Marine Biodiversity Hub

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Baseline genomic data collection and assisting natural recovery of seagrass meadows

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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)

May 2020

Milestones 2, 3 & 4

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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EXECUTIVE SUMMARY

The goal of Project E6 "*Assisting restoration of ecosystem engineers through seed-based and shoot-based programs in the Shark Bay World Heritage Site (WHS)"* is to work alongside the Malgana Traditional Owners to assist recovery of the dominant seagrasses, *Amphibolis antarctica* and *Posidonia australis* following the 2011 marine heat wave. Therefore, this project has been developed and implemented with consultation and collaboration between scientists from the University of Western Australia (UWA) and the Malgana people. 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 upcoming community events including the seagrass festival to be held in April 2020 in Denham, Shark Bay. All team members have been introduced, including team leaders from both UWA and the Malgana Aboriginal Committee (MAC), the Malgana Land and Sea Rangers and UWA students and volunteers.

Our project has been divided into two parts with each part developed and implemented with consultation and collaboration between UWA scientists and the Malgana people – (1) Collection of baseline restoration genetic diversity and connectivity estimates across the salinity gradient (within both gulfs) for the two dominant seagrass species – *Amphibolis antarctica* and *Posidonia australis*, and (2) Assisting natural recovery of seagrass meadows through the collection of reproductive and vegetative propagules for on-ground restoration activities.

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

Sourcing plant material from the most appropriate genetic provenance will be critical to the success in our restoration efforts in the steep environmental gradients of Shark Bay. Genetic data will be used to inform on movement of plant material for restoration activities. One notable outcome is that there are no plans to move any material between the eastern and western gulfs, as there are currently healthy meadows of both species in each gulf.

In addition to assessing genetic diversity and connectivity, we have undertaken research on population genomics to improve selection of seed sourcing, meta-omics to improve assessment and monitoring of restoration outcomes, and genome editing that can generate novel genotypes for restoration in challenging environments. Genomic DNA was extracted and bioinformatic analyses are currently being conducted to identify potentially adaptive DNA markers.

2. Assisting natural recovery of seagrass meadows

UWA scientists and the Malgana Land and Sea Rangers developed and implemented (i) ecological and restoration training and workshops and (ii) restoration practice to assist natural recovery of seagrass meadows. Ecological training exercises were conducted in water and on land and typically involved shared communication in our joint understandings of the Shark Bay marine environment, with a particular focus on seagrasses. UWA researchers gave in-field demonstrations of each of the species' habit and form, their functional role in the ecosystem, how they grow and when possible, showed how they reproduce. 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. Restoration practice was broken down into several steps based on methodology, species and life-history.

Replanting of adult plants and seedlings for both species were successfully trialled in several sites.

Next steps

(i) The early success of using biodegradable sandbags for facilitating *Amphibolis* recruitment means this technique is worth exploring in Shark Bay.

(ii) We are continuing to monitor the mortality and growth of seedlings and adult transplants at restoration sites in collaboration with the local community. We will also deploy underwater cameras to study the movement of invertebrates and fish of both restored areas and nearby vegetated and unvegetated locations, to assess return of ecosystem function. In addition, we will conduct training on how to collect small cores of sediment within both vegetated, transplanted and bare sediments and then train the community on how to identify key infauna as well as take organic Carbon samples for assessment of sediment Carbon content.

1. INTRODUCTION

Relatively little is known about the restoration potential of seagrass meadows in the Shark Bay World Heritage Area, Western Australia, 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 of 23% of seagrass cover in the bay (860 km2) had a flow on effect to mega-herbivores, fish, tourism and the commercial aquaculture and fisheries industries dependent on the ecosystem. There is a critical need to develop management actions to respond to such events and to prepare for predicted future events. Seagrass restoration has been explored at Useless Loop and on both sides of the Peron Peninsula near Denham and Monkey Mia over the past 6-8 years, resulting in an increased understanding of the factors required for successful seagrass restoration along the extreme salinity gradient found in Shark Bay. The Malgana people have responsibilities and cultural obligations for sea country in Gathaagudu (Shark Bay) and a strong connection to the land and inshore seas that make up the Shark Bay WHS.

This project is developing community-based seeding and shoot planting restoration practices in the Shark Bay World Heritage Site (WHS). Our collective vision is to scale up the existing restoration research to practice and assist recovery of the dominant seagrasses, *Amphibolis antarctica* and *Posidonia australis* following the 2011 marine heat wave. In this project, we work alongside the Malgana people, whom have responsibilities for sea country in Shark Bay and a strong connection to the land and inshore seas that make up the Shark Bay WHS.

There are two parts to this project – (1) Collection of baseline restoration genetic diversity and connectivity estimates across the salinity gradient (within both gulfs) for the two dominant seagrass species – *Amphibolis antarctica* and *Posidonia australis*, and (2) Assisting natural recovery of seagrass meadows through the collection of reproductive and vegetative propagules for on-ground restoration activities. The sourcing of plant material for assisting recovery will be directly informed by results from the population genetic study.

