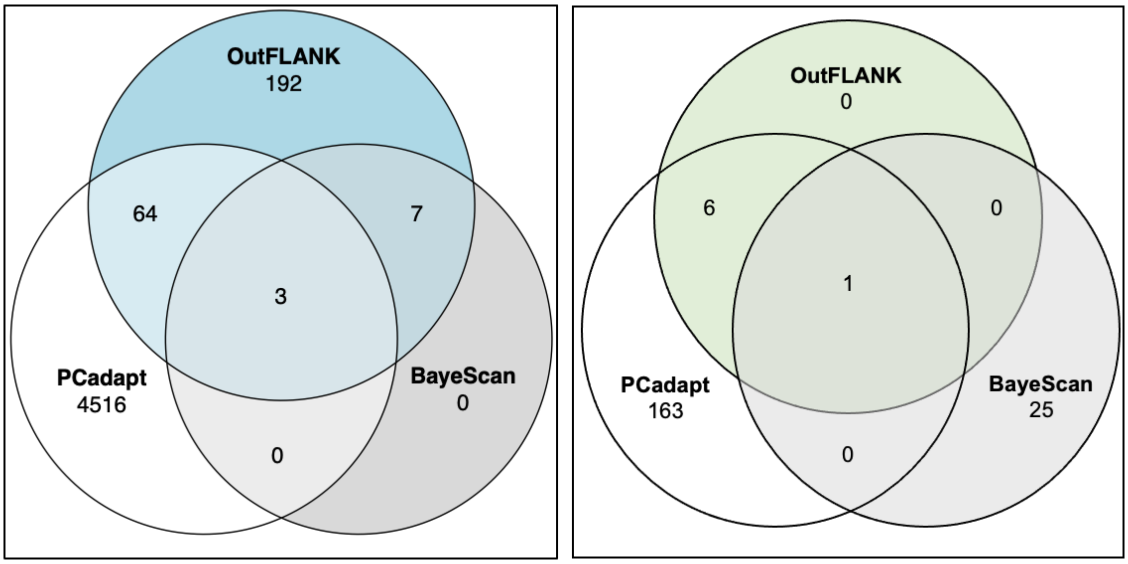

Figure 2 Number of outlier loci (or SNPs) detected using PCadapt, OutFLANK, BayesScan for (left) *Posidonia australis* and (right) *Amphibolis antarctica.*

3.3.2 Population diversity analyses

Initial population diversity statistics were estimated using Stacks: populations within the bioinformatics pipeline available at: (https://github.com/ascheben/RAD_analysis_workflow#Diversity-analysis-protocol; Severn-Ellis et al. 2020). Diversity statistics included mean frequency of the most common allele at each locus (P), the number of private or unique alleles (Private), observed (Ho) and expected heterozygosity (He), inbreeding coefficient (F_{IS}) and Tajima's nucleotide diversity (Pi). Population genomic diversity indices were calculated for *P. australis* for neutral and outlier loci, separately. Population diversity statistics for *A. antarctica* were estimated using GENALEX (Peakall & Smouse 2006, 2012). The statistics describing allelic diversity within a meadow were: percentage of polymorphic loci (%P), number of private or unique alleles (Private), observed (Ho) and expected heterozygosity (He) and inbreeding coefficient (F_{IS}). Diversity statistics for outlier loci in *A. antarctica* were not generated due to the low number of markers (n = 7).

Clonal richness, the number of genetically-different samples, *R,* = (MLG-1)/(N-1), where MLG = multilocus genotype, (Dorken & Eckert 2001) was calculated based on the whole data set for each species using the R package Poppr (Kamvar et al. 2014). Values range from zero to one, with values close to zero indicating high levels of clonality (samples belong to the same genetic individual) and 1 indicating all samples were from genetically different plants (high clonal diversity). A genotype accumulation curve was created using Poppr to identify the minimum number of loci required to confidently assign samples to a unique clone or multilocus genotype (MLG).

3.3.3 Population structure analyses

Population genomic structure was assessed using several approaches in both species - visually through a Principal Coordinate Analysis (PCoA), and statistically by determining whether there was significant structure among sampled meadows via genetic differentiation.

Different software packages were used for each species (*P. australis* and *A. antarctica*) due to the difference in size of the data sets for each species (number of SNP markers and samples).

Posidonia australis populations

A Principal Coordinate Analysis (PCoA) was used to visualise the spatial relationship among all sampled individuals using the packages SNPrelate v.1.23.0 (Zheng et al. 2012) and ggrepel v0.8.2 (Slowikowski 2020) in STACKS. An Analysis of Molecular Variance (AMOVA) was performed in Poppr (Kamvar et al. 2014) to partition genetic variability among individuals, among meadows, and among gulfs, with significance based on 999 permutations. Pairwise F_{ST} values were computed to determine genetic differentiation between pairs of sampled meadows using the population tool in STACKS, and pairwise p-values were computed using the R package Poppr (Kamvar et al. 2014).

Identification of the number of ancestral populations, *K*, and degree of admixture (= gene flow) among meadows was investigated using non-negative matrix factorization algorithms, computed on least-squares estimates of ancestry coefficients of populations using the program sNMF in R (Frichot et al. 2014). The sNMF program assumes that genetic data originates from the admixture of *K* parental populations, where *K* is unknown (Frichot et al. 2014). An estimate of ancestry proportions for each multilocus genotype (MLG) was computed. The number of distinct genetic clusters (*K*) was determined, based on 10 iterations per *K* value for *K* = 1 to 10.

Amphibolis antarctica populations

A Principal Coordinate Analysis (PCoA) was used to visualise the spatial relationship among all sampled meadows for neutral (n = 6527) and outlier (n = 7), separately, in GENALEX v6.5 (Peakall & Smouse 2006, 2012). All other population genetic structure analyses were based on neutral SNPs only. A hierarchical Analysis of Molecular Variance (AMOVA) was performed in GENALEX to partition genetic variability between gulfs, among meadows, among individuals, and variation within individuals. Significance was based on 999 permutations. Significant pairwise population genetic structure (F_{ST}) was based on 9999 permutations. Connectivity between meadows was assessed by calculating the effective number of migrants (Nm) in GENALEX.

A STRUCTURE analysis (Pritchard et al. 2000; Porras-Hurtado et al. 2013) was performed using an admixture model with correlated allele frequencies for *K* = 1 - 10. Ten iterations per performed per *K* value with a burnin period of 10,000 with 100,000 MCMC steps. The optimal *K* clusters was estimated using the maximum likelihood value of the second order rate of change of the posterior probability value of the simulations (Evanno et al. 2005; Porras-Hurtado et al. 2013). STRUCTURE results were visualized using the online program CLUMPAK (Kopelman et al. 2015).

3.4 Posidonia australis

A total of 130 out of 144 *P. australis* individuals remained after the bioinformatics pipeline was performed for quality control (SNP filtering) was completed and technical replicates were removed. The complete SNP data set consisted of 18,021 biallelic SNPs, 17,954 SNPs are regarded as neutral and 67 were identified as outlier (or putative adaptive) SNPs. There were minimal differences among the four technical replicates.

3.4.1 Within meadow diversity

All samples had unique SNP profiles (or multilocus genotype, MLG), thus clonal diversity, *R*, was 1.0. Genomic diversity statistics for each sampled meadow are presented for neutral (Table 2) and outlier markers (Table 3), separately. Neutral diversity statistics were remarkably similar for nine out of ten sampled meadows, characterized by high heterozygosity (Ho = 88.4 – 91.6%) and highly negative inbreeding coefficients (F_{IS} = -0.787 - -0.829) (Table 2). The high inbreeding coefficient is associated with a significant excess of heterozygotes (Ho is double He). Interestingly, private alleles were only present in two of these nine meadows, Dirk Hartog (DH, private = 87; salinity at normal sea water) and Faure Island (FI, private = 24; salinity at ~49 salinity units).

Guischenault Point has a moderate level of heterozygosity and is in Hardy-Weinberg equilibrium (Ho = He), and thus consistent with random mating. Guischenault Point was characterized by a high number of private alleles (Private = 164), 60% of all private neutral markers identified. Nucleotide diversity (Pi = 0.242) was half that of the other nine meadows (Pi = 0.474 - 0.487).

Genetic diversity estimates were much lower in all 10 meadows for the 67 outlier markers (Ho < 10%; Pi < 0.075; Table 3), as expected for markers under putative selection. The same nine out of the ten meadows had very few private alleles. The Guischenault Point meadow also had the highest number of private outlier alleles (private = 43), 64% of all private outlier markers identified.

Table 2 Genomic diversity estimates for ten sampled Posidonia australis meadows in Shark Bay, based on 17,954 SNPs (neutral): N = number of samples sequenced;P = frequency of the most common allele; Ho (%) = observed heterozygosity; He (%) = expected heterozygosity; Private = number of private alleles; *F*is = inbreeding coefficient; Pi = nucleotide diversity.

Pop	Abbrev.	N	P	Private	Ho (%)	He (%)	*Fis*	Pi
1	DH	14	0.545	87	90.8	46.4	-0.813	0.485
2	MB	12	0.553	0	88.9	45.6	-0.791	0.477
3	FC	12	0.556	0	88.4	45.5	-0.787	0.475
4	NB	14	0.549	0	89.8	46.3	-0.810	0.480
5	WH	14	0.541	0	91.6	46.8	-0.829	0.487

Pop	Abbrev.	N	P	Private	Ho (%)	He (%)	*Fis*	Pi
6	HB	14	0.549	0	90.0	46.3	-0.809	0.481
7	GU	13	0.818	164	24.7	23.1	0.016	0.242
8	MM	13	0.554	7	88.9	45.5	-0.801	0.474
9	DP	12	0.549	0	90.0	45.9	-0.804	0.480
10	FI	12	0.554	24	89.0	45.3	-0.797	0.475

Table 3 Genomic diversity estimates for ten sampled *Posidonia australis* meadows in Shark Bay, based on 67 SNPs (outlier): N = number of samples sequenced; P = frequency of the most common allele; Ho (%) = observed heterozygosity; He (%) = expected heterozygosity; Private = number of private alleles; *F*is = inbreeding coefficient; Pi = nucleotide diversity.

Pop	Abbrev.	N	P	Private	Ho (%)	He (%)	*Fis*	Pi
1	DH	14	0.982	0	3.6	2.0	-0.031	0.021
2	MB	12	0.978	0	0.6	3.8	0.155	0.040
3	FC	12	0.964	1	1.4	5.8	0.159	0.060
4	NB	14	0.973	0	3.0	3.8	0.027	0.039
5	WH	14	0.964	0	7.2	3.8	-0.064	0.040
6	HB	14	0.956	0	8.4	6.2	-0.042	0.065
7	GU	13	0.968	43	6.1	4.4	-0.027	0.046
8	MM	13	0.948	7	7.9	7.3	0.004	0.076
9	DP	12	0.948	0	10.4	5.4	-0.092	0.056
10	FI	12	0.999	0	0.1	0.1	0.000	0.001

3.4.2 Genomic structure among meadows

The Principal Coordinate Analysis (PCoA) shows the relationship among multilocus genotypes (MLGs) from different meadows. Similar genotypes are clustered together. Guischenault Point genotypes were very different from all other sampled meadows (Figure 3 top). Principal coordinate 1 (PC1) accounted for 35.9% of the variance, and separated Guischenault Point from the nine high heterozygosity meadows, which covered ~150km, two gulfs, and a salinity gradient from 35 - 49 salinity units. PC2 accounted for only 9.0% of the variance, with some clustering of MLGs by sampled meadow. PCoA of individual samples without Guischenault Point showed some spatial separation of genotypes by sampled meadows (Figure 3 bottom). Most MLGs from the two northern meadows clustered together (Dirk Hartog and Herald Bight), while most of the genotypes from the two southern meadows in the western gulf clustered together (White Islet and Nanga Bay). All other MLGs were clustered in a single group.

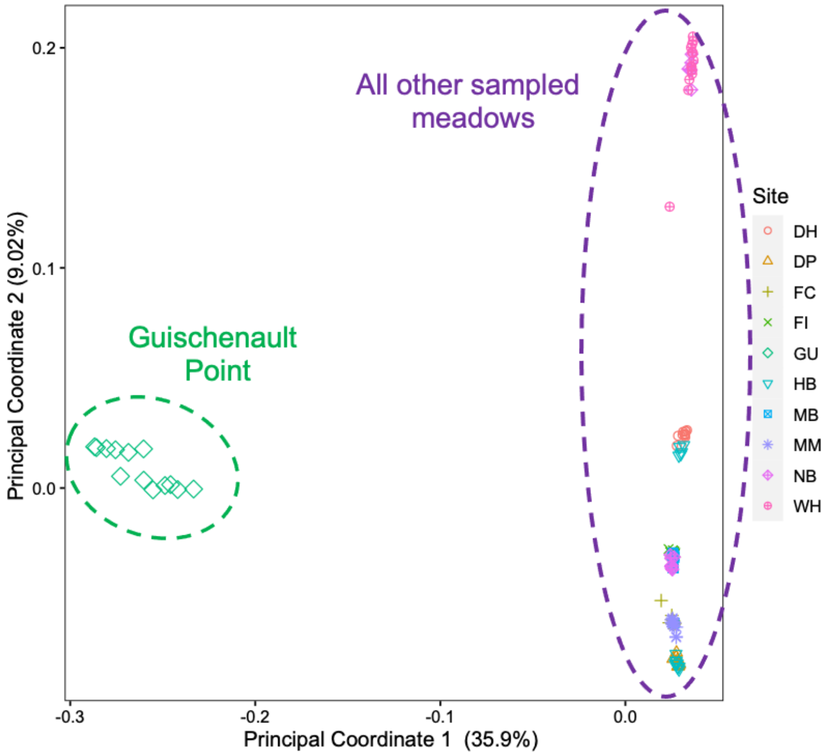

Figure 3 continued over

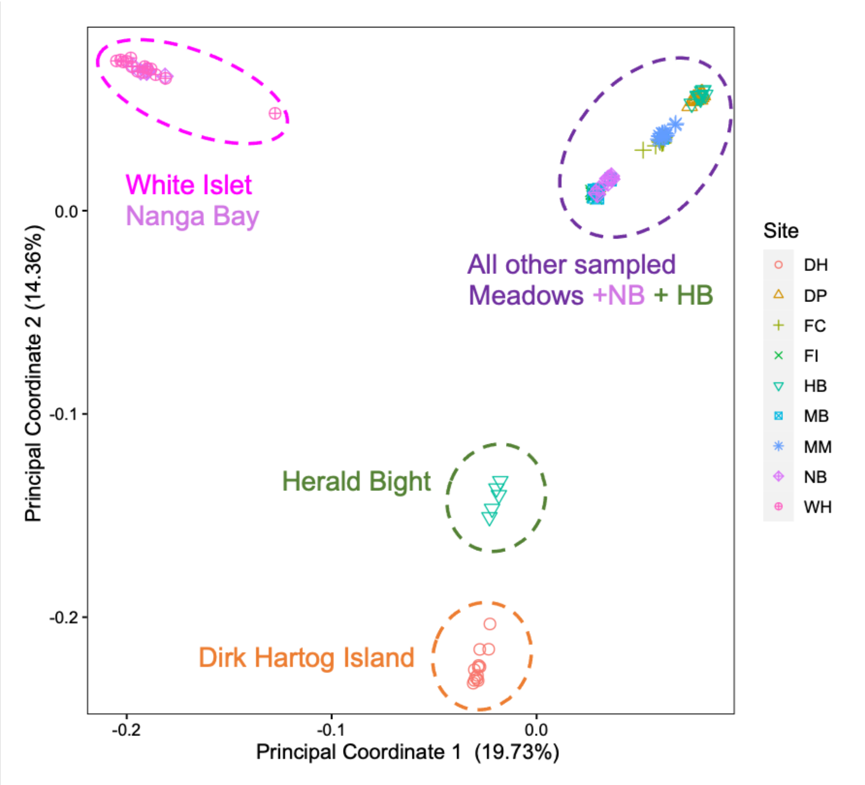

Figure 3 Principal Coordinate Analysis (PCoA) showing the spatial arrangement of samples for 10 sampled meadows based on 17,954 neutral SNPs (top) and 67 outlier SNPs (bottom). Meadow abbreviations are the same as Figure 1: western gulf: Dirk Hartog Island (DH), Middle Bluff (MB), Fowlers Camp (FC), Nanga Bay (NB), White Islet (WH); eastern gulf: Herald Bight (HB), Guischenault (GU), Monkey Mia (MM), Dubaut Point (DB) and Faure Island (FI).

A hierarchical Analysis of Molecular Variance (AMOVA) showed significant diversity was partitioned within and among meadows, but not by gulf, for both neutral and outlier markers (Table 4). Most of the variation was attributed to variation among meadows for neutral (62.1%) and outlier markers (92.8%). However, the amount of variation was much more evenly portioned within and among the nine high heterozygosity meadows (48.0% and 45.8%, respectively).

Table 4 Hierarchical Analysis of Molecular Variance (AMOVA) partitioning variation within and among ten sampled *Posidonia* meadows.

Source of variation	**d.f.**	**SS**	**Variance component**	**Estimated variance**	**Total variation (%)**	**P value**
Neutral markers (n = 17,954)						
Between gulfs	1	4194.8	4194.8	7.1	1.7	0.101
Among meadows	8	29620.1	3702.5	267.0	62.1	0.001
Within meadows	123	19159.9	155.8	155.8	36.2	0.001

Source of variation	d.f.	SS	Variance component	Estimated variance	Total variation (%)	P value
Total	132	52974.8	401.3	429.9	100.0	
Neutral markers without Guischenault Point						
Between gulfs	1	1145.6	1145.6	7.0	6.3	0.101
Among meadows	7	5135.0	733.6	51.1	45.8	0.001
Within meadows	111	5936.9	53.5	53.5	48.0	0.001
Total	119	12217.6	102.7	111.5	100.0	
Outliers markers (n = 67)						
Between gulfs	1	88.6	88.6	0.0	0.4	0.321
Among meadows	8	682.9	85.4	6.6	92.8	0.001
Within meadows	120	57.7	0.5	0.5	6.8	0.001
Total	129	829.3	6.4	7.0	100.0	

Significant differentiation was observed between Guischenault Point and the nine other meadows (GU comparison with others range, F_{ST} = 0.152 - 0.160, P < 0.001). There was no significant pairwise genetic differentiation among these nine meadows based on F_{ST} values for neutral SNPs (all negative F_{ST} values, effectively zero).

A STRUCTURE analysis based on neutral SNPs suggests there are five genetic clusters, with admixture among them (Figure 4 top), and based on 67 outlier markers there are K = 3 populations (or clusters) (Figure 4 bottom). In both cases, Guischenault Point is significantly different from all other meadows (consistent with PCoA).

