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Adelaide Seagrass Rehabilitation Project: 2017-2019

Jason E. Tanner and Mandee J. Theil

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SARDI Aquatics Sciences
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July 2019

Final report prepared for the Adelaide and Mount Lofty Ranges Natural Resources Management Board

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

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EXECUTIVE SUMMARY

The Adelaide metropolitan seagrass rehabilitation program commenced with an international workshop in 2002, and has investigated a range of rehabilitation techniques since. The major focus over the last 15 years has been using hessian sand bags to facilitate natural recruitment of *Amphibolis*, and this has resulted in several small patches of rehabilitated seagrass that are now over ten years old, and which have similar structure and ecosystem function to adjacent natural seagrass meadows.

The majority of the work conducted to date has focused on extremely small experimental scales, with most individual deployments only consisting of ten hessian bags. To investigate the potential for scaling up rehabilitation using this technique, we report here on results from three 1-hectare trials, two of 1,000 bags deployed in 2014 in a previous project, and one of 2,500 bags deployed in 2017 as a part of the current project. All three sites appeared to have been subject to external physical disturbance, with many marker stakes lost, making temporal comparisons difficult. There was some indication from spatial comparisons of higher *Amphibolis* densities inside some plots, whereas *Posidonia* showed a north-south gradient, suggesting that the bags may have had led to some increase in *Amphibolis* densities in the plots. Although not conclusive, we suspect that the most likely agent of disturbance was fishing and anchoring.

To complement previous work to determine the temporal availability of recruits, we investigated the use of beach surveys to establish when *Amphibolis* recruits were available. The previous bimonthly deployments of bags showed highest recruitment on bags deployed from May to August, but the logistics and cost of bag deployments meant that the temporal grain of observations was limited. Beach surveys were conducted 3-5 days per week, and in both 2017 and 2018, showed a distinct peak in recruit availability over a few weeks in July.

In an attempt to extend the applicability of the hessian bag technique to seagrasses other than *Amphibolis*, trials were also conducted with *Posidonia angustifolia*. Due to the different life-history strategy and morphology of the two genera, *Posidonia* had to be planted into the bags as seedlings by divers, as they do not naturally recruit to them. Continued surveys of seedlings planted in 2012 and 2013 showed substantial declines in leaf densities, which we again suspect is related to external physical disturbance. Overall results suggest that the type of fill used in the bags is not relevant, and that the easiest, 100% sand, is adequate. We also conducted trials of pre-planting seedlings into bags before deployment, which showed good success. This technique saves considerable time, and avoids the need for divers if bags do not need to be precisely placed on the seafloor.

As well as *in situ* trials, we also conducted a number of tank trials with *Posidonia*, primarily *P. angustifolia*, to further examine the role of sediment composition, as well as timing of fruit collection and how fruits and seedlings are handled after collection. These trials reinforced the conclusion from the field experiments that the substrate is not highly important. However, the window of opportunity for collection of fruits appears to be narrow, with fruits collected as little as 1 week before or after the best date underperforming. In 2017, the best date for collection was December 28, although collections were only made approximately weekly, so further work is needed to determine exactly how broad a window of opportunity for collection there is. Once collected, fruits that took more than a few days to dehisce produced seedlings that performed poorly, and seedlings needed to be planted within 10 days for best results. Very few *P. sinuosa* fruits washed up onto the beach during the collection period, and the resultant seedlings did not perform as well as those of *P. angustifolia*. Both the field and tank trials indicated that small seeds (<10 mm) should also be discarded, as they do not survive and grow well.

Finally, we undertook a preliminary assessment of how bag storage condition influenced their integrity. While the trials conducted so far have only used small numbers of bags, and did not require bags to be stored for an extended period, upscaling to larger deployments such as will occur in 2020 (~50,000 bags) will require some storage. We found that the fibers of bags stored outside and exposed to the elements had a lower breaking strain than those stored inside. Pallet wrapping only had a small influence on breaking strain, but bags became mouldy over 4 weeks, suggesting that their integrity may still have been compromised. Thus if bags need to be wrapped for transport, the wrapping should only remain on for as short a period of time as possible. The moisture content of the sand used to fill the bags did not appear to be important, however, this experiment was conducted with a single layer of bags on raised pallets, resulting in good airflow both over and below them, which led to the sand rapidly drying out. When bags are stored eight high on a pallet, as would be the case operationally, the results may differ.

There have been a number of key factors influencing success that have been identified through this and previous work:

1. Site location is important – sites need to no longer be exposed to the stresses that caused the initial loss of seagrass, and for recruitment facilitation, they need to be downstream of a source of recruits. Sites also need to be free from other external physical disturbances as much as possible. One of our key knowledge gaps is currently around the dispersal pathways for seedlings along the Adelaide coast.

2. Timing is crucial – peak recruit availability appears to occur in July off Adelaide. Bags deployed after this risk missing this event, while those deployed to soon may end up buried by longshore sand movement before recruits become available. Thus May/June is suggested as the best time for deployment.
3. An appropriate bag density needs to be determined to ensure that the patches of seagrass that recruit to each bag are not too isolated from each other to benefit from density-dependent feedback mechanisms that promote survival.
4. For deployments involving tens to hundreds of thousands of bags, bag handling and storage between filling and deployment need to be considered, to ensure that the bags don't degrade.

Overall, after showing promising early results, the performance of the previously established trials reported here declined, possibly as a result of physical disturbance. However, the original small-scale *Amphibolis* trials also performed poorly during the medium term, and it was only after 5-6 years that they started to improve. They have now produced flourishing healthy patches of seagrass that appear to act like natural seagrass patches. Given that the large-scale trials described here utilized lower overall bag densities, by factors of 4 to 10, it cannot be expected that they would show success in a shorter time-frame than what was observed for the small-scale trials. Instead, it is likely to be at least 5 years, and even 10 years, before we can truly say how well these trials have succeeded. The technique is, however, very cost-effective, with likely costs for large-scale operation rehabilitation on the order of $20,000-30,000 per hectare, compared to typical published median costs of restoration ~$600,000 per hectare.

Keywords: Seagrass, restoration, *Posidonia*, *Amphibolis*, hessian.

1. INTRODUCTION

1.1. Background

Since 1949, there has been a total loss of some 6,200 ha of seagrass from the Adelaide metropolitan coast. The majority of this loss (5,200 ha) occurred between 1949 and 2002, and was documented through *in situ* sampling and the analysis of aerial photography (Neverauskas 1987a, Shepherd et al. 1989, Hart 1997, Cameron 1999). A net loss of a further 1800 ha was documented in 2007 (Cameron 2008), with a net gain of ~ 800 ha then occurring up to 2013 (Hart 2013). Much of this loss has occurred in shallow waters, up to ~ 7 m depth, with seagrasses receding seaward, rather than the pattern frequently documented elsewhere of losses due to eutrophication commencing in deep water and proceeding shoreward (Westphalen et al. 2005). Some of this loss has also occurred within the seagrass meadows, associated particularly with sewage sludge discharges in the 1970s and 80s (Neverauskas 1987b, Shepherd et al. 1989, Bryars and Neverauskas 2004), and more recently, meadow fragmentation is occurring in the shallower remaining seagrasses in more wave exposed areas (Seddon 2002, Fotheringham 2008). The primary causes of loss are generally considered to be the overgrowth of seagrass by epiphytic algae that thrived as a result of anthropogenic nutrient inputs, and to a lesser extent, turbidity associated with stormwater runoff (Fox et al. 2007).

In response to these losses, and efforts by both SA Water and the Adelaide and Mount Lofty Ranges Natural Resources Management Board to substantially decrease anthropogenic nutrient and sediment inputs, the South Australian Research and Development Institute (SARDI) and the Coast and Marine Branch of the then Department of Environment and Heritage (DEH, now Department of Environment and Water – DEW), held the first Seagrass Restoration Workshop (Seddon and Murray-Jones 2002). This workshop brought together a range of Australian and international experts on seagrass restoration, along with local scientists and managers, to discuss ways to approach the development of restoration techniques suited to local conditions. Following on from this, the first phase of what has become a long-term program of research on seagrass rehabilitation was initiated. A further two workshops were held in 2008 (Murray-Jones 2008) and 2013 (Murray-Jones 2013) to review progress, benchmark activities against work being done elsewhere in Australia, and keep stakeholders informed of progress.

Initial efforts focused on adapting techniques used elsewhere, namely transplantation and the laboratory production of seedlings (Seddon et al. 2004, Seddon et al. 2005), but success was limited. Observations made during these trials, however, suggested that the use of hessian to facilitate natural recruitment of *Amphibolis* seedlings may be a feasible approach to rehabilitation (Seddon 2004). While the work documented here focuses primarily on *A. antarctica*, a small amount of *A. griffithii* also occurs along the Adelaide coast, and no distinction was made between recruits of the two species. Subsequent work trialed a range of different deployment options for hessian in 2004, and suggested that a double-layered hessian bag consisting of a standard hessian sack surrounded by a coarse-weave hessian mesh (Figure 1.1) and filled with around 20 kg of sand resulted in the highest recruitment rates (Wear et al. 2006, Wear et al. 2010). These double-layered bags, along with standard hessian bags, have formed the basis for all subsequent work, which has been aimed at refining the methodology, and understanding factors that may lead to spatial and temporal variation in success.

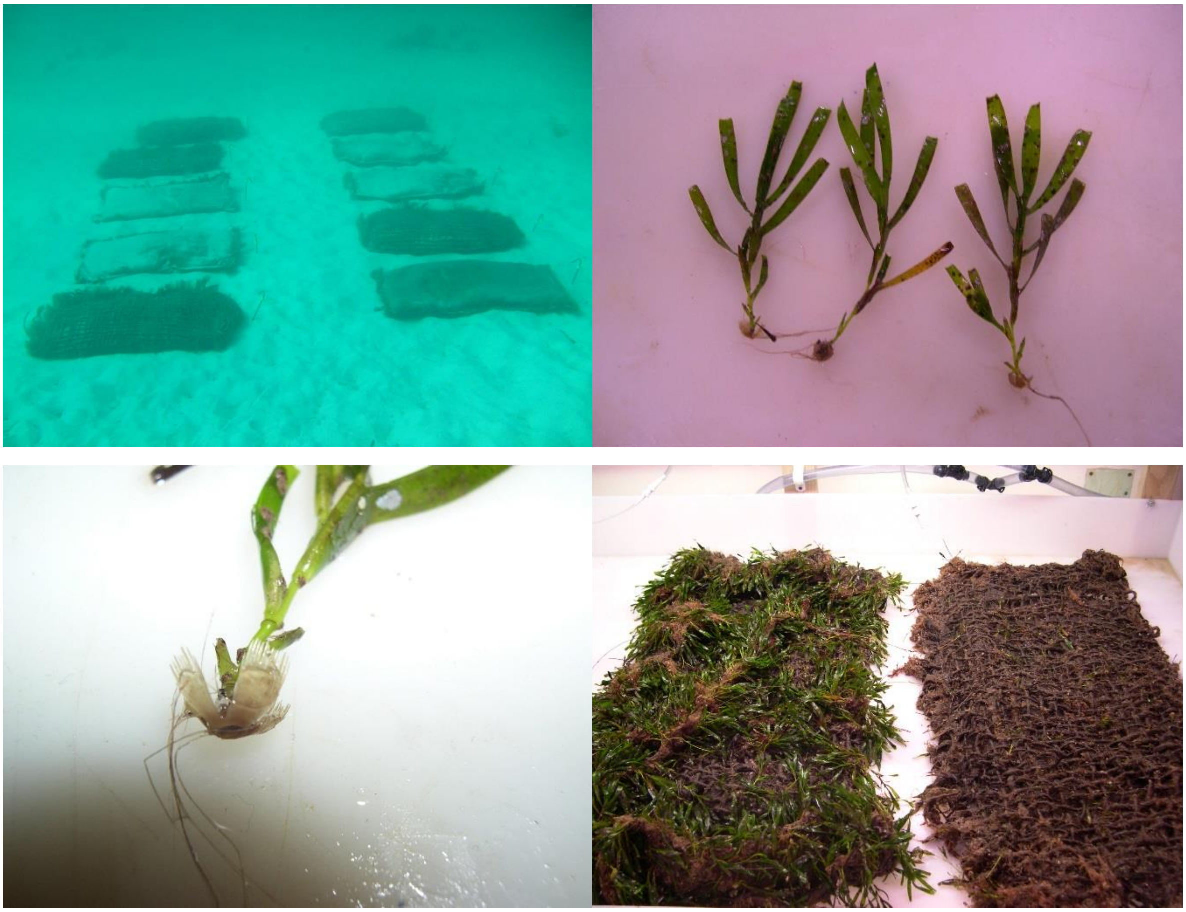

Figure 1.1: Bag layout for small-scale experiments on *Amphibolis* recruitment facilitation (top left), *Amphibolis* seedlings (top right), close-up of basal 'grappling hook' that allows seedlings to attach (bottom left), and examples of double-layered bags with and without seedlings attached (bottom right).

As the initial studies on hessian bags were only done at two sites and in a single year (Wear et al. 2006, Wear et al. 2010), it was important to examine spatial and temporal variability in recruitment in more detail. Bags were deployed at 12 sites in 2005, with an order of magnitude variability in recruitment between sites (3-122 seedlings per bag), and a 50-80% lower recruitment than in 2004 at the two repeat sites (Collings et al. 2007). While initial recruitment was strongly related to the density of nearby *Amphibolis*, this relationship broke down after 10 months, following 80-100% mortality. The structural longevity of the bags deployed in 2005 appeared to be considerably less than that of those deployed in 2004, leading to increased seedling mortality.

An initial trial of a 1-hectare rehabilitation plot was undertaken in 2006, with 1000 bags deployed in a 100 x 100 m area (Bryars 2008, Collings 2008). Initial recruitment was low (0-12 seedlings per bag 4 months after deployment), although average seedling densities then changed little until they increased the following year with a new cohort of recruits. This plot extended 100 m shorewards of the existing seagrass edge, and there were no differences in recruitment success with distance from the edge. Two additional 1-hectare trials were established in June 2014 (Tanner and Theil 2016). Initial recruitment onto these bags was lower than onto bags deployed in small-scale trials in the winter of previous years, but after 20 months, stem densities were within the range of what was found 20 months after previous small-scale deployments. A similar pattern was found with stem lengths.

An issue with all trials conducted up to 2006 was uncertainty around when was the best time of year to deploy bags in order to maximize recruitment of *Amphibolis*. Anecdotal evidence suggested late winter/early spring. In 2007, a concerted effort to identify the timing of reproduction and recruitment commenced, with bimonthly deployments of bags and collection of adult plants at four sites along the Adelaide coast (Brighton, Grange – the main study site over time, Semaphore and Largs Bay). Deployments covered the periods November 2007 to October 2009 and January 2011 to March 2013 (Irving 2009c, b, Delpin 2014, Tanner 2015). These studies showed May to August to be the best period for bag deployment to maximize recruitment success, and showed that *Amphibolis* structural characteristics (stem density and length) were similar to those in natural meadows 5 years after bag deployment. Interannual variation in recruitment was present, but only explained 15.5% of the variation in recruitment, compared to 81.1% for month of deployment. Whilst previous studies had pointed towards double-layered bags being the best for recruitment, analysis of the long-term data (Tanner 2015) showed no difference between double-layer and single-layer bags.

Continued monitoring of bags deployed in small-scale experiments between 2007 and 2013 showed that bags deployed during winter continued to support densities of *Amphibolis* similar to those found in adjacent natural meadows, but that stem lengths could reach up to double those found in the natural meadow (Tanner and Theil 2016). Early deployments have coalesced into larger patches, where the locations of individual bags can no longer be distinguished. Interestingly, there was a major increase in stem densities on some deployments at the final survey in February 2016, with some patches having stem densities up to ten times those found naturally. While this was accompanied by a small decline in average stem length, this decline was not sufficient to suggest that this result was due to a major influx of new recruits in the winter of 2015.

As well as examining temporal variation in recruitment, Irving (2009c) examined the consequences of using different fill types (sand vs sand and 20 mm quartzite aggregate), and layouts (single bags vs clustered). Neither factor was found to influence recruitment.

One of the issues experienced throughout the program has been the rapid deterioration of some batches of bags. To address this, a series of trials were undertaken with Flinders University to develop coatings that would increase the durability of the hessian (Irving 2009b, c, Delpin 2014, Paterson et al. 2016). While these trials showed some promising results with respect to decreased bacterial loading on some treated bags (Paterson et al. 2016), and increased recruitment on these bags both initially and after 12 months (Delpin 2014), the logistics and costs associated with treating bags meant that this approach was not pursued further.

Not only do the bags provide a mechanism for the successful facilitation of *Amphibolis* recruitment, but the resultant *Amphibolis* patches appear to be providing a similar ecosystem function to natural seagrasses. Epifaunal richness and abundance reached that present in natural seagrasses 1 year after *Amphibolis* recruitment, although assemblage structure took 3 years, the same time as seagrass structure took to recover (McSkimming et al. 2016). Infaunal assemblages recovered within 2 years (McSkimming 2015). Anecdotally, both *Zostera* and *Posidonia* seagrasses have been observed to recruit into patches of restored *Amphibolis*, and larger fauna such as syngnathids also utilize the restored habitat.

In an attempt to extend the applicability of the hessian bag technique to seagrasses other than *Amphibolis*, trials have also been conducted with *Posidonia* (Tanner and Theil 2016). Due to the different life-history strategy and morphology of the two genera, *Posidonia* were planted into the bags as seedlings by divers, as they do not naturally recruit to them. Seedlings planted in 2012 survived and grew well over the subsequent four years, and produced multiple shoots. Seedlings planted in 2013 performed less well, possibly because they were held in aquaria for 2 months prior to planting out. Bags filled with a mix of sand and clay performed better than those with sand only, and the addition of organic matter had no effect on seedlings. Seedlings from larger seeds (> 13 mm) had better survival than those from smaller, and the number of surviving seedlings did not depend on the number initially planted, suggesting density-dependent survival of individual seedlings.

To date, and to our knowledge, the bags have been used three times in South Australia outside the Adelaide metropolitan region, although they have also been adapted for use in Western Australia. DEW deployed a set of bags at Beachport, but due to poor visibility were never able to relocate them, and it is presumed that they failed (Fotheringham pers. com.). The second SA trial was in Yankalilla Bay, south of Adelaide, where recruitment to bags ranged from 0-107 (mean ± se: 14 ± 2.3), although there was no long-term follow-up of survival (Irving 2009a). Finally, the bags have also been used at American River on Kangaroo Island, where they were unsuccessful, apparently due to the lack of nearby *Amphibolis* to provide a source of recruits (McArdle pers. com.). Following initial success in Adelaide, John Statton (University of Western Australia), has used sand-filled hessian bags (termed grow-bags) to transplant *Posidonia australis* seedlings into in Cockburn Sound (Oceanica Consulting Pty. Ltd. 2011). He found good survival in his first trial, with 100% of bags still supporting seedlings the following summer, however, subsequent trials were hampered by rapid deterioration of the hessian used, which broke down in 2-3 months compared to ~ 9 months in the first trial.

As with any rehabilitation project, an important consideration for success is that the original causes of loss have been ameliorated sufficiently to allow rehabilitation to occur. Anthropogenic nutrient inputs have been identified as one of the major causes of seagrass loss along the Adelaide coast (Fox et al. 2007). In 2003, there were ~ 2,400 tonnes of nitrogen introduced into the system from wastewater treatment plants, stormwater runoff and industrial discharges (Fox et al. 2007). In 2011, this had reduced to ~ 1,800 tonnes due to efforts to reduce inputs from all three sources (Van Gils et al. 2017). A further 600 tonne reduction was achieved in 2013 through the closure of the Penrice soda ash plant (Van Gils et al. 2017). In combination, these factors have thus led to a ~ 50% decrease in nitrogen loads to the Adelaide coastal waters, substantially reducing one of the major impacts that caused the original seagrass loss.

Overall, sand filled hessian bags deployed at small-scales during winter are an effective means for rehabilitating patches of *Amphibolis* with minimal intervention (Figure 1.2), provided that there is a nearby source of recruits. The larger scale trials still need to be monitored for a few more years to ascertain their success. While initial recruitment to them was disappointing, the results at 20 months are more promising, and in line with results for small-scale experiments at 20 months which then went on to establish patches that have so far lasted for up to ten years. These older small-scale patches now appear to be functioning the same as nearby natural meadows. The key issue that needs to be resolved before any large-scale rehabilitation becomes operational is the best way to handle bags prior to deployment to ensure they retain their integrity. Early large-scale deployments should then be used to investigate the role of factors such as bag layout and density on establishment success. For

Posidonia, early trials are promising, but the method is much more labour intensive, and so may only be applicable at smaller scales.

A list of all reports and papers that have resulted from the program (and associated projects) is provided in Table 1.1, with site details presented in Table 1.2 and Figure 1.3.

Figure 1.2: Examples of *Amphibolis* restoration showing progression of establishment from 12 months (top left), 41 months (top right), 58 months (bottom left) and 8 years (bottom right).

Table 1.1: List of publications arising from the seagrass rehabilitation program and directly associated projects since inception.

SARDI Reports
Seddon, S., D. Miller, S. Venema, and J. E. Tanner. 2004. Seagrass rehabilitation in Metropolitan Adelaide I. Transplantation from donor beds. SARDI Aquatic Sciences, Adelaide.
Seddon, S., R. J. Wear, S. Venema, and D. J. Miller. 2005. Seagrass rehabilitation in Adelaide metropolitan coastal waters II. Development of donor bed independent methods using *Posidonia* seedlings. SARDI Aquatic Sciences, Adelaide.
Wear, R. J., J. E. Tanner, and S. Venema. 2006. Seagrass rehabilitation in Adelaide metropolitan coastal waters III. Development of recruitment facilitation methodologies. Prepared for the Coastal Protection Branch, Department of Environment and Heritage. SARDI Aquatic Sciences Publication No. 04/0038-3. SARDI Aquatic Sciences, Adelaide.
Collings, G., S. Venema, R. J. Wear, and J. E. Tanner. 2007. Seagrass rehabilitation in metropolitan Adelaide IV. Geographic and interannual variability of recruitment facilitation. Prepared for the Coastal Protection Branch, Department for Environment and Heritage. SARDI Aquatic Sciences Publication No. F2007/000268-1. SARDI Aquatic Sciences, Adelaide.
Collings, G. 2008. Seagrass rehabilitation in Adelaide metropolitan coastal waters V. Large scale recruitment trial. Prepared for the Coastal Management Branch, Department for Environment and Heritage. SARDI Publication No. F2008/000077. SARDI Aquatic Sciences, Adelaide.
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.
Irving, A. 2009a. Reproduction, recruitment, and growth of the seagrass *Amphibolis antarctica* near the Bungala and Yankalilla rivers, South Australia. Final report prepared for the Coastal Management Branch of the Department for Environment & Heritage SA and the Adelaide & Mount Lofty Ranges Natural Resources Management Board. SARDI Publication Number F2009/000468-1. South Australian Research and Development Institute (Aquatic Sciences), Adelaide.
Irving, A. D. 2009b. Reproduction and recruitment ecology of the seagrass *Amphibolis antarctica* along the Adelaide coastline: Improving chances of successful seagrass rehabilitation. Final report prepared for the Coastal Management Branch of the Department for Environment & Heritage SA and the Adelaide & Mount Lofty Ranges Natural Resources Management Board. SARDI Publication No. F2009/000496-1. SARDI Aquatic Sciences, Adelaide.
Irving, A. D. 2009c. Seagrass rehabilitation in Adelaide's coastal waters VI. Refining techniques for the rehabilitation of *Amphibolis* spp. Final report prepared for the Coastal Management Branch of the Department for Environment and Heritage SA. SARDI Publication No. F2009/000210-1. SARDI Aquatic Sciences, Adelaide.
Tanner, J.E., and Theil, M.J. (2016). Adelaide Seagrass Rehabilitation Project: 2014-2016. Final report prepared for the Adelaide and Mount Lofty Ranges Natural Resources Management Board. South Australian Research and Development Institute (Aquatic Sciences), Adelaide. SARDI Publication No. F2009/000210-2. SARDI Research Report Series No. 914. 43pp.
Other reports
Delpin, M. W. 2014. Enhancing seagrass restoration: Improving hessian durability in marine environments. Final report to industry partners. ARC Linkage Grant LP0989354. Flinders University, Adelaide.
Seagrass Restoration Workshop Proceedings
Seddon, S., and S. Murray-Jones. 2002. Proceedings of the seagrass restoration workshop for Gulf St Vincent 15-16 May 2001. Department for Environment and Heritage and South Australian Research and Development Institute, Adelaide.
Murray-Jones, S. 2008. Proceedings of the second seagrass restoration workshop. Adelaide. April 2008. Department for Environment and Heritage, Adelaide.
Murray-Jones, S. 2013. Proceedings of the Third Seagrass Restoration Workshop. Adelaide. March 2013. Department for Environment, Water and Natural Resources, Adelaide.
Theses
Dobrovolskis, A.F. 2014. Reproduction in seagrasses and its potential implications for seagrass rehabilitation in Gulf St Vincent. Honours thesis, The University of Adelaide, Adelaide.
McSkimming, C. 2015. Stability and recovery of coastal ecosystems to local and global resource enhancement. PhD thesis, The University of Adelaide, Adelaide.
Papers
Seddon, S. 2004. Going with the flow: Facilitating seagrass rehabilitation. Ecological Management & Restoration 5:167-176.
Irving, A. D., J. E. Tanner, S. Seddon, D. Miller, G. J. Collings, R. J. Wear, S. L. Hoare, and M. J. Theil. 2010. Testing alternate ecological approaches to seagrass rehabilitation: links to life-history traits. Journal of Applied Ecology 47:1119-1127.