Genetic diversity has been recognized as an important component of ecosystem resilience (Bernhardt and Leslie, 2013). Several studies have shown that genetic diversity can benefit the resistance and recovery potential of seagrasses (e.g. Ehlers et al., 2008; Hughes and Stachowicz, 2011; Reynolds et al., 2012; Sinclair et al., 2013). However, the implementation of genetic diversity in resilience-based seagrass management and restoration is often lacking (Massa et al., 2013; York et al., 2017). The current genetic data available for three *P. australis* meadows in Shark Bay (Sinclair et al., 2016; Sinclair et al. unpublished data) suggest genetic structure occurs geographically across the Shark Bay World Heritage Site. Preliminary observations from a reciprocal transplant trial currently underway as part of an ARC Discovery grant, suggest there are different growth rates and likely adaptive differences among *P. australis* plants growing in different salinities (Sinclair et al. unpublished observations 2018). No such data are currently available for *A. antarctica*.

This report describes (Milestone 2): Collection of seagrass samples for the two dominant temperate seagrass species – *A. antarctica* and *P. australis*. These samples will be used to assess baseline genomic diversity and connectivity estimates across the salinity gradient in the eastern and western gulfs of Shark Bay, Western Australia. The sourcing of plant material for assisting seagrass recovery (vegetative shoots, seeds, seedlings) will be directly informed by results from this population genomics study; (Milestone 3): Ecological and restoration training and workshops; and (Milestone 4): Restoration practice between UWA and the Malgana Indigenous Rangers to assist natural recovery of seagrass meadows during field trips which took place in August 2019, November 2019 and February/March 2020.

2. MEETINGS AND ENGAGEMENT WITH TRADITIONAL OWNERS

This project was developed in consultation with Malgana woman, Bianca McNeair, who is the Aboriginal Program Coordinator at the Northern Agricultural Catchments Council (NACC) in Geraldton. We initially met through attending the Hamelin Station Science Fair hosted by Bush Heritage in August 2018. However, the Malgana Traditional Owners were awarded Native Title of their Land (Gathaagudu) on 4th December 2018 (https://ymac.org.au/media-release-malgana-people-celebrate-native-title-win/), after submission of the project proposal. Relationships needed to be built with the newly appointed Malgana Aboriginal Corporation in order to conduct seagrass research On Country.

Dr Elizabeth Sinclair and Jane Edgeloe, a Masters student at UWA, met with Sean McNeair (Malgana Land and Sea Management Ranger program Coordinator) and Marika Oakley (newly appointed Chair of the Malgana Aboriginal Corporation) during our first field trip in March 2019. Permission was sought and given to remove seagrass samples from Country for our genetics research. We are now continuing to develop these relationships with Malgana Board members, elders and the newly certified rangers. They are keen for seagrass research to continue and we are working with rangers to train them in seagrass restoration and monitoring activities (see Milestone 3).

Professor Gary Kendrick, Dr Elizabeth Sinclair, Dr John Statton, Amrit Kendrick, Dr Siegy Krauss, Dr Martin Breed, as well as students Jane Edgeloe, Ankje Frouws, Rachel Austin met with Sean McNeair (Malgana Land and Sea Management Ranger program Coordinator) and were introduced to the Malgana Land and Sea Rangers: Pat Oakley, Nick Pedrocchi, Alex Dodd, and Nykita McNeair in August 2019. This in-field introduction provided an opportunity for an official Welcome to Country provided by Nick Pedrocchi, an informal meet and greet and an in-field restoration demonstration trial and ecological training exercise.

Dr John Statton, Rachel Austin, Kate Dawson and Maria Jung met with Sean McNeair and the Malgana Land and Sea Rangers; Richard Cross and Marika Oakley in November 2019 to discuss the breadth of restoration methodologies and logistics as well as in field ecological training.

Prof. Gary Kendrick, Dr John Statton, Amrit Kendrick, Rachel Austin and Brenna Waite met with Sean McNeair and the Malgana Land and Sea Rangers; Nick Pedrocchi, Richard Cross, Marika Oakley (Chair of the MAC Board), Alex Dodd and Nykita McNeair in February/March 2020 to work through land and field based training activities, workshops, and restoration at two locations in Shark Bay.

3. MILESTONE 2. COLLECTION OF BASELINE RESTORATION GENETIC DIVERSITY AND CONNECTIVITY ESTIMATES ACROSS THE SALINITY GRADIENT (WITHIN BOTH GULFS) FOR THE TWO DOMINANT SEAGRASS SPECIES – *AMPHIBOLIS ANTARCTICA* AND *POSIDONIA AUSTRALIS*.

3.1 Site selection for sampling seagrass meadows

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 or 'two waters'). We focused on selecting locations in which both *P. australis* and *A. antarctica* meadows were in close proximity (~35 – 58 PSU), accessible, and healthy (i.e. not showing signs of degradation as a result of the 2010/2011 heatwave). Consideration of tidal influence and feasibility of sampling within the field trip timeframe was also taken into account.

Seagrass meadows for sampling were identified based on our prior knowledge of distribution and abundance, and on the most recent distribution maps generated from monitoring data by the Western Australian Department of Biodiversity, Conservation, and Attractions (DBCA). Assistance was provided by DBCA scientists Drs Simone Strydom and Kathy Murray (GIS mapping expert). Ten meadows were selected for sampling of *A. antarctica* and *P. australis*, with alternatives also listed as some of the distribution data was more than 10 years old. All sampling meadows were located within the Shark Bay Marine Park.