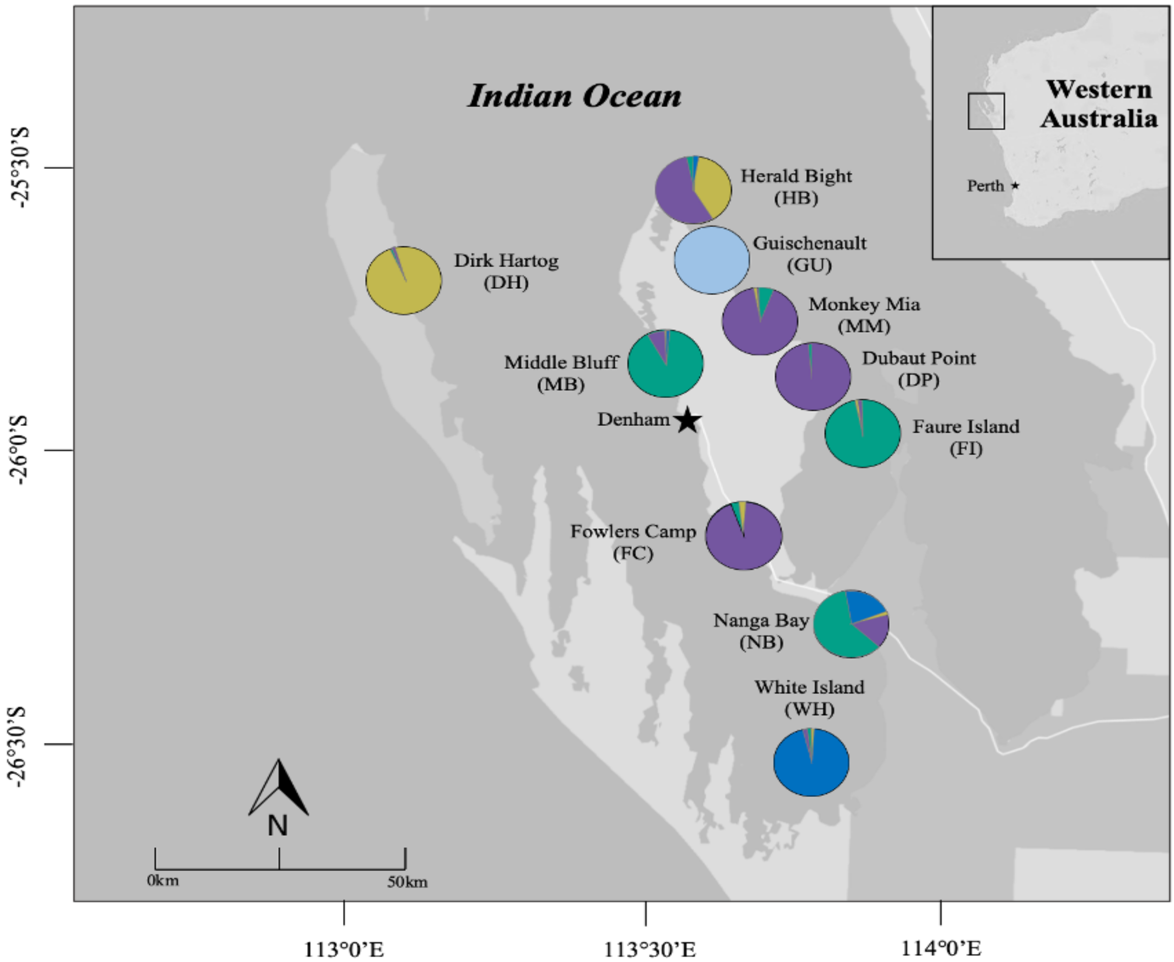

Figure 4 continued over

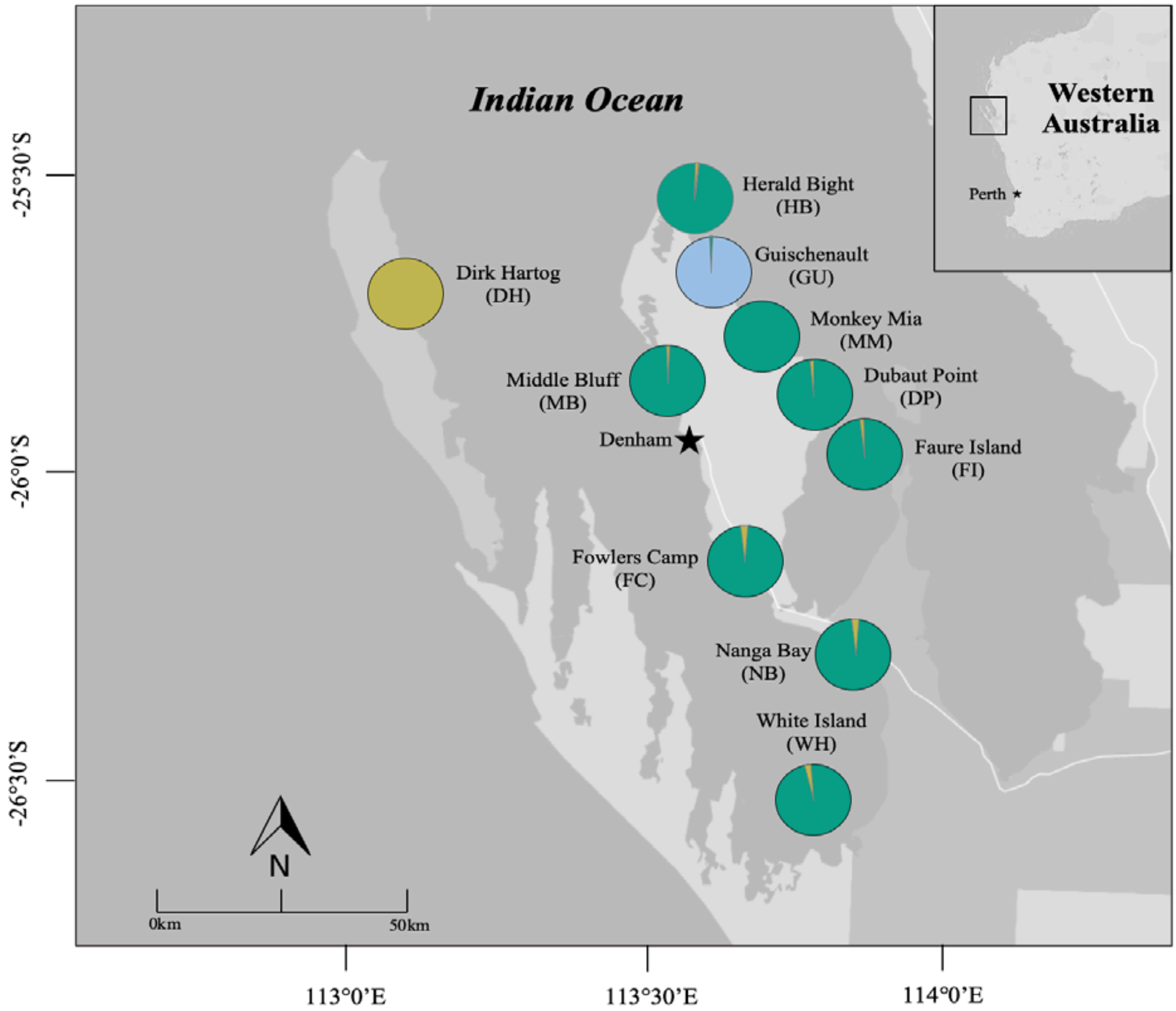

Figure 4 Allele frequency pie charts showing membership of population clusters from STRUCTURE analyses for 17,954 neutral markers K = 5 (top) and 67 outlier markers K = 3 (bottom). Meadows with similar colour composition belong to the same population or cluster.

These results essentially separated *Posidonia* meadows into two groups, which is consistent with what we currently know about seed production in Shark Bay: reproductive (Guischenault Point produces viable seeds) and non-reproduction meadows (all other sampled meadows in this study). This absence of genetic structure among most *Posidonia* meadows using outlier markers suggests no support for local adaptation associated with the salinity gradient. These *Posidonia* plants have a high genetic diversity (observed heterozygosity and nucleotide diversity) and appear to be tolerant to the highly variable environmental conditions across the Bays (diurnal fluctuations in temperature and salinity in a tidal, wind-driven environment).

3.4.3 Key results for *Posidonia australis*

- All 130 samples have unique genetic sequences.
- High heterozygosity and nucleotide diversity present in nine *Posidonia* meadows.
- PCoA and STRUCTURE analyses show significant structure among sampled *Posidonia* meadows.
- Guischenault Point produces viable seed and is genetically distinct from all other sampled meadows which appear to be non-reproductive.
- There is less genetic structure among outlier loci (*K* = 3) with a large number of loci present only in Guischenault Point.
- There does not appear to be significant genetic structure associated with the two gulfs or the salinity gradient.

3.5 *Amphibolis antarctica*

A total of 184 out of 192 *A. antarctica* individuals remained (17 - 20 samples per meadow) after the bioinformatics pipeline was performed for quality control (SNP filtering) was completed. The complete SNP data set consisted of 6,534 biallelic SNPs, of which 6,527 were regarded as neutral and seven were identified as outlier (or putative adaptive) SNPs.

3.5.1 Within meadow diversity

Each sample was identified as a unique MLG when analysed using the complete set of 6,534 SNPs (clonal diversity R = 1.0). A genotype accumulation curve (not shown) plateaued at around 100 loci, indicating a minimum of 100 loci were necessary for confident assignment of MLG's in this dataset. Overall, similar levels of genetic diversity were identified across all sampled *A. antarctica* meadows, with the exception of Herald Bight (HB) and Faure Island (FI), which had a lower proportion of polymorphic markers (Table 5). Very few private alleles were identified in meadows (Private = 0 - 3). Observed heterozygosity among meadows ranged from 26.9% at L'Haridon Bight (LB) to 41.8% at Monkey Mia (MM) (mean = 30.4%). All meadows had negative *F*is values, indicating a higher than expected number of heterozygotes. This is consistent with obligate outcrossing in a dioecious species (separate male and female plants).

Table 5 Genomic diversity estimates for ten sampled *Amphibolis antarctica* meadows in Shark Bay, based on 6527 neutral SNPs: N = number of samples sequenced; %P = proportion of polymorphic markers; Private = number of private alleles; Ho (%) = observed heterozygosity; He (%) = expected heterozygosity; *F*is = inbreeding coefficient; Pi = nucleotide diversity.

Pop	Abbrev.	N	%P	Private	Ho (%)	He (%)	*F*is	Pi
1	DH	19	87.0	0	28.8	27.0	-0.054	0.278
2	MB	17	86.6	0	27.5	27.1	-0.018	0.279
3	FC	19	79.9	0	29.0	25.4	-0.101	0.262
4	NB	18	79.5	2	30.1	24.2	-0.165	0.249

Pop	Abbrev.	N	%P	Private	Ho (%)	He (%)	*F*is	Pi
5	WI	20	86.5	0	28.0	25.7	-0.050	0.264
6	HB	18	66.0	0	31.7	21.4	-0.341	0.220
7	MM	19	81.9	1	41.8	26.8	-0.376	0.276
8	DP	17	80.7	0	29.9	26.5	-0.111	0.276
9	FI	18	54.5	0	30.6	21.7	-0.361	0.223
10	LB	19	80.1	0	26.9	25.2	-0.055	0.259
	Overall	184	78.3		30.4	25.1	-0.051	0.259

3.5.2 Genomic structure among meadows

The Principal Coordinate Analysis (PCoA) shows multilocus genotypes (MLGs) from meadows in the western gulf are more similar to each other than those from meadows in the eastern gulf (Figure 5). This plot shows that genotypes from meadows in the eastern gulf are not as closely related to each other (more spatially spread) than those in the western gulf (closed symbols more tightly clustered by meadow and location). The first and second coordinates for a PCoA based on population means of neutral SNPs accounted for 42% of the total variation (Figure 6 top). Eight out of ten sampled *A. antarctica* meadows clustered together, rather than by gulf. Herald Bight (HB) and Dubaut Point (DB) in the eastern gulf were the most differentiated. All meadows clustered in close proximity, with the exception of Herald Bight (HB) and Faure Island (FI) (Figure 6 below). The tight clustering of meadows suggest that these markers are not associated with local adaptation across the salinity gradient, as meadows that persist in a range of salinities (~35 - 52 psu) cluster together.

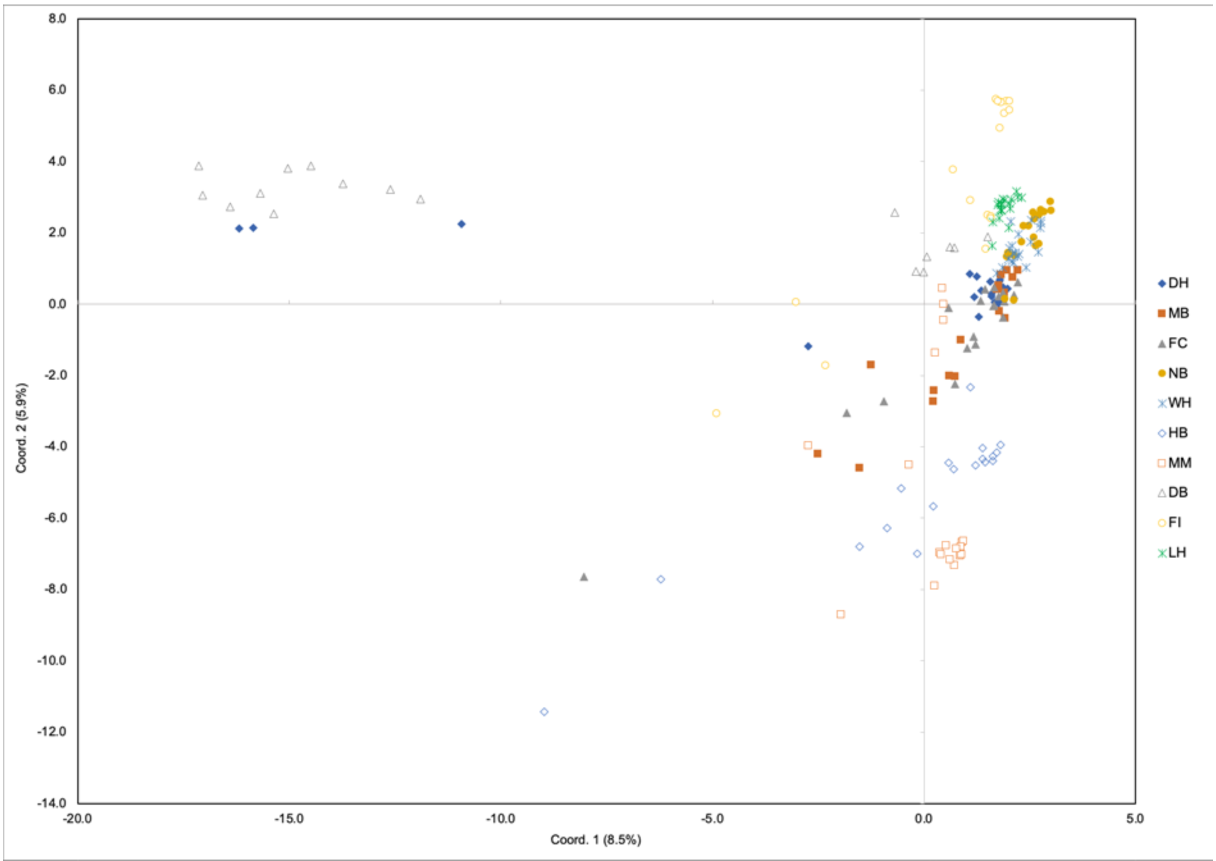

Figure 5 Principal Coordinate Analysis (PCoA) of *Amphibolis antarctica* individuals sampled from the ten meadows in Shark Bay, based on 6,527 neutral markers. Meadows in the western gulf (solid symbols): Dirk Hartog Island (DH), Middle Bluff (MB), Fowlers Camp (FC), Nanga Bay (NB), White Islet (WH); eastern gulf (open symbols): Herald Bight (HB), Monkey Mia (MM), Dubaut Point (DB), Faure Island (FI) and L'Haridon Bight (LB).

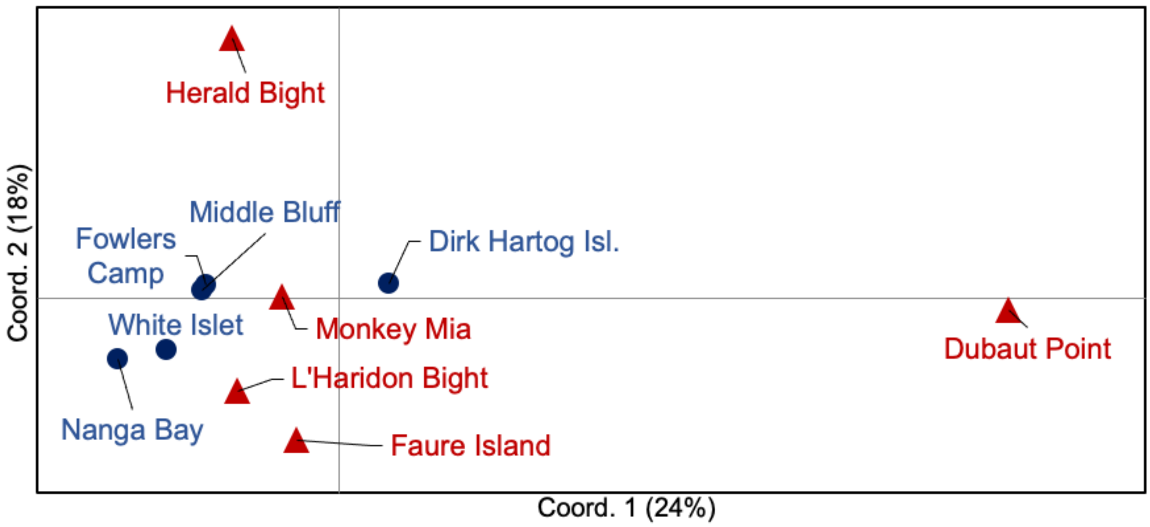

Figure 6 Principal Coordinate Analysis (PCoA) of population means among the ten sampled *Amphibolis antarctica* meadows in Shark Bay based on 6527 neutral markers (top) and 7 outlier markers (bottom). Meadows in the western gulf are represented by blue circles, and eastern gulf by red triangles.

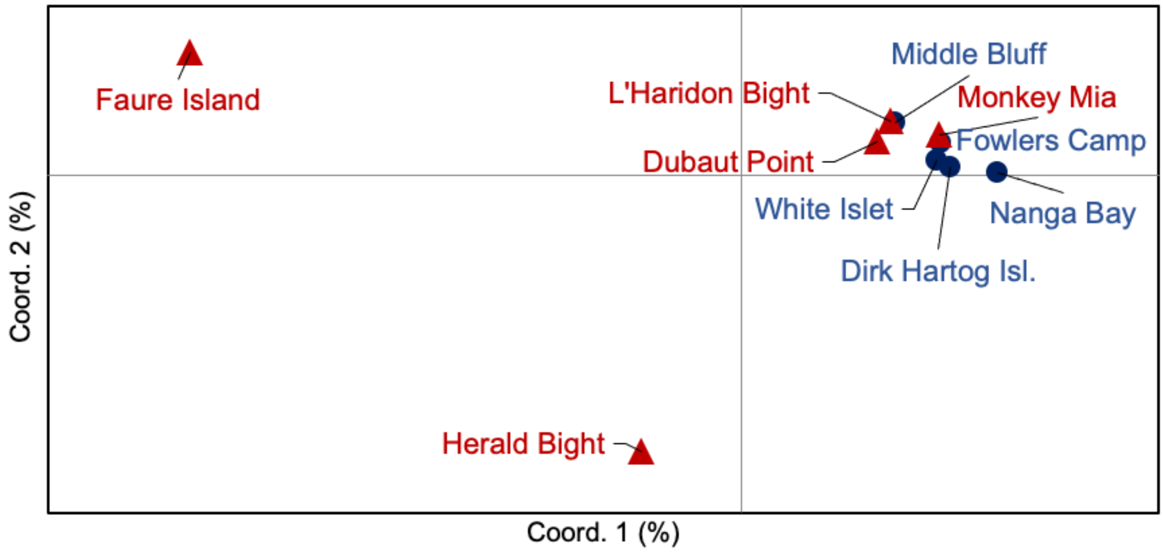

Figure 6 (continued)

A hierarchical Analysis of Molecular Variance (AMOVA) showed most of the variation was attributed to variation within individuals (72%; Table 5). Significant diversity was partitioned among individuals 11%) and among meadows (16%). Very little diversity was attributed to difference among gulfs (1%).

Table 6 Hierarchical Analysis of Molecular Variance (AMOVA) partitioning variation within and among ten sampled *A. amphibolis* meadows based on neutral markers.

Source of variation	d.f.	SS	Variance component	Estimated variance	Total variation (%)	P value
Between gulfs	1	10708.3	10708.3	11.9	1%	0.0299
Among meadows	8	68054.0	8506.8	198.3	16%	0.0001
Among Individuals	174	211118.7	1213.3	146.6	11%	0.0001
Within Individuals	184	169297.0	920.1	920.1	72%	0.0001
Total	367	459178.1		1276.9	100%	

The STRUCTURE analysis based on neutral SNPs suggested an optimal number of five populations or clusters (second order rate of change of the posterior probability $K = 5$ clusters). Meadows in the western gulf were less differentiated from each other than those in the eastern gulf. The five clusters are Herald Bight (HB), Monkey Mia (MM), and Faure Island

(FI) in the eastern gulf (Figure 7). The two southern meadows of Nanga Bay (NB) and White Islet (WH) cluster separately from the three northern meadows in the western gulf.

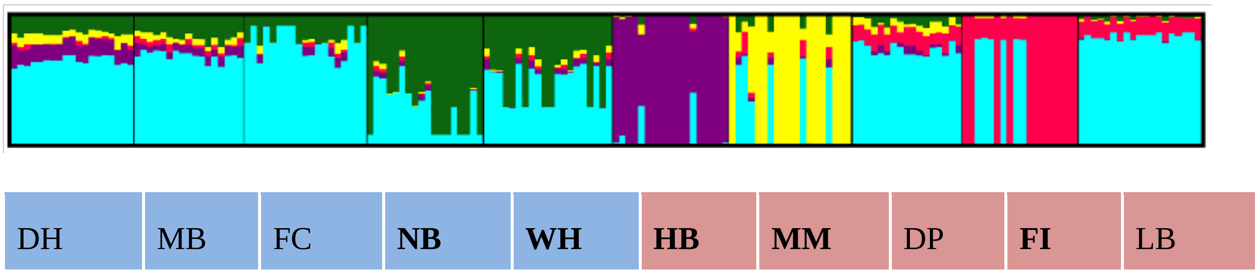

Figure 7 STRUCTURE analysis based on neutral SNPs only for ten sampled *Amphibolis antarctica* meadows. Each individual is represented by a single vertical line broken into colour segments, where segments are proportional to the membership coefficient for each of the population clusters where $K = 5$, mean Ln P(K) = -930,856.8. Site abbreviations are for western gulf: Dirk Hartog Island (DH), Middle Bluff (MB), Fowlers Camp (FC), Nanga Bay (NB) and White Islet (WH); eastern gulf: Herald Bight (HB), Monkey Mia (MM), Dubaut Point (DB), Faure Island (FI) and L'Haridon Bight (LB).

There was significant pairwise genetic differentiation among all meadows (Table 7). Differentiation was highest among meadows within the eastern gulf and in the southern part of the western gulf (Nanga Bay NB and White Islet WH).

Table 7 Heat map showing pairwise genetic differentiation (F_{ST}) among ten sampled *Amphibolis antarctica* meadows in Shark Bay. All pairwise values were significantly different at P < 0.001.

Meadow	DH	MB	FC	NB	WH	HB	MM	DP	FI	LB
DH		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
MB	0.076		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
FC	0.092	0.074		0.001	0.001	0.001	0.001	0.001	0.001	0.001
NB	0.137	0.122	0.143		0.001	0.001	0.001	0.001	0.001	0.001
WH	0.099	0.101	0.116	0.141		0.001	0.001	0.001	0.001	0.001
HB	0.141	0.137	0.158	0.215	0.191		0.001	0.001	0.001	0.001
MM	0.145	0.138	0.159	0.207	0.173	0.201		0.001	0.001	0.001
DP	0.123	0.163	0.176	0.224	0.197	0.218	0.202		0.001	0.001
FI	0.163	0.149	0.175	0.215	0.187	0.226	0.209	0.21		0.001
LB	0.121	0.117	0.139	0.171	0.137	0.2	0.182	0.192	0.158	

The effective number of migrants (Nm) between sampled meadows was used as a genetic estimate of genetic connectivity in the absence of demographic data (measuring actual dispersal of seedlings, which is challenging). A widely accepted rule of thumb suggests '*one migrant per generation*' is an appropriate level of gene flow among populations to maintain neutral genetic diversity (Mills & Allendorf 1996). All Nm estimates were close to or greater than 1 (Table 8). Nm was higher among meadows in the western gulf (Nm = 0.9 - 3.2) relative to meadows in the eastern gulf (Nm = 0.9 - 1.3).

