Seagrass Rehabilitation in Adelaide Metropolitan Coastal Waters
II. Development of Donor Bed Independent Methods using *Posidonia* seedlings

Prepared for

Coastal Protection Branch
Department for Environment and Heritage

by

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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. Current improvements to wastewater management, particularly in terms of nutrient loads, have led to the view that seagrass rehabilitation may now be possible in some areas. The main objectives of this study were (1) to investigate the use of seagrass seeds and/or seedlings as a source of propagules for seagrass rehabilitation, and (2) to assess the success of transplanting seagrass seedlings as a means of fast-tracking the natural regeneration of seagrass beds and accelerating the process of succession from fast-growing colonising species (such as *Halophila* spp. and *Heterozostera tasmanica*) to slower-growing, and more persistent climax species (i.e. *Posidonia* spp.).

Sexual reproduction in *Posidonia* was monitored during November, December and January in 2001/02, 2002/03, 2003/04 and 2004/05. Reproduction varied annually with high numbers of fruits observed along the Adelaide metropolitan coast during November, December and January in 2001/02, 2003/04 and 2004/05, but not during 2002/03, despite extensive searches. This observed variation in the timing, location and intensity of flowering and fruiting is consistent with studies on *Posidonia* elsewhere and maybe associated with varying water temperatures (e.g. below average temperatures over the previous year may reduce the incidence of flowering) and other environmental factors such as light availability and nutrient status, highlighting the need for further research to establish causal links.

Preliminary investigations to explore the possibility of culturing seedlings for use in seagrass rehabilitation began in summer 2001/02 (prior to the start of Phase I of this research program). From opportunistic collections of *Posidonia* spp. fruits sourced from beaches and directly from seagrass meadows, the seedlings released from these fruits were planted into biodegradable Jiffy pots and cultured in a flow-through seawater system at the South Australian Aquatic Sciences Centre (SAASC), resulting in over 95% survival six weeks after planting into pots. These seedlings successfully grew for approximately 11 months in culture with the shoots remaining in good condition and the roots well developed and aerated. Following on from these initial results, seedling culture was investigated in more detail during the summer of 2003/04.

In contrast to the results from the 2001/02 summer, seedling survival in 2003/04 was low (8% after 11 months in culture) and for those that survived, growth was variable, though generally slow. The reduced seedling survival is most likely related to effects of excessive algal growth in combination with the level of shading over the tanks, where 90% shade cloth was used to simulate the light environment at the proposed planting sites. Additionally, in an attempt to reduce the biomass of epiphytic algae, the seedlings were cleaned manually and various invertebrate grazers were introduced into the tanks, both of which may have contributed to poor

seedling survival.

Comparing the results from the two trials in 2001/02 and 2003/04, it is apparent that the main issue of focus regarding the successful culture of *Posidonia* seedlings is the low survival rate of the seedlings rather than their inability to grow. If trials of *Posidonia* seedlings are to be continued at some stage in the future, it is recommended that:

- Nutrient intake from the SAASC seawater supply is controlled (e.g. lowering flow rates and using pumps to provide water circulation and/or employing a system that can strip out excess nutrients such as an algal scrubber).
- Excessive algal growth is controlled with the use of appropriate invertebrate grazers rather than manual cleaning (e.g. tanks should be “seeded” with invertebrate grazers, with a preference for algae over seagrass, 3 to 6 months before seedlings are introduced).

At this stage, establishing a nursery to hold seedlings in preparation for planting-out at selected rehabilitation sites along the Adelaide Metropolitan coast appears to be an impractical approach to wide-scale seagrass rehabilitation. Nevertheless, the ability to collect fruits once washed up on the metropolitan beaches and hold the resulting seedlings in culture for future use could be very beneficial. For example cultured seedlings could be used to augment natural recruitment in some areas, or to accelerate (fast-track) the natural process of succession in areas that are starting to be recolonised by fast-growing species such as *Heterozostera tasmanica* and *Halophila* spp. In this respect, the development of rehabilitation techniques utilising seagrass seedlings potentially represents an important component of coastal management.

1. BACKGROUND

Seagrass meadows along the Adelaide metropolitan coast have suffered considerable declines over recent decades (Shepherd *et al*. 1989; Hart 1997; EPA 1998). The overall pattern of decline has occurred through losses on the inner edge of seagrass meadows (i.e. meadows have receded seaward), in combination with fragmentation of beds leading to ongoing erosion (Figure 1; EPA 1998; Fotheringham 2002). These losses have commonly 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).

Recognition of the need to improve water quality in Gulf St Vincent through reductions in nutrient loads and stormwater discharge has led to considerable ongoing improvements in catchment and sewage wastewater management. SA Water is undertaking an environmental improvement program aiming to reduce their discharge of nitrogen into Gulf St Vincent by 71% (Hamilton 2002). These improvements are likely to enhance seagrass growth and survival in the future and signs of seagrass recolonisation on previously impacted areas have recently been reported (Bryars & Neverauskas 2002; Bryars & Neverauskas 2004). It is largely as a result of these improvements in water quality that research and development toward seagrass rehabilitation in the Adelaide metropolitan region is now worthwhile.

In May 2001, SARDI Aquatic Sciences and the Coast and Marine Branch initiated and held Australia's first workshop on seagrass restoration to review the current status and knowledge in Australia and overseas (Seddon & Murray-Jones 2002). The workshop demonstrated that to date, techniques used in seagrass restoration and rehabilitation[1] are extremely labour intensive or require the use of expensive machinery designed for major dredging operations where large areas of seagrass need to be salvaged and transplanted (e.g. van Keulen & Paling 2002) and are therefore not ideal for large-scale seagrass rehabilitation off Adelaide. Following the workshop, a three-phase seagrass rehabilitation research and development program over nine years was developed (as outlined in Seddon *et. al.* 2004). The ultimate objective of the program is to develop reliable techniques for seagrass rehabilitation suitable for application along the Adelaide metropolitan coast.

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[1] Seagrass 'restoration' refers to returning a meadow to its pre-existing condition (i.e. same species composition, distribution, abundance and ecosystem function); whereas seagrass 'rehabilitation' is a more general term implying the return of seagrass to an area where seagrass meadows previously existed (but not necessarily the same species, abundance or ecosystem function).

There are three different approaches to rehabilitation that are being investigated in the first phase of the program, these include:

1. Donor dependant methods: These methods involve the collection of mature seagrass from donor meadows for transplanting to areas of seagrass loss (reported in Seddon *et al.* 2004).

2. Donor independent methods: In the context of this study, this method involves the collection of seagrass fruits and their subsequent culture and grow-out as seedlings in preparation for planting at appropriate rehabilitation sites. Planting seedlings, if successful, is a very promising method as their use avoids damaging existing seagrass meadows and potentially provides a reliable and ongoing source of seagrass material for the future. This method would also be more amenable to community participation.

3. Recruitment facilitation methods: These techniques will be aimed at maximising the level and success of natural recruitment events.

This report focuses on ‘donor independent methods’, and outlines early research into the use of *Posidonia* seedlings in rehabilitation along the Adelaide coastline.

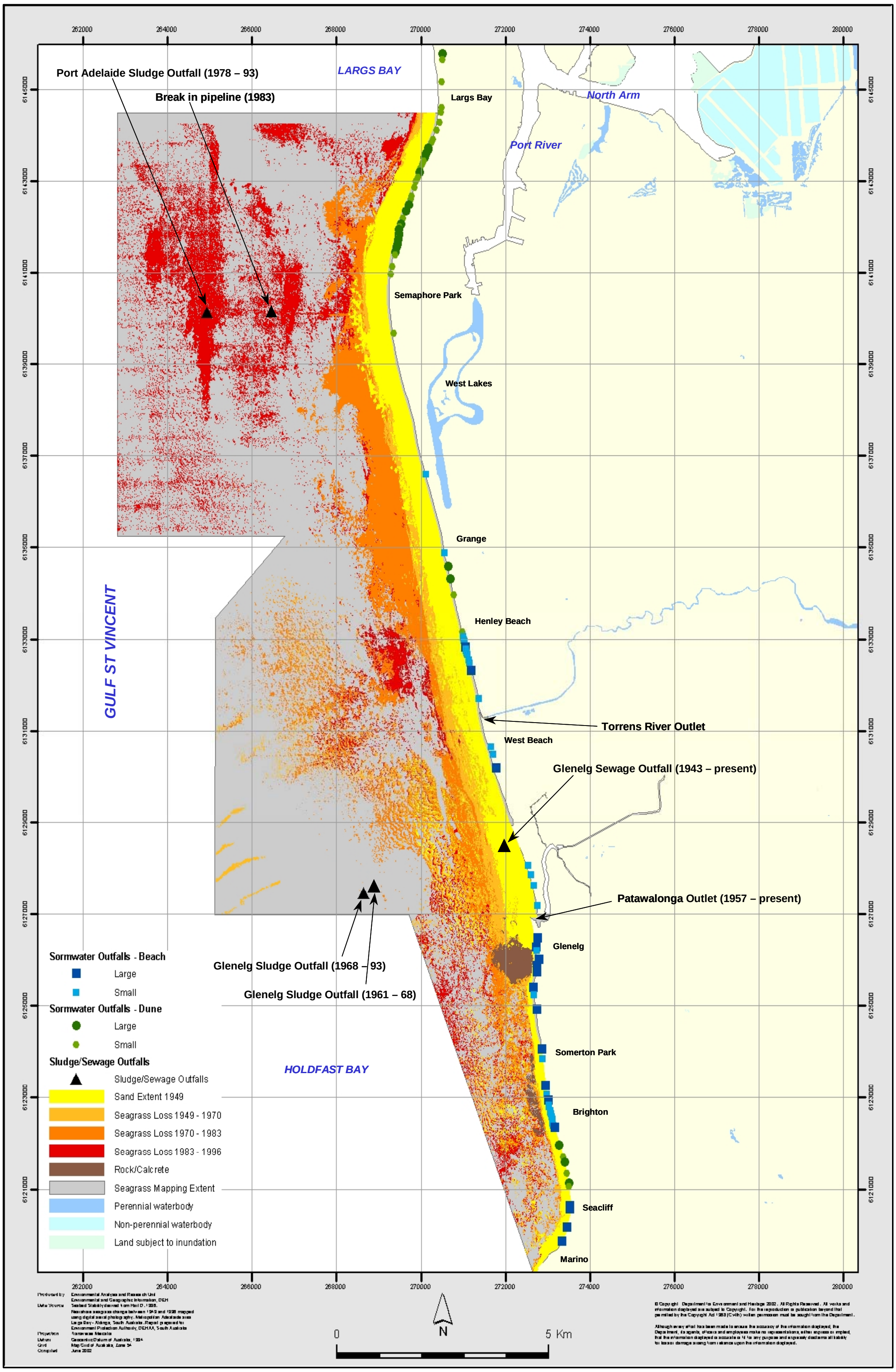

Figure 1. Map of the Adelaide metropolitan coast showing the extent of seagrass loss between Largs Bay and Marino from 1949 to 1996 with grey areas representing the presence of seagrass. The location of input sources is also indicated. Image from Seddon (2002) constructed by Tim Noyce care of the South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

2. INTRODUCTION

Seagrasses have a variety of important functions. Not only do they increase the stability of the seafloor through the growth of extensive rhizome mats (Fonseca & Fisher 1986), but they play a critical role in primary production, including the harnessing and cycling of nutrients (Hillman *et al.* 1989), in addition to providing valuable habitat for a diverse array of marine organisms (Bell & Pollard 1989; Short & Wyllie-Echeverria 1996; Connolly *et al.* 1999; Duarte 2002). Unfortunately there have been significant losses of seagrass habitat worldwide (e.g. Short & Wyllie-Echeverria 1996), which has led to an increase in research focusing on the development of techniques for seagrass restoration and rehabilitation. While there has been considerable activity invested in restoration efforts over 20-30 years in some locations (see Fonseca *et al.* 1998), research of this kind in South Australia is far more recent, despite losses along the Adelaide coast being recorded as far back as 1968 (Figure 1; Shepherd 1970). Across Australia in general, much of this research has centred on various species of *Posidonia*, primarily because various species within this genus have sustained significant losses in temperate regions (e.g. Kirkman 1978a; Cambridge & McComb 1884; Cambridge *et al*. 1986; Walker *et al*. 1989; Seddon *et al*. 2000) and perhaps also because these 'climax' species are considered more valuable in terms of maintaining seabed stability and their long-term persistence.

To date, restoration and rehabilitation efforts using *Posidonia* have had variable success. In most cases these techniques have assessed the survival of the transplanted seagrass, which in many instances, has been poor over the long-term. For example, in a study by Paling *et al.* (2003), the survival of *P. coriacea* transplants was over 90% four months following transplantation, but dropped to between 9% and 40% after 14 months. Similarly, in Botany Bay the survival of *P. australis* transplants varied between 60 to 70% in the first three months; however, after six months most of the transplants had washed away (West *et al*. 1990). Nevertheless not all studies have reported such poor transplant survival, for example Paling *et al*. (2001) recorded survival rates of 77% and 76% for *P. sinuosa* and *P. coriacea* respectively two years after transplantation. Transplant success has also proven to be site dependent. Over the short term (< 6 months), Meehan and West (2002) found that survival of *P. australis* transplants was high at four out of five of their planting sites, although over the longer term (16 months), transplants only survived at three of these sites. However at these three sites the transplants grew quite well, with those at the most successful sites showing a three-fold increase in the total shoot number. Meehan and West (2002) attributed poor survival at the remaining two sites to factors that caused seagrass loss at these sites in the first place (e.g. seabed instability and erosion). From these studies it seems that variability in the survival of transplants reflects a combination of factors including planting technique, local conditions and whether the causes for the original loss of seagrass are still present in the study area.

