

Seagrass Rehabilitation in Adelaide
Metropolitan Coastal Waters
III. Development of Recruitment
Facilitation Methodologies

Prepared for
Coastal Protection Branch,
Department for Environment and Heritage

by
Rachel Wear, Jason Tanner and Sonja Venema
SARDI Aquatic Sciences

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EXECUTIVE SUMMARY

Over recent decades, the Adelaide metropolitan coast has lost more than 5,200 ha of seagrass habitat due to increasing anthropogenic pollution and coastal development. This loss of seagrass has contributed to considerable and ongoing erosion of the Adelaide foreshore and marine environment, and has reduced the amount of habitat available for many local species of flora and fauna. Seagrass rehabilitation has been considered and investigated in an effort to encourage the return of the numerous functions provided by seagrasses in this state. Previous research in South Australia has focused on methods developed elsewhere, including the transplantation of mature seagrass units and the culturing and planting out of seagrass seedlings. The use of these methods has proven difficult, and results have generally been poor owing to the exposed nature of the Adelaide metropolitan coast. An alternative approach is to facilitate the natural recruitment of seagrass seedlings *in situ*. This report outlines the results of an experiment that was conducted to assess the ability of various artificial substrates to aid in facilitating the natural recruitment of *Amphibolis antarctica* (Labillardière) Sonder & Ascherson and *A. griffithii* (Black) den Hartog seedlings.

A range of biodegradable hessian bags, strips and mats in various configurations were deployed at two sites along the Adelaide metropolitan coast in September 2004. Approximately five weeks after deployment a total of 16,514 seedlings, or the equivalent of 157.2 seedlings m⁻², had recruited onto all experimental units. Significant differences in seedling recruitment were observed between the two sites and are likely to reflect the composition of the surrounding seagrass beds, depth and local hydrodynamic regime. The retention of seedlings on the experimental units declined over time, but after a one-year period 31.4% of seedlings remained. Recruitment and retention of seedlings varied between methods, and while coarse weave hessian strips were particularly effective at recruiting seedlings, survival was poor on this method. The most effective method in terms of seedling density at the end of the experimental period was hessian bags covered with a coarse weave hessian layer, while this method together with large hessian bags were amongst the most cost-effective. Surviving seedlings grew considerably over this time, as was evident by a 2.6 fold increase in above ground-biomass and a 6.4 fold increase in below-ground biomass. If the seedlings remain and continue to grow, the facilitation of *Amphibolis* recruitment via the provision of suitable substrates may represent a new method of seagrass rehabilitation. The methods developed offer significant advantages to other methods of restoration, particularly as they are non-destructive, cost effective, and may easily be deployed over large spatial scales. Furthermore, facilitating the natural recruitment of *Amphibolis* seedlings into areas of previous seagrass loss may fast-track the natural regeneration of other, later successional seagrass genera, such as *Posidonia*. Extrapolating from the costs of the small-scale trial conducted here, it will cost in the order of \$10,000 to rehabilitate one hectare of seagrass, which compares favourably with other methods, and the estimated economic value of seagrasses (\$12,635 to \$25,270 ha⁻¹yr⁻¹).

1. INTRODUCTION

1.1. Background

Seagrass meadows have long been recognised for their ecological and economic importance, and together with coral reefs and mangroves, are thought to represent one of the world's most productive coastal habitats (Short and Wyllie-Echeverria, 1996). Seagrass beds not only play a critical role in primary production (Borum et al., 2006) and nutrient cycling (Hillman et al., 1989; Romero et al., 2006), but they also provide habitat for a diverse array of marine organisms (Bell & Pollard, 1989; Short & Wyllie-Echeverria, 1996; Connolly et al., 1999; Duarte, 2002), and increase the stability of the seafloor through the growth of extensive rhizome mats (Fonseca & Fisher, 1986). Despite overwhelming research that consistently demonstrates the importance of seagrasses, seagrass beds are currently undergoing worldwide declines. While natural loss of seagrass is apparent in some regions (Robblee et al., 1991; Preen et al., 1995; den Hartog, 1996; Seddon et al., 2000), the majority of losses can be attributed to anthropogenic factors (Orth and Moore, 1983; Short and Wyllie-Echeverria, 1996; Seddon et al., 2003; Walker et al., 2006). Unfortunately with the ever-increasing growth in the world's population and the trend to live along the coastal margin, the threat posed by humans to seagrass meadows continues.

Recent estimates suggest that over the past two decades, approximately 18% (or 33,000 km²) of the world's documented seagrass area has been lost as a result of direct and indirect human impacts (Walker et al., 2006). While reports of seagrass losses are escalating, the recovery of seagrasses appears to be rare. One of the reasons for the unbalanced loss and recovery of seagrasses is that these processes take place over fundamentally different time scales. Wide scale losses of seagrass beds may occur rapidly following environmental perturbations. Conversely, recovery of seagrasses from disturbance either via clonal expansion (lateral growth of rhizomes) or natural recruitment is often very slow and can take decades to centuries (Kirkman, 1985; Clarke and Kirkman, 1989; Kirkman and Kuo, 1990; Hastings et al., 1995; Marbà and Walker, 1999; Meehan and West, 2000; Bryars and Neverauskas, 2004).

Increasing documentation of seagrass losses around the globe, and recognition of the importance of seagrasses to coastal protection, biodiversity, and productivity, has prompted researchers and resource managers to investigate ways to protect existing beds and restore disturbed communities. Management practices likely to benefit seagrass communities in terms of preventing degradation may include improvement of wastewater management, setting aside areas of seagrass habitat for preservation, and the prohibition or regulation of destructive and unsustainable activities. While the implementation of such management practices is likely to assist with the long-term conservation of seagrass communities, in some cases the revision of traditional management methods may not be enough to prevent

seagrass loss or facilitate recovery. Losses to date have weakened many of these systems beyond their ability to rebound, and as such, management must move beyond simple restoration of the physio-chemical environment, and address the need for accelerated recolonisation. Having said this, restoration is still dependent upon reparation of the situation which caused the original loss.

The restoration of previously disturbed marine ecosystems, including seagrass meadows, is an area of research that has received increasing attention over the past few decades (Rinkevich, 1995; Field, 1998; Yap, 2000; and references therein). With growing research and development into seagrass restoration and rehabilitation techniques, this relatively new conservation strategy is beginning to gain more widespread acceptance, particularly as such techniques may speed up the natural recovery process and result in a more immediate return of these vital yet threatened ecosystems.

Numerous rehabilitation techniques have been developed and tested over the years. The most common seagrass rehabilitation techniques to date have involved the transplantation of adult ramets, perhaps because they result in an immediate presence of the plant community. Some of these methods have involved transplantation of seagrass and associated sediments, whilst others transplant only the seagrass itself (Fonseca et al., 1998; Calumpong and Fonseca, 2001). The success of transplant methods is highly dependent upon the species used, site characteristics, and the experience of those undertaking the transplanting. Unfortunately, the majority of transplant trials have resulted in limited survival and coverage (Thorhaug, 1986; Paling et al., 2003; West et al., 1990), and in some cases have resulted in the net loss of seagrass habitat (West et al., 1990). Notwithstanding, some remarkable successes have been achieved. For example in Biscayne Bay, Florida, approximately 48 ha of seagrass, consisting of *Halodule wrightii*, *Syringodium filiforme* and *Thalassia testudinum* have been restored following the transplantation of over one million seagrass units (Thorhaug, 1987). In Australia, the restoration of such expansive areas is yet to be achieved and the survival of transplants has typically been poor (eg. Kirkman, 1998; Seddon et al., 2004), owing predominantly to the hydrodynamically turbulent environment existing in transplanted areas. The recent development of a mechanical seagrass transplanting machine (ECOSUB 2), capable of transplanting 75, 0.55 m² seagrass sods per day has increased transplant survival to 70%, albeit at considerable expense, with transplants showing signs of expansion and the restored area showing signs of natural infilling by seagrass seedlings (Paling et al., 2001a; Paling et al., 2002).

Despite recent endeavours to improve the capabilities for seagrass transplantation in Australia, including the development of mechanical techniques, the practice is generally not suitable over large scales. As pointed out by Seddon (2005), assuming the maximum predicted rate of 75 sods per day and approximately one metre between sods, ECOSUB 2

would take approximately 44 days to plant just one hectare of seagrass. Furthermore, the transplantation of seagrasses relies on the destruction of otherwise healthy meadows, which, as outlined earlier, are reported to have extremely slow recovery rates (Kirkman & Kuo, 1990; Meehan & West, 2000; Cambridge et al., 2002), and is generally labour intensive and expensive (Lewis et al., 1998; Spurgeon, 1998; Calumpong and Fonseca, 2001; Paling et al., 2001a).

An alternative to the transplantation of vegetative stock from one location to another, and an area that has received far less attention (Fonseca et al., 1998), is the planting out of seagrass seeds and seedlings. The use of seeds and seedlings for rehabilitation has recently been endorsed by experts within the field (Seddon, 2005; Orth et al., 2006), as such methods have major advantages with transplantation techniques. Firstly, the utilisation of seeds avoids the destruction of seagrass beds, although at this point in time little research is available on the role seeds and seedlings play in the maintenance of seagrass bed health. The use of seedlings has also been reported to encourage genetically heterogeneous seagrass beds that grow faster, produce more seeds and have better rates of germination compared with transplantation methods (Williams, 2001). Furthermore, many seagrass species produce copious quantities of propagules that can be easily harvested and stored. Despite the obvious benefits of using seeds in restoration programs, at this point in time this type of restoration appears only to be applicable in low energy environments and where seed predators are sparse (Fonseca et al., 1998). Techniques to reduce mortality associated with high energy regimes have included embedding seeds in biodegradable mesh, and planting seedlings in biodegradable peat blocks or Growool blocks (Kirkman, 1998). Results have been variable, and without doubt the use of seedlings in rehabilitation requires more research before any large-scale trials are likely to succeed, especially in moderate to high wave energy environments.

Facilitating the natural recruitment of seagrass ramets or seedlings and encouraging their survival and growth, may represent an additional means of seagrass restoration. While research into such methods currently remains unexplored, in a recent paper, Di Carlo et al. (2005) identified that rubble, back-filled into a dredge trench, facilitated the vegetative recruitment of *Posidonia oceanica*. Furthermore, in a recent trial in Adelaide, *Amphibolis* seedlings were observed recruiting onto hessian matting used to stabilise transplanted cores and springs of seagrass (Seddon, 2004). Facilitating the recruitment of seagrass ramets or seedlings as a means of restoration, such as observed by Di Carlo et al. (2005) and Seddon (2005), is likely to offer considerable advantages compared with transplantation of seagrass ramets and the planting out of seagrass seedlings and, if successful, may significantly reduce the cost involved with restoring seagrass beds, particularly if such efforts eliminate the need for SCUBA divers.

1.2. Objectives and Study Site

The objective of this paper is to describe the success of techniques being trialled for seagrass rehabilitation along the Adelaide metropolitan coast, South Australia. In particular it focuses on the development of seedling recruitment facilitation techniques for *Amphibolis antarctica* and *A. griffithii*. Both of these species are endemic to temperate Australian waters and produce seedlings on the parent plant that have a comb-like grappling apparatus at their base to facilitate entanglement. Seedlings are released annually between July and December (Robertson, 1984).

