Marine Environment
and Ecology

# Reproductive and recruitment ecology of the seagrass *Amphibolis antarctica* along the Adelaide coastline: Improving chances of successful seagrass rehabilitation

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
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September 2009

Government
of South Australia

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## ACKNOWLEDGEMENTS

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## EXECUTIVE SUMMARY

Seagrass meadows off the metropolitan coast of Adelaide have experienced considerable decline over the last 60 years, with current research aimed at developing methods to facilitate the rehabilitation of this critical coastal habitat. The research described here presents the latest findings of studies of the reproductive ecology and recruitment dynamics of the common seagrass *Amphibolis antarctica*, as well as tests of possible improvements to promising methods of rehabilitation.

The specific aims of this research were to:

1) Quantify spatio-temporal patterns in the structure of *A. antarctica* meadows (shoot density, biomass, epiphyte cover, etc.) along Adelaide's metropolitan coast
2) Test for spatio-temporal patterns in the reproductive output of *A. antarctica* meadows along Adelaide's metropolitan coast
3) Quantify temporal patterns in the recruitment of juvenile *A. antarctica* to hessian bags deployed off Adelaide's metropolitan coast, and test whether recruitment varies between hessian bags of different design.
4) Test whether methods to improve the longevity of hessian bags by using polymer coatings affects their capacity to facilitate recruitment of *A. antarctica* off Adelaide's metropolitan coast.

The meadow structure and reproductive output of *A. antarctica* was assessed ~ monthly from February – June 2009 by harvesting natural populations at four sites off the metropolitan coast (Henley, Grange, Semaphore, and Largs Bay). Meadow structure was generally similar among sites and sampling times, though some relatively minor spatio-temporal variability was apparent for shoot density, biomass, and height, as well as the biomass of attached epiphytes. A total of 367 juveniles were observed in harvested samples, with up to 15 individuals on a single shoot, but there were no obvious spatial or temporal trends in their abundance. Juvenile abundance was positively correlated with adult biomass and shoot height, but only weakly (~ 2.5 % and 7.3 %, respectively), suggesting other factors play a greater role in determining juvenile abundance on adult shoots.

The recruitment of juvenile *A. antarctica* to artificial recruitment units (hessian bags) was generally low from February – April (~ 5 individuals m$^{-2}$), but increased substantially from May - June (~ 112 individuals m$^{-2}$), corresponding with the annual recruitment peak also observed in 2008. Greatest recruitment was typically observed on hessian bags with a coarse outer-weave (double-layer bags) than without (single-layer bags), which matches patterns observed in other research off the coast of Normanville on the Fleurieu Peninsula.

In August 2008, hessian bags were coated with biodegradable organo-silicon based polymers (organosilane and polyurethane) to test whether such coatings could improve the longevity of hessian recruitment units or affect the recruitment of *A. antarctica*. Polymer coatings did not affect the recruitment, growth, and morphometrics of *A. antarctica* recruits to any substantial degree, suggesting they are a potentially useful tool for enhancing seagrass rehabilitation efforts.

The knowledge gained from this research will help maximise the chances of success for future large-scale seagrass rehabilitation efforts by identifying times and places where rehabilitation efforts should be concentrated (e.g. coinciding the deployment of hessian bags with natural peaks in recruit availability), as well as the methods that are most promising (e.g. deploying double-layer bags coated with a polymer). While some key advances have been made, addressing remaining gaps in knowledge will further increase chances of successful rehabilitation and ultimately pay dividends given the substantial environmental and economic benefits of healthy seagrass meadows.

## 1. INTRODUCTION

Habitat preservation and rehabilitation has become a key aspect of environmental management policies in many countries around the world (McCay et al. 2003, Edgar et al. 2007, Elliott et al. 2007), particularly those that have experienced substantial habitat loss because of numerous anthropogenic impacts (Benedetti-Cecchi et al. 2001, Connell 2007). In shallow coastal margins, seagrass meadows are often susceptible to anthropogenic impacts that reduce water quality. In Adelaide's metropolitan waters, increases in sedimentation, turbidity, and nutrient concentrations have been identified as primary agents of seagrass loss (Bryars et al. 2006, Collings et al. 2006a, 2006b), with an estimated decline of ~ 5200 ha since the 1950s (Seddon 2002). The subsequent impacts on the multitude of marine species that use seagrasses as habitat and food (Edgar 1990, Heck et al. 1995, Connolly 1997), which include commercially important species (e.g. King George whiting: Connolly 1994), are difficult to estimate given the lack of baseline data, but are likely to be considerable.

Numerous seagrass rehabilitation efforts have relied on labour-intensive methods such as translocating adult plants from healthy 'donor' meadows to impacted ones, often with mixed success (e.g. Fonseca et al. 1996a, b). Another approach is to enhance the recruitment and retention of seagrass seeds and juveniles in impacted areas such that viable recruits are not lost from the system and so currently healthy meadows are not damaged in the search for donor plants. Seagrass rehabilitation efforts that focus on enhancing recruitment require a solid understanding of natural reproductive cycles of the target species, the ecology of their early life-history stages, as well as how these key aspects may vary over time and space. With such knowledge, the long-term chances of successful rehabilitation can be maximised by, for example, coordinating the deployment of recruitment structures with known peaks in reproductive output and/or recruit availability.

Off the metropolitan coast of Adelaide, a peak in the recruitment of the common seagrass *Amphibolis antarctica* (also known as wire weed) to artificial recruitment structures has previously been observed from June/July – September 2008 (Irving et al. 2009). However, this peak has only been observed once and there is a great need to continue sampling to determine whether it is a predictable annual event, or essentially a random occurrence. In addition, sampling natural populations of adult plants can help us understand spatio-temporal patterns in the production of juveniles, as well as identify possible relationships between seagrass condition (density, biomass, etc.) and reproductive output. Such aspects are poorly understood for Adelaide's seagrass meadows, but may be of considerable benefit for planning rehabilitation programs because locations and times of greatest likely success can be identified.

For rehabilitation efforts that use artificial structures to enhance recruitment, considerable testing of materials and techniques is often required to design structures that not only enhance recruitment, but are also relatively cheap, easy to use, and leave minimal or no further environmental impact. For Adelaide's *A. antarctica* meadows, biodegradable sand-filled hessian bags have proven to be a promising structure because the basal grappling hook structure of juveniles easily entangles with the hessian fibres (Fig. 1) (Wear et al. 2006, Collings et al. 2007, Collings 2008). Trials are currently underway to improve the longevity of the hessian bags by coating them with organo-silicon based polymers to slow microbial degradation, but it is not known whether these coating treatments may affect the recruitment of *A. antarctica*, and hence the utility of the hessian bag technique.

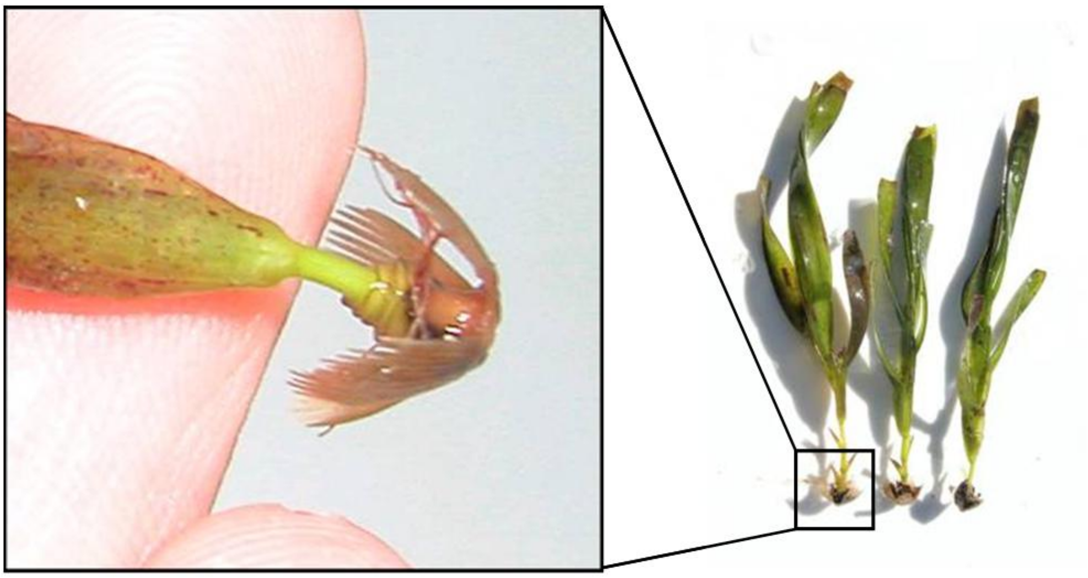

**Figure 1.** Three juvenile *A. antarctica* (right) with a close-up of photograph of the grappling hook apparatus (left) (photographs by Rachel Wear).