Rehabilitation efforts targeting the use of mature *Posidonia*, such as those outlined above, not only tend to generate variable results, but they are also labour intensive and therefore expensive. As a result, these techniques are really only suitable for application in relatively small areas i.e. several hectares at most (Seddon 2004). Such methods also rely on sourcing seagrass from existing *Posidonia* beds, which are reported to have extremely slow recovery rates, if they recover at all (Kirkman & Kuo 1990; Cambridge *et al*. 2000; Meehan & West 2000). While research to date has suggested that *Posidonia* seedlings are extremely slow growing (e.g. Meehan & West 2004), the use of seedlings in restoration efforts offers advantages in that it avoids impacting donor beds and large quantities of propagules (in this case seedlings) can be quickly and easily obtained. While preliminary studies have been done in Western Australia and overseas, studies of this nature have not been undertaken in South Australia.

The successful use of *Posidonia* seedlings in rehabilitation depends upon information about the reproductive biology and ecology of this genus. Unfortunately, there is very little data of this type in South Australia and consequently little is known about the timing and frequency of *Posidonia* flowering and fruiting. Most of the information we do have is from Western Australia, and as pointed out later in this report, observations of the timing and frequency of flowering do not necessarily concur with those in South Australia. Regrettably, the pioneering paper by Clarke (1988) reporting important observations and experimental results of *Posidonia* seedlings in Holdfast Bay was never resubmitted for publication and we have been unable to locate a copy of the unpublished manuscript (including searching through the author’s files and contacting the editor of *Aquatic Botany*). Consequently, most of our current knowledge on seagrass reproduction in South Australia (particularly timing and location of flowing and fruiting) is summarised by Robertson (1984).

From these limited sources of information, it appears that most species of *Posidonia* start flowering by late winter or early spring and then release fruits in summer (Table 1). If fruits are to be collected and utilised for seagrass rehabilitation it is essential to determine when fruits are ready for release. Kirkman (1998) invested considerable effort in studying the timing of fruit maturity in Perth metropolitan waters before starting pilot trials to grow and plant-out seedlings. For this current study, searches were carried out in October 2001 through to January 2004 to identify if and where fruiting was occurring in Adelaide metropolitan waters.

This report outlines early observations and research into the collection and grow-out of *Posidonia* seedlings for use in rehabilitation along the Adelaide coastline. The main objectives of the project were to investigate the use of seagrass seedlings as a source of propagules for seagrass rehabilitation, and to assess the effectiveness of transplanting seagrass seedlings as a means of accelerating the successional process.

Table 1. General timing of seagrass flowering and fruiting for species commonly found along the Adelaide metropolitan coast (shown as pale grey bars). Data compiled from Robertson (1984).

	June	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
Amphibolis antarctica												
Amphibolis griffithii												
Posidonia angustifolia												
		**										
Posidonia australis				***		14th *						
Posidonia coriacea							5th *					
		**										
Posidonia sinuosa					***		10th *					
Halophila ovalis												
Halophila australis												
Heterozostera tasmanica												
Zostera capricorni												

* Mature fruits collected in Western Australia (Kirkman 1998)
<u>**</u> *P. australis* and *P. sinuosa* **flowering** in Western Australia (Smith & Walker 2002)
<u>***</u> *P. australis* and *P. sinuosa* **fruiting** in Western Australia (Smith & Walker 2002)

3. SUMMER 2001/02

3.1. Methods

Observations of fruiting along the Adelaide metropolitan coast were made from November 2001 through to January 2002. During this time, *Posidonia* fruits were opportunistically collected from Henley and Grange beaches on the 27th November 2001 and the 8th January 2002. Fruits were also collected directly from seagrass meadows at Kingscote, Kangaroo Island, on the 27th of November 2001 (B. McDonald and J. Tanner, pers. comm.) and at Port Pirie on the 18th of December 2001. Once collected, all *Posidonia* fruits were kept in seawater and transferred to fibreglass holding tanks (500 L) supplied with flow-through seawater at the South Australian Aquatic Sciences Centre (SAASC). Fruits were sorted into species and maintained in separate floating plastic containers within the holding tanks (Figure 2). Each holding tank was positioned in direct sunlight and shaded with 70% shade cloth covers (see Section 4.1.1). Fruits were monitored for dehiscence[2], after which the seeds were kept in the containers until developing shoots had grown at least several centimetres long and there was evidence of root development (Figure 3).

Figure 2. Beach collected *Posidonia sinuosa* fruits enclosed within a floating container suspended in one of the 500 L holding tanks in the flow-through aquarium facility at the South Australian Aquatic Sciences Centre.

[2] Dehiscence is defined as the spontaneous opening of the seagrass fruit to release the seed.

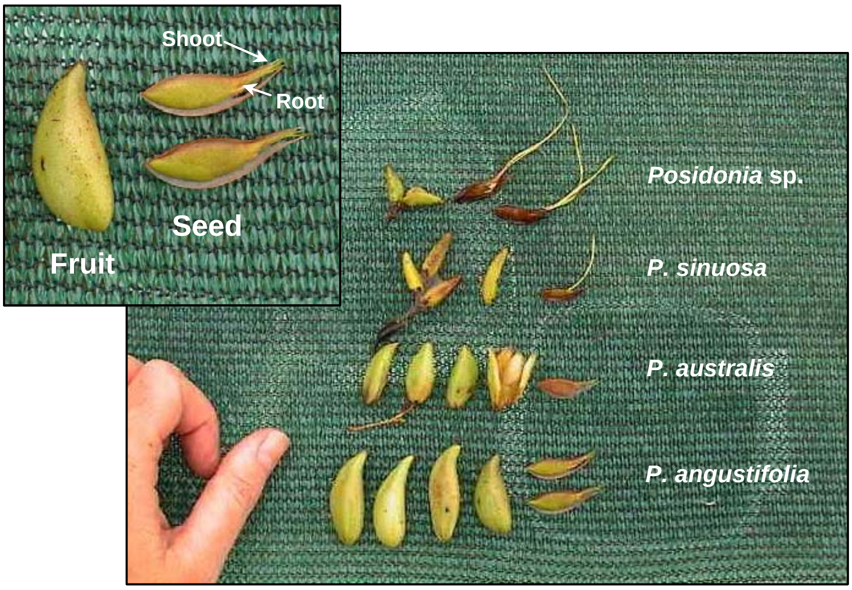

Figure 3. *Posidonia* fruits, seeds and seedlings collected from the metropolitan beaches in December 2001. Insert: A fruit and two germinated seeds showing developing shoots (originating from the plumule consisting of several leaf primordia) and roots (originating from the root primordia).

On the 9th of January, a sub-set of *P. australis* seedlings were planted into biodegradable square-conical Jiffy pots (6 x 6 x 15 cm; Jiffy International AS, Drobak, Norway) with beach sand in order to test the suitability of these pots for culturing seedlings (Figure 4). Three seedlings were planted in each pot and a total of 30 pots were used (90 seedlings in total). Seedlings were planted in groups of three rather than individually on the assumption that the greater volume of roots would oxygenate the sediments more effectively, thus encouraging better root growth and development.

Figure 4. Square-conical Jiffy pots supported in a plastic tray (photograph courtesy of Jiffy International).

Once the pots were positioned in the holding tanks, two 100 L h^{-1} submersible pumps (Project 100, Italy) connected to timers were installed at opposite ends of each tank to provide alternating reversed currents to simulate tidal movement (i.e. 6 hr on/off cycle). After 7 weeks (49 days) the seedlings were assessed for survival, number of leaves per seedling and seedling height.

In addition to the experimental seedlings, on the 22 January 2002 surplus seedlings were planted into two other types of Jiffy pot, supplied to us as samples by the manufacture: Nine round pots (10 cm diameter x 10 cm height) with 7 seedlings per pot; and 17 square-squat pots (5 x 5 x 5 cm) with 4 seedlings per pot. While the seedling experiments were discontinued at the end of February 2002, in order to test the durability and suitability of the Jiffy pots for long-term use in seawater (i.e. Jiffy pots are designed for use on land not underwater!), all pots (square-conical, square-squat and round) complete with seedlings were maintained in the flow-through aquarium facility at SAASC for an additional 267 days (approximately 11 months in total), after which observations of their growth and the state of the Jiffy pots were recorded.

3.2. Results and Discussion

The period from November 2001 through to January 2002 was a time of prolific fruiting of *Posidonia* in South Australia. Extensive quantities of fruits formed a large part of the wrack washed-up along the metropolitan beaches, particularly following strong onshore winds. As a result, a total of 1,317 *Posidonia* spp. fruits were collected on the 27th November from Henley and Grange beaches and Kingscote (Kangaroo Island). Additionally several hundred fruits were opportunistically collected at Port Pirie (Fifth Creek) on the 18th December and metropolitan beaches on the 8th January.

The percentage of fruits that dehisced varied substantially depending on date and site of collection. Dehiscence of fruits collected at the end of November ranged from 0.5% to almost 50% (Table 2). By contrast, the majority of *P. angustifolia*, *P. sinuosa* and *P. australis* fruits collected in mid December 2001 and early January 2002 successfully dehisced, releasing viable seedlings (Table 2). There are a number of factors that are likely to have contributed to variation in the percentage of fruits that dehisced, including the condition of beach collected fruits and fruit maturity. Controlled experiments would be required to determine the effects of various factors, such as desiccation and heat exposure, on fruit dehiscence and seedling viability. But perhaps more significantly, since dehiscence was more successful for fruits collected later in the reproductive season (December and January) it is possible that a high proportion of the fruits collected earlier in the season were immature.

Table 2. Observations of the time taken for *Posidonia* fruits to dehisce since collection. Fruits were collected either from the beach or directly from meadows using a beam trawl (nd = no data). Most = most fruits dehisced but a percent value was not obtained. *****Three replicate containers per tank, accordingly standard errors (SE) are given.

Collection Site / Spp.	**n**	**Date collected**	**# Days after collection**	**% Fruits dehisced (± SE)**
Posidonia australis				
Henley/Grange Beach (from beach)	300*	27-Nov-01	0	0
			6	34 (0.04)
			10	45 (0.02)
			43	46 (0.00)
Posidonia sp.				
Henley/Grange Beach (from beach)	529*	27-Nov-01	0	0
			6	5.33 (0.01)
			10	9.33% (0.03)
Posidonia sinuosa				
Henley/Grange Beach (from beach)	198	27-Nov-01	0	0
			6	0.5
			10	0.5
Posidonia australis				
Henley/Grange Beach (from beach)	33	27-Nov-01	0	0
			6	3
			10	9
Posidonia australis				
Kingscote, Kangaroo Island(beam trawl)	257	27-Nov-01	0	0
			21	13
Posidonia angustifolia?				
Fifth Ck, Port Pirie (beam trawl)	nd	18-Dec-01	0	0
			17	most
Mixed *P. australis* & *P. sinuosa*				
Metropolitan coast (from beach)	nd	08-Jan-02	0	0
			45	most

Fruits from two of the containers where the fruit densities were high rotted rather than dehisced over the Christmas break. Interestingly, however, the seedlings contained within these fruits were still viable, although the shoots were paler green compared to seedlings in other containers, presumably due to lack of light. Accordingly the developing seedlings were divided into smaller groups in separate containers to allow for effective water circulation and greater exposure to light. These results suggest that there are two possible methods for seed release; firstly, dehiscence and secondly, rotting away of the fruit (i.e. pericarp) from around the seed. The second method makes ecological sense since fruits washed-up on the beach, or into blowouts and depressions, often get buried amongst piles of seagrass mat and other debris, providing ideal conditions to promote fruit rotting. If seedlings can survive a period of burial, then they have the potential to remain viable until the debris is eventually washed away during strong swell or high tides.

Approximately six weeks (49 days) after planting the seedlings into Jiffy pots, seedling survival of *P. australis* was just over 95%, with 86 out of the original 90 seedlings surviving and appearing healthy. On average seedlings had 2.9 leaves and varied between 7.3 cm and 7.9 cm in height (Table 3), with a maximum height of 12 cm.

Table 3. Growth of *Posidonia australis* seedlings in the flow-through aquaria at SAASC, after planting into square-conical jiffy pots. Values are means, SE = standard error, n = 10.

Group	# Leaves / Seedling	SE	Seedling Height (cm)	SE
1	2.89	0.00	7.26	0.01
2	2.97	0.00	7.85	0.01
3	2.86	0.00	7.41	0.01

After an additional 267 days in culture, the seedlings were examined primarily to assess the suitability of Jiffy pots for further trials. Observations of the roots growing through the base of some pots (Figure 4a and b) and aerated sediments within the pots (Figure 5a and b), demonstrated that the seedlings were capable of successfully growing in the Jiffy pots. The fact that the roots were able to grow through the biodegradable material strongly indicates that if the whole Jiffy pot and seedlings were planted *in situ*, their roots (and eventually rhizomes when developed) would be able to grow through the jiffy pot into the surrounding sediments, producing minimal stress and disturbance to the developing roots. While there seemed to be little difference in the appearance of the shoots between seedlings growing in the square-squat pots compared with the longer square-conical pots, the square-conical pots encouraged deeper root growth and therefore better anchorage.