Figure 1 Sampled meadows for *Posidonia australis* (blue circles) and *Amphibolis antarctica* (red circles) in the eastern and western gulfs of Shark Bay, Western Australia. These meadows cover the full geographic range across the salinity gradient (~ 35 - 58 PSU). Restoration sites (green dots) located at Dubaut Point (Site name Wyrrya - S 25º51.135'; E 113º45.612') and Middle Bluff (Site name Muga – S 25º49.452'; E 113º27.841'). Demonstration restoration trial site was located at Fowlers Camp (Orange dot).

3.2 Seagrass sampling

Two field trips to Shark Bay were conducted to complete the genetic sampling, one in March 2019 and a second in August 2019. Sampling was completed across ten meadows, five in the western gulf, ranging from Sandy Point to White Island, and five in the eastern gulf ranging from Herald Bight to L'Haridon Bight (Figure 1, Table 1). Fresh *A. antarctica* were collected from all ten meadows, while fresh *P. australis* samples were collected from eight meadows. Samples for two *P. australis* meadows (from Guischenault and Monkey Mia) were included from earlier collections to reduce the total amount of seagrass being collected.

Table 1 Sampled meadows for collection of *P. australis* and *A. antarctica* shoot material for genetic assessment.

Location	Latitude (S)	Longitude (E)	Coll. date	No.	Water temp (C)	Depth (m)	Salinity (PSU)
Posidonia australis							
Sandy Point	-25.71063	113.07661	15/3/19	30	25.4	4.2	35.7
Middle Bluff	25 49.446	113 27.837	12/3/19	30	25.3	1.1	39.6
Fowlers Camp	-26.10549	113.61285	10/3/19	30	27.9	0.6	40.5
Nanga Bay	-26.24116	113.77155	18/3/19	30	23.7	0.5	49.1
White Island	-26.45465	113.76843	12/8/19	30	18.0	2.9	35.0
Herald Bight	-25.58981	113.53467	13/3/19	30	25.8	0.8	38.7
Guischenault	-25.59965	113.58147	14/11/12	30	-	0.5	-
Monkey Mia	-25.78797	113.7199	15/11/12	30	-	<3.0	-
Dubaut Point	-25.85239	113.76082	17/3/19	30	25.3	1.2	44.7
Faure Island	-25.90925	113.83094	14/3/19	30	25.6	2.8	45.4
Amphibolis antarctica							
Sandy Point	-25.71690	113.07727	15/3/19	30	26.9	2.3	35.0
Middle Bluff	-25.82466	113.46389	12/3/19	30	25.3	1.1	39.6
Fowlers Camp	-26.09997	113.60976	10/3/19	30	26.9	1.1	40.6
Nanga Bay	-26.24116	113.77155	16/3/19	30	23.6	0.8	48.6
White Island	-26.45553	113.76801	12/8/19	30	18.0	2.4	35.0
Herald Bight	-25.59125	113.53484	13/3/19	30	25.8	0.8	38.7
Monkey Mia	-25.77772	113.78951	17/3/19	30	25.1	2.2	42.1
Dubaut Point	-25.85324	113.75616	17/3/19	30	25.3	1.2	44.7
Faure Island	-25.92709	113.82803	14/3/19	30	24.7	1.2	47.4
L'Haridon Bight	-26.03899	113.73726	14/3/19	30	24.4	1.6	51.9

All meadows were accessed by boat, excluding the Nanga Bay meadow which was accessed from beach at low tide. Meadows were located using GPS coordinates established prior to collecting. The sampling protocol of McMahon et al. (2018) was used to ensure a representative sample was collected in an efficient manner. Each species was sampled separately from mostly monospecific meadows. Identification of each species was performed on arrival at a meadow by snorkelers. A dive flag with weight attached was deployed to mark the centre point for sampling (Figure 2). A sampling site was defined as a circular area 50 m in diameter of continuous seagrass where possible. A team of 3-4 SCUBA divers measured out 25 m transect lines within the 50 m circular area, orientated on pre-defined compass bearings (Figure 3). A total of six samples were collected along each of five transects, for a total of 30 shoot samples per species per site. If there was no seagrass present at a predefined point, then the closest shoot was sampled, with sampling kept at a minimum of 2 m apart. The new sample location was measured and recorded. The Nanga Bay meadow was inshore and highly fragmented, so samples were randomly collected at least 2 m apart.

A *P. australis* shoot sample consisted of a single shoot meristem with no rhizome attached. An *A. antarctica* sample contained three stems with rhizome, each with multiple meristems (Figure 4). SCUBA divers placed individual seagrass samples into uniquely labelled calico bags which were recorded on a data sheet. Samples were kept in catch-bags during the sampling process, and transferred onto the boat intermittently during sampling. Samples were kept on ice and transported back to the land based laboratory and refrigerated immediately. Samples were

individually processed consistent with Sinclair et al. (2014) protocol. The *P. australis* meristem (white) was exposed then cut longitudinally into two pieces (a and b sample) and placed into a labelled 2 ml microcentrifuge tube prior to freezing at -20ºC. The *A. antarctica* meristems are much smaller than *P. australis*, so multiple greenish meristems (n = 6 – 8) were collected from a single stem and placed into a single sample tube for DNA extraction (Figure 5). The two outer leave sheaths were removed to reduce the amount of epiphyte contamination in DNA extractions. All sample tubes were transferred to a -80ºC freezer on return to Perth and stored at Kings Park Science until DNA extraction.