Table 8 Heat map showing the effective number of migrants per generation (Nm) among ten sampled *Amphibolis antarctica* meadows.

Meadow	DH	MB	FC	NB	WH	HB	MM	DP	FI	LB
DH										
MB	3.0									
FC	2.5	3.2								
NB	1.6	1.8	1.5							
WH	2.3	2.2	1.9	1.5						
HB	1.5	1.6	1.3	0.9	1.1					
MM	1.5	1.6	1.3	1.0	1.2	1.0				
DP	1.8	1.3	1.2	0.9	1.0	0.9	1.0			
FI	1.3	1.4	1.2	0.9	1.1	0.9	0.9	0.9		
LB	1.8	1.9	1.5	1.2	1.6	1.0	1.1	1.1	1.3	

Significant genetic structure was observed among *A. antarctica* meadows within Shark Bay, with more structuring in the eastern gulf, where restricted water movement has created a stronger salinity gradient than in the western gulf. The identification and sharing of very few outlier loci across meadows does not provide strong support for local adaptation. Instead, the relationships among meadows likely reflect that of a widely described phenomenon among many marine species at local to regional scales - 'chaotic genetic patchiness' (Broquet et al. 2013, e.g. Sinclair et al. 2014), that is structure is likely the result of different cohorts of seeds or seedlings recruiting in different places at different times. The use of hydrodynamic modelling for in-water column movement of *Amphibolis* seedling dispersal may improve our understanding of the processes driving this pattern.

3.5.3 Key results for *Amphibolis antarctica*

- All 184 samples have unique genetic sequences.
- There were moderate and similar levels of heterozygosity within all sampled meadows (heterozygosity ~30%, few private alleles).
- There was significant genetic differentiation (as measured by F_{ST}) among all pairs of sampled meadows.
- Genetic structure among meadows in the eastern gulf was higher than the western gulf.
- The stronger genetic structure in the eastern gulf is likely associated with restricted water movement, which also creates a stronger salinity gradient.
- Effective migration rates (Nm) were close to (eastern gulf) or greater than one (western gulf), consistent with gene flow among meadows associated with effective dispersal of seedlings.
- The identification of seven outlier SNPs (putative adaptive markers under selection) suggests there is not strong local adaptation.

3.6 Genomic diversity in other seagrass species

The genomic data collected during this study show there is genetic diversity within and among the sampled meadows in Shark Bay for both species. Both species show higher levels of diversity than those previously detected using a range of markers for *P. australis* (Waycott 1998; Sinclair et al. 2020) and *A. antarctica* (Waycott et al. 1996; van Dijk et al. 2018). These data show a capacity for identifying higher levels of diversity using the ddRAD method, as a much higher proportion of the genome is sampled. Interestingly, observed heterozygosity in the reproductive *Posidonia* meadow at Guischenault Point was very similar to that obtained from microsatellite markers (Ho = 17.9 - 25.9%, Sinclair et al. 2020, Sinclair et al. unpublished data) and SNPs (Ho = 24.7% SNPs, this study).

The significantly elevated heterozygosity levels in nine *Posidonia* meadows is explained by the presence of additional alleles. Plants may acquire variation over time through various mechanisms, including somatic mutations (Schoen & Schultz 2019). Additional alleles were also identified in a northern hemisphere seagrass, *Zostera marina,* and these were attributed to somatic mutation(s) (Reusch & Boström 2011). Reusch & Boström (2011) were able to sample and genotype individual branching rhizome that underwent somatic mutation on a cellular level, resulting in genetic variation occurring at a particular locus. SNP markers were used to further examine the genetic basis for somatic mutations, where sexual reproduction was rare or absent (Yu et al. 2020). This process may explain the variation among samples within an individual meadow (sampling distance < 50 m). However, alternative explanations are being explored to determine the origin of widespread high heterozygosity among *Posidonia* meadows in Shark Bay.

Currently, there are few published studies on population genomic diversity and structure in other seagrasses using SNP markers (e.g. Hernawan et al. 2017; McMahon et al. 2017; Phair et al. 2019, 2020). Our data for Shark Bay show considerably more genomic diversity than that observed across the whole species range for *Zostera capensis* in southern Africa, which was highly clonal with almost no genetic structure (Phair et al. 2019). The tropical seagrass *Halodule uninervis* had low clonal diversity (R = 0.19; McMahon et al. 2017) in two southern range edge meadows sampled from Shark Bay. Shark Bay is the meeting place for tropical and temperate species, thus although the species diversity is high for seagrasses at 12-13 species, levels of genetic diversity within species/meadows may be lower the more northerly or southerly meadows away from the range edges of respective tropical and temperate species.

There was a higher number of adaptive markers identified in *Posidonia* meadows than *A. antarctica* (67 versus 7, respectively). No significant geographic structuring appears to be present in either species across Shark Bay. Further examination of the location of the adaptive markers in the *Posidonia* genome will be required to determine their function once an annotated genome is available.

3.7 Genetic-based recommendations for sourcing plant material

Understanding the patterns of genetic variation in seagrasses is an important aspect of planning restoration activities, to ensure that donor material is genetically compatible for the restoration site and existing natural meadows adjacent to the restoration site, and that the genetic integrity of the existing meadows is maintained. Genetic-based guidelines for transplantation within Shark Bay have been developed, based on genomic diversity and connectivity analyses of mature ribbon weed (*Posidonia*) and wire weed (*Amphibolis*) meadows in Shark Bay. We have also drawn from additional genetic information on the mating system in ribbon weed from Sinclair et al. (2016, 2020).

Specific recommendations by species are below:

3.7.1 Ribbon weed (*Posidonia australis*)

Adults (non-reproductive plants)

Locally sourced plants are the easiest option – but this approach will maintain low (local) clonal diversity. Most genotyped plants were very closely related within a meadow.

Collections of shoots should be spaced at least 2 m apart to increase the number of different plants used for transplanting.

Collecting plants from within the (west or east) gulf is recommended if local meadows are unavailable (plants are unhealthy or absent).

We caution against moving transplants large distances (not moving plants across the extremes of the salinity gradient). The implications of this on long term plant health and reproduction have not been assessed.

Seeds (reproductive plants - Guischenault Point and Red Hill Bay)

Ribbon weed is monoecious - male and female parts on the same flower, however, plants prefer pollen from a different genetic plant to pollinate and grow a viable seed.

The outcrossing rate is ~50% and seed viability is mixed (Sinclair et al. 2020).

Half the seeds are self-pollinated (identical to the parent plant), so there is lower genetic diversity.

The use of seeds for restoring *Posidonia* in Shark Bay is a limited option. We have found viable seeds from two sites only and their abundance is highly variable between years (Kendrick et al. 2019). Our new research project is exploring the reasons for low seed production in *Posidonia* meadows in Shark Bay, and whether there are opportunities to increase outcrossing rates (and therefore seed production) in the future.

3.7.2 Wire weed (*Amphibolis antarctica*)

Adult plants (reproductive – dioecious plants)

Locally sourcing plants is the easiest option – levels of genetic diversity were similar in all meadows.

Collections of shoots should be spaced at least 2 m apart to increase the number of different plants transplanted. There is no way to identify male and female plants when not reproductive. A mix will be required, especially if restoring remote meadows. Female plants will have visible seedlings attached from March – July.

Collecting plants from within each (west or east) gulf is recommended if local meadows are unavailable (plants are unhealthy or absent),

Caution against moving transplants over large distances (not moving plants across the extremes of the salinity gradient), particularly in the eastern gulf where meadows are genetically differentiated.

Seedlings

Genetic structure in adult populations suggest seedlings are dispersing widely, particularly in the western gulf.

Restoration relying on natural recruitment using hessian tubes will attract seedlings from neighbouring meadows, as well as some from further afield.

Dispersing seedlings can be collected from the water for attachment to hessian tubes, if there are no local meadows producing seedlings.

Further research is required to determine how long the seedlings float, and how long the season for floating seeds is.

4. TRAINING WORKSHOPS – CONTINUING TRANSFER OF KNOWLEDGE TWO-WAYS (MILESTONE 3)

A series of four joint training workshops were conducted on Malgana Country between UWA researchers and Malgana Land and Sea Rangers. Six rangers participated in the workshops: Richard Cross, Alex Dodd, Nykita McNeair, Marika Oakley, Pat Oakley, and Nicholas Pedrocchi. The last training workshop, significantly delayed due to COVID-19 regional closures in Western Australia preventing access to field sites, was conducted between 11-13 August 2020. This workshop was done in conjunction with Maryka Gray from Geraldton TAFE and fulfils part of the Certificate III training in Conservation and Land Management for Malgana Rangers. The workshops and dates were as follows:

1. Welcome to Country, an introduction to seagrasses and facilitated recruitment trial at Fowlers Camp (August 2019)
2. Collecting *Posidonia* fruit for seed-based restoration (October 2019)
3. Seagrass theory and restoration practice at Middle Bluff (western gulf) and Dubaut Point (eastern gulf) (March 2020)
4. Facilitating *Amphibolis* seedling recruitment using a novel approach at Dubaut Point (eastern gulf) and Denham (western gulf) (August 2020).

The following map shows the location of all restoration sites within Shark Bay, including sites that were established with Rangers specifically for this project, as well as other more established sites which have been developed through our other research programs (Figure 8).

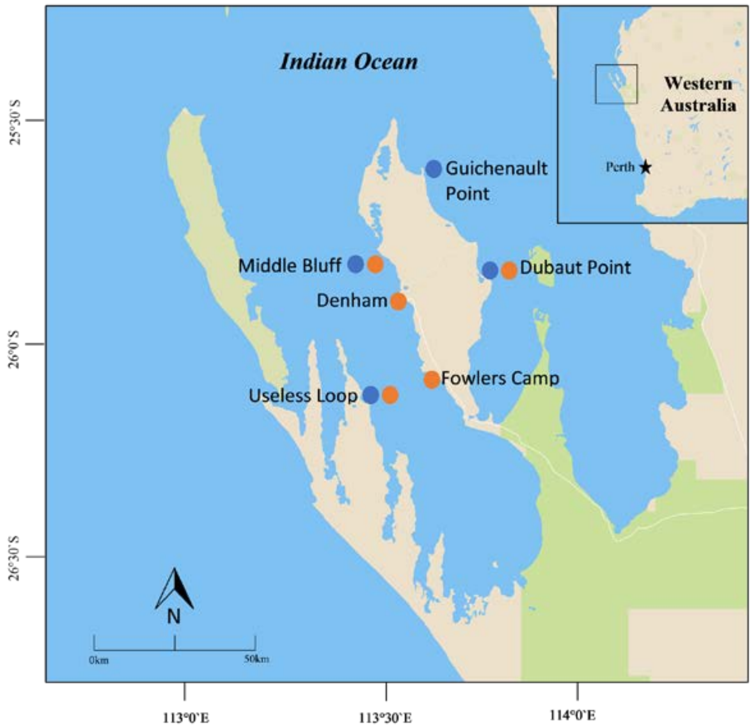

Figure 8 Map showing the location of all seagrass restoration sites in Shark Bay by species: *Posidonia australis* (blue circles) and *Amphibolis antarctica* (orange circles). *Posidonia australis* was established at Middle Bluff and Dubaut Point in April (30 months old) and August (26 months old) 2018. Both *P. australis* and *A. antarctica* were established within Malgana Ranger restoration sites at Dubaut Point and Middle Bluff in March 2020 Workshop 3. Both *P. australis* and *A. antarctica* were established in Useless Loop by University of W.A. researcher in 2015. Experimental sites for survival and return to ecosystem function were Useless Loop - 60 months old; Middle Bluff and Dubaut Point - 30, 26 and 8 months old. Demonstration restoration trial site was located at Fowlers Camp Workshop 1 (Orange circle) for *A. antarctica* seedlings and sand-filled hessian bags. *Posidonia australis* fruit collection at Guichenault Point Workshop 2 (Blue circle; October 2019). Facilitating *Amphibolis antarctica* seedling recruitment using biodegradable, sand-filled hessian tubes at Dubaut Point and Denham (August 2020).

4.1.1 Workshop #1: Welcome to Country, an introduction to seagrasses and facilitated recruitment trial at Fowlers Camp (August 2019)

UWA researchers gave in-field demonstrations of each of the species, their functional role in the ecosystem, how they grow, and when possible, showed how they reproduce. We found great symmetry with how our contemporary scientific understanding of the various seagrass species was underpinned by contemporary and historical traditional knowledge based on how the Malgana people interacted with the seagrass. For example, *A. antarctica* has a darker colour signature above the water than that of *P. australis*. This knowledge allowed each species to be targeted differently for fishing practices, and we know from a scientific perspective each species provides a different forage and structural habitat, therefore influencing species abundance.

In another example, UWA scientists demonstrated how the *Amphibolis* seedlings have a grappling hook appendage for attaching to the seafloor, but spend time floating on the surface after they have been released. While important for the recovery of *Amphibolis* in Shark Bay, *Amphibolis* can be problematic during net fishing, getting caught up in fishing nets. However, the timing of *Amphibolis* seedling release can change from year to year, and we discovered that having local, real-time knowledge of the timing of seedling release will be crucial for future restoration of this species.

During this training exercise UWA researchers also demonstrated how it's possible to artificially create a substrate for *Amphibolis* seedlings to attach to, and which biodegrades over the course of a year while the seedlings establish. This involved a team effort of filling 14 hessian and 12 jute sandbags with beach sand and walking out to the trial site at Fowlers Camp for deployment (Figure 9). Sandbags were placed in pairs of hessian and jute. Following bag deployment, we collected over 100 *Amphibolis* seedlings and placed six seedlings on each bag using their grappling hook as the point of attachment (Figure 9). Survival was monitored during a subsequent trip in February/March 2020.

Figure 9 Deployment of (a) Jute and (b) Hessian bags. White arrows indicate *Amphibolis antarctica* seedlings attached to the bags.

4.1.2 Workshop #2: Collecting *Posidonia* fruit for seed-based restoration (October 2019)

In October 2019, the aim of this training exercise was to identify mature *P. australis* flowers and fruit, conduct flower counts and then demonstrate a well-established technique to collect and extract the fruit (Statton et al. 2013). The approach is to initially count flower heads 0.5 m either side of a 10 m transect tape and replicate 10 times. Flower heads are then collected to count the number of viable fruit, aborted fruit and unfertilised flowers on randomly collected flower heads. Collection involves knocking fruit off the flower head and as they float to the surface they are collected in large nets. Collected fruit are then placed within a large aquaculture tank where the water is agitated with aeration and a pump to promote the splitting open of fruit and release of the seed.

In previous research we had identified the most suitable location, Guischenault Point (Figure 8), to collect *P. australis* fruit (Kendrick et al. 2019). However, the timing of this years' expedition also coincided with an extreme low, astronomical low-tide event in late spring. Consequently, large expanses of *P. australis* meadows at Guischenault Point were exposed to the air and were desiccated by the heat and dry air (Figure 10). There appeared to be a gradient in impact, with meadows that were exposed along the shallower margins bleached white (Figure 10a), those partially submerged had brown leaves (Figure 10b) while meadows that were much deeper tended to have green leaves with brown tips (Figure 10c) or didn't appear impacted (Figure 10d). Some meadows of *Posidonia* showed evidence that flowers had been produced (Figure 10e), but fruit development was aborted on every flower observed (Figure 10f) and flower counts were often less than 1 m^{-2} (c.f. 80 m^{-2} in 2017, Kendrick et al. 2019).

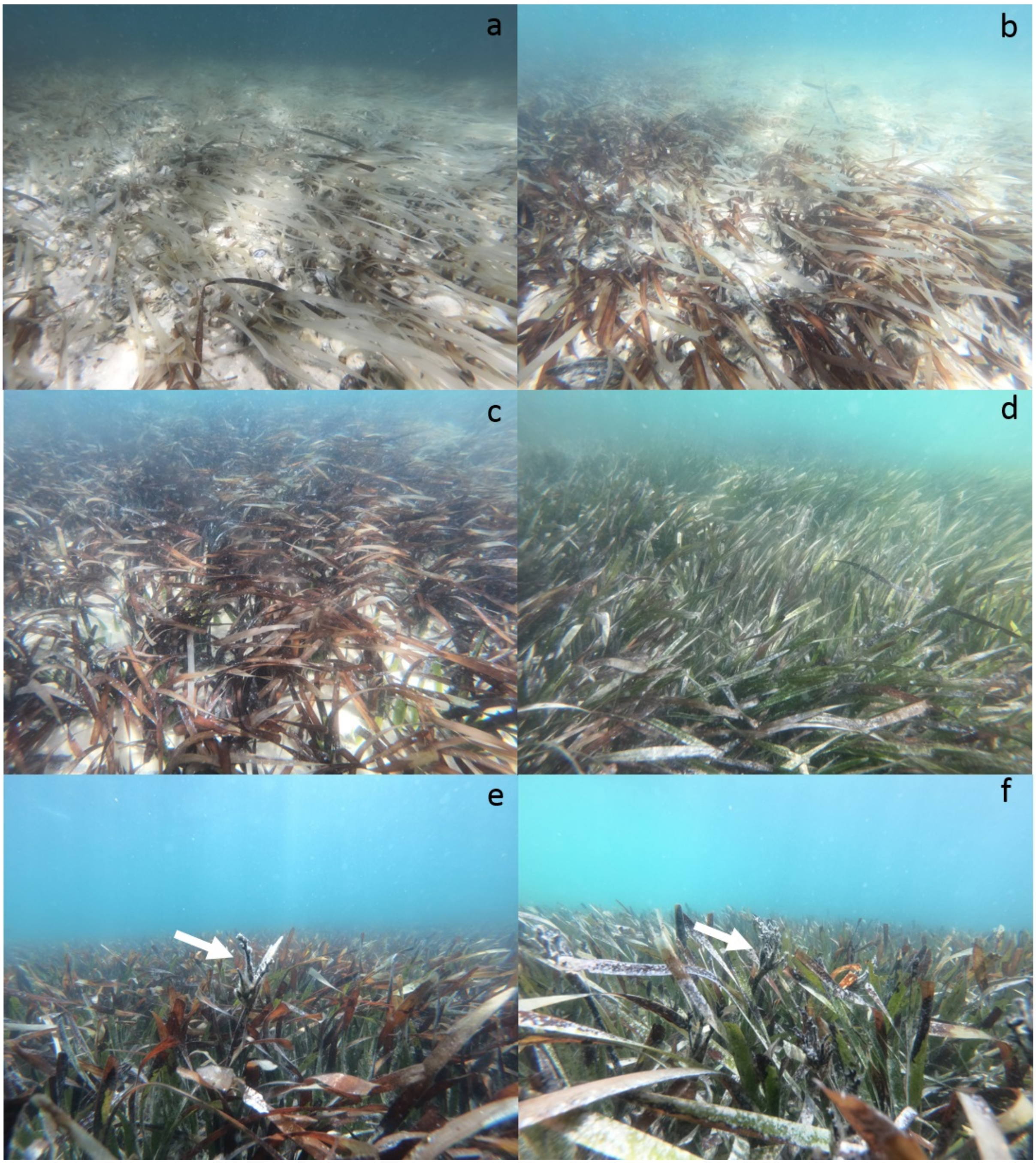

Figure 10 *Posidonia australis* meadow at Guischenault Point, Shark Bay showing (a) complete bleaching of leaves and death of the meadow, (b) mix of bleached and brown leaves with no green leaves, (c) brown leaves with some green leaves, (d) healthy green meadow. Meadows that remained submerged during the low-tide event but were (e) desiccated with brown leaf tips or were in deeper waters with minimal leaf tip browning retained flowers but all fruit were aborted. Photos by John Statton

4.1.3 Workshop #3: Seagrass theory and restoration practice at Middle Bluff (western gulf) and Dubaut Point (eastern gulf) (March 2020)

During February/March 2020, UWA researchers demonstrated both land and water-based seagrass-focussed training activities. In the water the Malgana Land and Sea Rangers worked within research sites where *P. australis* had previously been transplanted (these plants were 19 and 23 months old at the time and formed part of the genetics translocation research - see Milestone 2). The Malgana Land and Sea Rangers initially learnt how to identify a living transplant based on locating a coded tag attached to each plant, then shoot counts were conducted on each transplant to later assess shoot density of plants (Figure 11). The next activity was to establish restoration trials at these two sites; Dubaut Point (S 25°51.135'; E 113°45.612') and Middle Bluff (Malgana name Muga – S 25°49.452'; E 113°27.841'). At each site we set-up two 25 m^2 plots adjacent to each other. One plot we transplanted 36 adult plants of *A. antarctica* and the other 36 adult plants of *P. australis* (Figure 12).

Figure 11 Eighteen month old *Posidonia australis* transplants being assessed for individual transplant survivorship and counting the number of shoots on each surviving transplant.

To undertake the restoration trials UWA researchers conducted training on how to collect, prepare and plant *P. australis* and *A. antarctica* transplants. For both species, the Malgana Land and Sea Rangers were shown how to identify suitable shoots for collection which are found along the leading edge of a meadow and how to easily remove these shoots by tracing the rhizome 4-6 shoots back and harvesting the rhizome fragment. Plants were placed in bags and returned to the boat for preparation. Preparation involved removing excess roots and ensuring the rhizomes were not damaged beyond where the rhizome had been excised from the meadow. To plant the transplants, a small furrow was made in the seafloor with a blunt tool, the rhizome was buried 3-5 cm below the surface, a wire peg then anchored the transplant and the sand was pushed in to fill the furrow and cover the rhizome. Transplants were planted at a distance of 1 m apart.