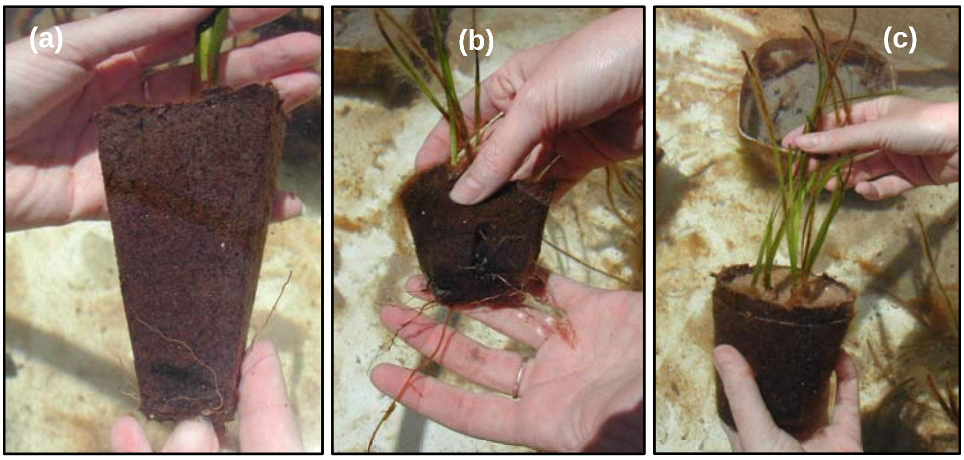

Figure 4. *Posidonia* seedlings after approximately 11 months in culture planted into; **(a)** square-conical Jiffy pot, **(b)** square-squat Jiffy pot, and **(c)** round Jiffy pot. Note that the roots are able to grow through the biodegradable material.

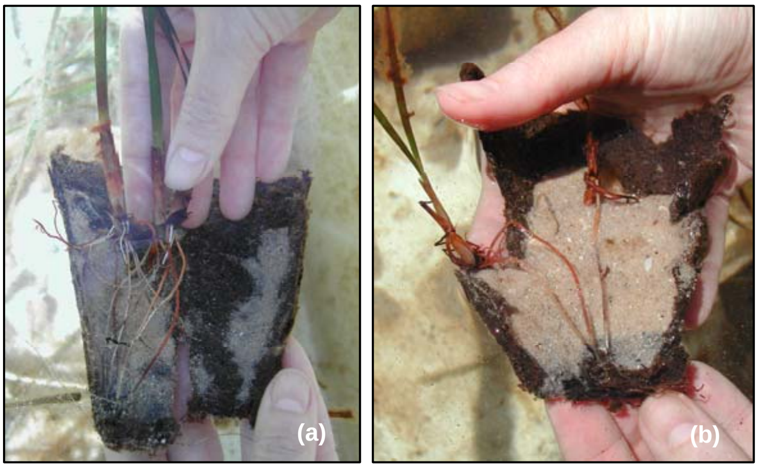

Figure 5. *Posidonia* seedlings after 316 days (9 Jan – 21 Nov 2002) grown in square-conical Jiffy pots showing; **(a)** vertical growth of healthy aerated roots, and **(b)** highly aerated sediments with only a narrow band of slightly anoxic sediments (grey) at the base of the pot.

Seedlings in the round pots also appeared healthy (Figure 4c); however if these pots were used on a large-scale, they would require more sediment, making them heavier to handle and more difficult to transport. By comparison, the longer square-conical pots allow maximum depth for root growth in minimum sediment. In addition square conical pots are more economical with respect to space, and are therefore the better choice for use in future seedling trials.

The focus on techniques to promote healthy root development may be one of the key factors contributing to successful seedling growth and survival. The roots are vital for providing a secure anchorage for the seedling in the face of strong currents and wave action. It is therefore important that the roots can be encouraged to grow vigorously into the sediments to increase the chances of the seedling remaining anchored. However this is only possible if there is adequate light to produce enough energy to sustain root growth and to create enough photosynthetically produced oxygen for aerating the roots and the surrounding sediments (Smith *et al*. 1984). Within the first 12 months of culture, these seedlings achieved good root growth and were clearly able to oxygenate the surrounding sediments. These results demonstrate that it is possible for *Posidonia* seedlings to be grown in culture for a significant period, which is advantageous because they can be held in culture until the conditions are optimal for planting-out. This would most likely be in spring, after the winter storm season (May to September) is over and turbidity is typically greatly reduced, thereby maximising chances of survival.

4. SUMMER 2002/03

4.1. Methods

4.1.1. Seagrass Nursery

In response to the initial success of culturing *Posidonia* seedlings over the 2001/02 reproductive season, and in preparation for the 2002/03 reproductive season, a more extensive outdoor flow-through seawater system was designed at SAASC. This system was intended for large numbers of seedlings that could be held in culture for a year, or more if required, until conditions were suitable for planting the seedlings out at select rehabilitation sites.

The flow-through seawater system was installed in November 2002 comprising five fibreglass tanks (500 L) arranged in a series orientated north-south (Figure 6). The tanks were situated in full sunlight and shaded by 90% shade cloth covers fixed within an aluminium frame (1.08 x 1.07 m).

Figure 6. The seagrass nursery at SAASC comprised five 500 L fibreglass tanks in full sunlight, each shaded by 90% shade cloth.

In the first seedling trial 70% shade-cloth was used (Section 3.1), whereas 90% shade cloth was used for this trial to better simulate the light conditions experienced by seagrasses at the potential planting sites (i.e. depth of approximately 6-7 m). Light availability at the depth of the these seagrass beds ranged from 12-27% (mean 18%) surface irradiance at the West Beach planting site and 7-22% (mean 15%) surface irradiance at the Henley Beach planting site (from preliminary instantaneous measurements made by Seddon *et al*. 2004). Therefore the shade cloth covers needed to reduce incident light by approximately 85% in order to simulate the level of light attenuation experienced at the planting sites. From instantaneous light measurements made using a LiCor Quantum Sensor underwater in the tanks on 11/12/03, it was found that the degree of shading provided by a 70% shade cloth cover was 73%, whereas the degree of shading for a 90% shade cloth was 88% which more closely approximated the level of light attenuation measured at the planting sites.

Tanks were supplied with filtered seawater by a system of PVC pipes set-up to produce alternating bi-directional flow to simulate natural tidal water movements. This was achieved by installing seawater outlets at opposite sides of each tank and only allowing flow from one side at a time (Figure 7). An auto timer connected to two solenoids acting as flow switches controlled seawater flow. When one solenoid was on, the other remained off, then after a set period of six hours, the timer switched the first solenoid off and the second one on for the next six hours and so on.

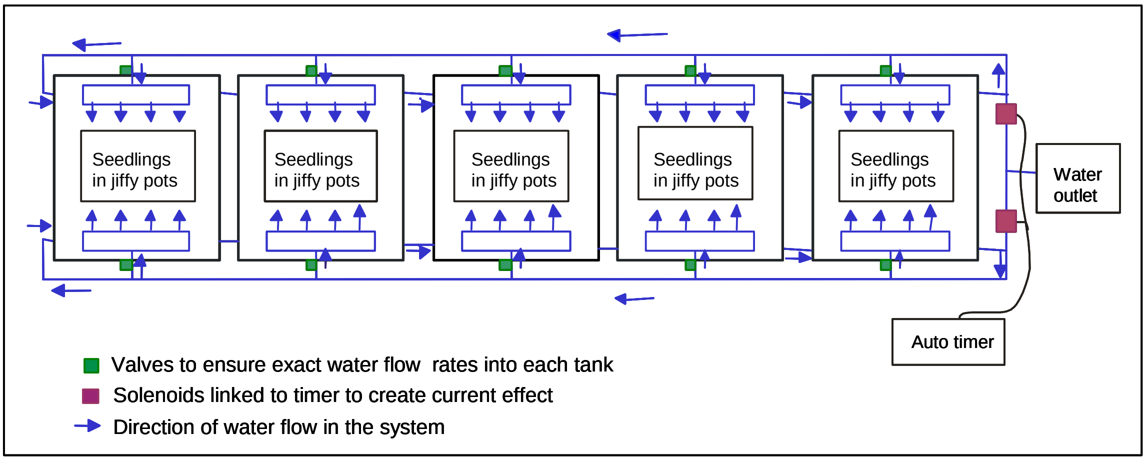

Figure 7. Schematic diagram of the flow-through seawater system for culturing seagrass seedlings at SAASC.

Valves attached to the individual seawater supply pipes of each tank ensured flow rates were comparable between tanks. Outlets consisted of PVC pipe with 20 holes, 8 mm in diameter drilled on one side designed to create enough pressure to force seawater out of the pipe in a series of jets, producing currents perpendicular to the outlet pipe that flowed across the tank at the height of the growing seedlings (Figure 8). This system did not require the use of electric pumps as those used in the first seedling trial to produce an alternating bi-directional water flow (Section 3.1).

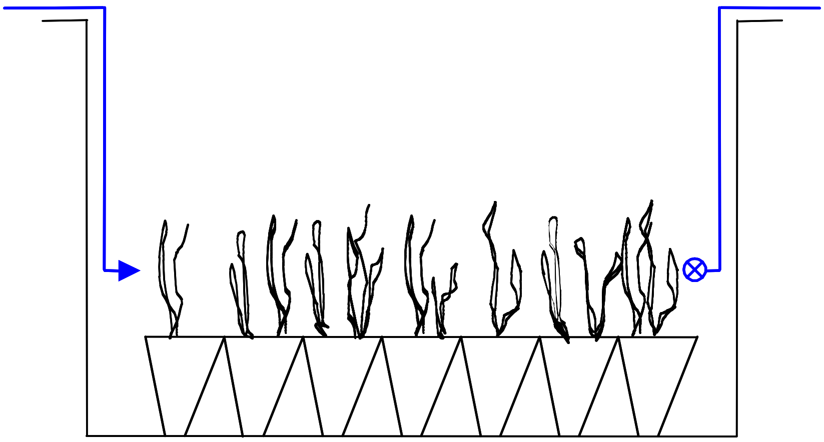

Figure 8. Schema showing the direction of seawater flow into the tank and across the seedlings. A unidirectional flow is maintained from one outlet (left) for a period of 6 hrs, after which a timer connected to a solenoid opens the outlet on the other side reversing the direction of flow.

4.1.2. Flowering and Fruiting Observations

In order to evaluate the consistency of the observations of flowering and fruiting made during the 2001/02 season, additional observations monitoring the timing of flowering and fruit maturity of *Posidonia* spp. were made over the 2002/03 summer using three methods; beach surveys, remote video transects and diver searches. Beach surveys were conducted on a regular basis from October 2002 to February 2003 along the shore from Largs Bay to Semaphore Jetty. During the surveys seagrass wrack was examined and a search made for seagrass fruits and seedlings. Remote video transects and diver surveys were undertaken between St. Kilda and West Beach during November and December 2002 (Table 4 and Figure 9).

Table 4. Summary of field trips related to finding seeds along the Adelaide metropolitan coast (in addition to these surveys, daily beach surveys were conducted throughout the season).

Date	Objective of field trip
7 Nov 2002	Search for flowering/ fruiting (diver & remote video)
29 Nov 2002	Search for flowering/ fruiting (diver & remote video)
13 Dec 2002	Search for fruiting (diver & remote video)
17 Jan 2003	Planting site selection and search for fruiting/seeds

In addition to the surveys, an e-mail was sent to personnel from SARDI and other South Australian organisations working in the South Australian marine environment. Recipients were provided with information on the reproduction of *Posidonia* species, together with photographs of fruits and were asked to report sightings of *Posidonia* spp. flowering and/or fruiting. A database was established to record ongoing observations of seagrass flowering and fruiting along the Adelaide metropolitan coast and regional south Australia and includes sightings from community groups such as Coastcare and Reef Watch (see Appendix).

