Reproduction, recruitment, and growth of the seagrass *Amphibolis antarctica* near the Bungala and Yankalilla rivers, South Australia

Prepared for the Coastal Management Branch of the
Department for Environment & Heritage SA
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Management Board

Andrew Irving

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South Australian Research and Development Institute
SARDI Aquatic Sciences
2 Hamra Avenue
West Beach, SA 5024

Telephone: (08) 8207 5400
Facsimile: (08) 8207 5406
http://www.sardi.sa.gov.au

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Author(s): Andrew Irving

Reviewers: Sue Murray-Jones & Sunil Sharma

Approved by: Jason Tanner

Signed:

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EXECUTIVE SUMMARY

Potential impacts of the Bungala and Yankalilla rivers (Fleurieu Peninsula, South Australia) on the reproduction, recruitment, and growth of the seagrass *Amphibolis antarctica* were tested from March – May 2009. Two river (impact) and two control sites were established and sampled each month.

Reproductive output was assessed by harvesting adult plants from natural meadows and quantifying the number of attached juveniles. Few juveniles were recorded (a total of 14 individuals for the entire study) and no differences were detected between river and control sites. Furthermore, juvenile abundance did not correlate with adult shoot density, biomass, or height.

Recruitment was assessed by quantifying the number of juveniles attaching to artificial recruitment units comprising sand-filled hessian bags deployed on the sea floor. Recruitment was considerable at all sites during the study, but no consistent effect of rivers was observed. Recruitment to two different types of hessian bag (single vs double-layered bags) was tested, with the double-layered bags typically supporting greater numbers of recruits, which is consistent with similar tests off the metropolitan coast of Adelaide.

The growth of adult *A. antarctica* was assessed through the production of new leaves, while growth of juveniles was assessed as changes to the average height of recruits on hessian bags. In both cases, no consistent effect of river was detected, though a north-south spatial gradient in growth of adult plants was apparent.

Overall, the data suggest the Bungala and Yankalilla rivers had no appreciable effect on the reproduction, recruitment, and growth of *A. antarctica* during the study period. This outcome is not unexpected given the intermittent connectivity of these two rivers with the sea, and that both rivers were observed to be open to the sea only after the arrival of winter rain in late June/early July. As such, it would be worthwhile repeating the study during winter months when river-sea connectivity is likely to be more frequent and hence any potential impacts on marine life are most likely.

While this study sampled only a discrete period of time, such information may contribute to baseline data for this coastal region that is expected to undergo considerable growth of human populations in the coming years, and is therefore potentially at greater risk of human-mediated impacts. Additionally, these results show for the first time that rehabilitation efforts using the hessian bag technique are feasible outside of Adelaide's metropolitan waters.

1. INTRODUCTION

Human impacts on marine habitats have been widely publicised (Vitousek et al. 1997, Jackson 2001, Harley 2006), with many countries making concerted efforts to minimise further impacts and promote rehabilitation (McCay et al. 2003, Edgar et al. 2007, Elliott et al. 2007). One of the more promising and cost-effective methods for the rehabilitation of damaged marine biogenic habitats (e.g. coral reefs, kelp forests and seagrass meadows) is to enhance natural rates of recruitment by entraining and/or retaining recruits that would otherwise be lost from the system (Reed et al. 2004). Unfortunately, the likelihood that rehabilitation programs geared towards early life-history stages will be successful is often compromised because far less is known about their biology and ecology relative to adult life-history stages (Schiel & Foster 2006).

The solution is to obtain a thorough understanding of the reproductive cycle and early life-history of the target species. With such information at hand, rehabilitation projects can be timed for suitable periods (e.g. natural peaks in the reproductive output of the target species) and targeted at suitable locations (e.g. environments with suitable water quality) that will collectively improve the chances for success.

While a good understanding of reproductive and recruitment biology can help direct rehabilitation programs, it is important to understand how early life-history stages can be affected by human activities and impacts (e.g. reduced water quality). Indeed, it is usually because of some human impact that there is the need to rehabilitate damaged habitats in the first place. Knowing whether, for example, peaks in reproductive output and survival of recruits change in conditions of high turbidity or increased nutrient levels may not only redirect rehabilitation efforts to different times and places, but can also be useful for identifying future ‘at risk’ sites.