As part of an ongoing long-term research program aimed at gaining the knowledge and tools needed for successful large-scale rehabilitation of Adelaide's lost and damaged seagrass meadows (Wear et al. 2006, Collings et al. 2007, Collings 2008, Irving et al. 2009), the specific aims of this research were to:

1) Quantify spatio-temporal patterns in the structure of *A. antarctica* meadows (shoot density, biomass, epiphyte cover, etc.) along Adelaide's metropolitan coast
2) Test for spatio-temporal patterns in the reproductive output of *A. antarctica* meadows along Adelaide's metropolitan coast
3) Quantify temporal patterns in the recruitment of juvenile *A. antarctica* to hessian bags deployed off Adelaide's metropolitan coast, and test whether recruitment varies between hessian bags of different design.
4) Test whether methods to improve the longevity of hessian bags by using polymer coatings affects their capacity to facilitate recruitment of *A. antarctica* off Adelaide's metropolitan coast.

## 2. METHODS

### *2.1. Study sites*

All research was done from August 2008 – June 2009 in the shallow waters off Adelaide's metropolitan coast, specifically at sites offshore from Henley, Grange, Semaphore, and Largs Bay (Fig. 2). Work was done within or near natural meadows of seagrass that included *A. antarctica* as a major floristic component, sometimes mixed with *Posidonia* spp. Sampling at each site was done between depths of 7 – 10 m.

### *2.2. Seagrass meadow structure*

Spatio-temporal patterns in the structure of *A. antarctica* meadows were quantified by ~ monthly harvesting of all biomass within ten 0.25 × 0.25 m quadrats haphazardly placed at all four sites. Within each quadrat, shoots were cut at the level of the sand and collected in plastic bags prior to processing in the laboratory. The specific dates of sampling were the 26$^{th}$ of February, 9$^{th}$ of April, 20$^{th}$ of May, and 18$^{th}$ of June 2009.

Processing each sample involved first counting the number (density) of shoots and recording the wet biomass of the entire sample. Five shoots were then randomly selected and had their height measured before having all macro-epiphytes scraped off with a scalpel (epiphytes such as encrusting coralline algae were not removed). The wet biomass of the five shoots and epiphytes were recorded before being oven-dried at 65 ºC for 72 hours. The dry biomass of the five shoots and epiphytes was then recorded, and the dry biomass of

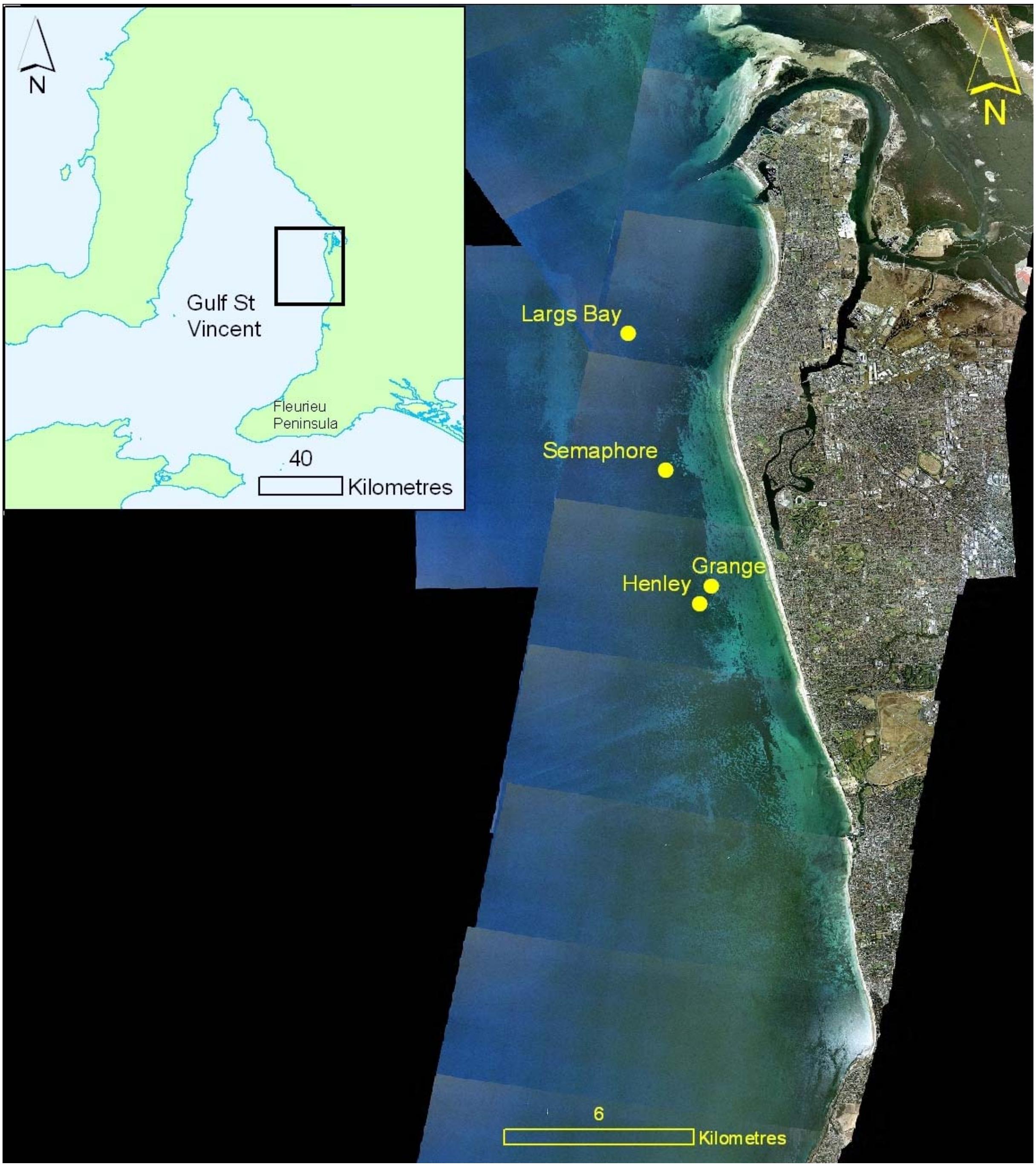


**Figure 2.** Composite aerial photograph of the Adelaide metropolitan coastline and the locations of the four study sites. Inset: Map of Gulf St Vincent and the Fleurieu Peninsula highlighting the Adelaide coast.

*A. antarctica* and epiphytes in the entire sample was finally estimated by multiplying the ratio of dry to wet biomass of the five shoots or epiphytes by the initial wet biomass of the entire sample. In total, the variables of shoot density, biomass, height, and epiphyte biomass were quantified from each replicate. Data analysis involved two-way ANOVA for individual variables, while the overall meadow structure was assessed in multivariate space using two-way PERMANOVA and non-metric ordination (Clarke & Warwick 2001; Anderson et al. 2008).

