New Life for our Coastal Environment Seagrass Rehabilitation Project: 2019-2022

J E. Tanner

SARDI Publication No. F2022/000383-1
SARDI Research Report Series No. 1167

SARDI Aquatics Sciences
PO Box 120 Henley Beach SA 5022

March 2023

Final Report to the Department for Environment and Water

Government of South Australia
Department of Primary Industries and Regions

Government of South Australia
Department for Environment and Water

SARDI
SOUTH AUSTRALIAN RESEARCH AND DEVELOPMENT INSTITUTE

New Life for our Coastal Environment Seagrass Rehabilitation Project: 2019-2022

Final Report to the Department for Environment and Water

Jason E. Tanner

SARDI Publication No. F2022/000383-1
SARDI Research Report Series No. 1167

March 2023

The South Australian Research and Development Institute respects Aboriginal people as the state's first people and nations. We recognise Aboriginal people as traditional owners and occupants of South Australian land and waters. We pay our respects to Aboriginal cultures and to Elders past, present and emerging

This publication may be cited as:
Tanner, J.E. (2023). New Life for our Coastal Environment Seagrass Rehabilitation Project: 2019-2022. Final Report to the Department for Environment and Water. South Australian Research and Development Institute (Aquatic Sciences), Adelaide. SARDI Publication No. F2022/000383-1. SARDI Research Report Series No. 1167. 71pp.

DISCLAIMER

The authors warrant that they have taken all reasonable care in producing this report. The report has been through the SARDI internal review process and has been formally approved for release by the Research Director, Aquatic and Livestock Sciences. Although all reasonable efforts have been made to ensure quality, SARDI does not warrant that the information in this report is free from errors or omissions. SARDI and its employees do not warrant or make any representation regarding the use, or results of the use, of the information contained herein as regards to its correctness, accuracy, reliability, and currency or otherwise. SARDI and its employees expressly disclaim all liability or responsibility to any person using the information or advice. Use of the information and data contained in this report is at the user's sole risk. If users rely on the information, they are responsible for ensuring by independent verification its accuracy, currency, or completeness. The SARDI Report Series is an Administrative Report Series which has not been reviewed outside the department and is not considered peer-reviewed literature. Material presented in these Administrative Reports may later be published in formal peer-reviewed scientific literature.

© 2023 SARDI
This work is copyright. Apart from any use as permitted under the *Copyright Act* 1968 (Cth), no part may be reproduced by any process, electronic or otherwise, without the specific written permission of the copyright owner. Neither may information be stored electronically in any form whatsoever without such permission.

Author(s):	J.E. Tanner
Reviewer(s):	Kathryn Wiltshire (SARDI) Jason Nicol (SARDI), Alison Turner, Cristina Vicente, and Judith Kirk (DEW)
Approved by:	S. Goldsworthy Science Leader – Marine Ecosystems
Signed:	
Date:	06 March 2023
Distribution:	DEW, SARDI Aquatic Sciences, Parliamentary Library, State Library and National Library
Circulation:	OFFICIAL

ALL ENQUIRIES

South Australian Research and Development Institute - SARDI Aquatic Sciences
2 Hamra Avenue West Beach SA 5024
PO Box 120 Henley Beach SA 5022
P: (08) 8207 5400 F: (08) 8207 5415
E: pirsa.sardiaquatics@sa.gov.au W: http://www.pir.sa.gov.au/research

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

We thank Mandee Theil, Ian Moody, Leonardo Mantilla, Kathryn Wiltshire, Sharon Drabsch and many others for their assistance with undertaking field and laboratory work. Comments on an earlier draft of this report from Kathryn Wiltshire and Jason Nicol from SARDI and Alison Turner, Cristina Vicente and Judith Kirk from DEW, and the editorial oversight of Simon Goldsworthy, are greatly appreciated. Funding was provided by the Department of Environment and Water, through the New Life for our Coastal Environment initiative.

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 sandbags 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. While several isolated 1-hectare deployments have previously been undertaken, these have used a low density of bags. The major focus of this study is to scale-up the hessian bag method for restoring *Amphibolis* seagrass, by deploying 50,000 bags over ten 1-hectare plots along the Adelaide coast. As the optimal bag density is not currently known, this study was set up as an experiment, with plots ranging from 0 to 10,000 bags per hectare.

A pilot study indicated that the best locations for restoration appeared to be in the Semaphore and Grange region. Sites north of this, and especially those south, had low recruitment of *Amphibolis* seedlings to 30 bags put out at each of 15 sites in 2019 and 10 sites in 2020. Subsequently, 27,800 bags were deployed over five plots between Semaphore and Tennyson in 2021 and a further 22,200 were deployed over four plots in 2022. Monitoring 2-4 months after deployment indicated that recruitment was lower than seen at Grange in most years, with 3-20 seedlings per bag. There was no effect of bag density on initial recruitment, which instead varied spatially and between years.

In addition to the large-scale deployment of hessian bags for *Amphibolis* restoration, further work was undertaken to develop alternative restoration techniques that could be used off Adelaide, especially for *Posidonia* seagrasses. The first technique was inspired by observations that *Posidonia* seedlings would often collect around foreign objects (e.g. crab traps) that had been lost on the seafloor and provided some protection from water movement. To mimic this at a slightly larger scale, and with natural materials, 50-65 mm rock ballast was deployed in both summer (for *Posidonia*) and winter (for *Amphibolis*), with the idea that the interstices between the rocks would provide protected microsites for seedlings to accumulate and establish. However, the technique proved unsuccessful. A second technique trialed was planting and scattering *Posidonia* seedlings into already established patches of *Amphibolis* seagrass, which was inspired by the observation that some *Posidonia* did naturally re-establish on the edge of restored *Amphibolis*. Again, this proved unsuccessful. The third field trial was to determine whether it is necessary to glue *Posidonia* seedlings into bags before deploying bags from a boat. While some 95% of seedlings glued in survived the deployment

process in place, only about 25% of unglued seedlings remained in the bags after deployment, indicating the importance of securing the seedlings into the bags.

A number of laboratory trials were undertaken to better understand the requirements for using beach-cast *Posidonia* fruit in restoration. The timing of release of mature viable fruits tended to be more erratic than in previous studies, with some years having multiple peaks of high-quality fruit. There were also some years in the current study with little to no fruits washing onto the beach at West Beach, or large gaps in fruit availability. Periods with no fruit in late December and early January coincided with a lack of sea breezes, and in 2020/21 with strong southerly winds. It is hypothesized that these conditions meant that fruits either stayed out at sea or were washed into northern Gulf St Vincent, although it is also possible that fruits weren't released. Substrate conditions appeared to have little influence on seedling survival and growth, as previously established. Shading also had little influence on survival, although seedling size tended to peak at intermediate to high light. However, after 4-5 months seedlings still appeared to be in good condition even under extreme levels of shading (>99% light reduction), probably reflecting that they were relying primarily on stored reserves in their seeds rather than photosynthesis.

The final area of work was to further assess the impact of storage conditions on bag structural integrity, to determine the feasibility of building up a reserve of bags prior to the commencement of large-scale deployments. This showed that bags could be stored for up to 1 month either inside or under cover with no detrimental effects, but that bags exposed to the elements (sun and rain) had deteriorated after 1 month.

Further recommendations for research include:

1. Following the success of the one-hectare plots described here over time, in order to quantify the trade-off between bag density (= cost) and time to restore a meadow,

2. Further work to establish if scattering *Posidonia* seedlings could be a viable approach, without the need for actual planting.

3. Determining the influence of the spatial pattern of restoration. For example, can plots be restored in a checkerboard fashion, with the unrestored checkers naturally recolonizing?

4. Determining if the hessian bag method is a viable means for stabilizing erosion scarps in *Posidonia* meadows, thereby reducing ongoing loss of seagrass.

5. Establishing whether the use of *Posidonia coriacea* provides enhanced outcomes in more exposed areas, as it naturally occurs in areas with higher wave activity than *Posidonia angustifolia*.

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 following 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 for 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.

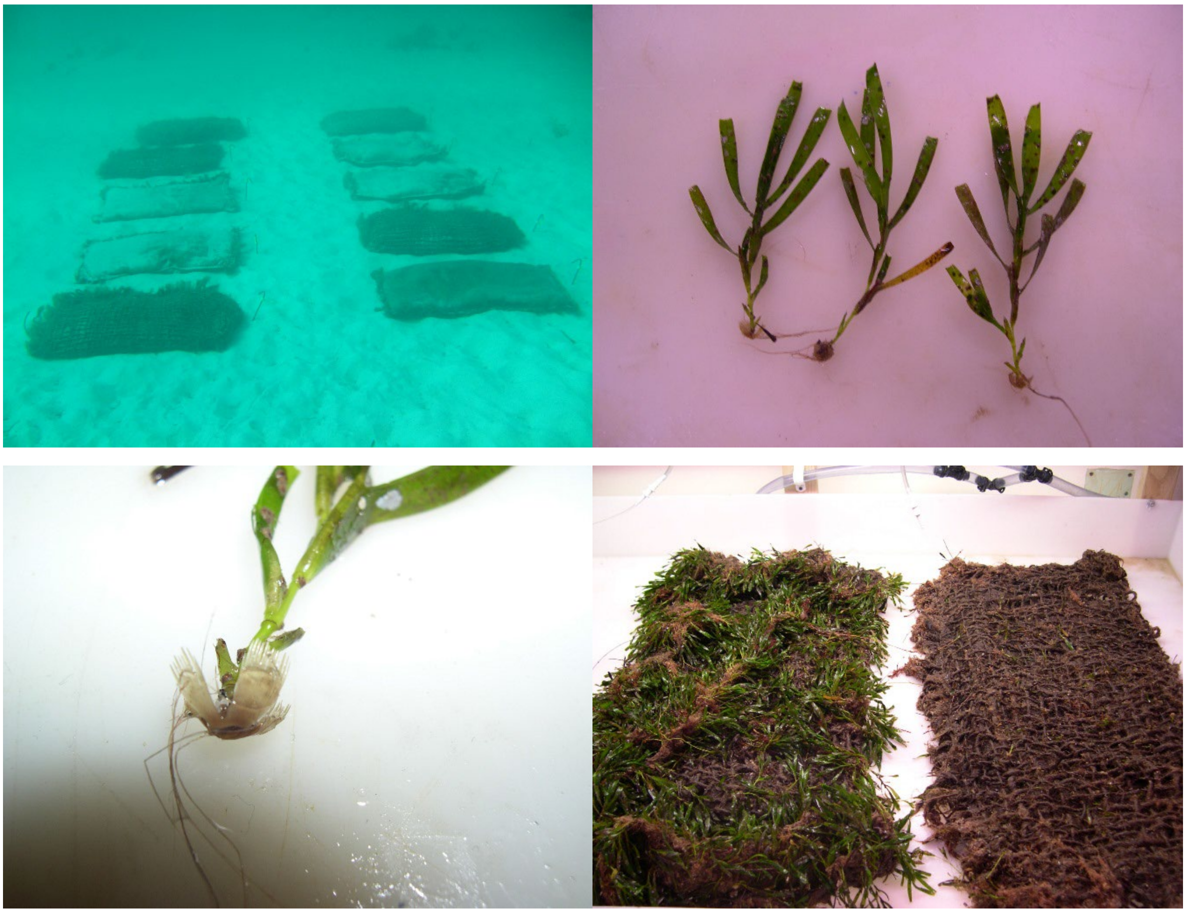


Figure 1-1: Bag layout for small-scale experiments on *Amphibolis* recruitment facilitation (top left), *Amphibolis* seedlings (top right), close-up of basal 'grappling hook' that allows seedlings to attach (bottom left), and examples of double-layered bags with and without seedlings attached (bottom 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 of seedlings on sandbags. 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. A fourth 1-hectare trial was established in 2017, this time with 2500 bags (Tanner and Theil 2019). Both this and the two 2014 trials were subject to extensive physical disturbance that removed many of the marker stakes used for monitoring, which made further temporal comparisons difficult. However, spatial comparisons showed some indications of higher *Amphibolis* densities inside the plots in 2018 and 2019, whereas *Posidonia* showed a north-south gradient.

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 in the final number of stems 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). Deployments from earlier years 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.

To further assess the timing of recruit availability in *Amphibolis*, beach surveys were undertaken in 2017 and 2018 (Tanner and Theil 2019). Surveys were undertaken 3-5 days a week and showed a distinct peak in beach-cast seedlings over a few weeks in July of both years.

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, 2019). 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 in the short term (2-3 years), but there was no influence of fill type in the long term (5-6 years), 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. A trial of planting and supergluing seedlings into bags prior to deployment was successful, and more time-effective than divers planting into bags after deployment (Tanner and Theil 2019).

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 (Tanner and Theil 2019). 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 the window of opportunity for collection 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 (Tanner and Theil 2019). While the trials conducted prior to the current study only used small numbers of bags and did not require bags to be stored for an extended period, upscaling to larger deployments, as described in this report requires 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.

To date, and to our knowledge, the hessian bag method has 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 in this high wave environment with frequent sediment resuspension, they 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 also showed some indications of success, although physical disturbance interrupted the intended monitoring before long-term success could be established. While initial recruitment to bags in the larger-scale trials 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.

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 too early in the year 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.

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.
Tanner, J.E., and Theil, M.J. (2019). Adelaide Seagrass Rehabilitation Project: 2017-2019. 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. F2009/000210-3. SARDI Research Report Series No. 1025. 77pp.
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
Tanner & Theil 2019	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 April 2019 and October 2022. In particular, we look at four main components:

- Pilot studies to determine the best location along the Adelaide coast for large-scale trials;
- A large-scale trial with ten one-hectare deployments ranging from 0 to 10,000 bags;
- Pilot trials of an alternative technique for restoring both *Amphibolis* and *Posidonia*;
- Field and laboratory trials with *Posidonia* seagrass, to try and establish a low-cost technique for the rehabilitation of this genus.

1.3. Blue Carbon

Following the release of the South Australian Government's *Blue Carbon Strategy for South Australia*, there has been increased interest in the capacity of seagrass and other vegetated coastal ecosystems to sequester organic carbon. The availability of several patches of restored *Amphibolis* of different ages, as described above, provided an excellent opportunity to assess the implications of *Amphibolis* restoration for blue carbon storage. In 2020, in collaboration with Edith Cowan University, we undertook an assessment of blue carbon stocks and accumulation rates in several of the restored *Amphibolis antarctica* seagrass patches, with two specific objectives:

1. To determine the standing stock, and if possible, the rate of accumulation, of blue carbon in *Amphibolis antarctica* meadows off the Adelaide coast; and
2. To assess how well restored *Amphibolis antarctica* patches capture and store carbon relative to the adjacent natural meadow.

Sediment cores were collected from five *Amphibolis* habitats at the Grange site, approximately 1.5 km offshore from Grange on the Adelaide coast: a natural (undisturbed) meadow; three restored patches (established in 2008, 2009 and 2011); and a disturbed but never recovered area (bare). The cores were analysed to determine their carbon stocks and dated using ^{210}Pb method to establish the carbon accumulation rate in the seagrass soils. Up to 10 Sediment Elevation Rods were also placed into each of the sites to allow ongoing assessment of sediment accumulation rates in the meadows.

The soil C_{org} stocks in all sites were low compared to mean stocks in Australian seagrass meadows. In the top 10 cm, the mean C_{org} stocks ranged from 0.05 ± 0.01 (SD) kg C_{org} m^{-2} in the bare treatment to 0.3 ± 0.2 kg C_{org} m^{-2} in the 2011 revegetated treatment. Stocks were larger in the top 50 cm, ranging from 0.7 ± 0.09 kg C_{org} m^{-2} to 1.4 ± 0.2 kg C_{org} m^{-2}. When compared over appropriate soil depth to other seagrass meadows, including other *Amphibolis* meadows, the sites at Grange, including the undisturbed meadow, had about 5.5 times less carbon stock. Over the top 10 cm of sediment, which is the most appropriate depth for comparisons, there was no statistically significant difference in the carbon stocks of the Natural, Restored or Bare meadows.

Full details of this work are presented separately (Lavery et al. 2022).

2. PILOT STUDIES FOR LARGE-SCALE SITE SELECTION

There is currently limited information on the spatial variability in availability of *Amphibolis* recruits along the Adelaide coastline. A single study was undertaken in 2005 to examine recruitment at six locations from Largs Bay to Seacliff (see Figure 1-3), at depths of 8 m (all sites), 10 m and 12 m (three sites only) (Collings et al. 2007). There was substantial variability in both recruitment and survival among sites, with Grange and to a lesser extent Henley performing best. Southern sites (Seacliff and Brighton) had very low recruitment, while northern sites (Largs and Semaphore) had moderate to good recruitment but poor survival. This study was only followed for 12 months, and the bags used broke down relatively quickly compared to those used in other deployments, which is likely to have had a negative influence on survival. The deployments were also all in areas of seagrass, and not in bare sand areas requiring rehabilitation. Consequently, while Collings et al. (2007) provides useful initial information on spatial variability in recruit availability, it is not adequate for selecting sites for the proof of application of the hessian bag technique of rehabilitation.

In this chapter, we describe the pilot experiments undertaken in 2019 and 2020 to help select the final sites for ten 1-hectare deployments of sandbags.

2.1. Methods

Pilot site selection

The selection of pilot sites was undertaken following a workshop between relevant scientists from SARDI, EPA, SA Water, DEW and Adelaide University. At this workshop, a number of existing data sets were collated and interrogated to help determine an initial shortlist of sites. These data sets were:

- EPA Aquatic Ecosystem Condition Reporting results for seagrass change in cover from 2010 to 2017
- SA Water model outputs showing predicted suitability for *Amphibolis*
- DEW rod line data showing seabed stability as well as seagrass species and cover at each rod site
- Adelaide University hyperspectral mapping of current seagrass cover

During the workshop, a set of 49 potential pilot sites were chosen, ranging from near Bolivar in the north to Seacliff in the south (Figure 2-1).

2019

Potential pilot sites north of Outer Harbor were eliminated, as travel time to cover them as well as those off the metropolitan coast was excessive. The remaining sites were then reduced to the planned 15 (Figure 2-2), largely by eliminating sites in close proximity and ensuring a roughly even spread. Sites less than 5 m in depth, or greater than 8 m, were also eliminated.