Given the propensity for human populations to cluster around coastal areas (Edwards 2003), and particularly along estuaries, these environments are prime candidates for exposure to a multitude of human impacts. The goal of this research was to understand how estuaries in rural catchments of the Fleurieu Peninsula, South Australia, may affect patterns of reproduction, recruitment, and growth of the common coastal meadow-forming seagrass *Amphibolis antarctica*. The two estuaries tested (Bungala and Yankalilla rivers) may be generally classified as being in moderate to poor condition based on their elevated concentrations of nutrients such as nitrate, nitrite, ammonia, and orthophosphate (Elsdon et al. 2009 and their unpublished data). Increased delivery of nutrients to seagrasses is often considered a major threat to seagrass abundance because of the overgrowth of seagrass

leaves by epiphytes and the subsequent reduction in seagrass photosynthesis and survival (Borowitzka & Lethbridge 1989, Shepherd et al. 1989). Even if only open intermittently, as the Bungala and Yankalilla estuaries are (Gillanders et al. 2008), there is still the potential for impacts on seagrass if, for example, pollutants such as heavy metals accumulate in coastal sediments during periods of connectivity (Burton et al. 2004, Lee et al. 2006). Furthermore, adult *Amphibolis* plants are reported to sustain developing juveniles for up to 8 – 12 months before releasing them (Kuo & den Hartog 2006), which allows substantial time for impacts not just on adult populations but also the next generation.

The specific aims of this work were to

1) Test whether the production of juvenile *A. antarctica* on adult plants differs between river (impact) and nearby control sites
2) Test whether the recruitment of juvenile *A. antarctica* to artificial recruitment units (hessian bags) differs between river and control sites
3) Test whether the growth of *A. antarctica* differs between river and control sites.

Results of this work provide some indication of the level of impact of the Bungala and Yankalilla rivers on *A. antarctica* populations, and also contribute baseline data in a coastal area where substantial human population growth is forecast. Additionally, this work improves our fundamental understanding of the ecology of early life-history stages of *A. antarctica*.

2. METHODS

2.1. Study sites

The study was done in shallow waters near the coastal town of Normanville on the Fleurieu Peninsula, South Australia. Two river (impact) and two control sites were chosen along the coast and were at least 1 km away from each other (Fig. 1). Both the Bungala and Yankalilla rivers were closed for the duration of the study (March – May 2009), but were observed to have opened in late June/early July with the arrival of the first substantial winter rain.

The seagrass meadows off Normanville primarily comprise *Posidonia* spp., *A. antarctica*, and *A. griffithii*, sometimes in a mixed-species configuration. Sand blowouts, as evidence of prior disturbance, occur within meadows though they are usually relatively small (between 2 – 5 m across) and reasonably infrequent (pers. obs.). All research was done between ~ 3 – 5 m depth where natural populations of *A. antarctica* could be found. Although *A. antarctica* occurs at shallower depths closer to the rivers of interest and therefore any potential impacts, this depth range was chosen so that experimental materials had less chance of being removed by storms and to allow workable conditions for SCUBA divers.