*2.3. Reproductive output*

Most aquatic plants produce seeds or spores as part of their reproductive cycle, but *A. antarctica* instead reproduces viviparously, and releases free-living seedlings (Kuo & den Hartog 2006). As such, it can be easy to quantify patterns in reproductive output of *A. antarctica* because the juveniles can be readily observed when attached to the adult plants.

For this study, spatio-temporal patterns in the reproductive output of *A. antarctica* were quantified by counting the number of juveniles present on the branches of plants that were harvested for quantifying seagrass meadow structure (as described in the previous section). All plants from each sample were spread on a tray and searched for attached or loose juveniles before being processed. The number of juveniles observed was then compared among sites and sampling times using two-way ANOVA to identify spatio-temporal patterns in reproductive output, and was also correlated to adult shoot density, biomass, and height using linear regression to test if these factors related to reproductive output.

*2.4. Recruitment*

Temporal patterns in the recruitment of *A. antarctica* were quantified at the Grange site using sand-filled hessian bags to sample recruitment intensity (Wear et al. 2006, Collings et al. 2007, Collings 2008). On the 26$^{th}$ of February 2009, ten hessian bags were positioned on the sandy sea floor adjacent to an extensive meadow of *A. antarctica*. Five single-layer and five double-layer hessian bags were used to test which type of bag facilitated greater recruitment (Fig. 3; also see Wear et al. 2006 for a detailed description of bag types). Bag deployment was repeated in this fashion on the 9$^{th}$ of April and the 20$^{th}$ of May 2009.

On each day of bag deployment, the number and height of *A. antarctica* recruits on all previously deployed bags were quantified (note that bags deployed on the 20$^{th}$ of May were sampled on the 18$^{th}$ of June). Height was sampled by haphazardly selecting three individuals (or the maximum available) on each bag and measuring the distance from the base to the tip, with the mean of all three measurements used as a single replicate for analyses. Observations regarding the condition of hessian bags were also made at this time. For both density and height, repeated-measures ANOVA was used for the 26$^{th}$ of February and 9$^{th}$ of April deployments, while standard ANOVA was used for the 20$^{th}$ of May deployment since these bags had only been sampled once by the time of preparing this report. Note that for repeated-measures analyses, the Greenhouse-Geisser epsilon-adjusted *P*-value was used for 'within subjects' tests to account for any departures from sphericity among the data and to compensate for inflated Type I error rates (Myers & Well 2003).

**Figure 3.** Photograph of single-layer (top) and double-layer (bottom) hessian bag types used to facilitate the recruitment of *A. antarctica* juveniles. Double-layer bags consist of a single-layer bag encased in a coarse outer weave of hessian. Before deployment, bags are filled with clean builder's sand to enhance rigidity and structural integrity (photograph by the author).

### *2.5. Hessian coating trial*

On the 18$^{th}$ of August 2008, a test of the ability of non-toxic organo-silicon polymer coatings to reduce degradation of hessian bags and improve their longevity was established off Grange (in collaboration with Drs Jamie Quinton and Stephen Clarke, Flinders University). In this experiment, bags were coated with one of two types of ultra-thin polymer, either organosilane (of form $R'\text{-}Si(OR)_3$) or polyurethane, or were left as uncoated controls ($n$ = 30 per treatment).

A key prediction of this test was that the ultra-smooth microscopic surface of the polymers would prevent the adhesion of microorganisms as small as bacteria, inhibiting the formation of a biofilm on the hessian and consequently reducing its rate of degradation (results of this test are being prepared by Dr Kirsten Benkendorff, Flinders University). For this report, the point of interest was whether polymer coatings would affect the ability of hessian bags to facilitate recruitment of *A. antarctica* seedlings, or their subsequent growth.

The number and height of recruits on all bags were sampled as described in section 2.4 on the 30th of October 2008, and on the 19th of January, 9th of April, and 20th of May 2009. Tests of differences among times and coating treatments were done using repeated-measures ANOVA (using Greenhouse-Geisser epsilon-adjusted *P*-values for 'within subjects' tests as described in section 2.4). Additionally, three bags of each treatment were collected on the 26th of February 2009, with the density, biomass, shoot height and root length of attached seedlings quantified in the laboratory as described in section 2.2. Each variable was analysed separately using standard ANOVA, while the overall morphology of seedlings on bags was analysed in multivariate space using PERMANOVA and non-metric ordination.

## 3. RESULTS

### *3.1. Seagrass meadow structure*

Seagrass meadow structure was defined in this study as the density, biomass, and height of *A. antarctica*, as well as the biomass of attached epiphytes. Analysis of the data from seagrass harvests revealed effects of sampling time, sometimes in conjunction with site, for all variables. Multivariate analysis of the collective suite of variables sampled tested for spatio-temporal patterns in the overall structure of *A. antarctica* meadows. The major effect was that differences in structure over time were dependent on the site sampled (Table 1: Site × Time interaction). Essentially, meadow structure was generally similar over time at all sites except at Largs Bay, and to a lesser extent at Henley, where the structure in February was different to all other times. Non-metric ordination of the data does not provide the clearest representation of these statistical differences (Fig. 4), yet the separation of Largs Bay and Henley data points in February is evident.

The density of *A. antarctica* averaged between ~ 385 and 582 shoots $m^{-2}$ over the duration of the study, but was greater at Henley than all other sites for sampling done in April (Fig. 5a, Table 2a: Site × Time interaction). Both the biomass and height of *A. antarctica* generally increased over time at all sites (Fig 5b & c), with overall values in February less than at other times (Table 2b & c: Time effect). Lastly, the biomass of attached epiphytes exhibited substantial variation among sites and sampling times (Fig. 5d). Analysis of these data showed that epiphyte biomass in June was greater at Largs Bay than other sites, and that within Largs Bay, epiphyte biomass was greater in April and June than February and May (Fig. 5d, Table 2d: Site × Time interaction).

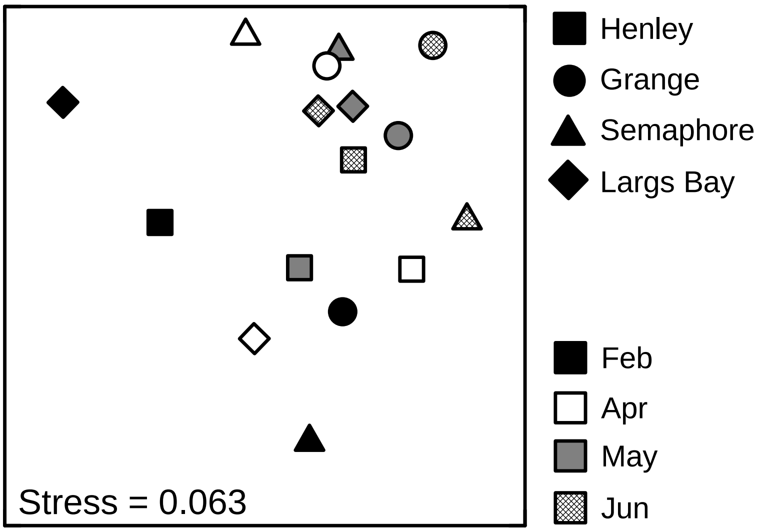


**Figure 4.** nMDS plot of *A. antarctica* meadow structure among sites and sampling times. Each point represents a centroid value of 10 replicates. A stress value of 0.063 indicates a good reduction of multidimensional data into two dimensions (Clarke 1993).