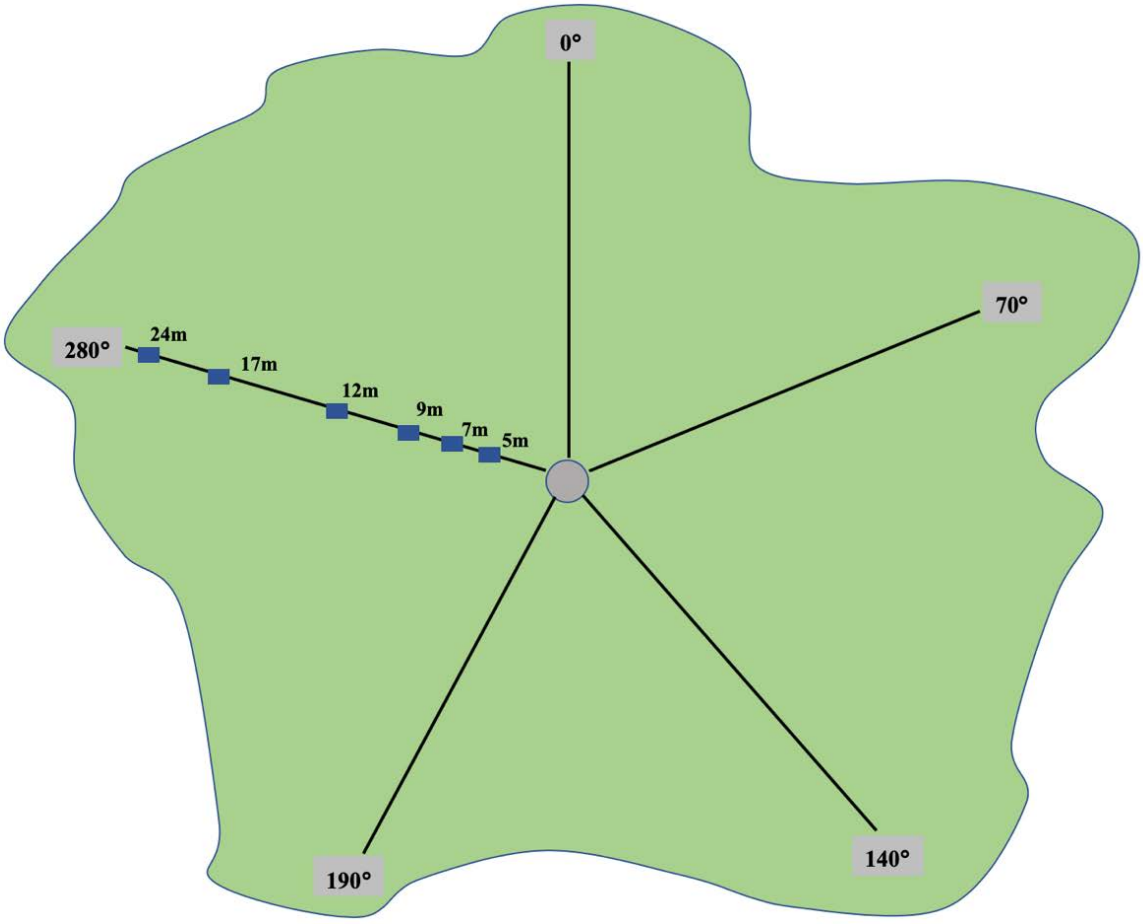

Figure 2 Sampling design for seagrass meadows. The centre point was marked with a dive flag, with five transect lines 25 m in length. Shoot sampling locations are indicated in blue at predefined bearings and distances from the centre point. Six samples were collected per transect for a total of 30 samples per species at each sampled meadow.

Figure 3 SCUBA diver measuring out a 25 m transect for sampling in a *P. australis* meadow. Five 600 x 600 mm grid plots were photographed to measure percentage cover at each of the sampled meadows. Photo Rachel Austin.

Figure 4 Ankje Frouws sampling *Amphibolis antarctica* at L'Haridon Bight. Each sampling unit contained three mature stems. Photo Rachel Austin.