Holdfast Bay, the site of the current study, is located adjacent to Adelaide (138°35'55"E, 34°55'42"S), South Australia. The Bay contains *Posidonia angustifolia*, *P. australis*, *P. sinuosa*, and *Amphibolis antarctica* beds interspersed with small patches of *A. griffithii* (Shepherd and Robertson, 1989). *Heterozostera tasmanica* and *Halophila australis* also occur throughout, often as an understorey component to larger species, although *Heterozostera* may occur more densely in deeper areas (Shepherd and Robertson, 1989). The seagrass community once dominated the soft-bottom coastline to within several hundreds of metres of the shore. However, since the 1940s, Holdfast Bay has lost more than 4,000 ha of seagrasses (Figure 1; Hart, 1997). The spatial and temporal pattern of seagrass losses adjacent to Adelaide correlates with the pattern of coastal development and anthropogenic inputs into coastal waters (Neverauskas, 1987; Shepherd et al., 1989; EPA, 1998; Seddon, 2002; Bryars and Neverauskas, 2004) and have previously been attributed to sewage and stormwater discharges into the gulf (Neverauskas, 1987; Shepherd et al., 1989; EPA, 1998; Seddon, 2002), particularly during the 1970s when the greatest rates of seagrass loss occurred (Hart, 1997). However, in recent times significant revisions to storm- and waste-water management have been put into place (Hamilton, 2002). Apparently as a result, seagrass losses along the metropolitan coast have slowed down in some areas and ceased in others. This makes the Adelaide metropolitan coast a potential candidate for a successful rehabilitation project.

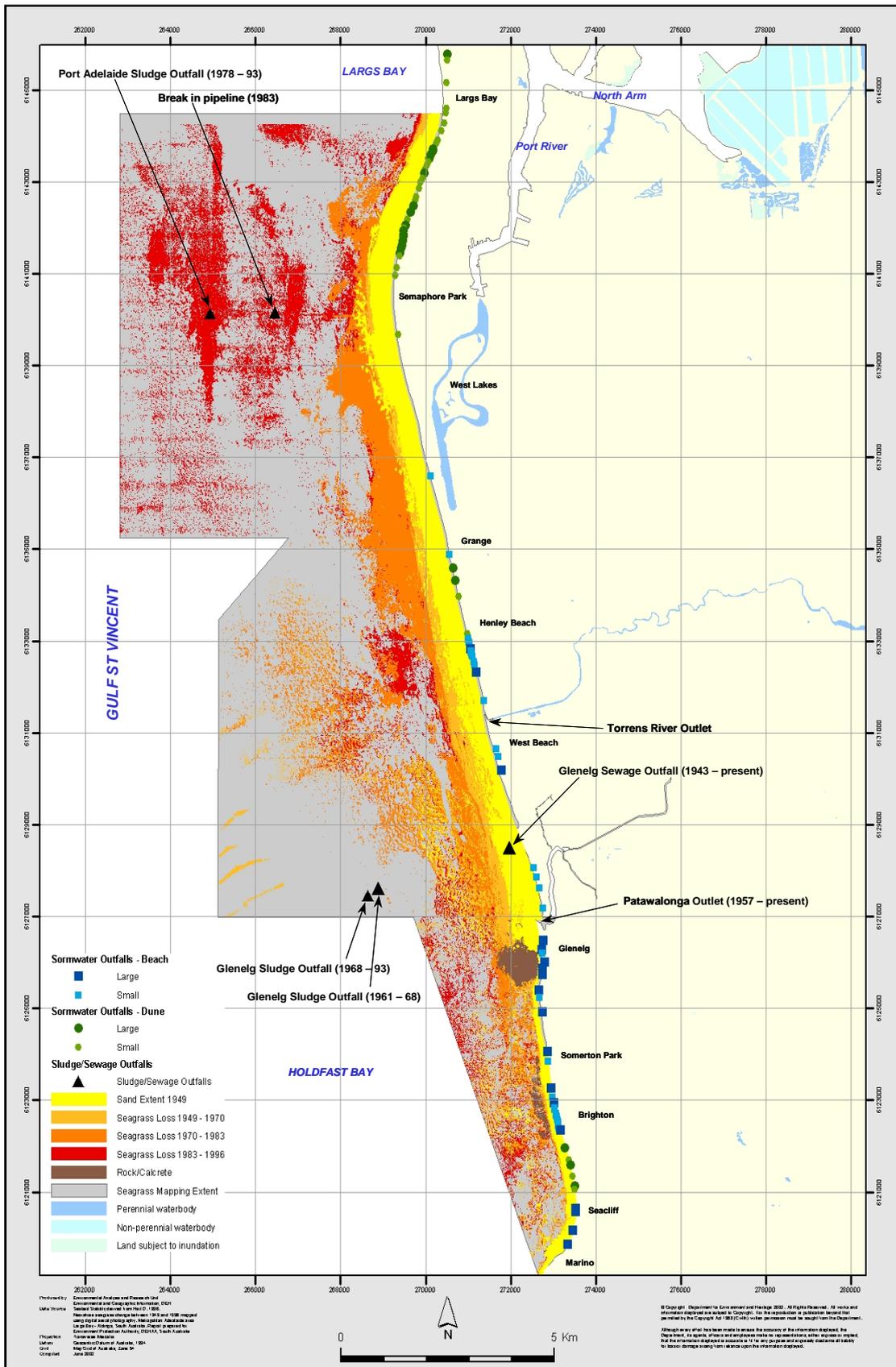


Figure 1. Map of the Adelaide metropolitan coast showing the extent of seagrass loss between Largs Bay and Marino between 1949 and 1996. The location of input sources is also indicated. Image from Seddon (2002) constructed by Tim Noyce care of the South Australian Department of Environment and Heritage.

2. METHODS

A seagrass rehabilitation experiment aimed at determining the best method for facilitating the natural recruitment of *Amphibolis* seedlings was carried out at two sites along the Adelaide metropolitan coast. Both sites were located approximately two kilometres from the shore in unvegetated areas previously dominated by seagrass, and were surrounded by dense seagrass beds consisting largely of *Amphibolis*. The first site ($138^{\circ}28'03.5''\text{E}$, $34^{\circ}54'01.9''\text{S}$) was situated adjacent to Grange in approximately 8 m of water, while the second was located further north ($138^{\circ}27'47.9''\text{E}$, $34^{\circ}52'20.1''\text{S}$), adjacent to Semaphore Park in approximately 6.5 m depth (Figure 2).

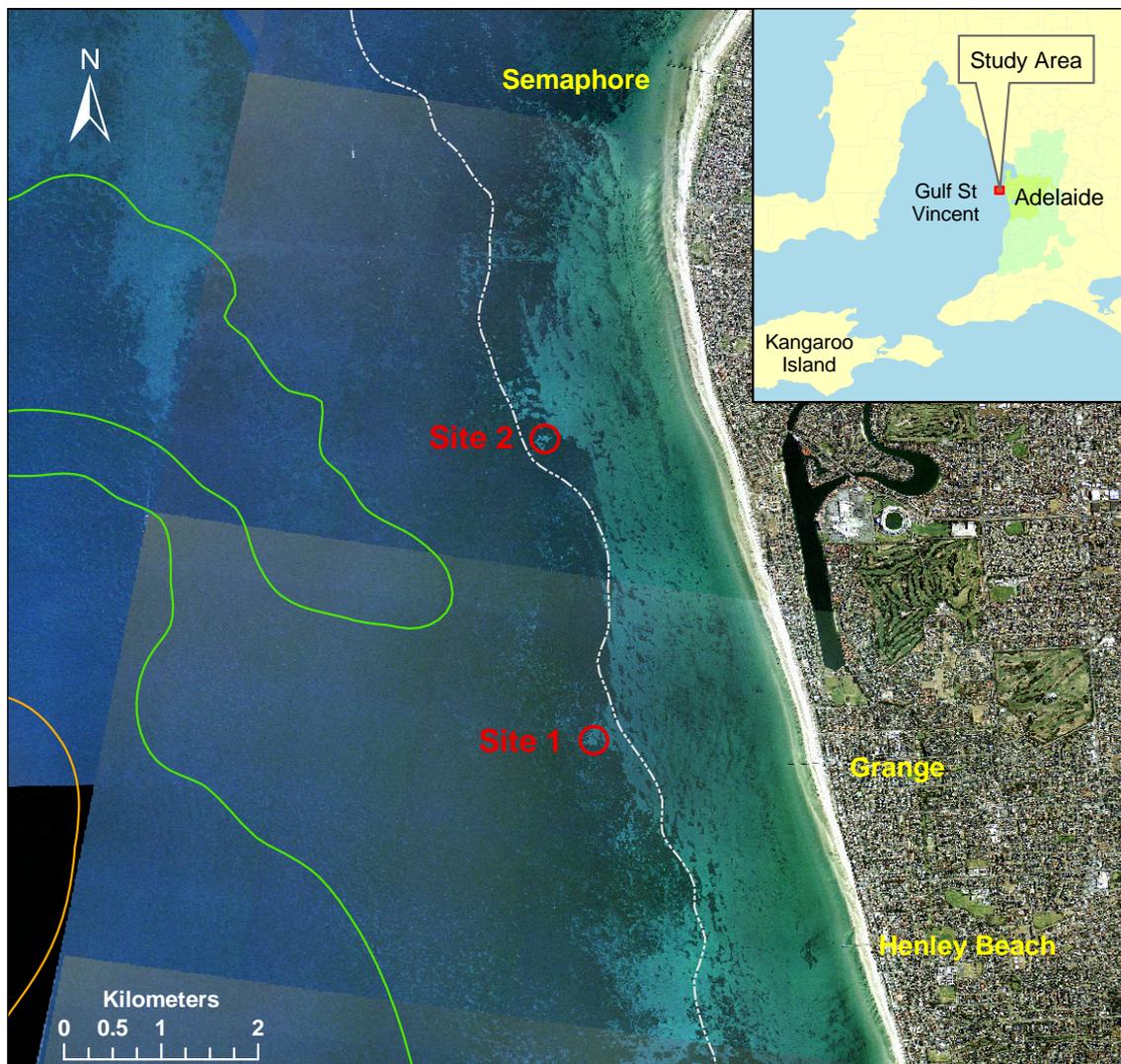


Figure 2. Location of the two study sites at which the experiment aimed at facilitating the natural recruitment of *Amphibolis* seedlings was carried out. White, green and orange lines represent the 5, 10 and 15 m depth contours, respectively.

2.2. Site Characteristics

The success or otherwise of any attempt at restoration will be closely related to the physical environment in which that attempt occurs. It thus becomes important to have a good understanding of the physical conditions of the environment if comparisons between studies are to be made. While such comparisons are not done here, we present below a description of the environment to facilitate such comparisons in the future.

2.2.1. Environmental conditions

Inshore surface water temperature within the area varies seasonally between 11.0 °C and 26.6 °C, while total dissolved solids generally varies between 33.0 and 38.8 µgL⁻¹. Other water quality parameters are provided in Table 1.

Table 1. Surface water quality in Holdfast Bay. Data collected off jetties between 1996 and 2003 by the Environment Protection Authority, South Australia.