Wear, R. J., J. E. Tanner, and S. L. Hoare. 2010. Facilitating recruitment of *Amphibolis* as a novel approach to seagrass rehabilitation in hydrodynamically active waters. Marine and Freshwater Research 61:1123-1133
Irving, A. D., J. E. Tanner, and G. J. Collings. 2014. Rehabilitating Seagrass by Facilitating Recruitment: Improving Chances for Success. Restoration Ecology 22:134-141.
Tanner, J. E., A. D. Irving, M. Fernandes, D. Fotheringham, A. McArdle, and S. Murray-Jones. 2014. Seagrass rehabilitation off metropolitan Adelaide: a case study of loss, action, failure and success. Ecological Management & Restoration 15:168-179.
Tanner, J. E. 2015. Restoration of the Seagrass *Amphibolis antarctica* - Temporal Variability and Long-Term Success. Estuaries and Coasts 38:668-678.
McSkimming, C., S. D. Connell, B. D. Russell, and J. E. Tanner. 2016. Habitat restoration: Early signs and extent of faunal recovery relative to seagrass recovery. Estuarine Coastal & Shelf Science 171:51-57.
Paterson, J. S., S. Ogden, R. J. Smith, M. W. Delpin, J. G. Mitchell, and J. S. Quinton. 2016. Surface modification of an organic hessian substrate leads to shifts in bacterial biofilm community composition and abundance. Journal of Biotechnology 219:90-97.
York PH, TM Smith, RG Coles, SA McKenna, RM Connolly, AD Irving, EL Jackson, K McMahon, JW Runcie, CDH Sherman, BK Sullivan, SM Trevathan-Tackett, KE Brodersen, AB Carter, CJ Ewers, PS Lavery, CM Roelfsema, EA Sinclair, S Strydom, JE Tanner, KJ van Dijk, FY Warry, M Waycott & S Whitehead. 2017. Identifying knowledge gaps in seagrass research and management: An Australian perspective. Marine Environmental Research. 127: 163-172.

Table 1.2: Details of locations of all study sites used for seagrass rehabilitation off the Adelaide metropolitan coast. Mapped in Figure 1.3.

Study	Site	Year	Latitude	Longitude	Map Name
Seddon et al. 2004	Henley Beach	Feb/Mar 2003	-34.9154	138.4789	T'plant HB
	West Beach	Feb/Mar 2003	-34.9581	138.4887	T'plant WB
Wear et al. 2006	Multimethod 1	Sep 2004	-34.9005	138.4676	Multi 1
	Multimethod 2	Sep 2004	-34.8723	138.4633	Multi 2
Collings et al. 2006	Seacliff 8 m	Sep 2005	-35.0309	138.501	Sea 8m
	Brighton 12m	Sep 2005	-35.0286	138.4892	Bri 12m
	Brighton 10m	Sep 2005	-35.027	138.4945	Bri 10m
	Brighton 8m	Sep 2005	-35.023	138.5022	Bri 8m
	Henley 12m	Sep 2005	-34.9072	138.4331	Hen 12m
	Henley 10m	Sep 2005	-34.9091	138.4625	Hen 10m
	Henley 8m	Sep 2005	-34.9093	138.4674	Hen 8m
	Grange 12m	Sep 2005	-34.8999	138.4292	Gr 12m
	Grange 10m	Sep 2005	-34.9004	138.4376	Gr 10m
	Grange 8m	Sep 2005	-34.9008	138.4675	Gr 8m
	Semaphore 8m	Sep 2005	-34.8713	138.4579	Sem 8m
	Largs Bay 8m	Sep 2005	-34.8324	138.4472	Lar 8m
Collings et al. 2008	Lg-scale	Aug 2006	-34.9042	138.4708	Lg 2006
Irving 2009b	Grange	2009	-34.904	138.4708	Grange
Irving 2009c	Brighton	Sep 2007	-35.023	138.5022	Bri
	Grange	2007-2008	-34.904	138.4708	Grange
	Semaphore 8m	Sep 2007	-34.8713	138.4579	Sem 8m
	Largs Bay 8m	Sep 2007	-34.8326	138.4473	Lar 8m
Delpin 2014	Grange	2008-2013	-34.904	138.4708	Grange
Tanner 2015	Grange	2007-2013	-34.904	138.4708	Grange
Tanner & Theil 2016	2014 Lg scale 1	June 2014	-34.8987	138.4708	Lg1 2014
	2014 Lg scale 2	June 2014	-34.8701	138.4650	Lg2 2014
This study	2017	June 2017	-34.8663	138.4635	2017

Figure 1.3: Map of all sites used for seagrass rehabilitation research off the Adelaide metropolitan coast between 2003 and 2018. Details of each site are presented in Table 1.2.

1.2. Objectives

This report details experiments undertaken on seagrass rehabilitation off Adelaide's metropolitan coast between February 2016 and April 2019, as well as longer-term results from some of the earlier studies documented above. In particular, we look at three main components:

- The ongoing outcomes of two 1-hectare plots established in June 2014, and a third in June 2017, targeting *Amphibolis*, along with the utility of beach surveys for understanding the timing of recruit availability;
- Extension of the hessian bag technique to *Posidonia* seagrass;
- Assessment of how moisture content and bag storage influence the structural integrity of hessian sand bags prior to deployment.

2. *AMPHIBOLIS* LARGE-SCALE TRIAL

Apart from a single 1-hectare trial established in 2006, the research on seagrass rehabilitation off Adelaide to date has all been at very small scales. As the deployments have all been experimental in nature, most have consisted of 5-10 bags, with the second largest being 100 bags. All of these smaller deployments involved divers precisely positioning bags in close proximity to each other, and within ~ 50 m of the edge of a natural *Amphibolis* bed, so as to both maximize chances of success and facilitate future monitoring. However, this approach would be logistically difficult for larger-scale operational rehabilitation, and negates one of the major strengths of the hessian bag method, which is the ability to deploy the bags over the side of a boat and leave them where they fall without any diver intervention or associated costs. The 2006 1-hectare trial deployed 1,000 bags off the back of the RV Ngerin in a 100 x 100 m area to determine if this method of deployment did in fact produce similar results to those found in the smaller-scale diver deployments. Recruitment to these bags was relatively low, with only 1.35 recruits per bag after 4 months (Collings 2008), compared to densities up to 319 recruits per bag reported by Tanner (2015) for nearby small-scale deployments. However, the large scale deployment occurred at the end of August, based on the understanding at the time that recruitment was likely to peak in spring (Collings 2008). Following more detailed study of recruitment patterns throughout multiple years, we now know that recruitment peaks in winter, and can decline substantially by the end of August (Tanner 2015). There can also be substantial annual variability, although no small-scale experimental deployments were made in 2006 to determine whether it was a good or poor year for recruitment.

Following further detailed work between 2006 and 2014 on *Amphibolis* recruitment, two additional 1-hectare scale trials were established in 2014, each with 1,000 bags, to assess the feasibility of scaling up the hessian bag method to an operational scale (Tanner and Theil 2016). In addition, we established a fourth 1-hectare trial in 2017 with 2,500 bags, as part of the current study, to undertake a preliminary assessment of whether a greater bag density might enhance *Amphibolis* recruitment and establishment.

2.1. Methods

As with the 2014 deployments, the trial site was selected on the basis of aerial photography available from Google Earth, and the known distribution of *Amphibolis* along the Adelaide coast (Bryars 2008). The site was predominantly bare sand immediately inshore of the current seagrass line, and in relatively close proximity to the main small-scale experimental site, maximizing the likelihood that environmental conditions were suitable for seagrass rehabilitation. As in the previous deployments, the site was 100 x 100 m, but in this case 2,500 bags were deployed. As per normal, single bags were filled with 20 kg of clean silica-based builder's sand. Approximately 100 bags were dropped off the back of the RV Ngerin (Figure 2.1) as it steamed along each of eleven north-south and east-west transect lines spaced 10 m apart on each of 6 & 7 June 2017. Subsequent inspection by divers on 19 June 2017 showed that this produced distinct lines of bags, with some clumping along each line. Most bags landed flat, although some landed on their side. At this time, three east-west transects were marked through the site, with 16 bags on each pegged with two tagged stainless steel pegs to allow future re-identification. In addition, ten bags were deployed in a row ~ 0.5m apart at each of this site, the two 2014 sites, and the main small-scale site at Grange. These bags were located within 5-10 m of the seagrass edge, mimicking previous small-scale deployments, and were intended to allow direct comparison between recruitment rates at all 4 sites.

Figure 2.1: Pallets of bags ready for deployment on the RV Ngerin (top left), and typical images of deployment.

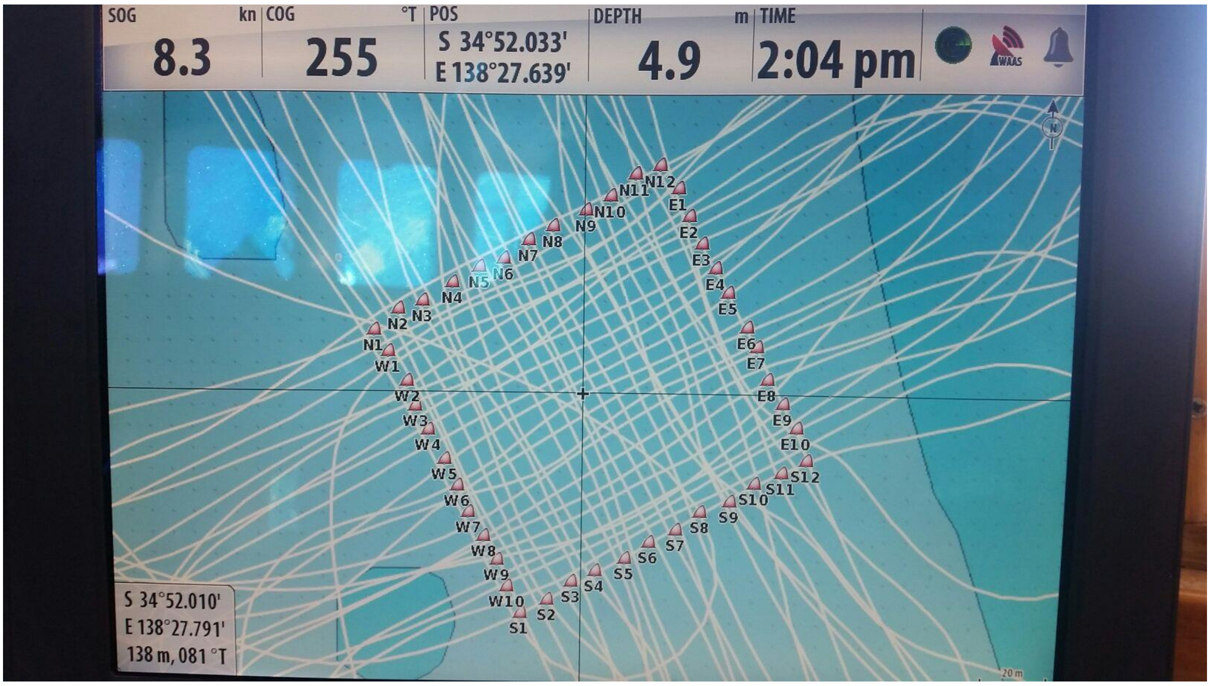

Figure 2.2: Vessel tracks during the deployment of 2,500 sandbags in June 2017 showing the lines along which bags were deployed.

Recruitment to these bags was first surveyed in January 2018 along the previously established east-west transects. Monitoring of these bags followed standard protocols, with the number of seedlings/stems being counted, and the length of three haphazardly selected seedlings/stems being measured. Surveys were attempted on the two 2014 trials at the same time, but the majority of marker pegs had been lost, making it impossible to survey individual bags as planned. These earlier trials were also surveyed in February 2017. As an alternative, in August 2018, and again in January 2019, a series of 3 randomly located 50 m long east-west transects were surveyed inside each plot, as well as north and south of each plot. Each transect was 1 m wide, and all *Amphibolis* stems and *Posidonia* leaves were counted to compare densities inside and outside the rehabilitation plots.

Data on stem counts on the 2014 trials between February 2015 and February 2017 were analysed using multivariate repeated measures analysis of variance (rmANOVA) in SPSS (ver 24), with plot and transect nested within plot as random factors, distance from the offshore edge of the plot as a covariate, and census date as the repeated measure. A similar approach was taken with stem length, except that as there were multiple measurements per bag at each census, bag was nested within transect, and as stems to measure were haphazardly chosen at each census, census was an orthogonal factor rather than a repeated measure (i.e. the analysis was a standard nested ANOVA). This extends the analysis of Tanner and Theil (2016) by a further year. For the transect data, a Generalised Linear Model with Poisson link function was used, with plot, transect location (north, inside, south) and census as orthogonal factors.

2.2. Results

The number of stems present on individual bags in the 2014 trials varied with time, with this variation being idiosyncratic between transects (rmANOVA: $F_{12,249}$=4.08, P<0.001). As can be seen in Figure 2.3, most of this variation occurred within plot 1, while plot 2 showed little change between transects or over time. Overall, there were 6.2 (± 0.4 se) stems per bag at the first census 8-9 months after deployment, increasing to 7.2 (± 0.9) and 9.2 (± 0.8) after an additional 6 and 12 months respectively. A further 12 months later, in February 2017, this had declined to 3.1 (± 0.5). Initial recruitment (after 8 months) was very low compared to that found on small-scale winter deployments in previous years, and similar to that found on summer deployments (Figure 2.4a). After 20 months, however, stem abundances were similar to those found after 20 months on bags deployed in the winter of 2011, although still lower than those found on bags deployed in the winters of 2008 and 2009 (Figure 2.4b). A further 12 months later, stem abundances were again comparable to those of summer deployments of the same age (Figure 2.4c). There was a significant effect of distance from the edge of the plot on stem abundances (rmANOVA: $F_{1,83}$=5.7, P=0.19, Figure 2.5), unlike what was found for the first 3 surveys (Tanner and Theil 2016). In 2017, higher values of stem abundance occurred further from the existing seagrass line, although variability also increased (Figure 2.5).

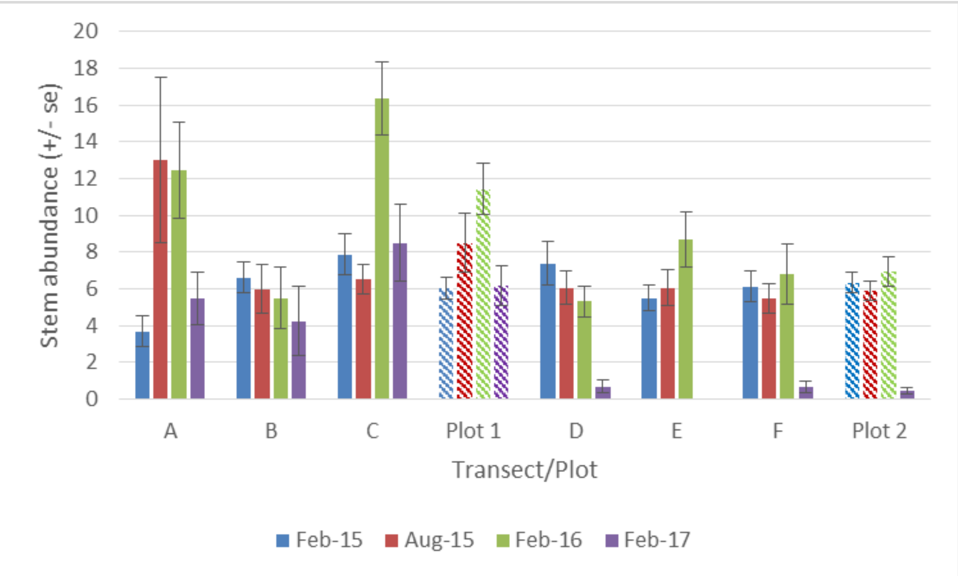

Figure 2.3: Mean *Amphibolis* stem abundances on bags at 8 to 32 months after deployment in June 2014 in two 1-hectare scale seagrass rehabilitation plots. Plot 1 is the average of transects A-C, and plot 2 of D-F.

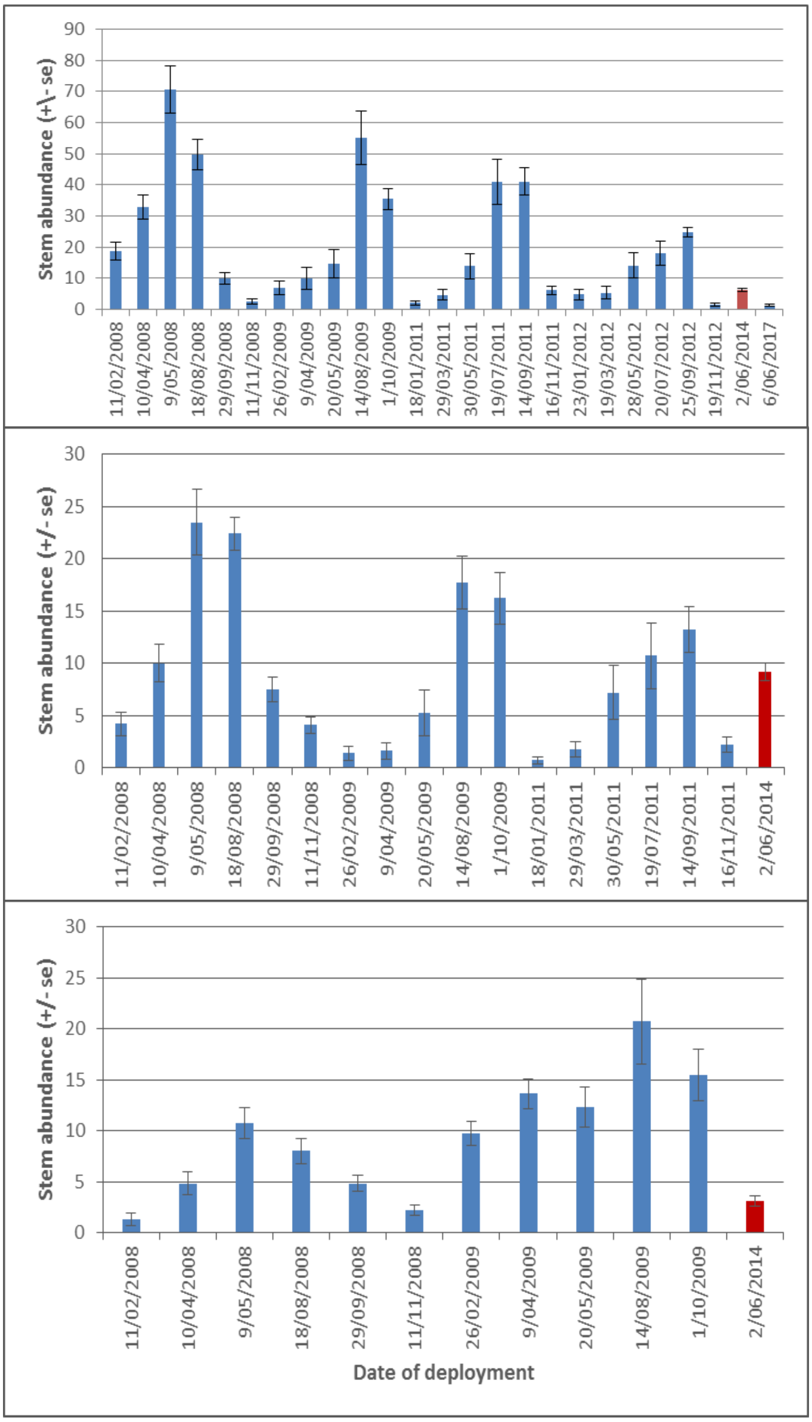

Figure 2.4: *Amphibolis* stem abundances on bags a) in January/February of the year following deployment (top); b) in January/February of the subsequent year (middle); and c) January/February 3 years later (bottom). Red bar indicates results from the 2014 large-scale trials. The final (purple) bar in the top panel indicates results from the 2017 large-scale trial.

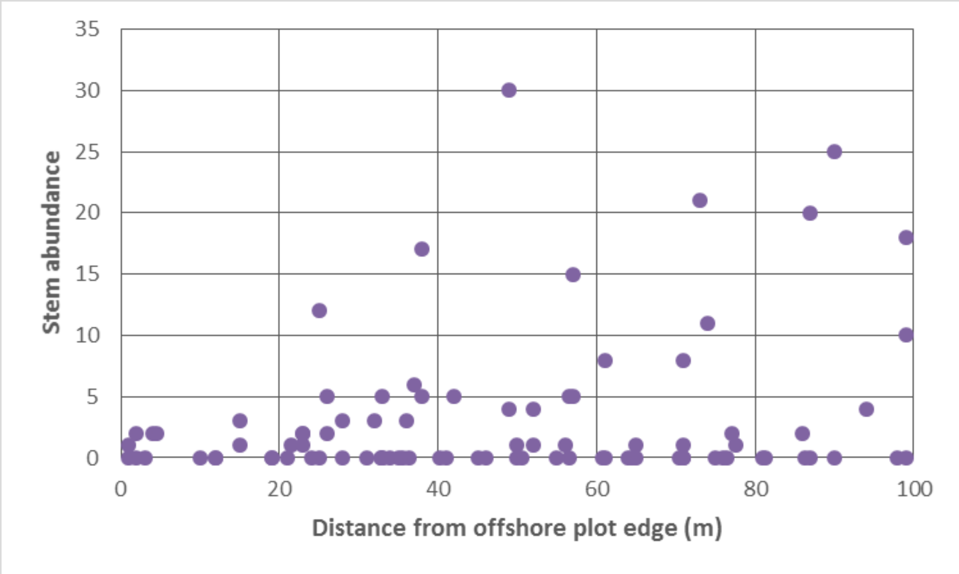

Figure 2.5: Influence of distance from the offshore margin of the plot on *Amphibolis* stem abundance 32 months after deployment in June 2014 in two 1-hectare scale seagrass rehabilitation plots.

Stem length in the 2014 trials varied between bags differently between censuses, although did not vary between plots (Table 2.1). For the most part, length increased over time, with some minor exceptions (Figure 2.6). Eight months after deployment, mean stem length was 12 ± 0.3 cm, which was relatively low compared to previous small-scale deployments (Figure 2.7a). After 20 months, stem length had increased to 16.1 ± 0.5 cm, and was starting to catch up to what was found in previous small-scale deployments (Figure 2.7b). A further 12 months later, it was 20.2 ± 1.8 cm, and still trailing what was found in the small-scale deployments (Figure 2.7c).

Table 2.1: ANOVA table for *Amphibolis* stem length in two 1-hectare rehabilitation trials.