**Table 1.** Results of two-way PERMANOVA testing for multivariate differences in the structure of *A. antarctica* meadows among sites (Henley vs Grange vs Semaphore vs Largs Bay) and sampling times (February vs April vs May vs June). Effects judged as significant are shown in bold type.

| Source | df | MS | *F* | *P* |
|---|---|---|---|---|
| Site | 3 | 1132.95 | 1.30 | 0.2904 |
| Time | 3 | 2527.75 | 6.16 | **0.0002** |
| Site × Time | 9 | 872.29 | 2.13 | **0.0056** |
| Residual | 144 | 410.02 | | |

**Table 2.** Results of two-way ANOVAs testing the effects of site (Henley vs Grange vs Semaphore vs Largs Bay) and sampling time (February vs April vs May vs June) on the (a) shoot density, (b) biomass (g dry weight), and (c) height of *A. antarctica* harvested from natural meadows, as well as (d) the biomass of attached epiphytes (g dry weight). Effects judged as significant are shown in bold type.

| Source | df | MS | *F* | *P* | MS | *F* | *P* |
|---|---|---|---|---|---|---|---|
| | | (a) density | | | (b) *Amphibolis* biomass | | |
| Site | 3 | 70577.07 | 0.70 | 0.5771 | 519133.16 | 3.56 | 0.0604 |
| Time | 3 | 15656.53 | 0.50 | 0.6822 | 1145267.44 | 7.48 | **0.0001** |
| Site × Time | 9 | 101314.13 | 3.24 | **0.0013** | 145731.63 | 0.95 | 0.4828 |
| Residual | 144 | 31249.07 | | | 153147.99 | | |
| | | (c) height | | | (d) epiphyte biomass | | |
| Site | 3 | 142.45 | 1.62 | 0.2522 | 19448.94 | 1.88 | 0.2030 |
| Time | 3 | 514.25 | 7.38 | **0.0001** | 9450.48 | 1.85 | 0.1416 |
| Site × Time | 9 | 87.88 | 1.26 | 0.2634 | 10331.40 | 2.02 | **0.0413** |
| Residual | 144 | 69.71 | | | 5121.72 | | |

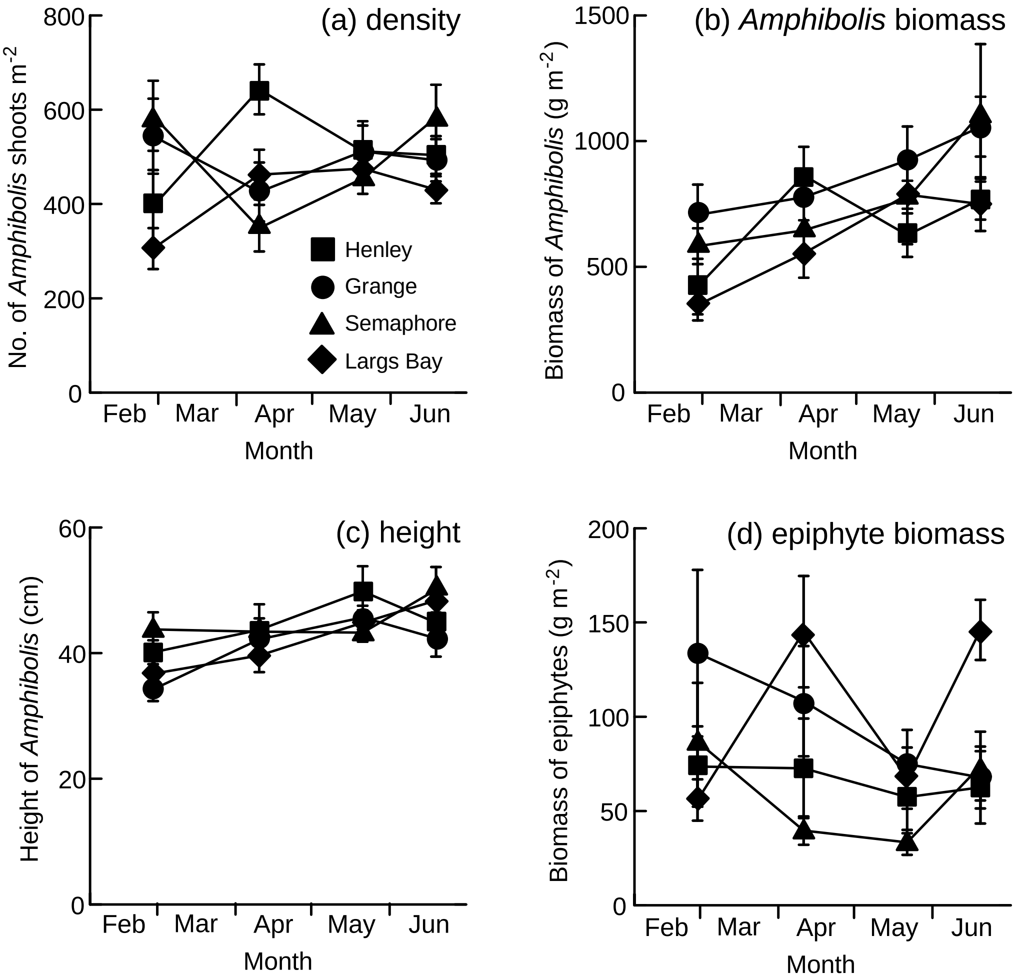


**Figure 5.** Spatio-temporal patterns in the mean (± SE) (a) density, (b) biomass (g dry weight), and (c) height of *A. antarctica* shoots, as well as (d) the biomass of attached epiphytes.

## *3.2. Reproductive output*

Over the duration of the study, a total of 367 juvenile *A. antarctica* were sampled on harvested adult plants. Within any particular site, the number of juveniles sampled was often quite variable among replicates, such that formal analysis detected no differences among sites or times of sampling for the duration of the study (Fig. 6, Table 3). Furthermore, there was no evidence of any obvious spatial or temporal trend in juvenile production; north-south gradients were not observed, and the greatest number of sampled juveniles occurred at different sampling times among sites (Fig. 6). When juvenile *A. antarctica* were observed in quadrats, it was usually only in low numbers (i.e. 10 or fewer juveniles per quadrat; Fig. 7). However, greater numbers were observed on five occasions, peaking at 31 individuals in a quadrat from Semaphore in June (Fig. 7). Although individual adult shoots most frequently supported 1 – 3 juveniles, abundances as high as 15 juveniles were observed, including the occurrence of ‘twins’, whereby two juveniles grew from one node (Fig. 8).

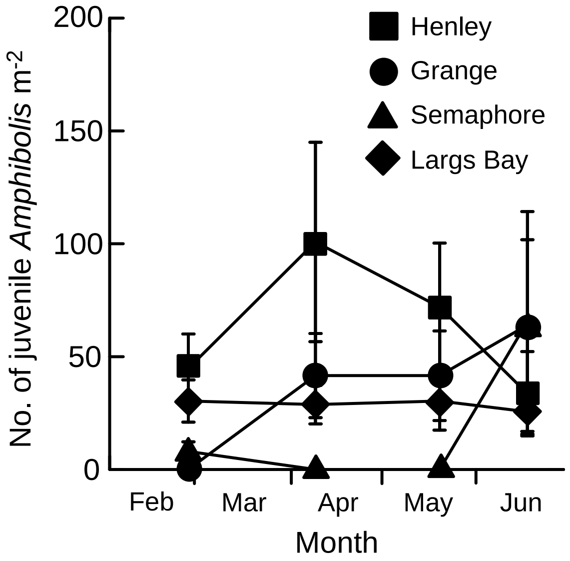


**Figure 6.** Mean (± SE) number of juvenile *A. antarctica* observed on adult plants among sites on Adelaide's coast over time.