The former are considered less likely to be suitable due to exposure to wave action, while the later impose greater limitations on dive time. Keeping depths to a narrow range also eliminates depth as a confounding factor in interpreting success. The majority of the final sites are in areas of large-scale loss, but the two southernmost are erosion scarps to test the applicability of the hessian bag method for scarp stabilisation these. Three existing sites were also chosen to provide some temporal comparisons, including the main short-term study site directly off Grange jetty.

Thirty hessian sandbags were deployed at each of the 15 pilot sites from the RV Ngerin on 12June 2019. These were all ‘standard’ hessian bags, as used in previous work, and with 20 kg of fill. An additional 30 bags were also deployed at the Grange site (S21 in Figure 2-2) with 15 kg of fill. This was to assess the potential to use slightly lighter bags to reduce manual handling issues that will be experienced with the deployment of large numbers of bags. These bags were rearranged by divers on the 19-21 June 2019 to facilitate monitoring of recruitment to them. Poor visibility hampered the relocation of the bags, and 15 bags (1 – 2 at each of 14 sites) could not be relocated. In addition, no bags at S13 (Largs) could be relocated. The GPS used to mark the exact location that the bags were deployed lost the satellite signal as the bags were being tipped off the boat, meaning that a precise location was not obtained for this site. Visibility at this site was also poor, being only 1-2 m, compared to 5+ m at other sites dived the same day. The surface sediments at this site were very fine and easily resuspended, and as a result, as soon as the divers reached the bottom, visibility declined to <0.5 m. These conditions made searching for the bags exceptionally difficult, and also suggest that the site is poorly suited to rehabilitate seagrasses, although some small patches of mature seagrass were present. All other sites showed evidence of some seagrass present, either as extensive but sparse cover, or small dense patches, although none were in seagrass meadow. At virtually all sites, there were a small number of *Amphibolis* recruits that had already attached to the bags, which is consistent with small numbers concurrently washing up onto the beach at West Beach.

The bags at each site were surveyed to count the number of *Amphibolis* recruits on each in September 2019 (10th & 11th), and again in January 2020 (29th & 30th). Better visibility allowed the lost bags at S13 to be relocated, and these were counted in both surveys, although as the bags sometimes overlapped, the data is not strictly consistent with that for the other sites.

To determine if there were any differences between 15 kg and 20 kg bags, count data were analysed using generalized linear mixed effects models with the package lme4 (Bates et al. 2015) in R (ver 4.2.1). Weight and survey date were included as fixed effects, with an interaction, bag was included as a random effect, and a Poisson distribution was used.

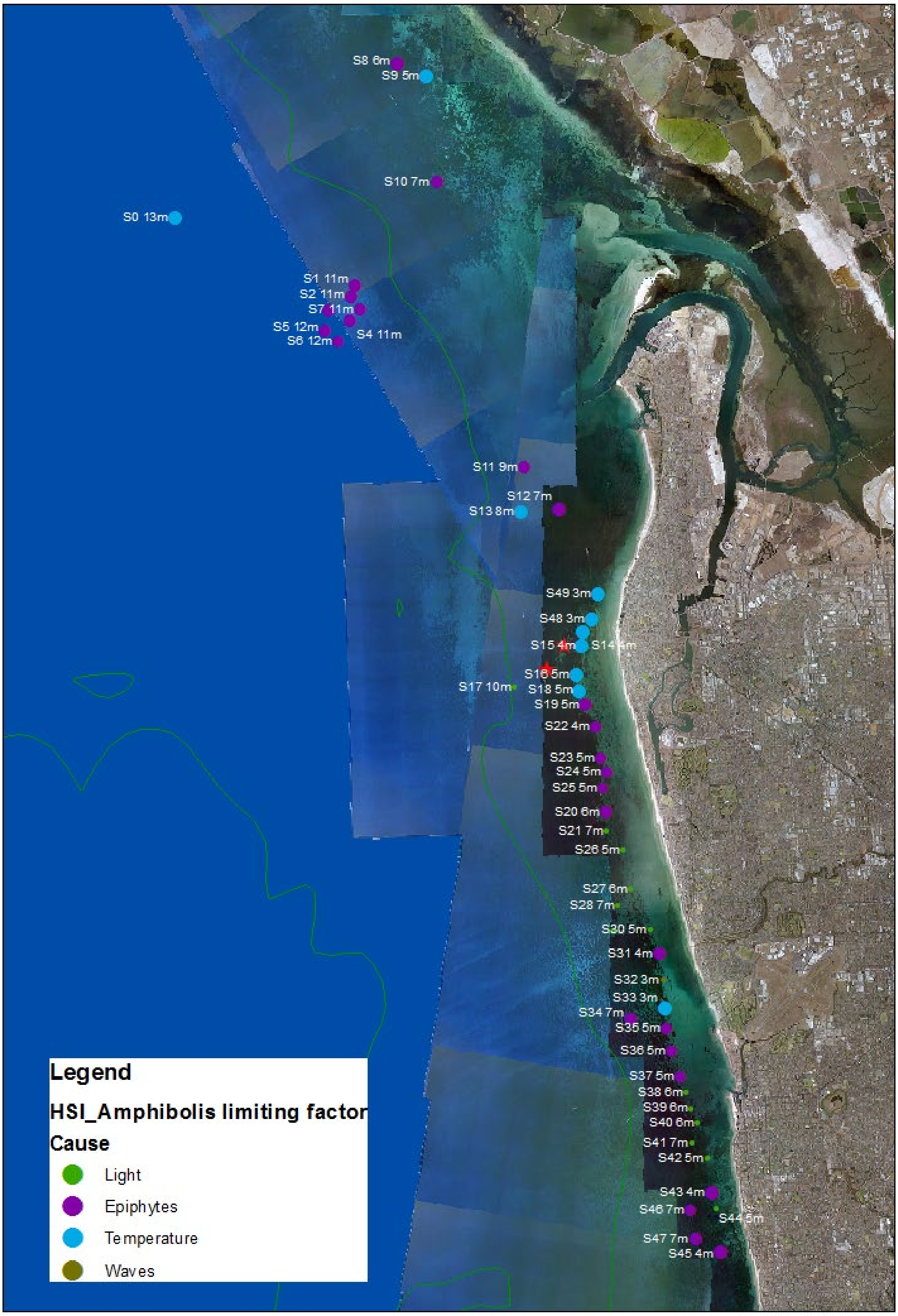


Figure 2-1: Location of original 49 candidate pilot sites. Colour coding indicates the main limiting factor preventing *Amphibolis* re-establishment as predicted by the SA Water model. Red stars indicate EPA carbon coring sites. Green lines indicate the 10 & 20 m depth contours.

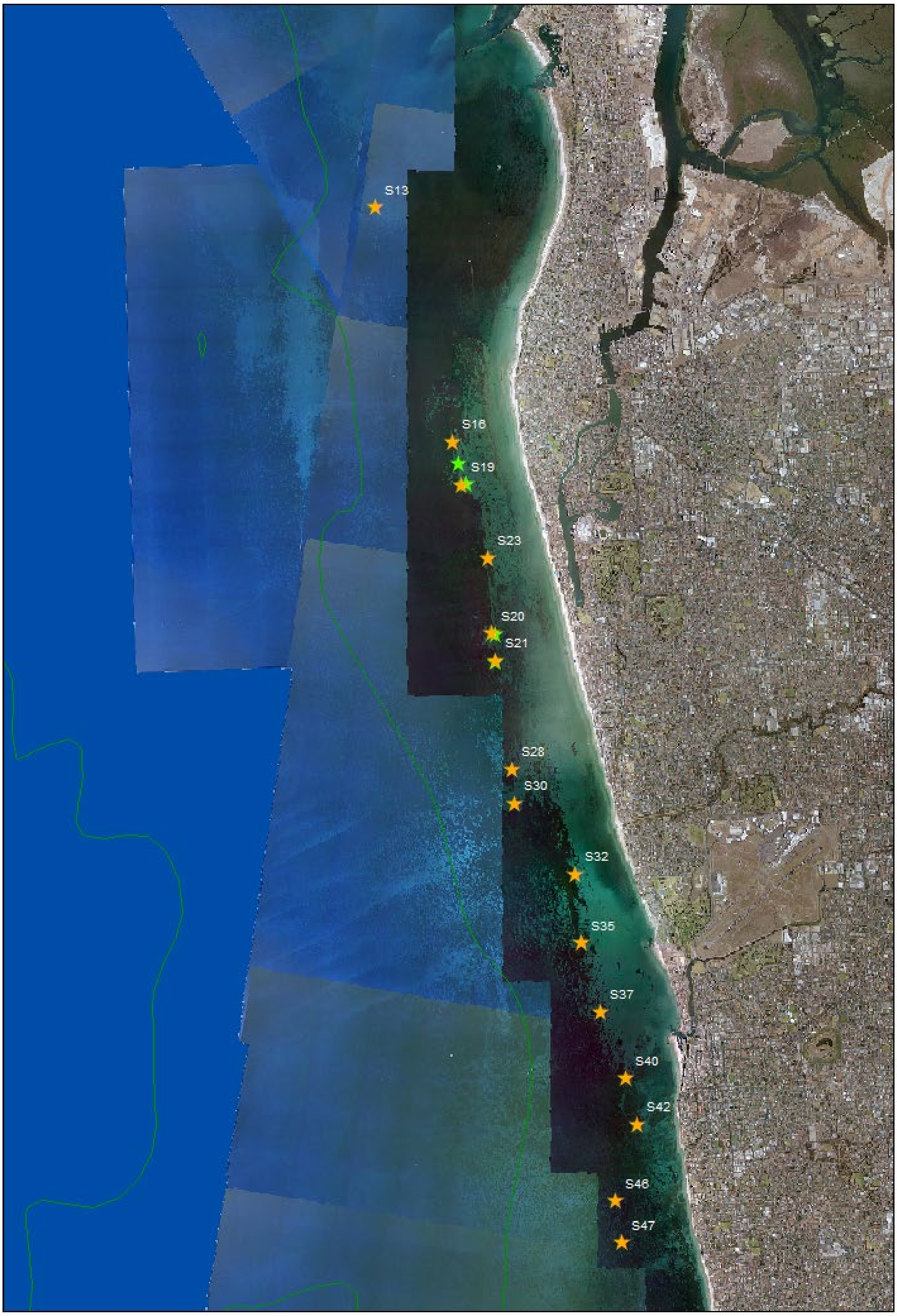


Figure 2-2: Location of final 15 pilot sites for hessian bag deployment in 2019 (orange stars). Green stars indicate existing rehabilitation sites.

2020

The main 10-hectare deployment was originally scheduled for 2020, however was delayed due to COVID19. Consequently, there was an opportunity to undertake a second pilot trial, focusing especially on sites north of Outer Harbor, but with some of the southern sites used in 2019 to ensure that any differences were spatial and not temporal (Figure 2-3). Two of the northern sites targeted areas of loss further offshore, and consequently were in deeper water than the sites surveyed in 2019 (10-12 m compared to 5-8 m). Deployments mimicked those in 2019, with 30 bags at each site deployed on 17 June 2020, although in this case bags were deployed by Maritime Constructions using the Frederick G. All bags were 20kg. Bags were then rearranged by divers on the 7th & 8th July 2020. Bags were surveyed to determine the abundance of recruits on the 9th and 10th of September 2020.

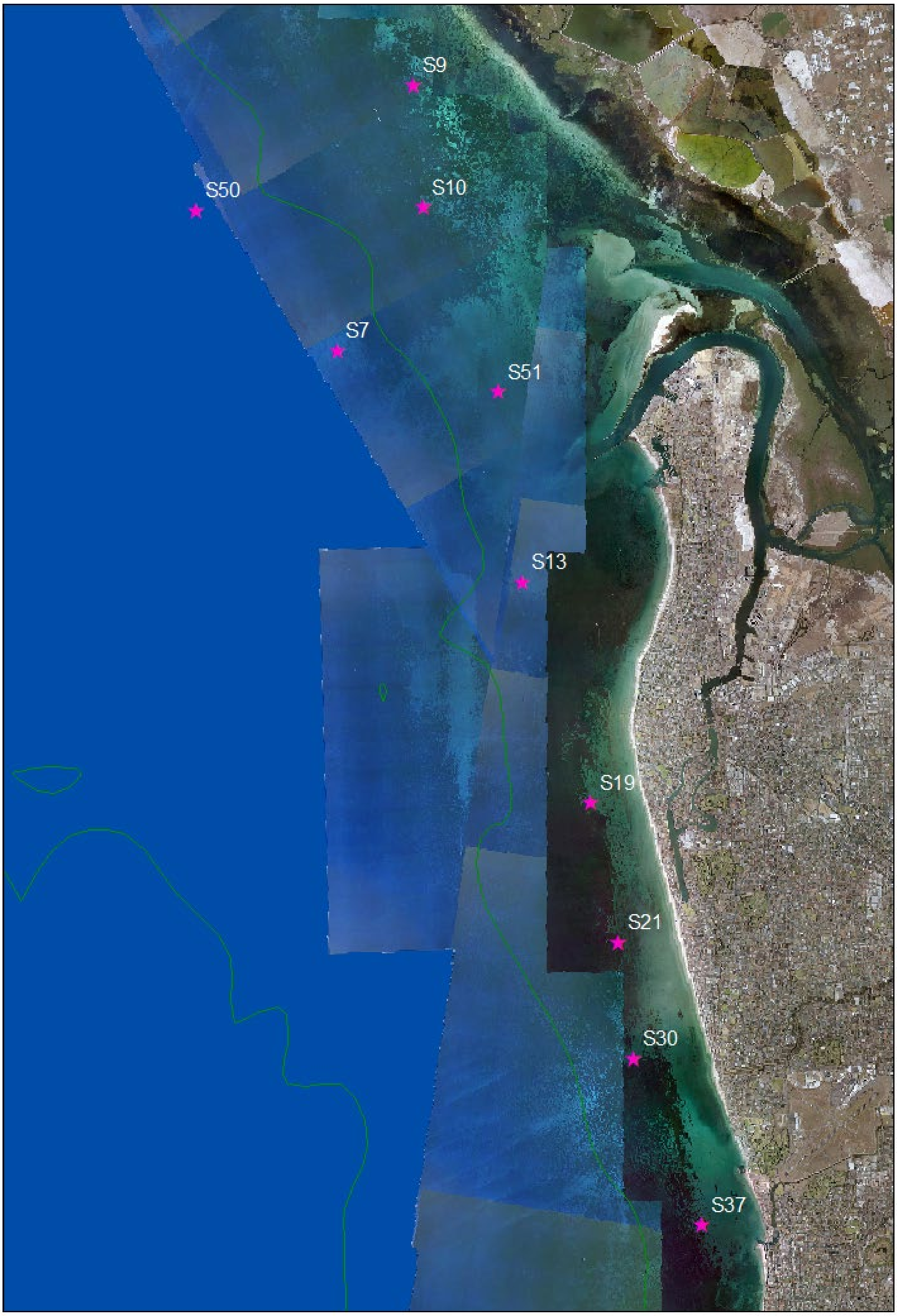


Figure 2-3: Location of 10 pilot sites for hessian bag deployment in 2020.

2.2. Results

2019

The 2019 pilot study showed high levels of recruitment and survival between Semaphore and Grange, with very low levels at the northernmost site, and south of Grange (Figure 2-4). Whilst the data for S13 (Largs) are not consistent with that for the other sites as the bags were not re-arranged by divers after deployment, the extent of the difference between this site and the sites from Semaphore to Grange still indicates that recruit supply was low.

There were no differences in counts between the 15 kg and 20 kg bags at either census date (GLMER: P=0.71, see Figure 2-4).

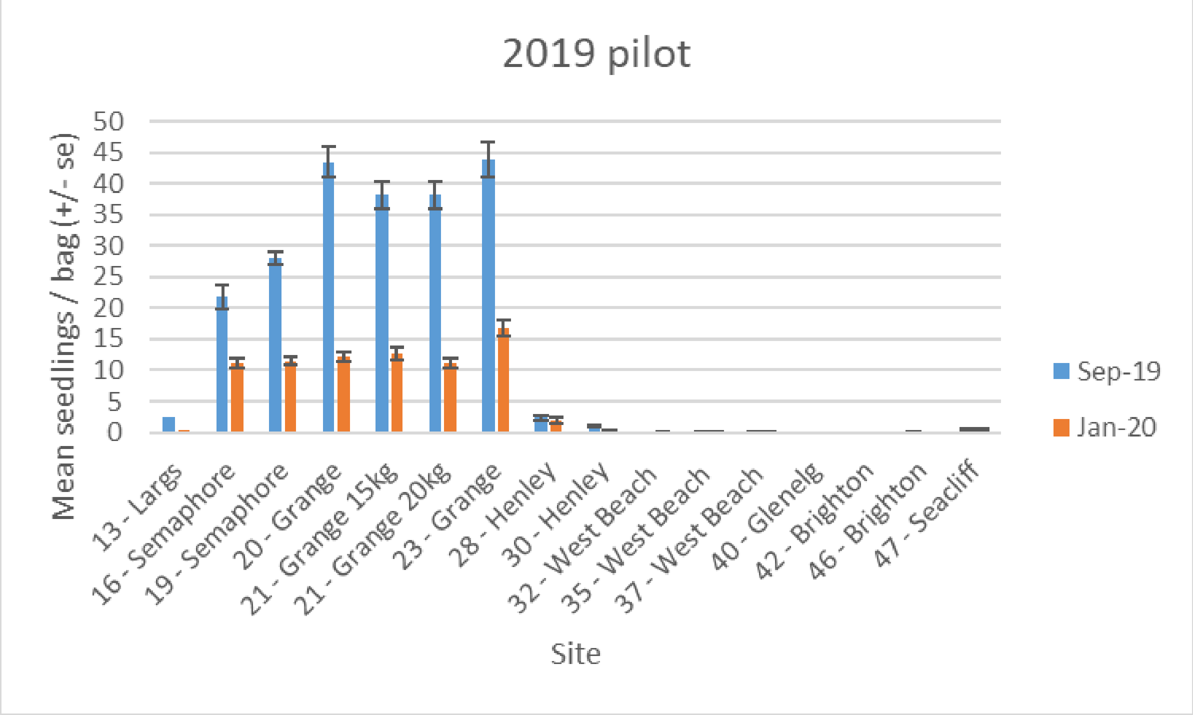


Figure 2-4: Total recruitment onto 30 bags during the 2019 pilot study 3 months after deployment, and survival after 7 months.