Figure 12 Transplanting *Posidonia australis* adult shoots at Middle Bluff

On the land, the Malgana Land and Sea Rangers developed their theoretical understanding of the science underpinning the seagrass species within Shark Bay, and this work was led by Amrit Kendrick. The workshop activities included developing an understanding of the biology of several seagrass species in Shark Bay, as well as their ecology, ecosystem function and restoration approaches. The Malgana Land and Sea Rangers were encouraged to ask questions to broaden their understanding of the different ecological aspects of the seagrasses. Many different forms of educational material were used, including pre-made booklets, posters, powerpoint presentations and videos demonstrating different techniques used to restore seagrasses across Australia. Rangers also looked at a body of scientific literature that included 900 papers written about molluscs, fish, dugongs and turtles that live in the seagrass ecosystem.

4.1.4 Workshop #4: Facilitating *Amphibolis* seedling recruitment using a novel approach at Dubaut Point (eastern gulf) and Denham (western gulf) (August 2020).

In this final of four workshops, the researchers and rangers worked on developing and trialling an innovative facilitated seagrass recovery technique. The rangers also completed their conservation and land management training, which includes seagrass habitat restoration.

An *Amphibolis antarctica* seedling facilitation technique involving biodegradable sand-filled hessian tubes (2.5 m long, 80 mm diameter) was trialled at Denham and Dubaut Point. After the success of the hessian bags the previous year (see Workshop #1), we used a hessian bag design which increased the hessian surface area but was also lighter. Long, sand-filled hessian tubes were determined to be the best technique after they were successfully trialled in Corner Inlet, Victoria by UWA researchers to act as baffles to slow water flow and enable *P. australis* seedlings to establish. Hessian tubes can be filled with local beach sand (Figure 13). Ninety sand-filled hessian tubes were deployed at two locations, Denham and Dubaut Point, close to existing wire weed meadows. Hessian tubes can be transported and deployed from a vessel as the vessel drifts (Figure 14). The hessian tubes can also be manoeuvred into place or oriented so that they are perpendicular to the prevailing currents (Figure 15a). *Amphibolis* seedlings naturally attach to the hessian tubes or can be manually attached after floating seedlings are collected (Figure 15b). Seedlings are firmly attached to the hessian tube by their grappling hook appendage and can remain attached for months while the seedling puts down a root to enhance anchorage (Figure 16).

This method is simple, cheap, and easy, but timing is critical. The hessian tubes must be in place before the major release of seedlings begins. Ongoing work by Malgana Rangers on Sea Country means hessian tubes in coming seasons can be appropriately timed. The hessian tubes should last about 18 months: long enough for new seedlings to establish.

Figure 13 (a) UWA researchers and Malgana Land and Sea Rangers filling biodegradable sand-filled hessian tubes with beach sand. (b) sand-filled hessian tubes loaded on the boat ready for deployment (Images: Elizabeth Sinclair)

Figure 14 (a) Malgana rangers prepare to deploy sand-filled hessian tubes at a restoration site. (b) Sand-filled hessian tube deployed over the side of the vessel (Images: Gary Kendrick)

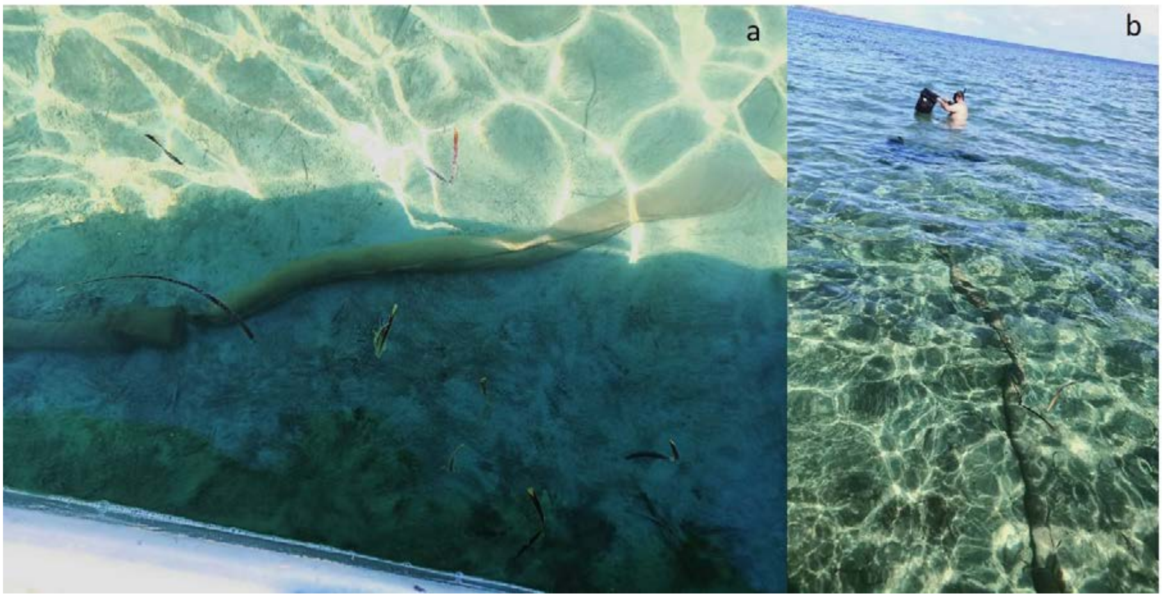

Figure 15 (a) Biodegradable sand-filled Hessian tubes deployed and manoeuvered on the seafloor near Denham, and (b) with Malgana rangers and UWA researchers attaching *Amphibolis* seedlings.

Figure 16 Biodegradable sand-filled Hessian tube with *Amphibolis* seedlings still attached and producing roots after 4 months

5. SEAGRASS ASSISTED RECOVERY (MILESTONES 4 & 5)

5.1 Background

Restoration research in Australia has focused on small-scale experimental tests using a variety of techniques ranging from the planting of sprigs (seagrass fragments) or plugs (seagrass cores) to seed-based restoration (Tan et al. 2020). The majority of seagrass restoration trials to date have used shoot-based techniques. Seed-based approaches are becoming increasingly more widespread with some early successes (Tan et al. 2020; Sinclair et al. 2021). Survival of transplanted seagrass fragments or cores have had variable success in many studies, although transplant unit survival can be high, eventually establishing and growing to form large patches or meadows with similar shoot densities to those found in naturally occurring meadows (e.g. Bastyan & Cambridge 2008).

Within Australia and globally, seed-based approaches are becoming more common and successful. Collecting, processing and remotely delivering seeds of *Posidonia australis* have seen some early successes within the citizen science program ‘Seeds for Snapper’ (Sinclair et al. 2021). The use of *Amphibolis antarctica* seedlings in restoration is more well-established, especially in the use of hessian bags which act as a substrate for *Amphibolis* seedling recruitment. Long-term trials involving the use of hessian bags placed on the ocean floor to aid natural seedling recruitment started in 2004, with many showing long-term survival (Irving et al. 2010; Tanner 2015). The restoration successes seen in Australia today largely come from studies on *Posidonia* and *Amphibolis* (Tan et al. 2020). In this project, we trialled these approaches to test the efficacy of each technique.

While these studies have contributed to the overall knowledge of restoration and our growing confidence that restoration is possible (Tan et al. 2020; Sinclair et al. 2021), Shark Bay has not been extensively tested as a restoration location. Since 2011 restoration research has been carried out within a 120 ha area at Useless Loop, Shark Bay. Using shoot transplant techniques, this research has demonstrated that successful restoration within Shark Bay is possible. Here, we compare three 5 year old restoration sites within Useless Loop to sites in this project: Middle Bluff (western embayment) and Dubaut Point (eastern embayment). This comparison will serve to demonstrate the potential long-term restoration outcomes possible with our newly established sites in terms of transplant growth, fish and invertebrate diversity, and carbon storage.

5.2 Seed-based approaches to assisting recovery of seagrasses

5.2.1 *Amphibolis antarctica* (Wire Weed) seedlings

In August 2019, *Amphibolis* seedlings were collected from ocean drift material near Fowlers Camp (Figure 8). Seedlings, with their grappling hook appendage, were attached to 14 Hessian and 12 Jute sandbags (see 4.1 for details). Typically, seedlings naturally attach to the sand bags with 10’s to 100’s attaching over the reproductive period, however, for this demonstration restoration trial we attached six seedlings directly to each sandbag via the seedlings grappling hook appendage (Figure 17a). Seedling presence/absence was monitored 8 months later in March 2020. Eight-six percent (12 out of 14) of Hessian and 83 percent (10 out of 12) of Jute sandbags had at least one *Amphibolis* seedling present. One Jute sandbag had retained three seedlings and several had two seedlings attached with roots developing and penetrating the sandbags (Figure 17b). All Hessian and Jute sandbags remained intact after 8 months and showed signs of sediment accretion around the bags suggesting good integration within the site.

Figure 17 *Amphibolis antarctica* seedlings initially attached to Jute sandbags (a) and then after eight months (b) at Fowlers Camp, Shark Bay.

We considered each sandbag a planting unit, and this trial was highly successful in recruiting and establishing *Amphibolis* seedlings after 8 months.

The early success of using hessian or jute sandbags for facilitating *Amphibolis* recruitment (see Workshop #1 for details) suggests this technique is worth exploring in Shark Bay. While hessian bags are a good technique, they have a low surface area to their overall mass meaning they can only collect seedlings over a small area. We have designed and trialled 2.5 m long hessian tubes in another project with good success. Hessian tubes are similar in weight to a standard square hessian bag, but have a far greater surface area for attachment of dispersing *Amphibolis* seedlings. We commissioned an upholsterer to produce the 2.5 m long hessian sandbag tubes. These were shipped to Shark Bay in June/July 2020 in time for the Malgana Land and Sea Rangers to fill with local sand. Hessian tubes were deployed at restoration sites in Denham and Dubaut Point at the peak season for release of *A. antarctica* seedlings in August (see Workshop #4 for details).

This method is simple and cheap and could be scaled effectively, but timing is critical. The hessian tubes must be in place before the major release of seedlings begins. We were able to demonstrate natural seedling attachment, but with the delay in implementing these trials due to COVID-19 set-backs, it appeared the hessian tubes were deployed at the end of seedling release and so only a few seedlings attached to few hessian tubes across both restoration locations (see Figure 16). Ongoing work by Malgana Rangers on Sea Country means hessian tubes in coming seasons can be appropriately timed. The hessian tubes should last about 18 months: long enough for new seedlings to establish.

5.2.2 *Posidonia australis* (Ribbon Weed) fruit and seeds

Posidonia australis fruit become available in early November in Shark Bay. As part of the training exercises with the Malgana Land and Sea Rangers we anticipated collecting floating fruit using dip nets as well as removing fruit from the parent plants directly during the November 2019 reproductive period. However, an extreme low-tide event that was predicted in October 2019 had caused complete flower and/or fruit abortion of many of the shallow *P. australis* meadows (see Training for details). We were also unable to find successful fruit production in deeper *P. australis* meadows in Shark Bay which has typically been the case and not related to the low-tide event. Therefore, no restoration trials were established in 2019 using *P. australis* seeds. We question the value of continued efforts with *P. australis* seeds, as they are rare throughout Shark Bay with high levels of seed abortion (Sinclair et al. 2020), and we would not recommend this as a viable method for restoration in this system.

5.3 Shoot-based restoration

Transplanting adult shoots has been a successful and well-known technique used around Australia (Bastyan & Cambridge 2008; Statton et al. 2012; Tan et al. 2020). Since 2015, restoration trials have been conducted at Useless Loop, Shark Bay using transplant methods for both *P. australis* and *A. antarctica.* These trials have shown that both species can be successfully established at Useless Loop, Shark Bay.

For a previous ARC Discovery funded program, two experimental *P. australis* field plots were established as separate experiments in April and August 2018 (Dubaut Point, Middle Bluff). The transplants are between 26 months and 30 months old for Dubaut Point and Middle Bluff. In February/March 2020 this NESP project E6 established a further two plots at each site with 36 plants of each of *P. australis* and *A. antarctica* collected, processed (trimmed to four shoots) and transplanted in adjacent plots (see section 4.3 for details).

In this report, we compare the NESP E6 transplants to these longer term (26 - 30 months) established transplant plots and 3 plots at Useless Loop that are 60 months old). This will enable temporal and spatial comparisons between *P. australis* (and when present *A. amphibolis*) restoration to provide a greater level of understanding of the establishment success and changes in shoot density over time longer than the 2 year E6 program length, as well as how biodiversity and carbon capture may change (see Milestone 6).

5.3.1 Survivorship

All transplants for both species showed high survivorship suggesting transplants are effective as a restoration methodology in Shark Bay. After 8 months the NESP E6 transplants had >95% survival for each species at Middle Bluff and Dubaut Point for both *P. australis* and *A. antarctica*.

The medium term plots for *P. australis* only show generally high survival rates for up to 30 month, for both Middle Bluff (western gulf) and Dubaut Point (eastern gulf) (Figure 18; Figure 19).

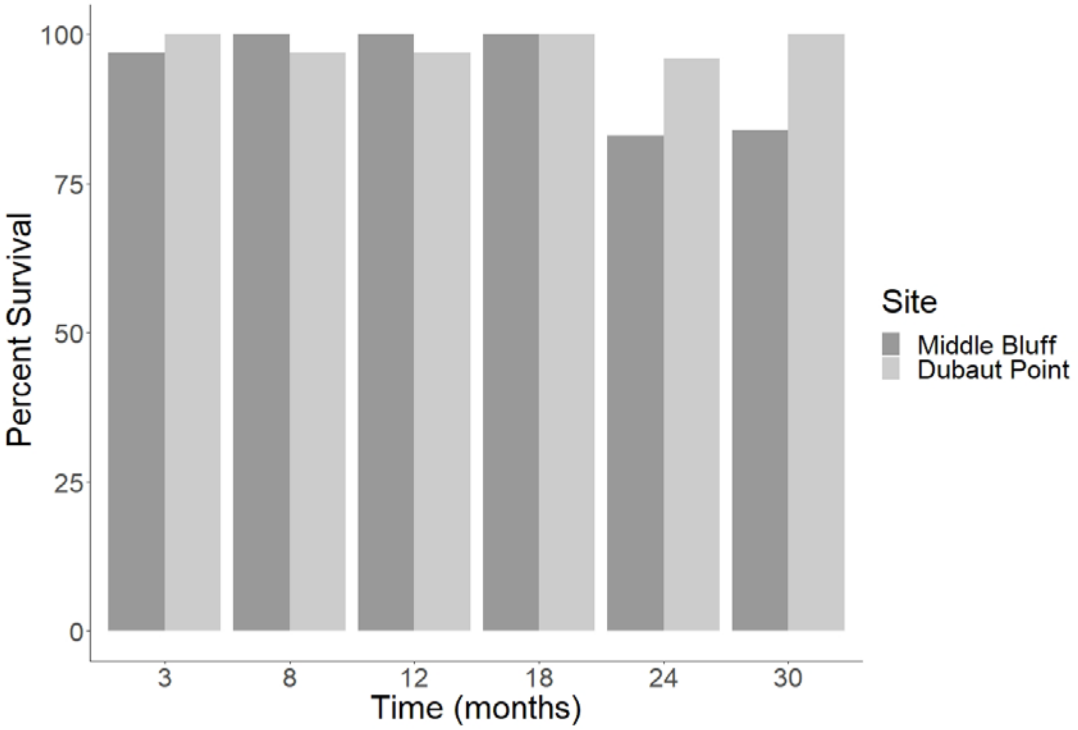

Figure 18 Transplant survival of *P. australis* for experimental plots established at Middle Bluff and Dubaut Point in April 2018 (i.e. survival after 30 months)

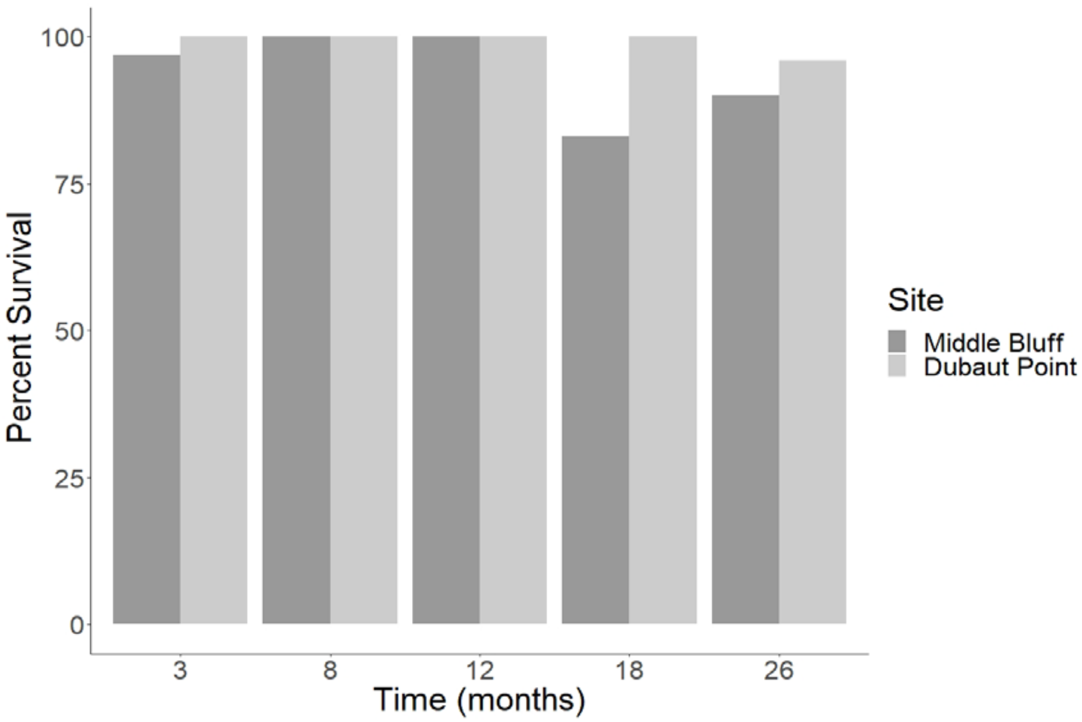

Figure 19 Transplant survival of *P. australis* for experimental plots established at Middle Bluff and Dubaut Point in August 2018 (i.e. survival after 26 months).

5.3.2 Shoot counts and density (*Posidonia australis*)

In October 2020, we assessed shoot counts for *P. australis* for 8 month old NESP E6 transplants, and for medium-term (26 and 30 month old) transplant plots at Middle Bluff and Dubaut Point (Figure 20). We also assessed shoot density (shoot count per m^2) of *P. australis* for long-term transplant plots at Useless Loop.

Figure 20 Established transplants of (a) 8 month old A*mphibolis antarctica* and (b) 26 month old *Posidonia australis* at Dubaut Point (by Gary Kendrick). Note the smaller *Halodule uninervis* is growing around the *Posidonia* plants.

Transplants at Dubaut Point tended to maintain the same number of shoots they were transplanted with (4 shoots per transplant) and showed a three-fold increase in shoot numbers at 26 months old and almost a four-fold increase for 30 month old transplants. Despite the high survivorship of transplants at both Dubaut Point and Middle Bluff, Middle Bluff tended to show shoot loss (one shoot) after 8 months. At 26 and 30 months, transplants continued to persist (see survivorship) but showed no increase in the number of shoots per transplant (Figure 21).

At Useless Loop, although transplants had a 10- to 70-fold increase in shoot density since the time of planting, shoot densities across the area depended on water depth (Figure 22) which also coincided with proximity to the Denham Channel which is nearest the deepest site. Transplants planted into deeper water (7 m) had clearly grown slower (~50 shoots per m^2), followed by plants at 5 m (~110 shoots per m^2) and the highest shoot density was found at 3 m (~280 shoots per m^2). At 60 months, *P. australis* transplant shoot density per m^2 was 60-70% that of adjacent established meadows (~450 shoots per m^2).

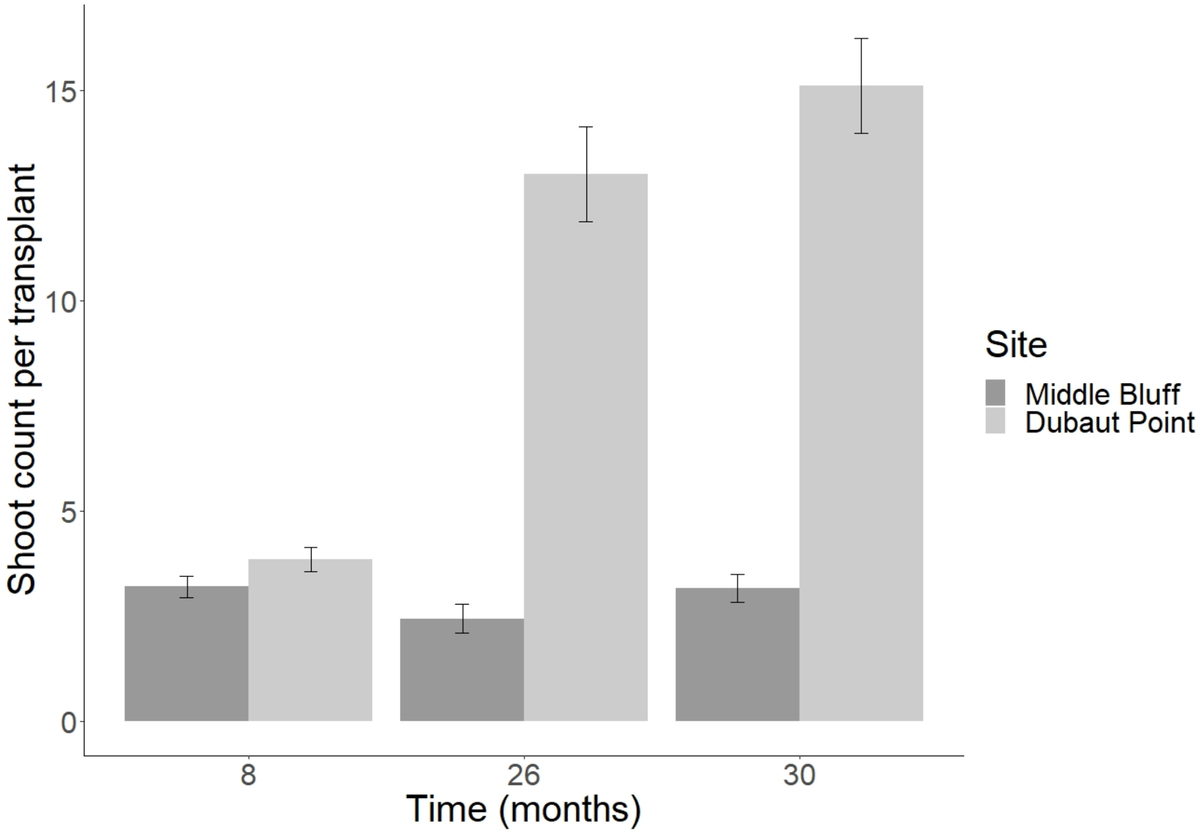

Figure 21 Shoot count per transplant for 8, 26 and 30 month old *Posidonia australis* transplants at two sites, Middle Bluff and Dubaut Point.