**Table 3.** Results of two-way ANOVA testing for differences in the abundance of juvenile *A. antarctica* on adult plants among sites (Henley vs Grange vs Semaphore vs Largs Bay) and sampling times.

| **Source** | **df** | **MS** | ***F*** | ***P*** |
|---|---|---|---|---|
| Site | 3 | 85.10 | 2.95 | 0.0909 |
| Time | 3 | 13.93 | 0.74 | 0.5276 |
| Site × Time | 9 | 28.87 | 1.54 | 0.1385 |
| Residual | 144 | 18.72 | | |

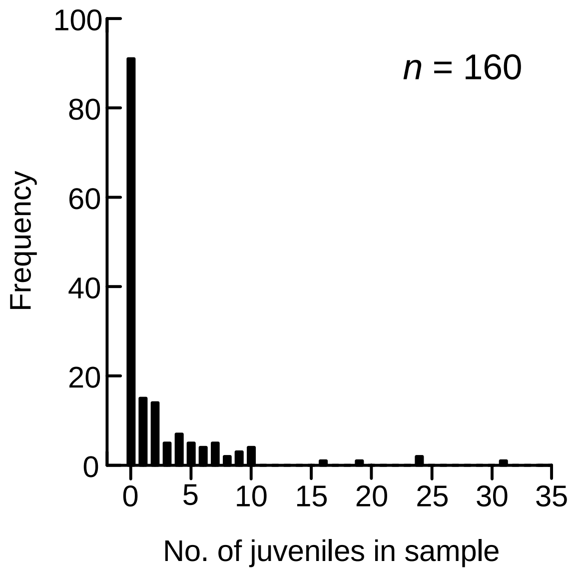


**Figure 7.** Frequency distribution of the number of juvenile *A. antarctica* sampled in quadrats (0.25 × 0.25 m). Note that data from all sites and sampling times have been pooled.

**Figure 8.** Photograph of three juvenile *A. antarctica*, observed as a single individual (right) and 'twins' (left) (photograph by Mandee Theil).

Given the absence of any site or time effect on juvenile abundance (Table 3), data were pooled for regression of juvenile abundance against adult shoot density, biomass, and height. These tests revealed no significant relationship of juvenile abundance with adult density (Fig. 9a), but did reveal weak positive correlations with adult biomass and height (Fig. 9b & c) whereby only 2.5 % and 7.3 % of the variance in juvenile abundance could be attributed to these factors, respectively. Examination of the data showed that juveniles occurred across the range of sampled values of all three factors, with a possible exception of those few samples exhibiting the greatest adult biomass ( > ~ 150 g; Fig. 9b).

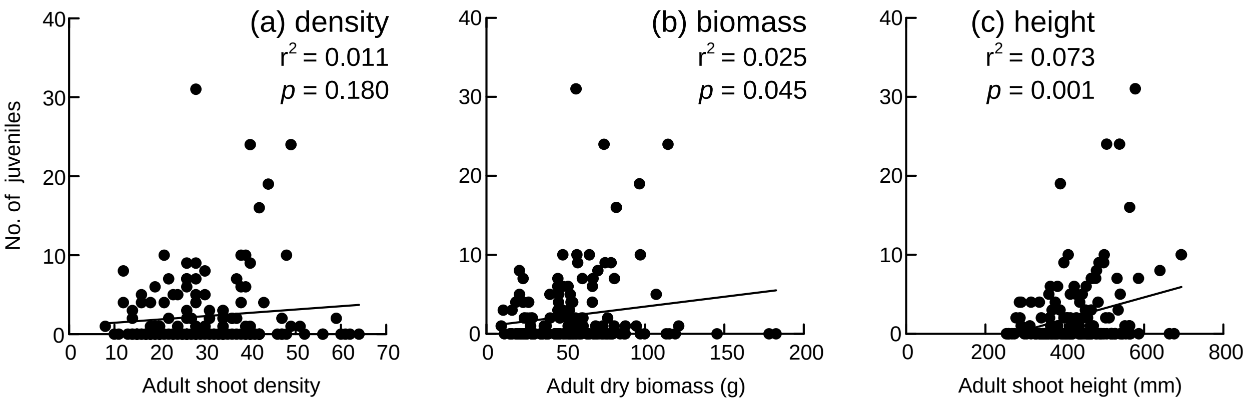


**Figure 9.** Linear regression of the abundance of juvenile *A. antarctica* found in samples (0.25 × 0.25 m quadrats) against (a) adult shoot density, (b) biomass, and (c) height. Note that data from all sites and sampling times have been pooled.

*3.3. Recruitment*

Recruitment of juvenile *A. antarctica* to hessian bags was negligible early in the study period (February – April), but increased substantially during May and June (Fig. 10a). The greatest recruit densities were observed in June for double-layer bags that were deployed in May (~ 266 individuals $m^{-2}$). After April, double-layer bags usually supported greater numbers of recruits than single-layer bags (Fig. 10a, Table 4a: Time × Bag interactions). Notably, single-layer bags deployed in May supported greater recruit densities than bags deployed at other times (Fig. 10a).

The height of *A. antarctica* recruits on hessian bags generally did not vary significantly between bag types, nor over time (Fig. 10b, Table 4b). Across treatments, the height of recruits averaged ~ 6.4 cm.

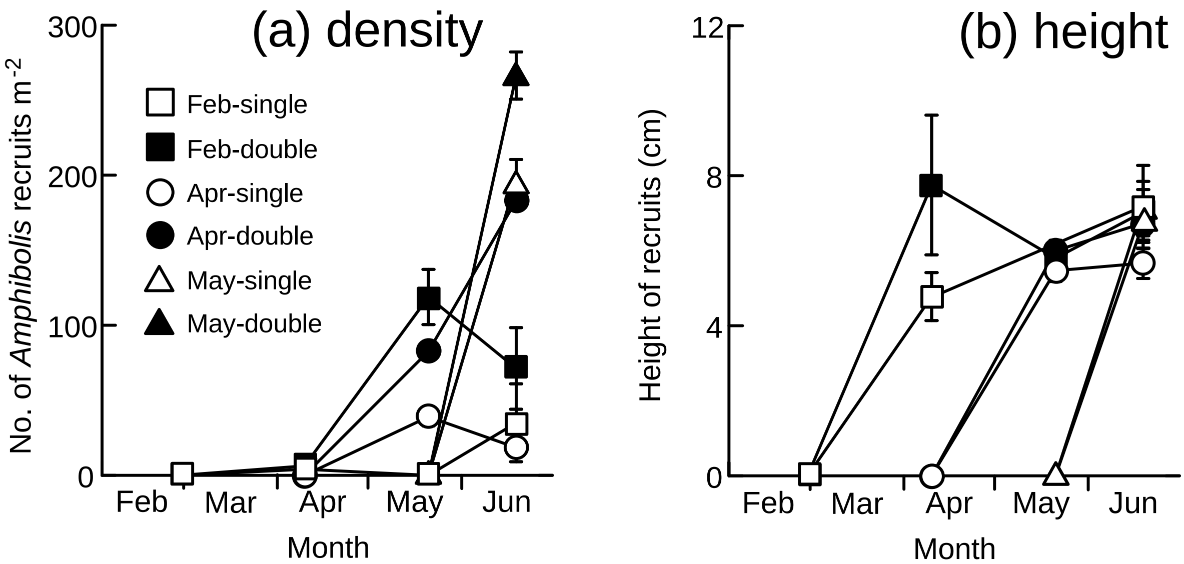


**Figure 10.** Mean (± SE) (a) density and (b) height of *A. antarctica* recruits on single vs double-layer bags over time.