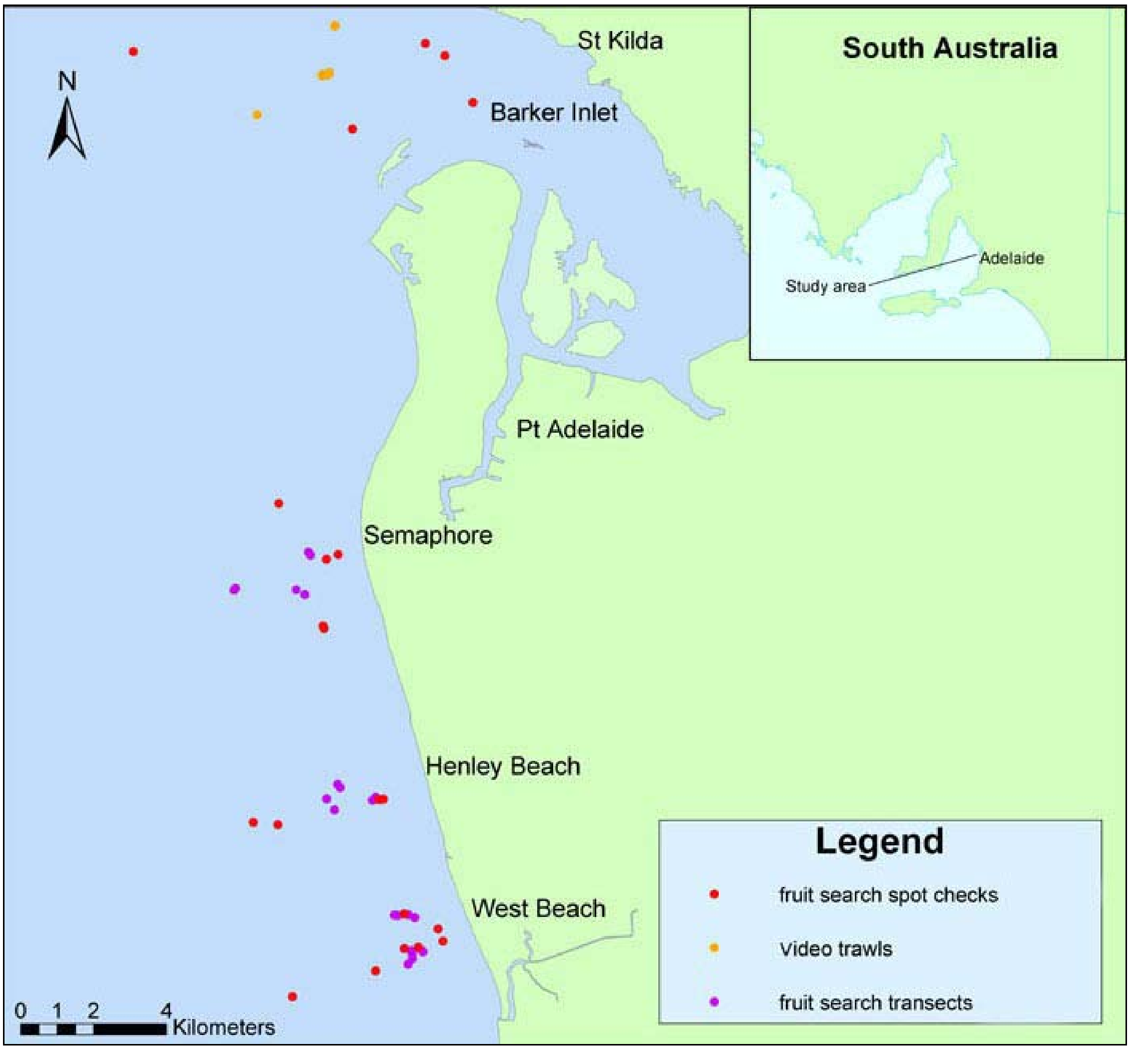

Figure 9. Map of the Adelaide metropolitan coastline showing the location and timing of video and diver transects in an effort to identify seagrasses fruiting.

4.2. Results and Discussion

Despite extensive beach surveys, remote video transects and diver searches along the Adelaide metropolitan coast (Figure 9), no evidence of *Posidonia* spp. flowering or fruiting was found during the 2002/03 reproductive season. In addition, no observations of *Posidonia* flowering or fruiting were reported by government personnel working in the marine environment at other locations in South Australia (e.g. Gulf St Vincent and Kangaroo Island), suggesting that 2002/03 was an extremely poor reproductive season for *Posidonia* species. These findings are in contrast to observations made over the 2001/02 reproductive season, when *Posidonia* fruiting was prolific along the metropolitan coast and was observed in other areas of the state including Port Pirie and Kingscote (see Section 3).

The observed variation in sexual reproduction of *Posidonia* species between the 2001/02 and 2002/03 seasons along the Adelaide coastline is consistent with literature on the reproduction of *Posidonia* species elsewhere, which suggests that sexual reproduction in *Posidonia* species is highly variable both temporally and spatially (Inglis & Smith 1998; Campey *et al*. 2002; Balestri & Cinelli 2003; Balestri & Vallerini 2003; Balestri 2004). Campey *et al.* (2002) recorded small-scale interannual variation in the sexual reproduction of *P. coriacea* on Success Bank, Western Australia. They authors found significant differences in flowering intensity over the three-year study period, with a greater flowering density observed in 1996 and 1997 compared with 1998. Similarly, Balestri and Vallerini (2003) identified significant interannual variation in the flowering frequency of *P. oceanica*, with two years of abundant flowering separated by several years with little or no flowering.

Unfortunately, little is known about interannual variation in reproduction of the species most commonly found along the Adelaide metropolitan coast, including *P. australis*, *P. angustifolia* and *P. sinuosa*. Kirkman (1998) made observations of the reproductive development of these three species over a period of approximately ten years in Perth metropolitan waters. Throughout this time, *P. australis* and *P. angustifolia* flowered profusely each year, whereas reproduction in *P. sinuosa* occurred on an annual basis in some areas (eg. Cliff Head), but not in other areas (Kirkman 1998). In 1995, Marbà and Walker (1999) also observed flowering of these species in Western Australia, and although most of their observations were consistent with those of Kirkman (1998), others were not. For example, Marbà and Walker (1999) reported spatial variations in the flowering of *P. sinuosa* (flowering was observed at Geographe Bay and not Penguin Island) and while they observed flowering in *P. australis*, they did not observe flowering for *P. angustifolia*. In contrast to both of these studies, Inglis and Smith (1998) observed flowering of *P. australis* across a broad range of sites in New South Wales in 1993, but a complete absence of reproductive shoots at the same time in 1992. In South Australia, *P. sinuosa* is thought to flower biannually (S. M. Clarke, pers comm.), although until a comprehensive survey monitoring *P. sinuosa* beds over a broad spatial scale for at least four or more reproductive seasons, this remains conjecture. Nevertheless, all of these studies clearly demonstrate that sexual reproduction in *Posidonia* species is spatially and temporally variable.

The reasons for such high variability in the reproductive ecology of *Posidonia* species are not well known. In other seagrasses, particularly those growing in the tropics, spatial and temporal variation in sexual reproduction has been correlated with variations in water temperature (e.g. Phillips *et al.* 1983; Pettit 1984; Inglis & Smith 1998; Rollón *et al.* 2003). For example, Phillips *et al*. (1983) identified a latitudinal gradient in the timing of floral bud initiation in *Zostera marina* along both the east and west coasts of North America, which was consistent with seasonal changes in water temperature. Other environmental parameters such as light availability and photoperiod have also been linked with variation in sexual reproduction in seagrasses (Inglis & Smith 1998; Rollón *et al.* 2003). For example, following examination of the intensity of flowering and fruiting of *Enhalus acoroides* at a range of sites in NW Philippines, Rollón *et al.* (2003)

concluded that temporal variation in flowering intensity was correlated with mean water temperature, while spatial differences were correlated with available light as affected by turbidity and water depth. Inglis and Smith (1998) also suggested that flower production is associated with regional changes in environmental conditions, such as temperature and photoperiod. It is possible that some of these reasons for variation in the reproductive ecology seagrasses may also apply to species of *Posidonia* growing in southern Australia, such as temperature and light availability – although photoperiod can not explain interannual variation at the same locations, or variation between locations of a similar latitude.

The availability of light is one of the most important factors affecting growth and survival of seagrases, and indeed all photosynthetic organisms. The amount of light reaching seagrass meadows can be affected at several levels. For example, the amount of cloud cover at any given time can affect short-term rates of photosynthesis, whereas high cloud cover over a long period of time may even affect the ability for seagrasses to produce enough energy to direct toward reproduction. Regardless of the amount of light reaching the surface of the water, a considerable amount of light can be attenuated due to high turbidity owing to factors such as increased suspended solids, phytoplankton blooms etc. In addition to this, the light reaching seagrasses can also be blocked directly due to excessive growth of epiphytic algae or sediments smothering the leaves. Considerable energy is required for reproduction and as Cambridge and Hocking (1997) found, the production of flowering shoots and fruits, expressed as a proportion of annual leaf production, can range up to 17-26% for *P. sinuosa* and 12-15% for *P. australis*. For this reason alone it is possible that flowering may be poor or not occur at all in years following harsh environmental conditions or chronic exposure to anthropogenic stress. A period of prolonged poor weather and storm induced turbidity could result in inadequate carbohydrate storage in seagrasses to enable flowering – even for species that typically flower annually. Fitzpatrick and Kirkman (1995) investigated the effect of shading to less than 10% incident light on *P. australis* and found that not only did shading significantly reduce leaf growth rates after as little as one month, and shoot densities after 3 months, but no flowering occurred in seagrasses shaded for 8 – 9 months, or those shaded for 6 months followed by 2 – 3 months recovery (i.e. no shade). In addition, the authors of this study found only a few flowers in seagrass shaded for 3 months followed by 5 – 6 months recovery, while the control plots of seagrass flowered in August and October.

Did low light levels contribute to the lack for flowering and fruiting over the 2002/03 reproductive season? Unfortunately there are no continuous underwater light attenuation data available from anywhere in the metropolitan region to investigate this hypothesis. In fact these type of data are very rare and are only recently being collected as part of the Adelaide Coastal Waters Study, but we do have some data available on the amount of cloud cover leading up to and during the time that observations were made on flowering and fruiting (Figure 10). From these data it is apparent that the average cloud cover over the winter of 2001 was relatively high but was followed by a prolific reproductive season over spring and summer of 2001/02, whereas the total cloud cover over 2003 was the lowest of all the years compared (Figure 11) and this was followed by a successful flowering and fruiting season for 2003/04. So there does not appear to be a relationship between cloud cover leading up to a reproductive season and success of flowering and fruiting. Although with respect to the amount of incident light reaching the seagrass beds, we can not comment on the role that turbidity induced light attenuation may have played in the lack of flowering and fruiting over 2002/03.

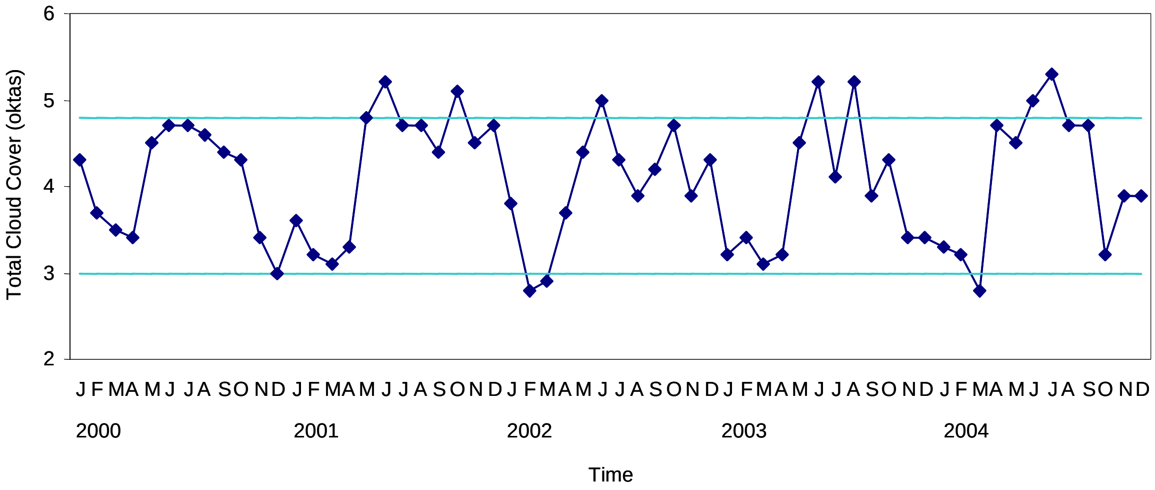

Figure 10. Average monthly total amount of cloud cover recorded at the Adelaide airport between 2000 and 2004. Pale blue lines represent the average maximum and minimum amount of cloud cover recorded over the past 50 years. Values based on synoptic observations. Data gathered by the Bureau of Meteorology.

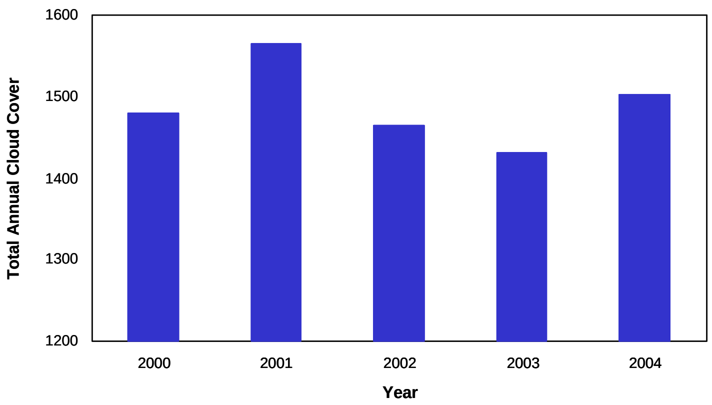

Figure 11. Total annual cloud cover calculated from the sum of the average daily cloud cover in oktas (excluding 29 February on leap years). Data gathered by the Bureau of Meteorology.

If water temperature had a significant effect on either the initiation or abundance of *Posidonia* flowering and fruiting, we would expect that temperatures during 2001 would be different from those years when *Posidonia* flowering and fruiting was observed. Again there are no continuous underwater temperature data available for the years of interest, but maximum and minimum air temperatures (Figures 12 and 13) and sea surface temperatures (Figure 14) from 2000 to 2004, have been recorded by the Bureau of Meteorology.