	Largs Bay	Semaphore	Grange	Henley Beach	Glenelg
Temperature (°C)	11.0 – 26.0	11.0 – 25.0	11.0 - 26.6	11.0 – 26.4	11.0 – 25.5
TDS (g L ⁻¹)	33.7 – 38.8	26.3 – 38.8	33.0 – 38.2	34.0 – 38.2	33.0 – 38.3
pH	7.88- 8.3	7.88- 8.3	7.46 – 8.35	7.76 – 8.33	7.79 – 8.38
Turbidity (NTU)	0.35 - 31	0.386 - 21.6	0.337 - 24	0.404 – 25.1	0.341 - 26
Ammonia (mg L ⁻¹)	0.005 – 0.2	0.005 - 0.164	0.005 – 0.19	0.005 – 0.216	0.007 – 0.19
Nitrate (mg L ⁻¹)	0 - 0.069	0 - 0.105	0 - 0.178	0 - 0.159	0 – 0.13
Nitrite (mg L ⁻¹)	0.005 - 0.01	0.005 - 0.01	0.005 - 0.02	0.005 - 0.014	0.005 - 0.018
Phosphorus (mg L ⁻¹)	0.013 – 0.32	0.011 – 0.14	0.018 – 0.68	0.014 – 0.091	0.013 – 0.165

2.2.2. Habitat mapping

To determine the spatial distribution of habitats surrounding the two sites chosen for this study, eight transects radiating from the centre of each site were established. Habitat types (sand, *Amphibolis*, *Posidonia*, *Heterozostera* and *Halophila*) were characterised along each transect using the Line Intercept Transect method (LIT) (English et al., 1997). In most cases at least fifty metres of habitat was recorded beyond the edge of the sand patch in which the study site was located, and as a consequence the length of each transect varied between 73 m and 100 m.

The habitats surrounding the two sites selected for this study differed (Figure 3). Site one was characterised by a larger sand patch (radius of 41.8 ± 3.50 m, mean \pm standard error – conversion used throughout) than site two (radius of 30.9 ± 3.39 m), and was surrounded almost entirely by monospecific meadows of *Amphibolis antarctica* (97.3%). The seagrass community surrounding site two consisted of dense *Amphibolis antarctica* beds (53.9%) and *Posidonia sinuosa* and *P. angustifolia* beds (44.2%).

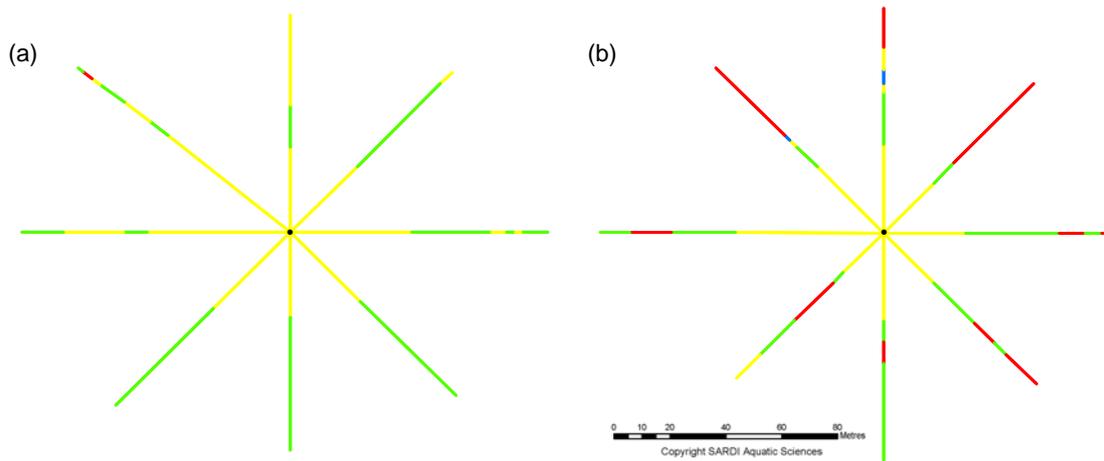


Figure 3. Spatial distribution of habitats surrounding site one (a) and site two (b). Yellow lines represent sand patches, green lines *Amphibolis antarctica* and *A. griffithii*, red lines *Posidonia sinuosa* and *P. angustifolia*, and blue lines *Heterozostera* and *Halophila* species.

2.2.3. Light climate

To quantify the light climate at each site, Odyssey light loggers (Dataflow Systems Pty Ltd, New Zealand) were attached to star pickets approximately 1 m above the sediment surface at the centre of each site in July 2005. The light loggers were programmed to record Photosynthetically Active Radiation (PAR, $\mu\text{Em}^{-2}\text{s}^{-1}$) every half an hour and were replaced on a fortnightly basis to avoid the build up of sediments and the growth of algae on the sensor element.

The average daily light availability at the two sites differed over time. As expected, the shallower of the two sites (site two) generally received more light during August, September and October. The opposite trend was observed during July (Figure 4). This result is consistent with the findings of the Adelaide Coastal Waters Study, which identified that during storm periods nearshore, turbid waters can substantially reduce seabed light availability in these shallower areas (S. Bryars, personal communication).

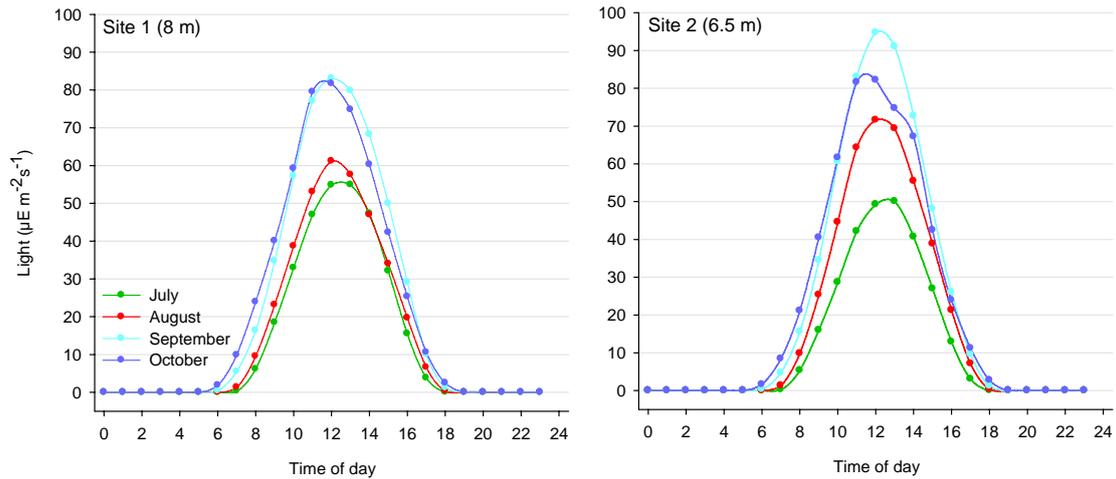


Figure 4. Average light availability at both sites, during July, August, September and October 2005.

2.3. Recruitment Facilitation Methodologies

In an attempt to enhance the natural recruitment of *Amphibolis* seedlings, a range of biodegradable substrates in various forms were deployed at the two study sites. Three different biodegradable materials were used in creating the recruitment facilitation methods; a fine weave hessian¹, a coarse weave hessian, and mats constructed from interwoven seagrass (Figure 5). Using these materials, ten different recruitment facilitation techniques were trialed, including six different hessian bags, three different strips and a seagrass mat, each of varying size and configuration (Table 2; Figure 6).



Figure 5. Fine weave hessian (a), coarse weave hessian (b) and seagrass mats (c) were the three biodegradable materials used to create a range of bags and strips used in an effort to facilitate the natural recruitment of *Amphibolis* seedlings.

¹ Hessian, otherwise referred to as jute or burlap, is a 100% biodegradable material made from the stems of the tropical herb *Corchorus* (*C. olitorius* and *C. capsularis*).

Table 2. A description of the different bags and strips deployed to enhance *Amphibolis* recruitment.

Method		Description / dimensions
Hessian Bags	Hessian Bag	Hessian bag with 10 oz weave, measuring ~ 76 x 46 cm and filled with 25.26 (\pm 0.86) kg of sand (n = 5). 10 replicates at each site.
	Hessian Bag Half Buried	As above, but half buried at deployment.
	Hessian Bag Double-layered	As above, but covered with a coarse layer of hessian and filled with 23.54 (\pm 0.56 se) kg of sand (n = 5) (Figure 6a, b).
	Hessian Bag with Flap	As above, but sewn in half lengthways and with one half filled with 24.82 (\pm 0.58 se) kg of sand (n = 5) (Figure 6c).
	Hessian Bag with Mat	As above, but with a 'seagrass' mat sewn to the top and filled with 28.70 (\pm 1.42 se) kg of sand (Figure 6f).
	Large Hessian Bag	Hessian bag with 10 oz weave, measuring ~ 107 x 74 cm and filled with 70.22 (\pm 2.51 se) kg of sand (n = 5) (Figure 6d).
Hessian Strips	Hessian Strip NS	Hessian strip made out of coarse weave hessian and bound with calico on three sides to prevent fraying, measuring 3 m x 60 cm. Deployed in a north-south orientation (Figure 6g). 10 replicates at each site.
	Hessian Strip EW	As above, but deployed in an east-west orientation (Figure 6g).
	Weighted Strip	Three metre lengths of chain, wrapped with four hessian bags secured in place with cable ties. The weight of the chains averaged 30.81 (\pm 0.93 se) kg (n = 8) (Figure 6h). Deployed in an east-west orientation. 8 replicates at each site.
Seagrass Mat	Mat	'Seagrass' mat made out of interwoven seagrass. Measuring 35 x 60 x 2 cm and secured to the substrate with four steel pegs (Figure 6e). 10 replicates at each site.



Figure 6. Photographs of the different types of hessian bags, strips and mats deployed in an effort to facilitate the natural recruitment of *Amphibolis* seedlings; double-layered hessian bag (a and b), hessian bag with flap (c), large hessian bag (d), seagrass mat (e), seagrass mat sewn onto a hessian bag (f), making the hessian strips (g), the coarse weave hessian used to make the hessian strips (h), and weighted strips (i).

The hessian bags and weighted strips were deployed on the 7th September 2004. In the week that followed (15th – 16th September) divers rearranged the bags and strips in a predetermined randomised manner. Bags were randomly placed on either side of four transects, at least one metre apart, radiating in north, south, east and west directions from the centre of each site, while strips were randomly positioned along transects radiating in north-west, north-east, south-east and south-westerly directions (Figure 7). During the same period, the hessian strips and the seagrass mats were set out by divers along each transect. The three-metre long hessian strips were planted by digging a trench approximately 30 cm deep and pegging the hessian into the substrate at each end and in the middle. The trench was then filled in, burying the bound edges but leaving approximately 30 cm of the hessian exposed. Strips were laid in two different orientations (east-west and north-south) to assess the potential for long-shore currents to enhance recruitment. A three metre gap separated each strip. The seagrass mats were anchored to the sediment with four steel tent pegs, one in each corner. Bags filled with sand were sufficiently heavy so as not to need staking down. Following deployment, all bags, strips and mats were labeled. At each site between eight and ten replicates of each method were deployed.