Term	df	MS	F	P
Census	3	1014	4.15	0.14
Plot	1	1543	10.82	0.075
Transect(Plot)	4	11.4	0.34	0.85
Bag(Transect(Plot))	90	92.4	1.56	**0.005**
Census * Plot	3	242	2.45	0.11
Census * Transect(Plot)	11	105	1.82	0.052
Census * Bag(Transect(Plot))	214	59.7	1.53	**<0.001**
Residual	584			

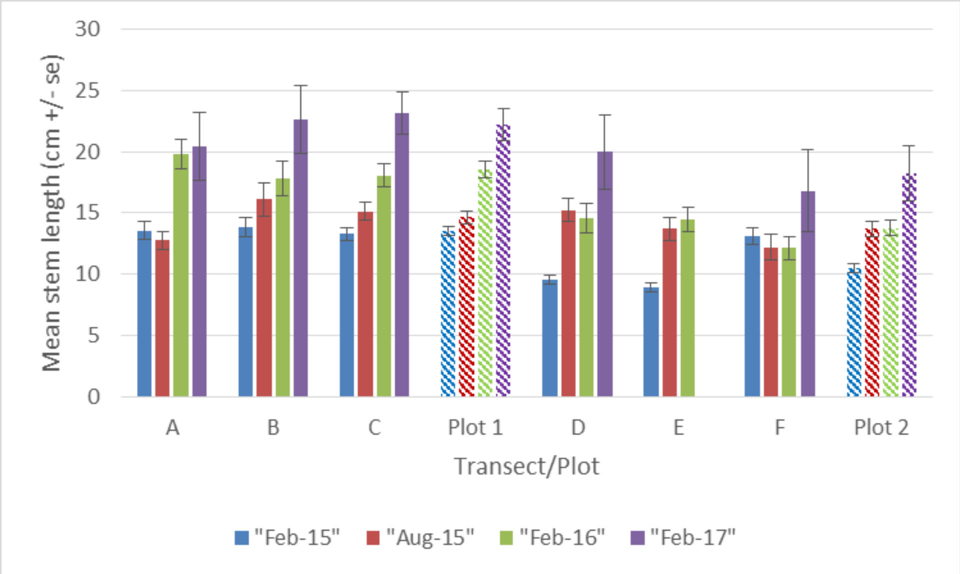

Figure 2.6: Mean *Amphibolis* stem length on bags at 8 to 32 months after deployment in June 2014 in two 1-hectare scale seagrass rehabilitation plots. Plot 1 is the average of transects A-C, and plot 2 of D-F.

The 2017 trial performed very poorly in terms of stem abundance per bag, having only 1.2 ± 0.3 stems per bag the following February (Figure 2.4a). This was lower than even any of the summer deployments, which occurred outside the main recruitment season. Those seedlings that did attach and survive grew at a relatively typical rate, however, with stem lengths being about the same as those on the 2014 trials at a similar age, and only slightly less than those in the smaller-scale trials (Figure 2.7a).

Due to the extensive loss of marker stakes, it was not possible to measure *Amphibolis* abundance on the bags in 2018 or 2019. Instead, belt transects were used to assess differences between each of the 3 plots, and immediately north and south of each. For *Amphibolis*, there was a highly significant 3-way interaction between plot, location (north, inside, south) and census (Poisson GLM, χ^2_3=35.1, p<0.001). Breaking this down further, there were no effects of either location or census for 2014 plot 1, while the interaction between these two variables was significant for 2014 plot 2 and 2017. For the former, there was slightly higher recruitment inside the plot in 2018, and significantly higher densities of *Amphibolis* inside in 2019 (Figure 2.8). For the later, densities were higher inside the plot in 2018, but to the north of the plot in 2019. For *Posidonia*, there was also a 3-way interaction between plot, location and census (Poisson GLM, χ^2_4=112, p<0.001). In this case, the interaction between location and census was significant for all 3 plots. There were no consistent differences between inside and outside, with the main patterns instead being north-south gradients, especially in 2019 (Figure 2.8).

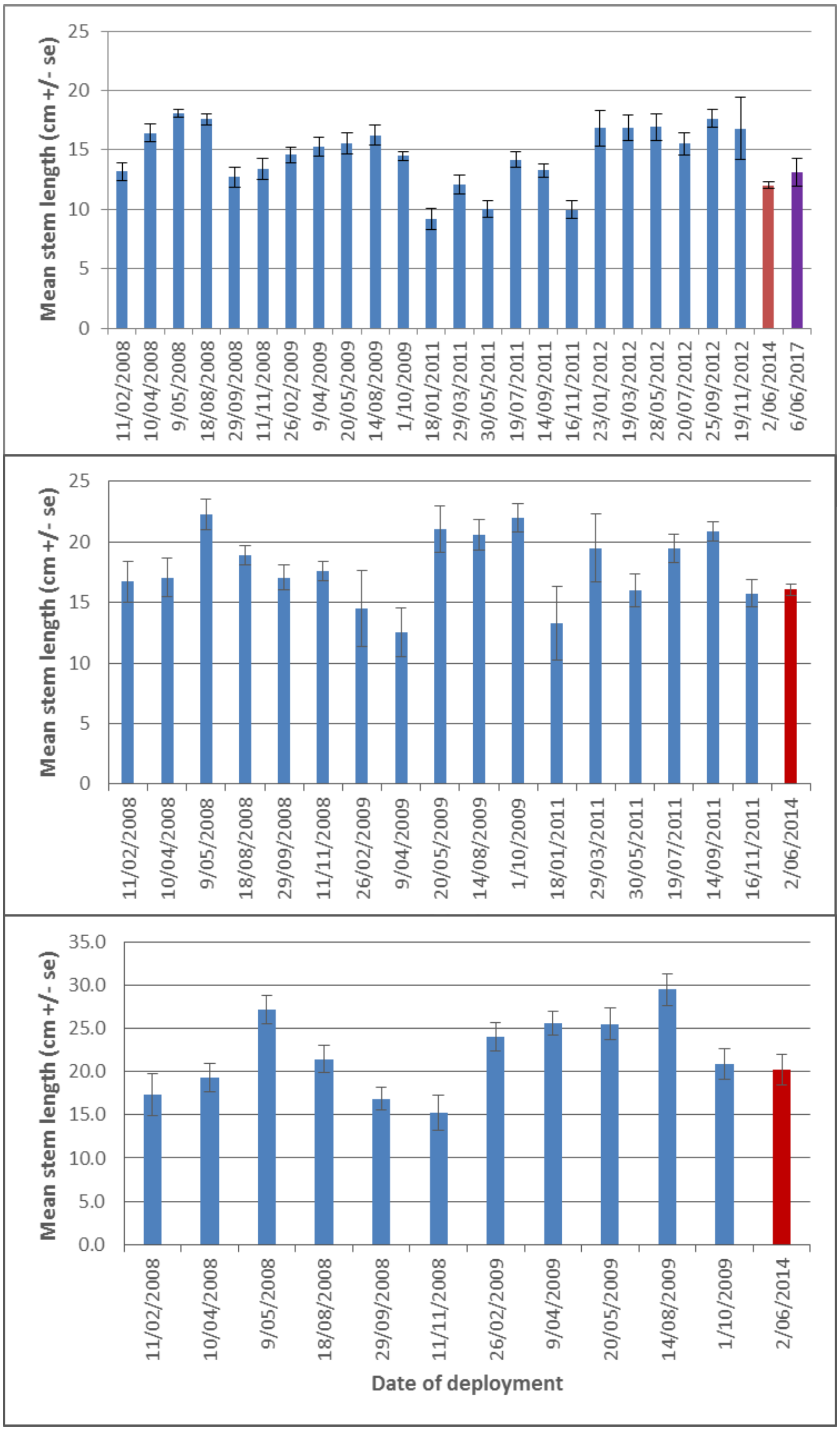

Figure 2.7: *Amphibolis* stem lengths on bags a) in January/February of the year following deployment (top); b) in January/February of the subsequent year (middle); and c) January/February 3 years later (bottom). Red bar indicates results from the 2014 large-scale trials. The final (purple) bar in the top panel indicates results from the 2017 large-scale trial.

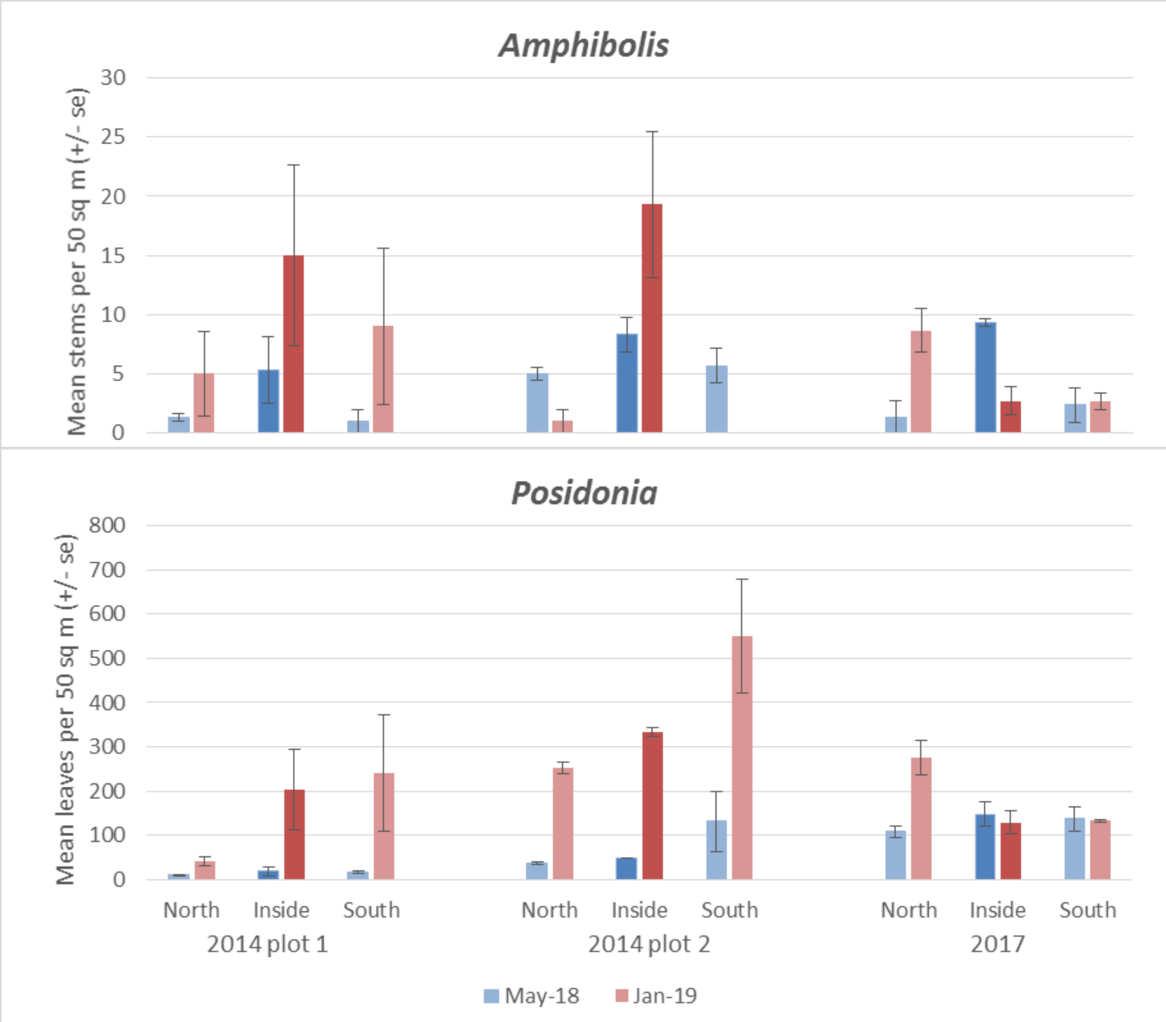

Figure 2.8: Stem and leaf densities for *Amphibolis* and *Posidonia* in 50 m^2 belt transects at each 1-hectare plot.

In June 2017, as well as deploying a 1-hectare trial, 10 additional bags were deployed at each of the three large-scale sites discussed here, as well as at the main small-scale experimental site at Grange. These bags were set up to mimic previous small-scale experiments, to better determine if low recruitment onto the large-scale sites was due to recruit supply, or differences in bag deployment methods/layout. When surveyed in January 2018, the bags at the 2014 plot 1 site (Lg1 2014 on Figure 1.3) had similar numbers of recruits to those at Grange, while those at 2014 plot 2 had ~ half as many, and those at the 2017 site had only 10% (Figure 2.9). A year later, all seagrass had been lost from the bags at Grange and 2014 plot 1, while only a few stems remained at the other 2 sites. Grange and 2014 plot 1 are only ~ 600 m apart, while 2014 plot 2 and 2017 are ~ 400 m apart, with ~ 3.2 km between the two pairs of sites.

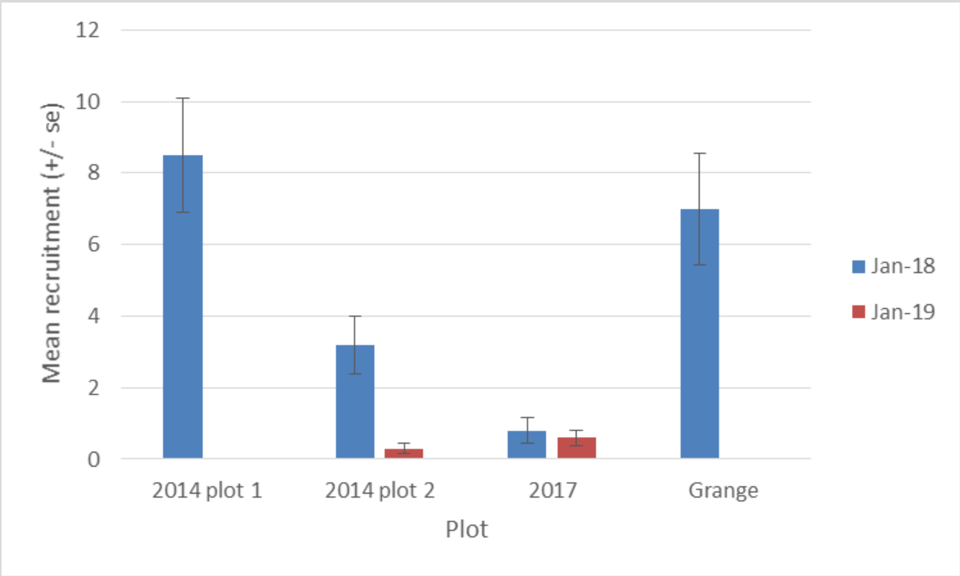

Figure 2.9: Results of small-scale deployments of 10 bags in June 2017 at the large-scale sites, and the Grange small-scale experimental site.

2.3. Discussion

Both the 2014 and 2017 1-hectare trials performed poorly relative to the earlier small-scale trials undertaken at Grange. While the 2014 trials showed a greatly improved performance after 20 months relative to the small-scale trials, after an additional 12 months they had again declined to very low stem abundances of *Amphibolis*. Small-scale trials at each site mimicking the Grange trials suggested that the poor performance in 2017 was not due to differences in deployment methodology, but rather appeared to be a feature of the site chosen. Recruitment to these small-scale trials did not reflect the differences in initial recruitment to the 2014 1-hectare trials, with both plots having similar recruitment levels in 2014, but plot 2 having much lower levels in 2017. Those plants that did survive on each of the 1-hectare plots did show close to the level of growth found in the earlier small-scale trials, however, suggesting that the issues experienced revolved around recruitment and/or survival, rather than conditions for growth. Despite this, there were greater *Amphibolis* densities in some of the plots than outside the plots, whereas the same was not found for *Posidonia*, suggesting that the bag deployment did lead to some increase in *Amphibolis* density.

There are 4 factors that may have contributed to the poor performance of these large-scale plots compared to previous small-scale experimental work:

a) Site location. The limited work we have done to assess spatial variability in restoration success suggests that it can be highly variable between sites even a few kilometres apart.

This is supported by the high variability between the small-scale deployments undertaken at each of the large-scale plots, and the main Grange study site used for previous small-scale experimental work, as reported in the previous milestone. We would recommend that a combination of modelling studies and small-scale *in situ* trials be used to select sites for larger scale restoration in the future. Having a greater diversity of sites along the coast will also help to determine what the key factors limiting success might be, and if there is a requirement for further improvement in habitat conditions along parts of the coast before rehabilitation can be implemented.

b) Bag density. All three 1-hectare trials used relatively low bag densities (either 1,000 or 2,500 bags), in comparison to the small-scale trials which were equivalent to ~ 10,000 bags per hectare. Higher bag densities are likely to have a greater effect in ameliorating water and sand movement, thus leading to greater rehabilitation success. Conversely, higher bag densities mean less area rehabilitated for a given cost. We would suggest that multiple 1-hectare plots with different bag densities be considered, to help decide on the best bag density to use in any future rehabilitation efforts.

c) Suspected disturbance by fishing and/or anchoring activity. For all three trials, we were unable to locate sufficient numbers of our marker pegs to resurvey individual bags in either 2018 or 2019. This observation suggests that the sites were exposed to a substantial physical disturbance. Each of the monitored bags was marked by two 54 cm long stainless steel pegs, bent over on top and individually numbered, pushed 40-45 cm into the sediment. Most of these were completely missing, although a few were located lying on the seafloor, sometimes 50 m or more from where they were deployed. In addition, the bag monitoring transects were marked by star pickets spaced 20-25 m apart, and driven in at least 50 cm, or until solidly lodged in the underlying calcrete. Some of these pickets were also missing.

Although the timing of stake loss has not been recorded, it does not appear to be focused around the occurrence of major storm events. Instead, it is thought that fishing and boating activity is most likely to be the cause. The trials described here have all been conducted along the boundary between the remaining seagrass and the inshore bare sand. This area is also fairly heavily fished. While line fishing is unlikely to have much impact, crab nets and pots are likely to become hooked on the stakes and pull them up when retrieved, and boat anchors would also pull them out if dragged across restoration plots. If this is occurring, then these activities may also be negatively affecting naturally recruited seagrass seedlings.

d) Bag deterioration. Anecdotal evidence from some previous deployments suggests that bags may deteriorate if left exposed to the air for considerable periods between filling and deployment. A series of trials to examine the influence of storage conditions and time after filling is detailed in Chapter 6.

It also needs to be remembered that the small-scale trials conducted previously (Tanner and Theil 2016) took at least 5-6 years before showing signs of success, and it cannot be expected that these larger-scale trials, with lower bag densities, would succeed in any less time than this. Indeed, the lower densities suggest that it may actually take longer, potentially in the vicinity of ten years, before success can be truly established.

In a recent review of marine restoration, (Bayraktarov et al. 2016) identified that the median cost of seagrass restoration was on the order of $US400,000 per hectare. Similarly, (Busch et al. 2010) identified costs ranging between $US80,000 and $US3 million per hectare for transplantation, although seed-based methods ranged from $US6,000 to $US170,000. While it is difficult to assess costs of operational rehabilitation using the hessian bag technique, we have recently calculated costs for experimental deployment of ~$A60,000 per hectare (or ~$US40,000). Moving to an operational phase would potentially further reduce costs by a factor of 2 or 3. These figures suggest that this technique is one of the most cost-effective methods of seagrass rehabilitation currently available, which is particularly due to the removal of divers from the operation. One of the trade-offs for this low cost, however, is that it is also a long-term process, which may take 5-10 years to produce fully functioning seagrass meadows.

3. *AMPHIBOLIS* BEACH SURVEYS

Previous work to establish the timing of *Amphibolis* seedling availability has focused on regular deployment of hessian bags throughout the year (Tanner 2015, Tanner and Theil 2016). This showed that the best time to deploy bags was over the winter months, both for initial recruitment, and for long-term success. However, because of the logistics and costs of deploying and monitoring bags, this was done at most bimonthly, and thus cannot provide finer temporal scale information on recruit availability Here we present the results of preliminary trials of beach surveys to help provide greater clarity on the timing of seedling release.

3.1. Methods

In the winters of 2017 and 2018, SARDI hosted interns to undertake beach surveys for *Amphibolis* seedlings immediately in front of the South Australian Aquatic Sciences Centre, West Beach. In 2017, surveys were undertaken between the 29th of May and the 20th of July. In 2018, surveys commenced on the 18th of June and went until the 3rd of September. In general, there were 3-4 surveys done per week. On each survey, the number of seedlings present along the latest high tide strand line was counted over a set section of beach (Figure 3.1). Whilst the same section of beach was surveyed every day, the results should only be considered semi-quantitative, as there was no attempt to ensure every single seedling was counted if there was a large amount of wrack washed up onto the beach. There was also no standardization for tidal phase. The general patterns, however, can be considered indicative of when recruits are available.

Figure 3.1: Map showing section of beach (red line) surveyed for *Amphibolis* seedlings in 2017 and 2018.

3.2. Results and Discussion

In 2017, seedlings first appeared on the beach in early July (Figure 3.2). While there is an apparent peak on the 19th of July, surveys ceased the day after, and so it is not known how long seedlings continued to wash ashore for. In 2018, there was a slower build up, with much higher peaks on the 9th and 12th of July, followed by a slow decline, with only a single seedling washing up from the 21st of August until the cessation of surveys on the 3rd of September.

Both surveys indicate that the primary recruitment season is in July, with some seedlings available in June and August. This suggests that May and June are likely to be the best months to deploy hessian bags to facilitate recruitment of *Amphibolis*.

An attempt was made to grow seedlings in the outdoor tanks at SARDI in both years, with little success. While seedlings were collected soon after high tide, it is possible that even a short period of exposure on the beach resulted in sufficient dehydration to limit their viability. Future attempts to grow *Amphibolis* seedlings in the lab may therefore need to involve collecting them while still in the water, as has been successfully done by Burnell et al. (2014).

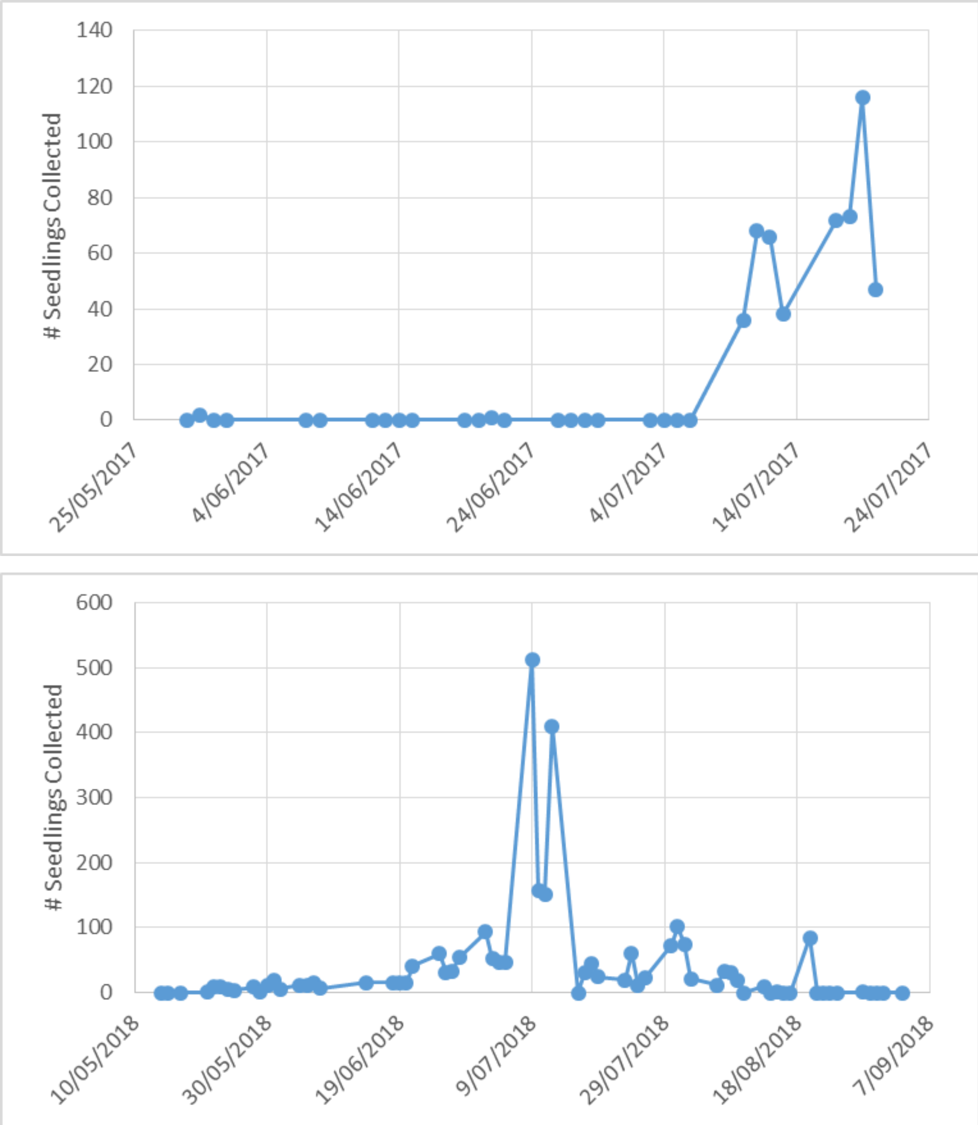

Figure 3.2: Abundance of *Amphibolis* seedlings washed onto the beach at West Beach in winter of 2017 and 2018.