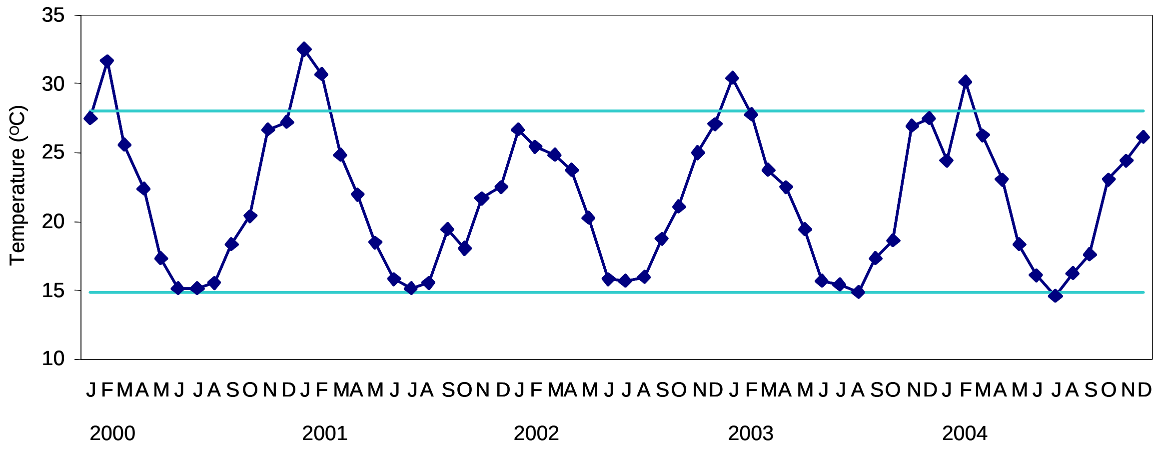

Figure 12. Monthly average maximum air temperature recorded at the Adelaide airport between 2000 and 2004. Pale blue lines represent the average maximum and minimum maximum air temperatures recorded over the past 49 years. Data gathered by the Bureau of Meteorology.

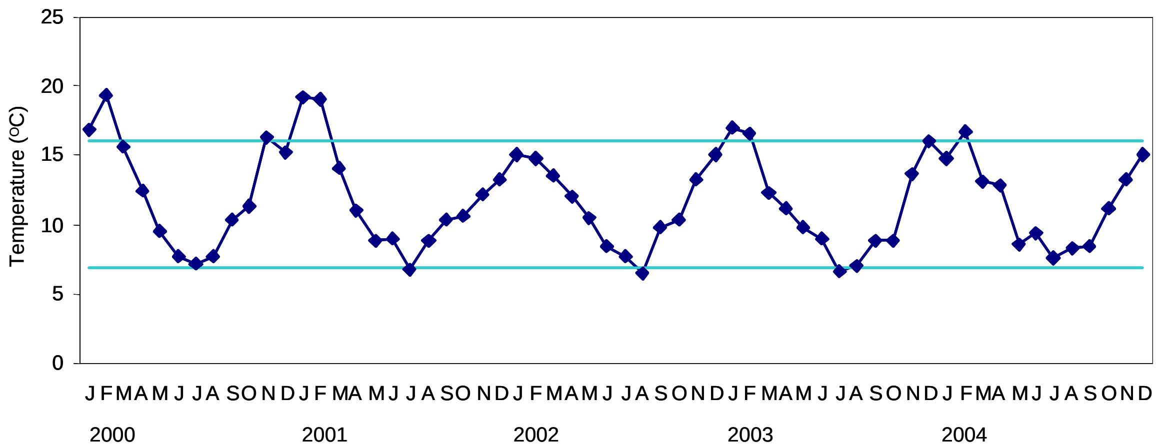

Figure 13. Monthly average minimum air temperature recorded at the Adelaide airport between 2000 and 2004. Pale blue lines represent the average minimum and maximum minimum air temperatures recorded over the past 49 years. Data gathered by the Bureau of Meteorology.

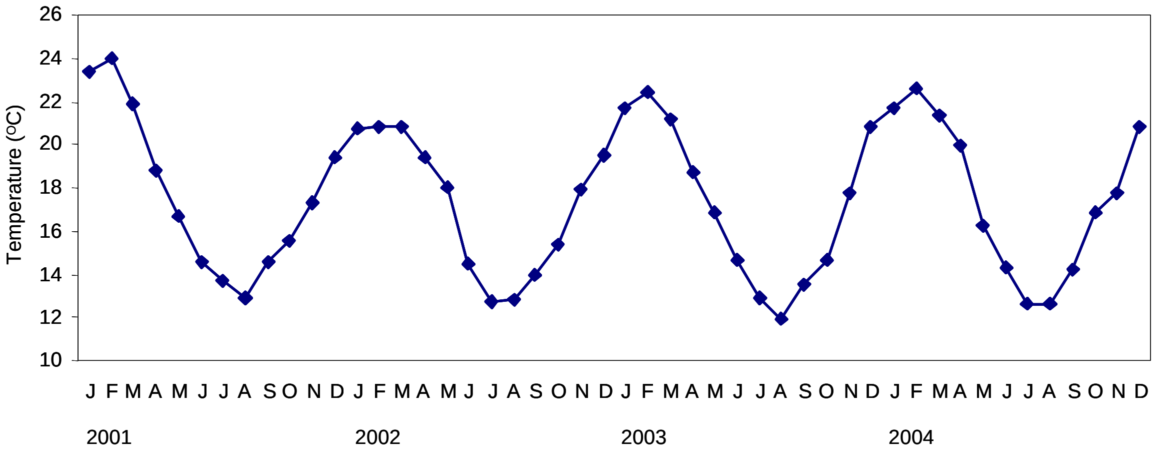

Figure 14. Sea Surface Temperatures in the Adelaide metropolitan area between January 2001 and December 2004. Data extracted from the 4 km resolution MODIS Terra sensor data held by the Physical Oceanography DAAC (Sensor was launched in October 2000).

Given that *Posidonia* flowering can begin as early as August in southern Australia (Robertson 1984), it is likely that temperatures prior to August and possibly even during the previous summer, are relevant to the onset of flowering. While there are only slight variations in average maximum temperatures from year to year (Figure 12), the summer leading up to the unsuccessful 2002/03 season was on average cooler than the previous two and following two summers. A similar trend is evident for the average minimum temperatures (Figure 13), in particular, low average minimum temperatures occurred in both January and February of 2002. A clearer trend is evident in the sea surface temperature (SST) data where SSTs were the highest in 2000/01, the summer preceding the prolific fruiting season, and conversely SSTs were lowest in 2001/02, the summer leading up to the season with no fruiting. From these data it appears plausible that below average temperatures over the previous summer may negatively affect the incidence of flowering leading up to the next reproductive season.

While efforts to find any signs of flowering or fruiting of *Posidonia* species during the 2002/03 season failed, it is possible that they did flower, but in extremely low abundances. During the same period, *Posidonia* fruiting was observed in Western Australia, although not as extensively as observed during the 2001/02 season (M. van Keulen, pers. comm.). Alternatively, if our observations gave a good indication of the lack of fruiting in South Australia, we can conclude that there are differences in the sexual reproduction of *Posidonia* species between South and Western Australia. This conclusion is likely, especially considering the degree of variability in sexual reproduction of species observed over a range of spatial and temporal scales in other studies (e.g. Inglis & Smith 1998; Campey *et al*. 2002; Balestri & Cinelli 2003; Balestri & Vallerini 2003; Balestri 2004).

The lack of evidence of flowering and fruiting at the locations visited in South Australia during the 2002/03 season has brought to light further research needs. These include a need to identify the spatial and temporal variability in sexual reproduction of *Posidonia* seagrasses, both within South Australia and across other states, and to identify potential reasons for this variation, including changes in large and small-scale environmental characteristics such as temperature, light and possibly nutrients. Information resulting from such studies will not only increase our understanding of seagrass biology and ecology in general, but will have important consequences for rehabilitation efforts. In particular, the success of various recruitment facilitation techniques currently being investigated at SARDI (Wear *et al*. in prep) will be influenced by the frequency and intensity of flowering and fruiting events and the subsequent supply of seedlings to rehabilitation sites.

5. SUMMER 2003/04

5.1. Methods

5.1.1. Flowering and Fruiting Observations

Staff at SARDI continued the program of regular monitoring of the Adelaide metropolitan beaches for signs of flowering or fruiting to determine the timing of *Posidonia* fruiting in South Australia during the 2003/04 reproductive season. In addition, a number of groups that work in the marine environment, including relevant government departments and local community, were asked to report occurrences (e.g. Reef Watch). Records of these observations were added to an ongoing ‘seagrass fruiting and flowering’ database managed by SARDI.

5.1.2. Seedling Culture

The flow through seawater system as described in Section 4.1.1, complete with 90% shade cloth covers and a seawater flow rate of approximately 11 L min^{-1}, was operational as of December 2003. Water quality parameters (temperature, salinity, pH and dissolved oxygen) were gathered every few months in the morning and afternoon throughout the grow-out period. This information revealed that, over this period, water temperature ranged from 13.2ºC to 19.1ºC (mean 14.9ºC ± 0.1) salinity averaged 36.4 ‰ (± 0.01), pH averaged 6.77 (± 0.01) and dissolved oxygen 9.89 mg L^{-1} (± 0.64) (all means ± standard error).

Posidonia angustifolia fruits were collected directly from seagrass beds by divers (Figure 15a) and from wrack washed up on the metropolitan beaches (Figure 15b). Collections by divers were made from *Posidonia* beds at West Beach and Seacliff on the 8^{th} December 2003. Whole fruits with no sign of discolouration or herbivory were collected. Approximately 900 fruits were collected in about 30 minutes at each location. Two weeks after the collection of fruits by divers, *Posidonia* fruits were collected from metropolitan beaches following strong onshore winds. Once collected, beach and diver-collected fruits were divided into groups of 150 and placed in floating containers in holding tanks at SAASC (Figure 2). All fruits were monitored once a week until the majority had dehisced and the seedlings released had produced shoots (Figure 16). In cases where the fruits had dehisced, but not released the seed, the seeds were manually removed. The number and origin of seeds that were manually removed in this way was recorded.

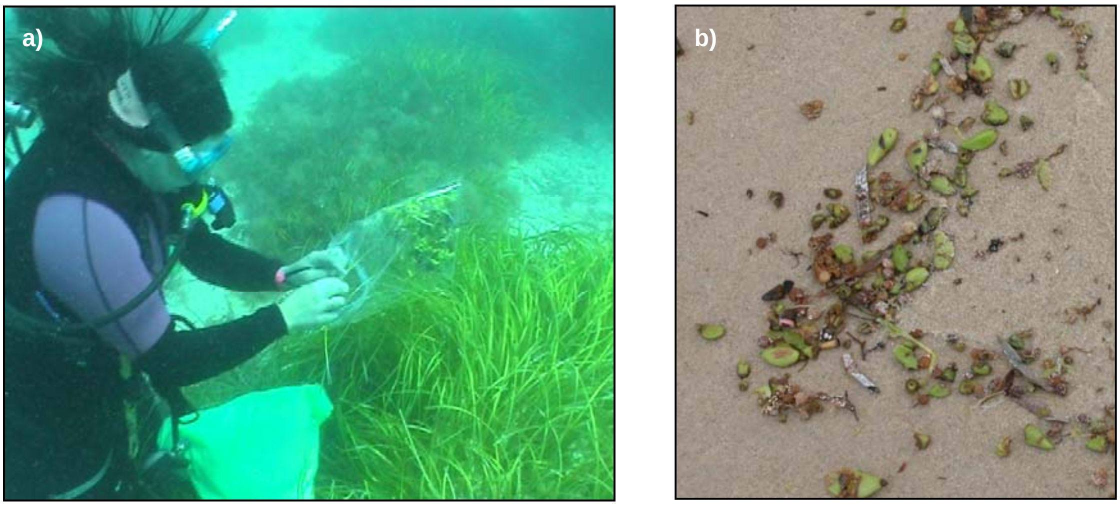

Figure 15. Methods of collecting fruits for the seedling experiments. **(a)** Diver hand collecting *Posidonia angustifolia* directly from a meadow, and **(b)** fruits washed-up on the beach at West Beach.

Figure 16. *Posidonia* fruits and seedlings in a floating container at SAASC.

In early February 2004, the seedlings were planted into square-conical Jiffy pots (as described in Section 4.1) in groups of two. A total of 160 seedlings were randomly positioned in each of four tanks, consisting of 80 beach and 80 diver-collected seedlings. The seedlings were held in culture for 11 months, during which time they were randomly rotated around the tanks. Approximately every two months seedling growth (number and length of shoots) and survival was monitored. At the end of the holding period the number of leaves, leaf length, number of roots and root length of each seedling was recorded. During the 11 month grow-out phase a high level of epiphyte growth occurred, particularly during the warmer months. In order to limit the effects of epiphytic growth on the seedlings, blades were manually cleaned once a week during high algal growth periods. A range of invertebrates collected from seagrass meadows at

Kingston Park, including palaemonid shrimps, isopods and amphipods, were also added to the tanks to control algal growth.

5.2. Results and Discussion

5.2.1. Flowering and Fruiting Observations

The first reports of *Posidonia* flowering were made in the upper sections of both Spencer Gulf and Gulf St Vincent in October 2003, after which both flowering and fruiting were observed in abundance along the metropolitan coast from mid November 2003 (see Appendix). High numbers of *Posidonia* fruits along Adelaide's metropolitan beaches coincided with storm activity during the middle of December 2003 and the beginning of January 2004. By the 19th of January 2004, seagrass fruits were no longer found along metropolitan beaches. While we have previously observed that the abundance and distribution of *Posidonia* is highly variable, the timing of fruiting of *Posidonia* spp. in the Adelaide metropolitan region generally coincides with the same species in Western Australia, except the season in South Australia appears to start and finish slightly later (e.g. *P. sinuosa*, Table 5). To confirm these trends in timing of fruiting, more extensive surveys are required.