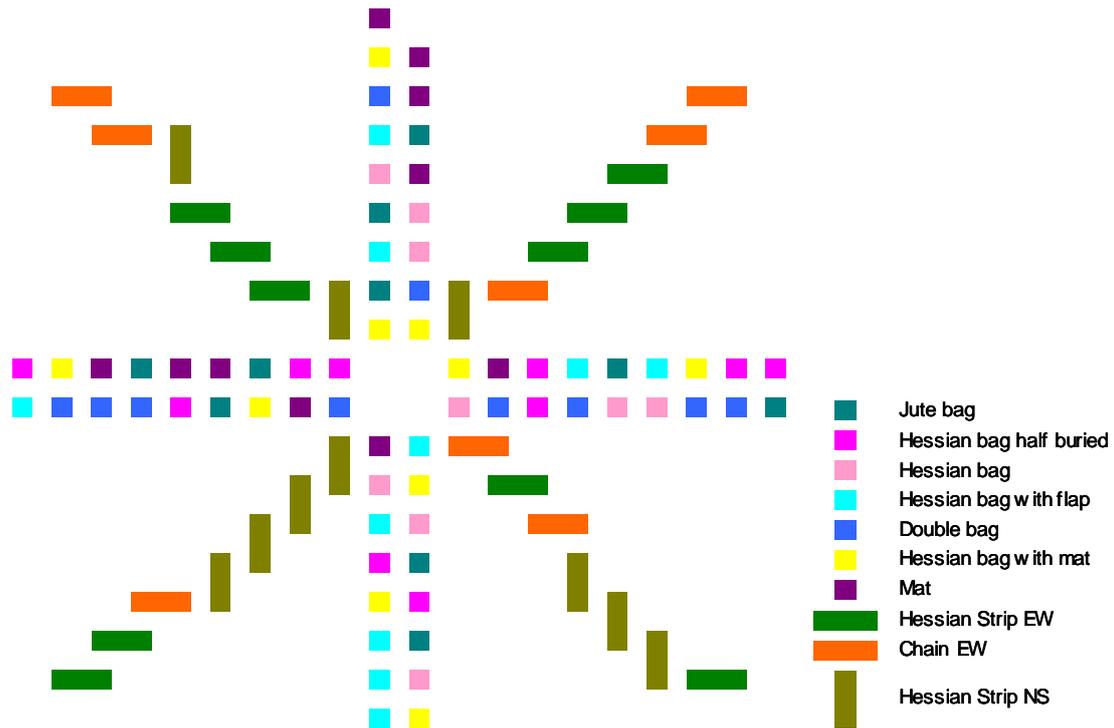


Figure 7. Schematic diagram of the random layout of the hessian bags and strips at Site 1. A similar design was employed at Site 2.

To determine the effectiveness of the different recruitment facilitation methods, the number of seedlings on each unit was monitored over time. Monitoring was undertaken shortly after deployment on 12 October 2004 and subsequently in December 2004 and February, April, June and September 2005. To compare the effectiveness of the different methods in capturing and maintaining seagrass seedlings, seedling numbers were converted to seedlings m^{-2} for analysis.

To determine if seedling numbers (seedlings m^{-2}) varied over time with either method or site, a Multivariate Repeated Measures Analysis of Variance test was undertaken in SPSS (Version 13.0, SPSS Inc., Illinois), with time (6 levels) as the within-subject factor and method (10 levels) and site (2 levels) as fixed between-subject factors. Data were not normal (as determined by QQ plots), and required transformation (fourth root). Variances were heterogeneous (Levene's test, $p < 0.05$) and as a consequence the Pillai's Trace multivariate test statistic was used in the analysis, as this statistic is reported to be more robust than other statistics to violations of model assumptions (Olson, 1974). A significance level of $\alpha = 0.05$ was used.

To more closely investigate differences in seedling densities between methods and sites, at both the start and end of the experimental period, a two-way Analysis of Variance (ANOVA) was performed in SPSS on fourth root transformed data for each census. In cases where a

significant interaction of site and method was found, one-way ANOVAs were performed with transformed data for each site separately. Pairwise *a-posteriori* comparisons between methods were performed using LSD tests ($p < 0.05$).

2.4. Cost of Recruitment Facilitation Methods

The cost involved in making and deploying the various recruitment facilitation units was calculated by combining the costs of materials, construction time and deployment for each method and dividing by the total by the number of units deployed. This provides a value for cost per deployed unit. For analysis, this cost per unit was used to calculate number of seedlings recruited for every \$100 spent by dividing the number of seedlings on the unit by the average cost per unit and multiplying by 100. While it would have been preferable to analyse cost per seedling data, this was not possible as some replicates (units) retained no seedlings which would return an infinite value for cost per seedling, making analysis impossible. Subsequent to analysis, means were transformed to provide an average cost per seedling for use in discussion.

To determine if the number of seedlings per \$100 varied with either method or site, separate two-way ANOVAs were performed at the beginning (5 weeks) and end (53 weeks) of the experimental period. A comparison of these analyses gives some indication of how sensitive the results are to what is essentially an arbitrary decision on how long the experiment runs for. Method and site were used as fixed factors. Data were not normal (as determined by QQ plots), and required transformation ($\ln(x + 1)$). Whilst the assumption of homogeneity was not met (Levene's test, $p < 0.05$), ANOVA is reported to be robust to deviations from this assumption (Underwood, 1997). A significance level of $\alpha = 0.05$ was used. In cases where a significant interaction of site and method was found, a one-way ANOVA was performed with transformed data for each site separately. Pairwise *a-posteriori* comparisons between methods were performed using LSD tests ($p < 0.05$).

2.5. *Amphibolis* Seedling Growth

To determine the growth of *Amphibolis* seedlings over time, 15 seedlings were randomly collected from the hessian units at each site in December 2004 and February and October 2005, to represent seedlings that were approximately 14, 23 and 57 weeks old. Seedlings that had been recently released from the parent plant were also collected in September 2005, and these were assumed to represent age 0. Divers collected the seedlings in plastic bags, which were then transported to the laboratory where morphological measurements were undertaken. Morphological measurements included seedling height, the number of shoots,

the number of short shoots², and the number and length of all roots. Above- and below-ground dry weight were also recorded for each seedling following overnight desiccation at 65°C.

To determine spatial and temporal variation in *A. antarctica* seedling morphological characteristics, permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001a; b) was performed, with time being treated as random and site fixed. The data were range-standardized, as the different variables were measured with different units and scales, and the analysis was based on Euclidian distances. Probability values were calculated using 4999 permutations of the raw data under a reduced model (Anderson, 2001a; b). Pairwise *a-posteriori* comparisons were performed for factors found to be significant ($\alpha = 0.05$) in the PERMANOVA.

² A 'short shoot' is the term given to a cluster of approximately 8-10 leaves at the end of a branch. The term has previously been used by Marbà and Walker (1999).

3. RESULTS

3.1. Recruitment Facilitation Methodologies

3.1.1. Seedling densities at 12 months

Hessian substrates appear to be extremely effective in facilitating the natural recruitment and survival of *Amphibolis* seedlings (Figure 8). At the final census (53 weeks after deployment) 53.45 ± 3.92 (mean \pm se) seedlings m^{-2} remained on all bags, strips and mats at both sites. Site one was more effective in recruiting *Amphibolis* seedlings, with an average 238% greater seedling density than site two (221.6 ± 18.49 and 92.9 ± 8.10 seedlings m^{-2} , respectively). The relative effectiveness of the various recruitment facilitation methods varied between sites, as indicated by a site by method interaction (Two-way ANOVA: $F_{9,175} = 2.215$, $p = 0.023$). The double-layered hessian bags supported the highest density of seedlings at both sites (site 1, 141.8 ± 32.84 seedlings m^{-2} ; site 2, 95.6 ± 14.48 seedlings m^{-2}), although significant differences between sites were apparent (Figure 9c and d). At site one, although mean seedling density was greatest on the double-layered hessian bags, it did not differ significantly from that on many of the other substrates, including the east-west oriented strips, weighted strips, hessian bags, large hessian bags, half buried hessian bags, hessian bags with mats and bags with a flap ($p > 0.05$) (Figure 9c). Interestingly, at this site the north-south oriented strips retained far fewer seedlings than did the east-west oriented strips ($p < 0.001$). At site two, however, the double-layered hessian bags retained significantly more seedlings than any other method ($p < 0.05$) (Figure 9d). At the end of the experimental period, the seagrass mats differed significantly to all other methods ($p < 0.001$) and remained the most ineffective method in terms of seedling recruitment and survival, with an average of 15.2 ± 8.48 seedlings m^{-2} at site one and 6.7 ± 4.44 seedlings m^{-2} at site two (Figure 9).

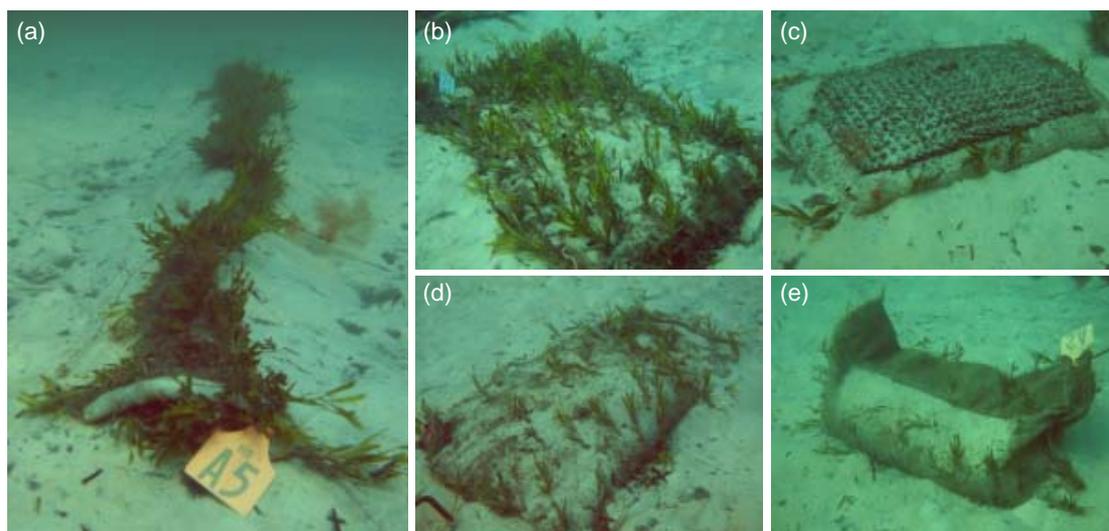


Figure 8. Photographs of a hessian strip (a), double-layered hessian bag (b), hessian bag with a mat attached (c), large hessian bag (d), and hessian bag with a flap (e) on 12/10/04, approximately five weeks after deployment.

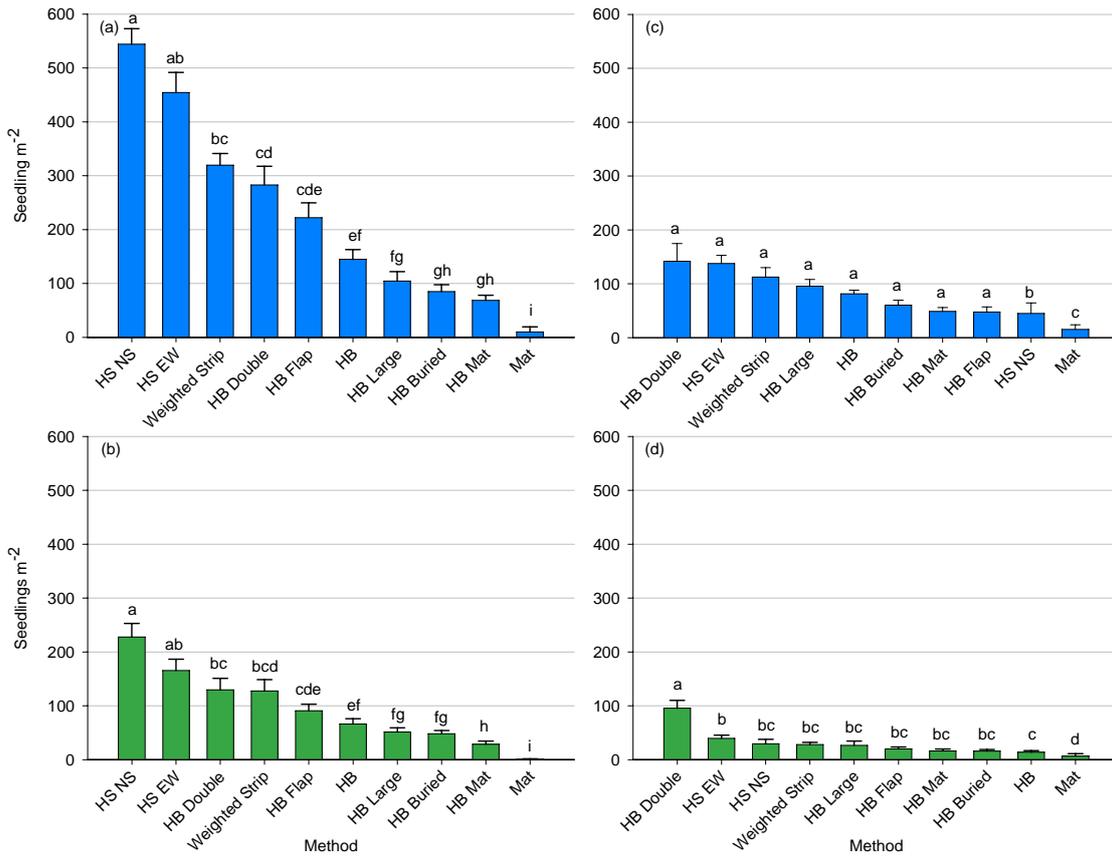


Figure 9. Seedling density (\pm se) at site one (a and c) and site two (b and d) during the first (a and b) and final census (c and d). First census 12/10/04 and final census 13/9/05. Letters indicate the results of post hoc tests, with different letters indicating a significant difference between methods.