4. *POSIDONIA* FIELD EXPERIMENTS

With the exception of the initial studies on transplantation and use of seedlings for rehabilitation (Seddon et al. 2004, Seddon et al. 2005), all of the work on seagrass rehabilitation off the Adelaide coast has focused on *Amphibolis*, as this is the only genus with a life history strategy that suits the use of hessian bags to facilitate recruitment. While *Amphibolis* has experienced major declines in the last 50 years, and appears to be the most sensitive genus to reductions in water quality, there have also been major declines in *Posidonia* (Bryars 2008, Bryars et al. 2011). It is thus of interest to determine if the hessian bag method of rehabilitation can be adapted for use with *Posidonia*. Oceanica Consulting Pty. Ltd. (2011) have undertaken some initial trials with *P. australis* in Western Australia, with good short-term success in the first, but problems with hessian quality in subsequent trials, and only short-term (< 1 year) monitoring. As this genus reproduces via the production of fruits in which seeds germinate just prior to release from the parent plant, and the resultant seedlings lack any apparatus that would promote entanglement in hessian, fruits were collected and dehisced in the laboratory prior to being manually planted by divers into bags on the seafloor.

4.1. Methods

An initial experiment planting *Posidonia* seedlings into hessian bags was conducted in January 2012, at the main small-scale rehabilitation site used for *Amphibolis* experiments. Fruits of *Posidonia angustifolia* were harvested directly from the parent plants by divers on the 21st of December 2011, offshore from Brighton, in ~ 9 m water depth. At this time, fruits had started to wash up onto the adjacent beaches, suggesting that they were ready, and many harvested fruits released from the parent plants with only the slightest touch. Fruits were returned to the laboratory at West Beach, where they were maintained in mesh bags (mesh size < 1 mm) in flow through seawater until they had dehisced and were ready for planting. Every 2-3 days, these bags were sorted to remove dehisced fruits and seedlings, with the former discarded and the later transferred to new bags. At the same time, all bags of seedlings were resorted to remove any dead material, and any amphipods that may have grazed the seedlings. Remaining seedlings were planted into bags on the seafloor on the13th of January 2012.

This experiment focused on examining the effect of different substrate mixes on *Posidonia* seedling growth and survival, with bags being filled with different mixes of sand and clay. The ratios of sand:clay used were 30:70 (n=9), 50:50 (n=9), 70:30 (n=8) and 100:0 (n=5). The last is the same as has been used for the majority of the *Amphibolis* trials. Three hundred grams of dried chopped *Posidonia* seagrass was mixed into each bag to provide a source of organic matter. All other aspects of the bags and how they were deployed, were the same as for the *Amphibolis* trials in Chaper 2. A diver haphazardly selected 10 seedlings from the pool for each bag, and manually planted them into the bag in a roughly uniform arrangement, maintaining a 10 cm unplanted border around the edge of the bag. The weave of the bag was gently teased apart to create a small planting hole, the seedling inserted so that the leaf shoot projected from the bag, and then the weave teased back together to hold the seedling in place. Bags were surveyed approximately every 2 months until May 2013, then 6-monthly until February 2016, and then annually until January 2019. For the first 3 surveys, only the number of surviving seedlings on each bag were counted. Subsequently, the number of leaves on each seedling, and maximum leaf length, were also measured. Once individual seedlings started to produce multiple shoots, shoots were counted as it was not possible to determine which shoots belonged to the same plant without causing excessive disturbance. By February 2015, sediment depth over the bags was sufficient that it was sometimes difficult to allocate leaves to individual shoots, and hence only leaves were counted.

A second set of experiments were established in February 2013. Fruits were again collected from *P. angustifolia* offshore from Brighton by divers, this time on the 6th of December 2012, and maintained as per the previous experiment until planting out on the 8th of February 2013. Four different experiments were established in 2013. The first replicated the 2012 experiment, with 10 bags per treatment (9 for the 70:30 mix). The second examined the influence of different levels of organic matter in the sand mix, with 0, 300, 600 or 900 g of dried chopped *Posidonia* per bag. Experiment 3 looked at the influence of seed size, with seeds being < 10 mm, 10-13 mm or > 13 mm. The final experiment examined planting density, with either 5, 10 or 16 seedlings per bag. Apart from the parameter of interest, all other details for each experiment were as per the 2012 experiment. Bags were surveyed on 19/3/2013 and 29/5/2013, then 20/2/2015, 28/8/2015, 12/2/2016, 8/2/2017, 18/1/2018, and 15/1/2019.

A third deployment of bags with *Posidonia* seedlings was undertaken in January 2017. This experiment aimed to determine if it was feasible to pre-plant seedlings into bags prior to deployment. Fresh fruit were collected off the beach at West Beach in late December on this occasion (Figure 4.1), and kept as for the previous experiments until planting on the 25th of January 2017. Ten bags were planted once deployed, as per previous experiments, ten were planted in the boat immediately prior to deployment, and ten were planted onshore prior to being transported to the deployment site. Seedlings in pre-planted bags were glued into place by running a bead of super-glue around them. Pre-planted bags were dropped over the side of the boat with the seedlings on the upper surface, and were then relocated underwater to facilitate monitoring. All bags received ten seedlings. These bags were surveyed immediately following deployment and relocation on the seafloor, ~ 2 weeks later, and then 1 and 2 years later. Leaf length was not measured on the second survey, as 2 weeks was insufficient time for appreciable growth to be expected.

Figure 4.1: Example of *Posidonia angustifolia* fruits offshore from Brighton (left) and collected from West Beach on the 14th of December 2017 (right).

Data on seedling and leaf number for individual surveys in each experiment were analysed using generalized linear mixed effects models with a log link-function (number), while those on leaf length were analysed using linear mixed effects models, with the package lme4 (Bates et al. 2015) in R (ver 3.3.0). In all cases, fill and date were treated as fixed effects with an interaction term, while bag was included as a random effect. Generalised linear models were used to examine the results for individual years. When needed, Tukey's post-hoc tests were undertaken using the package multcomp (Hothorn et al. 2008).

4.2. Results

2012 Experiment

The number of seedlings present generally declined over time, although for the 50:50 sand:clay mix, this trend reversed after ~ 1 year, while for the 30:70 mix it reversed after ~ 2 years (Figure 4.2a). This reversal is likely to indicate that the original seedlings had started to produce rhizomes with new shoots. The mixes with higher proportions of sand (100% and 70%) continued to decline over time. After three years, it was difficult to confidently identify individual seedlings in any fill type, and seedling counts were discontinued. Seedling counts differed significantly between treatments at the final reliable seedling count in August 2014 (GLM: $\chi^2_{3,26}$=21.2, P<0.0001), with post-hoc tests indicating that the 100% sand mix had significantly fewer seedlings than the 50:50 and 30:70 mixes.

Summer leaf counts consistently increased from 2012 to 2016, although there were declines in the winter of 2014, and especially 2015 (Figure 4.2b). With decreased light availability and water temperature, a loss of leaves over winter is normal for this species. After 2016, summer leaf counts declined to a minimum in 2018, with only a small recovery in 2019. Through the middle part of the study, leaf counts were highest on the 50:50 bags. At the final census, there was a significant difference between fill types (GLM: $\chi^2_{3,27}$=81, P<0.0001), with post-hoc tests showing 50:50=70:30>100:0>30:70.

Leaf length increased up to February 2015, and then declined somewhat, especially in the 100% sand and 70:30 mixes in 2017 (Figure 4.2c). There were no differences between treatments at the final census (GLM: $\chi^2_{3,315,}$=0.70, P=0.87).

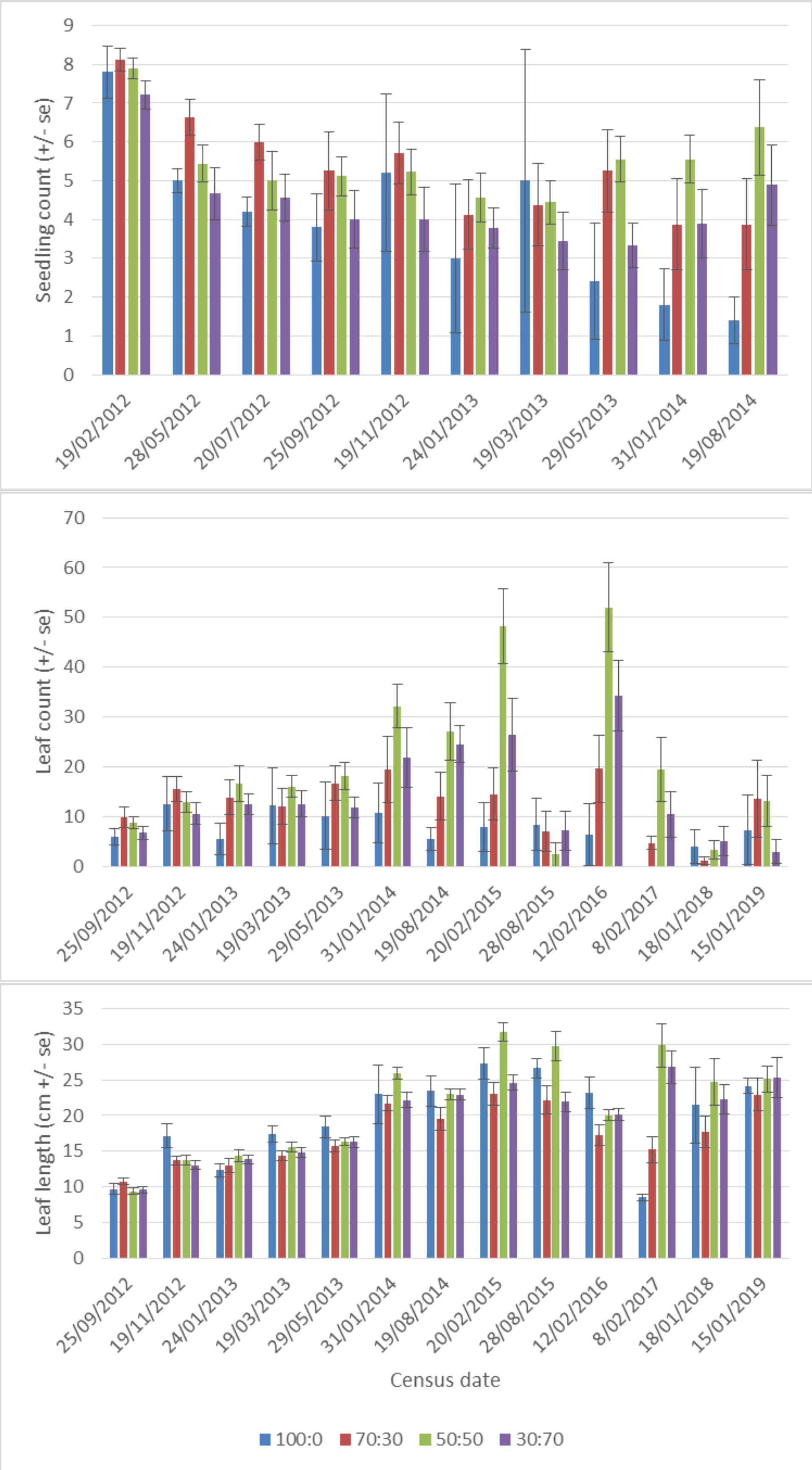

Figure 4.2: Influence of fill type on a) survival (top), b) number of leaves (middle) and c) leaf length (bottom) of *Posidonia* seedlings planted into hessian bags with different proportions of sand:clay (legend) in 2012.

2013 Experiments

Fill type

There was a consistent decline in seedling abundance over time, with no clear differences between fill types (Figure 4.3a). At the final census, there were no differences between fill types (GLM: $\chi^2_{3,33}$=1.92, P=0.59). Leaf count fluctuated, and tended to be low in winter, except in 2015. In summer 2017, 2018 and 2019, abundance was low but relatively stable (Figure 4.3b). At the final census, the 30:70 (sand:clay) mix had the highest leaf count, with the other 3 treatments being equal (GLM: $\chi^2_{3,39}$=547, P=0.012). Leaf length increased over time, before dropping slightly at the final census (Figure 4.3c), with 100% sand being lower than 70%, but no other differences between treatments (GLM: $\chi^2_{3,38}$=1696, P=0.025).

Organic matter addition

There was again a consistent decline in seedling abundance over time, with no differences between fill types (Figure 4.4a). At the final census, there were no differences between fill types (GLM: $\chi^2_{3,28}$=3.46, P=0.33). Leaf count reached a minimum in August 2014, and then increased and fluctuated to the final census (Figure 4.4b), differences between treatments varied over time, and at the final census the highest leaf counts were for the 0 and 900 g addition treatments (GLM: $\chi^2_{3,39}$=80.2, P<0.0001), although apart from the 600g and 300g treatments, all treatments differed. Leaf length increased over time, although declined slightly at the final census (Figure 4.4c), with no differences between treatments (GLM: $\chi^2_{3,72}$=473, P=0.27).

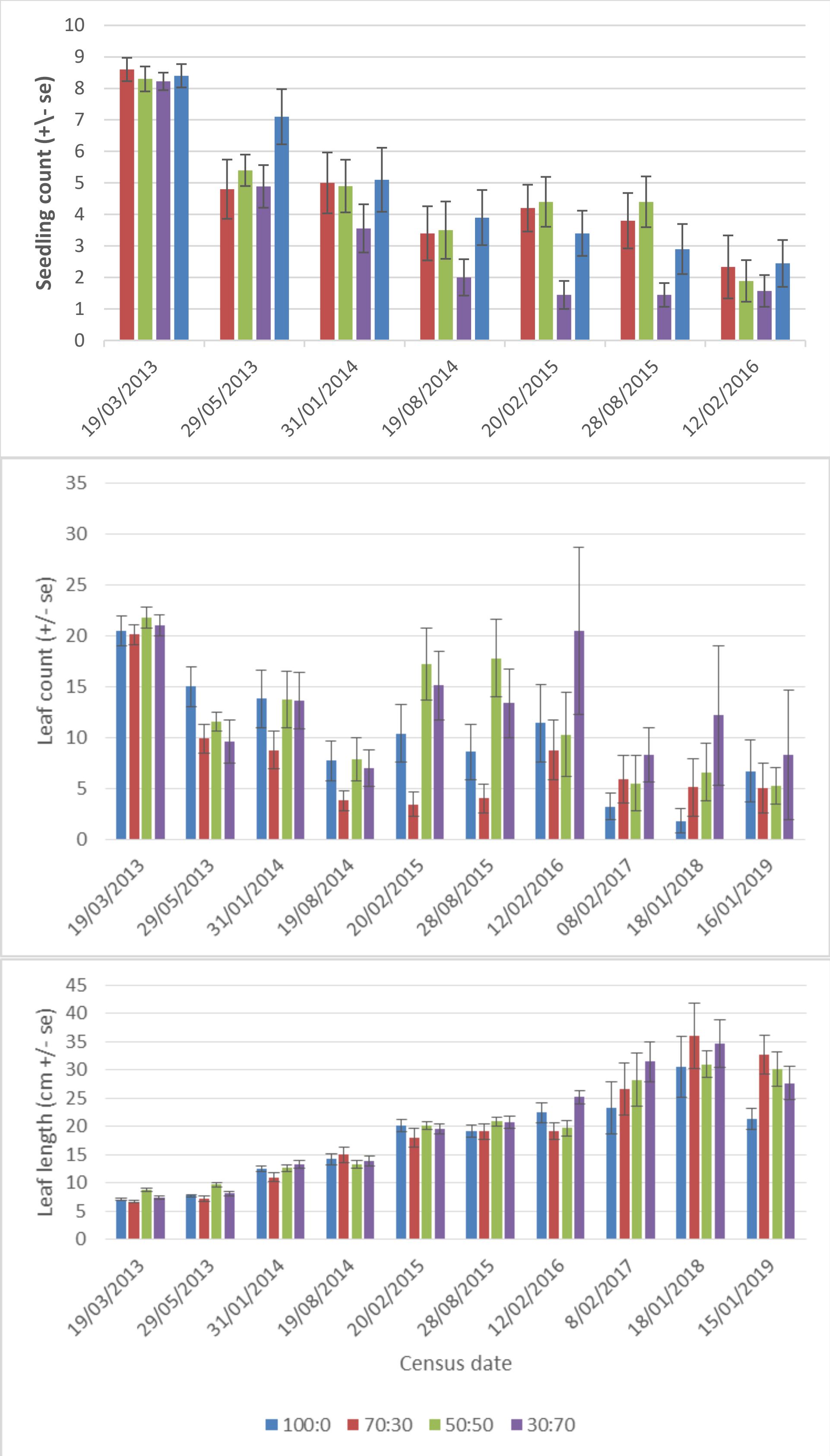

Figure 4.3: Influence of fill type on a) survival (top), b) number of leaves (middle) and c) leaf length (bottom) of *Posidonia* seedlings planted into hessian bags with different proportions of sand:clay (legend) in 2013.

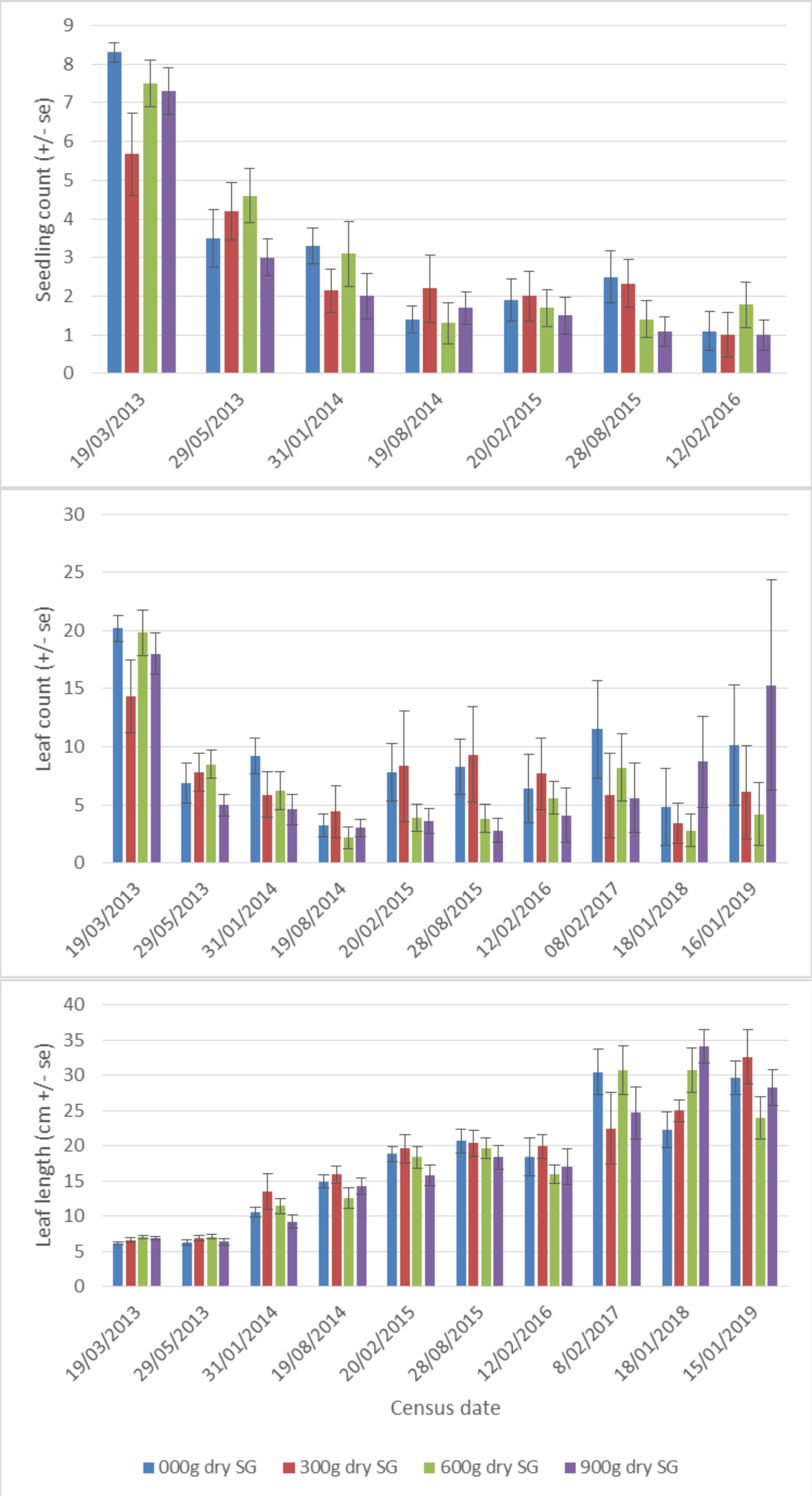

Figure 4.4: Influence of organic matter addition on a) survival (top), b) number of leaves (middle) and c) leaf length (bottom) of *Posidonia* seedlings planted into hessian bags with different amounts of dried chopped *Posidonia* leaf matter added in 2013. Legend indicates amount of organic matter added in grams.

Seed size

There was again a consistent decline in seedling abundance over time (Figure 4.4a), and while large seeds (> 13 mm long) appeared to show better survival in the final 2 years of the study, there were no significant differences at the final census (GLM: $\chi^2_{2,25}$=5.29, P=0.071). Leaf abundance again reached a minimum in August 2014, and then fluctuated but was generally higher by the final census (Figure 4.4b), when medium seeds produced more than twice as many leaves as large seeds, which in turn produced twice as many as small seeds (GLM: $\chi^2_{2,28}$=60, P<0.001). Leaf length increased over time, stabilizing over the final 3 years of the study (Figure 4.4c), with no differences between treatments (GLM: $\chi^2_{2,67}$=3.64, P=0.16).

Seedling planting density

While there were initially greater numbers of seedlings on bags which were planted with greater numbers, this difference disappeared within a year (Figure 4.5a), and there were no differences at the final census (GLM: $\chi^2_{2,21}$=1.15, P=0.56). Leaf abundance showed the same pattern, and was consistently low throughout the final five years of the study (Figure 4.5b). At the final census, bags planted with 16 seedlings had ~ twice as many leaves as those planted with 5 (GLM: $\chi^2_{2,29}$=10.5, P=0.005). Leaf length increased over time (Figure 4.5c), with bags planted with 16 seeds having shorter leaf lengths than those planted with 10 (GLM: $\chi^2_{2,42}$=1522, P=0.021), although this was unique to the final census.