2020

The 2020 pilot study showed similar results to the 2019 study for sites south of Outer Harbor that were included in both years. Recruitment was highest at Grange and then Semaphore, while there was low recruitment at Largs, and virtually no recruitment at West Beach or Glenelg (Figure 2-5). Recruitment was ~ 3 times higher in 2020 than in 2019. The new sites north of the Outer Harbor shipping channel experienced little or no recruitment, with the exception of the furthest north site off Port Gawler, which had ~32 recruits per bag.

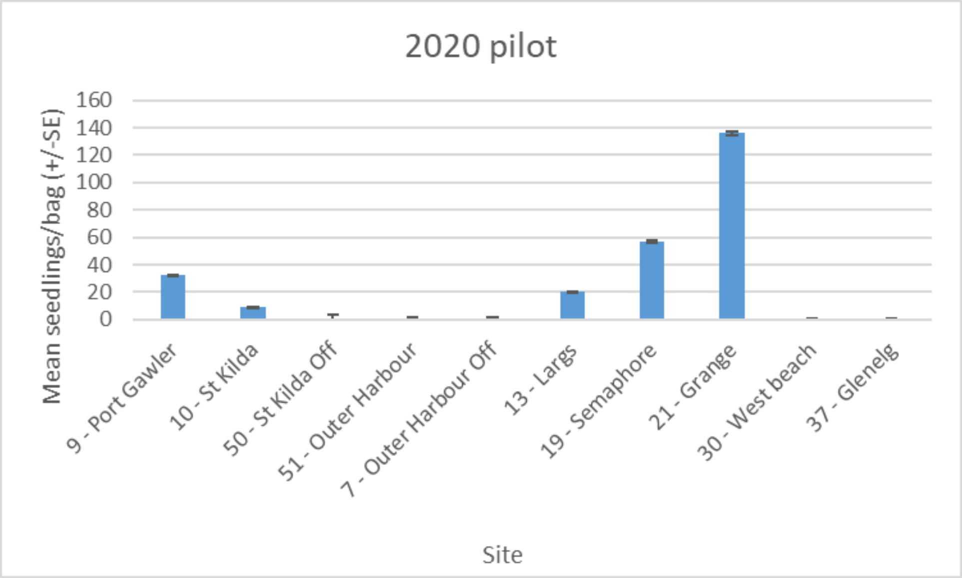


Figure 2-5: Total recruitment onto 30 bags during the 2020 pilot study 3 months after deployment.

2.3. Discussion

Across both years, sites between Semaphore and Grange received good recruitment of *Amphibolis* seedlings, indicating that there is an upcurrent *Amphibolis* meadow producing recruits, and that they survive in at least the short-term. Sites further south had very low numbers of recruits, which could indicate a lack of an upcurrent source, although it could also indicate that recruits were present but did not survive until surveys in September. Mapping in 2006/7 indicated that *Amphibolis* cover was very low in the vicinity of these southern sites (Bryars 2008), which is supported by DEW rod line data (A. Turner *pers. com.*). These southern sites are more exposed to swell coming in through Investigator Strait, so the effects of wave action on the bags would be higher, which can be expected to make it more difficult for recruits to survive. For the immediate purposes of selecting a site at which to undertake the main deployment, the cause of the poor recruitment at these sites is irrelevant. However, if a method for restoring seagrass in these areas is to be developed, it becomes important to understand why there was no recruitment to the bags. The first step would be to use particle dispersal models to assess whether the known *Amphibolis* meadows along the Adelaide coast are well connected to this southern region or not. If not, then recruit supply is an issue, and adding recruits to the system may be required. If they are connected, then it is more likely that wave action on the bags is the issue, and alternative approaches to ameliorating this will need to be investigated.

Low recruitment north of the Outer Harbor approach channel is more likely to be a supply issue. These sites are more sheltered from swell, and some are in deeper water (12-14 m versus 6-10 m) where wave action would be expected to have a lesser effect. Less is known about the species level distribution of seagrasses in this region, so it may not be possible to use particle tracking models to assess their connectivity to potential sources of recruits.

Fifteen-kilogram bags had the same number of recruits as 20 kg bags after three and seven months. This suggests that lighter bags would be suitable in areas where wave activity is limited. Using lighter bags reduces manual handling issues and increase the number of bags that can loaded onto the vessel by a third, reducing the cost of deployment.

3. LARGE-SCALE DEPLOYMENT

3.1. Methods

Based on the pilot studies showing high recruitment between Grange and Semaphore, a series of ten 1-hectare sites (100 x 100 m) were selected in this region. Sites were focused on the northern part of the region to reduce transit times, and thus decrease overall cost. Sites were selected along the inshore margin of the existing seagrass meadow, and to avoid as much as possible having any existing seagrass in them. Once selected, bag densities were randomly assigned to sites, ranging from 0 to 10,000 bags per hectare. A series of evenly spaced north-south and east-west transects were then established in each plot, with each transect corresponding to a single pallet of 50 bags.

Figure 3-1: Location of the ten 1-hectare rehabilitation sites (green boxes) off Semaphore and Tennyson, with pilot study sites marked in yellow. Sites are numbered 1-10 from north to south.

Table 3-1. Details of the rehabilitation sites. Sites are numbered from 1 in the north to 10 in the south.

Plot #	# Bags	# Pallets	Year
1	5550	111	2021
2	10000	200	2021
3	1100	22	2021
4	4450	89	2022
5	0 (control site)	0	2021
6	8900	178	2022
7	6650	133	2022
8	3350	67	2021
9	7800	156	2021
10	2200	44	2022

Deployments commenced on the 11 May 2021 using the SARDI research vessel Ngerin, and due to crew availability, were intermittent to the 6 July 2021. Deployments recommenced on the 24 May 2022 and continued to the 21 June 2022. The vessel navigated along each transect line, with bags being rolled off the pallet loaded onto a tilt table at the back of the vessel (Figure 3-2), or manually dropped over the sides. As much as possible, bags were evenly distributed along each transect.

Figure 3-2: RV Ngerin loaded with pallets of sandbags ready for deployment (left) and bags being deployed off the tilt table (right).

Monitoring transects were established at each of the five sites at which bags were deployed in 2021 on the 10 September 2021. For 2022 deployments, monitoring transects were established on the 17 October 2022. Each transect consisted of 2 rows of 10 bags which were moved to be evenly spaced between star pickets so that they could be easily relocated, even if the bags are covered by sand. In addition, a similar set up was established in 2021 at the control site (#5) where no bags had been deployed. Seedlings were counted on each bag, and at the control site. A generalized linear model with Poisson distribution was used to assess the influence of bag density (as a continuous variable), placement along the coastline, and deployment year, on initial recruitment.

3.2. Results

Amphibolis seedling recruitment onto the bags 2-4 months after the end of deployments ranged from 3.05 to 20.1 seedlings per bag (Figure 3-3). No recruits were found at the control site. Recruitment was influenced by a complex interaction between all factors tested (Table 3-2), however, order from north to south along the coast was the most important, followed by deployment year. Bag density had only a relatively minor effect on recruitment, only explaining an additional 3% of the variability after year and order were taken into account.

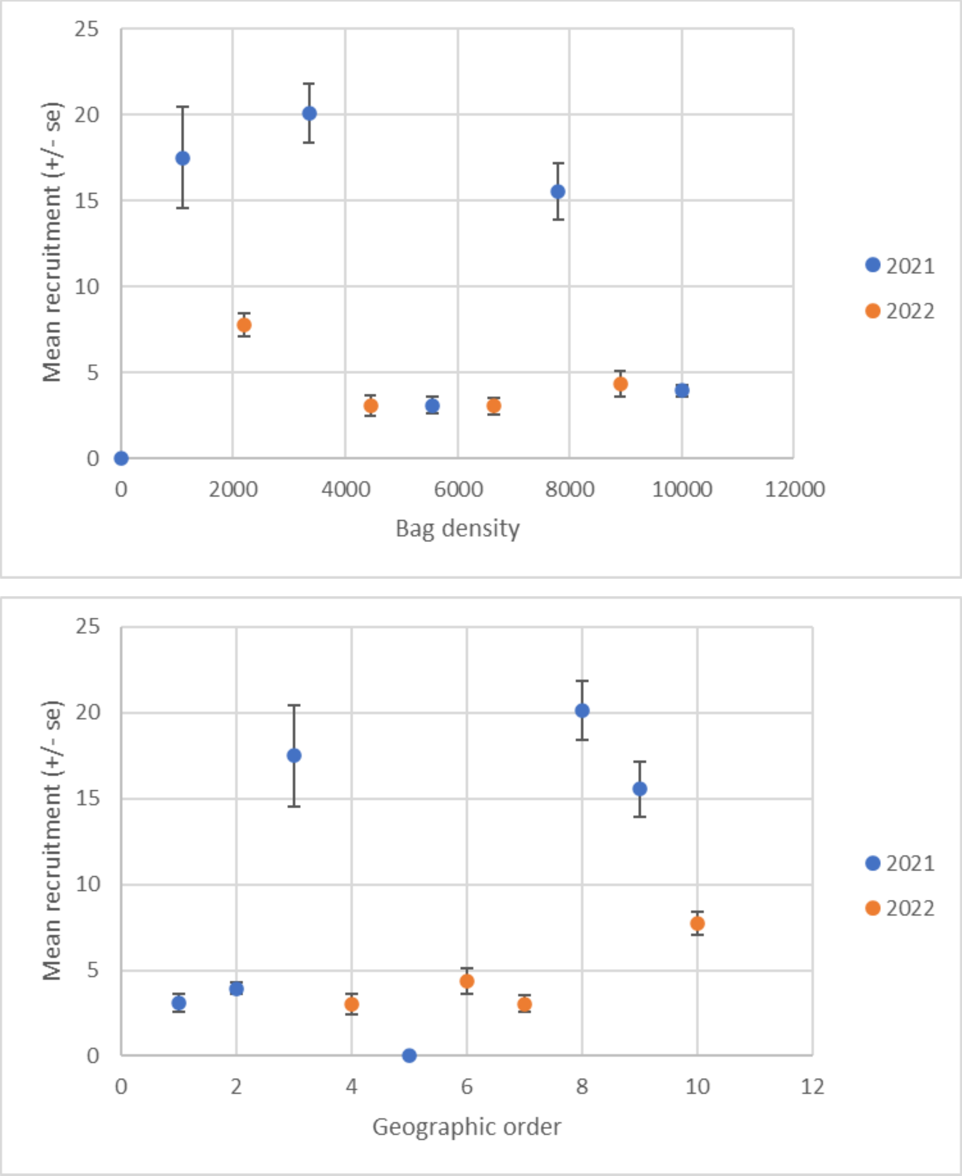


Figure 3-3: *Amphibolis* recruitment onto hessian sandbags as a function of bag density (top) and geographic order (north to south) (bottom).

Table 3-2: ANOVA table for Poisson GLM showing influence of deployment year, bag density and site order on *Amphibolis* seedling recruitment to hessian bags.

	Df	Deviance	Resid. Df	Resid. Dev	Pr(>Chi)
NULL			199	1652.5	
Year	1	197.878	198	1454.6	**< 2.2e-16**
Bag Density	1	15.249	197	1439.3	**9.42E-05**
Order	1	298.185	196	1141.2	**< 2.2e-16**
Year:Bag Density	1	0.354	195	1140.8	0.5517
Year:Order	1	0.259	194	1140.5	0.6105
Bags:Order	1	22.462	193	1118.1	**2.14E-06**
Year:Bags:Order	1	17.366	192	1100.7	**3.08E-05**

3.3. Discussion

While there was a statistically significant influence of bag density on recruitment, this was relatively minor, only accounting for 3% of the variation in recruitment. Consequently, there is no indication that bag density plays an important role in determining *Amphibolis* recruitment to hessian sandbags, although the presence of bags always led to *Amphibolis* recruitment, which was not detected at the site without bags. Instead, even though the entire study area only extends over 3 km, there appears to be an important influence of spatial variation, with southern sites generally having higher recruitment than northern (noting that site 3 is an exception). Temporal variation also played an important role, with all sites having >10 seedlings per bag being deployed in 2021, while sites deployed in 2022 had <8 seedlings per bag. At least part of this difference could be due to surveys occurring later in 2022 than in 2021, due logistical challenges. There was also a 3-way interaction between year, density, and order, suggesting that the influence of bag density varied both spatially and temporally, although this becomes difficult to tease out with only 10 sites. The original intention was for the entire deployment to be undertaken in a single year, however logistical issues prevented this, making it more difficult to confidently assess the roles of geographic variation and bag density on recruitment.

4. ROCK DEPLOYMENTS

The hessian sandbags used for most of the field experiments reported here provide a stable microsite that allows both *Amphibolis* and *Posidonia* seedlings to become established. There have also been anecdotal reports that *Posidonia* seedlings will establish around other objects, such as discarded crab traps that provide some protection from hydrodynamic activity and sand movement. These reports have motivated the idea of using rock rubble to provide protected interstices that will allow seedlings of both genera to settle and become established.

4.1. Methods

On each of 17th June and 8th December 2020, five bulka bags of 800 kg (0.5 m^3) of 50/65 rock ballast were deployed at Grange. These deployments were made just prior to the *Amphibolis* and *Posidonia* recruitment seasons respectively. The vessel was held as stationary as possible for each deployment, and the bottom of each bag was cut open to allow the rock to disperse over a constrained area of ~5-10 m^2 for each bag.

June deployments were surveyed on 10th September 2020, and both were surveyed on 10th February 2021 (Figure 4-1) and 11th January 2022. On the last date, 630 *Posidonia* seedlings were manually scattered by a diver on four randomly selected rock drops. These seedlings were 1-2 weeks old, with a well-developed shoot and had commenced root formation. The success of these seedlings was assessed on 18th October 2022.

Figure 4-1: Example rock drop deployment in February 2021, 8 months after deployment.

4.2. Results and Discussion

No seedlings of either *Amphibolis* or *Posidonia* were observed on any of the deployments up to and including the 18th October 2022. This technique did not prove to be suitable for encouraging natural recruitment of either genus, nor to enhance establishment of manually dispersed seedlings.

5. *POSIDONIA* FIELD EXPERIMENTS

In addition to the experiment described above trialing the use of rock rubble to provide suitable microsites for *Posidonia* settlement and establishment, several other small field experiments were undertaken to try and further refine the methodology for *Posidonia* restoration. The first of these was based on the observation that *Posidonia* had self-established on the periphery of long-term *Amphibolis* restoration plots. In an attempt to fast-track this process, *Posidonia* seedlings were both scattered and planted into quadrats in plots of restored *Amphibolis* that were established in 2008-2011. The second experiment was to assess the need to glue *Posidonia* seedlings into the bags if they were being pre-planted prior to deployment as per Tanner and Theil (2019). This was particularly motivated by the fact that the Seeds for Snapper community restoration project (https://ozfish.org.au/projects/seeds-for-snapper-south-australia/) has been based on planting seedlings into bags without gluing.

5.1. Methods

To determine if *Posidonia* seedlings planted or scattered into established *Amphibolis* had greater survival than those on bare sand, a series of permanent quadrats were marked in and around *Amphibolis* patches that were restored between 2008 and 2011. Each quadrat was 30 cm by 30 cm and marked by weighted electrical conduit frames which were securely pegged in place. Nine quadrats were established in the *Amphibolis* plots, and nine outside. Three quadrats in each habitat had 100 *Posidonia* seedlings loosely scattered in them, with no attempt to plant them or otherwise weight them down. A further three quadrats had 20 seedlings carefully planted so that their roots were buried but the shoot emergent. A further three quadrats did not have any seedlings added and acted as controls. The experiment was established on 15th February 2019 and surveyed on 30th January 2020.

To determine of *Posidonia* seedlings need to be glued into bags when pre-planting them prior to deployment, a total of 30 hessian sandbags were deployed on 13th January 2022, and each had 10 seedlings planted. To facilitate resurveying, these bags were haphazardly arranged into three rows of ten immediately after deployment and mapped. Ten bags were planted by a diver after deployment, ten were planted on the boat, and the seedlings were affixed to the bag with a small dab of superglue, and ten were planted on the boat with no gluing. In all cases, a small planting hole was created for each seedling by gently pushing the bags fibers apart, and then reclosed around the seedling by pushing them back together. All bags were surveyed for survival immediately after deployment (and planting of those planted by diver).

5.2. Results and Discussion

Of the 600 scattered seedlings, and 120 planted seedlings, only a single one survived for the 12-month duration of the experiment. While this seedling was established in bare sand, no conclusions can be drawn about differences between treatments.

Posidonia seedlings that were not glued into the bags prior to deployment had a significantly lower survival rate immediately after deployment than those that were glued or were planted by a diver (ANOVA: $F_{2,27}=3.35$, $p<0.001$, Figure 5-1). Given the relative survival rates, almost four times as many seedlings need to be planted into bags if they are not glued in place compared to if they are. Although not formally measured, planting each seedling by a diver takes approximately five times as long as gluing prior to deployment. Thus, planting extra seedlings not only requires more seedlings, but also more time, and is not recommended. It could not be determined if seedling loss occurred through seedlings being washed out of the bags while they were falling through the water column, or if it was because seedlings were pulled completely into the bags. If the latter, it is possible that some would still manage to grow back out through the bag, although the weave is likely too close to allow this to happen easily.

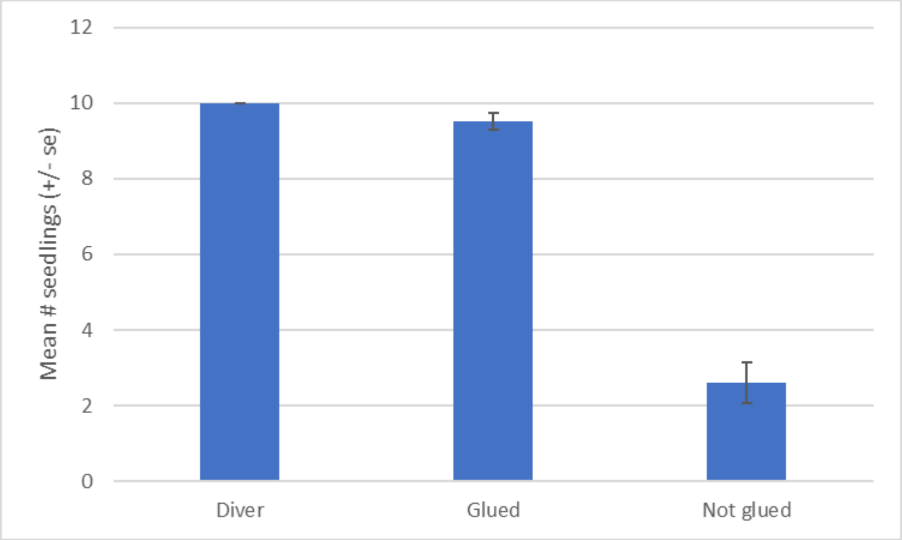


Figure 5-1: Influence of gluing *Posidonia* seedlings into bags on survival immediately after deployment.