3.1.2. Trend over time

The relative success of the different methods was not consistent across site or time, as indicated by a time by method by site interaction (Repeated measures ANOVA; $F_{45,875} = 1.613$, $p < 0.001$; Figure 9). At the first census, approximately five weeks after deployment, substantially more seedlings had recruited onto the recruitment facilitation units at site one than at site two (221.6 ± 18.49 and 92.9 ± 8.10 seedlings m^{-2} , respectively; Figure 10). Whilst at this time, the relative effectiveness of the various recruitment facilitation units varied between sites (Two-way ANOVA: $F_{9,176} = 3.452$, $p = 0.001$), pairwise *a-posteriori* comparisons between methods at each site, revealed that the most successful methods in terms initial recruitment at both sites were the hessian strips (Figure 9), recruiting an average of 104.0 and 73.4 seedlings m^{-2} at site one and two respectively. While the hessian strips were significantly more effective at recruiting seedlings than all other methods ($p < 0.05$), the weighted strips, double-layered hessian bags and the hessian bags with a flap also recruited large numbers of seedlings at both sites. The seagrass mat was the most ineffective method of recruiting *Amphibolis* seedlings, averaging just 5.5 ± 4.74 seedlings m^{-2} at both sites. Notwithstanding this, it is worth noting no attached seedlings were observed on adjacent sandy substrates.

Seedling densities declined over time (Figure 10). Between five and twelve weeks after deployment, seedling density increased slightly, before 64% of seedlings were lost during the following 10 weeks. Following this, seedling density varied little, and after 53 weeks, approximately 77.8 ± 6.28 and 29.1 ± 3.20 seedlings m^{-2} remained on all bags, strips and mats at sites one and two respectively (Figure 10). While 31.4% of all seedlings survived the experimental period, seedling survival over time varied between methods and sites, and methods that were particularly effective at recruiting seedlings initially, were not necessarily the most effective after a period of 53 weeks (Repeated Measures ANOVA, time x site x method interaction; $F_{45,875} = 1.613$, $p = 0.007$). For example, while the hessian strips were the most effective method at recruiting seedlings, their survival was relatively poor, with only 10.6% and 27.1% of seedlings on north-south and east-west oriented strips surviving (Figure 12). This compares to the double-layered hessian bags, which experienced an average of 62.0% seedling survival (Figure 12).

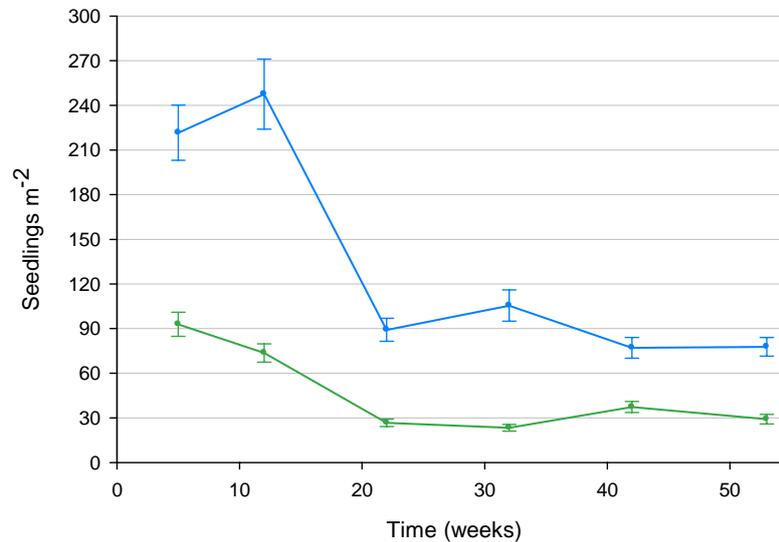


Figure 10. Seedling density (\pm se) for all recruitment facilitation methods at site one (blue) and site two (green), over the 53-week experiment.

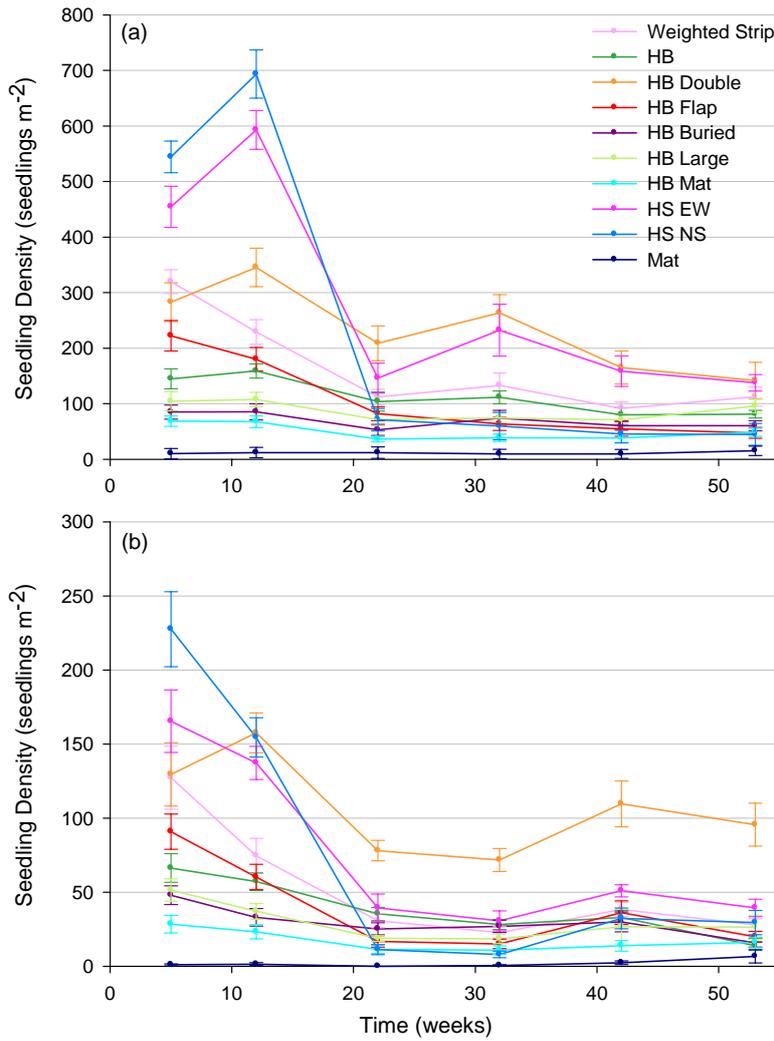


Figure 11. Seedling density for each method at site one (a), and site two (b) for each recruitment facilitation method, over the 53-week experiment. Note the scale differences between sites.

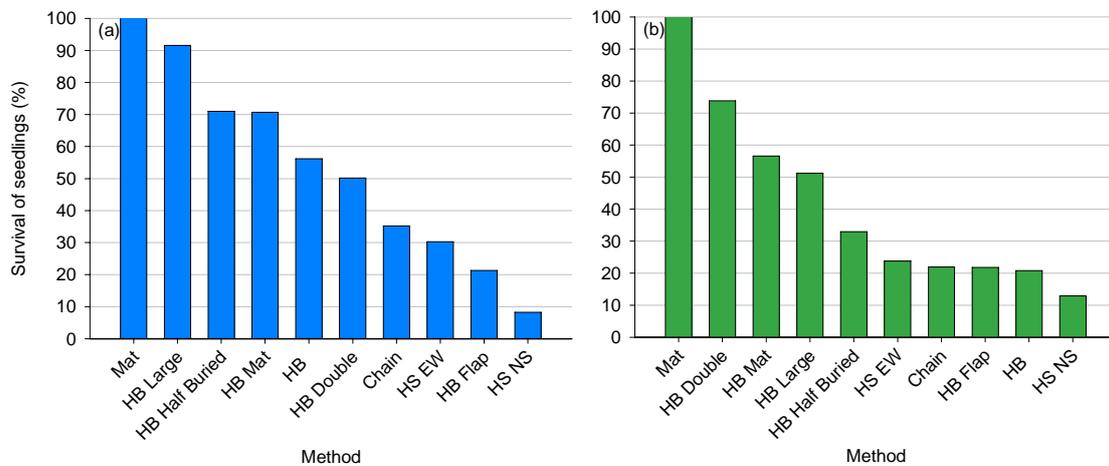


Figure 12. Seedling survival after 53 weeks at site one (blue) and site two (green).

3.2. Cost of Recruitment Facilitation Methods

3.2.1. Cost per unit

The costs involved with constructing and deploying the various recruitment facilitation units varied substantially, and were strongly influenced by construction costs for those methods that involved alterations prior to deployment, and deployment costs for those methods that required considerable dive time for deployment (Table 3). The cheapest method to construct and deploy was the hessian bag costing a total of \$8.69 each (Table 3). The large hessian bag, hessian bag with a flap, and the double-layered hessian bag were also relatively cheap, each costing less than \$20 to construct and deploy. Quoted costs for all these methods are based on deploying bags from a boat without any aid from divers. Diver-deployed methods (hessian strips, half buried hessian bag and seagrass mats) were considerably more expensive, with hessian strips being the most expensive (\$172.13 each) (Table 3).

Table 3. The cost of materials and cost associated with constructing and deploying each recruitment facilitation unit.

	Materials	Construction	Deployment	Total
BAGS				
Hessian Bag	\$1.74	\$1.95	\$5.00	\$8.69
Large Bag	\$4.71	\$3.90	\$5.00	\$13.61
Hessian Bag with Flap	\$1.74	\$7.81	\$5.00	\$14.56
Double Bag	\$1.97	\$11.70	\$5.00	\$18.67
SG Mat on Bag	\$3.24	\$14.95	\$5.00	\$23.19
Hessian Bag Half Buried	\$1.74	\$1.95	\$38.94	\$42.64
STRIPS				
Weighted Strip	\$1.89	\$19.50	\$5.00	\$26.39
Hessian Strip	\$11.20	\$19.50	\$141.43	\$172.13
MATS				
SG Mat	\$14.90	\$0.00	\$33.94	\$48.84

NOTE: Costs do not include research-based costs such as those associated with redistributing the units into the predetermined randomised manner, or tagging and monitoring each unit.