Table 5. Timing of fruiting of *Posidonia* species reported in South Australia from 2002 to early 2005 (present study) compared with Western Australia (Kirkman 1998; Smith & Walker 2002).

Species	South Australia	Western Australia
Posidonia angustifolia	November – January	November – December
Posidonia australis	? – December	September – December
Posidonia coriacea	? – December	November – December
Posidonia sinuosa	November – January	October – December

5.2.2. Seedling Culture

Of all of the fruits collected, 93.5% dehisced. There was little variation in the numbers of fruits that dehisced between tanks and collection method, with fruit dehiscence exceeding 90% (natural plus manual) in all cases (Figure 17). While the majority of the fruits collected dehisced, there was a notable difference between manual and natural dehiscence (the seed completely released from the fruit on its own) of beach and diver-collected fruits. The natural dehiscence of beach-collected fruits was almost 80%, whereas those collected by divers was just 34% (Figure 17). This difference is very likely to be related to fruit maturity. Diver collected fruits were gathered two weeks prior to the collection of fruits from Adelaide beaches and as a result, fruits may not have been mature and ready for release from the plant. It is also likely that the process of washing up onto the beach not only exposes the fruits to increased light and wave energy, but also desiccation, all of which could promote dehiscence. Observations of desiccated fruits on the beach support this hypothesis. Only a small percentage of fruits rotted, releasing the seedling (Figure 17).

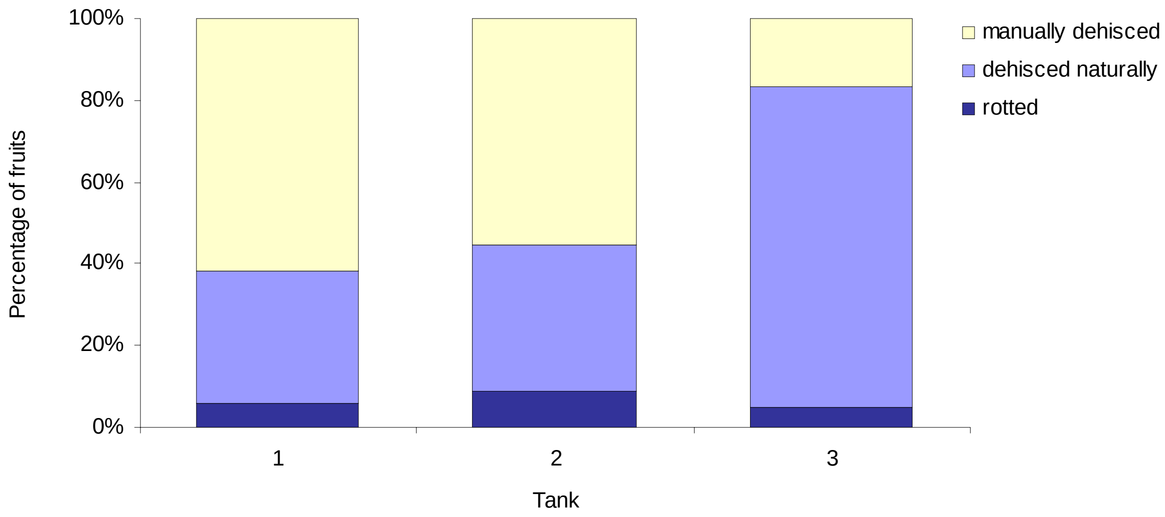

Figure 17. The percentage of fruits that rotted (dark blue), dehisced naturally (pale blue) and dehisced manually (yellow) at the end of the holding period (16/1/04). Tank 1 and 2 contained fruits that were hand collected by divers from West Beach and Seacliff, while tank 3 contained fruits that were collected on the beach at West Beach.

Despite the high dehiscence of the 2,700 *Posidonia* fruits collected from along the Adelaide metropolitan coastline (1800 beach collected; 900 diver collected), after 6 – 8 weeks (beach and diver collected respectively) only 1,315 seedlings were suitable for culture, of which 640 were planted into square-conical Jiffy pots for the experiment. Of the 640 planted seedlings, only 8% survived the 11 month grow-out period (51 seedlings; Figure 18), which was not enough seedlings to initiate an *in situ* planting-out trial. This unexpectedly low seedling survival could be related to excessive algal growth during the warmer months of the year. High epiphytic loads reduce photosynthetic efficiency and growth and have been implicated in seagrass losses (Sand-Jensen 1977; Silberstein *et al*. 1986; Shepherd *et al.* 1989; Short *et al.* 1995; Bryars *et al.* 2003). In this experiment, epiphytic algae potentially had two detrimental effects on the seedlings. Firstly, the growth of epiphytic algae on seagrass leaves limits the amount of light reaching the chloroplasts within the epidermis of the seagrass blades, and possibly also restricts gas exchange (Borowitzka & Lethbridge 1989) and nutrient uptake (Hauxwell *et al*. 2001). Secondly, as a result of the excessive epiphytic growth and in an effort to limit its impact on the seedlings, the leaves were manually cleaned once a week during high algal growth periods. While the cleaning process reduced epiphyte cover on the leaves and presumably increased the amount of light available for photosynthesis, it did cause some disturbance to the seedlings and occasionally resulted in the leaves breaking off or the entire seedling itself being dislodged; a factor likely to have contributed to poor seedling survival. In addition, the cleaning process temporarily created turbid conditions within the tanks potentially compounding the problem of reduced light levels associated with the epiphytic algae, further stressing the seedlings.

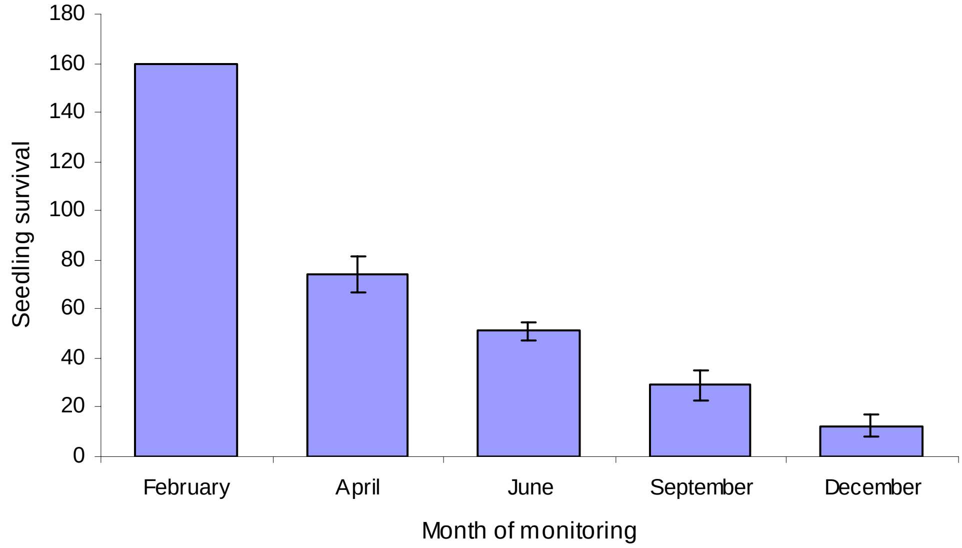

Figure 18. Average survival of *Posidonia* seedlings (± standard error) over an 11 month period in four tanks at the SAASC. Seedlings were collected from Seacliff and West Beach, Adelaide. Note that seedlings resulting from beach and diver-collected fruits were randomly dispersed across tanks. Error bars represent standard errors (n=4).

Given that epiphytes often dominate the diets of herbivorous epifauna (Klumpp *et al*. 1989) and have been shown to be effective in reducing epiphyte cover (Hily *et al.* 2004), a range of invertebrates was added to the tanks, including palaemonid shrimps, isopods and amphipods. Initially, the addition of the invertebrates appeared to be extremely successful at reducing the epiphytic algae. However once the abundance of epiphytic algae in the tanks had been reduced, we observed evidence of grazing on seagrass leaves including serrations on the sides of leaves, and in some cases, the entire leaf had disappeared leaving behind the remains of a green serrated leaf base. This damage and loss of leaves associated with invertebrate grazing is likely to have contributed to poor seedling survival. While several species of amphipods have been shown to graze upon seagrass leaves (e.g. Kirkman 1978b), we have found at least one amphipod species that successfully grazes on algae but does not appear to graze seagrass leaves (S. Seddon pers. obsv.), which warrants further investigation.

Excessive growth of epiphytic algae and invertebrate grazing are two problems commonly encountered when growing seagrass seedlings in tanks over an extended period. Kirkman (1978b) found that the growth of epiphytic algae on *Zostera capricorni* could be successfully reduced by excluding light from the tanks for three days “whenever epiphytes built-up”. This process successfully removed all of the epiphytes from the tanks and Kirkman (1978b) reported that the seedlings appeared healthy, although without finer-scale measurements on factors such as growth rates, photosynthesis, sugar and starch content, it is not possible to confirm whether or not the seedlings were affected by one or more episodes of total light deprivation. One of the main reasons why the exclusion of light has such a rapid effect on algae but not on seagrasses, even though the minimum light requirement for algae is generally much lower than seagrass, is that seagrasses can mobilize reserves of carbohydrate stored in their leaves, roots and particularly rhizomes (e.g. Zimmerman *et al.* 1995a; Burke *et al*. 1996). Studies have shown that the accumulation of carbohydrate reserves by seagrasses during favourable conditions provide a critical source of energy for various metabolic functions, growth and survival during prolonged periods of low light (Kraemer & Alberte 1993; Fitzpatrick & Kirkman 1995; Zimmerman *et al*. 1995a; Burke *et al*. 1996; Carbello-Pasini *et al*. 2002). Depending on species, it is generally believed that the average minimum light requirement for seagrasses varies between 8 to 18% surface irradiance – given 6 hours of light saturating irradiance (generalisation by Cabello-Pasini *et al*. 2002 based on work by Duarte 1991; Dunton 1994; Zimmerman *et al*. 1995b and Olesen 1996). However, due to the higher energy requirements for growth and repair of transplanted seagrass, the recommended minimum light requirement is at least 25% surface irradiance, (Fonseca *et al*., 1998). While there do not appear to be any studies specifically addressing the light requirements of seedlings, it is likely that they are more vulnerable to periods of reduced light than mature seagrass, particularly once nutrient reserves are exhausted in the attached hypocotyl (after 8 months; Kuo & Kirkman 1996) since seedlings have not yet grown an extensive system of rhizomes for storing carbohydrates. For these reasons, a one-off application excluding light to *Posidonia* seedlings for several days may be helpful if algal epiphytes were excessive and unable to be controlled through the use of appropriate invertebrate grazers, although the effects of repeated applications of light exclusion on the carbohydrate content and growth of seedlings would need to be closely monitored.

At the time that the seedlings were planted into Jiffy pots (i.e. when they were 6 or 8 weeks old - beach and diver collected respectively), the average number of leaves per seedling was 2.1; however by the end of the 324 day grow-out period, the number of leaves had decreased to 1.8 (Figure 19a). The drop in number of leaves is most likely attributed to the grazing of leaves by invertebrates and the need to manually clean epiphytic algae from the seedlings causing the removal of some leaves. Over the same period the average maximum leaf length increased by 55 mm (from 43 mm to 98 mm), and the average number of roots increased from 2 to 3 (Figure 19b and c). While the length of the roots was not measured at the time of planting, by the end of the grow-out period the average root length was 39 mm, with a maximum root length of 181 mm recorded. These results show that, for the seedlings that managed to survive the 11 month period, on average there was a two to three-fold increase in maximum leaf length. Interestingly, seedlings from fruits that were collected from the beach appeared to grow more quickly over this period than those collected directly from meadows by divers in terms of maximum leaf and root lengths (Figure 19b and d). Nevertheless it is important to note that