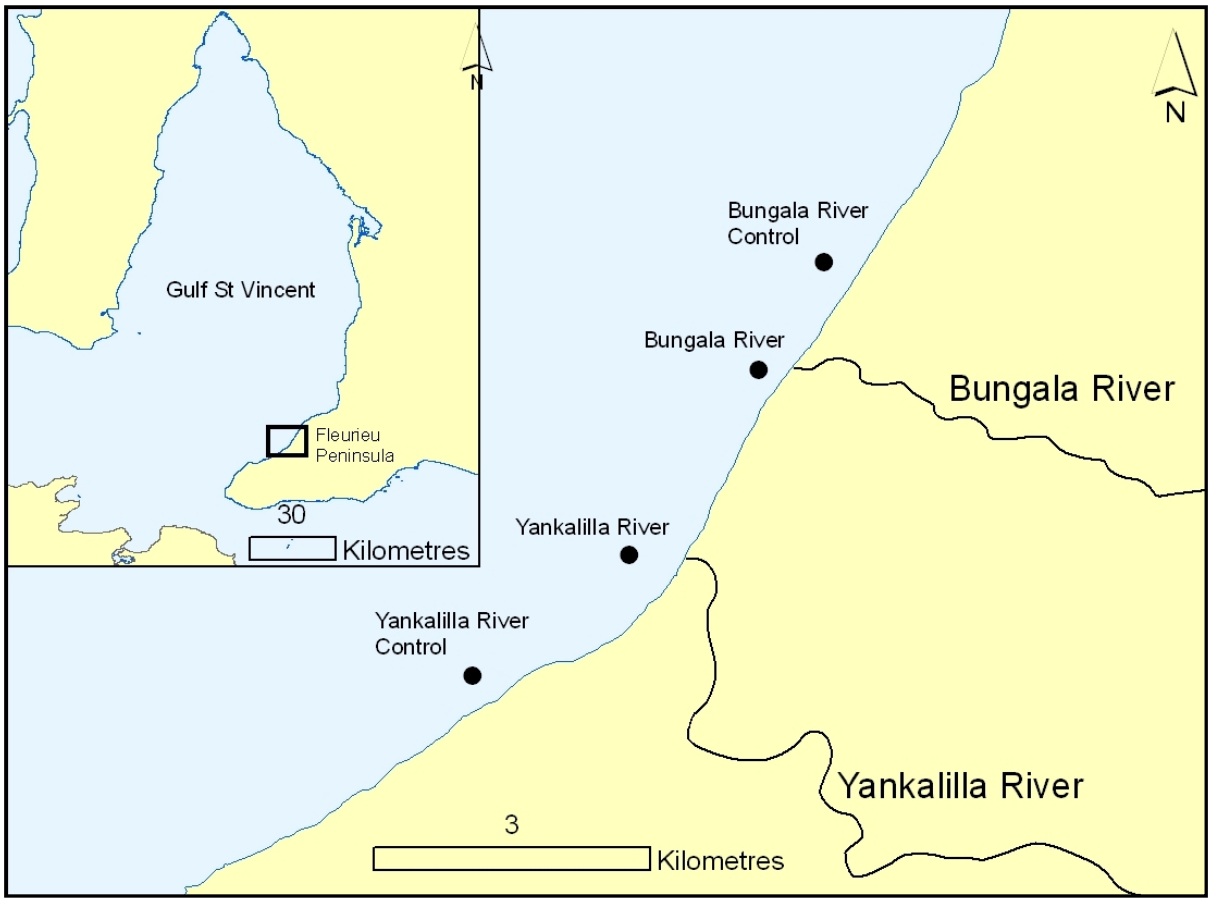

Figure 1. Map of the study region showing locations of the Bungala and Yankalilla rivers and the study sites (closed circles). Inset: map of Gulf St Vincent and the Fleurieu Peninsula, with the study region highlighted.

2.2. Reproductive output

Rather than producing seeds, *A. antarctica* reproduces by nurturing seedlings along its branches and then releasing them as free-living individuals, a process known as vivipary that is quite rare among aquatic plants (Kuo & den Hartog 2006). As such, it is relatively 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, patterns in the reproductive output of *A. antarctica* between river and control sites were assessed by harvesting natural populations of adult plants on a monthly basis and subsequently searching for attached juveniles. At each site, ten replicate quadrats (0.25 × 0.25 m) were haphazardly placed within natural *A. antarctica* meadows and all individuals within were cut at their base and collected in plastic bags. Samples were then processed in the laboratory by laying all adult plants on a tray and counting all attached or loose juveniles. At this time, the density, height, and epiphyte-free biomass of adult *A. antarctica* shoots sampled in each quadrat were also quantified to test whether these variables correlated with the observed abundance of juveniles.

2.3. Recruitment

Recruitment patterns between river and control sites were studied using artificial recruitment units that capture juvenile *Amphibolis* (both *A. antarctica* & *A. griffithii*). Sand-filled hessian bags were used as they have been shown to successfully facilitate recruitment by allowing the basal grappling hook structure present on juvenile *Amphibolis* to become entangled with the hessian fibres (Wear et al. 2006, Collings et al. 2007, Collings 2008). At each site, a patch of sand surrounded by seagrass was located and five single-layer and five double-layer hessian bags were positioned on the sea floor (Fig. 2; also see Wear et al. 2006 for a detailed description of bag types).

Each subsequent month, the number and height of *Amphibolis* recruits was quantified on all bags. Height was sampled by haphazardly selecting three individuals 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 (i.e. sampling three individuals allowed for within-bag variation in height to contribute to a single mean estimate of height per bag). Observations regarding the condition of hessian bags were also made at this time. While it was not possible to differentiate juvenile *A. antarctica* and *A. griffithii* in the field, it is likely that most, if not all, recruits on hessian bags were *A. antarctica* given this species occurred in far greater abundance at the study site than *A. griffithii*. Nevertheless, results for this test will be presented as the response of *Amphibolis* spp.

2.4. Growth

Differences in the growth of *A. antarctica* between river and control sites were primarily tested using leaf marking techniques (e.g. Short & Duarte 2001). Such techniques require the relocation of marked individuals through time, and it was therefore not feasible to mark juveniles that had recruited to hessian bags since they can be precariously attached until they establish a root system. Instead, the growth of adult plants in natural meadows was sampled as they are well-established and can be reliably relocated.