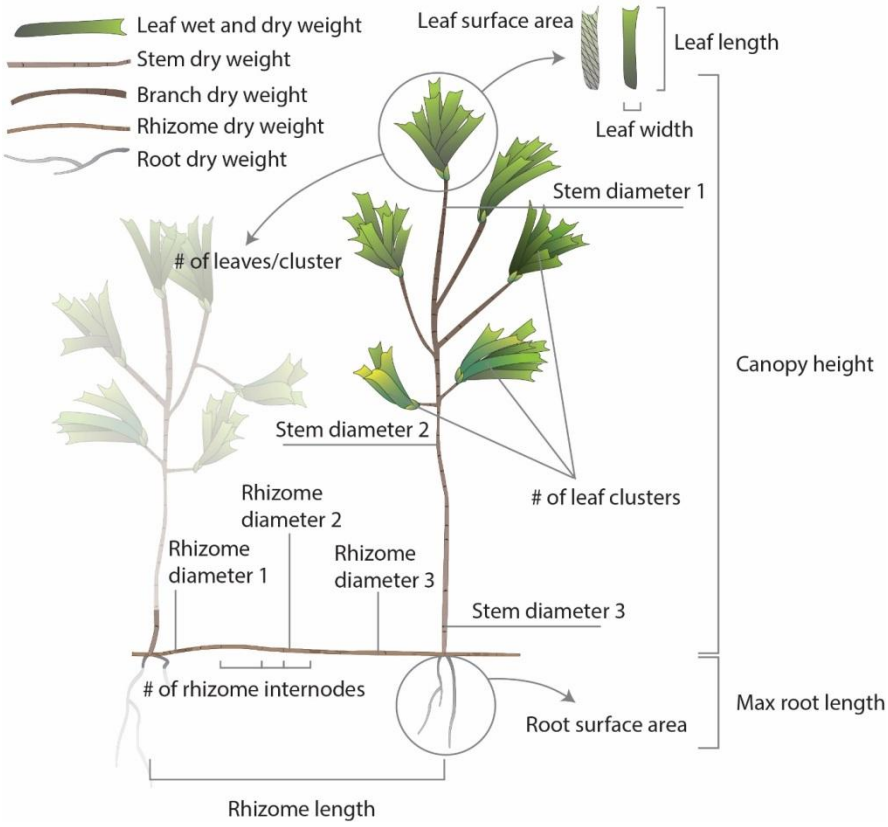

Figure 5 Phenotypic measurements taken from each of the ten populations of *Amphibolis antarctica* to analyse phenotypic diversity along the salinity gradient (N=15 per population).

3.3 Genomic data collection

Emerging genomics tools offer the potential to improve our understanding of seagrass biology. These tools include population genomics to improve selection of seed sourcing, meta-omics to improve assessment and monitoring of restoration outcomes, and genome editing that can generate novel genotypes for restoration in challenging environments (Breed et al., 2019). Next Generation Sequencing (NGS) methods, such as restriction site-associated DNA sequencing (RAD-seq) and double-digest RAD-seq (ddRAD-seq) are promising, cost effective methods that can be used to explore genomic diversity and structure within and among populations (Arafa et al., 2017), especially in 'non-model' organisms (Maroso et al., 2018). These genomic technologies generate thousands of markers, provide an opportunity to explore a much wider portion of the genome, relative to microsatellite DNA loci which are regarded as neutral markers. They can be used to identify underlying adaptive variation in geographically and genetically distinct populations, in return largely revealing how populations adapt to changing climatic conditions (Davidson and Davidson, 2012). There is currently very little published literature on population genomic diversity in seagrasses (e.g. Hernawan et al., 2017; McMahon et al., 2017; Phair et al., 2019, 2020), so we have chosen to collect genomic data, rather than use microsatellite DNA loci as originally intended.

Genomic DNA was extracted from a subset samples (*P. australis* n = 14/meadow; *A. amphibolis*, n = 19-20/meadow) using a Qiagen DNeasy→ Plant Pro Kit (Qiagen, Germany). Extraction protocols were modified 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 a reduced representation of each of the *P. australis* and *A. amphibolis* genomes. DNA libraries were prepared in the Batley genomics laboratory at UWA, following the protocols outlined in Severn-Ellis et al. (2020). HiseqX10 whole genome sequencing (x30 coverage) was conducted at the Garvan Institute of Medical Research (New South Wales). Bioinformatic analyses are currently being conducted, whereby the quality of the sequencing reads is being assessed and Single Nucleotide Polymorphism (SNP) marker discovery will be subsequently performed. SNP filtering will then be performed to identify neutral genetic markers and those potentially under selection (adaptive markers).

3.4 Additional research on *Amphibolis antarctica*

Phenotypic diversity has not been assessed in *A. antarctica*. However, variability in form has been observed in South Australia (Bryars, 2008) and Western Australia (M. Fraser, Sinclair et al., personal observations), with a wide range in plant form, particularly in relation to plant height and pigmentation. The remaining two stems from *A. antarctica* are currently being used for analysis of phenotypic diversity along the salinity gradient. The phenotypic measures will include leaf length and width (Lavery et al., 2009), leaf cluster density, stem length, internode length, and above and below ground biomass (Marbà and Walker, 1999) (Figure 5). Leaf material from a single leaf cluster was cleaned of epiphytes and retained for pigment composition analysis by HPLC (as per Davey et al., 2018).

4. MILESTONE 3 – WORKSHOPS AND TRAINING IN SEAGRASS ECOLOGY, SEED COLLECTING AND RESTORATION METHODOLOGIES WITH MALGANA PEOPLE

4.1 Workshops and Training

In August 2019, both UWA researchers and Malgana Land and Sea Rangers participated in an ecological training exercise that involved cross-communication in our understandings of the Shark Bay marine environment, with a particular focus on seagrasses. There was a strong emphasis on identifying the main impacted species, *A. antarctica* as well as the other dominant species in the system (*P. australis, Halodule uninervis*, *Halophila ovalis* and *Cymodocea serrulata*) from a scientific perspective, but also from a cultural perspective. UWA researchers gave in-field demonstrations of each of the species form, 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 decades to centuries of 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 (Figure 1). Sandbags were placed in pairs of hessian and jute, with the exception of one pair of hessian bags in a shallow site that would remain submerged even during low tide. 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 6). Survival was monitored during a subsequent trip in February/March 2020.

Figure 6 Deployment of (a) Jute and (b) Hessian bags. White arrows indicate *Amphibolis antarctica* seedlings attached to the bags.