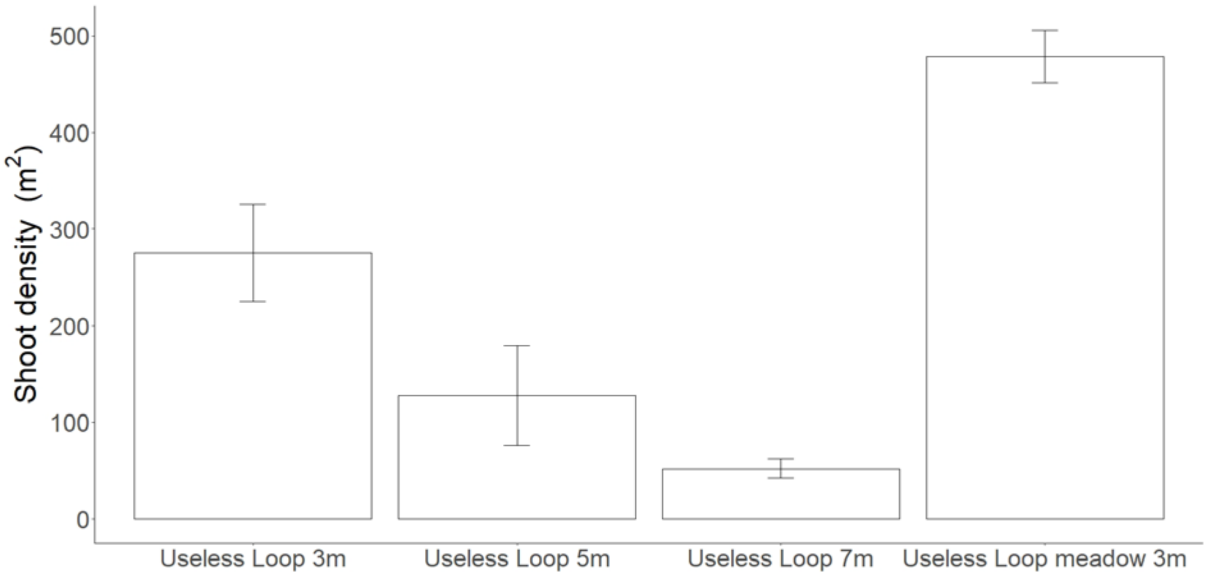

Figure 22 Shoot density per m^2 for 60 month old *Posidonia australis* transplants at three sites at Useless Loop (3 m, 5 m and 7 m water depth) and compared to an established meadow in Useless Loop at 3 m water depth.

5.3.3 Shoot counts and density (*Amphibolis antarctica*)

In October 2020, we assessed shoot counts for *A. antarctica* for 8 month old NESP E6 transplants at Middle Bluff and Dubaut Point (Figure 20). We also assessed shoot density (shoot count per m^2) of *A. antarctica* for long-term transplant plots at Useless Loop.

Transplants at Dubaut Point tended to show ~7 shoots after 8 months, an increase of 2 – 3 shoots compared to the number of shoots they were transplanted with (4 shoots per transplant). Middle Bluff tended to retain the same number of shoots they were planted (Figure 23).

At Useless Loop, transplants had a 55- to 85-fold increase in shoot density since the time of planting, shoot densities across the area also appeared to be influenced by water depth or proximity to the Denham Channel, but were in the opposite direction to *P. australis* transplants (Figure 24). *A. antarctica* transplants planted into deeper water (7 m) had clearly grown faster (~320 shoots per m^2), followed by plants at 5 m (~240 shoots per m^2) and the highest shoot density was found at 3 m (~220 shoots per m^2). At 60 months, *A. antarctica* transplant shoot density per m^2 was nearly 90% that of adjacent established meadows (~360 shoots per m^2).

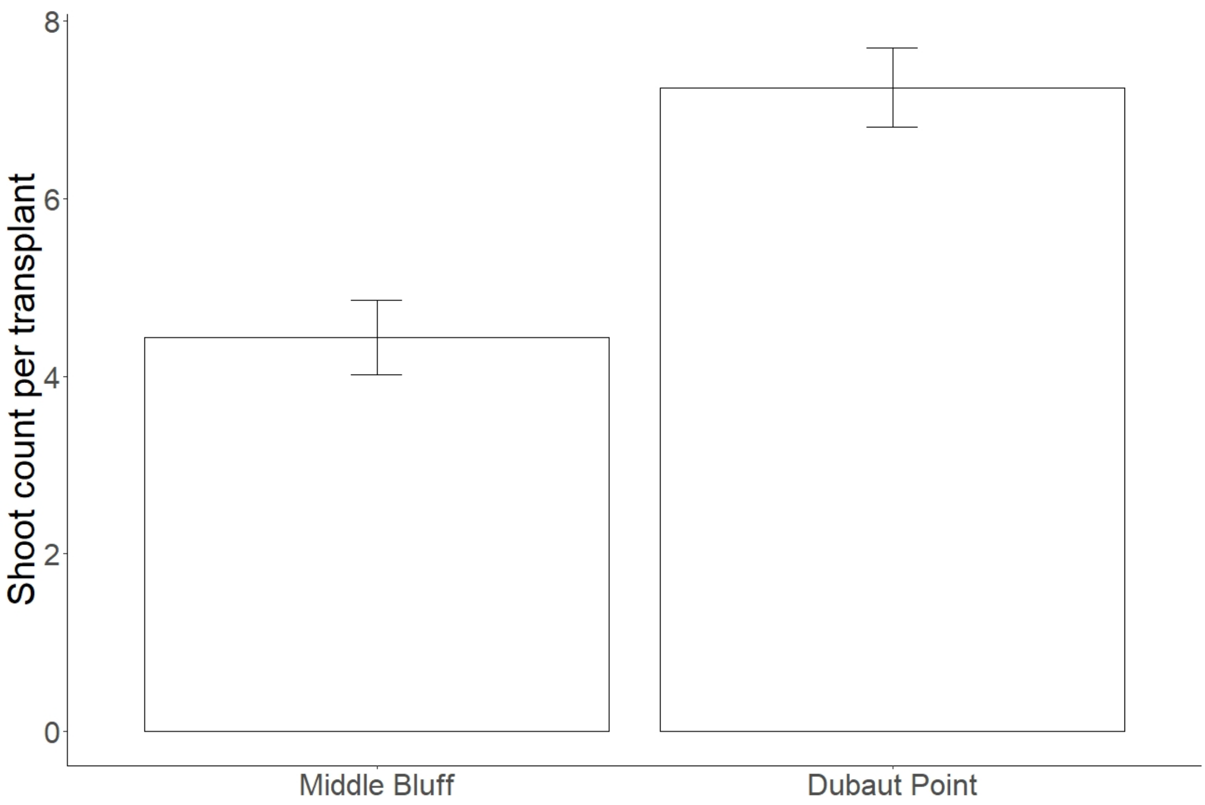

Figure 23 Shoot count per transplant for 8 month old *Amphibolis antarctica* transplants at two sites, Middle Bluff and Dubaut Point.

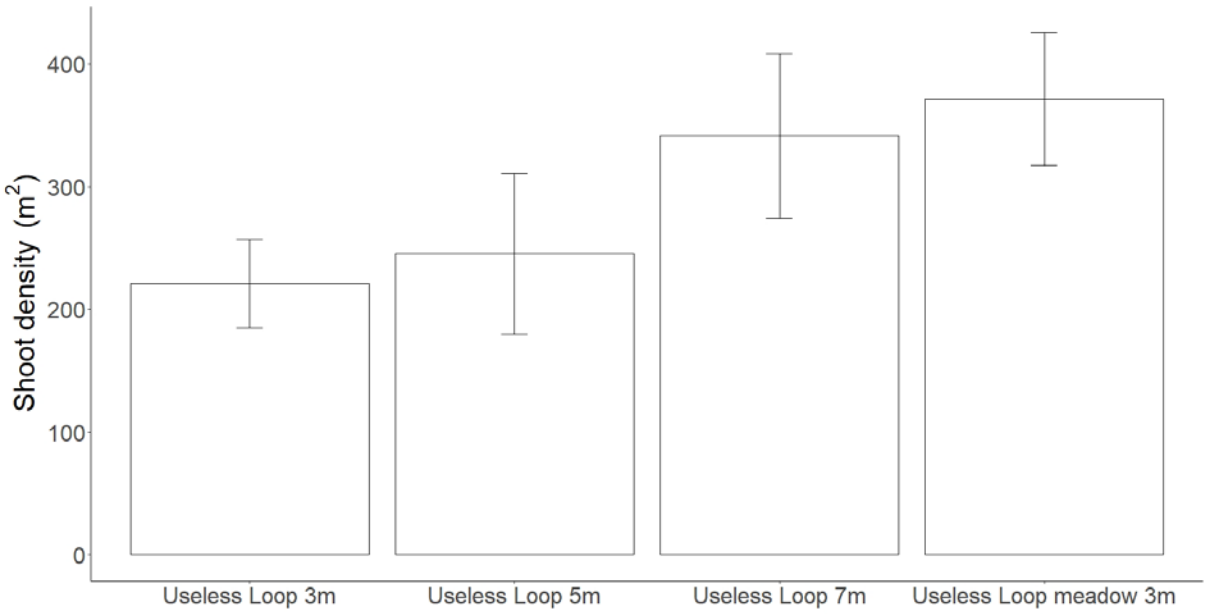

Figure 24 Shoot density per m^2 for 60 month old *Amphibolis antarctica* transplants at three sites at Useless Loop (3 m, 5 m and 7 m water depth) and compared to an established meadow in Useless Loop at 3 m water depth.

6. ASSESSMENT OF 'RETURN TO ECOSYSTEM FUNCTION' (MILESTONES 6 AND 7)

6.1 Carbon capture and sampling

Seagrasses and mangroves are some of the more important and efficient carbon storage ecosystems on the planet (Macreadie et al. 2017). Although mangroves, seagrass meadows, and salt marshes represent a much smaller area than terrestrial forests, their total contribution to long-term C sequestration is comparable to C sinks in terrestrial ecosystem types (Mcleod et al. 2011). The marine heatwave during 2010/11 resulted in the release of up to an estimated 9 million tonnes of CO_2 (Aria-Ortiz et al. 2018). It is important to understand how long it takes for a restoration site to start sequestering carbon at similar rates to natural meadows (pre-heatwave conditions).

Larger seagrass plants store more carbon, but it takes time for plants to recover, and for newly establishing transplants in a restoration site to establish and store carbon (Marbà et al. 2015). Sampling for the content of carbon in bare sand and natural seagrass meadows (*Posidonia australis, Amphibolis antarctica, Halodule uninervis, Halophila ovalis*) establish what the baseline carbon content is in the sediment. We then compared carbon content in different aged restoration sites to determine how long it takes for carbon to start accumulating. Carbon content was assessed at Dubaut Point, Middle Bluff and Useless Loop restoration sites (Figure 8).

6.1.1 Laboratory processing

Three replicate samples were collected using 35 ml syringes (Figure 25) from bare sand, restored seagrass meadows, and natural meadows. Restored seagrass meadows ranged in age from 8 months (Malgana Ranger sites), 26 months, 30 months and 5 years experimental sites). All samples were brought back to the basecamp and were sliced every 0-0.5 cm, 0.5-1 cm, 1-2 cm and 2-3 cm (Figure 26). Subsamples were stored in labelled plastic containers and kept frozen until further analysis in Perth. Samples were defrosted, dried in the oven (60°C) until constant weight and grounded into powder in the laboratory at UWA. Approximately every 2 gr samples were acidified using HCl 4% in 50ml conical tubes to remove carbonate prior to elemental analysis. About 20 ml of HCl were gently added to the sample in the fume hood until CO_2 went out and were left overnight. Samples were centrifuged at 2000g for 15 minutes and the supernatant was retrieved gently using pasteur pipette to keep the pellet undisturbed. The pellet was then rinsed twice using milli-Q water (distilled water), centrifuged at 2000 g for a further 20 minutes and supernatant was removed gently. The tubes were dried in an oven at 60°C with caps open until the contents were completely dried. After cooling down, tubes were weighed and the contents were homogenized using a glass rod. All samples were subsequently analysed using Elemental Analyser for total carbon (C), organic carbon (Corg), total nitrogen (N), stable isotope composition ($\delta^{13}C_{org}$ and $\delta^{15}N$).

Figure 25 Sediment samples collected in the field using 35 ml syringes.

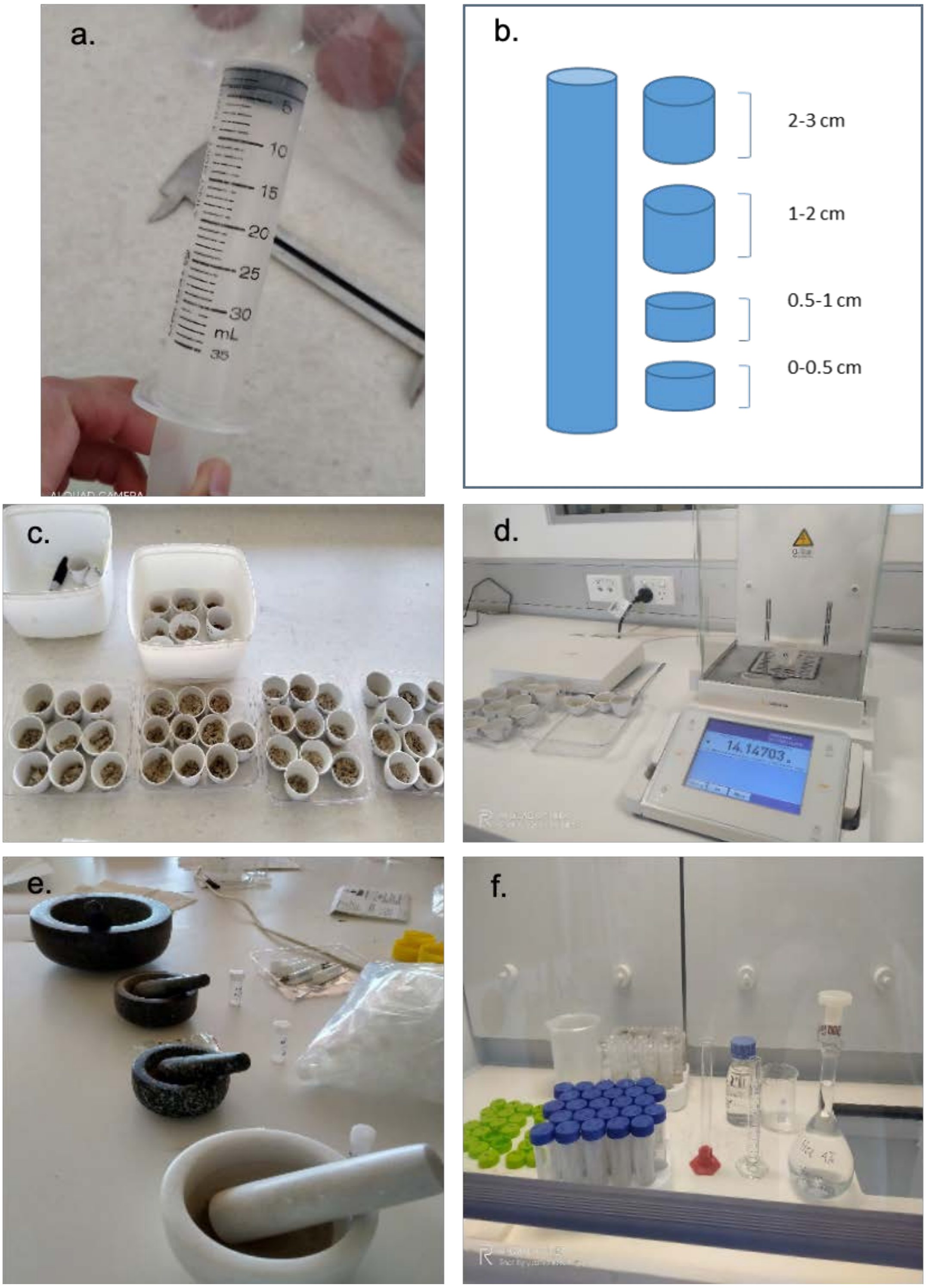

Figure 26 Collection and processing of sediment cores: a. 35 ml syringe used to get the sediment samples; b. Illustration showing subsampling of cores; c. Samples were ready for drying in the oven; d. Samples weighing before and after drying; e. Samples were ground using mortar and pestle; f. Acidification procedure in the fume hood.

6.1.2 How long does it take for restoration sites to start acquiring measurable Carbon in the substrate?

Restored sites are similar to bare sand and have not started accreting carbon. Useless Loop shows a different pattern and maybe a feature of its seagrass loss legacy. For example, at the shallow sites where the bare sand and meadow samples were collected, bare sand had similar Carbon stocks to meadows. The bare sand area had supported extensive seagrass meadows until the 1980’s after which they were degraded from bitterns discharge from the salt mine. The remnant seagrass matte is still present just below the sediment surface (Morrison 2009).

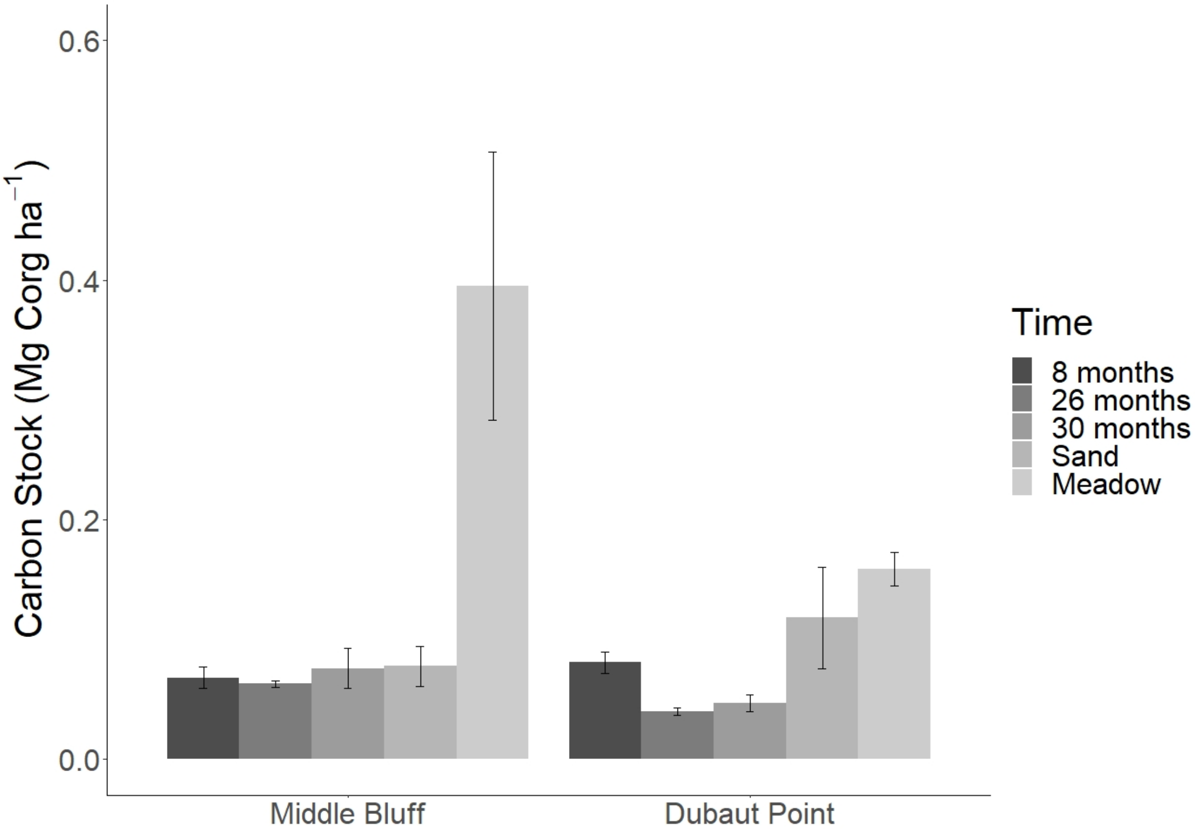

Figure 27 Carbon stocks at Dubaut Point and Middle Bluff for 8, 26, and 30 month old transplant plots, bare sand and existing seagrass meadows

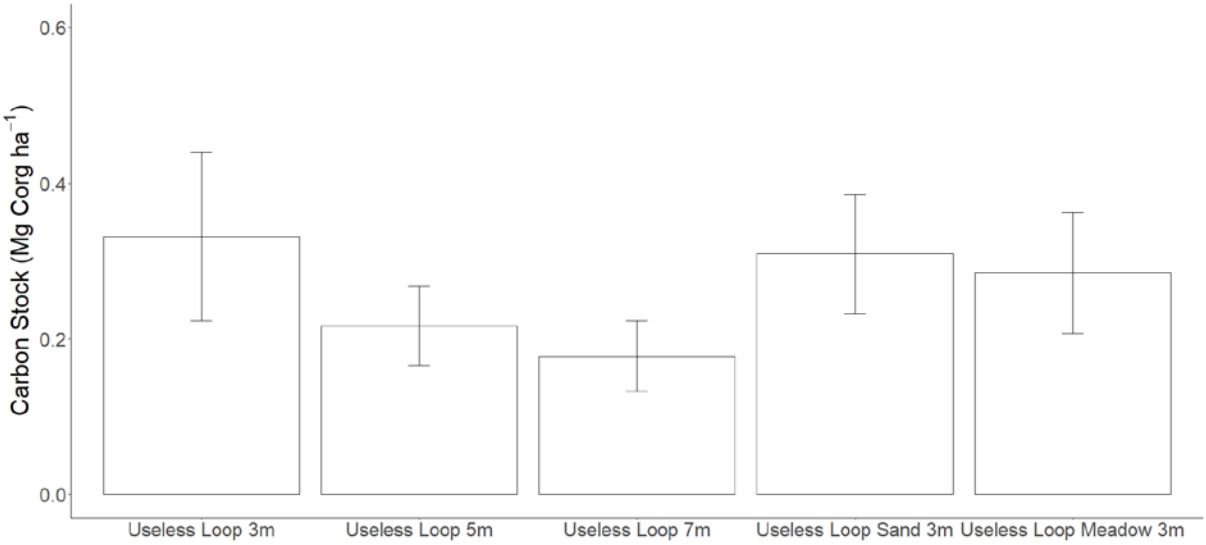

Figure 28 Carbon stocks at Useless Loop within 60 month old restoration plots at 3, 5 and 7 m water depth, bare sand and existing seagrass meadows at 3 m water depth.