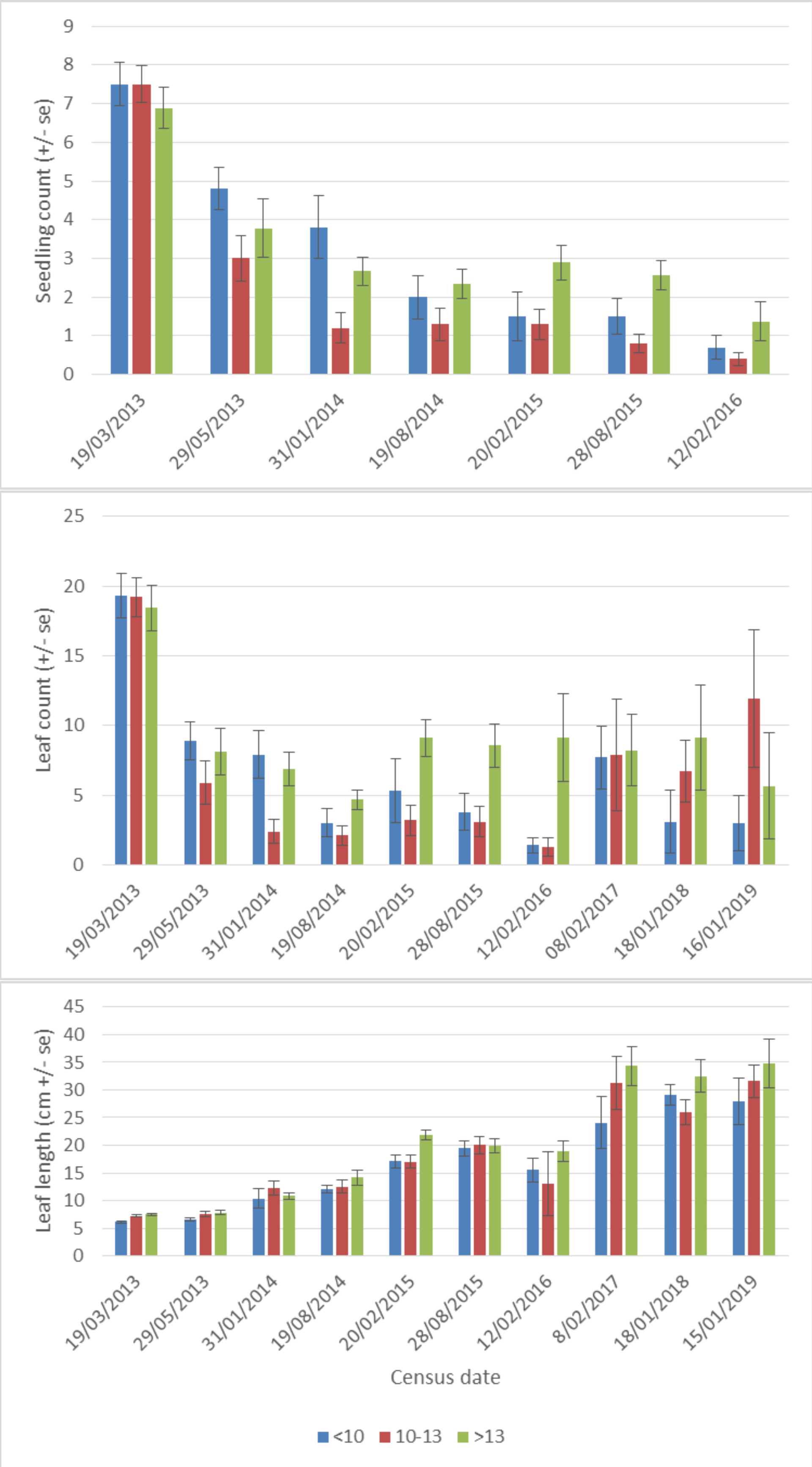

Figure 4.5: Influence of seed size on a) survival (top), b) number of leaves (middle) and c) leaf length (bottom) of *Posidonia* seedlings planted into hessian bags in 2013. Legend indicates seed size in mm.

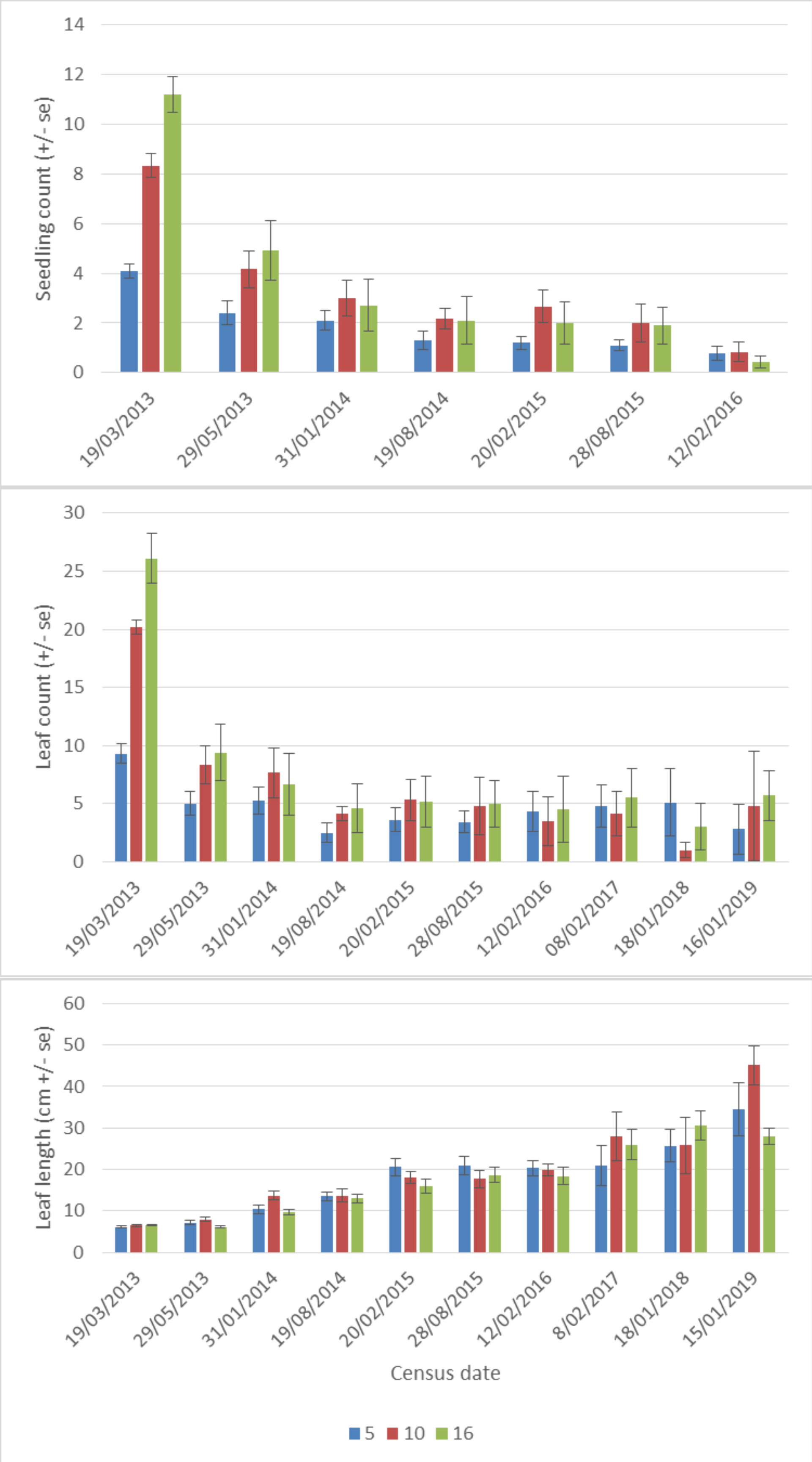

Figure 4.6: Influence of seedling planting density on a) survival (top), b) number of leaves (middle) and c) leaf length (bottom) of *Posidonia* seedlings planted into hessian bags in 2013. Legend indicates number of seedlings sown into each bag.

2017 experiment

Seedling abundance declined consistently over time across all three treatments (Figure 4.7). Overall, there was no difference between bags planted on shore and those planted on the boat, although those planted in the water had slightly lower survival. For leaf length, there was an interaction between treatment and date (LMER: $P<0.001$), although there were no differences between treatments at the final census (LMER: $P=0.41$).

Table 4.1: Linear mixed effects model results for seedling count in the pre-planting experiment.

	Df	**AIC**	**LRT**	**P(χ)**
Null		486		
Treatment	2	490	12.1	**0.002**
Date	3	651	175	**<0.001**
Treatment x Date	6	482	8.0	0.24

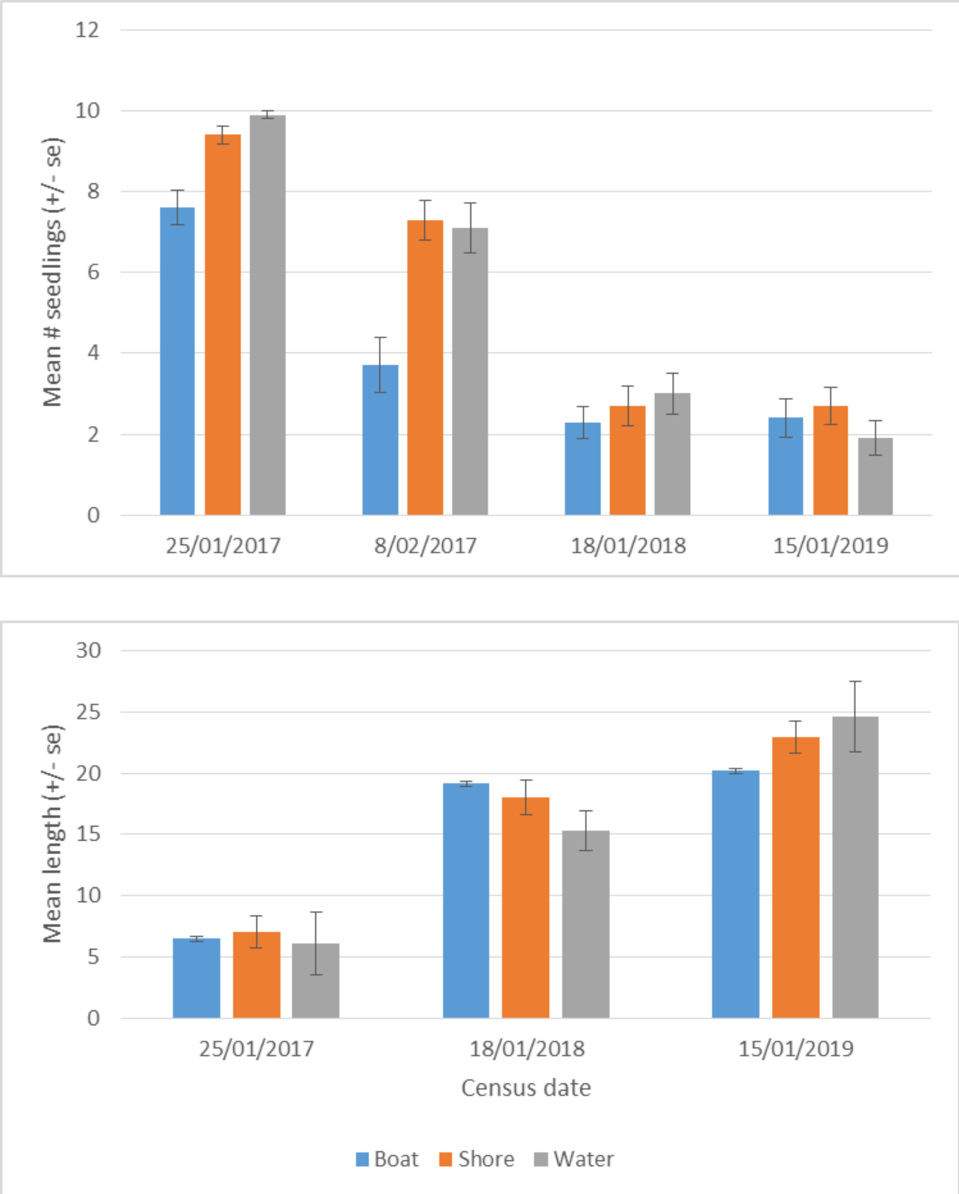

Figure 4.7: Influence of pre-planting on a) survival (top), and b) leaf length (bottom) of *Posidonia* seedlings planted into hessian bags in 2017. Legend indicates where the bags were located when planted.

4.3. Discussion

Overall, there were few consistent patterns in seedling survival across the range of experiments undertaken. For fill type, in the 2012 experiment the intermediate mixes of sand and clay (70% and 50% sand) performed best after 7 years, but a year earlier they were the worst performers. In the 2013 experiment, the 30% sand treatment performed best at the end (after 6 years), but was only marginally better than 100% sand, while the two intermediate mixes were the worst. Interestingly, but probably coincidently, both experiments showed the same results after 6 years. For organic matter addition, high levels were best at the end (6 years), with intermediate levels worst, but zero addition was best after 4 years and high the worst. For seed size, the 10-13 mm seeds were best after 6 years, but the > 13 mm seeds were best after 2-5 years. The < 10 mm seeds were consistently the worst, except after 4 years when all seed sizes were equal. Planting a greater number of seedlings led to higher leaf counts at the end, but less than proportionately, and again this pattern was not consistent over time.

The addition of organic matter to the substrate had no detectable influence on growth or survival of *Posidonia*. The levels used correspond to 1.5, 3 & 4.5% organic matter in the sediment. *Posidonia* along the Adelaide coast has previously been found to consist of ~ 37.5% carbon by dry weight (Tanner, unpublished data), so these values correspond to 0.56, 1.12 & 1.79% carbon. The sediment organic carbon content in restored *Amphibolis*, natural *Amphibolis* and bare sediments adjacent to the rehabilitation site is all < 0.1% (McSkimming 2015), so the lack of a response is not related to unrealistically low levels of organic matter addition. It may be, however, that other sources of organic matter are more readily utilized by seagrasses, as seagrass material is known to take a long time to degrade. Oceanica Consulting Pty. Ltd. (2011) reported better growth of tank grown *P. australis* in sediments with 1.5% dried seagrass after 7 months, although only in unsorted carbonate and silica sediments, and not in sorted silica. They did not follow these seedlings any further, or look at responses in the field. Addition of inorganic nutrients via Osmocote fertilizer did not affect seedling growth in those trials, but did increase the proportion of shoot biomass compared to root. However, the study was terminated at 4 months, before all the seed nutrient reserves were utilized. It is thus still unclear if *Posidonia* seedlings benefit from the addition of nutrients/organic matter to the bags.

The general impression is that external influences are overwhelming any real differences between the treatments, and that which treatment is better at any given time is generally a matter of chance. This is despite the bags within each annual deployment being randomly interspersed, so that in theory, any external influence should be distributed randomly across treatments. These external influences are likely to include fishing and boating disturbances as described in section 2. It is also likely that any influence of the bag fill would only be short-term, and would become less relevant as the roots of the seagrass penetrated through the bottom of the bag into the underlying natural sediment, and as the bags disintegrate and the fill becomes mixed with the natural sediments.

For those seedlings that did survive, there were very few differences in performance in terms of leaf length. While those in 100% sand were shorter at the end in the 2013 experiment, as were those planted into bags with 16 seedlings, both differences were unique to the final census. Rather, leaf length increased consistently over time regardless of treatment until it reached a plateau of ~ 30 cm after about 5 years.

Interestingly, pre-planting *Posidonia* seedlings into bags prior to deployment resulted in a higher survival rate of seedlings than did planting them once the bags had been deployed and placed into their final position. Planting underwater involved teasing a small hole in the hessian bag, planting the seedling, and then pushing the individual strands of hessian back into place to close the hole up and hold the seedling in. This process can be somewhat cumbersome, especially in a current, and it is likely that some seedlings were poorly secured. Planting above water involved the same process, except that the hessian around the seedlings was then superglued in place to prevent the seedlings being washed out as the bag fell to the bottom and was then moved into its final location. Whilst the survey on the day of deployment indicated that this was not entirely successful, especially for those bags planted on the boat, over the longer term it appears that seedlings that had been glued into place were less likely to be washed out of the bags by water movement, and thus more likely to survive in place. As planting underwater is time-consuming, and requires the expense of divers, this result suggests that pre-planting is feasible for any ongoing rehabilitation with *Posidonia*. While the bags used here were relocated by divers after deployment, this was purely for the purposes of making it easier to monitor them, and so may not be required in an active rehabilitation program.

The 2013 experiments produced lower leaf counts in the long term than the 2012 experiment, although after 2 years, both had ~ 4 seedlings per bag, which was double that of the 2017 deployments after 2 years. Apart from natural environmental variation between years, a clear difference was the length of time seedlings were held in aquaria (23 days in 2012 versus 64 in 2013, and ~ 30 in 2017). In Chapter 5, we show that this is not ideal, and that seedlings should preferably be planted within 10 days of dehiscing. During this time, seedlings were kept shaded and grouped together in mesh bags, which is unlikely to be ideal for their maintenance. Future work should seek to minimize this holding time to ensure that seedlings are in the best condition possible when planted.

Although at no stage in the 6-7 years of monitoring did any of the *Posidonia* patches established start to coalesce and begin to form a coherent patch, in February 2016 when leaf densities were at their peak across the whole study, there were small clumps comprised of multiple shoots that had originated from a single seedling (Figure 4.8). This shows that it is possible to establish *Posidonia* seedlings using the hessian bag technique. However, it is important that they be protected from disturbance, as even after 4-5 years they can experience substantial set-backs. It will likely be at least several more years before we know if these set-backs can be overcome by the plants that have established, or if they will slowly dwindle and eventually disappear.

Figure 4.8: Example of *Posidonia* rehabilitation at time of planting (left - January 2012), after 2 years (middle - February 2014) and 4 years (right - February 2016).

Combined, the results for seedling abundance, leaf count and leaf length suggest that the type of fill used in the bags is of little importance in determining the overall establishment rate of *Posidonia* seedlings planted into the bags. Instead, the easiest mix to use, 100% sand, is adequate for future work. Particularly small seeds should probably be discarded, as they are less likely to survive, and there appears to be no advantage to planting large numbers of seedlings into a bag, although due to the time taken to plant seedlings, the range of densities explored was small. With the success of pre-planting, it may be useful to further explore the planting density question with higher densities, which can be much more quickly planted into bags on shore.

5. *POSIDONIA* TANK EXPERIMENTS

In addition to the *in situ* experiments with *Posidonia* detailed in Chapter 4, a series of tank experiments were undertaken in early 2018 to help refine the optimal conditions for growing *Posidonia* from beachcast fruit. In total, 10 different experiments were conducted using fruits that had been collected off the beach at West Beach, and dehisced in flow through seawater tanks at the South Australian Aquatic Sciences Centre (SAASC).

5.1. Methods and Results

All fruits were collected shortly after high tide, and appeared to be fresh and were thus considered to have been stranded that day. Unless specified otherwise, all fruits were from *P. angustifolia*. After collection, fruits were returned to SAASC and placed in plastic floating trays with a flyscreen mesh base, and floated in 2300 L tanks of flow through seawater. Fruits from each collection date were kept separate. Every few days, fruits were sorted, with dehisced seedlings removed and placed in immersed plastic containers with flyscreen sides, and dehisced pericarps discarded. After dehiscing, seedlings were planted into individual seedling pots (forestry tubes 50 mm square by 120 mm high), with either beach sand, or other substrate as specified for each individual experiment below. Trays of 50 pots were kept in low (50 cm water depth) 1,900 L flow-through tanks under 75% shadecloth (equivalent to ~ 7-8 m water depth off Grange, Figure 5.1). Seedlings in each experiment were randomly interspersed, with separate experiments generally being kept in separate trays. Throughout each experiment, seedlings were manually cleaned of epiphytic algae by gently running their leaves between the fingers as needed, and trays were moved around the tank to accommodate any differences in light availability and water flow. At the conclusion of each experiment, seedlings were harvested to determine the number of leaves, length of the longest leaf, number and length of roots, and total weight. Poisson GLM, using R (ver 3.5.1, R Core Team 2018) was used to assess any difference in survival between treatments in each experiment. PERMANOVA (Anderson 2001), using the PERMANOVA+ add on in Primer (Anderson et al. 2008), was used to determine if there were any significant differences in the performance of surviving seedlings between treatments in each experiment. Due to variables being measured on different scales, each was scaled by its maximum. Resemblance matrices were then calculated using Euclidean distances. For single factor analyses, we used 9,999 unrestricted permutations of raw data, while for multifactor analyses we used 9,999 permutations under a reduced model. When necessary and appropriate, pairwise tests were conducted following the main analysis to determine which levels differed for significant factors.

Figure 5.1: *Posidonia* tnak experiment set-up showing a tray of 50 pots planted with *Posidonia* seedlings (left) and a tank used for holding the seedlings (right).

a. Effect of time of collection

Fruits were collected from the beach on a regular basis from the 8th of Dec 2017 to the 4th of Jan 2018. Fruits were held for 10 days to dehisce, after which ten seedlings from each collection date were planted into individual seedling pots filled with beach sand. Seedlings from each date were allowed to grow for 71 days from planting to harvest. There was a significant difference in survival of seedlings between collection dates (GLM, p=0.04), with survival increasing steadily to a peak for fruit collected on the 28th of December, followed by a rapid decline (Figure 5.2). There were significant differences between collection dates (PERMANOVA: $F_{5,18}$=2.64, p=0.018), with pairwise tests indicating that those collected on the 28th of December performed better than those collected on the 8th of December, the 22nd of December and the 4th of January (Figure 5.3). These seedlings had a mean of 3.3 leaves and 3.3 roots, with the longest of each being ~120 mm. Root development was more affected by time of collection than was leaf development.

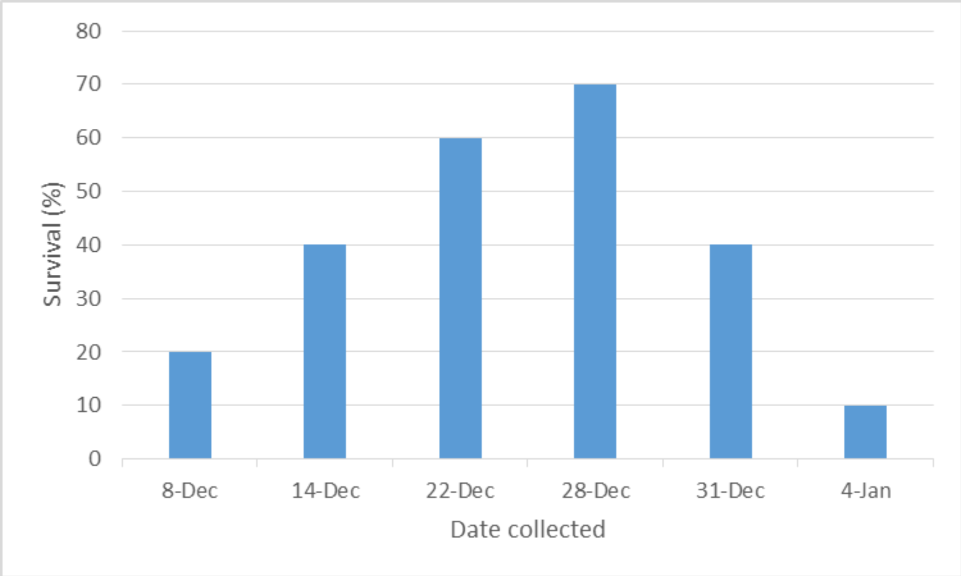

Figure 5.2: Influence of date of collection on survival of *Posidonia* seedlings over 71 days.