**Table 4.** Results of repeated measures ANOVA (February and April bag deployments) and ANOVA (May bag deployment) testing the effects of hessian bag type (single-layer vs double layer) on the (a) density and (b) height of juvenile *A. antarctica* recruiting to hessian bags over time. Effects judged as significant are shown in bold type.

| Response | Deployment | Source | df | MS | *F* | *P* |
|---|---|---|---|---|---|---|
| (a) density | February | Within-subjects | | | | |
| | | Time | 2 | 8801.24 | 7.45 | **0.013** |
| | | Time × Bag | 2 | 8984.15 | 7.61 | **0.013** |
| | | Residual | 16 | 1180.93 | | |
| | | Between-subjects | | | | |
| | | Bag | 1 | 20618.52 | 10.19 | **0.013** |
| | | Residual | 8 | 2024.20 | | |
| | April | Within-subjects | | | | |
| | | Time | 1 | 7959.34 | 74.03 | **< 0.001** |
| | | Time × Bag | 1 | 18340.56 | 170.58 | **< 0.001** |
| | | Residual | 8 | 107.52 | | |
| | | Between-subjects | | | | |
| | | Bag | 1 | 54282.63 | 338.74 | **<0.001** |
| | | Residual | 8 | 160.25 | | |
| | May | Bag | 1 | 12062.81 | 11.16 | **0.010** |
| | | Residual | 8 | 1081.38 | | |
| (b) height | February | Within-subjects | | | | |
| | | Time | 1 | 8.30 | 1.69 | 0.250 |
| | | Time × Bag | 1 | 12.23 | 2.50 | 0.175 |
| | | Residual | 5 | 4.90 | | |
| | | Between-subjects | | | | |
| | | Bag | 1 | 4.02 | 0.64 | 0.459 |
| | | Residual | 5 | 6.26 | | |
| | April | Within-subjects | | | | |
| | | Time | 1 | 1.09 | 3.73 | 0.089 |
| | | Time × Bag | 1 | 0.36 | 1.22 | 0.302 |
| | | Residual | 8 | 0.29 | | |
| | | Between-subjects | | | | |
| | | Bag | 1 | 3.20 | 3.91 | 0.084 |
| | | Residual | 8 | 0.82 | | |
| | May | Bag | 1 | 0.54 | 0.56 | 0.476 |
| | | Residual | 8 | 0.97 | | |

*3.4. Hessian coating trial*

Recruitment of juvenile *A. antarctica* to hessian bags in the coating trial was considerable, averaging ~ 213 individuals m$^{-2}$ across treatments by the first sampling date (30$^{th}$ of October, ~ 2.5 months after bags were deployed). Formal analysis using repeated-measures ANOVA revealed an effect of coating type that was dependent on time (Table 5a). At early stages of the experiment, bags coated with polyurethane supported fewer recruits than other treatments, but such differences did not exist at later times (Fig. 11a). The decline in recruit density over time that is seen across all treatments is consistent with juvenile mortality following initial recruitment.

*A. antarctica* recruits across treatments generally exhibited an increase in height over time (i.e. growth), reaching an average of ~ 17 – 18 cm during the study period (Fig. 11b). No differences in recruit height among treatments were detected until the final sampling date (Table 5b: Time × Coating interaction), when recruits on bags coated with polyurethane were slightly taller than those on organosilane-coated or uncoated bags.

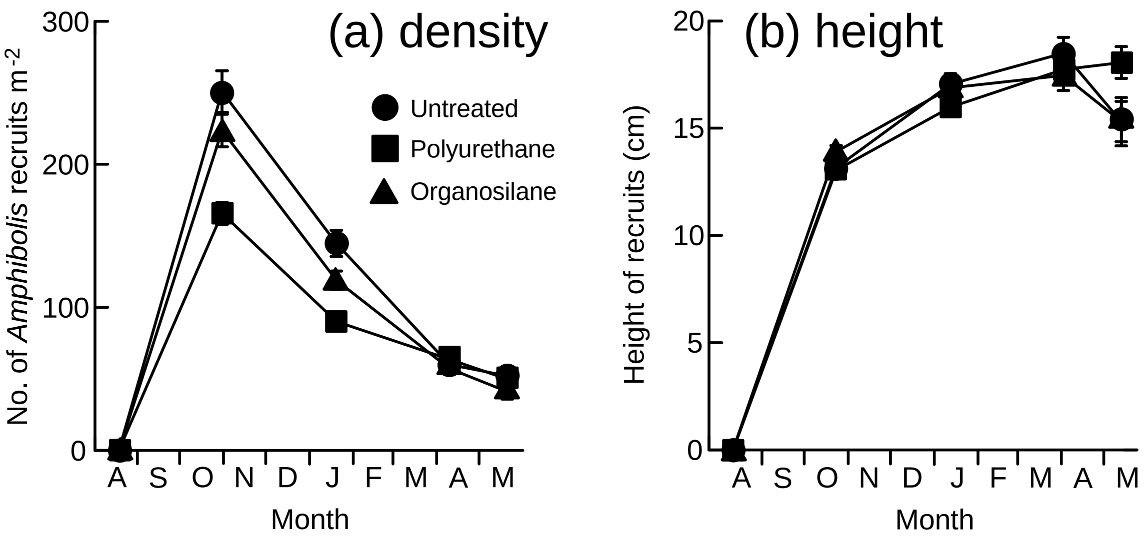


**Figure 11.** Mean (± SE) density (a) and height (b) of *A. antarctica* juveniles sampled on hessian bags coated with either polyurethane or organosilane, or left untreated, in 2008 and 2009.

**Table 5.** Results of repeated measures ANOVA testing the effects of hessian coating treatment (untreated vs polyurethane vs organosilane) on the (a) density and (b) height of juvenile *A. antarctica* recruiting to hessian bags over time. Effects judged as significant are shown in bold type. Note that the Greenhouse-Geisser epsilon-adjusted *P*-value was used for 'within subjects' tests to account for any departures from sphericity among the data and to compensate for inflated Type I error rates (Myers & Well 2003).

| **Response** | **Source** | **df** | **MS** | ***F*** | ***P*** |
|---|---|---|---|---|---|
| (a) density | Within-subjects | | | | |
| | Time | 3 | 503640.89 | 350.10 | **< 0.001** |
| | Time × Coating | 6 | 14883.38 | 10.35 | **< 0.001** |
| | Residual | 234 | 1438.57 | | |
| | Between-subjects | | | | |
| | Coating | 2 | 35428.44 | 15.14 | **< 0.001** |
| | Residual | 78 | 2339.87 | | |
| (b) height | Within-subjects | | | | |
| | Time | 3 | 299.61 | 30.70 | **< 0.001** |
| | Time × Coating | 6 | 29.42 | 3.02 | **0.019** |
| | Residual | 222 | 9.76 | | |
| | Between-subjects | | | | |
| | Coating | 2 | 1.80 | 0.154 | 0.857 |
| | Residual | 74 | 11.69 | | |

Multivariate analysis of the density and morphometrics of recruits attached to hessian bags sampled in February revealed no differences among hessian coating treatments (PERMANOVA: $F_{2,6}$ = 3.39, $P$ = 0.1092). This similarity among treatments was reflected in non-metric ordination of the data (Fig. 12), where considerable dispersion of replicates from each treatment is apparent.

Univariate analysis of each variable, however, showed that the average root length of recruits was greater for bags coated with polyurethane than other treatments (Fig. 13d, Table 6d). Consistent with multivariate patterns, no differences were detected among treatments for all other variables (Table 13a-c), although density of recruits was marginally non-significant (Table 6a). Notably, bags that were coated with either polyurethane or organosilane consistently ranked as the greatest for all variables (Fig. 13), while uncoated control bags ranked lowest for all variables except shoot height (Fig. 13c).

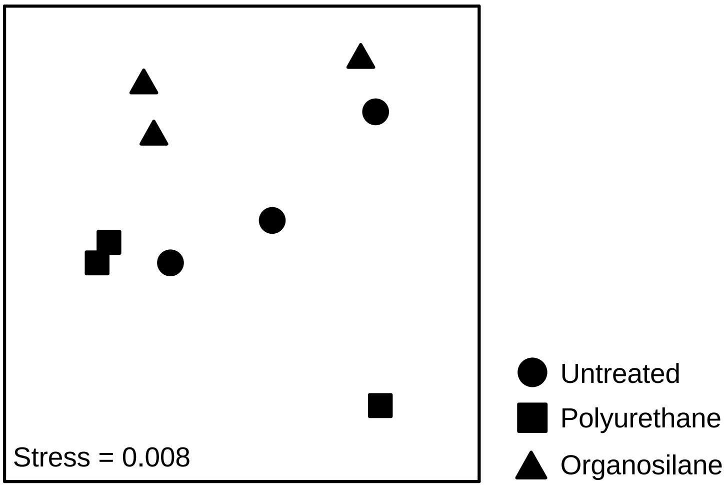


**Figure 12.** nMDS plot of the morphological structure (including density) of *A. antarctica* recruits sampled on hessian bags with different coatings. Each point represents a replicate hessian bag sampled in February 2009. A stress value of 0.008 indicates an excellent reduction of multidimensional data into two dimensions (Clarke 1993).