Given that manually dispersed *Posidonia* seedlings did not establish in either established *Amphibolis* patches or in the rock drops described in the previous chapter, it is not clear whether seedlings raised in the lab for several weeks prior to being dispersed are capable of establishing without being planted. This question warrants further laboratory experiments to assess the success of seedlings of different ages in being able to get their roots into the sediment.

6. *POSIDONIA* TANK EXPERIMENTS

In addition to the *in-situ* experiments with *Posidonia* detailed in Chapter 5, a series of tank experiments were undertaken to help refine the optimal conditions for growing *Posidonia* from beach-cast fruit. These 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). Experiments conducted during the 2017/18 summer (Tanner and Theil 2019) showed that the time of collection of fruits was very important, with a small window of opportunity in late December. Fruits that dehisced within a few days of collection also produced better results than those that took some time to dehisce, and once dehisced, seedlings should ideally be planted within ten days. Finally, small seeds (<10 mm long) performed poorly compared to larger. External factors, including substrate composition, water flow through the substrate and exposure to air for up to 30 minutes prior to planting, all appeared to be unimportant.

6.1. 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 small: 50 mm square by 120 mm high; large: 65 mm square by 160 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% shade cloth (equivalent to ~ 7-8 m water depth off Grange, Figure 6-1). 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 and later, 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.

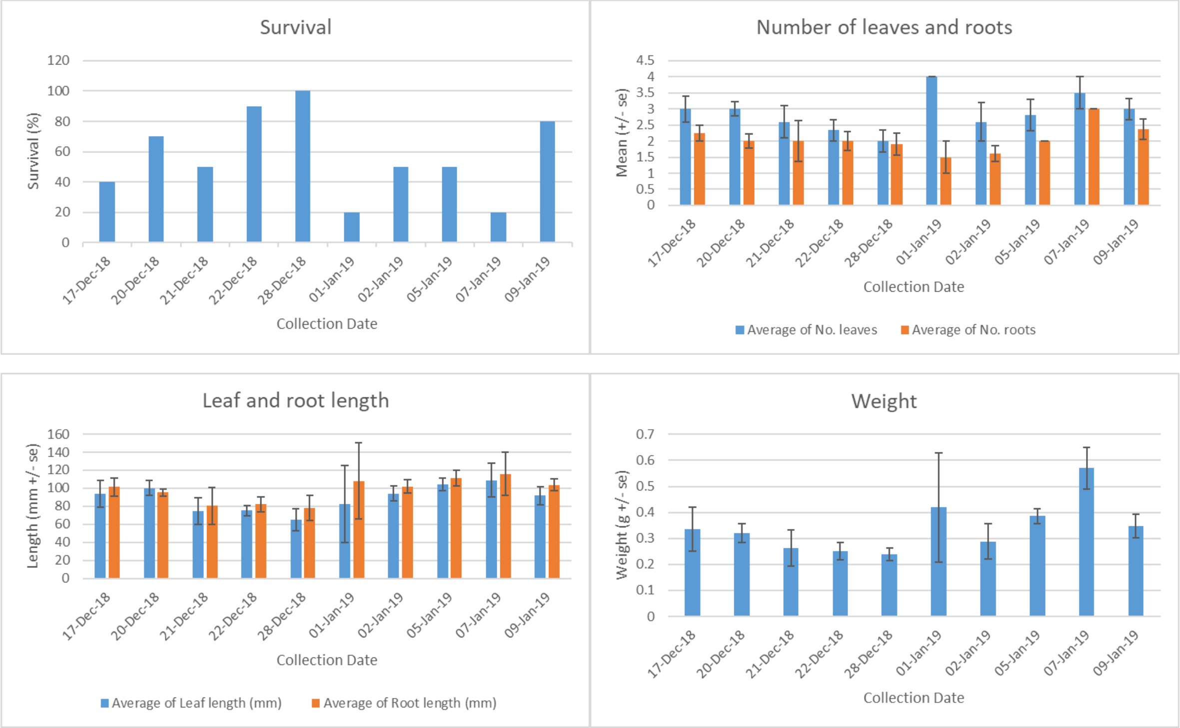
Figure 6-1: *Posidonia* tank experiment set-up showing a tray of 50 pots planted with *Posidonia* seedlings (left) and a tank used for holding the seedlings (right).

a. Effect of time of collection

2018-19

Fruits were collected from the beach on a regular basis from the 17th of December 2018 to the 9th of January 2019. Fruits were held for up to 4 days to dehisce, although where possible seedlings that had dehisced after 1 day were used. Once ten seedlings were available from a collection date, they were planted the following day into individual seedling pots filled with beach sand. Seedlings from each date were allowed to grow for 94 days from planting to harvest.

There was a significant difference in survival of seedlings between collection dates (GLM: $p<0.001$), with survival increasing steadily to a peak for fruit collected on the 28th of December, after which it became erratic, although with high survival also from fruits collected on the 9th of January, the last day on which sufficient fruits were available on the beach to undertake this experiment (Figure 6-2). It is possible that these last fruits were actually *P. coriacea* but not recognized as this species, rather than *P. angustifolia*. There were no significant differences between collection dates in seedling morphology after 94 days (PERMANOVA: $F_{9,56}=1.36$, $p=0.16$, Figure 6-2). However, the general patterns appeared opposite to that for survival, with fruits collected on days with poor survival producing seedlings with higher growth than those collected on days with good survival.


Survival

Number of leaves and roots

Leaf and root length

Weight

Figure 6-2: Effect of collection date (2018-19) on *Posidonia* seedling survival and growth over 94 days.

2019-20

Fruits were collected from the beach on a regular basis from the 25th of December 2019 to the 11th of January 2020. When possible, fruits were collected in both the morning and afternoon. In addition to *P. angustifolia*, fruits of both *P. sinuosa* and *P. coriacea* were collected if present, although numbers were small, and it was not always possible to obtain the standard 10 seedlings. *Posidonia angustifolia* overwhelmingly dominated the fruits on the beach, accounting for well over 99.9% of those present, and was present throughout the collection period. *Posidonia sinuosa* was only present up until January 1, while only a single collection of *P. coriacea* was obtained on the final day of collecting (January 11). Seedlings that had dehisced were planted two days following collection, into individual pots filled with beach sand. Seedlings from each date were allowed to grow for 126 days from planting to harvest. As there were different temporal patterns in the availability of each species, the data were split into three different sets for analysis: 1) *P. angustifolia* only across the whole collection period; 2) *P. angustifolia* and *P. sinuosa* for the collections which included both species; 3) *P. angustifolia* and *P. coriacea* for the only collection which included the later.

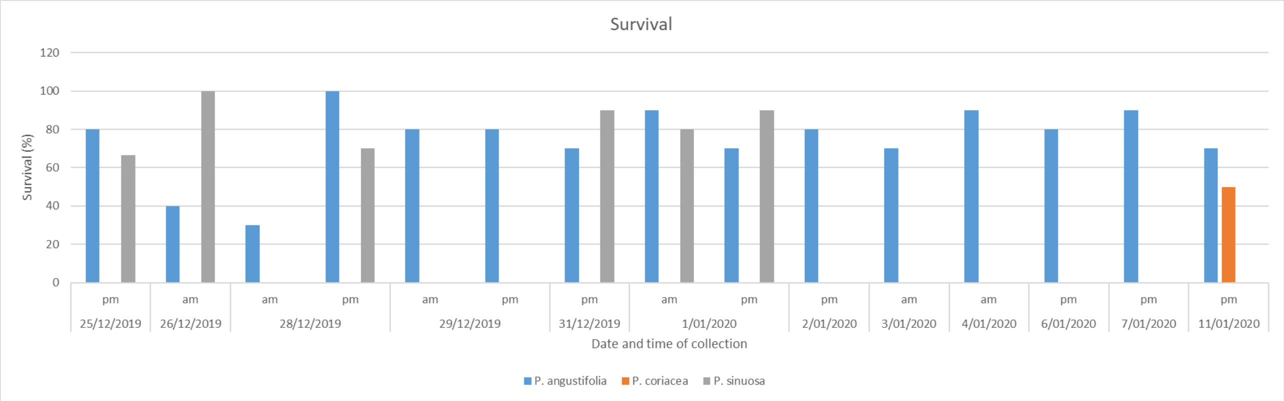
For *P. angustifolia* only, there was a significant interaction between date and time of day on survival (Table 6-1), particularly driven by the large difference between morning and afternoon on the 28th December (Figure 6-3). There was a collection date effect on growth (Table 6-2), although no consistent patterns over time (Figure 6-3). For the comparison between *P. angustifolia* and *P. sinuosa*, there was a species by date interaction on survival (Table 6-1), particularly driven by the higher survival of *P. sinuosa* on the 26th December (Figure 6-3), and a species effect on growth (Table 6-2) due primarily to decreased root length in *P. sinuosa* (Figure 6-3). There were insufficient data to test the Date x Time or 3-way interactions. Finally, there were no differences in survival or growth of *P. angustifolia* and *P. coriacea* for the single collection when both were obtained (Table 6-1, Table 6-2).

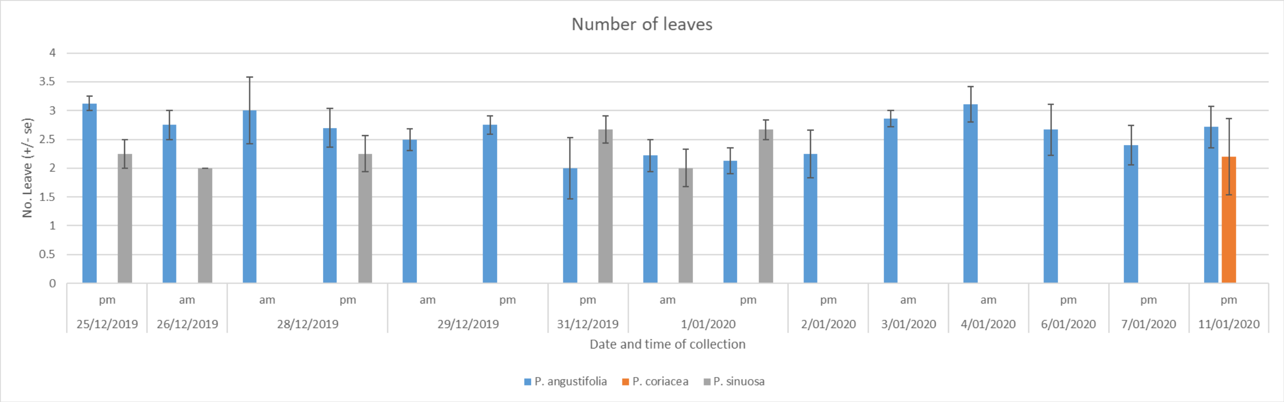
Table 6-1: Influence of collection date, time of day and species on survival of *Posidonia* seedlings over 126 days in 2019-20.

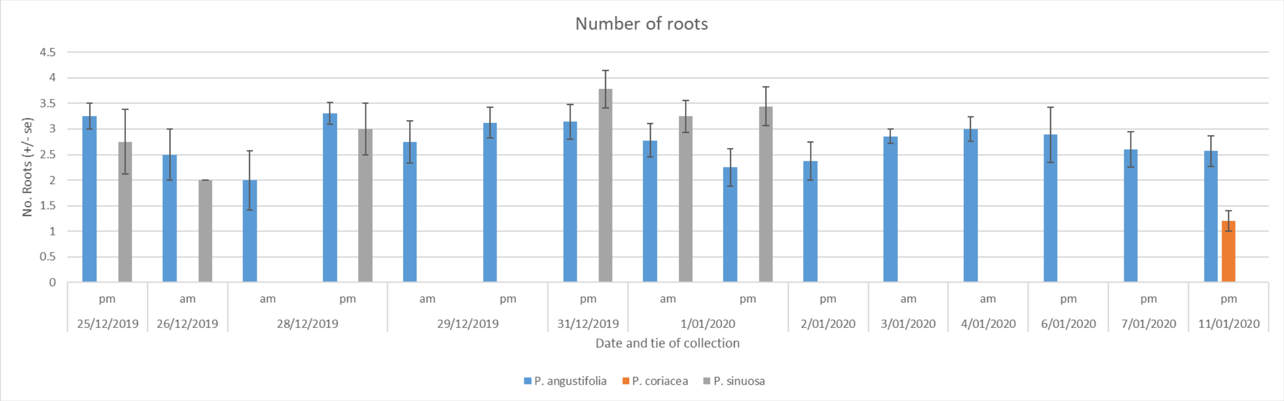
	df	Deviance	P
P. angustifolia			
Date	11	10.72	0.47
Time	1	2.31	0.13
Date x Time	2	12.66	**0.002**
Residual	135	144.09	
P. angustifolia* vs *P. sinuosa			
Date	4	5.76	0.22
Time	1	0.17	0.68
Species	1	0.06	0.80
Date x Time	0	0	
Date x Species	4	9.62	**0.047**
Time x Species	1	1.52	0.22
Date x Time x Species	0	0	
Residual	96	97.27	
P. angustifolia* vs *P. coriacea			
Species	1	0.84	0.36
Residual	18	26.08	

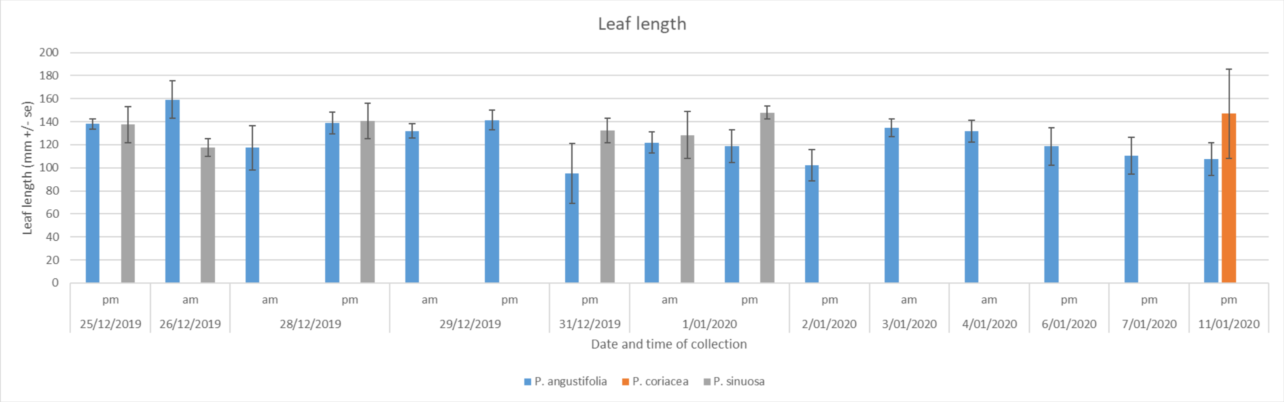
Table 6-2: Influence of collection date, time of day and species on growth of *Posidonia* seedlings over 126 days in 2019-20.

	df	**SS**	**Pseudo-F**	**P**
P. angustifolia				
Date	11	32288	1.75	**0.017**
Time	1	1325	0.79	0.46
Date x Time	2	2869	0.86	0.50
Residual	99	165990		
P. angustifolia* vs *P. sinuosa				
Date	4	6379	0.85	0.57
Time	1	667	0.36	0.79
Species	1	5556	2.97	**0.043**
Date x Time	0	0		
Date x Species	4	13418	1.80	0.065
Time x Species	1	1584	0.85	0.44
Date x Time x Species	0			
Residual	74	138270		
P. angustifolia* vs *P. coriacea				
Species	1	6537	2.36	0.085
Residual	10	27757		


Survival
Survival (%)
0
20
40
60
80
100
120
pm
am
am
pm
am
pm
pm
am
pm
pm
am
am
pm
pm
pm
25/12/2019
26/12/2019
28/12/2019
29/12/2019
31/12/2019
1/01/2020
2/01/2020
3/01/2020
4/01/2020
6/01/2020
7/01/2020
11/01/2020
Date and time of collection
P. angustifolia
P. coriacea
P. sinuosa


Number of leaves
No. Leave (+/- se)
0
0.5
1
1.5
2
2.5
3
3.5
4
pm
am
am
pm
am
pm
pm
am
pm
pm
am
am
pm
pm
pm
25/12/2019
26/12/2019
28/12/2019
29/12/2019
31/12/2019
1/01/2020
2/01/2020
3/01/2020
4/01/2020
6/01/2020
7/01/2020
11/01/2020
Date and time of collection
P. angustifolia
P. coriacea
P. sinuosa


Number of roots
No. Roots (+/- se)
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
pm
am
am
pm
am
pm
pm
am
pm
pm
am
am
pm
pm
pm
25/12/2019
26/12/2019
28/12/2019
29/12/2019
31/12/2019
1/01/2020
2/01/2020
3/01/2020
4/01/2020
6/01/2020
7/01/2020
11/01/2020
Date and tie of collection
P. angustifolia
P. coriacea
P. sinuosa


Leaf length
Leaf length (mm +/- se)
0
20
40
60
80
100
120
140
160
180
200
pm
am
am
pm
am
pm
pm
am
pm
pm
am
am
pm
pm
pm
25/12/2019
26/12/2019
28/12/2019
29/12/2019
31/12/2019
1/01/2020
2/01/2020
3/01/2020
4/01/2020
6/01/2020
7/01/2020
11/01/2020
Date and time of collection
P. angustifolia
P. coriacea
P. sinuosa