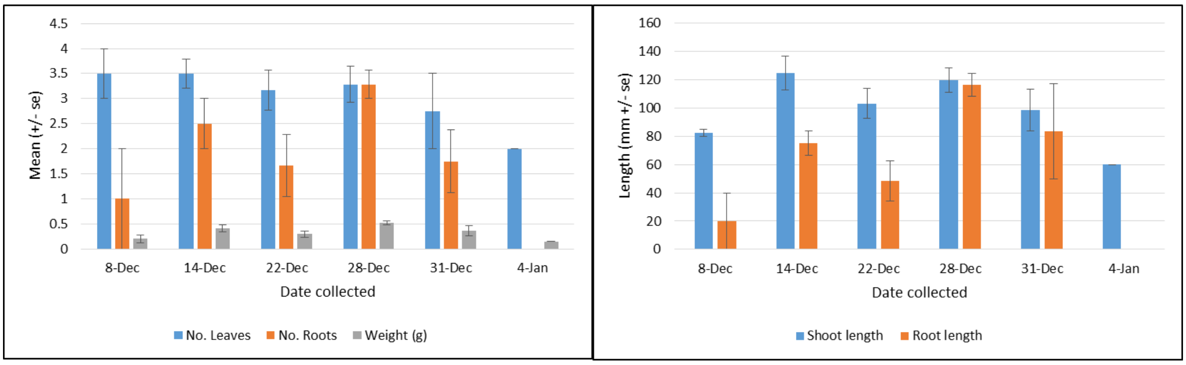

Figure 5.3: Influence of date of collection on *Posidonia* seedling growth over 71 days.

b. Effect of initial seedling length and seed burial

To test the influence of initial seedling size, and whether the actual seed is fully buried at planting or just half buried has an influence on growth, ten replicate seedlings for each combination of these factors were planted as above on the 3rd of January from fruits that were collected on the 20th of December and which dehisced on the 22nd of December. These seedlings were harvested and measured 55 days after planting. Long seedlings had 51 mm long shoots on planting (se = 2.5 mm), while short seedlings had 10.1 mm long shoots (se = 0.5 mm). Survival was lower for seedlings with short shoots than those with long shoots, but did not differ between planting regimes (Table 5.1, Figure 5.4). Of those that survived, there were no significant differences for either factor (Table 5.2), although with only a single short exposed seedling surviving, the power of this analysis would be very low. Indeed, that single survivor was one of only two seedlings that did not grow any roots, and was only half the weight of the next lightest surviving seedling. Short seedlings were on average 40.9 mm shorter than long seedlings at planting, but only 18.1 mm shorter at harvest (Figure 5.5), indicating that they had made up over half the difference in 55 days.

Table 5.1: GLM results for survival of *Posidonia* seedlings as a function of length and burial depth.

	df	Deviance	P
Length	1	8.42	**0.0037**
Burial	1	0.13	0.72
Length x Burial	1	3.37	0.066
Residual	36	43.43	

Table 5.2: PERMANOVA results for *Posidonia* seedling growth as a function of length and burial depth.

	df	SS	Pseudo-F	P
Length	1	6,581.2	2.3036	0.086
Burial	1	2,007.9	0.70282	0.5225
Length x Burial	1	5,298.6	1.8546	0.1631
Residual	15	42,854		

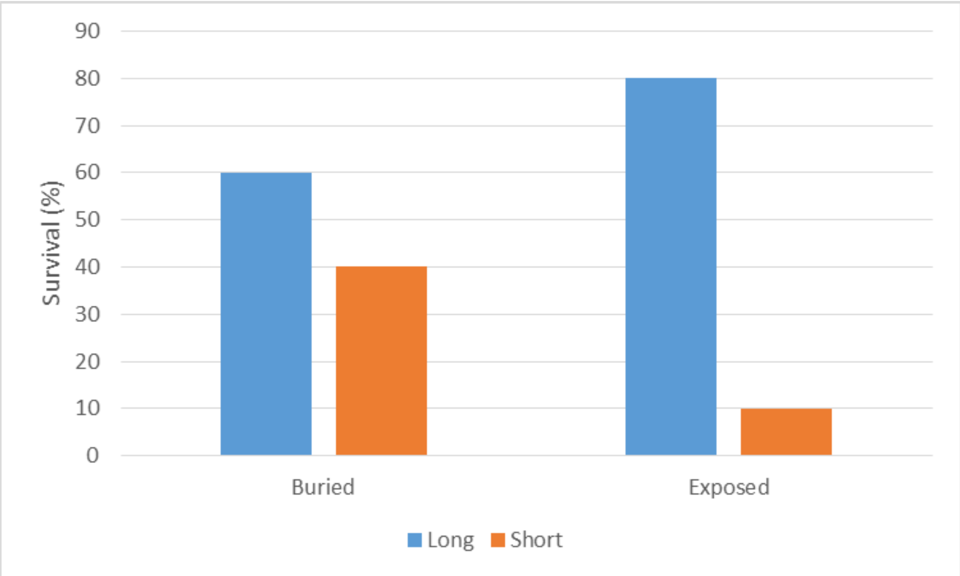

Figure 5.4: Influence of initial seedling length and planting depth on survival of *Posidonia* seedlings after 55 days.

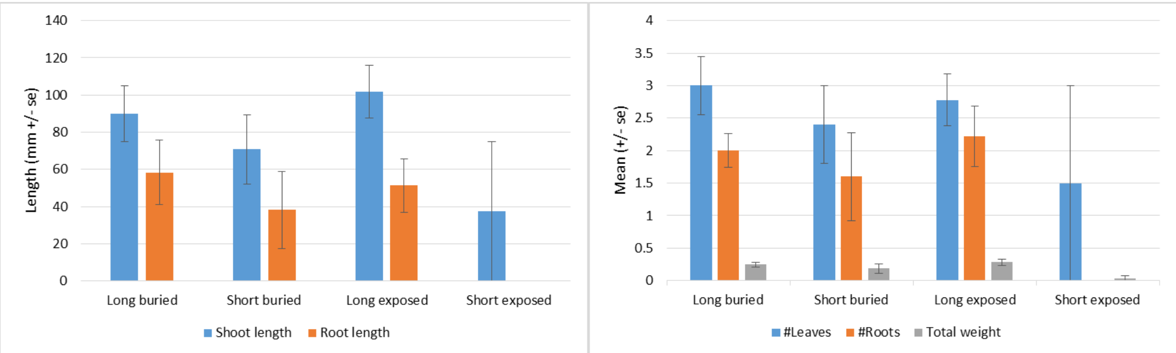

Figure 5.5: Effects of initial seedling length, and planting depth, on *Posidonia* seedling development over 55 days.

c. Influence of exposure to air

To determine if seedlings are negatively affected by exposure to air during transport and planting, 10 seedlings were removed from the holding tank and exposed for each of 0, 5, 10, 15, 20, 25 & 30 min in the shade immediately prior to planting. Air temperature at the time was 21°C. Fruits were collected on the 20th of December, dehisced on the 24th, planted on the 4th of January and harvested 56 days later. Neither survival (GLM: P=0.94) nor growth (PERMANOVA: $F_{6,39}$=0.70, P=0.83) were affected by exposure time, indicating that short-term exposure to air under moderate temperatures is not detrimental to the seedlings.

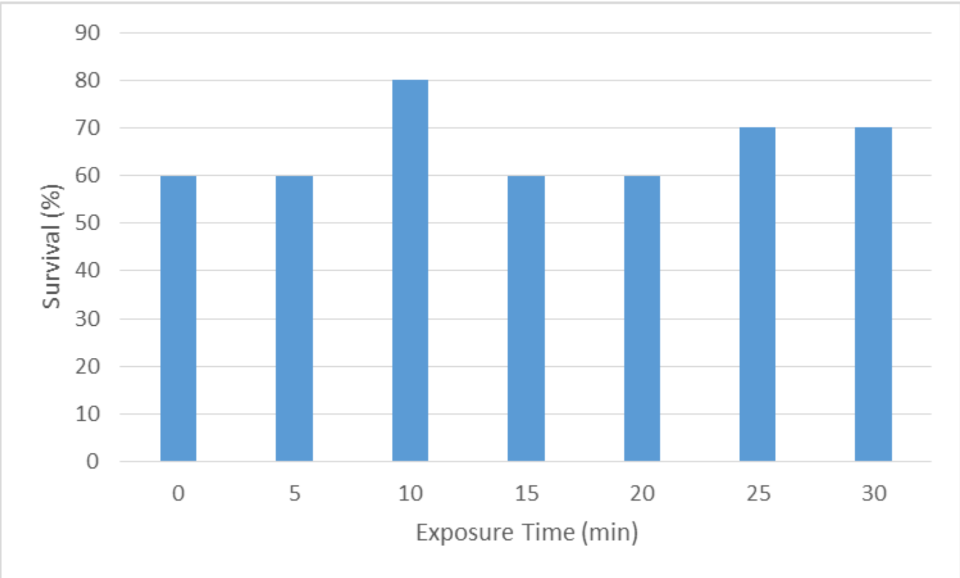

Figure 5.6: Influence of exposure to air on survival of *Posidonia* seedlings after 56 days.

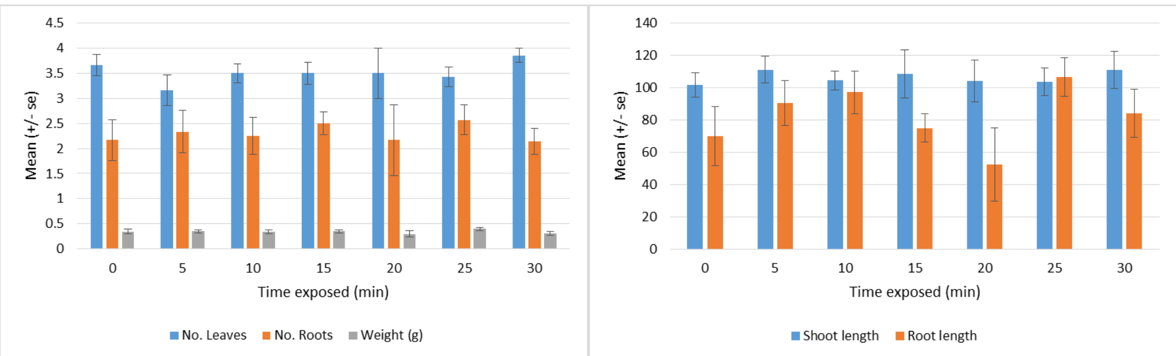

Figure 5.7: Effects of exposure to air prior to planting on *Posidonia* seedling development over 56 days.

d. Time taken to dehisce

To determine if the time taken for the seed to dehisce (be released from the fruit) influenced seedling growth, fruits were collected on the 20th of December, and seedlings that had dehisced were separated out each day. These seedlings were then either planted immediately, or held for six or 12 days before planting, and harvested 61 days after dehiscing. Seeds that had sprouted, but not dehisced, were also planted out 9 days after collection with the fruit still intact. Survival was influenced by the time to dehisc, but not by time since dehiscing or their interaction (Table 5.3). Those seedlings that dehisced within 4 days had greater survival than those that took longer to dehisce (Figure 5.8). Those seedlings that did not dehisce also showed low survivorship. The growth of surviving seedlings was influenced by both factors (Table 5.4). Again, those that took longer to dehisce tended to perform worse, while there was also a decline in performance for those seedlings that were planted longer after dehiscing, especially when measured as total weight (Figure 5.9).

Table 5.3: GLM results for survival of *Posidonia* seedlings as a function of time to dehisce and time since dehiscing.

	df	Deviance	P
Time to dehisce	5	16.78	**0.005**
Time since dehiscing	2	0.65	0.72
Interaction	4	1.83	0.77
Residual	107	145.5	

Table 5.4: PERMANOVA results for survival of *Posidonia* seedlings as a function of time to dehisce and time since dehiscing.

Source	df	SS	Pseudo-F	P
Time to dehisce	4	16,850	2.2478	**0.0152**
Time since dehiscing	2	18,044	4.8142	**0.0001**
Interaction	4	12,196	1.6269	0.0702
Residual	50	93,703		

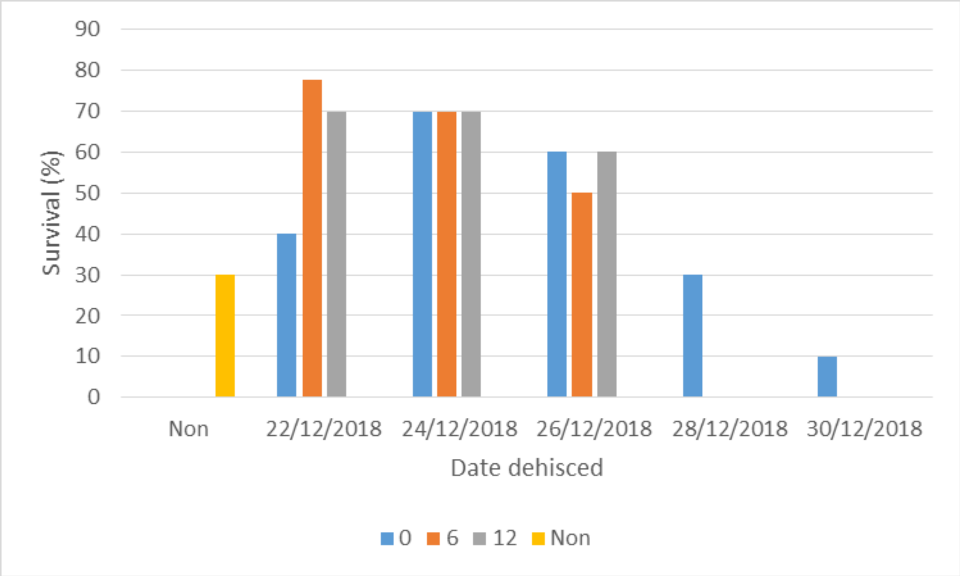

Figure 5.8: Influence of time to dehisce and time since dehiscing on survival of *Posidonia* seedlings after 61 days. Non refers to fruits that had not dehisced, but had still sprouted.

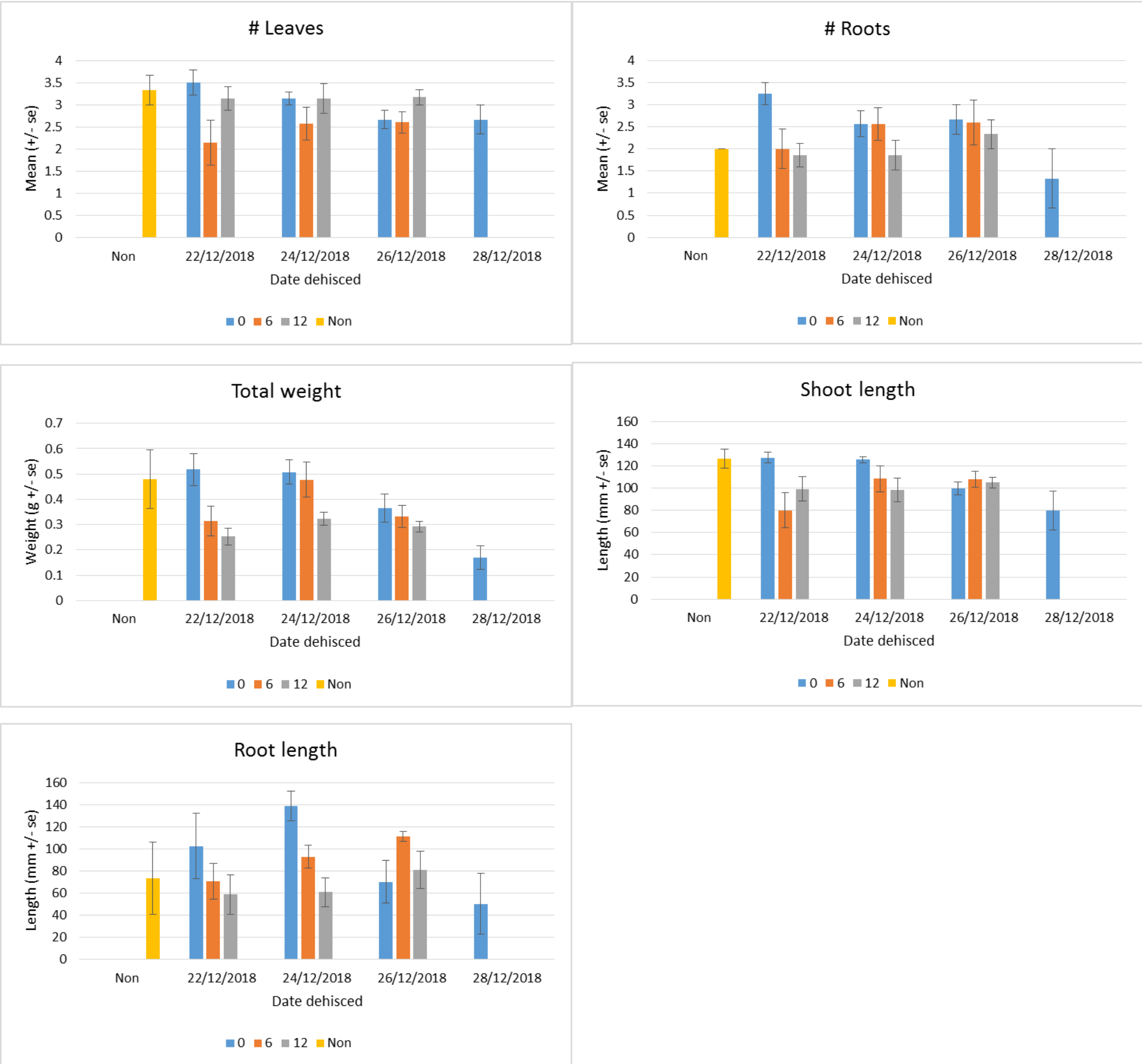

Figure 5.9: Influence of time to dehiscing (seedling release from the fruit), and time from dehiscing to planting, on *Posidonia* seedling growth over 61 days. Non refers to fruits that had not dehisced, but had still sprouted.

e. Time since dehiscing

This experiment examined a wider range of times since dehiscing in isolation. Fruits were collected on the 20th of December, and dehisced on the 21st, with the resulting seedlings being held for up to 30 days prior to planting. Harvest was 97 days after dehiscing. Survival was not influenced by how long seedlings were held before planting (GLM: P=0.63; Figure 5.10). There was, however, a significant influence on growth (PERMANOVA: $F_{6,47}$=2.49, P=0.003). Pairwise tests indicate that seedlings planted within the first 10 days perform similarly, while those planted from 15-30 days also perform similarly. Those in the first group have longer roots and leaves, and higher weights, than those in the second group (Figure 5.11).

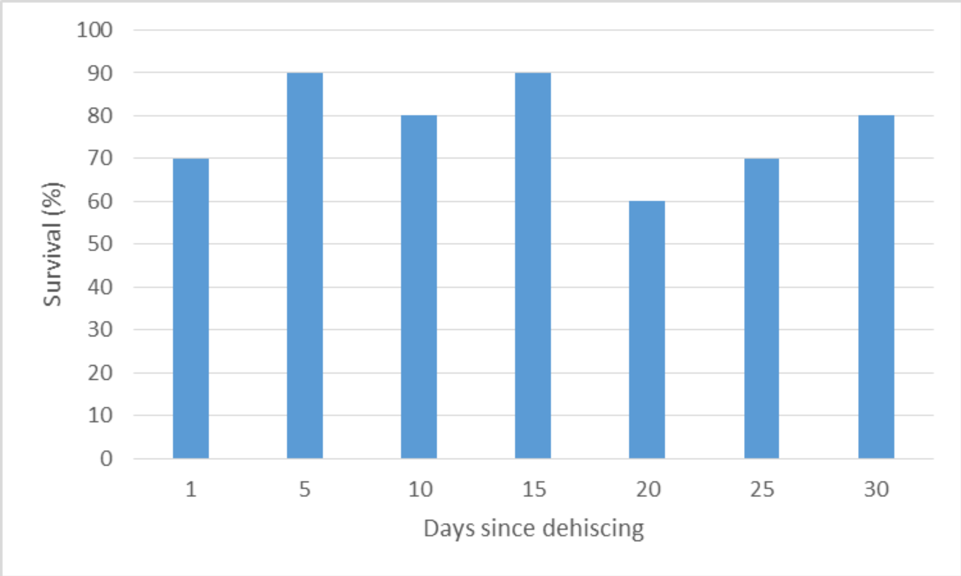

Figure 5.10: Influence of time since dehiscing on survival of *Posidonia* seedlings after 97 days.

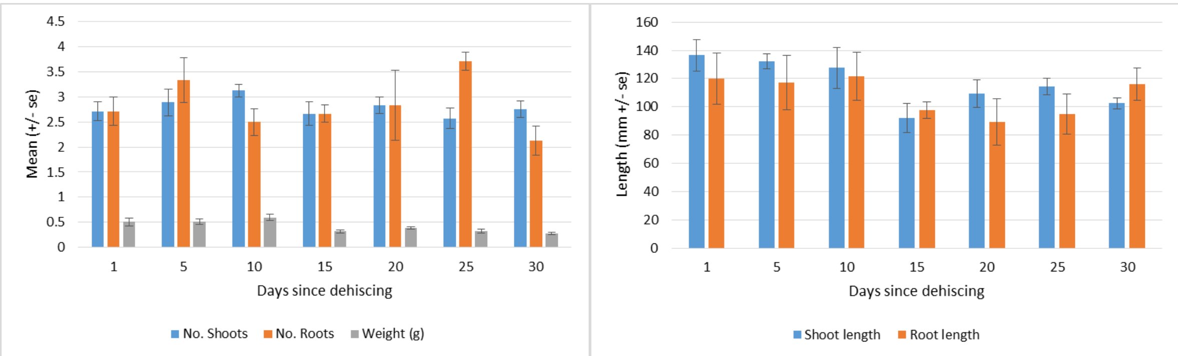

Figure 5.11: Influence of time since dehiscing (seedling release from fruit) on *Posidonia* seedling growth over 97 days.

f. Clay content of substrate

Different mixes of sand and clay were used in previous in situ experiments, and suggested that a 50:50 mix produced the best survival and growth of *Posidonia*. To investigate whether this mix is best for young seedlings, we set up 10 replicate pots with each of a range of different clay and beach sand mixes, as well as with straight builder's sand. Fruits were collected on the 20th of December, and seedlings that dehisced on either the 22nd or 25th of December were planted 6 days later. Seedlings were harvested 41 days after planting. Survival was not influenced by substrate, but was influenced by time to dehisc (Table 5.5), with those taking 2 days to dehisce having higher survival than those taking 5 days (Figure 5.12). Similarly, growth of surviving seedlings was not affected by the substrate, but was by the time to dehisce

(Table 5.6), with those taking 5 days to dehisce generally being smaller after 41 days (Figure 5.13).

Table 5.5: GLM results for survival of *Posidonia* seedlings as a function of substrate and time to dehisce.

	df	Deviance	P
Substrate	6	3.71	0.72
Time to dehisce	1	17.06	**<0.001**
Interaction	6	9.23	0.16
Residual	126	104.39	

Table 5.6: PERMANOVA results for growth of *Posidonia* seedlings as a function of substrate and time to dehisce.

Source	df	SS	Pseudo-F	P
Substrate	6	7,144	0.73	0.75
Time to dehisce	1	8,434	5.14	**0.007**
Interaction	6	5,313	0.92	0.92
Residual	100	164,020		

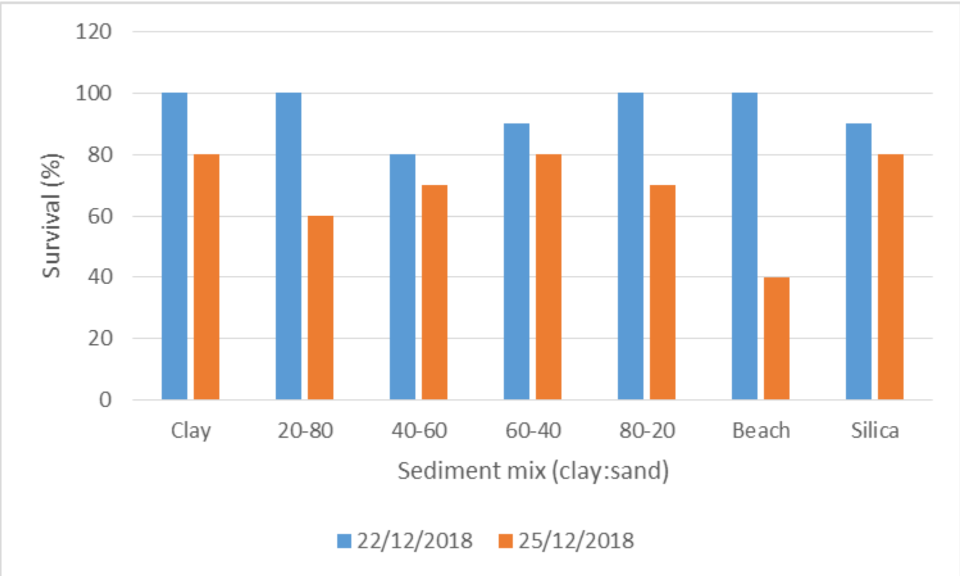

Figure 5.12: Influence of sediment mix and time to dehisc on survival of *Posidonia* seedlings after 41 days.