In November 2019, the aim of this training exercise was to identify mature *P. australis* flowers and fruit, conduct flower counts and then demonstrate a technique to collect and extract the fruit (Statton et al., 2013). Flower counts were conducted by counting flower heads 0.5 m either side of a 10 m transect tape and replicated 10 times. Flower heads were also 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 the 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 have identified the most suitable location, Guischenault Point (Figure 1), 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 7). There appeared to be a gradient in impact, with meadows that were exposed along the shallower margins bleached white (Figure 7a), those partially submerged had brown leaves (Figure 7b) while meadows that were much deeper tended to have green leaves with brown tips (Figure 7c) or didn't appear impacted (Figure 7d). Some meadows of *P. australis* showed evidence that flowers had been produced (Figure 7e), but fruit development was aborted on every flower observed (Figure 7f) and flower counts were often less than 1 m^{-2} (c.f. 80 m^{-2} in 2017 (Kendrick et al., 2019).

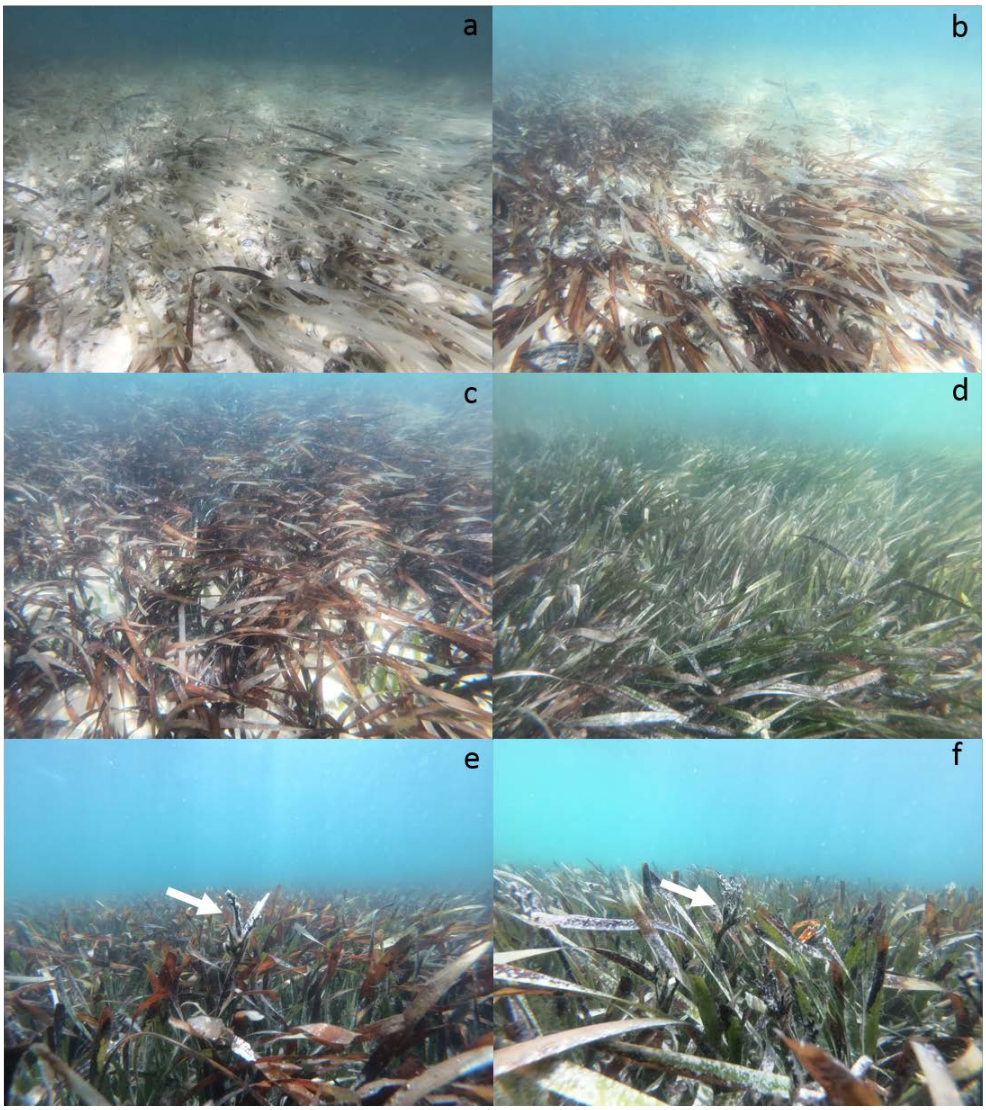

Figure 7 *Posidonia australis* meadow at Guischenault Point, Shark Bay showing (a) complete bleaching of leaves and death of the meadow, (b) mix of bleached and brown leaves with no green leaves, (c) brown leaves with some green leaves, (d) healthy green meadow. Meadows that remained submerged during 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.

During February/March 2020, UWA researchers ran both land and water-based 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 18 and 24 months old and form part of the genetics translocation research (see Milestone 2). The Malgana Land and Sea Rangers 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 growth of plants (Figure 8).

Figure 8 Eighteen month old *Posidonia australis* transplants being assess for individual transplant survivorship and counting the number of shoots on each surviving transplant.

Following survivorship and shoot counts of existing *P. australis* transplants, 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 breaking off 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.

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.