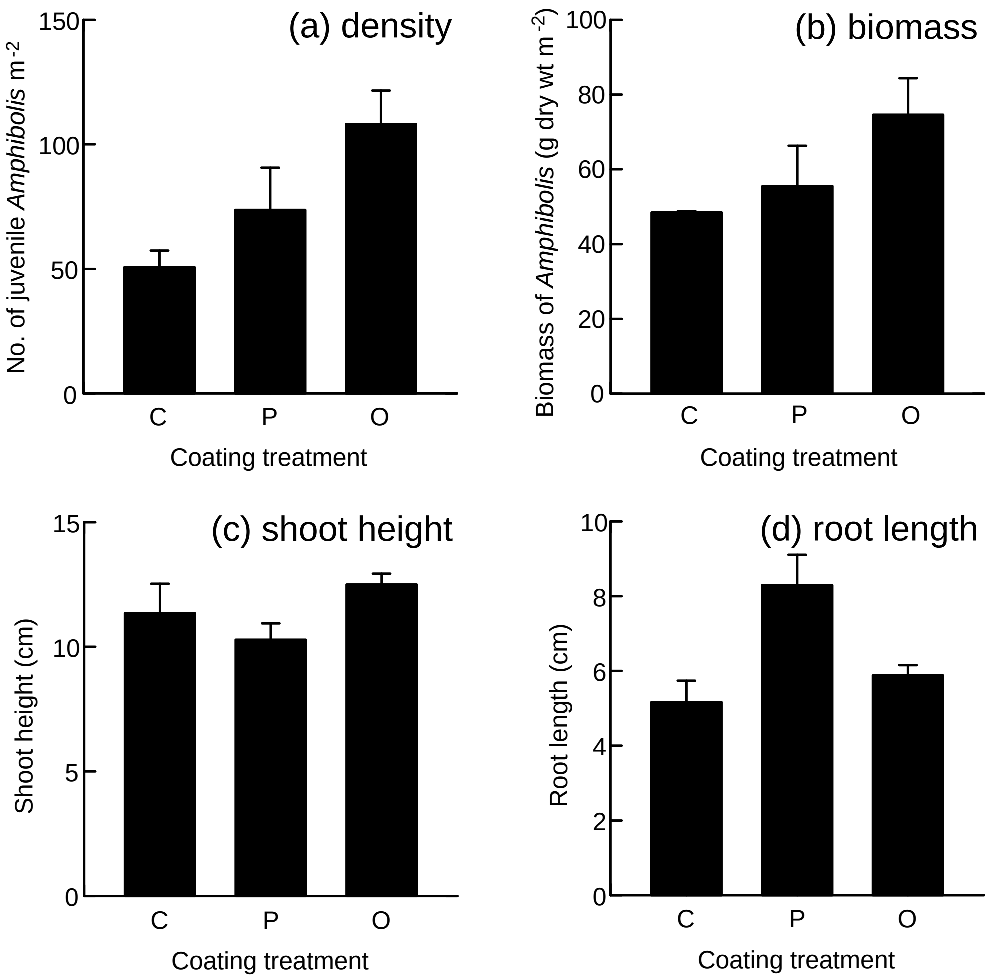


**Figure 13.** Mean (± SE) (a) density, (b) biomass (dry weight), (c) shoot height, and (d) root length of *A. antarctica* recruits attached to hessian bags coated with either polyurethane (P), organosilane (O), or left uncoated as a control (C). Samples were collected in February 2009, equating to ~ 6 months immersion time.

**Table 6.** Results of one-way ANOVAs testing the effects of hessian bag coating treatment (uncoated control vs polyurethane vs organosilane) on the (a) density, (b) biomass (g dry weight), (c) shoot height, and (d) root length of *A. antarctica* recruits attached to bags after ~ 6 months of immersion. Effects judged as significant are shown in bold type.

| **Source** | **df** | **MS** | ***F*** | ***P*** | **MS** | ***F*** | ***P*** |
|---|---|---|---|---|---|---|---|
| | | (a) density | | | (b) biomass | | |
| Coating | 2 | 2504.67 | 4.90 | 0.0547 | 549.33 | 2.60 | 0.1540 |
| Residual | 6 | 510.82 | | | 211.57 | | |
| | | (c) shoot height | | | (d) root length | | |
| Coating | 2 | 3.68 | 1.82 | 0.2405 | 8.05 | 7.60 | **0.0227** |
| Residual | 6 | 2.02 | | | 1.06 | | |

## 4. DISCUSSION

The metropolitan coast of Adelaide has lost over 5,200 ha of seagrass since the 1950s (Seddon 2002), with poor water quality resulting from high turbidity and excessive nutrient loads being identified as the primary causes (Bryars et al. 2006, Collings et al. 2006a, 2006b). Environmental conditions have improved but natural seagrass recovery is a slow process (e.g. in excess of 200 years for *Posidonia* spp.: Bryars and Neverauskas 2008) and so developing techniques to hasten this process is desirable. To this end, the work described herein presents the latest findings of on-going research to understand the ecology and recruitment dynamics of *A. antarctica*, as well as tests of improvements to methods used for rehabilitation (e.g. hessian bags).

The over-arching goal of this research is to maximise the chances of success for future large-scale seagrass rehabilitation efforts. To meet this goal, the focus has been placed on understanding the ecology and recruitment dynamics of early life-history stages of the target species, so that rehabilitation can focus on enhancing natural rates of recruitment, rather than on more unreliable and expensive methods such as adult transplants (Fonseca et al. 1996a, b). Key findings of this research include the observation that the production of juvenile *A. antarctica* is variable over scales ranging from cm (i.e. among quadrats) to km (i.e. among sites), but that juveniles were found at all sites at least some of the time. Furthermore, recruitment of juveniles to hessian bags was substantial and the greatest densities were sampled during May/June, which is consistent with observations from 2008 showing an annual recruitment peak beginning in June/July and lasting until September (Irving et al. 2009). Another important result was that the experimental polymer coatings used to enhance hessian longevity and therefore the potential for seagrass rehabilitation do not appear to inhibit the recruitment and growth of *A. antarctica*.

The seagrass meadows studied exhibited some spatio-temporal variability in shoot density, biomass, height, and the biomass of attached epiphytes, but such differences were often relatively minor or temporally inconsistent (e.g. greater density of *A. antarctica* shoots at Henley relative to all other sites was observed in April, but at no other time; Fig. 5a). Multivariate analysis of overall meadow structure (incorporating all four variables) indicated spatio-temporal patterns, but this was essentially restricted to differences at Largs Bay and Henley during February (Fig. 4). The biomass of attached epiphytes was certainly the most variable element studied, which is not unexpected given their often ephemeral nature (Borowitzka & Lethbridge 1989). Overall, the data suggest that meadows of *A. antarctica* from Henley to Largs Bay possess quite similar structure, and that findings may be generalized to this area. A possible exception, however, may be the abundance of epiphytes at Largs Bay, which shows considerably greater temporal variability than the other three sites. Given epiphytes usually respond positively to increased nutrient concentrations (Borowitzka & Lethbridge 1989, Shepherd et al. 1989), such variability may be party attributable to the proximity of Largs Bay to the mouth of the Port River and its associated delivery of nutrient-rich water to the coast (Fernandes et al. 2009).