A recent study for carbon accretion of restored *Posidonia australis* in Oyster harbour using an 18 year transplant study, showed that restored meadows require 7-10 years to become equivalent to established meadows (Marbà et al. 2015). In our study, the highest carbon stock within a restoration plot was in Useless Loop for 60 month old transplants at 3 m water depth (132 g C m^{-2}). The accumulated carbon stocks for *P. australis* since planting is on a similar trajectory to *P. australis* transplant plots in Albany at the same time but it is clear 60 months represents the beginning of a restored areas carbon accumulation (Figure 29a) and the predicted carbon burial rate (Figure 29b) potential.

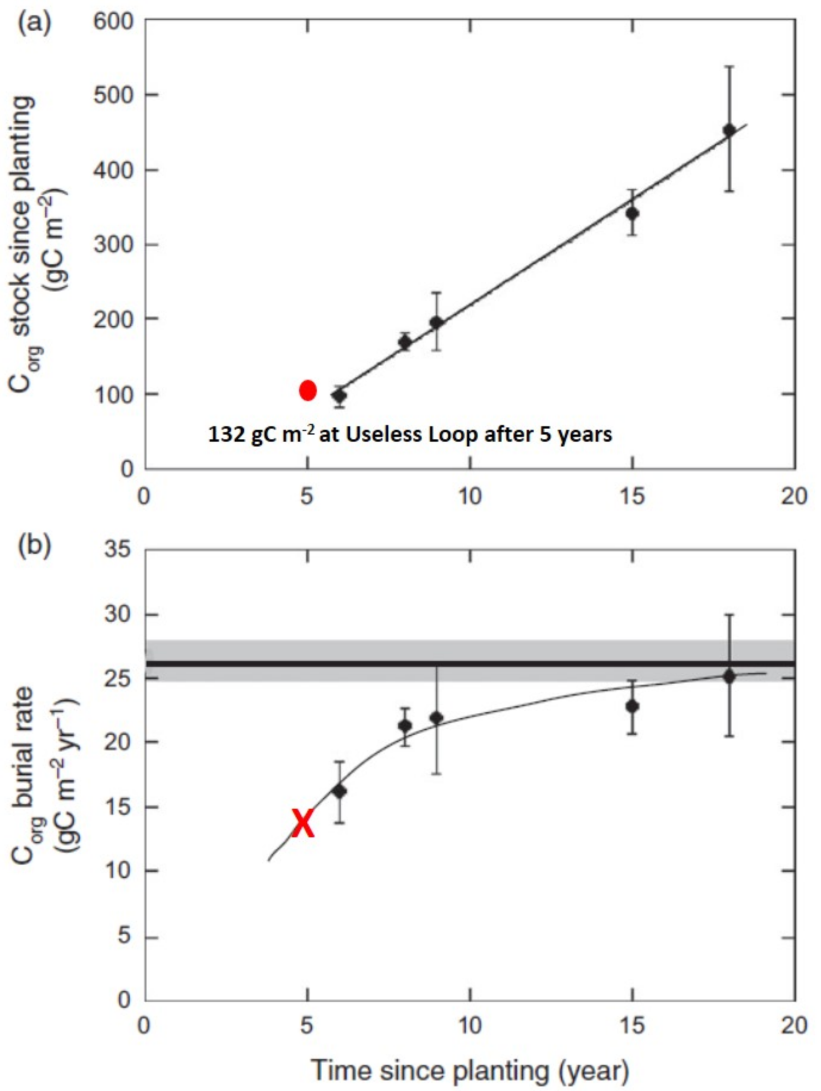

Fig. 5. Chronosequences of carbon stock accumulated (a) and carbon burial rate (b) across the revegetated plots at Oyster Harbour. The continuous line in panel A shows the fitted equation: $y = -64.02 + 28.19\ x$, $SE_{slope} = 1.36$; $R^2 = 0.99$, $P < 0.0005$. The continuous line in panel B shows the fitted equation: $y = 28.70 - (69.01/x)$; $SE_{slope} = 14.24$; $R^2 = 0.85$; $P < 0.05$. The horizontal line and grey area in panel B indicate, respectively, the average and 95% confidence limit of carbon burial rate in the continuously vegetated site. Error bars indicate the standard error.

Figure 29 (a) Comparison of carbon stock accumulated for restored *Posidonia australis* plots in Oyster Harbour, Albany (Black circles) versus Useless Loop, Shark Bay (Red circle); (b) Annual carbon burial rate in Oyster Harbour, Albany versus the predicted annual carbon burial rate after 5 years in Useless Loop, Shark Bay (Red X; Figure sourced from Marbà et al. 2015).

6.2 Animal abundance (not behaviour)

Seagrasses form the foundation of the Shark Bay ecosystem. Seagrass meadows are highly productive and provide important habitat for fauna including benthic invertebrates and fishes. Tracking the status and trends in seagrass cover and quality and biodiversity is a high priority for Shark Bay management.

In this program, we use a standardized set of measurements for characterizing the biodiversity of seagrass communities, specifically the community composition of invertebrate animals and fishes. We do this for seagrass communities at varying establishment stages, 8, 26, 30 and 60 months for *P. australis* and 8 and 60 months for *A. antarctica*. We compare these restoration communities with adjacent bare sand and established seagrass habitat.

6.2.1 Benthic invertebrate surveys

Benthic invertebrate assessments consisted of swimming three 5 m transect lines and looking 1 m either side of each transect line until we covered the entire 25 m^2 area of each restoration plot. Similarly, the same approach was undertaken within established meadows and in bare sand. Benthic invertebrates were broadly identified and were observed either on the surface of the sediment (Figure 30), on seagrass shoots/leaves or as evidence of presence of subsurface dwelling fauna (e.g. burrows of tubeworms).

Across all sites and both seagrass species we observed 13 different groups of invertebrates (See Appendix B). For *P. australis,* Dubaut Point and Useless Loop had the greatest number of invertebrates with a total of nine at each location. However, the greatest species richness was found at Useless Loop with seven species in any one site followed by Dubaut Point with six species.

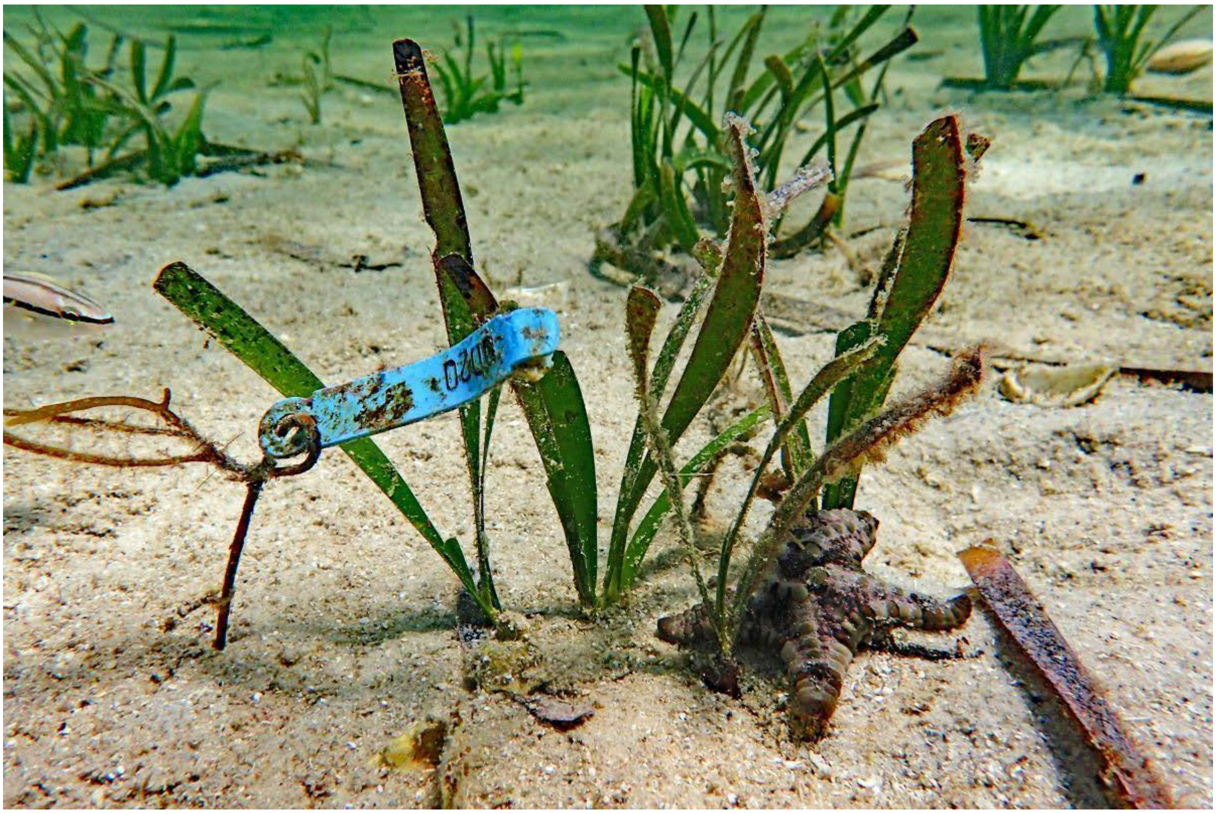

Figure 30 A sea-star forages amongst a 26 month old *Posidonia australis* transplant, with multiple transplants established in the background (Image: Rachel Austin).

At Dubaut Point we found a clear trend with restoration plot age and species diversity (richness by abundance, Figure 31a). Bare sand showed very low species diversity (almost zero), whereas at 8 months there were several invertebrates groups with a number of individuals observed within the *P. australis* plots. Diversity increased at 26 and 30 month old transplant plots with a slightly higher species diversity within established adjacent meadows. In contrast, we did not find a clear trend at Middle Bluff. With the exception of the 26 month old transplant plot, which had half the species diversity of Dubaut Point, we observed very low species diversity across different aged transplant plots as well as the established meadow. This site also tended to have poorer transplant growth across the different aged plots and the established meadows appeared in poorer health. We did not observe invertebrates within bare sand at this site.

At Useless Loop, we observed greater species diversity in *P. australis* within 60 month old plots compared to adjacent bare sand at 3, 5 and 7 m plots (Figure 31b). There was a trend towards lower species diversity from shallow to deeper water and this appeared to follow the same trend of reducing shoot density per m^2 with depth for *P. australis*. Invertebrate diversity within established meadow, measured at 3 m only, was greater than the 3 m plot but less than or equal to the 5 m and 7 m plots, respectively.

For *A. antarctica,* Useless Loop had the greatest number of invertebrate species, with a total of ten, Dubaut Point had five and Middle Bluff had two (See Appendix B). At Dubaut Point we found young restoration plots (8 months old) had greater species diversity than bare sand but less than adjacent established meadows (Figure 32a). In contrast, we found a total of two species at Middle Bluff with only ever one species found at bare sand, 8 month old plot and established meadows. Similar to *P. australis*, this site also tended to have poorer *A. antarctica* transplant growth across the different aged plots and the established meadows appeared in poorer health. We did not observe invertebrates within bare sand at this site.

At Useless Loop, we generally observed greater species diversity in *P. australis* 60 month old plots compared to adjacent bare sand at 3, 5 and 7 m plots (Figure 32b). The two deeper sites (5 m and 7 m) showed slightly higher species diversity than the shallow site, and like *P. australis*, this appeared to follow the same trend of lower species diversity with decreasing shoot density. Invertebrate diversity within established meadow, measured at 3 m only, was the greatest species diversity.

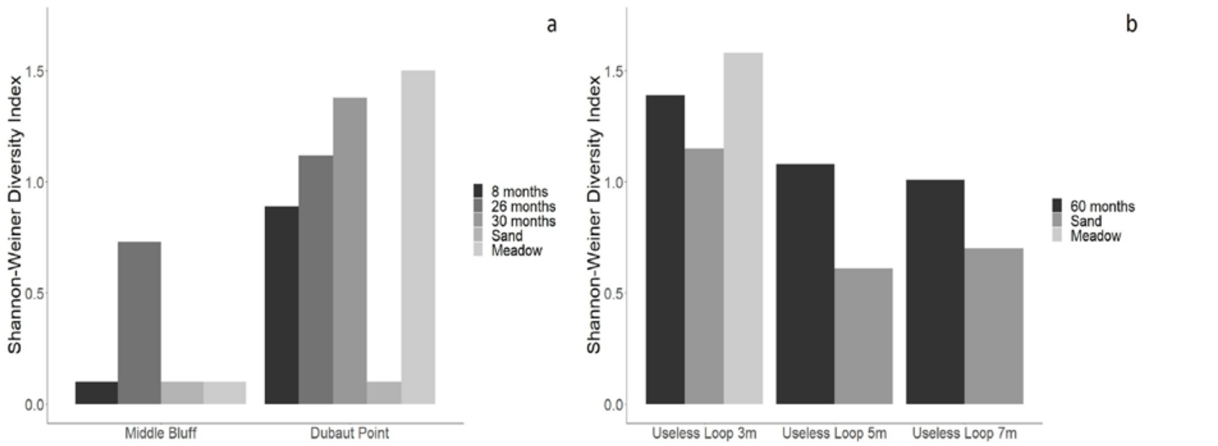

Figure 31 Invertebrate species diversity (Shannon-Wiener diversity) for (a) different aged (8, 26 and 30 months) transplant plots, bare sand and established meadows of *Posidonia australis* at Middle Bluff and Dubaut Point; and (b) for bare sand, *P. australis* meadow at 3 m depth and transplant plots at Useless Loop that are 60 months old and planted at 3, 5 and 7 m depth.

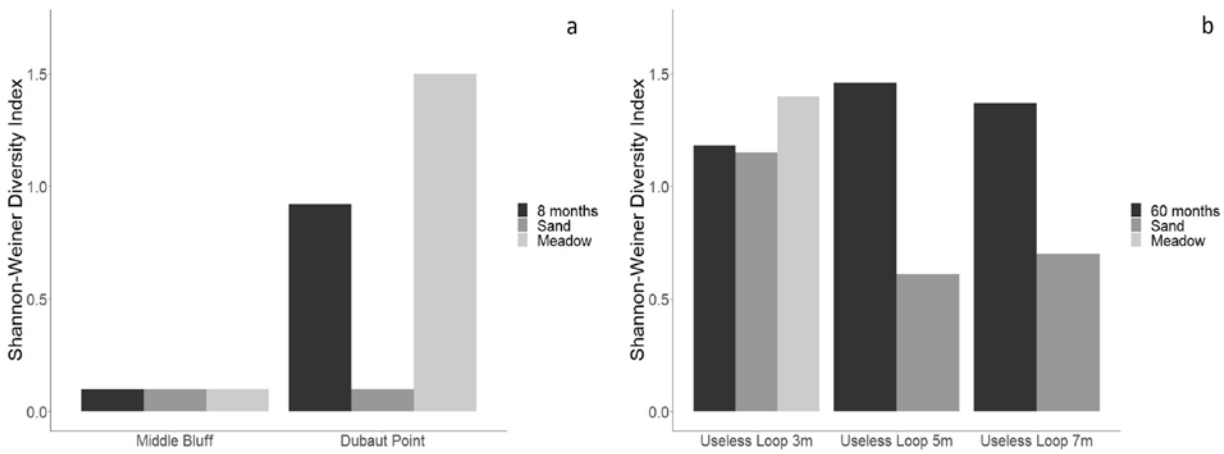

Figure 32 Invertebrate species diversity (Shannon-Wiener diversity) for (a) 8 month old transplant plots, bare sand and established meadows of *Amphibolis antarctica* at Middle Bluff and Dubaut Point; and (b) for bare sand, *Amphibolis antarctica* meadow at 3 m depth and transplant plots at Useless Loop that are 60 months old and planted at 3, 5 and 7 m depth.

6.2.2 Fish surveys

Fish assessments consisted of swimming three 5 m transect lines with a GoPro camera with a field of view 2 m wide. Three swims were conducted for each 25 m^2 plot to cover the entire plot which had four corner posts to mark the area. Similarly, the same approach was undertaken within established meadows and in bare sand. Fish were broadly identified by analysing the video imagery in the lab.

Across all sites and both seagrass species we observed a total of 8 different fish species (See Appendix B). For *P. australis* we observed a total of two species at each location; Dubaut Point, Middle Bluff and Useless Loop. Butterfish were one of the species that were observed at the transplant plots (Figure 33). Given the fish counts were so low we did not observe any clear trends at any site or aged transplant plot (Figure 34).

For *A. antarctica* we observed one species at Dubaut Point and Middle Bluff and only one individual of each resulting in a low species diversity at these locations (Figure 35a). The greatest number of species was observed at Useless Loop, with a count of seven. At Useless Loop, we observed greater species diversity in all 60 month old plots compared to adjacent bare sand and established meadows (Figure 35b).

Figure 33 Several butterfish exploring one large 30 month old *Posidonia australis* transplant (Image: Rachel Austin)

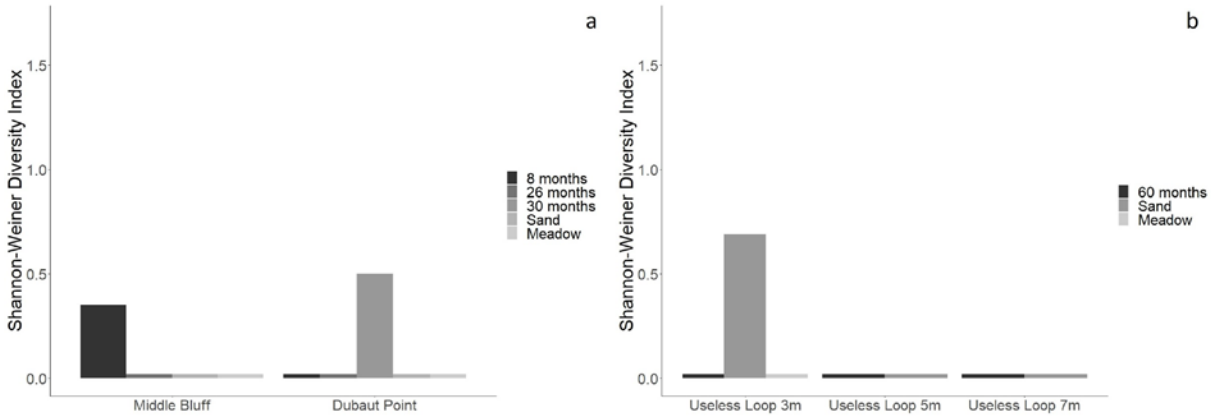

Figure 34 Fish diversity (Shannon-Wiener diversity) for *Posidonia australis* for (a) different aged (8, 26 and 30 months) transplant plots, bare sand and established meadows of *Posidonia australis* at Middle Bluff and Dubaut Point; and (b) for bare sand, *P. australis* meadow at 3 m depth and transplant plots at Useless Loop that are 60 months old and planted at 3, 5 and 7 m depth.

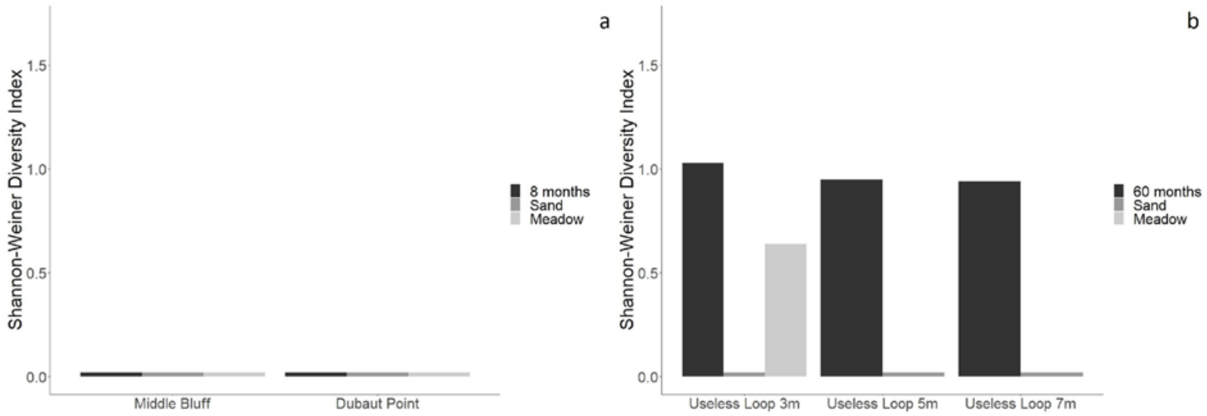

Figure 35 Fish diversity (Shannon-Wiener diversity) for *Amphibolis antarctica* for (a) 8 month old transplant plots, bare sand and established meadows of *Amphibolis antarctica* at Middle Bluff and Dubaut Point; and (b) for bare sand, *Amphibolis antarctica* meadow at 3 m depth and transplant plots at Useless Loop that are 60 months old and planted at 3, 5 and 7 m depth.