5. MILESTONE 4. ASSISTING NATURAL RECOVERY OF SEAGRASS MEADOWS, AMPHIBOLIS ANTARCTICA AND POSIDONIA AUSTRALIS, THROUGH THE COLLECTION OF REPRODUCTIVE AND VEGETATIVE PROPAGULES FOR ON-GROUND RESTORATION ACTIVITIES.

5.1 Seed-based Restoration

In August 2019, *Amphibolis* seedlings were collected from drift material near Fowlers Camp (Figure 1). Seedlings were planted into 14 hessian and 12 jute sand bags (see Training for details). Typically, seedlings naturally attach to the sand bags in 10's to 100's over the reproductive period, however, for this demonstration restoration trial we planted six seedlings directly to each sandbag via the seedlings grappling hook appendage. Seedling presence/absence was monitored 8 months later in March 2020. Twelve hessian and 10 Jute sandbags had one seedling present while one Jute bag had retained three seedlings (Figure 9).

Figure 9 Eight month old *Amphibolis antarctica* seedlings attached to Jute sandbags at Fowlers Camp, Shark Bay.

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

Posidonia australis fruit become available in early November in Shark Bay. We had anticipated collecting floating fruit using dip nets or removing fruit from the parent plants directly during November 2019. However, the 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. Therefore, no restoration trials were established in 2019 using *P. australis* seeds.

5.2 Shoot-based Restoration

Transplanting adult shoots has been a successful and well-known technique used around Australia (Statton et al., 2012). We have been trialling and conducting experiments using transplant methods for *P. australis* and *A. antarctica* in Useless Loop, Shark Bay since 2015 and these trials have shown that both species can be successfully established.

For this NESP project E6, four experimental *P. australis* field plots were established as separate experiments in April and August 2018 (Herald Bight, Dubaut Point, Middle Bluff, Fowlers Camp). The transplants are between 18 months and 2 years old. These plots show generally high survival rates (~80 - 90%, Figure 10). However, there are some important site-specific differences impacting survival including high herbivory and physical removal of transplants at Herald Bight. This approach can be conducted at any time of the year, although higher survival is generally achieved from Summer/Autumn plantings.

Figure 10 Transplant survival of *P. australis* for experimental plots established in A. April 2018 and B. August 2018.

During February/March 2020 field trip, the Malgana Land and Sea Rangers transplanted seagrass into new restoration sites. A restoration trial was established at two sites Dubaut Point (Malgana name Wyrrya - S 25º51.135'; E 113º45.612') and Middle Bluff (Malgana name Muga – S 25º49.452'; E 113º27.841'). At each site we set-up two 25 m^2 plots adjacent to each other. One plot we transplanted 50 adult plants of *A. antarctica* and the other 50 adult plants of *P. australis* (see Training for details on methods, Figure 11).

Figure 11 Transplanting *Posidonia australis* adult shoots at Middle Bluff

The combination of established transplant plots and experiments, along with these newly established restoration sites, will enable temporal and spatial comparisons between *P. australis* and *A. amphibolis* sites in order to understand the time required for a return of ecosystem function (through carbon capture and biodiversity assessment) for Milestone 6.

5.3 Next steps

The early success of using hessian or jute sandbags for facilitating *Amphibolis* recruitment means 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 which means they can only collect seedlings over a small area. We have designed and trialled 2.5 m long hessian sausages in another project with good success. Hessian sausages are similar in weight to a standard square hessian bag, but have a far greater surface area for attachment of dispersing *Amphibolis* seedlings. We have commissioned an upholsterer to produce the 2.5 m long hessian sandbags. These will be shipped to Shark Bay in June/July 2020 in time for the Malgana Land and Sea Rangers to fill with local sand and deploy at restoration sites prior to the peak reproductive season in August.

We aim to conduct another demonstration on how to identify viable *P. australis* fruit, collect, prepare and disperse seed at restoration sites in November 2020. The success of this approach

in Shark Bay will depend upon sourcing viable seeds from meadows that have recovered sufficiently from the October 2019 extreme low tide event.

We are continuing to monitor the mortality and growth of seedlings and adult transplants at restoration sites in collaboration with the local community. We will also deploy underwater cameras to study the movement of invertebrates and fish of both restored areas and nearby vegetated and unvegetated locations in order to assess return of ecosystem function. In addition, we will conduct training on how to collect small cores of sediment within both vegetated, transplanted and bare sediments, and then train the community on how to identify key infauna, as well as take organic Carbon samples for assessment of sediment Carbon content.

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7. REFERENCES

Arafa RA, Rakha MT, Soliman NEK, Moussa OM, Kamel SM, Shirasawa K (2017) Rapid identification of candidate genes for resistance to tomato late blight disease using next-generation sequencing technologies. PLoS ONE 12, e0189951.

Bernhardt JR, Leslie HM (2013) Resilience to climate change in coastal marine ecosystems. Annual Review of Marine Science 5, 371-392.

Breed MF, Harrison PA, Blyth C, Byrne M, Gaget V, Gellie NJC, Groom SVC, Hodgson R, Mills JG, Prowse TAA, Steane DA, Mohr JJ (2019) The potential of genomics for restoring ecosystems and biodiversity. Nature Reviews Genetics 20, 615–628.

Bryars S (2008) Restoration of Coastal Seagrass Ecosystems: *Amphibolis antarctica* in Gulf St Vincent, South Australia. South Australian Research and Development Institute, Aquatic Sciences, Adelaide.