Although a considerable number of juvenile *A. antarctica* were observed in harvests of adult plants (a total of 367 individuals), no clear spatio-temporal trends in juvenile abundance were detected. Abundances ranged from zero to a mean of ~ 100 juveniles $m^{-2}$ over the duration of the study, with substantial variability among replicates a characteristic feature (Fig. 6). Furthermore, individual sites exhibited their greatest abundance of juveniles at different sampling times, even though sometimes only separated by a few kilometres (e.g. greatest abundances at Henley were observed in April, and in June at Grange, even though these sites were only separated by ~ 0.7 km). Before accepting that spatio-temporal patterns in reproductive output do not exist, it is worth considering two points. First, sampling was only done for a discrete period of the year, and further sampling may reveal significant spatio-temporal phenomena (e.g. annual peaks in reproductive output; Irving et al. 2009). Second, the substantial variation in juvenile abundance among replicates (seen in Fig. 6) suggests that an increased sampling effort (i.e. greater replication) is required to reduce the influence of this inherent variability in formal analyses, which would improve the chances of detecting patterns if they exist (Underwood 1997).

Where they occurred, juvenile *A. antarctica* were most often found in densities of 1 – 10 individuals per quadrat (~ 16 – 160 individuals $m^{-2}$), though occasional samples supported densities up to 31 individuals per quadrat (~ 496 individuals $m^{-2}$). Casual observation revealed two interesting biological features of the distribution of juveniles. First, juveniles were often found only on a single or a few shoots in each quadrat, with these shoots often supporting few, if any, leaf clusters (i.e. possible 'dedicated' reproductive shoots). Second, juveniles were often observed as twins, where two juveniles grew from the same branch node (Fig. 8). Notably, several reviews covering the reproductive biology of the genus *Amphibolis* do not mention either observation (Kuo & McComb 1989, McConchie & Knox 1989, Walker et al 2001, Ackerman 2006, Kuo & den Hartog 2006).

Reproductive output in terrestrial and aquatic plants is often dependent on adult traits such as biomass and population density (Schiel & Choat 1980, Kunin 1997, Knight 2003, Goldenheim et al. 2008). For this study, the abundance of juvenile *A. antarctica* was found to correlate positively with adult biomass and shoot height, but only weakly (Fig. 9). In fact, these factors could only explain 2.5 % and 7.3 % of the variation in juvenile abundance, respectively, which hardly puts them in the category of a predictor variable. Other factors may be more closely linked to juvenile abundance, such as the degree of water motion and subsequent dispersal of pollen for fertilization, or the age of the plant itself, and warrant further study to assist with the planning of rehabilitation efforts (e.g. site selection based on the presence of nearby natural *A. antarctica* populations possessing traits likely to produce many juveniles).

Recruitment to hessian bags varied through time, but still reached substantial densities by the end of the study period (~ 266 individuals $m^{-2}$). The observed increase in recruitment to hessian bags in May and June (Fig. 10a) corresponds well with observations in 2008 that the annual peak recruitment period for *A. antarctica* at Grange begins in June/July (Irving et al. 2009). Additionally, it was evident that double-layer hessian bags typically facilitated greater recruitment than single-layer bags, most likely because the grappling hook apparatus of *A. antarctica* recruits become entangled with the coarse outer-weave of hessian more readily than the finer weave of single-layer bags (Wear et al. 2006). This difference in recruitment between bag types has also recently been quantified in separate tests off the coast of Normanville on the Fleurieu Peninsula (Irving 2009), suggesting it is a temporally and spatially general phenomenon.

The use of ultra-thin polymer coatings represents a novel approach to improving the longevity of hessian recruitment units, and ultimately the capture and establishment of *A. antarctica* seedlings. Analysis of hessian fibres immersed in seawater suggests polymer coatings may have substantial long-term benefits by inhibiting microbial degradation and prolonging the integrity of the hessian recruitment units (Campleman & Benkendorff 2009). Tests for effects of polymer coatings on the recruitment of *A. antarctica* showed some variability in recruitment at the beginning of the study whereby hessian coated with polyurethane supported fewer recruits, but numbers became similar among treatments as density declined over the following months (November – May: Fig. 11a). The high levels of recruitment at the October 30$^{th}$ sampling date probably reflect the very end of the 2008 annual recruitment peak (Irving et al. 2009), while the subsequent decline in density is consistent with juvenile mortality and a negligible supply of new recruits during this ‘off-peak’ recruitment period. Height of recruits, as an indicator of growth, generally remained similar among treatments over time.

Detailed sampling of *A. antarctica* recruits attached to hessian bags collected in February 2009 revealed few statistical differences among coating treatments for recruit density and morphometrics. Indeed, the only clear effect was for a greater length of roots for recruits growing on bags coated with polyurethane (Fig. 13d), which suggests better establishment of plants in this treatment. The general absence of univariate differences among treatments was reflected in the multivariate analysis of morphological structure (Fig. 12). However, in addition to the results for root length, it is worth noting that shoot height, biomass, and density of recruits were all ranked greatest for bags coated with organosilane (though not significantly), suggesting both coating types offer advantages for *A. antarctica* recruits over uncoated bags.

Overall, the polymer coatings did not appear to inhibit the recruitment, growth, or morphometrics of *A. antarctica* recruits to any substantial degree. Coupled with their ability to inhibit microbial attachment (Campleman & Benkendorff 2009), the data suggest that polymer coatings are an effective way to improve hessian longevity and thus enhance the potential for seagrass restoration.

In conclusion, this research contributes to our understanding of the reproductive biology and recruitment dynamics of *A. antarctica* in Adelaide’s metropolitan waters. Such information is useful not just for increasing our natural history knowledge of the species, but for designing rehabilitation programs with improved chances of success (Wear et al. 2006, Collings et al. 2007, Collings 2008, Irving et al. 2009). For example, there is now growing evidence that the best time to deploy hessian recruitment units is from ~ May – September, coinciding with the natural recruitment peak for *A. antarctica* observed in 2008 and probably 2009. Deploying hessian bags coated with an ultra-thin polymer (organosilane or polyurethane) appears useful since hessian longevity is increased and the recruitment of *A. antarctica* appears largely uninhibited. Furthermore, deploying double-layer hessian bags instead of single-layer bags seems the best option given they facilitate greater recruitment of juveniles (though it must be noted their ability to retain juveniles is currently unresolved: Wear et al. 2006).

While some key advancements have been made, it is important to note that some major gaps in knowledge still exist that may jeopardise successful long-term seagrass rehabilitation. Understanding annual and interannual variability in juvenile production and timing of recruitment are key pieces of information, and while some ground has been made in Adelaide’s coastal waters, continued research is needed both here and on regional coasts for more precise planning of rehabilitation programs. Furthermore, it is not known whether the hessian bag technique is suitable for rehabilitation outside of Adelaide’s metropolitan waters, though recent work on the Fleurieu Peninsula is promising (Irving 2009). Testing different hessian bag designs and arrangements will indicate the best conditions for recruitment of juveniles, while further development of polymer coatings will likely improve potential for the establishment of recruits and their long-term persistence. Another key but poorly understood question is whether the hessian recruitment units are suitable for other seagrass species (e.g. *Posidonia* spp.) that can be major floristic components of Australian seagrass meadows but have very different early life-history strategies to *A. antarctica*. The hessian bags likely provide a stable sediment environment that would encourage *Posidonia* spp. root growth and establishment, but since *Posidonia* spp. lack grappling hooks, they are highly unlikely to recruit naturally to bags and would perhaps instead need to be sewn in prior to bag deployment. Addressing these knowledge gaps will provide a solid foundation upon which the chances for successful seagrass rehabilitation are maximised, which will ultimately pay dividends given the substantial environmental and economic benefits of healthy seagrass meadows (Costanza et al. 1997).

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