7. TOOLS FOR RESTORATION (MILESTONE 7)

Stakeholder participation in the environmental decision-making process has been increasingly sought and embedded into national and international policy. The complex and dynamic nature of environmental problems requires flexible and transparent decision-making that embraces a diversity of knowledge and values (Reed 2008).

Eight features of best practice participation are then identified from a Grounded Theory Analysis of the literature (Reed 2008). These features emphasise the need to replace a ‘tool-kit’ approach, which emphasises selecting the relevant tools for the job, with an approach that emphasises participation as a process. It is argued that stakeholder participation needs to be underpinned by a philosophy that emphasises empowerment, equity, trust and learning.

In taking this on board, Project E6 has endeavoured to include Malgana Rangers in every step of this project. Initial project ideas were conceived through early conversations on Country (in August 2018). We have shared knowledge of seagrass restoration methods (some outlined in a recent review by Tan et al. 2020 and Sinclair et al. 2021) with Malgana Rangers through a series of four training workshops. Methods have been trialled and adapted to environmental conditions, skills, and working in a remote location, such as Shark Bay. Our approach reflects continued participation and knowledge sharing has resulted in an approach developed with Malgana Rangers and Elders over > 2 years.

The framework below (Figure 36) has been adapted from Miller et al. (2017). There are five key areas to consider when planning restoration. Scientific knowledge is incorporated into on ground restoration activities. Through these key areas it identifies when additional scientific research is required to achieve successful, long-term restoration.

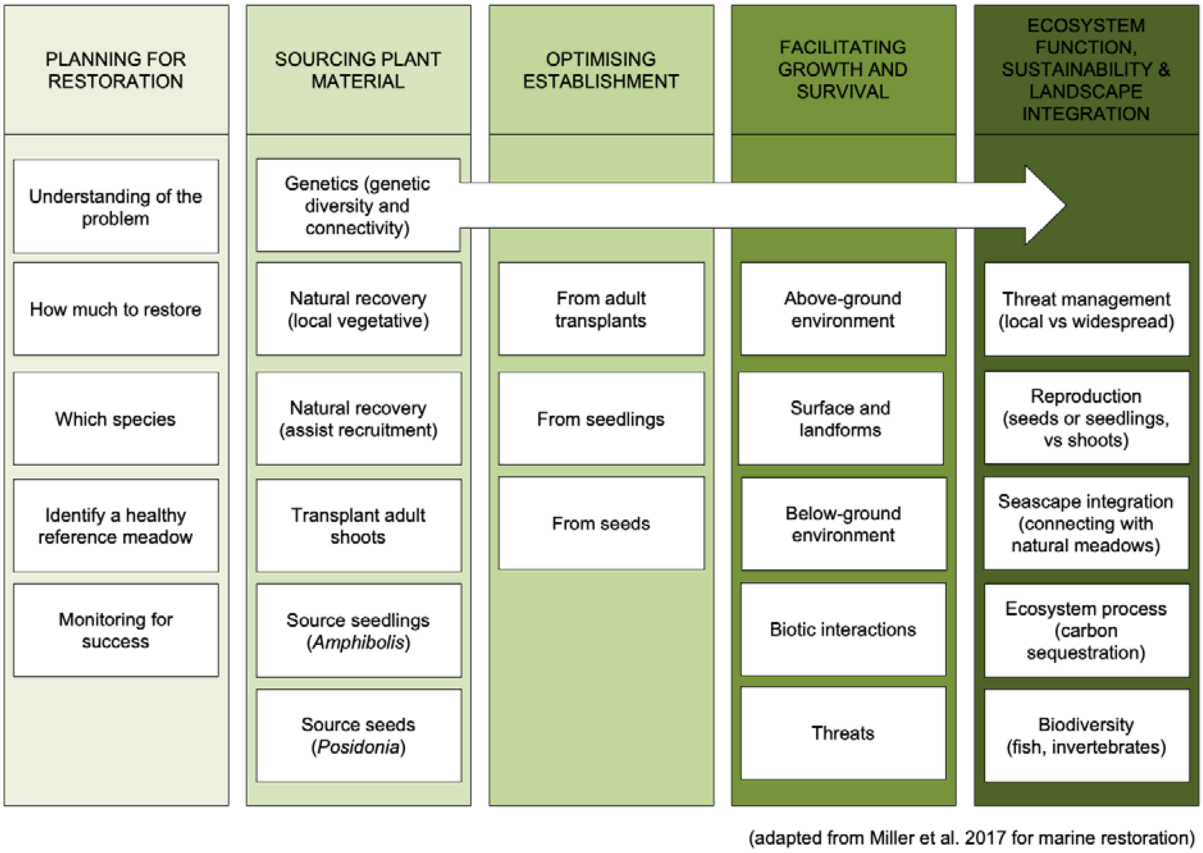

Figure 36 Framework as a practical guide to decision making for appropriate restoration activities. Adapted for marine restoration from Miller et al. 2017.

We work through this framework for restoration activities that were conducted during the NESP E6 project below (Table 9), but this approach can also be applied to other restoration activities. The Framework has been built under five broad themes; Planning for restoration, Sourcing plant material, Optimising establishment, Facilitating growth and survival, and Ecosystem function, sustainability and landscape integration. Each theme is a necessary part of restoration, keep in mind when planning and identify any research required

Table 9: The Restoration Framework applied to seagrass restoration in Shark Bay, through NESP 2 E6 and previous restoration activities. The themes are outlined in the framework shown in Figure 36, the key questions that should be asked to prepare a site for restoration. The Approach and Outcomes describe approaches taken to answer the questions and the outcomes from those approaches with the final column, Relevant references are cited where additional published information is available.

Steps	Questions	Outcome/Approach	Reference
1. Planning for restoration	Define the area of seagrass that has been impacted?	The marine heatwave in 2011 impacted the entire 13,500 km^2 Shark Bay ecosystem with 4,176 km^2 of seagrass-dominated banks and sills. 1,300 km^2 has been severely degraded or complete loss of seagrass, and requires restoration.	Kendrick et al. 2019; Strydom et al. 2020
	How much seagrass needs to be restored, and where is it?	Historical data sets have been catalogued for Shark Bay, mapping change in coverage. Priority areas can be identified (e.g. cultural significance, control erosion of sediment, fisheries habitat, bird feeding, tourism hotspots).	Strydom et al. 2020
	Which seagrass species have been most impacted?	Wire weed (*Amphibolis antarctica*) and ribbon weed (*Posidonia australis*)	Fraser et al. 2014; Thomson et al. 2016
	What was the cause of the loss and is the 'stress' still present?	Heat stress, turbidity and loss of light were the main cause of loss. There has been a return to average summer temperatures in the decade that has followed and there has been a clearing of turbidity.	Fraser et al. 2014; Thomson et al. 2016
	Is there a nearby reference meadow (non-impacted, healthy) for comparison (monitoring restoration success)	Reference meadow(s) should be located nearby, the same species and at a similar depth. Reference meadow percent cover should be 75% or greater.	National standards for ecosystem restoration: https://www.seraustralasia.com/standards/principle1.html
	Is there an alternative strategy for assisting recovery and restoration? Could different species or intermediate steps with alternative species assist recovery?	Warming waters may mean large temperate seagrasses (wire weed & ribbon weed) are replaced with tropical species (*Cymodocea spp*., or *Halodule sp.*) However, there are still large areas (300,000 hectares) of wire weed and ribbon weed seagrass that survived and persisted post-2011 marine heatwave. Natural	Hyndes et al. 2016; this report - Figure 20

Steps	Questions	Outcome/Approach	Reference
		recruitment of other species will occur.	
	What local resources are available to undertake restoration (people, skills, equipment, financial support)?	The Malgana Land and Sea Rangers have undertaken basic training on how to restore wire weed and ribbon weed using a range of suitable techniques. Local resources (marine vessels; vehicles; tools) are available and skills are developing or are well-developed. Additional SCUBA diving labour or skills developed and operating costs need to be sourced to undertake the assisted recovery/restoration work at the scale that is necessary.	this report - Section 4 Training workshops
	Plan for monitoring success	Restoration monitoring requires regular assessment, at least every 3-6 months initially. Metrics include individual plant survival, shoot counts, shoot growth and comparison with nearby reference meadow.	Tan et al. 2020
2. Sourcing plant material	Is there any information on genetic (or genomic) diversity and connectivity?	Yes, for ribbon weed and wire weed	Sinclair et al. 2016; Sinclair et al. 2020; this report - Section 3 Genomic diversity and connectivity
	Is there local healthy plant material available or do you have to collect elsewhere?	Yes for ribbon weed and wire weed. We do not yet know the long term implications of moving plants between meadows for restoration. A reciprocal transplant experiment has been established for *P. australis*. Results are currently being assessed for survival, growth rates and gene expression stress responses. We recommend that other experiments could be built into restoration activities. Records should be kept on where plants are sourced from. *Posidonia* and *Amphibolis* are long lived plants so it will take time to answer this question.	this report – Section 3

Steps	Questions	Outcome/Approach	Reference
	What type of the plant should be used (adult shoots, seedlings, seed)?	Seed availability is highly variable for *Posidonia* but adult shoots are abundant and successful propagation units. *Amphibolis* seedlings and adult shoots are common and also successful.	this report - Section 5
	How should genetically diverse source material be collected?	Adult shoot transplants - minimum 2 m apart to collect different plants.	this report Sections 3, 4, 5
3. Optimising establishment	What restoration methods are available?	There are multiple seagrass restoration methods available in the literature.	this report - Section 5; Tan et al. 2020 for review of methods; Sinclair et al. 2021 for field examples
	What is the most successful method?	The two most successful methods tested so far in Shark Bay include (i) harvesting, processing and replanting adult shoots of wire weed and ribbon weed; (ii) deployment of sand-filled, biodegradable Hessian tubes to facilitate the natural recruitment of wire weed seedlings.	this report - Section 5
	Are any additional treatments that could improve plant establishment?	The *timing of hessian tube deployment (just prior to seedling release) and orientation (perpendicular to current flow) should be considered to enhance wire weed seedling attachment to the hessian tubes.	*No data are available on the exact timing of wire weed seedling release. It would be useful to report when floating seedlings are observed to improve the success of future restoration activities.
4. Facilitating growth and survival	What needs to be monitored?	The first two years are critical for seagrass establishment. Monitoring during this time should include individual transplant survival, shoot counts (compare number planted with versus how many at each monitoring period), percent coverage of the restoration area/site and growth rates. This information is critical to projecting the success of the restoration and whether supplemental planting or infilling needs to	Bastyan & Cambridge 2008; this report - Section 5

Steps	Questions	Outcome/Approach	Reference
		occur. After 3 years we would expect that identifying individual transplants would be difficult (due to overlapping growth of rhizomes and shoots) so monitoring shoot density, changes in shoot loss/production (demographics), percent coverage and growth rates of a representative number of shoots (e.g. 20 per site) would be a more appropriate monitoring strategy to determine the longer term success of the restoration. A direct comparison of these parameters with existing, natural (nearby) meadows is essential. Finally, determine if there are differences in survival and growth of plants sourced from different locations.	
	Is there a need for additional planting or infilling?	The high survivorship we found in this study and previous restoration research within Shark Bay suggests not, although there may be site specific differences. If losses are high and consistent despite additional planting, choosing an alternative site could be a more productive approach until the causes of loss can be determined.	
	How long does monitoring need to continue?	Monitoring should match the timeframe that a seagrass should become self-sustaining (sustained positive growth rate, resembling a nearby reference meadow, and/or become reproductively mature). This will be beyond 5 years possibly a decade (10 years). Monitoring of restored areas should be included with routine monitoring in the bay once meadows demonstrate 75% coverage or shoot density of nearby existing meadows.	Bastyan & Cambridge 2008; Statton et al. 2012; van Katwijk et al. 2016
	Are there negative interactions with other species?	Herbivory (fish, dugongs, turtles) are known to eat seagrass, bioturbation (of the sand) by marine worms, sand dollars,	Statton et al. 2015; Bell et al. 2019

Steps	Questions	Outcome/Approach	Reference
		urchins may uproot transplants, predation of (*Posidonia*) seeds by crabs and fish may all impact success of a restoration site.	
5. Ecosystem function, resilience, and landscape integration	Are fish and invertebrates returning?	Invertebrates and fish can be observed in, and nearby restoration plots, and this may differ from bare sand areas. However, how much of this return is movement from existing meadows nearby is still unknown. Detailed studies and longer term assessments will be required to determine if faunal abundance and diversity within restored sites are similar to natural (reference) meadows. Recovery of biodiversity in restored wire weed may be quicker than for ribbon weed	Historical data sets have been catalogued for Shark Bay for major species of air-breathing megafauna and sharks (Nowicki et al. 2019), seagrass-dependant biota (Caputi et al. 2016; D'Anastasi et al. 2016; this report Section 6.2 McSkimming et al. 2016
	Is the water quality improving (reduced sediment resuspension)?	Yes	
	How long should the monitoring continue?	Restoration plots could take greater than 5 years to show some semblance of shoot density or coverage to an existing nearby meadow. This will have an influence on the associated infaunal abundance and diversity and carbon burial rate. Therefore, there is a strong case for longer term monitoring. Monitoring should continue beyond 5 years and possibly a decade. Restored sites should be compared with a natural (reference) meadow.	Bastyan & Cambridge 2008; Marbà et al. 2015
	Is carbon capture returning?	We have not shown that to be the case after 30 months nor 5 years. However, long term data sets for other restored ribbon weed restoration plots in Albany suggest that 7-10 years is required before meadows begin to show comparable carbon burial rates to an existing meadow.	Marbà et al. 2015 this report - Section 6.1; Data sets have been catalogued for Shark Bay carbon storage and loss (Arias-Ortiz et al. 2020; Fourqurean et al 2012), but not direct measurement of burial rates.

7.1 Knowledge sharing

Developing connections between western scientists and Traditional Owners requires spending time on Country. We are developing relationships to enable long term partnerships with the Malgana People and their Ranger Program. This has been a particularly rewarding part of this NESP-funded collaboration, however, not one which is readily captured by traditional 'milestones'. We have included a collection of outputs resulting from this project (Appendix C). This includes traditional academic writing, popular articles, and other opportunities for sharing information about seagrasses and related research with the broader community. The joint organisation of the Wirriya Jalyanu (seagrass) Festival will provide an opportunity to discuss results from Workshop #4 and ensure the restoration framework is useful.

8. NEXT STEPS

We outline some key elements which will assist with a way forward through ongoing two-way learning and seagrass ecosystem recovery. There will be more marine heat wave events that impact the health of the Shark Bay ecosystem in the future, in fact current conditions are very similar to 2010/11, with warming waters and La Niña rainfall events creating flood plumes from the Wooramel and Gascoyne Rivers. Establishing a plan for monitoring impacts as they unfold and assisted recovery is critical for the long-term health and this globally recognised World Heritage Site.

8.1 Scaling up restoration

Restoring seagrass meadows at appropriate scales will be challenging in Shark Bay (Shark Bay) given the scale of loss of seagrasses from a single marine heat wave in 2011 (approx. 1,300 km^2: Strydom et al. 2020). The frequency and intensity of such events is predicted to increase, so the time intervals between events are important to allow sufficient recovery. For example, shoot density in *Posidonia* meadows took approximately six years to recover to pre-heat wave levels (Kendrick et al. 2019).

We have shown that a combination of restoration methods including vegetative shoot transplants, engineering sediment stabilization to enhance seedling establishment in *Amphibolis* and opportunistic use of *Posidonia* seagrass seeds is an appropriate mix of local and regional scale approaches for the two large temperate species. We have demonstrated that with the right resourcing and logistic support, there are opportunities to fund both training and broad scale restoration by working closely with the traditional custodians of the land.

8.2 Planning for restoration

We have started to work with the NESP phase 1 “Earth Systems and Climate Change Hub” to develop a Malgana seasonal calendar. We envision the calendar would incorporate key seagrass life history attributes that are relevant for planning restoration activities. For example, understanding the timing of seedling release for *A. antarctica* will maximise effectiveness of seagrass hessian tubes. Seagrass hessian tubes should be deployed before the seedlings start dispersing. This has been discussed with Nick Pedrocchi (Malgana Director and Ranger) and flagged as an activity for the future.

8.3 Supporting an on-going role for Malgana Rangers

The Malgana Ranger Land and Sea Management program is fully supported by the Malgana Aboriginal Corporation. They are very proud of their Rangers and keen for seagrass research to continue. We are working to enable Ranger-lead seagrass restoration and monitoring activities to continue into the future. These activities will be impacted by whether permanent roles and funding support for Ranger positions can be secured.

9. ACKNOWLEDGEMENTS

We thank the Traditional Owners and custodians of Shark Bay, the Malgana People, for permission to conduct research on Sea Country. Permission was received from the Malgana Aboriginal Corporation to remove seagrass samples for research purposes in March 2018.

Sincere thanks go to our large field team and many volunteers: Rachel Austin, Martin Breed, Mitchell Booth, Matthew Fraser, Belinda Martin, Josh Revell, Mike Taylor, skippers - Kate Dawson, Corey Morgan, and John Statton, and indigenous liaison officer Amrit Kendrick; Andrea Zavala Perez, Sam Gustin-Craig for 2012 *Posidonia* collections at Guischenault and Monkey Mia; Simone Strydom and Kathy Murray (DBCA) for assisting with identification of suitable sampling locations for the genomic studies. All collection activities were performed under DBCA Regulation 4 permits (CE003942, CE005704). All restoration activities were performed under DBCA Regulation 4 permits (CE006109).

We thank the Batley Laboratory at UWA for access to equipment, training for student researchers, and assistance with bioinformatics data handling and analyses, in particular, Anita Severn-Ellis and Dr Philipp Bayer; Yusmiana Puspitaningsih Rahayu for processing the carbon cores.

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11. APPENDIX A – SHARING MALGANA STORIES AND WORDS

'Many indigenous peoples have been separated from their country, culture and language over time due to the lack of understanding of indigenous culture and lore since colonisation. However, indigenous ranger programs are enabling them to rediscover their identity.'

Nick Pedrocchi, Malgana Ranger and Director, April 2020

11.1 When Sea Country was Land

Malgana peoples are the Traditional Owners of Shark Bay, which they have inhabited for approximately 30,000 years. They are saltwater people, living alongside seagrasses (12 species, wirriya jalanyu), the rich habitat that provides food for dugongs (*Dugong dugon*; wuthuga), green and loggerhead turtles (*Chelonia mydas* and *Caretta caretta*; buyungurra), sharks (28 species, thaaka), and many more. Stories from Indigenous groups around Australia represent genuine and unique observations of the post-glacial rise in sea level, at time scales that range from about 13,070–7,250 years BP (Nunn & Reid 2016). Rising sea levels from the end of the Last Glacial Maximum mean that much of Malgana Country is now drowned, with cultural heritage preserved under the extensive seagrass meadows that thrive in the shallow waters. Stories have been shared by Malgana Rangers about their ancestors walking from Denham to Wirruwana (Dirk Hartog Island), and Bernier and Dorre Islands across the sandy bottom or at low tides, and knowing where the freshwater seeps were located. The implications of this extraordinary longevity of oral traditions demonstrate effective transgenerational communication in aspects of Aboriginal culture, is also present within the Malgana. Much of this submerged heritage is yet to be explored (as per Veth et al. 2019).

We are sharing language and stories as a means to improve communication and understanding between Malgana Peoples and recent immigrants. These next two stories were told by Auntie Topsy Cross, one of the last Malgana speakers, to a young friend who recounted them as best he could. They were reproduced with permission of the Malgana Working Group in Tindale (1966). These stories have been passed down through family groups.

11.2 The Thorny Devil

Well, before the saltwater in Shark Bay was salt water, it used to be freshwater. All the different animals used to drink the water, they all used to share and know how much of the water to drink, how much to take.

A long time ago the Thorny Devil (he never used to have thorns on him then), he used to drink from the water, too, the Mountain Devil.

The Mountain Devil was very greedy and he drank up nearly all of the fresh water and all the other animals told him off, and then told him he was really, really bad; what he did was really *greedy. He got very angry with the rest of the animals, and urinated. You must understand – those animals were different to what they are now, sort of spiritual, something like that. And he urinated on the fresh water, and that's how it became salt water. And after that he had some form of thorns.*

And now the Thorny Devil, he doesn't drink. You never see him go to a water hole and drink, or anything like that. He has a kind of pouch on his neck or on his back, well he takes in water from the environment and doesn't actually go to drink.

When you see that, you know that that's a reminder that if you get too greedy you won't be able to drink. And another thing is that the Thorny Devil has thorns on him which also reminds us of the story. And you're reminded now when you see the salt water not to be greedy and to share the things that live in there.

This story also reminds us that Shark Bay has only become Sea Country since rising sea levels started inundating the Bay at the end of the Last Glacial Maximum.

….the presence of freshwater seeps on Tamala Station explain why the salinity levels are much closer to normal seawater at the seagrass sampling site of White Islet, southwest of Nanga.

11.3 Buyungurra

The turtle, buyungurra, well he used to live on the land a long time ago. And how it got from being on the land to being in the sea was because it was chasing these particular berries. These were some berries or seeds washing out to the ocean. The turtle was eating these things and all the other animals said to it not to keep chasing the berries because it would end up in the ocean forever, in the deep forever. But that turtle, it kept ignoring, kept ignoring, kept ignoring, and not it's in the ocean forever.