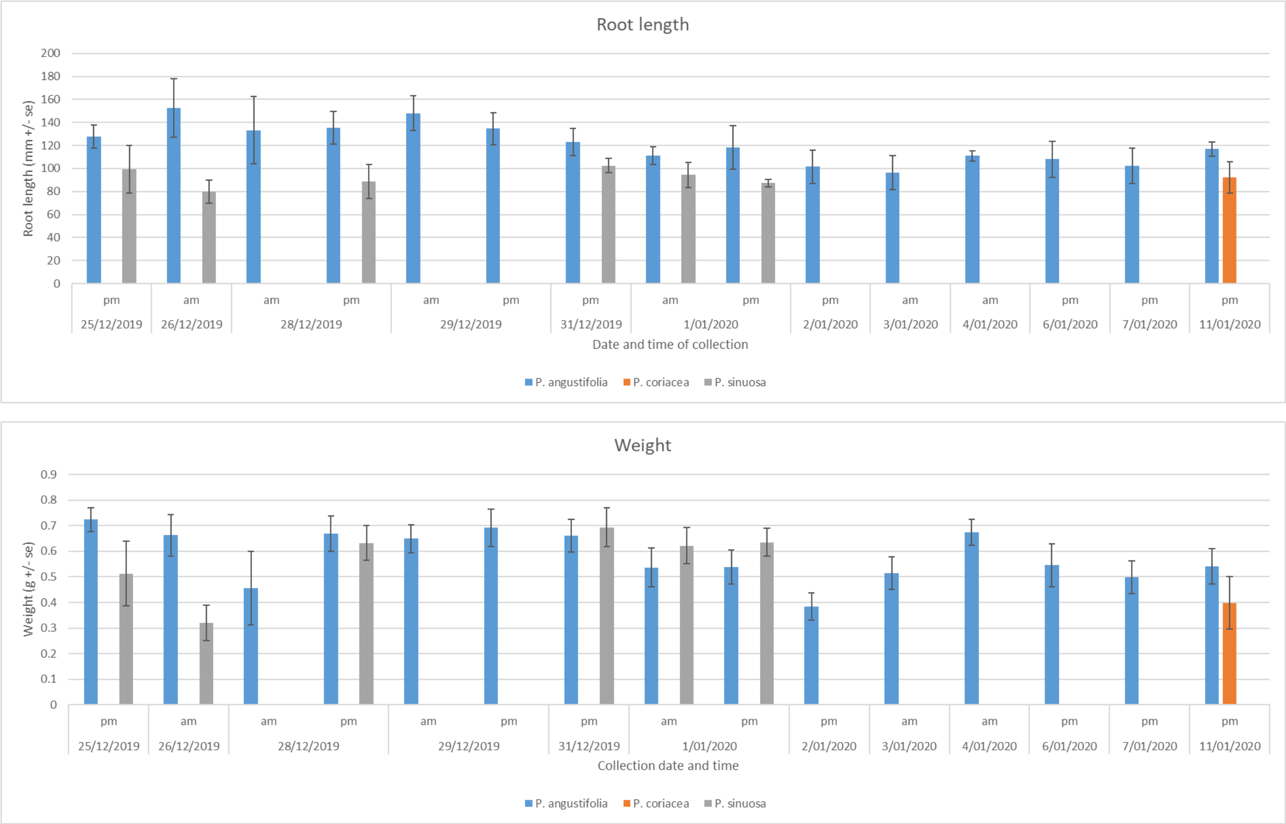


Figure 6-3: Influence of collection date, time of day and species on survival of *Posidonia* seedlings over 126 days in 2019-20.

2020-21

Immature fruits of Posidonia were first documented on the beach on 1st December 2020, with the first apparently mature fruits found on 22nd December. Daily surveys were then undertaken until 12th January 2021, and less regular surveys thereafter. Only extremely small numbers of fruits were ever present on the beach, and while collected, these were insufficient to undertake any meaningful experiments. Throughout this period, there were consistent strong northerly winds, and temperatures were relatively cool. The afternoon sea breezes that normally bring fruits onto the beach were not present. It is likely that fruits were instead dispersed into northern Gulf St Vincent, although it is also possible that there was a reproductive failure for the year.

2021-22

Fruits were collected from the beach on a regular basis from the 20th of December 2021 to the 12th of January 2022. Surveys, and if fruit were available, collections, were made in both the morning and afternoon from the 20th. Prior to this period, daily surveys indicated that any fruit washing up was immature. All mature fruit were *P. angustifolia*, although there was a high abundance of immature *P. coriacea* on the 5th of January. No *P. sinuosa* were observed. Large numbers of dehisced fruit were observed on the 11th, 12th and 14th January, suggesting that this was the primary period for release of fully mature fruits, which all released their seedlings prior to washing up on the beach. There was a lull in fruit washing up between the 23rd December and 2nd of January, possibly due to the lack of a sea breeze over this time. Early fruits (December) were held for 4 days prior to planting, to allow sufficient time to dehisce. Later fruits were only held for 2 days, as a large proportion dehisced within this timeframe. Once ten seedlings were available from a collection date, they were planted into individual seedling pots filled with beach sand. Seedlings from each date were allowed to grow for 75 days from planting to harvest.

There was a significant difference in survival of seedlings between collection dates (GLM: $p<0.001$), with peaks in survival from fruits collected on the 1st and 2nd of January, and again on the 6th and 7th (Figure 6-4). There were no differences between morning and afternoon collections (GLM: $p=0.58$), and no interaction ($p=0.56$). There were significant differences between collection dates in seedling morphology after 75 days (PERMANOVA: $F_{9,100}=2.52$, $p=0.001$), but no difference between morning and afternoon collection ($F_{1,100}=2.52$, $p=0.075$) and no interaction ($F_{4,100}=0.46$, $p=0.91$). Overall, the largest seedlings were obtained from fruits collected on the 24th December and 6th January (Figure 6-4).

Figure 6-4: Influence of collection date and, time of day on survival and growth of *Posidonia angustifolia* seedlings over 75 days in 2021-22.

b. Clay and organic matter content of substrate

Different mixes of sand and clay, and different levels of organic matter in pure sand, were used in previous *in situ* experiments, although neither variable had a clear and consistent influence on survival or growth (Tanner and Theil 2019). A previous tank experiment also looked at the influence of clay content on seedling survival and growth over 41 days, with no differences detected between treatments (Tanner and Theil 2019). To investigate whether there was an interaction between clay and organic matter content, we set up 10 replicate pots with each of a range of different clay (0-100%) and beach sand (100-0%) mixes, as well as with straight silica sand, crossed with different levels of organic matter (0, 5 & 10% by volume). Organic matter additions consisted of chopped and dried *Posidonia* seagrass leaf matter. Silica sand was commercial kiln dried sand obtained from a local hardware store. Fruits were collected from the 17th to 21st of December 2018, and seedlings that dehisced within three days were planted on the 3rd of January. Prior to planting, initial seed and shoot length, and weight, were recorded for each seedling. Seedlings were harvested 95 days after planting. Survival was not influenced by either clay or organic matter content of the substrate, but was positively influenced by initial shoot length (Table 6-3, Figure 6-5). Growth of surviving seedlings was not affected by either the sediment variables or initial size (Table 6-4, Figure 6-5).

Table 6-3: GLM results for survival of *Posidonia* seedlings as a function of clay mix, organic matter addition and initial size.

Source	df	Deviance	P
Initial Weight	1	0.20	0.66
Initial Seed Length	1	3.27	0.07
Initial Shoot Length	1	59.31	**<0.001**
Clay	6	4.14	0.66
Organic Matter	2	1.45	0.48
Interaction	12	8.85	0.72
Residual	186	207.73	

Table 6-4: PERMANOVA results for growth of *Posidonia* seedlings as a function of clay mix, organic matter addition and initial size.

Source	df	SS	Pseudo-F	P
Initial Weight	1	1850	1.05	0.35
Initial Seed Length	1	461	0.26	0.89
Initial Shoot Length	1	3904	2.21	0.08
Clay	6	6625	0.63	0.88
Organic Matter	2	1154	0.33	0.94
Interaction	12	30041	1.42	0.07
Residual	101	178060		

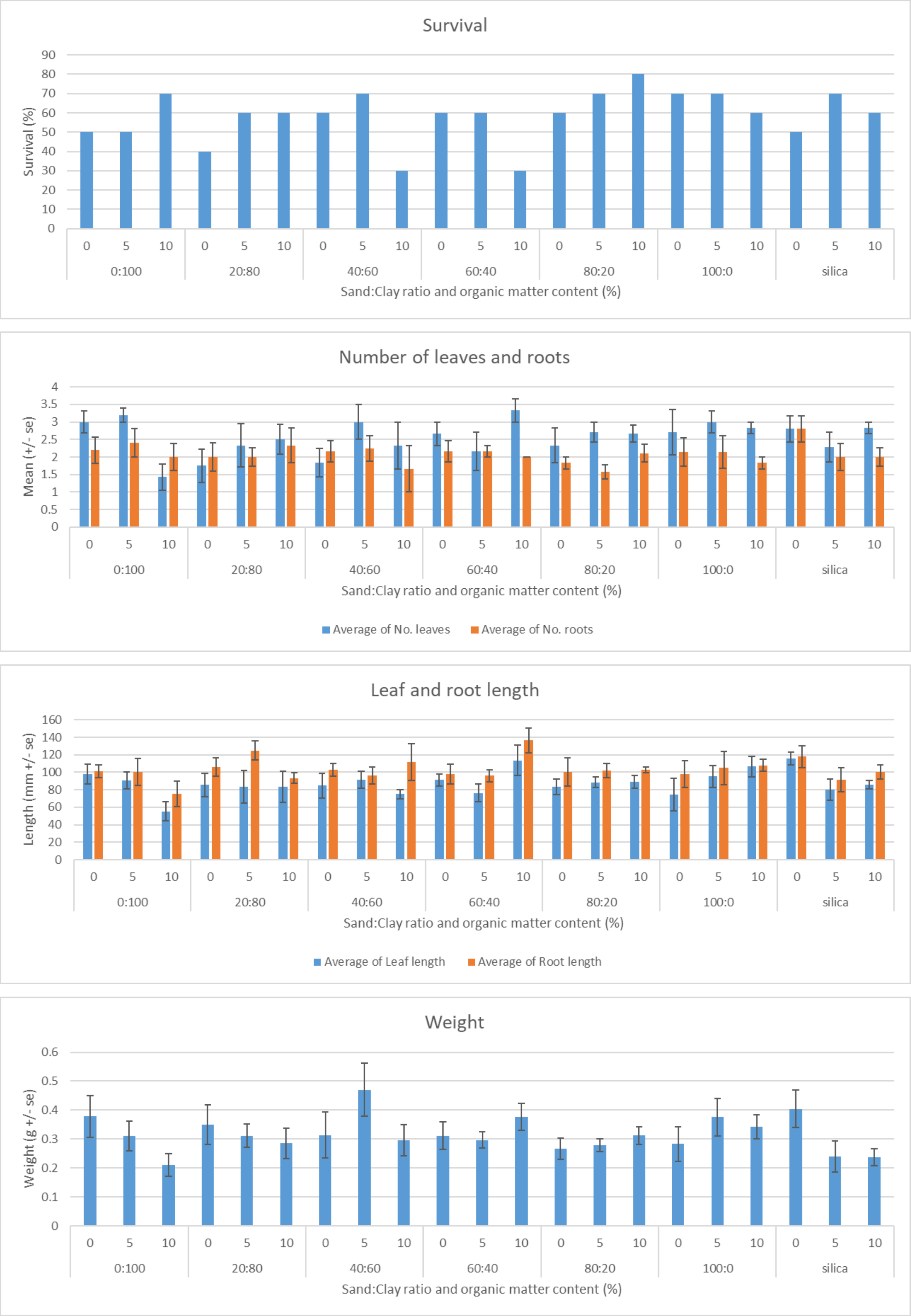


Figure 6-5: Effect of Sand:Clay ratio and organic matter content on *Posidonia* seedling survival and growth over 94 days.

c. 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, we repeated a previous experiment that showed no effect of water flow after 78 days (Tanner and Theil 2019), but for a longer time period. 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 25th of December, planted on the 3rd of January, and harvested either 98 or 175 days later.

There was no difference in seedling survival as a function of water flow, length of the experiment or the initial characteristics of the seedling (Table 6-5, Figure 6-6). There was, however, an effect of experimental duration on growth (Table 6-6, Figure 6-6), with older plants having fewer leaves but more roots, longer roots and greater weight.

Table 6-5: GLM results for survival of *Posidonia* seedlings as a function of water flow and harvest date.

	df	**Deviance**	**P**
Initial Weight	1	1.85	0.85
Initial Seed Length	1	0.03	0.95
Initial Shoot Length	1	0.004	0.89
Flow	2	0.13	0.94
Harvest Date	1	1.68	0.19
Interaction	2	0.13	0.94
Residual	51	79.18	

Table 6-6: PERMANOVA results for growth of *Posidonia* seedlings as a function of water flow and harvest date.

Source	**df**	**SS**	**Pseudo-F**	**P**
Initial Weight	1	1822	0.96	0.39
Initial Seed Length	1	3133	1.65	0.17
Initial Shoot Length	1	1315	0.69	0.54
Flow	2	3961	1.04	0.40
Harvest Date	1	12870	6.76	**<0.001**
Interaction	2	1849	0.49	0.82
Residual	22	41880		

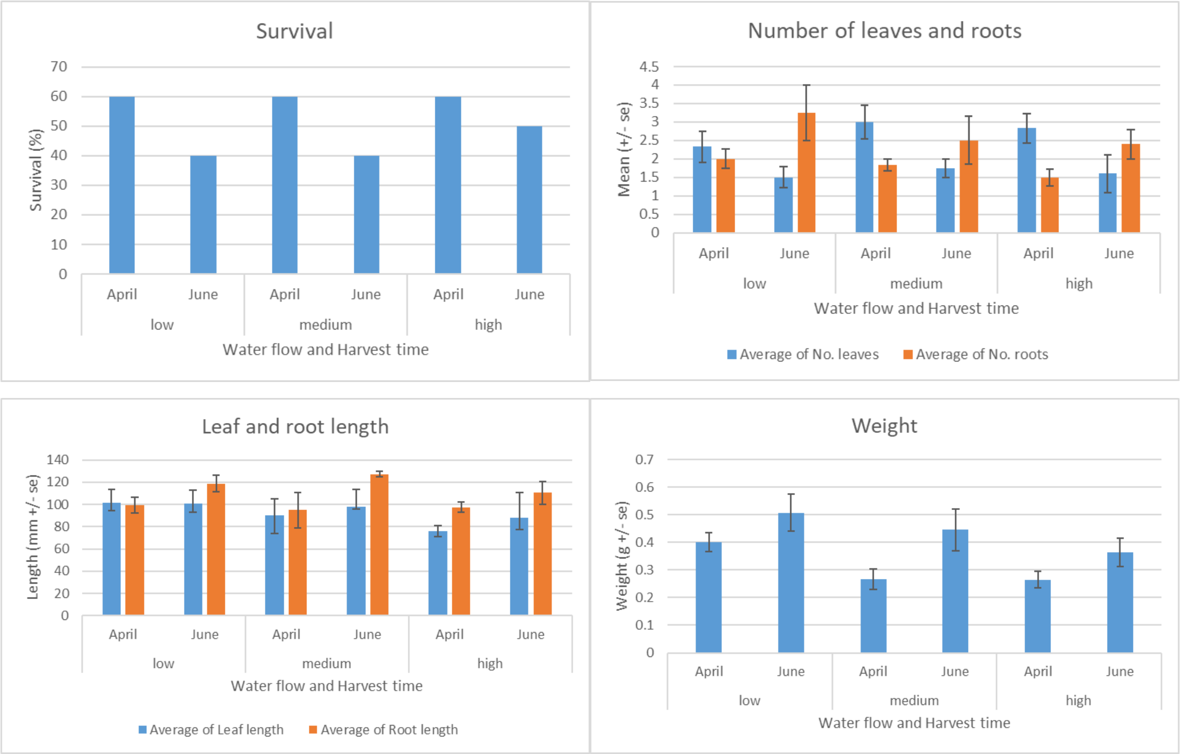


Figure 6-6: Effect of water flow and harvest date on *Posidonia* seedling survival and growth over 98 and 175 days.

d. Seed size

Previously we have demonstrated that larger seeds led to faster growing seedlings after 67 days, although seed size did not influence survival (Tanner and Theil 2019). Here we repeat the experiment over 92 and 170 days to see if this pattern holds over time. Fruits were collected on the 25th December, and seedlings planted on 7th January. Eighty seedlings each from small (<12mm), medium (12-13 mm) and large (>13 mm) seeds were planted, with half of each harvested after each time interval.

Survival decreased as initial weight increased, although it only explained ~2% of the variation in the data, and none of the other factors or covariates were significant (Table 6-7). Seedling size was influenced by both initial weight and initial shoot length, as well as time of harvest, but was not influenced by seed length (Table 6-8). Seedlings harvested later had fewer leaves than those harvested earlier, those with low initial weight tended to be smaller at harvest, and those with low initial shoot length also tended to have fewer leaves (Figure 6-7, Figure 6-8).

Table 6-7: GLM results for survival of *Posidonia* seedlings as a function of seed size and time to harvest.

Source	df	Deviance	P
Initial Weight	1	6.91	**0.009**
Initial Seed Length	1	2.85	0.091
Initial Shoot Length	1	0.24	0.62
Time	1	0.50	0.48
Size	2	0.24	0.89
Time x Size	2	2.70	0.26
Residual	231		

Table 6-8: PERMANOVA results for growth of *Posidonia* seedlings as a function of seed size and to harvest.