In April, ten adult plants at each site were marked by clipping the youngest leaf of the cluster present on the longest branch (as recommended by Short & Duarte 2001). Strands of flagging tape were then tied around the stem to facilitate relocation. On a subsequent visit (May), growth was quantified as the number of new leaves produced since the leaf that was clipped. Here, it was assumed that energy for growth would be evenly distributed among leaf clusters within each plant.

As a secondary estimate of plant growth, the average height of juveniles recruiting to hessian bags was sampled each month (see description above) and the change in height over time (i.e. growth) was compared among sites. This technique was chosen over leaf clipping because juveniles can be removed from bags by strong waves if they haven't established a root system, and so relocating leaf-clipped individuals over time is unreliable. Although the height of different individuals was probably measured each time, the data do provide an indication of the mean growth of juvenile *Amphibolis* on each bag, and any associated spatiotemporal variation.

Figure 2. 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.

3. RESULTS

3.1. Reproductive output

The overall abundance of juvenile *A. antarctica* sampled on adult plants was quite low, with only 14 individuals sampled over the duration of the study. No differences in the number of juveniles observed among sites and sampling times were observed (Fig. 3, Table 1), which is not surprising given the variation associated with such low numbers of juveniles.

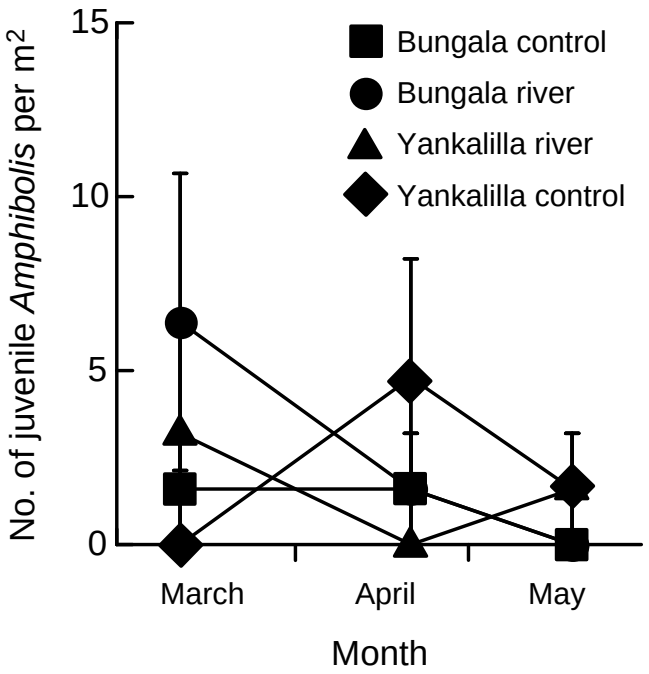

Figure 3. Mean (± SE) number of juvenile *A. antarctica* observed on adult plants among river and control sites over time.

Table 1. Results of two-way ANOVA testing for differences in the abundance of juvenile *A. antarctica* on adult plants among sites (Bungala control vs Bungala river vs Yankalilla river vs Yankalilla control) and sampling times.

Source	df	MS	*F*	*P*
Site	3	14.22	0.26	0.8521
Time	2	40.53	0.92	0.4019
Site × Time	6	54.76	1.24	0.2907
Residual	108	44.09		

When juvenile *A. antarctica* were observed in quadrats, only one or two individuals per quadrat were ever present (Fig. 4). Given the large number of samples containing no juveniles, it is not surprising that juvenile abundance did not correlate with adult shoot density, biomass, or height (Fig. 5, $P > 0.05$ for all tests). However, it is worth nothing that juveniles were never observed in samples with shoot densities greater than ~ 100 per 0.0625

m^2 (1600 per m^2; Fig. 5a) or when biomass was greater than ~ 75 g (dry) per 0.0625 m^2 (1200 g per m^2; Fig 5b).