And that's why the shape of this particular kind of berry looks a little bit similar to the turtle shell. So when people see the shell, they say that's they berry that came up, you see, and that's what happened.

…so was this berry a floating Posidonia fruit?

11.4 Malgana words

Yandani Gathaaguduni	Welcome to Shark Bay
Nyinda wula wujanu, nyinda yajala	You come a stranger, you leave a friend

Locations:

Duthuduguda	Broadhurst Bight
Thaamarli	Tamala Station
Wilyamaya	Tip of Heirisson Prong
Wirruwana	Dirk Hartog Island
Wulyibidi	Peron Peninsula
Muga	Middle Bluff

The environment:

baba	rain
barraja	land
birrida	salt pan
boolagooda	stromatolites
buyungurra	turtle (green *Chelonia mydas* and loggerhead *Caretta caretta*)
buthurru	sand
djiljit	fish
gurab	crab
irrabuga	bottlenose dolphin (*Tursiops aduncus*)
jurruna	pelican (*Pelecanus conspicillatus*)
mardirra	pink snapper (*Chrysophrys auratus*)
muga	deep water
mulgarda	mullet (*Mugil spp*.)
mulhagarda	whiting (*Sillago schomburgkii*)
thaaka	shark (28 species; tiger, *Galeocerdo cuvier*)
thalganjangu	tidal pool, lagoon
wabagu	sea eagle (*Haliaeetus leucogaster*)
wanamalu	cormorant or shag (4 species)
warda	pearl
wilya	shell
wilyaa	seagull (2 species)
wilyara	pearl shell (*Pinctada spp*.)
winthu	wind
wirriya	sea, salt water
wirriya jalyanu	seagrass (12-13 species)
wuthuga	dugong (*Dugong dugon*)

12. APPENDIX B – SUPPLEMENTARY RESULTS

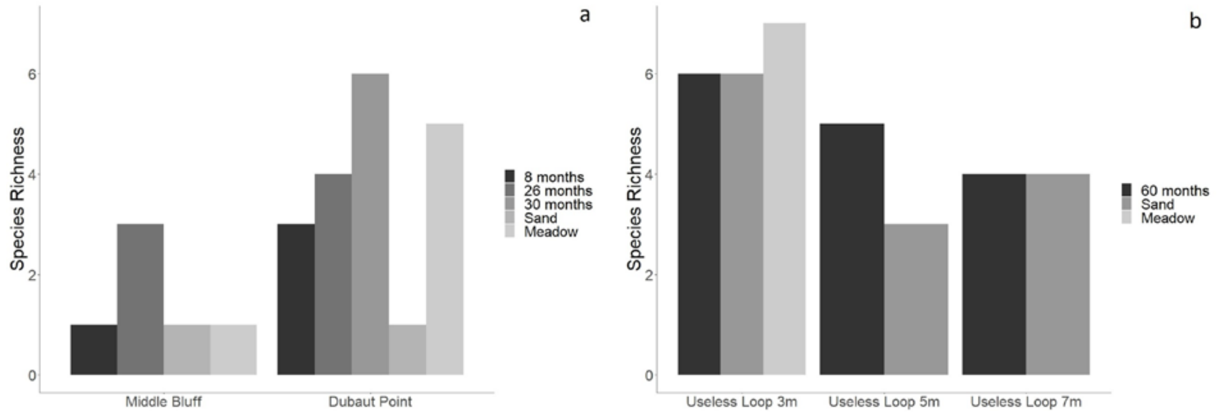

Figure S1 Invertebrate species richness *Posidonia australis* for (a) different aged (8, 26 and 30 months) transplant plots, bare sand and established meadows of *Posidonia australis* at Middle Bluff and Dubaut Point; and (b) for bare sand, P. australis meadow at 3 m depth and transplant plots at Useless Loop that are 60 months old and planted at 3, 5 and 7 m depth.

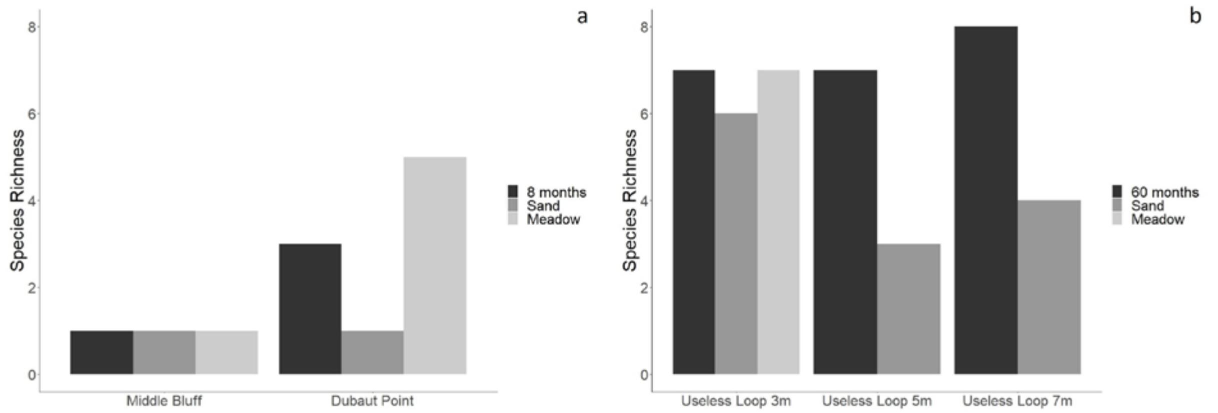

Figure S2 Invertebrate species richness *Amphibolis antarctica* for (a) 8 month old transplant plots, bare sand and established meadows of *Amphibolis antarctica* at Middle Bluff and Dubaut Point; and (b) for bare sand, *Amphibolis antarctica* meadow at 3 m depth and transplant plots at Useless Loop that are 60 months old and planted at 3, 5 and 7 m depth.

Table S1: Presence/absence of invertebrates in *Posidonia australis* at Dubaut Point, Middle Bluff and Useless Loop restoration sites, adjacent sand and meadows.

Invertebrate	Dubaut Point	Middle Bluff	Useless Loop
Gastropod snail	Y	Y	Y
Hermit crab	Y	N	Y
Mussel	Y	N	Y
Tube worm	Y	Y	Y
Clam	Y	N	Y
Scallop	Y	N	N
Seastar	Y	N	Y
Ascidian	Y	N	N
Pinna	Y	N	Y
Bivalve (other)	N	Y	N
Oyster	N	N	Y
Sea urchin	N	N	Y

Table S2: Presence/absence of invertebrates in *Amphibolis antarctica* at Dubaut Point, Middle Bluff and Useless Loop restoration sites, adjacent sand and meadows.

Invertebrate	Middle Bluff	Dubaut Point	Useless Loop
Gastropod snail	Y	Y	Y
Hermit crab	N	Y	Y
Mussel	N	Y	Y
Tube worm	Y	N	Y
Clam	N	N	Y
Seastar	N	N	Y
Ascidian	N	Y	N
Pinna	N	Y	Y
Oyster	N	N	Y
Sea urchin	N	N	Y
Decorator crab	N	N	Y

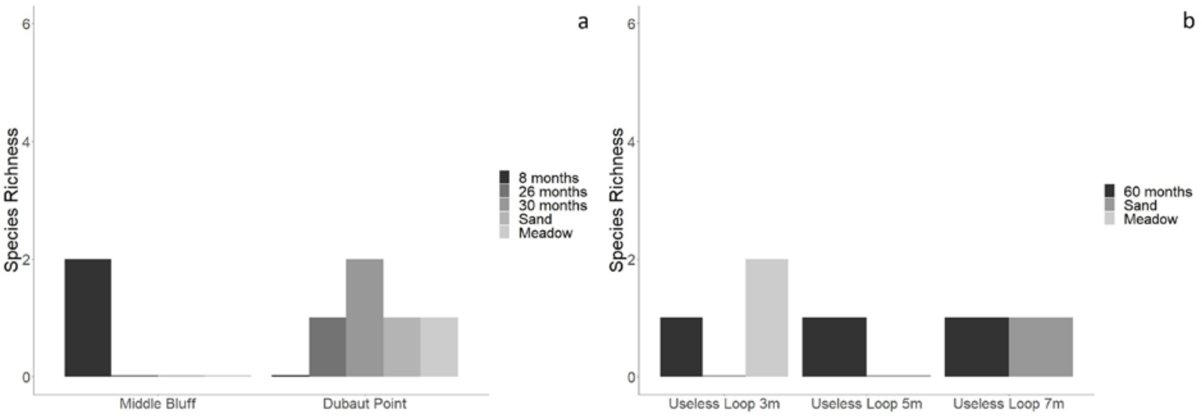

Figure S3 Fish richness for *Posidonia australis* for (a) different aged (8, 26 and 30 months) transplant plots, bare sand and established meadows of *Posidonia australis* at Middle Bluff and Dubaut Point; and (b) for bare sand, P. australis meadow at 3 m depth and transplant plots at Useless Loop that are 60 months old and planted at 3, 5 and 7 m depth.

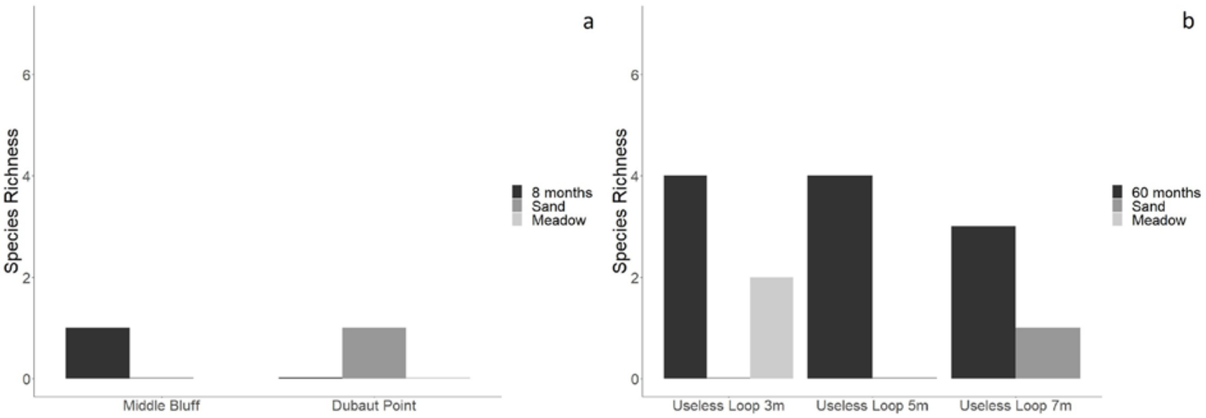

Figure S4: Fish richness for *Amphibolis antarctica* for (a) 8 month old transplant plots, bare sand and established meadows of *Amphibolis antarctica* at Middle Bluff and Dubaut Point; and (b) for bare sand, *Amphibolis antarctica* meadow at 3 m depth and transplant plots at Useless Loop that are 60 months old and planted at 3, 5 and 7 m depth.

Table S3 Presence/absence of Fish in *Posidonia australis* at Dubaut Point, Middle Bluff and Useless Loop restoration sites, adjacent sand and meadows.

Fish	Middle Bluff	Dubaut Point	Useless Loop
Butterfish	Y	Y	Y
Blennie	Y	Y	N
Brownfield wrasse	N	N	Y

Table S4 Presence/absence of fish in *Amphibolis antarctica* at Dubaut Point, Middle Bluff and Useless Loop restoration sites, adjacent sand and meadows.

Fish	Middle Bluff	Dubaut Point	Useless Loop
Butterfish	Y	Y	Y
Brownfield wrasse	N	N	Y
Yellowfin bream	N	N	Y
Fanbellied leatherjacket	N	N	Y
Tuskfish	N	N	Y
Bream	N	N	Y
Gobbleguts	N	N	Y

13. APPENDIX C – PROJECT OUTPUTS

13.1 Publications

Peer-reviewed publications

Sinclair EA, Sherman CDH, Statton J, Copeland C, Matthews A, Waycott M, van Dijk K-J, Vergés A, Kajlich L, McLeod IM, Kendrick GA (2021) Advances in approaches to seagrass restoration in Australia. *Ecological Management and Restoration* 22: 10-21.

We are in the process of developing several manuscripts for academic journals. Two are currently being led by student researchers and a third one has been accepted based on an abstract. Approximate author lines and titles:

Sinclair EA, Statton J, Austin R, Krauss SL, Pedrocchi N, Oakley P, McNeair S, Breed MF, Kendrick GA (in preparation) Restoration in extreme marine environments: a case study from Shark Bay (Gathaagudu). Abstract accepted for *Joint Special Feature on the Decade of Ecosystem Restoration*' in *Journal of Applied Ecology*

Edgeloe JM, Severn-Ellis AA, Bayer PE, Mehravi S, Krauss SL, Breed MF, Kendrick GA, Batley J, Sinclair EA (in preparation) Population genomic structure among Shark Bays' extensive *Posidonia* meadows. For submission to *Proceedings of the Royal Society London B*

Frouws A-M, Severn-Ellis A, Kendrick GA, Batley J, Lavery PS, McMahon KM, Sinclair EA (in preparation) Population genomic diversity in wire weed *Amphibolis antarctica* meadows across Shark Bay.

Student theses

Edgeloe, Jane M (2019-2020) '*Population genomics in Posidonia australis: assessment of adaptive variation across a natural environmental gradient in Shark Bay*'. Masters Degree in Marine Science, The University of Western Australia.

Supervisors: Dr Elizabeth Sinclair, Prof Jacqui Batley, Dr Janet Anthony

Jane was a finalist and Runner-up in the Australian Marine Science Association WA student prize night.

Frouws, Anna-Maria (Ankje) (2018-) '*Spatial and temporal patterns in genetic diversity in seagrass meadows and the implications of these patterns for resilience*'. Edith Cowan University. Supervisors: Dr Kathryn McMahon, Prof Paul Lavery, Dr Elizabeth Sinclair

Popular articles

Sinclair EA, Oxenham TJ, Lewandrowski W (2021) Seagrass science inspires Malgana Artist. For People and Plants Winter 114: 24-26

Workshopping seagrass (Wirriya Jalyanu) restoration in Shark Bay (Gathaagudu). An illustrated story of seagrass restoration for NAIDOC week, 6 November 2020 https://www.nespmarine.edu.au/news/workshopping-seagrass-wirriya-jalyanu-restoration-shark-bay-gathaagudu

Sinclair EA, Kendrick GA, Kendrick A (2020) Working together to assist seagrass recovery at Shark Bay. *Wetlands Australia* 32 February 2020. http://www.environment.gov.au/water/wetlands/publications/wetlands-australia/national-wetlands-update-february-2020/shark-bay

Report

Statton J, Sinclair EA, Kendrick A, McNeair S, Kendrick GA (2020) Assisting restoration of ecosystem engineers through seed-based and shoot-based programs in the Shark Bay World Heritage Site (WHS). Report on Milestone 2, 3 & 4 to the National Environmental Science Program, Marine Biodiversity Hub.

13.2 Published materials for the local community

Wirriya Jalyanu seagrass Festival – What did you learn? Inscription Post May 2021 https://www.sharkbaycrc.net.au/services1

A series of illustrated fun facts about seagrass are being published in the monthly Shark Bay Inscription Post, published by the Shark Bay Community Resource Centre, Denham. These are running from November 2020 until April 2021. This is being done to increase local knowledge about seagrass and generate some interest around the Wirriya Jalyanu (seagrass) Festival which was rescheduled for 7-8 April 2021.

Fact sheet: Seagrass (wirriya jalyanu): giving life to saltwater country of Shark Bay (Gathaagudu) March 2021

Media interviews

Dr Elizabeth Sinclair (UWA Researcher) and Nick Pedrocchi (Malgana Director and Ranger) spoke with ABC Pilbara's Susan Standen about seagrass restoration on Gathaagudu, 11 November 2020

Dr Elizabeth Sinclair (UWA Researcher) spoke with ABC Pilbara's Kelly Gudgeon regarding the Wirriya Jalyanu (seagrass) Festival, 10 Sept 2020

Anthony James from Clean State podcast series features Professor Gary Kendrick talking about *Blue carbon, conservation economies and the great seagrass restoration.* It can be heard here: https://www.cleanstate.org.au/podcast 22 September 2020.

Goolarri Media, an indigenous media group from Broome, Western Australia, made a short documentary about the Malgana Rangers for a new display at the Western Australian Museum. Interviews were conducted with Malgana Aboriginal Corporation Board chair, Bianca McNeair, UWA researchers and Malgana Rangers, as well filming of restoration activities during our last training workshop in August 2020 at Dubaut Point and Denham. A short film now resides in Boola Bardip Western Australian Museum.

Presentations

Sinclair EA (9 March 2021) was invited to give a research presentation to Malgana Aboriginal Corporation Board, Malgana Rangers and Elders at the NESP Climate Change Hub workshop. Online presentation.

Sinclair EA (October 2019) Project update. NESP Marine Hub workshop, Hobart

Sinclair EA (21 August 2020) was an invited guest speaker at the School of Isolated and Distance Education to talk about seagrass research and restoration for National Science week.

13.3 Other opportunities and activities

Sinclair EA (2020) was awarded a SCITECH – Inspiring Australia grant to host 'A Pinch of Science with a view' $2350. The evening will feature three speakers – Prof. Gary Kendrick, Malgana woman Bianca McNeair and Dr Ana Sequeira. The event was rescheduled along the Wirriya Jalyanu (seagrass) Festival until 7 April 2021.

UWA researchers, Malgana Elders and Rangers and Northern Agricultural Catchments Council (NACC) employees were treated to a cruise on the Aristocat-2 skippered by Nick Pedrocchi (Malgana Ranger and Director). This provided an opportunity to meet and share stories with Malgana people and observe seagrass meadows (wirriya jalyanu) and dugongs (wuthuga) on the outer banks, off-shore from Monkey Mia.

Dr Elizabeth Sinclair was an invited speaker at the Hamelin Station Science Fair: 'Managing seagrass families in Shark Bay', 11 August 2019.

Ms Ankje Frouws was a finalist (and Runner up) in the Asia-Pacific 3MT competition. The talk can be heard here: https://vimeo.com/showcase/7624763/video/464056807

Professor Gary Kendrick presented Malgana Elder Bobby Hoult and Benny Bellottie '*A Snapshot of 70 Years of Marine Research in Shark Bay: Ecological, Social and Economic'.* This book was compiled by Drs Jenny Shaw and Alicia Sutton, Western Australian Marine Science Institute. https://www.wamsi.org.au/news/70-years-marine-research-shark-bay-ecological-social-and-economic

Laboratory skills training for Malgana Artist and undergraduate student

Tiahna Oxenham (Malgana Artist and environmental science student at Murdoch University) is visiting Kings Park Science to work with Drs Elizabeth Sinclair and Wolfgang Lewandrowski. Tiahna developed tissue sectioning and microscope skills to generate cell images from seagrass leaves collected in Shark Bay (*Posidonia, Amphibolis, Halodule spp.*) (Figure S5).

These sections were used as the basis for art works. The art will be displayed at the Wirriya Jalyanu (seagrass) Festival in April 2021.

Figure S5 Wolfgang Lewandrowski and Tiahna Oxenham looking at a fresh leaf section image from *Amphibolis*.

Wirriya Jalyanu (seagrass) Festival, 7-8 April 2021

Organisers have conducted regular meetings via zoom throughout the second half of 2020 and into 2021. This has created an opportunity to share, learn cultural practice, and work together on the Art meets Science Festival. On-going weekly to monthly meetings via zoom were used to overcome COVID-19 travel bans and reduce time/expenses associated with long distance travel. The meetings, convened by our Indigenous liaison person, Amrit Kendrick, have involved researchers, Malgana Rangers - Pat Oakley, Nick Pedrocchi, Malgana woman Bianca McNeair, Bush Heritage staff – Michelle Judd and Lis Mclellan, Jade Pervan (UWA archaeologist and Malgana cultural educator), and Sabrina Dowling Giudici (transcultural artist from Aartworks). The Festival is composed of a range of activities, from science talks on the Wednesday evening to a Thursday full of celebrity chef cooking demonstrations, dance performance, seagrass ecology and restoration experts in conversation with the public.

Shark Bay Arts Council seagrass workshop, March 2021

Twenty local artists are creating a collaborative glass-mosaic artwork for the Wirriya Jalyanu (Seagrass) Festival for April 8 in Denham, WA. This joint initiative of Sabrina Dowling Giudici (Aartworks, https://www.aartworks.org) and Shayne Thomson (Future Glass) is being auspiced and led by the Shark Bay Arts Council. The artwork features three prominent Shark Bay seagrasses, *Posidonia*, *Halophila*, and *Amphibolis*. The patchwork of glass mosaic panels is being individually crafted in group workshops in Denham, Carnarvon, and Perth (Figure S6). The Carnarvon workshop included established Malgana artists Barry Bellotti and Carleen Ryder. One of the welcome surprises was the inter-generational sharing of Shark Bay family stories. Sharing the Seagrass Factsheet was a useful tool to generate dialogue about seagrasses, their habitat, and more cultural storytelling centred on fishing tales and approaches for artist interpretations. These glass mosaic workshops are still active, coming together to nurture a sense of belonging, bolster mental health in a difficult remote area, helping to connect with Country, and more yarning about seagrasses!

Figure S6 Carnarvon glass mosaic class being delivered via zoom from Perth (6 March 2021). Clockwise from top left: Malgana artists learning how to cut glass mosaic tiles (left to right around the table) Barry Bellotti Jr, Isabella Capewell-Randall, Pat Oakley (Malgana Ranger), Gail Bellotti, Carleen Ryder, and Barry Bellotti; Transferring artwork onto one of fourteen mosaic panels; Isabella Capewell-Randall applying blue glass mosaic pieces to a large *Posidonia* panel; Meet the Artists Pat Oakley, Isabella Capewell-Randall, Carleen Ryder, Barry Bellotti Jr, Gail Bellotti, and Barry Bellotti. Images: Sabrina Dowling Giudici.

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