Source	df	SS	Pseudo-F	P
Initial Weight	1	6406	3.41	**0.024**
Initial Seed Length	1	2242	1.19	0.28
Initial Shoot Length	1	6131	3.27	**0.029**
Time	1	4109	1.09	0.34
Size	2	39879	21.25	**<0.001**
Time x Size	2	7661	2.04	0.07
Residual	160	300320		

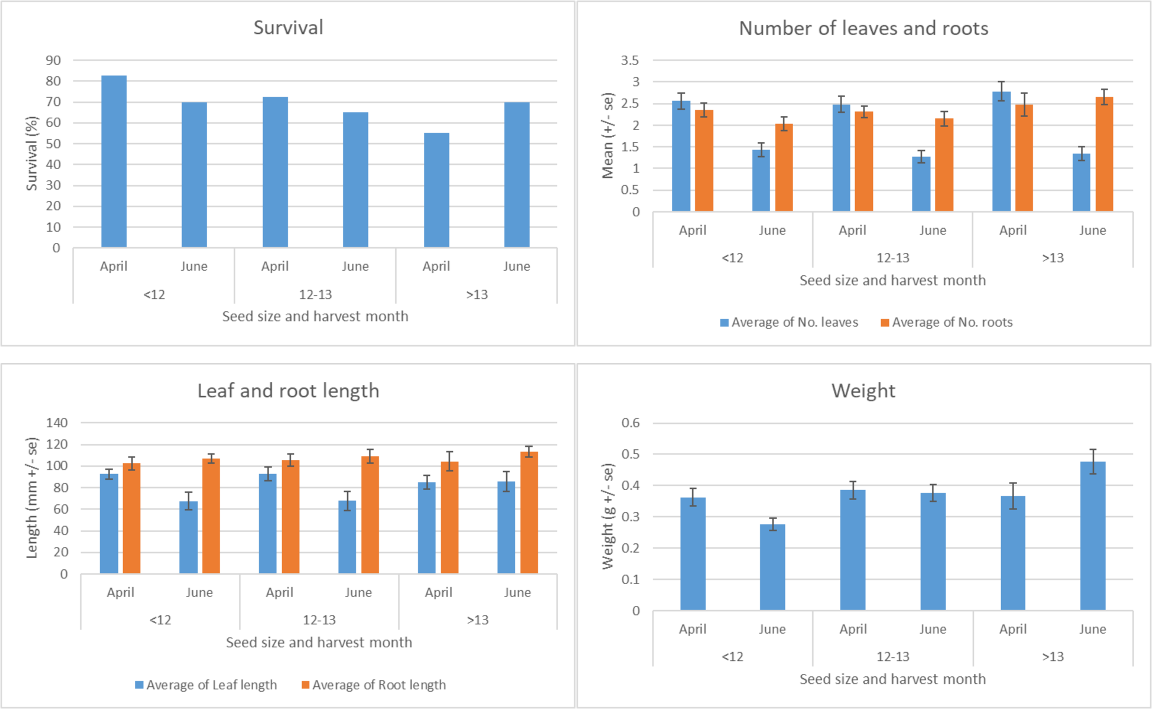


Figure 6-7: Effect of seed size and time to harvest on *Posidonia* seedling survival and growth over 92 and 170 days

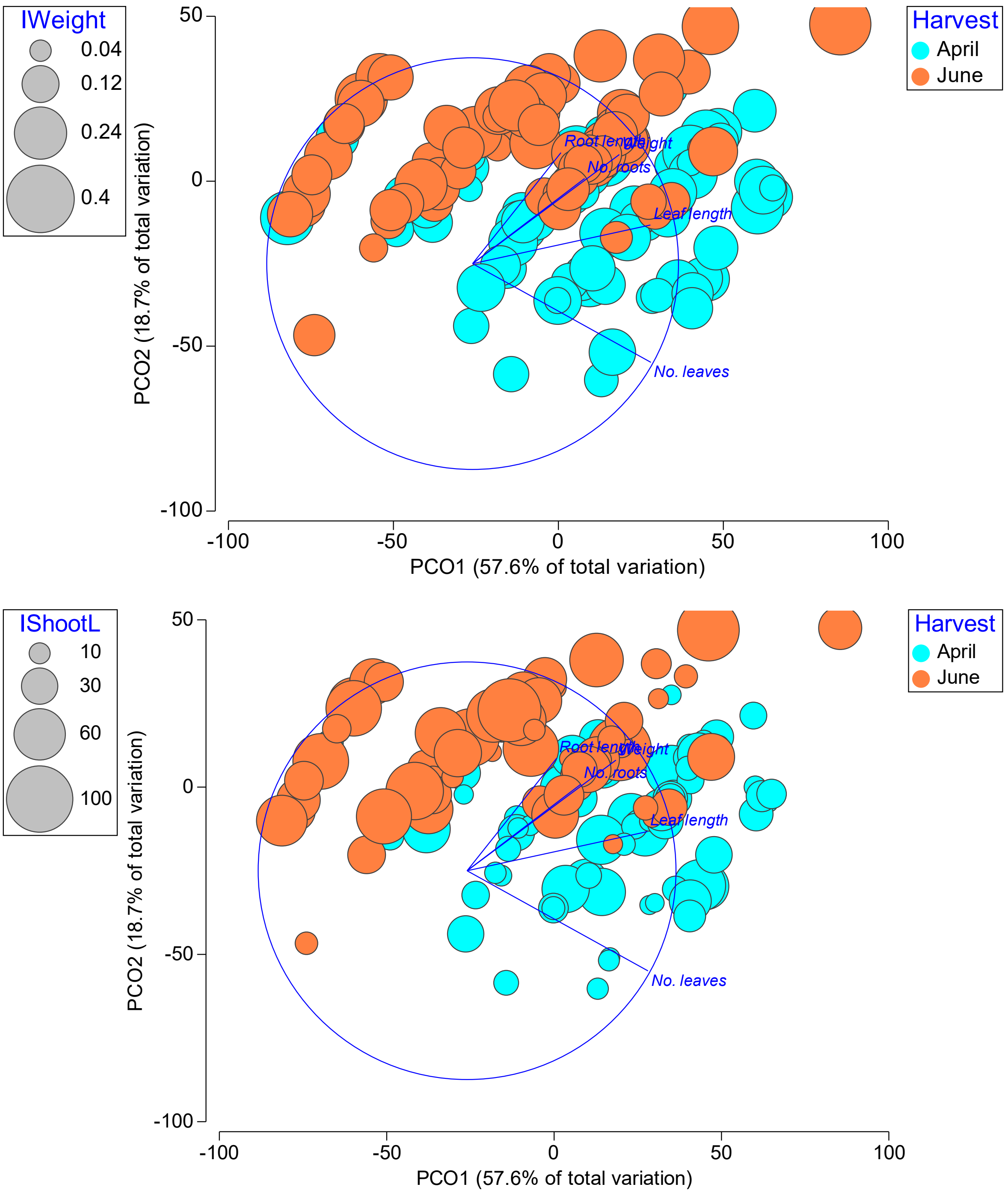


Figure 6-8: Principal coordinates analysis plots showing influence of harvest month on growth. In the top panel, symbols are scaled by initial weight, in the bottom by initial shoot length.

e. Time to dehisce, time since dehiscing, and size

Previously we have shown that seedlings that had dehisced from their fruit within 4 days of collection performed better than those that took longer to dehisce, and that growth was better in seedlings planted soon after dehiscing. Larger seeds were also found to produce better growth (Tanner and Theil 2019). Here, we examine all three factors in a multifactorial experiment using fruits collected on 2nd January 2019. Seedlings were separated into those that had dehisced after 1, 2 or 3 days, and on the basis of size (small: < 12 mm; medium: 12-13 mm; large: > 13 mm), and planted either 3, 10 or 20 days after dehiscing. Insufficient seedlings that had dehisced after 2 days were available to plant at 20 days, and there were only enough that dehisced after 3 days to plant 3 days later. In addition, only 5 seedlings from large seeds that dehisced after 3 days were available to plant 3 days later. Seedlings were harvested after 89 days.

Survival in this experiment was very low, with only 41 seedlings out of the initial 175 planted being alive at harvest. There was a clear interaction between time to dehisce and time to planting, with seedlings dehiscing after 1 day declining in performance the longer they were held before planting, whereas the opposite occurred for those that took 2 days to dehisce (Table 6-9,Figure 6-9). No factor influenced growth (Table 6-10, Figure 6-9).

Table 6-9: GLM results for survival of *Posidonia* seedlings as a function of seed size, time to dehisce, and time between dehiscing and planting.

Source	df	Deviance	P
Initial Weight	1	1.98	0.16
Initial Seed Length	1	0.05	0.83
Initial Shoot Length	1	1.10	0.29
Time to dehisce (TTD)	2	4.54	0.10
Size	2	5.51	0.06
Time since dehiscing (TSD)	2	9.15	**0.01**
TTD x Size	4	2.17	0.70
TTD x TSD	1	14.25	**<0.001**
Size x TSD	4	4.51	0.34
TTD x Size x TSD	1	5.08	0.079
Residual	154		

Table 6-10: PERMANOVA results for growth of *Posidonia* seedlings as a function of seed size, time to dehisce, and time between dehiscing and planting.

Source	df	SS	Pseudo-F	P
Initial Weight	1	1816	0.90	0.44
Initial Seed Length	1	1808	0.90	0.46
Initial Shoot Length	1	2610	1.30	0.28
Time to dehisce (TTD)	2	6579	1.64	0.13
Size	2	6484	1.61	0.14
Time since dehiscing (TSD)	2	3877	0.96	0.44
TTD x Size	4	5855	0.73	0.74
TTD x TSD	1	486	0.24	0.92
Size x TSD	4	1273	0.63	0.63
TTD x Size x TSD	1	1027	0.51	0.71
Residual	24	48284		

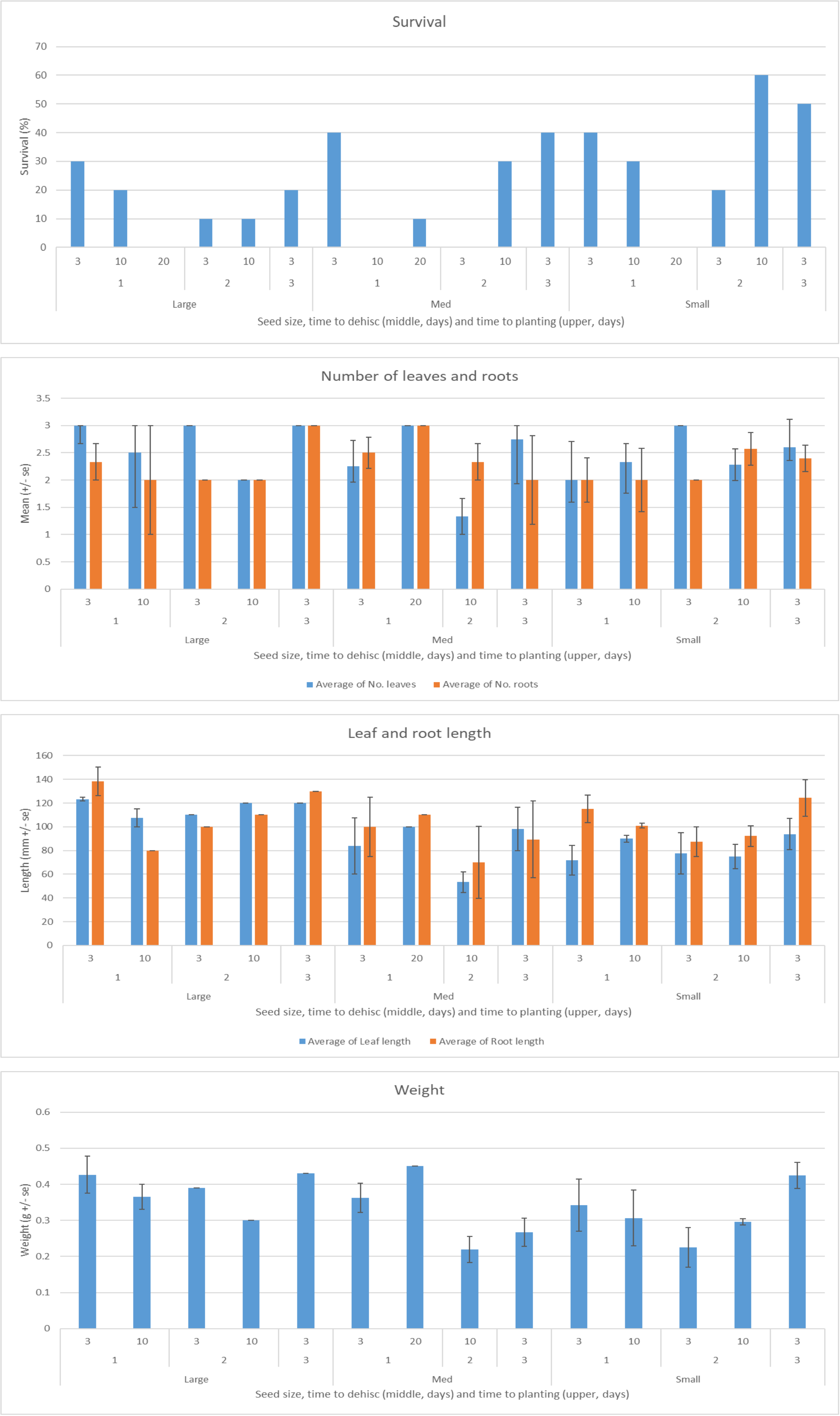


Figure 6-9: Effect of seed size, time to dehisce, and time between dehiscing and planting on *Posidonia* seedling survival and growth over 89 days

f. Planting method

All previous experiments have involved planting seedlings by inserting the seed (and any roots) into the substrate using tweezers. While this is relatively quick and easy in tank experiments, in field experiments the hessian weave of the sandbag has to be carefully teased apart, and then pushed back together after planting, which is time consuming. Here we test an alternative method of planting, which involve pre-gluing seedlings onto bamboo skewers, and then pushing the skewers into the pot to the point that the seed is just buried. *Posidonia angustifolia* seedlings from fruit collected on 11th January 2020, and which had dehisced 2 days later, were used for this experiment. Ten seedlings were individually glued onto bamboo skewers using superglue prior to planting, while another ten were planted normally. Seedlings were allowed to grow for 126 days prior to harvest.

There was no influence of planting method on either survival (GLM: p=0.25) or growth (PERMANOVA: $F_{1,14}$=0.68, p=0.55, Figure 6-10).

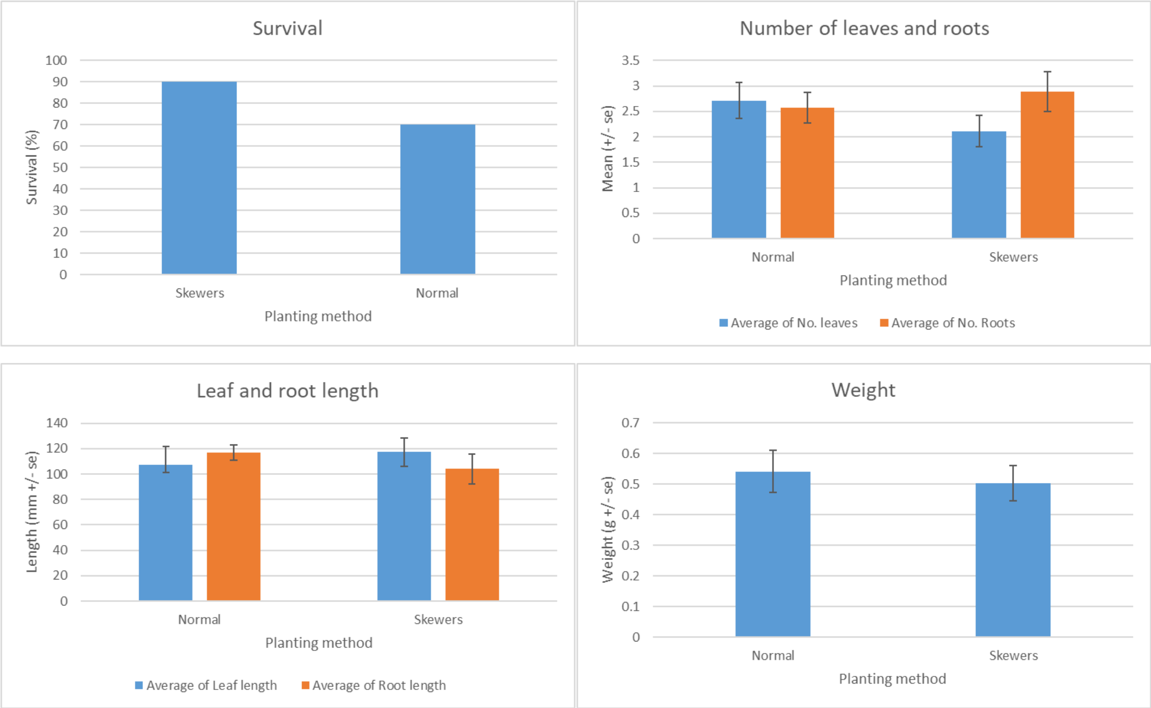


Figure 6-10: Influence of planting method (normal vs skewers) on survival and growth of *Posidonia* seedlings over 126 days.

g. Shading

2019-2020

To assess the response of *Posidonia* seedlings to differences in light levels, a shading experiment was established in January 2020. Three replicate trays of ten seedlings in individual pots were maintained in each of six different light levels. One set of trays were unshaded controls (with only a wire frame over them), a set had black fiberglass flyscreen over the wire frame, and green shade cloth was used over the other trays at either 50%, 70%, 90% or 99% nominal shading (the last obtained by using two layers of 90% shade cloth). Trays were split between two tanks. Seedlings were planted on 3rd January 2020 from fruits collected on 25th December 2019. Shades were replaced with clean ones as needed and trays randomly moved between tanks and locations within tanks at the same time. Odyssey light loggers were placed under a subset of shades to measure actual light reductions achieved, and recorded light intensity every 30 min. All seedlings were measured for seed and shoot length, and total weight, prior to planting, and harvested after 122 days on 5th May 2020. Over the period of the experiment, the flyscreen shade resulted in a 34% reduction in light between the hours of 8 am and 7 pm compared to the no shade treatment, 50% shade cloth led to a 52-57% reduction, 70% a 72% reduction, 90% a 83-92% reduction and 99% a 99.4-99.6% reduction (Figure 6-11).

The level of shading did not affect survival (Table 6-11), with very high survival found in all treatments (Figure 6-12). There was, however, an influence of shading on growth (Table 6-12), with pairwise tests showing differences between no shade and flyscreen, 90% shade and 99% shade, between flyscreen and 99%, and between 90% and 99% shade. The number of leaves and roots was higher at intermediate light levels, as was weight. Leaf length tended to increase as light decreased, with leaf growth peaking at 90% shade, while there was little influence on root length, although root growth was lowest at 99% shade (Figure 6-12).