3.2.2. Cost-effectiveness

Cost-effectiveness reflects both the number of seedlings recruited and the cost associated with that recruitment. After 12 months there is some difference between sites in the relative cost-effectiveness of different methods, as indicated by a site by method interaction, (Two-way ANOVA: $F_{9,176} = 2.928$, $p = 0.003$). However some clear trends are evident when the sites are examined separately (as required because of the interaction). There are four methods that are more cost-effective than the others (Figure 13). These are the large hessian bag, double-layered hessian bag, weighted strip and hessian bag. At site one, the cost of each seedling on these methods varied between \$0.18 (large hessian bag) and \$0.39 (weighted strip), while at site two costs varied from \$0.56 (double-layered bag) to \$1.81 (hessian bag) per seedling (Table 4). The seagrass mat was the least cost-effective method

after 53 weeks, with seedlings costing on average \$15.26 at site one and \$34.89 at site two. Remaining methods were of intermediate cost-effectiveness at both sites.

When the situation was analysed after only five weeks the same general trend was apparent. Far greater cost-effectiveness was apparent at site one than site two (two-way ANOVA: $F_{1,176} = 82.309$ $p < 0.001$), with the same four methods (large hessian bag, double-layered hessian bag, weighted strip and hessian bag) being the most cost-effective at both sites (Figure 13). The lack of an interaction term (two-way ANOVA: $F_{9,176} = 1.093$, $p = 0.371$) indicates that the relative cost-effectiveness of different methods is similar between sites.

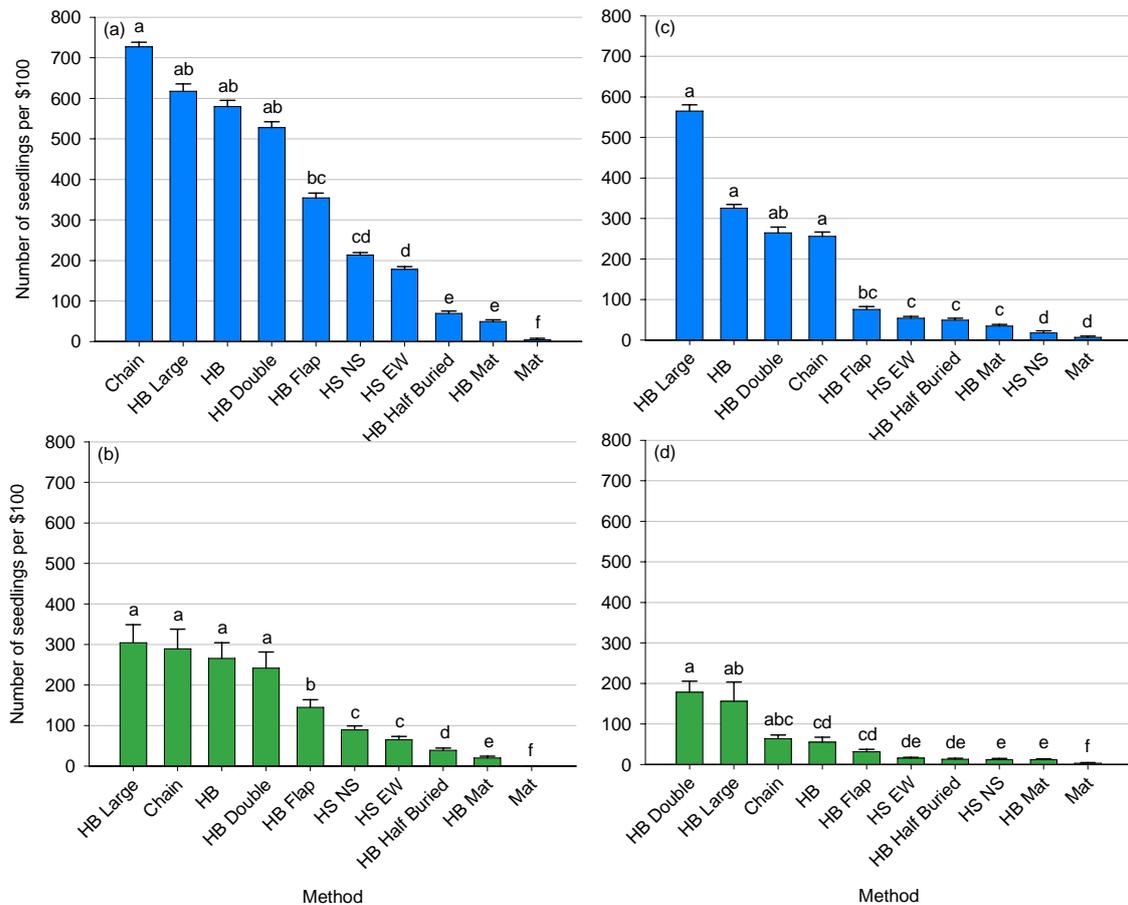


Figure 13. Average number of seedlings for every \$100 spent for various recruitment facilitation methods at site one (a and c) and site two (b and d) during the first (a and b) and final census (c and d). Letters indicate the results of post hoc tests, with different letters indicating a significant difference between methods.

Table 4. The cost effectiveness (as expressed as cost per seedling) of various recruitment facilitation methods at two sites at the first and final census.

Site	Method	Cost per seedling			
		Time 1	Time 6		
1	Hessian Bags	Hessian Bag	\$0.17	\$0.38	
		Large Bag	\$0.16	\$0.33	
		Hessian Bag with Flap	\$0.28	\$0.69	
		Double Bag	\$0.19	\$0.41	
		SG Mat on Bag	\$2.04	\$4.93	
		Hessian Bag Half Buried	\$1.44	\$2.55	
	Hessian Strips	Weighted Strip	\$0.14	\$0.35	
		Hessian Strip NS	\$0.47	\$1.12	
		Hessian Strip EW	\$0.56	\$1.54	
	Seagrass Mat	Mat	\$23.26	\$244.21	
	2	Hessian Bags	Hessian Bag	\$0.31	\$1.81
			Large Bag	\$0.18	\$0.64
Hessian Bag with Flap			\$1.32	\$3.17	
Double Bag			\$0.38	\$0.56	
SG Mat on Bag			\$2.89	\$8.72	
Hessian Bag Half Buried			\$2.03	\$7.75	
Hessian Strips		Weighted Strip	\$0.39	\$1.58	
		Hessian Strip NS	\$5.70	\$8.65	
		Hessian Strip EW	\$1.85	\$6.47	
Seagrass Mat		Mat	\$15.26	\$34.89	

3.3. *Amphibolis* Seedling Growth

The size of *Amphibolis* seedlings varied significantly as a function of a time by site interaction (PERMANOVA: $F_{3,112} = 4.3818$, $p < 0.001$) demonstrating that the effect of site on growth was not consistent across time. Notwithstanding this, some general patterns in seedling development were apparent (Figure 14). Above-ground biomass of *Amphibolis* seedlings increased substantially over time, owing to increased seedling height and an increase in the number of shoots and short shoots. Seedling height almost doubled over the 57 week period, increasing from approximately 104.4 mm to 198.5 mm, while the number of short shoots increased from 0 to 4.2. Secondary shoots were initially observed on seedlings approximately 23 weeks old and at 57 weeks, seedlings generally consisted of either 2 or 3 shoots. Below-ground biomass of *Amphibolis* seedlings increased almost seven fold over the 57 week period as a result of significant root development. Seedlings recently released from the parent plant generally only had 1 root, approximately 2.2 mm long, however, after 57 weeks seedlings had an average of 10.2 roots with a combined length of 639 mm. At this time, maximum root length had reached 213 mm and averaged 123.6 mm.

Pairwise *a-posteriori* comparisons indicated that seedling morphological characteristics were significantly different between sites at each census ($p < 0.05$), and while differences between parameters were apparent, in many cases seedling development was greater at site one.

While there were few differences among age 0 seedlings, those at site two had slightly greater above-ground biomass and number of roots (Figure 14). At 14 weeks, seedlings at site one had greatest development except that the number of shoots, total root length and maximum root length were similar between sites. This pattern persisted at 23 weeks of age, although total root length at site one was higher than at site two, and number of short shoots had converged at the two sites. At 57 weeks old, many of the measured parameters had converged, although site one still had greater above-ground biomass, seedling height and total root length.

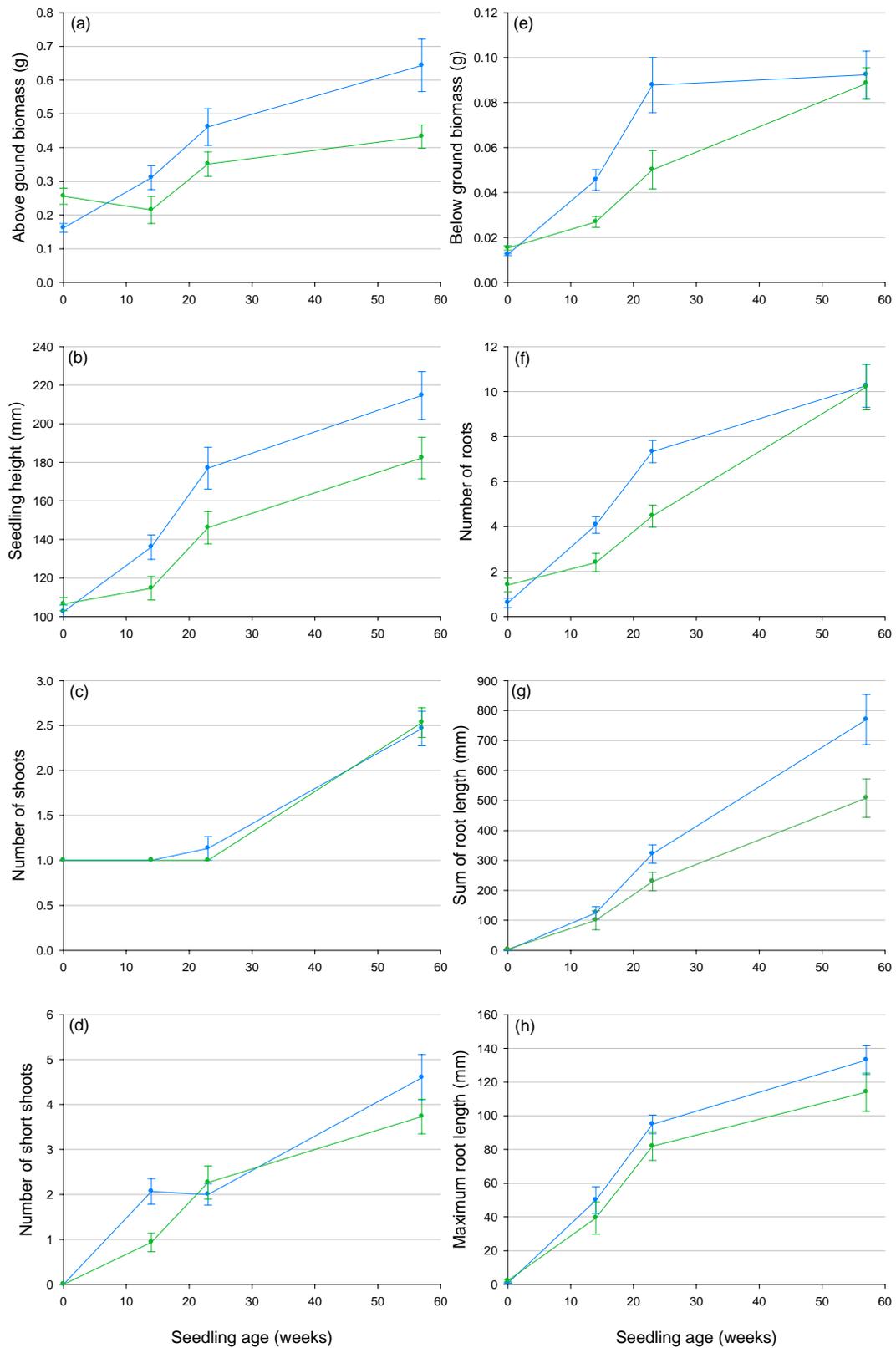


Figure 14. Morphological characteristics, including above-ground biomass (a), seedling height (b), number of shoots (c), number of short shoots (d), below-ground biomass (e), number of roots (f), total sum of root lengths (g) and maximum root length (h) of *Amphibolis antarctica* seedlings at different ages (mean \pm standard error, n = 15) at site 1 (blue) and site 2 (green).