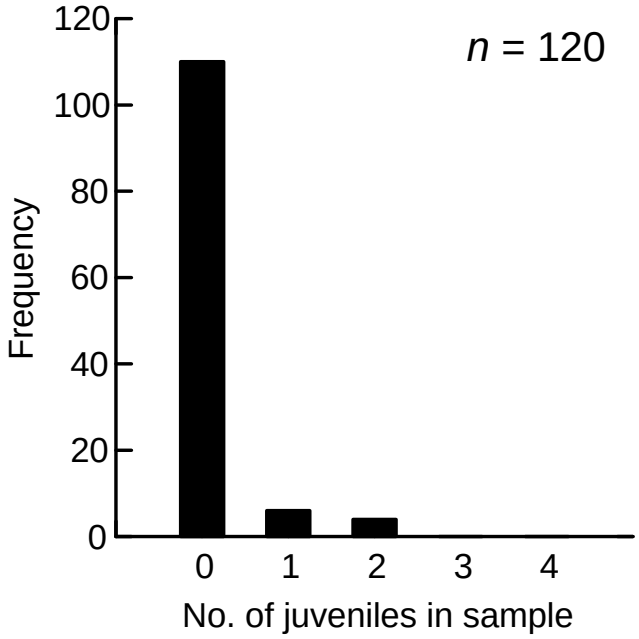

Figure 4. Frequency distribution of the number of juvenile *A. antarctica* sampled in quadrats (0.25 × 0.25 m). Note that data from all sites and sampling times have been pooled.

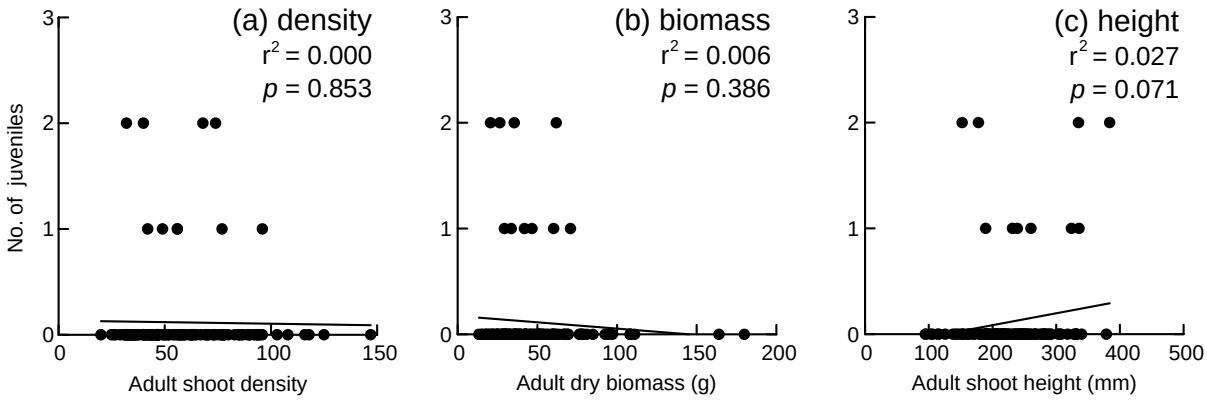

Figure 5. Linear regression of the abundance of juvenile *A. antarctica* found in samples (0.25 × 0.25 m quadrats) against (a) adult shoot density, (b) biomass, and (c) height. Note that data from all sites and sampling times have been pooled.

3.2. Recruitment

Recruitment of *Amphibolis* to hessian bags was observed at all sites, but was particularly strong at the Bungala control site, with densities averaging 236.5 ± 24.8 (SE) individuals per m^2 on double-layer bags (Fig. 6a). ANOVA detected interactive effects of bag type and site on recruit density, which was also dependent on time (Table 2a). In essence, no differences

were observed among sites or bag types during April (recruitment relatively low among all treatments), but during May, double-layer bags supported more seedlings than single-layer bags at all sites, with this difference particularly strong at the Bungala control site (Fig. 6a).

There was no evidence for an effect of river on *Amphibolis* recruitment. Even though final densities at both river sites were ~ 3 times less than at the Bungala control site, both were similar to the Yankalilla control site (~ 59 – 85 individuals per m^2) (Fig. 6a).

The height of seedlings on hessian bags varied between ~ 2 – 7 cm over the study (Fig. 6b). Seedling height was dependent on an interaction between site and bag type (Table 2b), whereby double-layer bags tended to support taller seedlings, especially at the Yankalilla control site (Fig. 6b). Again, there was no evidence for an effect of river on seedling height, with similar heights observed among sites within each bag type (~ 3 – 4 cm for single-layer bags, and 5 – 6 cm for double-layer bags).