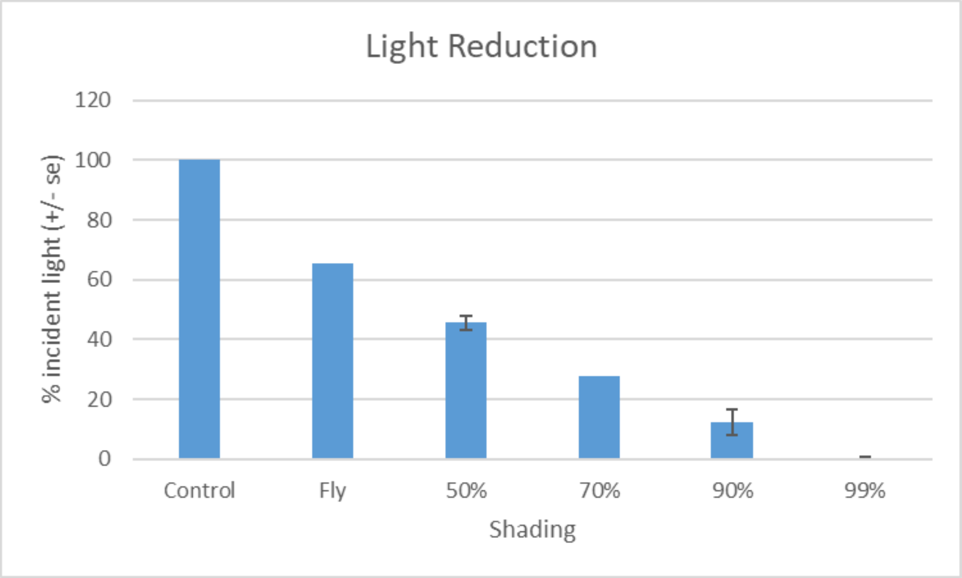


Figure 6-11: Average light reduction over the duration of the shading experiment for trays under each shading level in 2020. Reduction is measured against the control (note data only available from some trays).

Table 6-11: Influence of shading on *Posidonia* seedling survival after 122 days in 2020.

	Df	ChiSq	P
Shoot Length	1	14.53	**<0.001**
Weight	1	7.62	**0.006**
Seed Length	1	0.11	0.74
Tank	1	1.39	0.24
Shade	5	3.13	0.68
Tank x Shade	5	6.67	0.25
Residual	167		

Table 6-12: PERMANOVA results for growth of *Posidonia* seedlings as a function of shading in 2020.

Source	df	SS	Pseudo-F	P
Initial Weight	1	17400	11.88	**<0.001**
Initial Seed Length	1	8663	5.97	**0.002**
Initial Shoot Length	1	28410	18.95	**<0.001**
Shade	5	65250	5.84	**<0.001**
Tank	1	11976	5.46	**0.032**
Tank x Shade	5	25071	2.27	0.12
Tray (Tank x Shade)	6	13436	1.63	0.07
Residual	143	196810		

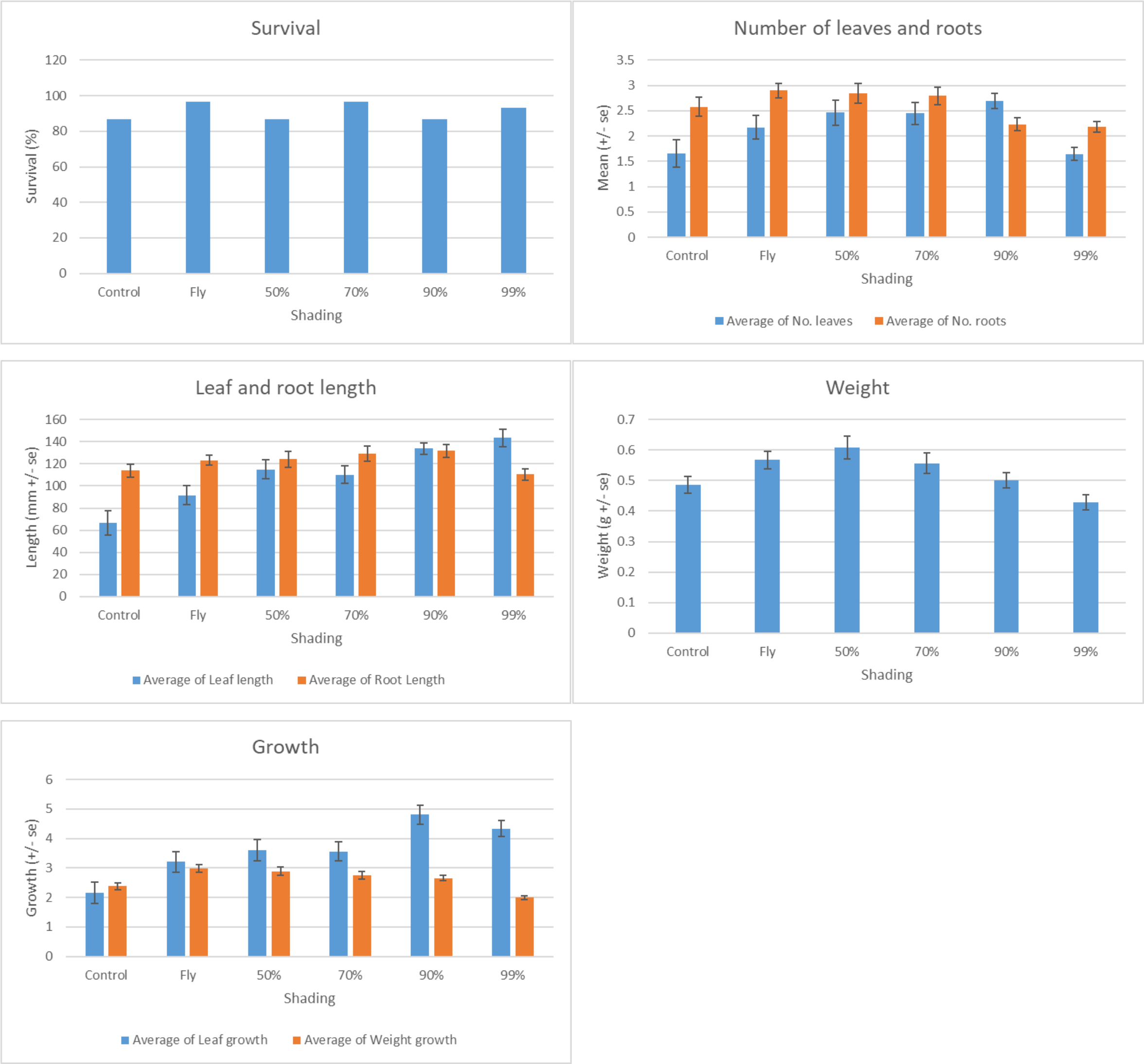


Figure 6-12: Influence of shading on survival and growth of *Posidonia* seedlings over 122 days in 2020.

2021-22

The shading experiment was repeated in 2021-22 because not all seedling trays had light meters in the 2019-20 experiments, and logistical issues resulted in a lack of cleaning. Consequently, light levels in several treatments were reduced and became similar to those at greater shade levels. All experimental procedures followed those from the previous year, except for changes as follows. Fruits were collected from the beach on 2nd January 2022, and allowed to dehisce before seedlings were planted on 6th January. Twenty seedlings were planted in each tray, and time constraints meant that size and weight were not measured at planting. All trays had both an Odyssey light logger, with a Hobo light logger as backup, set to record every 5 min, although only data from the Odyssey loggers are presented here. Light loggers were also placed in the middle of each tank, and adjacent to the tanks. All covers were cleaned on a fortnightly basis, and trays of seedlings were randomly re-arranged weekly. The experiment was terminated on the 19th and 20th May 2022, after 134 days, with all seedlings being harvested and measured as per the previous year.

Over the period of the experiment, control light loggers in the tanks received 39% of the incident light received by the logger outside the tanks (Figure 6-13). Compared to the in-tank control loggers, the shading control treatment experienced a 17-19% reduction in light over the period of the experiment, the flyscreen shades a 49-54% reduction, 50% shade cloth led to a 66% reduction, 70% a 77-80% reduction, 90% a 94-95% reduction and 99% a 99.5-99.6% reduction. Light reductions in excess of the nominal reduction from the shade cloth rating likely indicate algal fouling between cleanings.

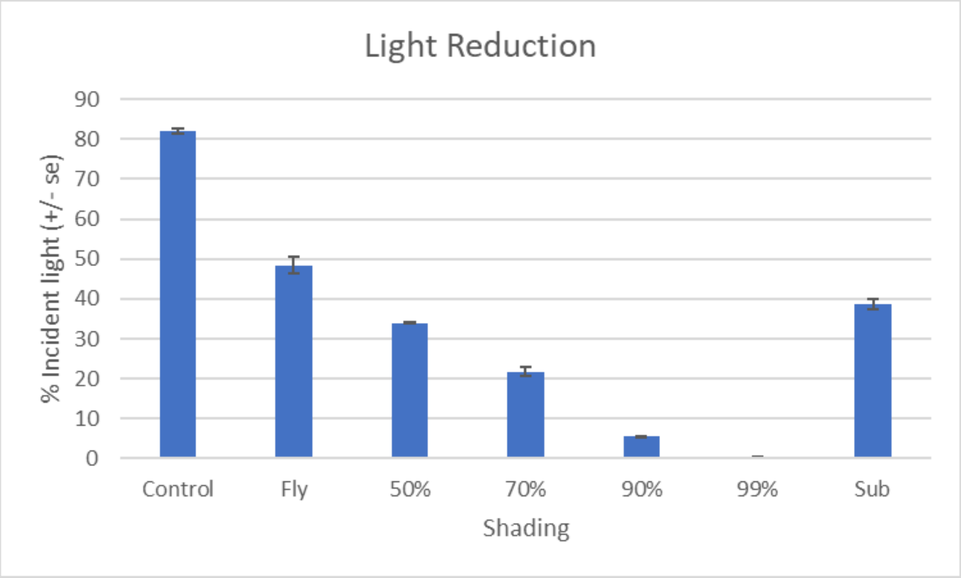


Figure 6-13: Average light reduction over the duration of the shading experiment for trays under each shading level. Sub indicates loggers in the center of each tank not subject to shading, and reduction in light is measured against a logger adjacent to the tanks. All other treatments are measured with respect to the Sub loggers.

Although there was a trend of increasing survival as the level of shading increased up to 90%, followed by a decline at 99% shading (Figure 6-14), this did not prove to be significant (Table 6-13). Growth, however, was significantly affected by level of shading (Table 6-14). The number of leaves peaked under 50% shade cloth (Figure 6-14), while the number of roots peaked at the lowest level of shading (flyscreen). Both leaf and root length peaked under 70% shade cloth. Seedling weight was consistent between the controls and two lowest levels of shading, and then declined consistently as shading further increased.

Table 6-13: Influence of shading on *Posidonia* seedling survival after 134 days in 2022.

	Df	ChiSq	P
Tank	1	0.23	0.63
Shade	5	1.83	0.87
Tank x Shade	2	0.47	0.79
Residual	351		

Table 6-14: PERMANOVA results for growth of *Posidonia* seedlings as a function of shading in 2022.

Source	df	SS	Pseudo-F	P
Tank	1	1766	1.12	0.32
Shade	5	45997	5.87	**0.0001**
Tank x Shade	2	4137	0.27	0.27
Tray (Tank x Shade)	9	14118	0.29	0.29
Residual	248	342500		

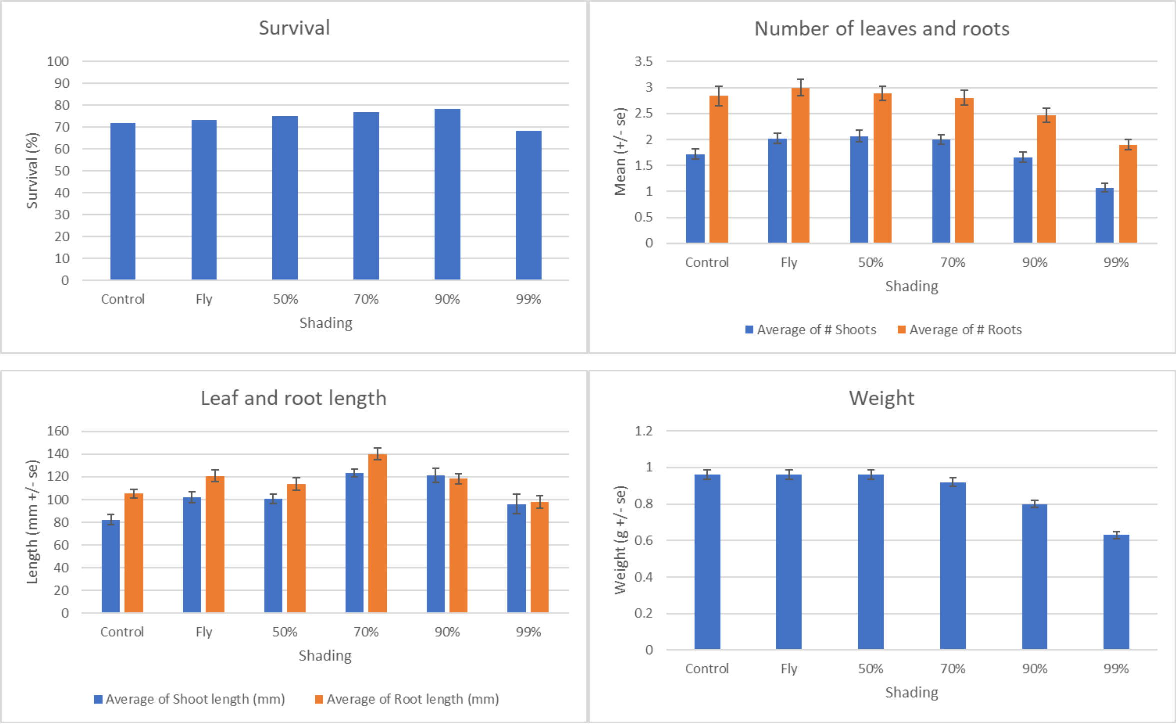


Figure 6-14: Influence of shading on survival and growth of *Posidonia* seedlings over 134 days in 2022.

6.2. Discussion

Initial results on the importance of timing of fruit release for survival and growth suggested a well-defined peak in survival over a few days in late December, with survival rapidly declining the further away from this peak that fruits were released (Tanner and Theil 2019). However, with each additional year of data collected, this pattern has become less apparent (Table 6-15). Whilst there were some peaks in later years, the pattern of rapid decline in survival away from these peaks did not exist, and instead it appears that the viability of fruits varies somewhat erratically through the peak season of fruit release, which can sometimes extend for 2-3 weeks. One possible factor that could not be controlled was how fresh fruit were when they were collected on the beach. Whilst fruits that were obviously sunburned or had lost turgor were avoided, it is likely that there was still variation in how long fruits had been drifting for and how long they had been washed up prior to collection, and this may have influenced their subsequent performance. Weather conditions also played an important role in fruit availability, with little to no fruit washed up onto the Adelaide beaches when there was no afternoon sea breeze. It is suspected that during cooler years, when winds are more southerly, that fruits are probably washed into northern Gulf St Vincent instead, although without data on *in situ* fruit abundance, it is possible that cooler years also lead to poor *Posidonia* reproduction.

As found previously (Tanner and Theil 2019), substrate composition and water flow through the substrate did not influence either survival or growth (Table 6-15). Seed length was also not found to influence seedling performance in this study, although it did in previous work. However, in the current study, seedlings that were smaller at planting remained smaller at harvest.

The role of the time taken for fruits to dehisce, and the time between dehiscing and planting, is currently unclear. Tanner and Theil (2019) generally showed that the sooner fruits dehisced the better they performed, but that the time to planting generally wasn’t important. Here, we found contradictory patterns in time to planting depending on whether fruits took 1 or 2 days to dehisce, although it should be noted that overall survival was very poor in that experiment (6.1e), suggesting that fruits from the collection used were of low quality and the results may not be generalizable.

Planting method did not have an influence on either survival or growth. One question that remains to be addressed, however, is if seedlings that are just dropped onto the sediment will establish once they are more than a few days of age. Several of the field experiments presented earlier were based on scattering seedlings onto the substrate, as this is much quicker than planting them, but none resulted in successful seedling establishment. It is possible that this is because seedlings only have the ability to sink their roots into the substrate without assistance for a few days, after which they will only establish if physically planted.

Table 6-15: Summary of outcomes of *Posidonia* seedling experiments. Note: 2017-18 results from Tanner and Theil (2019).

Trial	**Factor**	**Survival**	**Growth**
a	Time of collection		
	2017-18	Peak on Dec 28	Peak on Dec 28
	2018-19	Peak on Dec 28	No pattern
	2019-20	No Pattern	No pattern
	2021-22	Peak Jan1-2 & 6-7	Peak Dec 24 & Jan 6
b	Clay & organic matter content	Positive effect of shoot length only	No difference
	2017-18 Clay content	No difference	No difference
c	Water flow		
	2017-18	No difference	No difference
	2018-19	No difference	No difference
d	Seed length		
	2017-18	No difference	Large is better
	2018-19	No difference, but higher initial weight led to lower survival	No difference. Low initial weight and shoot length led to smaller seedlings.
e	Time to dehisce, time to planting and seed length	1 day to dehisce, survival decreased as time to planting increased. 2 days to dehisce led to opposite pattern.	No difference
f	Planting method	No difference	No difference
g	Shading		
	2019-20	No difference	Generally peaked at intermediate light levels
	2021-22	No difference	Peaked under intermediate to high light

Seedling survival over 4-5 months was not influenced by light availability. Even severe levels of shading (>99%) did not significantly affect survival. This result suggests that seedlings were primarily relying on their stored energy reserves for survival over this period. Growth, however, did decline under more intense levels of shading, which was obvious in both weight and the number of leaves and roots. Leaf and root length peaked at 70% shading, which may have been a trade-off between increased photosynthesis under low shading and increased elongation in search of light under high shading for the leaves. Off the Adelaide coast, *Posidonia* starts to peter out at around 18-20 m depth, where light levels are ~4% of subsurface light (Collings et al. 2006). This corresponds approximately to the 90% shading treatment, where light levels were 5-6% of the subsurface level. These results suggest that *Posidonia* seedlings are not limited by the light environment along the Adelaide coast during their first 4-5 months of life, and that recruitment and early establishment are restricted by other factors.

7. 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 an extended period of time. However, the current study required 50,000 bags to be deployed in a relatively short window of time, necessitating stockpiling, and storage for several weeks 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. Initial experiments indicated that bags should not be stored in pallet wrap, and should preferably be stored out of the elements, although moisture content of the sand did not appear to influence bag integrity (Tanner and Theil 2019). This earlier work only looked at bags stacked two high on a pallet, thus allowing greater air circulation than would be experienced by bags stacked the conventional eight high. In addition, bags were either stored indoors, or outdoors fully exposed to the elements.

Here, we describe an additional experiment which examined whether stacking bags higher on a pallet resulted in increased degradation due to moisture being trapped. In addition, we also look at whether storing bags outdoors under a tarp improved their structural integrity in comparison to those exposed to the elements or stored indoors. Storage under a tarp leads to reduced air circulation in comparison to storage indoors but protects bags from the sun and rain.