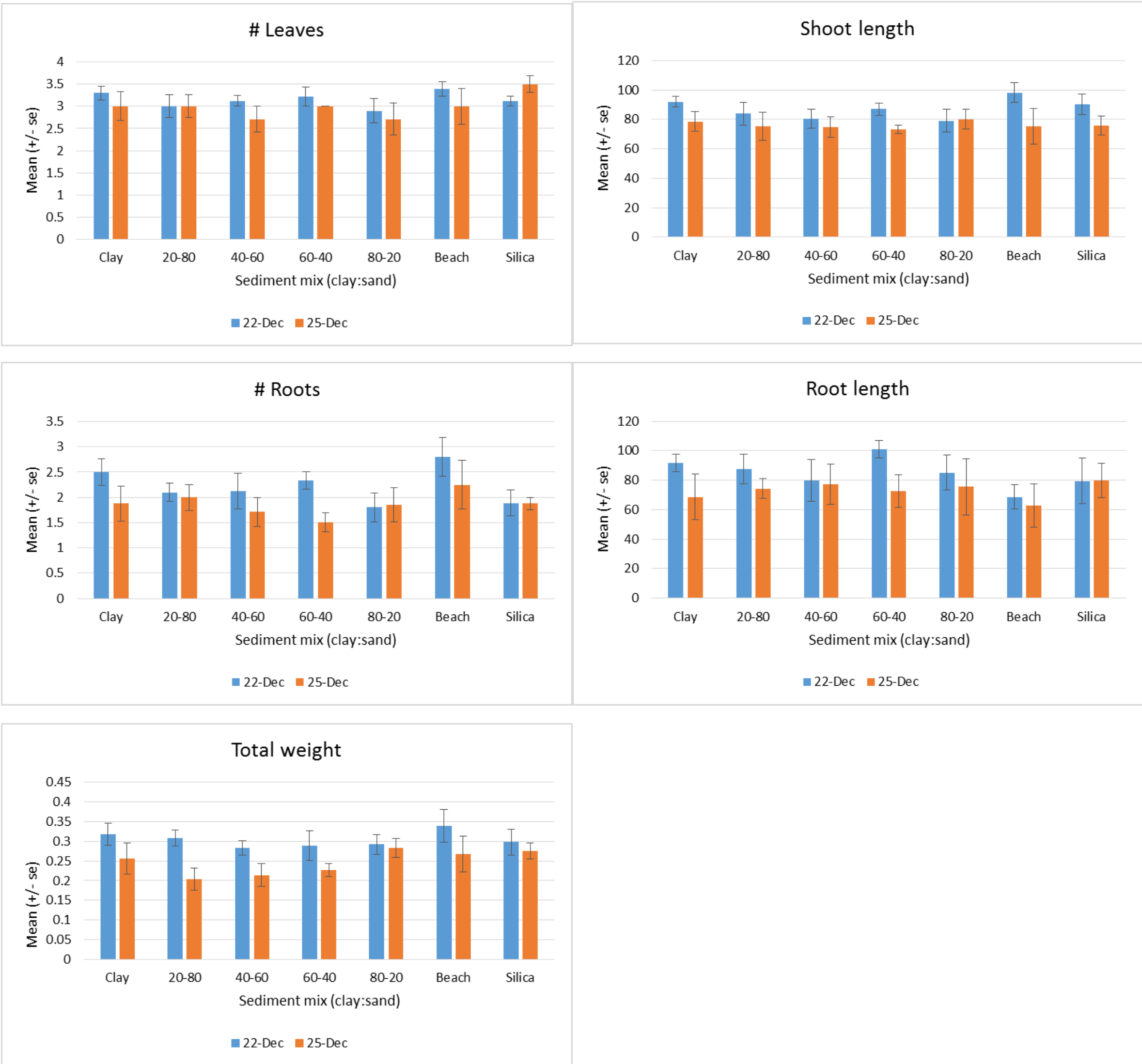

Figure 5.13: Influence of sediment mix and time to dehisce on *Posidonia* seedling performance over 41 days.

g. Seed size

To assess the influence of seed size on subsequent seedling performance, seed length of dehisced seedlings was measured, and seedlings categorised into small seeds (< 10 mm), medium (10-13 mm) and large (> 13 mm). Fruits were collected on the 20th of December, dehisced on the 22nd, and planted on either the 26th of December or the 1st of January, and harvested 67 days later. Neither seed size nor time since dehiscing affected survival (Table 5.7, Figure 5.14). Seed size did affect growth (Table 5.8), with large seeds performing better than smaller, especially in terms of leaf length and weight (Figure 5.15).

Table 5.7: GLM results for survival of *Posidonia* seedlings as a function of seed size and planting time.

	df	Deviance	P
Size	2	0.758	0.68
Time since dehiscing	1	0.522	0.47
Interaction	2	5.723	0.057
Residual	53	71.9	

Table 5.8: PERMANOVA results for growth of *Posidonia* seedlings as a function of seed size and planting time.

Source	df	SS	Pseudo-F	P
Size	2	29,614	11.25	**<0.001**
Time since dehiscing	1	3,082	2.34	0.067
Interaction	2	5,282	2.01	0.061
Residual	30	39,486		

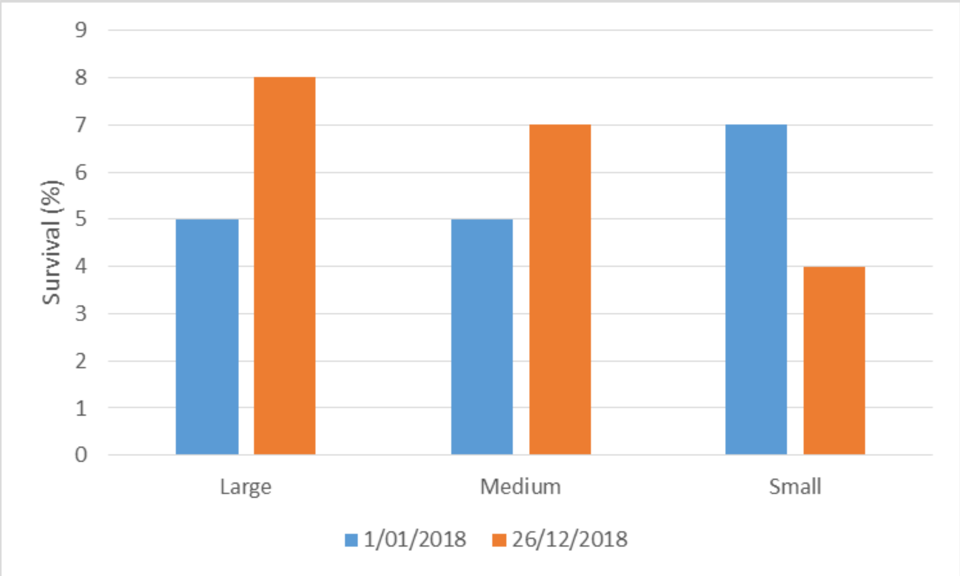

Figure 5.14: Influence of seed size and planting time on survival of *Posidonia* seedlings after 67 days.

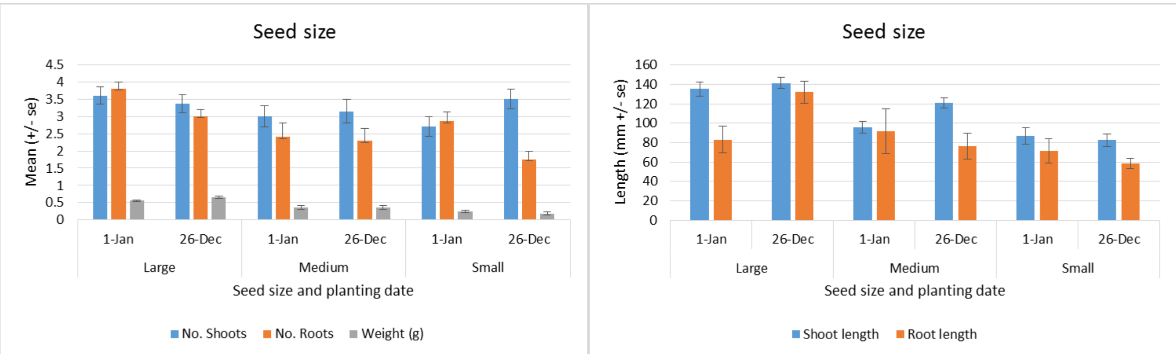

Figure 5.15: Influence of seed size and planting date on growth of *Posidonia* seedlings over 67 days.

h. Influence of hessian and gluing

To assess the viability of deploying planted hessian bags from the boat, rather than planting by divers once the bags have been deployed, in situ experiments have been undertaken gluing seedlings into bags (Chapter 4). To further investigate what influence this may have, seedlings were grown in pots that were either uncovered, covered with hessian and the seedlings inserted through a gap, but not glued, and covered with hessian and the seedlings glued in place once inserted. Fruits were collected on the 20th of December, dehisced on the 23rd of December, and planted on either the 28th of December or the 3rd of January, with harvest 33 days after dehiscing. There was no effect of either treatment or planting date on either survival (Table 5.9, Figure 5.16) or growth (Table 5.10, Figure 5.17).

Table 5.9: GLM results for survival of *Posidonia* seedlings as a function of hessian presence and planting time.

	df	Deviance	P
Treatment	2	4.54	0.1
Time since dehiscing	1	0	1
Interaction	2	1.84	0.4
Residual	54		

Table 5.10: PERMANOVA results for growth of *Posidonia* seedlings as a function of hessian presence and planting time.

Source	df	SS	Pseudo-F	P
Size	2	3,529	0.99	0.41
Time since dehiscing	1	4,710	2.66	0.06
Interaction	2	2,040	0.58	0.73
Residual	50	88,667		

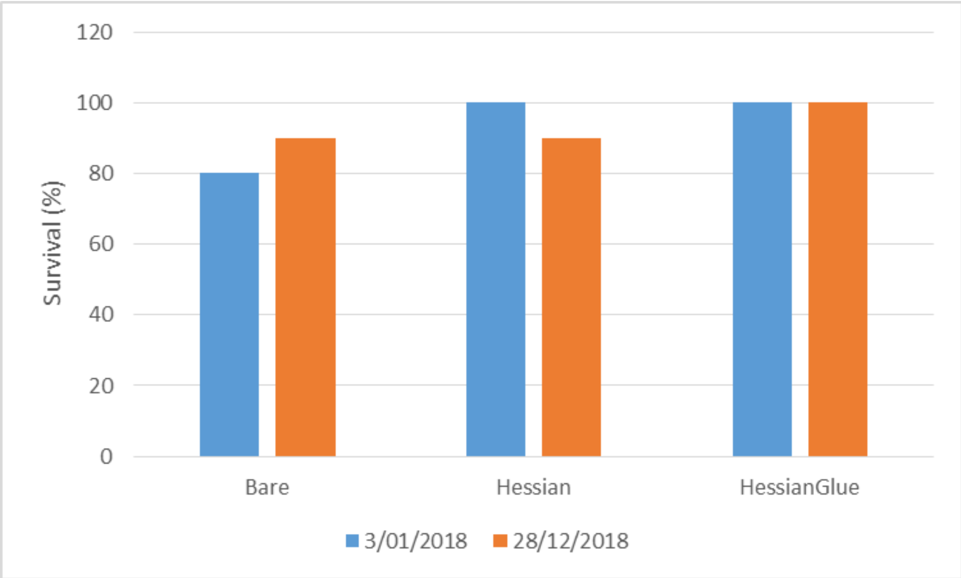

Figure 5.16: Effect of hessian covering and gluing on *Posidonia* seedling survival over 33 days.

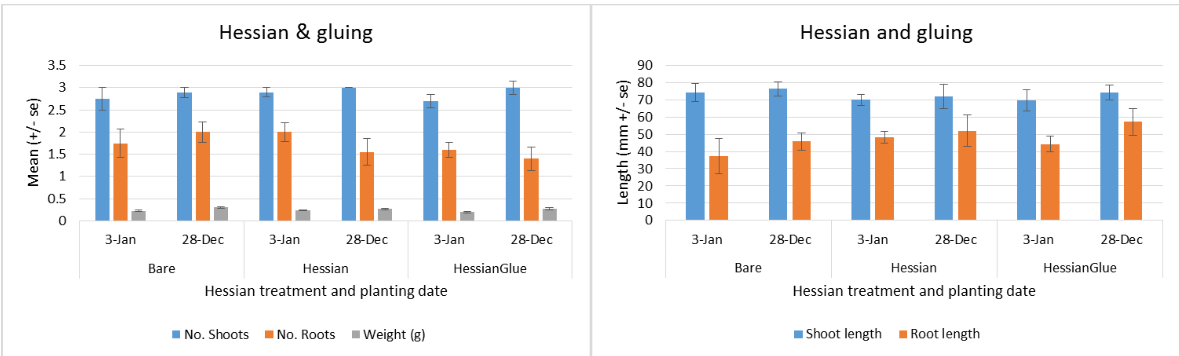

Figure 5.17: Effect of hessian covering and gluing on *Posidonia* seedling growth over 33 days.

i. Species and seedling age

To follow seedling growth through time, seedlings were harvested at ages ranging between 12 and 89 days. As a limited number of *P. sinuosa* fruits were washed up onto the beach, these were collected and also used in this experiment to examine differences between species. All fruits were collected on the 20th of December, dehisced on the 22nd of December and planted on the the 27th of December. After 3 weeks, survival declined, with only ~ 50% of seedlings surviving the full 13 weeks, and with no difference between species (Table 5.11, Figure 5.18). Growth generally increased over time (Table 5.12, Figure 5.19), although pairwise tests indicated that there was no difference between the final 2 harvests. *P. sinuosa* were consistently smaller than *P. angustifolia* throughout the experiment. The apparent cessation of growth may have been caused by overgrowth by epiphytic algae and subsequent disturbance involved in cleaning this off.

Table 5.11: GLM results for survival of *Posidonia* seedlings as a function of species and age.

	df	Deviance	P
Species	1	0.59	0.44
Harvest date	4	22.4	**0.0002**
Interaction	4	2.59	0.63
Residual	85		

Table 5.12: PERMANOVA results for growth of *Posidonia* seedlings as a function of species and age.

Source	df	SS	Pseudo-F	P
Species	1	22,899	20.0	**<0.0001**
Harvest date	4	94,022	20.5	**<0.0001**
Interaction	4	7,632	1.66	0.10
Residual	68	77,990		

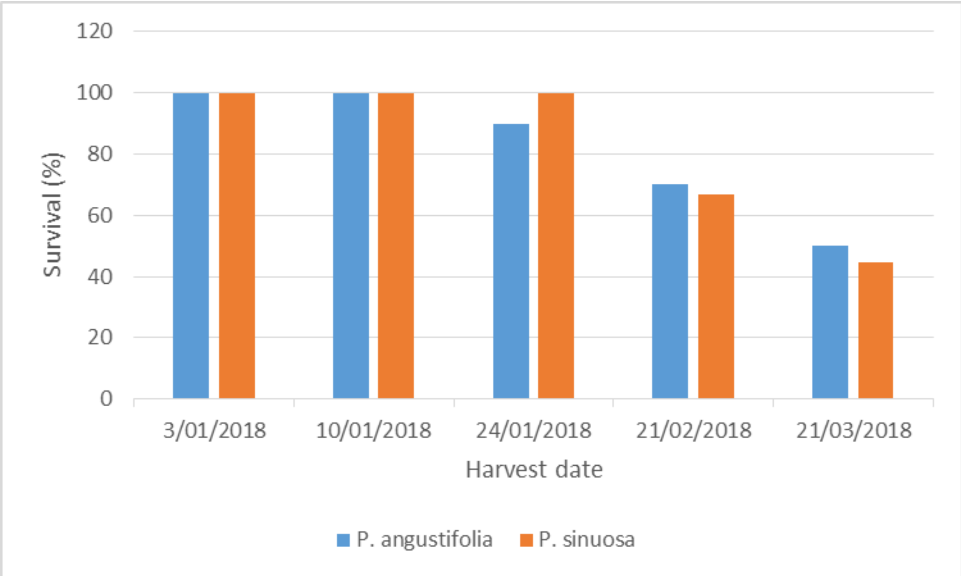

Figure 5.18: Effect of species and age on *Posidonia* seedling survival.

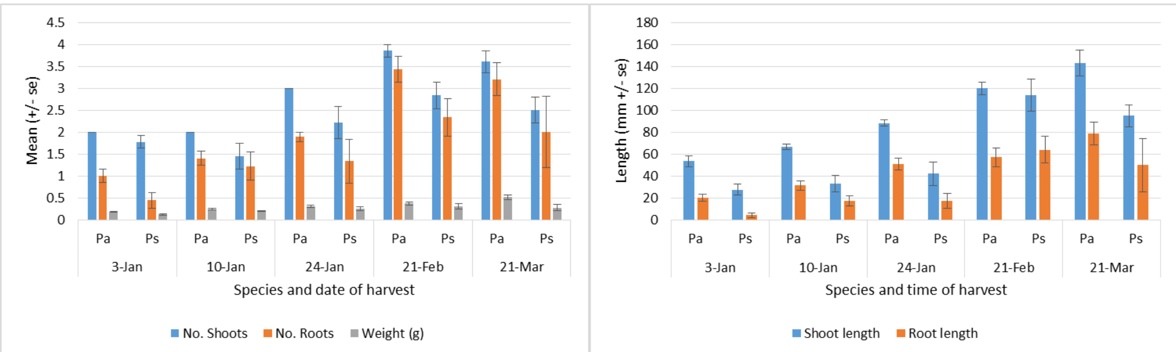

Figure 5.19: Effect of species and age on *Posidonia* seedling growth.

j. Water flow through the sediment

Water flow through the hessian bags may differ to that through the natural substrate, especially early on when the bags are still sitting above the surrounding seafloor. Restricted water flow in pots may also influence seedling growth in these tank trials. To assess the potential consequences of this, pots were set up with different levels of water flow through them. The standard pot used for all other experiments, with solid sides but a mesh base, was used for the medium flow treatment. These pots were entirely lined with a plastic bag for the low flow treatment, while for the high flow treatment a series of holes were drilled in the side of the pots. Fruits for this experiment were collected on the 20th of December, dehisced on the 23rd, and planted on either the 28th of December or the 3rd of January, and harvested 78 days later. There was no difference in either seedling survival (Table 5.13, Figure 5.20), or growth (

Table ***5.14***, Figure 5.21), as a function of either water flow or planting date.

Table 5.13: GLM results for survival of *Posidonia* seedlings as a function of water flow and planting date.

	df	Deviance	P
Flow	2	0.15	0.93
Planting date	1	1.21	0.27
Interaction	2	0.20	0.91
Residual	54	74.8	

Table 5.14: PERMANOVA results for growth of *Posidonia* seedlings as a function of water flow and planting date.

Source	df	SS	Pseudo-F	P
Flow	2	10,717	2.26	0.071
Planting date	1	4,435	1.87	0.15
Interaction	2	4,251	0.90	0.45
Residual	34	80,622		

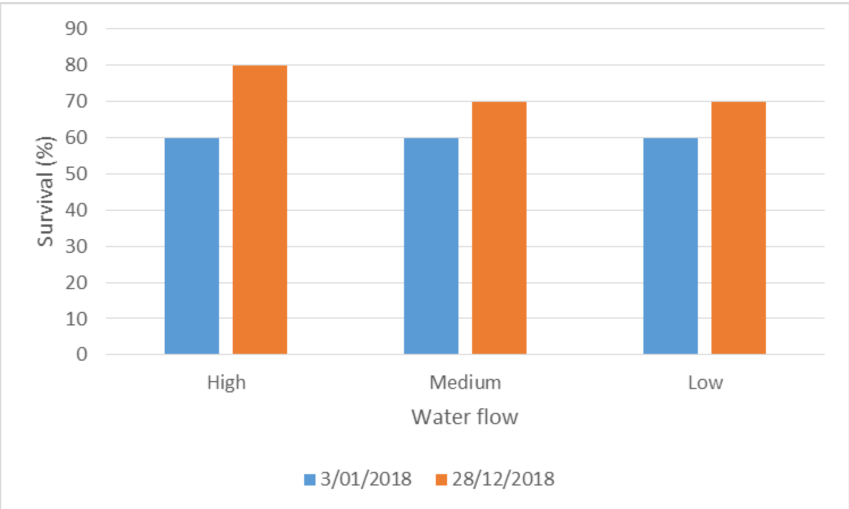

Figure 5.20: Effect of water flow and planting date on *Posidonia* seedling survival over 78 days.

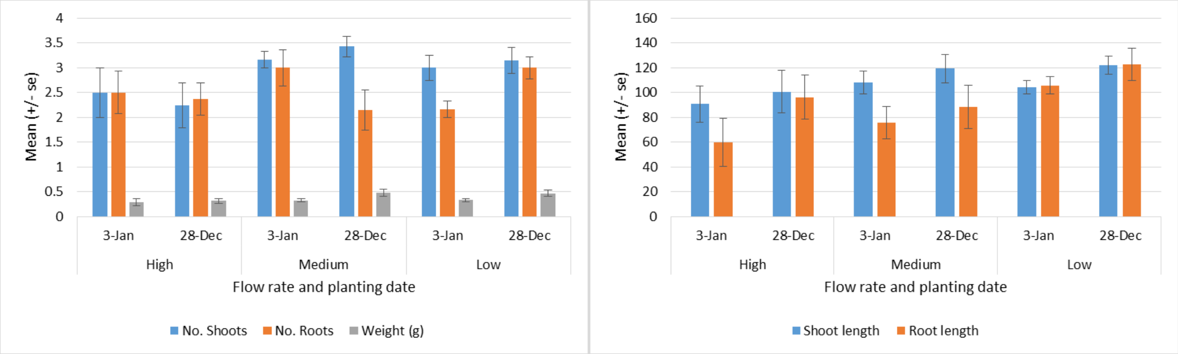

Figure 5.21: Effect of water flow and planting date on *Posidonia* seedling growth over 78 days.

5.2. Discussion

The general pattern (Table 5.15) emerging from this series of experiments is that when fruits are collected is highly important in determining their later success, with those fruits collected on the 28th of December 2017 producing better outcomes than those collected earlier or later. Fruits collected a week prior also had good survival, but had poor root growth, suggesting that they would not perform well under field conditions where disturbance levels would be higher than those present in these tank experiments.

The time taken for fruits to dehisce is also important, with those that dehisce within a few days of being washed up on the beach surviving and growing better than those that dehisce over a longer time period. This may be an indication of fruit maturity, with those taking longer to dehisce still being a little immature. Alternatively, *Posidonia* seeds actually germinate while still in the fruit, so delayed dehiscence may stunt the seedling in its early growth stages. The fruits used here were kept in floating trays under shadecloth, and so were exposed to very little mechanical agitation. In nature, as fruits are positively buoyant, they are likely to be exposed to some level of wave action and agitation, which may increase the percentage of fruit that dehisce in the first few days after release, although rougher conditions may also result in a greater proportion of fruit being released early.

Table 5.15: Summary of outcomes of *Posidonia* seedling experiments on survival and growth.

		Survival	**Growth**
a	Time of collection	Peak on 28 Dec	Peak on 28 Dec
b	Shoot length and burial	Long shoots better Suggestion that half-buried is worse	No difference Suggestion that half-buried is worse
c	Exposure to air	No effect	No effect
d	Time to dehisce and time since dehiscing	Quicker is better No difference	Quicker is better Sooner is better
e	Time since dehiscing	No difference	Best in first 10 days
f	Clay content and time to dehisce	No difference Quicker is better	No difference Quicker is better
g	Seed size and time since dehiscing	No difference No difference	Large is better than small No difference
h	Hessian and time since dehiscing	No difference No difference	No difference No difference
i	Species and age	No difference Survival decreases after 3 weeks	*P. sinuosa* is slower growing than *P. angustifolia* Growth stopped after ~ 7 weeks
j	Water flow and time since dehiscing	No difference No difference	No difference No difference

The time between the fruit dehiscing, and the seedling being planted, can also be important in determining the ultimate success of the seedling. While most of the experiments that looked at this factor showed no difference in performance, the single factor experiment where the time of planting covered a greater range did show that seedlings should be planted within 10 days of dehiscing for the best success. The multifactor experiments that included this variable all only included seedlings that were held for less than 10 days prior to planting. This finding has important implications for conducting field experiments, and any *in situ* rehabilitation work, as it suggests that holding seedlings for longer periods, as done for the experiments reported in chapter 4, is not ideal. Planting was delayed in those experiments due to logistical issues with fielding a dive team during the holiday season, and this needs to be considered in any further work on this species.