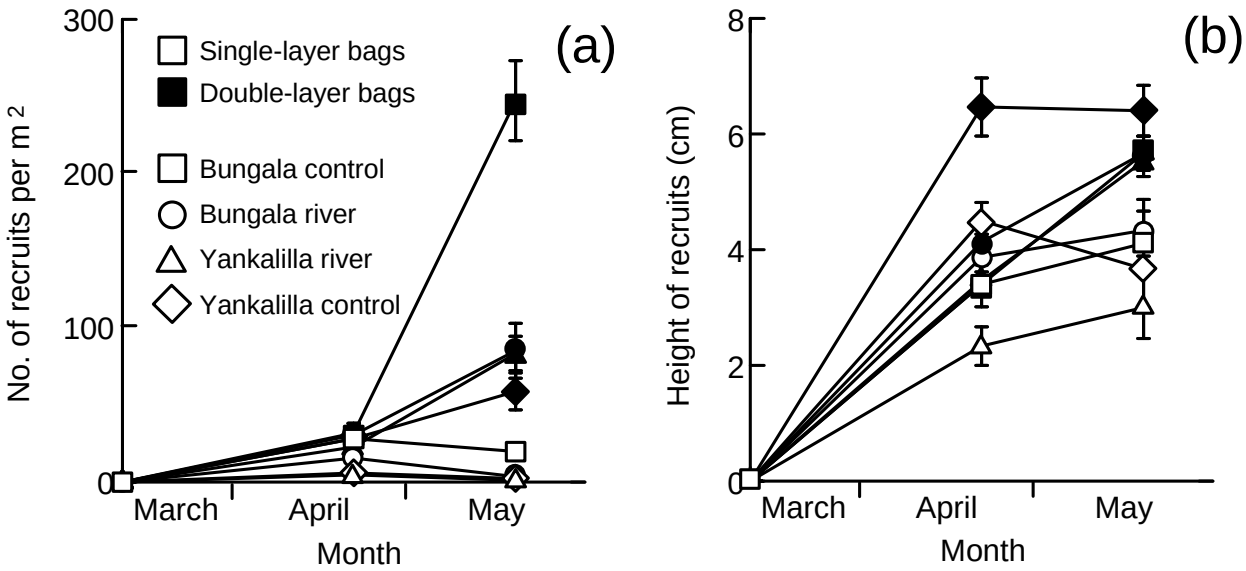

Figure 6. Recruitment (a) and height (b) of *Amphibolis* seedlings sampled on single and double-layer hessian bags at river and control sites. Note that single-layer bags are shown as open symbols, while double-layer bags are shown as filled symbols. Sites are represented as symbols of different shape (refer to legend).

Table 2. Results of repeated measures ANOVA testing the effects of site (Bungala control vs Bungala river vs Yankalilla river vs Yankalilla control) and bag type (single vs double-layer) on the (a) recruitment density, and (b) height of *Amphibolis* seedlings over time. Effects judged as significant are shown in bold type.

Response	Source	df	MS	*F*	*P*
(a) density	Within-subjects				
	Time	1	31837.37	82.50	**< 0.001**
	Time × Site	3	7941.35	20.58	**< 0.001**
	Time × Bag	1	43245.17	112.06	**< 0.001**
	Time × Site × Bag	3	8399.99	21.77	**< 0.001**
	Residual	32	385.90		
	Between-subjects				
	Site	3	13256.10	28.40	**< 0.001**
	Bag	1	73362.23	157.14	**< 0.001**
	Site × Bag	3	5645.40	12.09	**< 0.001**
	Residual	32	466.85		
(b) height	Within-subjects				
	Time	1	6.01	9.16	**0.006**
	Time × Site	3	1.89	2.89	0.059
	Time × Bag	1	6.01	9.16	**0.006**
	Time × Site × Bag	3	0.37	0.57	0.640
	Residual	22	0.66		
	Between-subjects				
	Site	3	4.05	7.37	**0.001**
	Bag	1	19.73	35.97	**< 0.001**
	Site × Bag	3	2.90	5.29	**0.007**
	Residual	22	0.55		

3.3. Growth

Production of new leaves (i.e. growth) by adult *A. antarctica* was observed at all sites, though the average number of new leaves produced differed among sites (Fig. 7, Table 3). Upon examination, a spatial gradient in leaf production was evident, with a clear decline from north to south (Fig. 7). The occurrence of this gradient, coupled with variability in the data, meant that the precise nature of differences among sites could not be unambiguously determined by *post-hoc* tests (Tukey pair-wise comparisons). Nevertheless, any consistent effect of river is not supported by these data.

As a secondary estimate of growth, changes to the average height of juveniles recruiting to hessian bags were generally positive (i.e. indicating growth) with the exception of recruits at the Yankalilla control site (Table 4). No evidence for an effect of river was observed, with differences among sites instead suggesting random spatial variation.