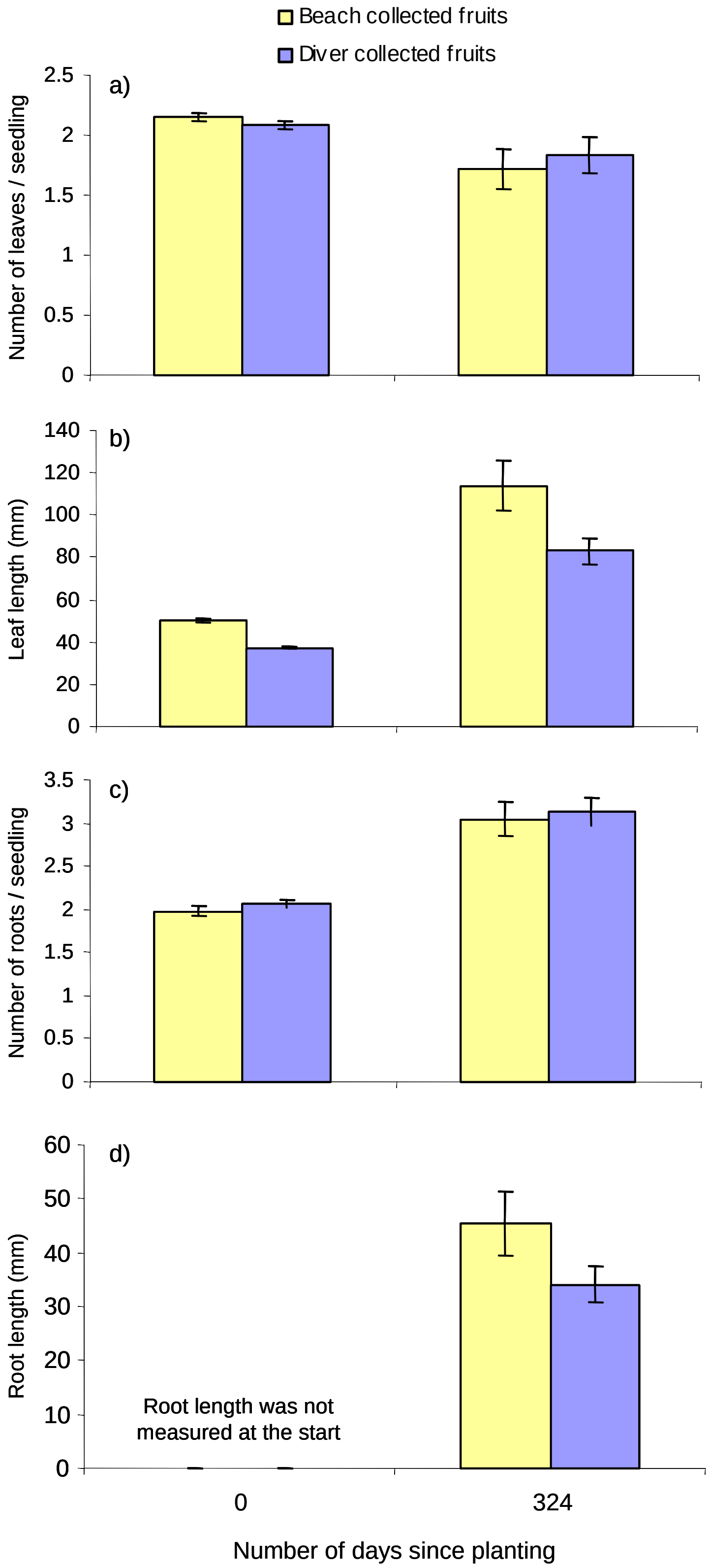

Figure 19. Measurements made on the *Posidonia* seedlings at the time of planting (2/2/2004) and at the end of the 324 day grow-out period (22/12/2004); **(a)** average number of leaves per seedling, **(b)** average maximum leaf length, **(c)** average number of roots per seedling, and **(d)** average root length. Note at the start of the grow-out period there were 320 diver and 320 hand-collected seedlings, whereas at the end there were only 21 beach and 30 diver-collected seedlings. All seedlings, whether diver-or beach collected, were randomly dispersed across the tanks. Error bars represent standard errors.

beach and diver fruits were collected at different times and at the end of the grow-out period only 21 beach and 30 diver-collected seedlings remained for comparison.

Our observations of the growth of *Posidonia* seedlings are consistent with other studies which show that *Posidonia* species have particularly slow growth rates relative to other species of seagrass (Kirkman & Kuo 1990; Marbà & Walker 1999; Meehan & West 2004). Evidence of their slow growth was identified by Marbà and Walker (1999), who found that *P. sinuosa* has an average leaf production rate of 2.6 leaves each year. Similarly, Meehan and West (2004) estimated that *P. australis* seedlings produce a second shoot at the age of 2.7 years, and thereafter produced one shoot each year.

5.2.3. Improving Methodologies

Given the differences in the proportion of seedlings surviving the 2001/02 and 2003/04 attempts at culturing *Posidonia* seedlings, it is clear that the methodologies used require further refining to produce more consistent results. This is not at all unusual in that it can take many years to develop reliable methodologies for culturing algae and many years to successfully grow-out various species of crustacean, mollusc and finfish, before it is possible to establish a commercially viable operation.

At this stage it is important to review our experiences over three reproductive seasons and to compare the results from the two trials in 2001/02 and 2003/04 to help identify which are the important factors contributing to good seedling survival. In both trials, the collection of ripe fruits and their subsequent dehiscence once held in tanks at SAASC proved highly successful. Similarly, the planting of seedlings into biodegradable Jiffy pots was also successful, and while growth was relatively slow, those seedlings that did manage to survive once planted into Jiffy pots, grew quite well. The primary issue of concern regarding the successful culture of *Posidonia* seedlings, particularly over long periods, is the low survival rate. Key factors that are likely to influence seedling survival, in addition to the effects of grazing and manual cleaning discussed above, include light availability and levels of nutrients.

One notable difference between the first and second trial is the nature and quality of the water supply. In 2001/02 the seawater flow rate was relatively low and flow within each tank was maintained by using electric pumps on alternating time cycles (Section 3), whereas in 2003/04, the flow rate was relatively high in order to maintain high water pressure, and hence good flow from the outlets within each tank. While this was a very elegant design, it is probable that more nutrients were delivered to the tanks than in the previous set-up simply because a greater volume of seawater was delivered to each tank over a 24 hour period. This may affect the rate of epiphyte growth; however for this system design, reducing water flow is not an option because this would cause a drop in pressure and hence the water flow across the seedlings within each tank would be insufficient.

The relationship between increased algal growth and increased nutrients, particularly during summer where there is more light and warmer temperatures, is well known (see Ralph *et al*. in press for a review). Contributing to the probable increase in dose of nutrients delivered to the tanks in the 2003/04 trial, is the location of the recently commissioned Barcoo Outlet (operational as of late November 2001), which is located 600 m south (i.e. upstream) of the SAASC seawater inlet. The proposed location of the Barcoo outlet was an issue for public discussion and also of particular interest to SARDI because the seawater collected by the SAASC inlet is filtered by sand filters, which remove suspended particulate matter but not nutrients, heavy metals or other dissolved compounds. While the first seedling culture trial started around this period, it is unlikely that the Barcoo Outlet would have contributed any significant discharge until the following autumn when the first major rainfall events occur; however by the start of the second trial in December 2003, two winter seasons with associated discharge had passed. The field team witnessed the effects of a significant rainfall event on the 20th of February 2003 during water quality monitoring near West Beach. On this occasion they recorded an extremely turbid layer of water associated with coastal outlets (Seddon *et al*. 2004), with divers noting that the visibility was greatly improved below this turbid layer.

As discussed earlier, the high algal growth necessitated manual cleaning of the tanks and seedlings as well as the addition of invertebrate grazers. There are two issues here: Firstly while at least one species of amphipod was found to be very successful at grazing algae and not seagrass in the first trial, the use of a more diverse group of grazers for the second trial clearly incorporated species that will also graze on seagrass. Secondly, the tanks take a while to ‘season’ in terms of establishing invertebrate communities (including grazers) on the sides of the tanks that help to keep algae to a minimum, greatly reducing the need for manual cleaning. The longer a tank can be ‘seasoned’ before use in a trial or experiment the better (e.g. at least three months, Seddon 2000) to establish an equilibrium between invertebrate fauna and flora. Unfortunately for the second trial we had little opportunity to ‘season’ the tanks before fruits were collected.

Another difference between the first and second trial was the density at which the seedlings were planted per Jiffy pot. This may seem like a relatively small difference regarding methodology, but in the 2001/02 trial three seedlings were planted per square-conical Jiffy pot, while in 2003/04 two seedlings were planted per pot since by that stage there were not enough viable seedlings available to plant three per pot. As discussed earlier in Section 3, it was hypothesised that a cluster of seedlings could be more beneficial than individual seedlings because of increased ability for seedlings to aerate their roots and the surrounding rhizosphere in addition to providing greater protection *in situ* if the seedlings were planted-out. Observations of the seedlings planted in the square-squat Jiffy pots (4 per pot) and circular Jiffy pots (7 per pot), both of which appeared to grow well over the 11 month period (Figure 4b and c) support the notion that clustering seedlings may be important for enhancing survival and growth.

Finally, and perhaps most significantly, it is possible that the greater degree of shading used in the second trial (i.e. 90% shade cloth) contributed toward reduced growth and survival of *Posidonia* seedlings. If this is the case, then there are potential implications for natural recruitment. At depths where light is reduced to 10% surface irradiance or less, light levels are likely to be too low for long-term seedling survival. Seddon (2002) pointed out the possible role of persistent high inshore turbidity, associated with storm water runoff and sediment resuspension, reducing light to below the minimal light requirements and therefore contributing to seagrass decline in the metropolitan region. From the limited light data collected by Seddon *et al*. (2004) it was suggested that the minimum light requirements for seagrass transplants (cores and sprigs), i.e. at least 25% surface irradiance, were not met at sites at West Beach or Henley Beach during the April or June sampling trips (Seddon *et al*. 2004) and it was concluded that good water clarity over the following spring/summer would be essential to enable the transplants to grow and repair any damage, in addition to providing enough carbohydrate reserves for the following winter. This concurs with the minimum light requirements of *A. antarctica*, *P. australis* and *P. sinuosa* reported by Dennison *et al*. (1993), which at ~25% surface irradiance, are higher than the average minimum light requirements for seagrasses in general, also suggesting that the light availability inshore at the Henley Beach and West Beach sites is, at best, marginal for long-term seagrass survival.

Studies investigating the effect of shading on various species of *Posidonia* elsewhere have shown similar responses to low light where, firstly, there is a reduction of leaf growth rates, followed by the reduction of leaf and shoot densities and eventually, wide-spread shoot mortality (e.g. Gordon *et al*. 1994; Ruiz & Romero 2001). Additionally the difference between adequate and insufficient incident light can be quite striking in terms of seagrass survival. For example, Ruiz and Romero (2001) recorded a significant difference in shoot survival for *P. oceanica* after 4 months of shading where they measured zero shoot survival in seagrasses exposed to ~10% surface irradiance, compared with 80% survival for seagrasses exposed to ~17% surface irradiance and no change in the controls (36% surface irradiance). In a longer-term study, Gordon *et al*. (1994) found that primary production and shoot densities of *P. sinuosa* were 10% that of unshaded seagrasses after 307 days of shading (treatments used: 80%, 88% and 99%) and suggested that continued chronic light reduction at these levels could lead to the collapse of meadows within two years. Clearly, 10% surface irradiance or lower is inadequate for mature *Posidonia* meadows, but the minimum light requirements for seedlings still needs to be established.

6. CONCLUSIONS AND RECOMMENDATIONS

One of the key findings from this early research into the use of *Posidonia* seedlings in rehabilitation along the Adelaide coastline is that the timing, frequency and distribution of *Posidonia* reproduction is highly variable, both spatially and temporally. While large amounts of fruits formed a high proportion of the wrack washed up along metropolitan beaches during the summers of 2001/02 and 2003/04, no fruiting was observed in 2002/03, despite being recorded in Western Australia. These findings and similar observations of variability in reproductive timing of *Posidonia* in the literature, demonstrate that there is still much to learn about the spatial and temporal variation of *Posidonia* fruiting and flowering in southern Australia and the mechanisms driving this variation. A greater understanding of these factors will greatly assist in future rehabilitation efforts using seedlings.

The project also identified that culturing *Posidonia* seedlings for extended periods of time can be problematic and while results from the first trial in 2001/02 were promising in terms of seedling survival, the second trial in 2003/04 showed poor seedling survival and relatively slow growth over the 11-month grow-out period. Poor seedling survival was attributed to insufficient light as a result of excessive growth of epiphytic algae and the use of 90% shade-cloth, in addition to the effects of invertebrate grazers in the tanks and possibly also increased supply of nutrients. The slow growth rate of the *Posidonia* seedlings is somewhat expected given that adult plants and seedlings of *Posidonia* are renowned for their slow growth (Kirkman & Kuo 1990; Marbà & Walker 1999; Meehan & West 2004), but may also be explained by the reduction in light as a result of epiphyte growth. As with many species undergoing trials for cultivation, whether plant or animal, considerable effort is required to establish reliable methodologies. While we know from our experience with this project and that of others in Western Australia and the Mediterranean, that cultivating seagrass seedlings is possible, more work is required to identify key factors influencing seedling survival so that we can increase the proportion of seedlings that survive the grow-out phase.

If trials of *Posidonia* seedlings are to be continued at some stage in the future, particularly with the view to supplying seedlings for fast-tracking succession at various rehabilitation sites (Seddon 2004), it is recommended that:

- Seedlings are cultured under an adequate light regime, at least 25% surface irradiance (i.e. 70% shade cloth), and that planting sites with a light regime that is lower that 25% are probably unsuitable for the long-term survival and growth of *Posidonia* seedlings (whether cultured or naturally recruited seedlings).
- If the seawater supply is sourced from an area that is potentially exposed to pulses of nutrients (e.g. storm water outlets), the level of nutrients in the seawater supply may need to be reduced by methods such as; lowering flow rates and using pumps to provide water

circulation and/or employing a system that can strip out excess nutrients (e.g. algal scrubber).

- Tanks should be seeded with appropriate invertebrate grazers with a preference for algae over seagrass and allowed to establish for a period of time (preferably 3-6 months) before the seedlings are introduced to minimise the need for cleaning the seedlings and walls of the tank manually.