Species and seed size were the final two factors that influenced survival and growth. *Posidonia sinuosa* grew more slowly than *P. angustifolia*. The former is less abundant along the Adelaide coast, and appears to produce very low numbers of fruit. Although not quantified, thousands of fruit were collected for these experiment, from which only 45 *P. sinuosa* seedlings were obtained, and even this required active searching for fruits of this species. It is estimated that something like 1 in 1000 fruits at West Beach are of *P. sinuosa*, and the few surveys that were undertaken on other Adelaide beaches suggests that this is probably typical. Seed size was also important for growth, although not survival, with small seeds performing poorly in comparison to large. There was no detectable difference between medium and large, with medium seeds making up about two thirds of seeds collected.

The range of external factors that were considered in these experiments did not have any detectable effects on seedling survival or growth. The clay content in the substrate, water flow, exposure to air for up to 30 minutes before planting, and the presence of a hessian covering (with our without gluing) were all unimportant. The only potential influence was whether the seeds were only half-buried or were fully buried when planted, with some indications that the former could lead to reduced growth and survival, although high variability in this experiment meant that the results were not significant. In a field situation, there may be a greater difference, as seeds that are fully buried would be less likely to be dislodged before being able to get their roots established. However, a typical naturally recruited seedling is most likely going to have settled onto the seafloor and established without burial, emphasizing the importance of calm conditions over the recruitment period in late December/early January.

The above results highlight a few key issue for further work. Firstly, how broad is the window of opportunity for collecting? The collections in this study were about a week apart, so future experiments should examine a finer temporal scale around the peak of fruit availability, although this is made somewhat more difficult by the fact that you don't know when the peak is until after it has occurred, and weather conditions mean that fruit availability on the beach is not consistent even throughout the period of peak release. Secondly, if a source of *P. sinuosa* fruits can be identified, then this species is currently understudied. There is also a need to develop techniques for keeping seedlings alive and healthy for longer periods of time. In the current study, overgrowth by algal epiphytes was a problem, and required frequent manual cleaning, which then resulted in mechanical damage to the seagrass. As an alternative, some very preliminary trials using dilute bleach to kill the algae have shown some promise, and this should be investigated further. The third option is to identify a suitable invertebrate grazer that consumes algae but not seagrass, as has been proposed by Seddon et al. (2005). Seddon et al. (2005) also suggested that planting seedlings in small groups, rather than individually, might be beneficial, based on some preliminary data from their experiments, and this would be worth pursuing.

Several implications for on ground rehabilitation with *Posidonia* can be drawn from these results. The first is that there is only a small window of opportunity for collecting good quality fruit, and along the Adelaide coast, this coincides with the Christmas/New Year holiday break, which complicates work with this species. Once fruit are collected, they should only be held for a few days to dehisce, and any that don’t dehisce in this time should be discarded, as they are likely to produce poor quality seedlings. Once dehisced, seedlings should preferably be planted within 10 days. While this time does not influence survival in tank conditions, it does influence growth, and in field conditions slower growing seedlings are less likely to establish before a disturbance potentially uproots them. Slower growing seedlings will also have less opportunity to develop an energy reserve before decreased light levels and temperatures over winter place a substantial stress on them.

6. BAG STRUCTURAL INTEGRITY

For small-scale deployments, and even single 1-hectare deployments of a few thousand bags, bags can be filled a few days before deployment, and thus there is no need to store them for any period of time. However, any operational rehabilitation using the hessian bag method would likely require tens if not hundreds of thousands of bags to be deployed in a relatively short window of time, necessitating stockpiling prior to deployment. It thus becomes important to understand how the integrity of the bags changes over time under different storage conditions to avoid the potential for them to tear apart as they are being deployed or shortly thereafter. In this chapter, we describe some initial experiments on how bag integrity, measured as the breaking strain of the hessian fibers making up the bag, changes over time under a variety of conditions.

6.1. Methods

Experiment 1: Storage location

To assess how storage location influenced bag degradation, 72 hessian sand bags filled with the standard 20 kg of sand were allocated across 6 pallets, with each pallet having 2 layers of 6 bags (Figure 6.1). Three pallets were stored indoors under ambient conditions (i.e. without climate control), while the other three were stored outdoors and exposed to the elements. The experiment started on the 16th of May 2018, and finished 4 weeks later on the 13th of June. A total of 21.2 mm of rain fell at the nearby Adelaide Airport during this time. At the same time as the bags were filled, ten 100g sub-samples of the fill were obtained to determine the initial moisture content. These were weighed and then dried at 60ºC until they reached constant weight, and had an average moisture content of 3.8% (± 0.3 se). At the end of the experiment, samples of sand were obtained from the top and bottom of each bag, and a single strand of hessian was also removed from the top and bottom of each.

As an index of bag integrity, the breaking strain of each strand of hessian was measured using a Sauter GmbH FH100 force gauge mounted to a Sauter GmbH TVL manual stand. Data on breaking strain were analysed using linear mixed effects models with the package lme4 (Bates et al. 2015) in R (ver 3.3.0).

Figure 6.1: Bag set-up for experiment 1.

Experiment 2: Moisture content of fill

To assess how the moisture content of the fill affected bag integrity over time, we filled 5 bags with sand of each of 4 different moisture levels on the 6th of June 2018. The sand used was sorted kiln dried sand, with 10 kg added to each bag. Five bags had the straight kiln dried sand, and 5 each had 100, 200 or 400 ml of water added. For each of the water additions, 10 kg of kiln dried sand was added to a bucket, and thoroughly mixed with the water prior to bagging. The 200 ml addition mimicked the moisture content of the sand in bags obtained from our normal commercial supplier for experiment one. Once filled, a 100 g sample of sand was taken from each bag to determine moisture content, and a single strand of hessian removed from both the top and bottom of each to assess breaking strain, both as per experiment 1. Bags were then randomly laid out over 2 pallets, with a single layer of bags on each. Plastic moisture barriers were placed between bags to prevent moisture seeping from one to another (Figure 6.2). Bags were stored indoors, under ambient conditions. Additional sampling occurred on days 7, 14 and 28, following the same procedure as for day 1. Analysis was as per experiment 1.

Figure 6.2: Bag set-up for experiment 2.

Experiment 3: Pallet wrapping

For safety and ease of transport, pallets of bags may be wrapped in pallet wrap. This may also have the potential to reduce exposure of bags to the elements during storage. However, it is also likely to trap moisture within the group of bags, which may enhance degradation. To examine this, we set up an experiment following the design used in experiment 1, except that all pallets were kept indoors, in the same room as used for the previous experiments, with 3 being wrapped and 3 not wrapped. This experiment started on the 20th of June 2018, and finished on the 17th of July.

6.2. Results

Experiment 1: Storage location

Both the storage location, and surface of the bag, significantly influenced the breaking strain of the hessian strands (Table 6.1). In particular, bags kept outside in the elements fared worse than those kept indoors, and there was a tendency for breaking strain to increase from the top of the pallet to the bottom for those kept outdoors (Figure 6.3). The moisture content of the bags kept indoors decreased to almost zero over the 4 week experiment, while that of those kept outdoors increased, especially at the top of the bags (Figure 6.3).

Table 6.1: LMER results for influence of storage location, location on pallet and bag surface on the breaking strain of hessian fibers from sand filled bags.

	df	AIC	P (Chi)
Location	1	1,051.8	**0.0003**
Position	1	1,040.9	0.15
Surface	1	1,043.6	**0.028**
Location x Surface	1	1,041.3	0.052
Location x Position	1	1,040.8	0.066
Surface x Position	1	1,037.8	0.58
Location x Surface x Position	1	1,039.5	0.78
Null	143	1,041.4	

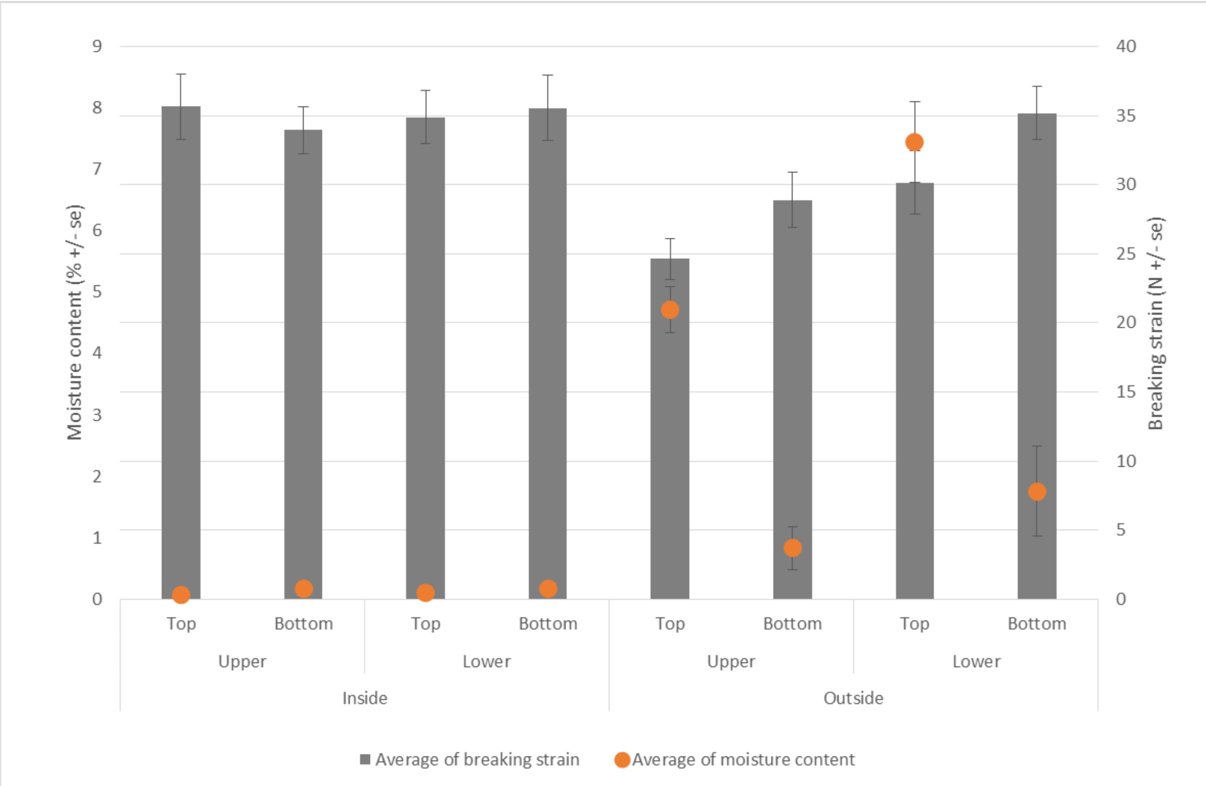

Figure 6.3: Influence of storage location, location on pallet and bag surface on the breaking strain of hessian fibers from sand filled bags.

Experiment 2: Moisture content of fill

The breaking strain of fibers from the bags varied as a function of the interaction between fill moisture content and day, but nothing else (Table 6.2). Whilst kiln dried sand consistently had some of the highest breaking strains, and showed little variation over time, the bags with different levels of moisture content all showed different patterns over time, with those having the highest initial moisture content generally, but not always, having the lowest breaking strain (Figure 6.4). There was no difference between the top and bottom of the bags. For all bags, the moisture content rapidly declined to almost zero over time, which may have been an artefact of the bags being kept as a single layer on the pallet, and thus having good air circulation over and below them.

Table 6.2: LMER results for influence of fill moisture content, location on bag and day of sampling on the breaking strain of hessian fibers from sand filled bags.

	df	AIC	P (Chi)
Moisture content	3	1,198.5	0.058
Surface	1	1,195.3	0.61
Day	3	1,196.5	0.14
Moisture x Surface	3	1,202.6	0.75
Moisture x Day	9	1,207.1	**0.039**
Surface x Day	3	1,202.4	0.81
Moisture x Surface x Day	9	1,207.4	0.64
Null	159	1,218.4	

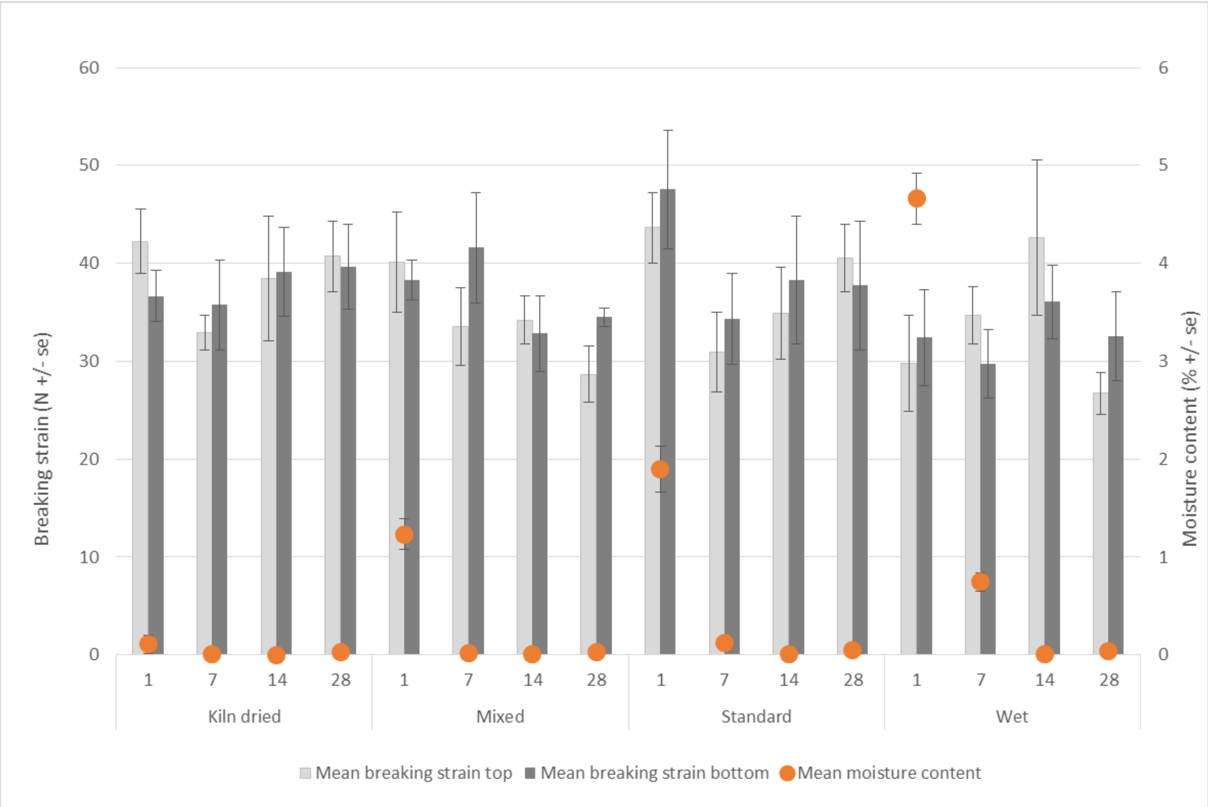

Figure 6.4: Influence of fill moisture content, bag surface and day of sampling on the breaking strain of hessian fibers from sand filled bags.

Experiment 3: Pallet wrapping

In this experiment, there was a significant interaction between pallet wrapping, location on pallet and the bag surface on breaking strain (LMER: $p<0.0001$). In particular, the upper surface of the top unwrapped bags had a relatively high breaking strain, while the lower surface of the bottom wrapped bags had a relatively low breaking strain (Figure 6.5). Except for the upper surface of the bottom bags, pallet wrapped bags had a lower breaking strain than unwrapped bags. In contrast to the results for bags kept indoors in the previous two experiments, moisture levels generally remained high, except for the top surface of the top unwrapped bags. In all cases, pallet wrapped bags had a higher moisture content than unwrapped bags. Pallet wrapped bags were also observed to be extremely mouldy and had a strong musty odour at the conclusion of the experiment.

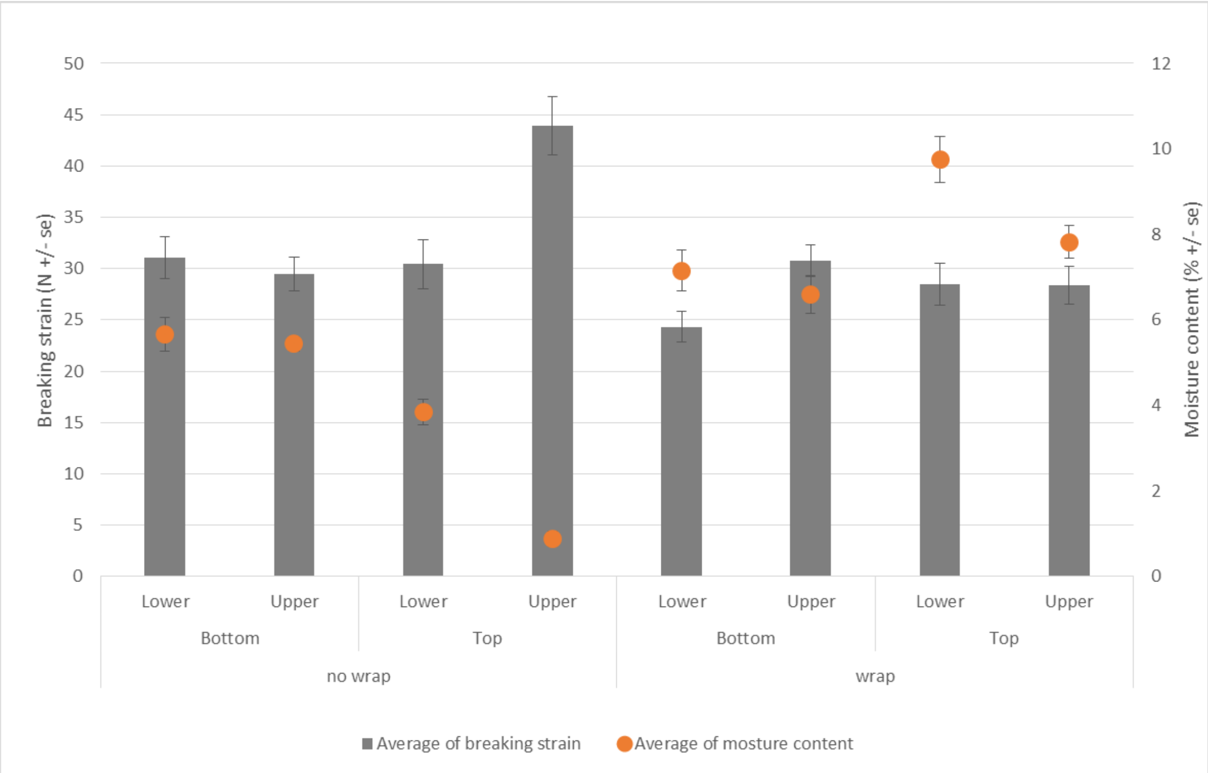

Figure 6.5: Influence of fill pallet wrapping, location on pallet and bag surface on the breaking strain of hessian fibers from sand filled bags.

6.3. Discussion

Overall, it appears that bags should not be exposed to the elements during storage, and if pallet wrap is required for transport purposes, it should only be applied immediately prior to transport and be removed immediately after. While storing pallets of wrapped bags only slightly reduced their breaking strain, it did result in bags becoming musty and mouldy, which may indicate a reduced lifespan. Filling the bags with moist sand did not appear to be an issue if they were not pallet wrapped, although this warrants further investigation, as only stacking bags 2 high allowed for all bags to be exposed to air movement. Typically, a pallet would have bags stacked 8 high (Figure 6.6), and so most bags would only be exposed to the air at their edges, which is likely to result in increased moisture retention and possibly increased degradation.

Figure 6.6: Fully stacked pallets of sand bags ready for deployment with (left) and without (right) pallet wrap.

7. GENERAL DISCUSSION

At a small scale, we have previously demonstrated that the hessian bag method is clearly successful for rehabilitating *Amphibolis* in areas where seagrass has been lost and the sediments have become mobile (Tanner 2015, Tanner and Theil 2016). In the short to medium term, the larger-scale deployments have not been so successful, although it should be noted that it took around 5 years before the small-scale experiments led to densities of *Amphibolis* equivalent to those found in natural meadows. While the slow nature of rehabilitation means that these plots should be surveyed for at least a few more years before a final conclusion as to their success is drawn, it is also important to start considering why they may not have been as successful as the small-scale studies suggested.

There are three main differences between the 1-hectare trials and the smaller-scale trials that may contribute to the difference in recruitment seen. Firstly, it could be a timing issue, with 2014 and 2017 being poor years for recruitment compared to the years when small-scale deployments were made. We have since instigated regular beach surveys of recruits, which may help us to address this question for future deployments. Second, it could be a spatial issue, with the larger plots being in areas where there is a poor supply of recruits compared to the small-scale site. Small-scale deployments in 2017 indicate that this is likely to be part of the problem, although sites with high recruitment in 2017 also performed poorly in the medium term, so it is not the sole factor at play. In 2019, small-scale deployments are being made at 15 sites along the Adelaide coast, to better investigate spatial variation in recruit availability and early establishment. Thirdly, it might be a bag density issue, with the high density of bags in a small area affecting water flow in such a way as to promote recruitment, and the low density of bags in the larger plots not having the same affect. This will be investigated in 2020, when trial 1-hectare deployments will be undertaken with bag densities ranging from 1,000 to 10,000 bags per hectare.

Another important issue is how to distribute the bags spatially. In the large-scale trials reported here, we deployed 1,000 bags per hectare, with the aim to have these approximately uniformly distributed throughout each plot. What actually happened due to the practicalities of bag deployment was that bags were laid out along 100 m long lines ~10 m apart. In scaling up, attempts should be made to determine if these two patterns produce different results or not. Other arrangements may include crossed lines (i.e lines running north-south crossed by east-west lines), and checkerboard (patches of high density alternating with patches of no bags).

The way bags are handled and stored prior to deployment may also affect success. Preliminary trials reported here suggest that the sand in bags rapidly dries if there is air flow around the bags, although this may not be the case when they are stacked 8 high on pallets.

Wrapping the pallets with pallet wrap does not appear to be a good idea, as moisture is retained and the bags start to go mouldy, although this did not appear to greatly affect their integrity over the 4 week study reported here. While pallet wrapping may be needed for transport, it should be removed for storage. Bags should also be kept out of the elements, as especially the top layer on pallets stored outdoors deteriorated faster than those stored indoors.

Using the hessian bag method for large-scale *Posidonia* rehabilitation is currently problematic from two perspectives. First, the technique does not seem as amenable to simply dropping the bags en masse off the side of the boat and leaving them, as can be done for *Amphibolis*. While survival of seedlings glued into bags prior to deployment was good, and planting before deployment is much quicker than after, it would still not be logistically feasible to plant large numbers of bags with *Posidonia* seedlings, especially considering the short window of opportunity to collect fruits and then plant them. Another consideration for *Posidonia*, at least around Adelaide, is the timing of fruit release. Mature fruits are released in late December or early January, and those collected much before or after this time performed poorly in tank growth trials, with high mortality rates. While beach collected fruits are viable, making collection easier, there needs to be a rigorous quality control process to ensure that only good quality fruits and seedlings are retained. Fruits should be collected as they are first washed up on the beach, at which time they are turgid, and if mature, yellow green in colour. Those that appear wrinkled or flaccid have dehydrated from being on the beach too long, while fruits with a large brown patch appear to have been sun scorched. Once apparently good quality fruits have been obtained, they should dehisce and release a seedling within a few days. Those that take longer should be discarded, as the seedlings show poor performance. Finally, while seedlings can be held for several months prior to planting, their performance starts to decline after around 10 days, should planting should occur by then if at all possible. Using the hessian bag technique for *Posidonia* rehabilitation is likely to only ever be a niche endeavor, suited to situations where there is a high priority for establishing even small patches of seagrass, possibly in locations where there is no supply of *Amphibolis* recruits to allow assisted recolonization of this genus.

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