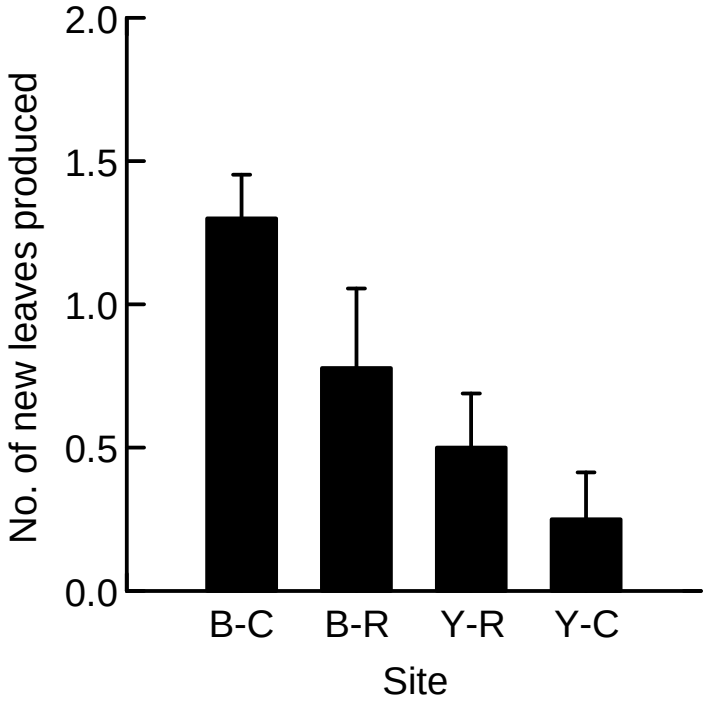

Figure 7. Production of new leaves by adult *A. antarctica* at different sites. Data are mean ± SE. B-C = Bungala control, B-R = Bungala river, Y-R = Yankalilla river, Y-C = Yankalilla control. Note that sites are plotted from the northern-most site (B-C) to the southern-most site (Y-C).

Table 3. Results of ANOVA testing the effect of site (Bungala control vs Bungala river vs Yankalilla river vs Yankalilla control) on the production of new leaves by adult *A. antarctica*. Effects judged as significant are shown in bold type

Source	df	MS	F	P
Site	3	1.84	5.12	**0.005**
Residual	31	0.36		

Table 4. Summary data of the change in the average height (cm) of juvenile *Amphibolis* recruiting to hessian bags. Data were pooled across bag types. Note that different individuals were likely sampled between times, such that apparent negative growth is possible if, for example, recruitment of small individuals occurred between sampling times.

	Bungala control	Bungala river	Yankalilla river	Yankalilla control
21st-April-2009	6.80	8.00	5.80	10.97
19th-May-2009	9.78	10.00	8.53	10.07
Change (cm)	2.98	2.00	2.73	-0.90
Change as %	43.79	25.00	47.13	-8.21

4. DISCUSSION

The results of this research suggest that the Bungala and Yankalilla rivers had no appreciable effect on the reproduction, recruitment, and growth of *A. antarctica* during the study period (March – May 2009), even though both the Bungala and Yankalilla rivers can be classified as being in moderate to poor condition (Elsdon et al. 2009). For all variables tested, the data from samples taken near rivers were typically within the range of data from control sites (Figs 3, 6 & 7). The absence of any river effect supports recent work showing nearby seagrass meadows posses good habitat structure (i.e. extensive and dense meadows of high quality species; Murray-Jones et al. in prep.), and that they appear to be in relatively good condition throughout the study area.

The absence of any significant effect of the Bungala and Yankalilla rivers in this study is perhaps not unexpected since the mouths of both rivers appeared to remain closed from the beginning of the study until the arrival of winter rain in late June/early July (author's pers. obs.), which is consistent with the intermittent nature of river-sea connectivity in this region (Gillanders et al. 2008). Thus, the flow of both rivers to the marine environment, and the delivery of any potential impact, was likely negligible during the sampling period (note that possible groundwater flow into the marine environment was not examined). It would be worthwhile repeating this study during winter months when increased rainfall may open both rivers to the sea for longer periods and readily transfer potential impacts. Rough weather and poor underwater visibility during this time would provide significant challenges (indeed, attempts to sample during winter were unsuccessful in the present study), but it nonetheless appears the most likely time to observe effects of these rivers on seagrass meadows, if they occur.

It is possible that when a river does open to the sea, inputs may occur that produce long-term impacts (e.g. accumulation of heavy metals in the sediment may produce impacts after the river has again closed; i.e. a 'protracted pulse' response as described in Glasby & Underwood 1996). However, the similarities between river and control sites in this study suggest that even if such long-term inputs are present at the study sites, they are not yet adversely affecting *A. antarctica* populations. Nevertheless, it is possible for such impacts to reveal themselves at a future date, and so the results of this study may contribute to baseline information about the ecology of *A. antarctica* meadows in this region.