Figure 15. Photographs of seedlings newly released from the parent plant approximately 104.4 ± 13.22 mm in height and a seedling taken from the bags at 23 weeks, measuring approximately 161.5 ± 40.18 mm.

4. DISCUSSION

Over the past few decades, researchers have been working towards developing cost effective methods of seagrass restoration suitable for implementation over large spatial scales. In Australia, the majority of early attempts to find such a method resulted in limited success, and sometimes resulted in a net loss of seagrass habitat (e.g. West et al., 1990; Kirkman, 1998; Paling et al., 2000; van Keulen et al., 2003). More recently, with increasing developments in restoration technology including the use of mechanical transplanters (e.g. ECOSUB 1 and ECOSUB 2), increased transplant survival and cover have been achieved (Paling et al., 2001a; 2001b). However, newly developed methods are still generally only feasible over relatively small spatial scales, and the transplantation of seagrasses raises concerns about donor bed recovery. We have shown here that the provision of suitable substrates can facilitate the natural recruitment of *Amphibolis antarctica* and *A. griffithii* seedlings, representing a new method of seagrass restoration that may be suitable for deployment over large spatial scales in moderate wave energy environments in temperate Australian waters.

4.1. Recruitment, Survival and Growth of *Amphibolis* Seedlings on Recruitment Facilitation Units

Following the deployment of 196 recruitment facilitation units, over 16,500 *Amphibolis* seedlings recruited at the two study sites. Seedling recruitment and retention over the 53-week experimental period varied significantly with method and site, and at the end of the experimental period ranged from 6.7 ± 4.44 to 141.8 ± 32.84 seedlings m^{-2} . Differences in the ability of different methods to recruit and retain *Amphibolis* seedlings are likely to reflect a number of factors including material type (weave of fabric), profile on the seabed and ability to withstand degradation. Seedling recruitment was generally highest on the coarse weave hessian fabrics (including the hessian strips and double-layered hessian bags), probably because the grappling hooks at the base of the seedlings become easily entangled in the hair-like nature of the fabrics' fibres. The coarse weave hessian used is in many ways similar to the below-ground fibrous root mat of *Posidonia* beds, and while no information is available on the preferred sites for natural recruitment of *Amphibolis* seedlings, large numbers can be found along the scarp edges of *Posidonia* beds following recruitment events (Kirkman, 1998). Fewer seedlings recruited onto the finer weave hessian fabrics, with recruitment particularly poor on the seagrass mats and the mats attached to hessian bags. Poor recruitment onto the seagrass mats may be explained by the fact that the interwoven seagrass making up the mats was coarse and inflexible, making it difficult for the seedlings to become entangled. Furthermore, the mats were situated close to the substrate and may have been more rapidly buried by sediments than other methods, reducing the time available for seedlings to attach. The half buried hessian bags are also likely to have been more rapidly buried, and recruited substantially fewer seedlings than their non-buried counterparts. The

observation of varying levels of *Amphibolis* seedling recruitment with different materials is consistent with results of other studies that have documented the preference of certain substrates over others for seedling recruitment. For example, Blanchette et al. (1999) identified that *Phyllospadix torreyi* seedling recruitment varied according to algal substrate.

Seedling densities under natural conditions vary considerably with species, and are likely to be related to their different life histories. The seedlings of larger and longer-lived seagrass species are generally less abundant than those of shorter-lived opportunistic species, which often rely on seed banks and seedling recruitment for seasonal regrowth and recovery after disturbances (Kenworthy, 2000). Notwithstanding this, through the facilitation of natural recruitment of *Amphibolis* seedlings, average seedling densities in this study were in many cases above those recorded for other more opportunistic seagrasses. For primary colonising species such as *Halophila decipiens*, *Zostera marina* and *Syringodium filiforme*, seedling densities have been reported to average between 0 and 28 m⁻² (Kenworthy, 2000; Peterson et al., 2002; Greve et al., 2005; Hammerstrom et al., 2006), although in some cases higher densities have been recorded (> 800 *Z. marina* seedlings m⁻²; Robertson and Mann, 1984). The larger, climactic species, tend to have much lower seedling densities. For example, in two separate studies average natural seedling densities in *Posidonia oceanica* have varied between 2.6 to 3.0 m⁻² (Piazzi et al., 1999) and 3.0 to 3.2 m⁻² (Balestri et al., 1998). Unfortunately little information is available on natural recruitment densities of *Amphibolis* seedlings. In the only documented study, Kirkman (1998) observed that large numbers of *Amphibolis* seedlings naturally recruit onto *Posidonia* bed blowouts, with approximately 700 seedlings collected by divers along a 100 m scarp edge, equating to densities of approximately 7 per m⁻¹. While detailed information on natural recruitment levels of *Amphibolis* seedlings is not available, a comparison of recruitment densities in other species of seagrass to the results of this study demonstrates that densities on the various recruitment facilitation units (average of 157.2 seedlings m⁻² for all methods, at both sites) in many cases exceed natural levels of seedling recruitment, and demonstrates the success of these methods. In addition, seedling densities on some of the methods developed in this study exceed those documented previously by Kirkman (1998), suggesting that some of the methods developed in this study may in fact be more effective than natural substrates in their ability to recruit seedlings of these species. Notwithstanding this, studies investigating the natural levels of recruitment in this system are needed before this statement can be verified.

The recovery of seagrass beds through the recruitment of seedlings is highly dependent upon survival and growth of the seedlings over time, and while recovery as a result of seedling recruitment has been observed (Preen et al., 1995; Kendrick et al., 1999; Whitfield et al., 2004; Olesen et al., 2004), in many cases, seedling survival is poor. The survival of seedlings in many studies has been documented over one to two year time scales, and these have shown that while large numbers of seeds and seedlings are produced, few seedlings recruit,

and even fewer survive over time (Kaldy and Dunton, 1999; Olesen et al., 2004; Whitfield et al., 2004). In a recent study by Olesen et al. (2004), survival of *Enhalus acoroides* and *Thalassia hemprichii* seedlings was 12.5% and 5.3% after one year and 7.5% and 0% after an additional year. Similarly, *T. testudinum* seedling survival at a number of sites after one year averaged 42%, and decreased to 20% a year later (Whitfield et al., 2004), while in another study, 22% survival of *T. testudinum* seedlings after 6 months was observed but decreased to 11% after an additional 6 months (Kaldy and Dunton, 1999). In some situations, higher seedling survival has been documented. For example, Piazzini et al. (1999) documented 53% survival of *P. oceanica* seedlings after two years along the Tuscan coast of Italy, and Balestri et al. (1998) documented that seedling survival in the same species can be as high as 66% after three years in some locations.

In many cases the restoration of seagrasses through the utilisation of seedlings has failed as a result of a high mortality (e.g. Kirkman, 1998; Bull et al., 2004). In one such case, after the planting out of *Phyllospadix torreyi* seedlings at two sites, survival of seedlings was just $2.3 \pm 1.3\%$ and $0.9 \pm 0.6\%$ after six months (Bull et al., 2004). Similarly, following the planting out of hundreds of *Posidonia* and *Amphibolis* seedlings in Western Australia, only a few seedlings survived for one year (Kirkman, 1998). Extremely poor survival of planted out one-year old *Posidonia* seedlings was also observed in a recent trial along the Adelaide metropolitan coast (unpublished data, SARDI Aquatic Sciences). The protection of *Z. marina* seeds, using burlap bags, has been found to have a positive effect on the survival of seedlings (Harwell and Orth, 1999), and in a recent experiment conducted in Adelaide, the concept of seedling protection was tested using *Posidonia* seedlings. In this experiment, *Posidonia* seedlings were planted into sand filled, hessian bags and while seedling survival was initially very poor, those that did survive the initial plant out phase remain and are showing significant signs of growth (unpublished data, SARDI Aquatic Sciences). The use of seed protection methods such as those investigated by Harwell and Orth (1999), and by SARDI Aquatic Sciences are offering promising results and may be worthy of further consideration for future restoration programs.

In the current study, 31.4% of initial seedlings survived the 53-week experimental period. Seedling survival varied significantly with method, and the most successful method in terms of initial seedling recruitment was not necessarily the most successful over the longer term. While seedling recruitment was highest on hessian strips, this method had the poorest survival, with 10.6% and 27.1% on north-south and east-west oriented strips, respectively. Conversely, more than 50% of seedlings survived on the half buried hessian bags, double-layered hessian bags, hessian bag with the mat and the large hessian bags. One of the main factors thought to affect seedling survival in the current study is the ability of the various units to withstand degradation. Seedling retention was poorest on the hessian strips, owing to the fact that many of these weakened at the sediment level and were lost along with attached

seedlings. Toward the end of the experiment, evidence of the hessian substrates breaking down was also observed on the hessian bags with flaps and the large hessian bags, although this did not seem to have a large impact on seedling survival.

Many other factors have been implicated in the loss of seedlings. For example, Whitfield et al. (2002) attributed the loss of *Thalassia testudinum* seedlings to the occurrence of a severe storm event. In another study, Whitfield et al. (2004) found high rates of recruitment in *T. testudinum*, but significant mortality, with only 20% of seedlings remaining after a period of two years. In this study the authors suggested that a reduction in temperatures over winter significantly affected seedling survival as winter temperatures fell below *T. testudinum*'s thermal limit. Herbivory was also recognised as a factor affecting seedling survival, with all seedlings collected showing evidence of leaf grazing (Whitfield et al., 2004). Herbivory or disturbance by crabs or fish was also thought to have contributed to the loss of *T. testudinum* seedlings in a study by Kaldy and Dunton (1999). Duarte and Sand-Jensen (1996) showed that nutrient deficiency is a source of mortality in seedlings in nutrient poor environments. Nutrient limitation, together with temperature and herbivory are unlikely to have contributed to the loss of seedlings over time in the current study. Both of the study sites are located in close proximity to sources of nutrients, experience temperatures well within the range experienced by *Amphibolis* beds and tolerated by *Amphibolis* seedlings (Walker and Cambridge, 1995), and no evidence of herbivory in terms of leaf scars was observed. Sediment instability (which is both a cause and a consequence of seagrass loss; Fotheringham, 2002) and disturbance from water movements and current, which have previously been implicated in seagrass transplant and seedling mortality in moderate to high energy environments (e.g. Paling et al., 2000; Paling et al., 2002), may have affected seedling survival in the current study. As most plants experience decreased mortality rates as size increases (Harper, 1977), it is expected that mortality rates in this experiment will decrease as the seedlings grow.

While the survival of transplanted seagrass units and/or seedlings is often used to determine the success of restoration efforts, it is also vital to monitor their growth. The growth of naturally recruited *Amphibolis* seedlings has not been studied although adult *A. antarctica* and *A. griffithii* are both relatively slow-growing (Marbà and Duarte, 1998; Marbà and Walker, 1999). However, all seedlings that survived the 53-week experimental period showed significant signs of growth in all morphological characteristics measured, with an average 61% increase in above ground-biomass and a 84% increase in below-ground biomass.