Davey PA, Pernice M, Ashworth J, Kuzhiumparambil U, Szabó M, Dolferus R, Ralph PJ. (2018) A new mechanistic understanding of light-limitation in the seagrass *Zostera muelleri*. Marine Environmental Research 134, 55-67.

Ehlers A, Worm B, Reusch THB (2008) Importance of genetic diversity in eelgrass Zostera marina for its resilience to climate warming. Marine Ecology Progress Series 355, 1-7.

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

Hughes AH, Stachowicz JJ (2011) Seagrass genotypic diversity increases disturbance response via complementarity and dominance. Journal of Ecology 99, 445-453.

Kendrick GA, Nowicki RJ, Olsen YS, Strydom S, Fraser MW, Sinclair EA, Statton J, Hovey RK, Thomson JA, Burkholder DA, McMahon K, Kilminster K, Hetzel Y, Fourqurean JW, Heithaus MR, Orth RJ (2019) A systematic review of how multiple stressors from an extreme event drove ecosystem-wide loss of resilience in an iconic seagrass community. Frontiers in Marine Science 6, 455.

Lavery PS, McMahon K, Mulligan M, Tennyson A (2009) Interactive effects of timing, intensity and duration of experimental shading on *Amphibolis griffithii*. Marine Ecology Progress Series 394, 21–33.

Marbà N, Walker DI (1999) Growth, flowering, and population dynamics of temperate Western Australian seagrasses. Marine Ecology Progress Series 184, 105-118.

Maroso F, Hillen JEJ, Pardo BG, Gkagkavouzis K, Coscia I, Hermida M, Franch R, Hellemans B, Van Houdt J, Simionati B, Taggart JB, Nielsen EE, Maes G, Ciavaglia SA, Webster LMI, Volckaert FAM, Martinez P, Bargelloni L, Ogden R (2018) Performance and precision of double digestion RAD (ddRAD) genotyping in large multiplexed datasets of marine fish species. Marine Genomics 39, 64-72.

Massa S, Paulino CM, Serrao EA, Duarte CM, Arnaud-Haond S (2013) Entangled effects of allelic and clonal (genotypic) richness in the resistance and resilience of experimental populations of the seagrass *Zostera noltii* to diatom invasion. BMC Ecology 13, 39.

McMahon KM, Evans RD, van Dijk K-J, Hernawan U, Kendrick GA, Lavery PS, Lowe R, Puotinen M, Waycott M (2017) Disturbance is an important driver of clonal richness in tropical seagrasses. Frontiers in Plant Science 8, 2026.

Peterson BK, Webster JN, Kay EH, Fisher HS, Hoekstra HE (2012) Double Digest RADseq: An inexpensive method for *de novo* SNP discovery and genotyping in model and non-model species. PLoS ONE 7, e37135.

Phair NL, Toonen RJ, Knapp I, von der Heyden S (2019) Shared genomic outliers across two divergent population clusters of a highly threatened seagrass. PeerJ 7, e6806.

Phair NL, Toonen RJ, Knapp ISS, von der Heyden S (2020) Anthropogenic pressures negatively impact genomic diversity of the vulnerable seagrass *Zostera capensis*. Journal of Environmental Management 255, 109831.

Reynolds LK, McGlathery KJ, Waycott M (2012) Genetic diversity enhances restoration success by augmenting ecosystem services. PLoS ONE 7, e38397.

Severn-Ellis AA, Scheben A, Neik TX, Saad NSM, Pradhan A, Batley J (2020) Genotyping for species identification and diversity assessment using double-digest restriction site-associated DNA sequencing (ddRAD-Seq). In: Jain M, Garg R (eds) Legume Genomics: Methods in Molecular Biology 2107, 159-187.

Sinclair EA, Verduin J, Krauss SL, Hardinge J, Anthony JM, Kendrick GA (2013) A genetic assessment of a successful seagrass meadow (*Posidonia australis*) restoration trial. Ecological Management and Restoration 14, 68-71.

Sinclair EA, Gecan I, Krauss SL, Kendrick GA (2014) Against the odds: complete outcrossing in a monoecious clonal seagrass. Annals of Botany 113, 1185-1196.

Sinclair EA, Statton J, Hovey RK, Anthony JM, Dixon, KW, Kendrick GA (2016) Reproduction at the extremes: pseudovivipary and genetic mosaicism in *Posidonia australis* Hooker (Posidoniaceae). Annals of Botany 117, 237-247.

Statton J, Cambridge ML, Dixon KW, Kendrick GA (2013) Aquaculture of *Posidonia australis* seedlings for seagrass restoration programs: effect of sediment type and organic enrichment on growth. Restoration Ecology 21, 250-259.

York PH, Smith TM, Coles RG, McKenna S, Connolly RM, Irving AD, Jackson EL, McMahon K, Runcie J, Sherman CDH, Sullivan B, Trevathan-Tackett S, Brodersen K, Carter AB, Ewers C, Lavery P, Roelfsema C, Samper-Villarreal J, Sinclair EA, Strydom S, Tanner J, van Dijk K-J, Warry F, Waycott M, Whitehead S (2017) Identifying knowledge gaps in seagrass research and management: an Australian perspective. Marine Environmental Research 27, 167-172.

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