The overall reproductive output of *A. antarctica* appeared quite low during the study period, with only 14 juveniles sampled from harvests of adult populations. In contrast, substantial recruitment of juveniles to hessian bags (up to a mean of ~ 236 individuals per m^2) indicates

an abundant supply of juveniles. This apparent mis-match between juvenile densities on bags compared to adults suggests that many juveniles had detached from adults and were dispersing through the meadow. By extension, it is possible that the deployment of hessian bags was fortuitously timed to 'sample' a recruitment pulse in the region (~ May), whereby harvesting of adults began shortly after they had released most of their juveniles. If so, the recruitment pulse would be asynchronous to that observed on Adelaide's coasts, which occurs from ~ June/July to September (Irving et al. 2009). Currently, reproductive and recruitment cycles of *A. antarctica* in the study region are poorly understood, and so it would be instructive to continue sampling the production of juveniles throughout the year and even over multiple years in this region to determine whether any consistent annual peaks in reproductive output occur. This knowledge would not only improve our grasp of the natural history of *A. antarctica*, but would also be critical if rehabilitation efforts are proposed for the region and such differences in timing of recruitment consistently occur over relatively short distances (Adelaide – Normanville).

When juveniles were found on adult plants, it was never more than two individuals per adult shoot (Fig. 4). This result contrasts with sampling off Adelaide's coast, where are many as 15 juveniles per adult shoot have been recorded (Irving, unpublished data). Plants off Adelaide are generally taller and larger (biomass) than those sampled in the present study, suggesting the quantity of juveniles may depend on adult size. To this end, no relationship was observed in the current study between the number of juveniles and either adult height or biomass (or density; Fig. 5). Since so few juveniles were observed, however, it is worth exercising caution with this interpretation, particularly if adults had just shed the majority of their juveniles (i.e. a recruitment pulse, as described above). Further sampling would be useful to establish the robustness of the current data.

While recruitment of *A. antarctica* to hessian bags appeared unaffected by rivers, a consistent pattern among sites was that double-layered bags facilitated greater recruitment than single-layered bags. Possessing greater structural heterogeneity, double-layered bags are probably more effective at capturing recruits because the coarse outer-weave of hessian appears to be an easier surface for the grappling hooks of juveniles to become entangled with. While this may be so, there is some concern that because the outer-weave can be somewhat loosened by wave action, the double-layered bags do not retain recruits as effectively as single-layered bags, which may negate any benefits of the initial increase in recruitment (Wear et al. 2006). Further development of the hessian bag technique will likely remedy this issue.

The level of recruitment observed in this study also suggests that the hessian bag technique would be an appropriate option for seagrass rehabilitation programs in formerly untested waters away from the metropolitan coast of Adelaide. Such results are promising for other rural coasts that have experienced substantial seagrass loss and may be strong candidates for rehabilitation programs using hessian bags in the near future (e.g. Beachport, South Australia).

Growth of adult and juvenile *A. antarctica* was observed at all sites except for juveniles at the Yankalilla control site (Table 4), which may represent an artefact of the sampling design (see Methods). For adults, a north-south gradient in growth was observed (growth increasing northwards) instead of any effects of either river (Fig. 7), which was not overly apparent for the other variables tested. While no data currently exist to explain this pattern, diver observations suggest that this gradient correlates with the overall abundance of *A. antarctica* meadows at the study site (greatest abundance northwards), which in turn appears to reflect a shift in substratum composition from predominantly sandy in the north to increasing abundance of large cobbles and reef further south. It is therefore possible that *A. antarctica* located in sandy substrata grow faster and are hence more abundant. This hypothesis is yet to be tested.

In conclusion, this study indicates that the Bungala and Yankalilla rivers had no effect on patterns of reproduction, recruitment, and growth of *A. antarctica* during the period of March – May 2009. Overall, the seagrass meadows of this region appear to be in quite good condition (Murray-Jones et al. in prep., and diver observations) and based on the current evidence, there is no reason to suspect the Bungala and Yankalilla rivers have had substantial short- or long-term impacts on *A. antarctica* populations of the region in recent times. Nevertheless, the region is forecast to experience substantial human population growth in the coming years, which will likely put increased strain on these rivers (e.g. increased nutrient loading) that could increase their likelihood of having some impact on seagrass populations. Therefore, the data presented in this study may contribute to baseline information on the ecology and condition of *A. antarctica* meadows of the region before such changes to population density occur.

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