7.1. Methods

Three pallets of sandbags were used in each of three treatments – indoors, outdoors under a tarp, and outdoors fully exposed to the weather. Each pallet was established on 11th July 2019 and contained 30 bags filled with 20 kg of sand arranged in five layers of six. Ten haphazard samples of ~150 g of the sand fill were obtained from the supplier at the same time as the bags, to determine moisture content. In addition, ten bags from the top layer were randomly sampled by extracting a thread of hessian, which was tested for breaking strain. Bags were stored for 32 days, after which three randomly chosen bags from each of the top middle and bottom layers of each pallet were sampled for moisture content and hessian breaking strain. A total of 52.6 mm of rain fell at the nearby Adelaide Airport during this time. For moisture content, a sample of ~150g of sand was taken from the upper side of each sampled bag. For breaking strain, three haphazardly selected strands of hessian were removed from the top surface of each sampled bag.

Moisture content of the sand samples was determined by weighing the sample and then oven-drying it at 60°C until constant weight. 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. Statistical analysis was undertaken in SPSS (v26). ANOVA was used to determine if breaking strain varied between storage locations and layers on the pallet, while linear regression was used to relate breaking strain to moisture content.

7.2. Results

Some mould developed on bags stored in all three locations (Figure 7-1), although the extent was not quantified. The breaking strain of the hessian strands varied between pallets within each storage location, as well as a function of the storage location by layer interaction (Table 7-1). Those bags stored outside exposed to the elements tended to have lower breaking strains than those stored inside or under a tarp, and this was especially noticeable for the top layer, which was fully exposed to the sun and rain (Figure 7-2). The breaking strains for bags stored inside or outside under a tarp were either the same or marginally higher than at the start of the experiment, while those exposed outside were either the same or marginally lower. There was no relationship between breaking strain and the final moisture content of the bags (linear regression: $F_{1,79}$=3.29, P=0.073, Figure 7-3).

Figure 7-1: Examples of bags located inside (top left), outside under a tarp (top right) and outside exposed to the elements (bottom).

Table 7-1: ANOVA results for hessian breaking strain.

Source	**SS**	**df**	**F**	**P**
Storage location	2216	2	3.72	0.12
Layer	52.8	2	0.20	0.82
Location x Layer	1562	4	3.96	**0.004**
Pallet(Location)	1191	4	3.02	**0.019**
Bag(Location x Layer)	2927	22	1.35	0.14
Residual	20118	204		

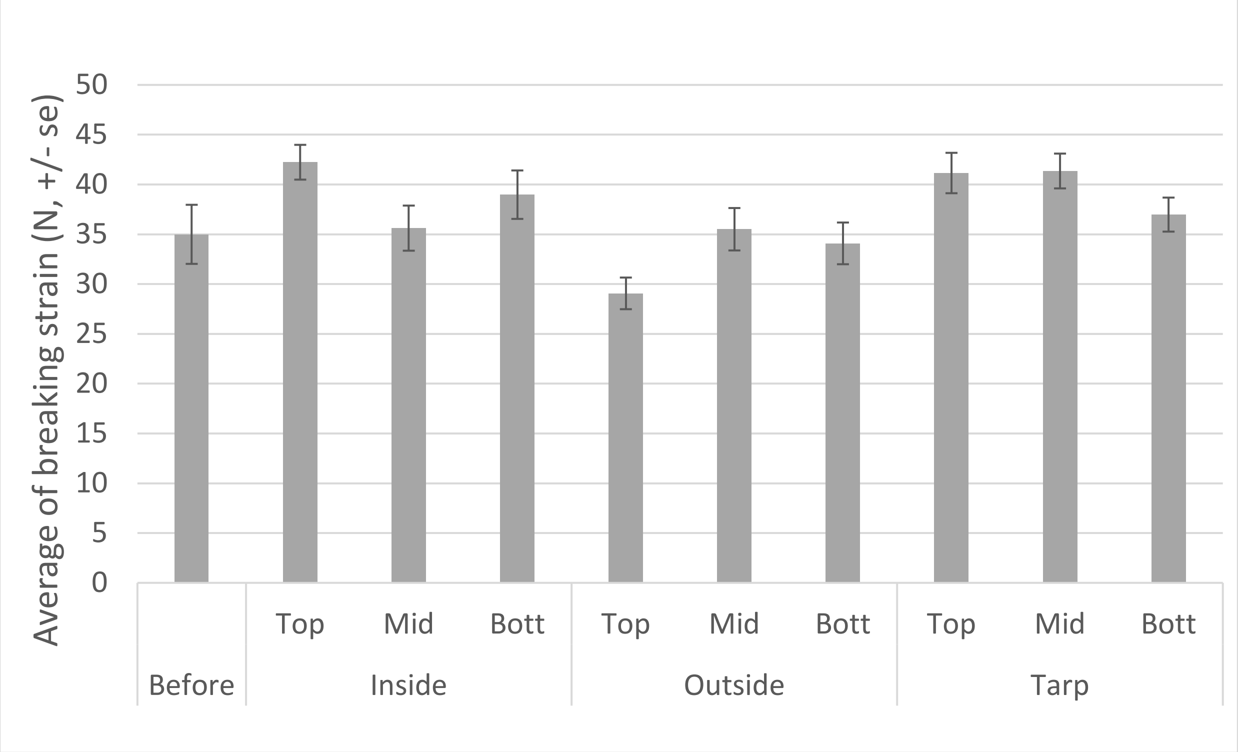


Figure 7-2: Average breaking strain of hessian strands from bags stored inside, outside (exposed to the elements) and under a tarp (but also outside) for 32 days, in comparison to that from freshly filled bags (before).

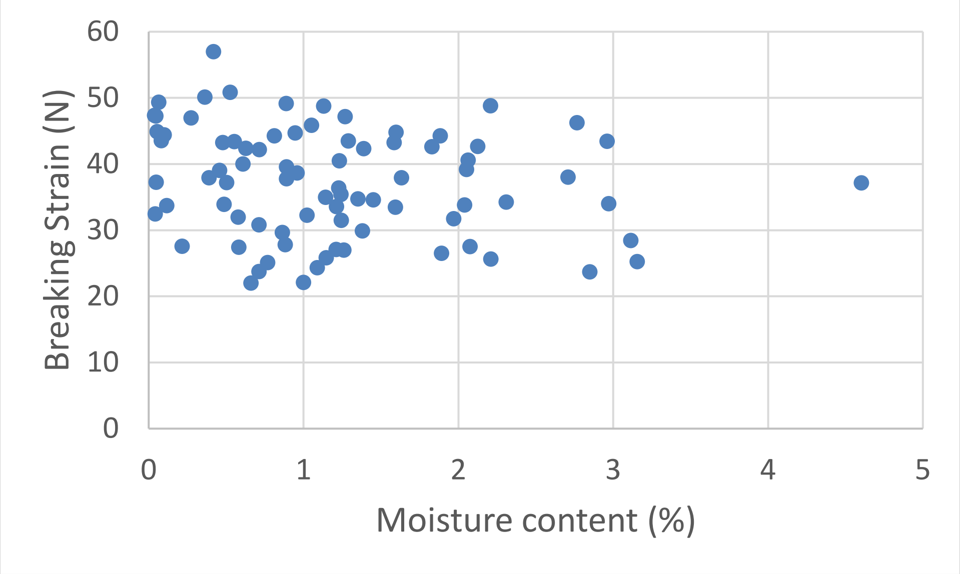


Figure 7-3: Relationship between hessian breaking strain and the final moisture content of the bags.

7.3. Discussion

Storage of bags for up to a month did not appear to have any effect on their structural integrity, provided that they were protected from the elements (sun and rain). Indeed, there is some suggestion that bag integrity actually increased over time. Importantly, this experiment more accurately mimicked the stacking of bags on pallets that is used for large-scale deployments, where there are generally 50 bags per pallet, than did the previous experiments which only used 12 bags per pallet (Tanner and Theil 2019). Consequently, a stockpile of bags can be built up prior to the commencement of deployments, and there is no need to rely on just-in-time delivery, with potential for delayed deliveries to delay the deployment process.

8. GENERAL DISCUSSION

Early results from the large-scale deployment reported here suggest that bag density does not have an effect on *Amphibolis* recruitment. Instead, annual and small-scale spatial variation had important influences. Unfortunately, logistical issues prevented all plots from being established in the same year, and so the annual variation may have played a role in obscuring any effects of bag density. Notwithstanding this, going forward it appears that there is no need to deploy high densities of bags to enhance initial recruitment and survival.

Based on previous work, it is likely to take 5+ years for these plots to become properly established, and for seagrass to start coalescing between the bags (Tanner 2015, Tanner and Theil 2016). Increased bag density will still likely lead to faster meadow formation, as there is less space between bags that needs to be filled in by seagrass growth. This leads to a tradeoff between area of meadow re-established for a given cost and the speed of re-establishment. Until further results on meadow re-establishment are obtained from this study, should funding become available, it is suggested that an intermediate bag density of ~ 5,000 bags per hectare would be appropriate going forward. Annual monitoring going forward will allow the long-term success of these plots to be established and should provide data to assess the trade-off between cost (= bag density) and time to re-establishment.

The pilot study also showed clear spatial variation in recruitment along the Adelaide coast. Semaphore and particularly Grange had very high levels of recruitment, while Henley Beach and sites further south had very low levels. It is not known if this is a recruit supply issue, with no up-current mature meadows to supply recruits to the southern sites, or if it is an environmental issue that leads to poor attachment and retention of recruits. There is a general trend of increasing wave energy moving south along the coast, which may play a role in determining this recruitment pattern, and it was observed that some of the bags at Brighton and Seacliff had been moved between when they were initially set up and when they were monitored. Presumably this movement was a result of wave activity. It is fortuitous that the initial work undertaken almost 20 years ago that ultimately led to the development of the hessian bag technique was in the vicinity of Grange. If it had been undertaken further south, then the technique may never have been developed.

The pilot study also compared 15 and 20 kg bags at Grange and showed no difference in survival and recruitment. This result is important, especially for areas further north with lower wave activity, as it means that lighter bags can be used. Lighter bags reduce manual handling issues and allow an increased number of bags to be deployed in a given time, as the main limitation on number deployed per trip is total weight.

Continued work on *Posidonia* restoration using seedlings suggests that the primary consideration for this species is that beach collection should occur regularly over the last week of December and first week of January (for Adelaide). It is not yet possible to predict on what days during this period fruits will wash up, although strong afternoon sea breezes increase the chances. Even when fruits do wash up, there is some temporal variability in their performance, and careful consideration needs to be given to fruit quality to ensure that effort is not being spent on fruits that will produce poor quality seedlings. It is not yet established what those fruit quality indicators are, although making sure that fruits have a yellow tinge indicates maturity, and fruits should also be turgid (=fresh) and free from major blemishes such as sunburn and crab bites. As discussed previously (Tanner and Theil 2019), the substrate composition does not appear to play an important role in initial seedling establishment, fruits that dehisce early after collection appear to produce the best seedlings, and seedlings should ideally be planted within 10 days of dehiscing, although success can be obtained with seedlings that are 20-30 days old. Light levels are also not important for early establishment, presumably because most nutriment is being obtained from the seed in the first 4-5 months. When planting seedlings into bags prior to deployment, it is important to secure them to the bag in some way, as 70% of seedlings that were not attached were lost on deployment. Further work on seedlings should seek to determine if older seedlings need to be planted to establish, or if they can simply be dropped onto the substrate and allowed to root themselves. There was little to no establishment of scattered seedlings in the field experiments that utilized this technique, which may indicate that they can only get their roots into the substrate in their first few days. It would also be useful to understand influences on longer-term establishment.

Continued work on seagrass restoration off Adelaide is currently following several avenues. Firstly, there will be a further increase in scale, with ~20 hectares to be restored off Port Gawler using 100,000 sandbags for *Amphibolis*. These bags will be over-sown with a small number (5,000) of bags sown with *Posidonia* seedlings, to trial multispecies restoration. There is also some ongoing work to determine if oyster reef restoration can enhance the outcomes of seagrass restoration. Further recommendations for research include:

1. Following the success of the 1-hectare plots described here over time, in order to quantify the trade-off between bag density (= cost) and time to restore a meadow,

2. Further work to establish if scattering *Posidonia* seedlings could be a viable approach, without the need for actual planting.

3. Determining the influence of the spatial pattern of restoration. For example, can plots be restored in a checkerboard fashion, with the unrestored checkers naturally recolonizing?

4. Determining if the hessian bag method is a viable means for stabilizing erosion scarps in *Posidonia* meadows, thereby reducing ongoing loss of seagrass.

5. Establishing whether the use of *Posidonia coriacea* provides enhanced outcomes in more exposed areas, as it naturally occurs in areas with higher wave activity than *Posidonia angustifolia*.

REFERENCES

Anderson, M. J. 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecology **26**:32-46.

Anderson, M. J., R. N. Gorley, and K. R. Clarke. 2008. PERMANOVA+ for PRIMER: Guide to software and statistical methods. PRIMER-E, Plymouth, UK.

Bates, D., M. Machler, B. M. Bolker, and S. C. Walker. 2015. Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software **67**:1-48.

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.

Bryars, S., and V. Neverauskas. 2004. Natural recolonisation of seagrasses at a disused sewage sludge outfall. Aquatic Botany **80**:283-289.

Cameron, J. 1999. Near shore seagrass change between 1989/90 and 1997/98 mapped using Landsat TM satellite imagery, Gulf St Vincent South Australia. Department for Environment, Heritage and Aboriginal Affairs, Adelaide.

Cameron, J. H. 2008. Near-shore seagrass change between 2002 and 2007 mapped using digital aerial photography orthophotography: Metropolitan Adelaide Area Port Gawler - Marino, South Australia. Department for Environment and Heritage, 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.

Collings, G., D. Miller, E. O'Loughlin, A. Cheshire, and S. Bryars. 2006. Turbidity and reduced light responses of the meadow forming seagrasses *Amphibolis* and *Posidonia*, from the Adelaide metropolitan coastline. ACWS Technical Report No. 12 prepared for the Adelaide Coastal Waters Study Steering Committee. South Australian Research and Development Institute (Aquatic Sciences) Publication No. RD01/0208-17., 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.

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.

Fotheringham, D. 2008. General background and patterns of loss. Pages 7-15 *in* S. Murray-Jones, editor. Proceedings of the second seagrass restoration workshop. Department for Environment and Heritage, Adelaide.

Fox, D. R., G. E. Batley, D. Blackburn, Y. Bone, S. Bryars, A. Cheshire, G. Collings, D. Ellis, P. Fairweather, H. Fallowfield, G. Harris, B. Henderson, J. Kampf, S. Nayar, C. Pattiaratchi, P. Petrusevics, M. Townsend, G. Westphalen, and J. Wilkinson. 2007. Adelaide Coastal Waters Study, Final Report, Volume 1, Summary of Study Findings, November 2007. CSIRO, Adelaide.

Hart, D. 1997. Near-shore seagrass change between 1949 and 1995 mapped using digital aerial orthophotography. Southern Adelaide area Glenelg-Marino South Australia. Department of Environment and Natural Resources, Adelaide.

Hart, D. 2013. Seagrass extent change 2001-13 - Adelaide coastal waters. Department of Environment, Water and Natural Resources, 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.

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.

Lavery, P., O. Serrano, J. Robinson, and P. Masque. 2022. Blue carbon capture and storage in restored *Amphibolis antarctica* meadows. Edith Cowan University, Perth.

McSkimming, C. 2015. Stability and recovery of coastal ecosystems to local and global resource enhancement. The University of Adelaide, Adelaide.

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 and Shelf Science **171**:51-57.

Murray-Jones, S. 2008. Proceedings of the second seagrass restoration workshop. Adelaide. April 2008. Department for Environment and Heritage, Adelaide.

Murray-Jones, S., editor. 2013. Proceedings of the Third Seagrass Restoration Workshop. Adelaide. March 2013. Department for Environment, Water and Natural Resources, Adelaide.

Neverauskas, V. P. 1987a. Monitoring seagrass beds around a sewage sludge outfall in South Australia. Marine Pollution Bulletin **18**:158-164.

Neverauskas, V. P. 1987b. Port Adelaide sewage treatment works sludge outfall - effect of discharge on the adjacent marine environment: final report. Engineering and Water Supply Department, Adelaide.

Oceanica Consulting Pty. Ltd. 2011. Seagrass research and rehabilitation plan: Synthesis report July 2003 to June 2010. Perth.

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.

R Core Team. 2018. R: A language and environment for statistical computing. Accessed on: 11/7/13. www.R-project.org/.

Seddon, S. 2002. Issues for seagrass rehabilitation along the Adelaide metropolitan coast: an overview. Pages 1-8 *in* S. Seddon and S. Murray-Jones, editors. Proceedings of the Seagrass Restoration Workshop for Gulf St. Vincent, 15–16 May 2001. Department of Environment and Heritage and SARDI Aquatic Sciences, Adelaide.

Seddon, S. 2004. Going with the flow: Facilitating seagrass rehabilitation. Ecological Management & Restoration **5**:167-176.

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., 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.

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.

Shepherd, S. A., A. J. McComb, D. A. Bulthuis, V. P. Neverauskas, D. A. Steffensen, and R. West. 1989. Decline of seagrasses. Pages 346-393 *in* A. W. Larkum, A. J. McComb, and S. A. Shepherd, editors. Biology of seagrasses. Elsevier, Amsterdam.

Tanner, J. E. 2015. Restoration of the seagrass *Amphibolis antarctica* - temporal variability and long-term success. Estuaries and Coasts **38**:668-678.

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., and M. Theil. 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.

Tanner, J. E., and M. Theil. 2019. Adelaide Seagrass Rehabilitation Project: 2017-2019. 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. F2009/000210-3. SARDI Research Report Series No. 1025. 77pp.

Van Gils, J., P. Erftemeijer, M. Fernandes, and R. Daly. 2017. Development of the Adelaide Receiving Model. Deltares, Delft, The Netherlands.

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.

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.

Westphalen, G., G. Collings, R. Wear, M. Fernandes, S. Bryars, and A. Cheshire. 2005. A review of seagrass loss on the Adelaide metropolitan coastline. South Australian Research and Development Institute (Aquatic Sciences) Publication No. RD04/0073, Adelaide.