Spatial variation in natural recruitment and survival of seagrass seedlings has commonly been documented in the literature (e.g. Inglis, 2000a) and the results of this study are consistent with this research. Substantial differences in the level of *Amphibolis* seedling recruitment were apparent between sites, with site one recruiting approximately 241% more

seedlings than site two. Differences in recruitment levels between sites may be explained by a number of factors including variation in the reproductive output of, proximity to, and density of, the surrounding seagrass beds. While there is a paucity of information on the reproductive output of *Amphibolis*, in other seagrasses, there are large fluctuations in reproductive output both spatially and temporally (e.g. Durako and Moffler, 1985; Inglis, 2000a; Rollon et al., 2001; Campey et al., 2002). Thus, while the sites in this study were located just 4 km apart, it is possible that the surrounding *Amphibolis* beds produced varying levels of seedlings. Further research, however, is needed in order to determine if there are differences in the reproductive output of *Amphibolis* beds along the Adelaide metropolitan coastline and if so, whether or not these differences may influence recruitment success at different sites. Examination of the habitats immediately surrounding each site revealed that site one consisted almost entirely of monospecific *Amphibolis* beds, whereas site two consisted of a mixed *Amphibolis* and *Posidonia* bed. While it is possible that such a difference would have resulted in variation in seedling recruitment between the sites, this assumes that nearby seagrass beds are the main source of seedlings, and that *Amphibolis* seedlings do not travel large distances away from the parent plant. While the distance travelled by fruits, seeds and seedlings has been estimated for other seagrass species, this information is not available for *Amphibolis* species, however, the morphological characteristics of *Amphibolis* seedlings and the location on the parent plant in which they grow, suggests that they are unlikely to travel great distances. For example, the grappling hooks located at the base of *Amphibolis* seedlings facilitate entanglement and are likely to limit seedling dispersal, rather than enhance it. Furthermore, the seedlings are neutrally buoyant, develop within the meadow (Verduin et al., 2002) and lack reserves of protein or starch, suggesting that seed dispersal may be more limited than other seagrass species with buoyant dispersal stages that can travel large distances (e.g. *Posidonia*), and/or have protein and starch reserves to permit an extended dispersal phase (Inglis, 2000b). However, dispersal is likely to be further than for species that release seeds very close to, or below, the sediment surface and are dispersed within centimetres of the parent plant (e.g. *Halophila*, *Cymodocea* and *Halodule*) (Orth et al., 2006).

Differences in recruitment levels between the two sites may also reflect differences in site characteristics such as depth, hydrodynamic conditions and sediment dynamics. Site one was located in about 8 m of water, approximately 1.5 m deeper than site two. The Adelaide metropolitan coast is considered to have moderate wave energies (Townsend, 2002) and a difference in depth of 1.5 m between sites is likely to have a significant effect on the influence of currents and wave attenuation, both of which are likely to be considerably higher at site two. Many studies have shown that seedling recruitment is highly dependent upon stable and calm environments, and survival of seagrass transplants and seedlings has been reported to be higher in low energy environments (see Orth et al., 2006).

4.2. Cost Effectiveness

The integration of economic aspects into restoration programs is often excluded (Edwards, 1998), making it difficult to assess the cost effectiveness of restoration and translate experimental research findings to large-scale management interventions. Fonseca et al. (1998) suggested that the average of cost of restoration efforts is approximately US\$37,000 per hectare (Fonseca et al., 1998), and following a review of restoration costs in the United States, Spurgeon, (1998) identified that the cost of restoration varies substantially with method and species between approximately US\$9,000 and US\$680,000 per hectare. In Australia, the cost of restoration has rarely been documented, however in a recent publication, Paling et al. (2001a) outlined that the capital cost of mechanical seagrass transplanters (excluding operating costs) was \$100,000 and the approximate cost per sod is \$200. The cost per seagrass unit in a study by Fonseca et al. (1994) was substantially lower than that documented by Paling et al. (2001a), and varied between US\$1.21 to US\$3.53 (Fonseca et al., 1994).

In the current study the cost to recruit an individual seedling at the end of the experimental period varied from \$0.33 to \$244.21, depending on method and site, with the two most cost effective methods costing an average of \$0.49 per seedling (double-layered and large hessian bags, sites combined). Differences in the number of seedlings that recruited between site one and two, led to differences in the cost effectiveness between sites. In all but one method, at the final census, site one contained higher seedling densities than site two and as a consequence was more cost effective, demonstrating the importance of site selection for recruitment facilitation as a means of restoration. The cost effectiveness of various methods was largely influenced by deployment method and subsequent costs. Methods that were remotely deployed were in most cases much more cost effective than those that required divers for deployment. The exception to this was the hessian bag with the mat, which had poor seedling recruitment. The strong influence that deployment costs had on the cost effectiveness of each method, demonstrates the benefits of remotely deployed methods and the removal of divers in transplantation efforts.

The estimated cost involved with the large-scale deployment of these methods will ultimately vary with unit density. However, if 1,000 units are deployed over a one hectare area in one day, and assuming that the recruitment facilitation methods developed in this study will over time lead to the complete recovery of a given area, the restoration of *Amphibolis* beds off the Adelaide metropolitan coast is likely to cost as little as \$8,960 per hectare depending on method. While the choice of method for any large-scale restoration trial is likely to reflect unit density and cost, seedling recruitment and retention should also be considered, as those methods that are cheapest may not necessarily yield a better outcome. To deploy 1,000 units ha⁻¹ of the two most cost effective methods in terms of cost per seedling, would cost approximately \$18,670 ha for the double-layered hessian bags and \$13,610 ha for the large

hessian bags. Further information likely to assist with choice of method over large scales includes knowledge of the longer-term survival of the seedlings on each method and whether or not initial recruitment success influences the rate of recovery. Regardless of which bag method is used, the estimated costs for deployment over a large scale are significantly lower than for other restoration methods costed in the literature, and compare favourably with estimates of the value of seagrasses in the range of AUS\$12,635 to AUS\$25,270 ha⁻¹yr⁻¹ depending on meadow density (Lothian, 1999).

4.3. Benefits of Recruitment Facilitation as a Means of Seagrass Restoration

Increasing documentation of worldwide seagrass losses, and recognition of the importance of seagrasses to coastal protection, biodiversity and productivity has led to an increase in research into the development of seagrass restoration techniques. Restoration efforts have focused primarily on the transplantation of seagrass ramets and more recently, but to a lesser extent, the planting out of seagrass seeds and seedlings. This study is the first study that has demonstrated that the facilitation of natural recruitment events via the provision of suitable substrates may represent an alternative method of seagrass restoration. Furthermore, the techniques developed, which encourage the natural recruitment of *Amphibolis* seedlings, offer significant advantages to those previously used and tested.

Unlike the transplantation of seagrass from one area to restore another, facilitating seedling recruitment avoids causing damage to donor beds, and is likely to have no significant impact on the long-term survival of existing seagrass beds. Furthermore, removing the need for SCUBA divers makes recruitment facilitation techniques that can be remotely deployed substantially more cost effective than other techniques, and offers significant savings in terms of time and labour. The majority of seagrass losses worldwide have occurred over hundreds or thousands of hectares, yet many of the successful restoration projects have only managed to restore comparatively small areas (no more than a few hectares). Given the likely cost savings of facilitating seedling recruitment as a means of restoration, and the avoidance of reliance upon the use of donor meadows, this approach to restoration may be applicable over broader scales. The use of seedlings in restoration programs has increased as the importance of seagrass seedlings in the recovery of previously disturbed seagrass beds has been recognised (Preen et al., 1995; Kendrick et al., 1999; Whitfield et al., 2004; Olesen et al., 2004; Bryars and Neverauskas, 2004). Previous restoration programs using seedlings have generally only been successful in low energy environments (Fonseca et al., 1998). While the success and use of recruitment facilitation methods developed in this study may be limited to areas containing *Amphibolis* seagrass beds, they are likely to be more successful in higher energy environments, as the hessian substrates not only facilitate recruitment but provide a stable environment in which seedlings can grow.

4.4. Future Research and Directions

Before recruitment facilitation methodologies may be employed over broader spatial scales as a means of seagrass restoration, it is important to determine the limitations of this type of restoration. Studies to investigate the limitations may include determining whether or not the proximity of adult *Amphibolis* beds affects seedling recruitment, and whether or not the effectiveness of such methods is affected by depth and location along the coast. Furthermore, it is essential to continue with monitoring the growth and survival of recruited seedlings to ensure that seedlings survive over a longer period and that the roots of the seedlings grow through the bags and the shoots develop beyond the edge of the bag and recolonise the bare substrates surrounding them. In addition to monitoring the growth of seedlings, it would also be beneficial to monitor growth of surrounding *Amphibolis* beds, the reproductive output of these beds and other aspects of the plants biology and morphology. Currently, trial sites have been established at six locations along the coast and three depths to ascertain the affects of these factors on recruitment. A trial to determine effect of distance from the nearest *Amphibolis* bed on recruitment was also established, but hessian bags deployed inshore from the current seagrass line were rapidly buried by shifting sand, suggesting rehabilitation may have to be incremental in nature.

Following experiments into the limitations of recruitment facilitation as a means of restoration and the identification of suitable sites for further trials, a large-scale deployment of bags may take place. Based on our current knowledge of the distribution of *Amphibolis* beds along the Adelaide coastline, and the likelihood that the success of this type of restoration will depend upon proximity to a source of seedlings, it is likely that large-scale trails will be most successful in the Henley Beach/Grange area. Furthermore, large-scale deployment of bags is likely to be more successful if the bags are placed in areas with relatively little sediment movement and in areas that are somewhat protected by existing seagrass beds.

The concept of habitat restoration is not only dependent upon the survival and growth of transplants or seedlings over time, but also whether or not the artificially restored habitat is providing the functions that natural systems offer. Therefore, while assessing the recruitment and survival of seedlings is important for determining the success of recruitment facilitation as a means of restoration, it may also be appropriate to investigate the return of function to newly restored areas.

The use of hessian in the current experiment not only appeared to facilitate the natural recruitment of *Amphibolis* seedlings but also seemed to aid seedling survival through the provision of a stable environment. Given that restoration efforts have often failed in moderate to high wave energy environments due to a lack of protection from waves and currents, it may be valuable to investigate the use of hessian bags in protecting other species of seagrasses in restoration efforts. In a recent pilot study in Adelaide, *Posidonia* seedlings

were planted into sand-filled hessian bags, and while seedling survival was initially very poor, those seedlings that survived have remained for approximately twelve months and are showing positive signs of growth.

5. CONCLUSION

This study shows that the provision of biodegradable fabrics in areas of seagrass loss can facilitate the natural recruitment of large numbers of *A. antarctica* and *A. griffithii* seedlings and aid in their survival and growth. Some of the recruitment facilitation methods trialled were more successful than others in terms of seedling recruitment, survival and cost effectiveness. Overall the most successful methods after approximately 12 months were the double-layered hessian bag and the large hessian bag. Indicative costs for restoring larger areas of seagrass using these methods are in the order of \$13,000 per hectare, which compares favourably with other methods, and the estimated economic value of seagrasses (\$12,635 to \$25,270 ha⁻¹yr⁻¹). Recruitment facilitation methods such as these are likely to speed up to the recovery of seagrasses in areas of loss and may represent a new, and cost effective means of seagrass restoration.

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