Given the unpredictable nature of sexual reproduction of *Posidonia* species in South Australia, the slow growth rate of *Posidonia* species in general, and the difficulties experienced in cultivating seedlings, the use of *Posidonia* seedlings in large-scale rehabilitation efforts in this way is unlikely to be practical. However there may be a role for using cultivated *Posidonia* seedlings to fast-track or augment the natural process of succession in areas that are starting to be recolonised by fast-growing species such as *Heterozostera tasmanica* and *Halophila* spp. (Seddon *et al*. 2004). Natural recruitment of such species may be significant because the survival rates of naturally recruited seedlings, regardless of species, are generally very low (e.g. Holbrook et al. 2002) and *Posidonia* is no exception. For instance, after monitoring naturally recruited *P. australis* seedlings over approximately 8 years, Meehan and West (2004) found that the abundance of *P. australis* seedlings at St Georges Basin (south-east Australia) is among the lowest for any species of seagrass. Similarly in southwest Australia, Kuo and Kirkman (1996) reported that the survival rate of naturally recruited *P. sinuosa* seedlings over a 13 month was very low (ranging from 0 – 14% depending on site). Clearly having the means to augment the number of seedlings recruiting to a given area and increasing their chances of survival (e.g. by enhancing protection) is likely to increase *in situ* seedling abundances and long-term survival. While the efficacy of encouraging the growth of colonising species then in-planting with seedlings of a climax species still remains largely untested, there have been some encouraging results overseas. In the Mediterranean, Balestri *et al*. (1998) demonstrated that approximately 70% of two month old cultivated *Posidonia oceanica* seedlings were able to survive for three years when secured into grids and planted-out on remnant seagrass mat compared with 66% survival for naturally recruited seedlings over the same period. Conversely, none of the cultivated seedlings fixed into grids and planted on pebbles survived beyond six months, demonstrating the importance of substrate type and protection on seedling survival.

There are advantages of using cultivated seedlings over seedlings collected *in situ*. For instance, seedlings can be held in culture until the conditions are optimal for planting-out (usually spring, when the photoperiod is increasing and the winter storms are over), thereby maximising chances of success for the amount of effort invested. Furthermore, as was shown in this study, not all years are good for sexual reproduction in *Posidonia* and consequently seedling recruitment, and in fact, for some years there may be no newly recruited *Posidonia* seedlings available at all. Until we understand the factors that control the distribution and abundance of flowering and fruiting in seagrasses along the metropolitan coast, the ability to collect fruits when they are available and to hold the resulting seedlings in a nursery until they have established an adequate root system is more desirable than having to immediately plant-out immature seedlings at times that are less than amenable to survival.

SARDI Aquatic Sciences and DEH are currently testing another method of rehabilitation using *Posidonia* seedlings (Wear *et al*. in prep). In this trial *Posidonia* fruits have been collected from metropolitan beaches, held in tanks until dehiscence and the resultant seedlings planted into sand-filled hessian bags. The idea was developed following observations of *Posidonia* seedlings naturally recruiting onto remnant root and rhizome mat at the edges of blow-out scars at Brighton. The hessian bags represent a biodegradable and portable alternative to the underlying root mat, which in many areas along the metropolitan coast has already eroded away (Fotheringham 2002), and may provide a stable environment long enough for the seedlings to develop roots. This method offers the benefit that the seedlings spend the first year of their life in natural conditions and are very low maintenance compared with those grown in tanks at SAASC.

In addition to the development of rehabilitation methods using *Posidonia* seedlings, DEH and SARDI Aquatic Sciences are currently investigating seedling 'recruitment facilitation' techniques (Wear *et al*. in prep). A variety of hessian types (including coarse and fine hessian and natural seagrass fibre), in the form of bags and strips have been placed at two sites along the metropolitan coast in areas of seagrass loss. These bags and strips have so far encouraged the natural recruitment of hundreds of *Amphibolis* seedlings. The sites have been surveyed three times since their placement in August 2004, and although the number of seedlings surviving has declined over this period, those seedlings that have survived and remained attached, have steadily grown. The early success of the natural recruitment facilitation technique may offer a more effective form of rehabilitation, given the variable success of the assisted recruitment methods described here.

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APPENDIX

Table 1. Observations of *Posidonia* flowering and fruiting made by staff or volunteers including those from the Department of Environment and Heritage, the South Australian Water Corporation, SARDI Aquatic Sciences and Reef Watch.

Date	Location	Species	Observation	Observer
Pre 2002				
08 Sep 1995	Tickera, Spencer Gulf	*P. australis*	flowering	Stephanie Seddon (Adelaide University)
29 Sep 1995	Port Broughton, Spencer Gulf	*P. australis*	flowering	Stephanie Seddon (Adelaide University)
14 Dec 1999	Semaphore Park	*Posidonia*	fruiting	Doug Fotheringham (DEH)
29 Oct 2001	Webb Beach	*Posidonia*	fruiting	Ron Sandercock (DEH)
06 Nov 2001	Tumby Bay	*Posidonia*	fruiting	Ron Sandercock (DEH)
08 Nov 2001	North Henley	*Posidonia*	fruiting	Sue Murray-Jones (DEH)
11 Nov 2001	North Henley	*Posidonia*	fruiting	Sue Murray-Jones (DEH)
12 Nov 2001	West Beach	*Posidonia*	fruiting	Scoresby Shepherd (SARDI)
13 Nov 2001	North Henley	*Posidonia*	fruiting	Sue Murray-Jones (DEH)
15 Nov 2001	Grange	*Posidonia*	fruiting	Sue Murray-Jones (DEH)
17 Nov 2001	South Henley	*Posidonia*	fruiting	Sue Murray-Jones (DEH)
17 Nov 2001	Glenelg South	*Posidonia*	fruiting	Joan Smolinski (DEH)
20 Nov 2001	South Henley	*Posidonia*	fruiting	Sue Murray-Jones (DEH)
21 Nov 2001	Grange	*Posidonia*	fruiting	Sue Murray-Jones (DEH)
22 Nov 2001	South Henley	*Posidonia*	fruiting	Sue Murray-Jones (DEH)
24 Nov 2001	C Elizabeth, Yorke	*Posidonia*	fruiting	Jon Emmett (DEH)
25 Nov 2001	Tennyson	*Posidonia*	fruiting	David Deane (DWLBC)
25 Nov 2001	Myoponga	*Posidonia*	fruiting	Jeremy Gramp
25 Nov 2001	West Beach & Henley	*Posidonia*	fruiting	Doug Fotheringham, Sue Murray-Jones (DEH), Scroesby Shepherd (SARDI)
01 Dec 2001	South Henley	*Posidonia*	fruiting	Sue Murray-Jones (DEH)
05 Dec 2001	South Henley	*Posidonia*	fruiting	Sue Murray-Jones (DEH)
12 Dec 2001	South Henley	*Posidonia*	fruiting	Sue Murray-Jones (DEH)
25 Dec 2001	South Henley	*Posidonia*	fruiting	Sue Murray-Jones (DEH)
2002/3 Season				
Nov 2002	Brighton - Semaphore	*P. angustifolia*, *P. sinuosa*	none	Stephanie Seddon (SARDI)
Nov 2002	Beachport	*P. angustifolia*, *P. sinuosa, P. coriacea*	none	Stephanie Seddon (SARDI)
Nov 2002	Largs Bay (beach)	*Posidonia* spp.	none	Sonja Venema (SARDI)
Nov 2002	Henley Beach (beach)	*Posidonia* spp.	none	Ron Sandercock (DEH)
Dec 2002	Metropolitan Adelaide	*Posidonia* spp.	none	Sonja Venema (SARDI)
Dec 2002	Metropolitan Adelaide	*Posidonia* spp.	none	Sonja Venema (SARDI)
Dec 2002	Largs Bay (beach)	*Posidonia* spp.	none	Sonja Venema (SARDI)
Dec 2002	Henley Beach (beach)	*Posidonia* spp.	none	Ron Sandercock (DEH)
16-18 Dec 2002	Penneshaw, Kangaroo Is. (beach & diving)	*Posidonia* spp.	none	Bryan McDonald (SARDI)
Jan 2003	Largs Bay (beach)	*Posidonia* spp.	none	Ron Sandercock (DEH)
Jan 2003	Henley Beach (beach)	*Posidonia* spp.	none	Bryan McDonald (SARDI)

16-18 Jan 2003	Nepean Bay, Bay of Shoals, Kangaroo Is. (beach)	*Posidonia* spp.	none	Bryan McDonald (SARDI)
Feb 2003	Largs Bay (beach)	*Posidonia* spp.	none	Ron Sandercock (DEH)
Feb 2003	Henley Beach (beach)	*Posidonia* spp.	none	Bryan McDonald (SARDI)
2003/4 Season				
05 Oct 2003	North Henley	*Posidonia*	none	Sue Murray-Jones (DEH)
06 Oct 2003	North Henley	*Posidonia*	none	Sue Murray-Jones (DEH)
29 Oct 2003	Cowell	*P. australis*	flowering	Kane Williams (PIRSA)
01 Nov 2003	Ardrossan	*Posidonia*	flowering	David Cowan (Reef Watch)
06 Nov 2003	Port Broughton	*Posidonia*	flowering	David Muirhead (Reef Watch)
13 Nov 2003	Henley Beach - Grange	*Posidonia*	flowering	Sonja Venema (SARDI)
13 Nov 2003	Tiddy Widdy	*Posidonia sinuosa*	fruiting	Ron Sandercock, Sue Murray-Jones (DEH)
13 Nov 2003	Tiddy Widdy	*Posidonia australis*	flowering	Ron Sandercock, Sue Murray-Jones (DEH)
14 Nov 2003	Seacliff Beach (2 m)	*Posidonia*	none	Sonja Venema (SARDI)
14 Nov 2003	Seacliff Beach (6.5 m)	*P. angustifolia*	flowering & fruiting	Sonja Venema (SARDI)
14 Nov 2003	West Beach (10 m)	*P. angustifolia & P. sinuosa*	flowering & fruiting	Sonja Venema (SARDI)
20 Nov 2003	Somerton Park	*Posidonia*	fruiting & flowering	Sandra Leigh (SARDI)
25 Nov 2003	Somerton Park	*P. angustifolia*	flowering & fruiting	Sonja Venema (SARDI)
26 Nov 2003	Kingscote	*P. sinuosa ?*	flowering & fruiting	Bryan McDonald (SARDI)
30 Nov 2003	Largs Beach	*Posidonia*	fruiting	Patricia Irvine (DEH)
01 Dec 2003	South Henley	*Posidonia*	none	Sue Murray-Jones (DEH)
02 Dec 2003	Brighton Beach	*Posidonia*	fruiting	Ron Sandercock (DEH)
02 Dec 2003	Port River channel	*Posidonia*	fruiting	Bruce Miller-Smith (SARDI)
02 Dec 2003	Glenelg	*Posidonia*	fruiting	Alison Eaton (DEH)
03 Dec 2003	Kingscote	*Posidonia*	flowering & fruiting	Bryan McDonald (DEH)
03 Dec 2003	South Henley	*Zostera ?*		Kristen Messenger (DEH)
05 Dec 2003	Webb Beach (Port Parham)	*Posidonia*	fruiting	Ron Sandercock (DEH)
05 Dec 2003	West Beach (shore)	*Posidonia*	fruiting	Sonja Venema (SARDI)
06 Dec 2003	Henley Beach	*Posidonia*	fruiting	Ron Sandercock (DEH)
08 Dec 2003	Seacliff (6.5 m)	*P. angustifolia*	fruiting	Sonja Venema (SARDI)
08 Dec 2003	West Beach (10 m)	*P. angustifolia & P. sinuosa*	fruiting	Sonja Venema (SARDI)
09 Dec 2003	North of St. Kilda	*P. australis*	fruiting	Tim Kildea (SA Water)
10 Dec 2003	Largs Beach Shores	*P. angustifolia & P. sinuosa*	fruiting	Sonja Venema & Stephanie Seddon (SARDI)
13 Dec 2003	Henley Beach	*Posidonia*	fruiting	Ron Sandercock (DEH)
13 Dec 2003	Largs Bay	*Posidonia*	fruiting	Kevin Smith
14 Dec 2003	Henley Beach South	*Posidonia*	fruiting	Sue-Murray Jones (DEH)
15 Dec 2003	Seacliff (6.5 m)	*P. angustifolia*	fruiting	Sonja Venema (SARDI)
15 Dec 2003	Henley Beach South	*P. sinuosa ?*	fruitng	Sue Murray-Jones (DEH)
15 Dec 2003	West Beach (10 m)	*P. angustifolia & P. sinuosa*	fruiting	Sonja Venema (SARDI)
22 Dec 2003	West Beach (shore)	*P. angustifolia*	fruiting	Sonja Venema (SARDI)
22 Dec 2003	West Lakes (shore)	*Posidonia*	fruiting	David Cowan (Reef Watch)
01 Jan 2004	West Beach (shore)	*Posidonia*	fruiting	Scoresby Shepherd (SARDI)
01 Jan 2004	West Lakes (shore)	*Posidonia*	fruiting	Wayne Hutchison (SARDI)
04 Jan 2004	Kangaroo Island (shore)	*Posidonia*	fruiting	Sandra Leigh (SARDI)
19 Jan 2004	Seacliff (6.5 m)	*P. angustifolia*	none	Sonja Venema (SARDI)

19 Jan 2004	West Beach (10 m)	*P. angustifolia & P. sinuosa*	none	Sonja Venema (SARDI)
2004/5 Season				
11 Aug 2004	West Beach boat ramp	*Posidonia*	flowering	Rachel Wear (SARDI)
11 Aug 2004	West Beach boat ramp	*Posidonia*	flowering	Rachel Wear (SARDI)
12 Oct 2004	Lacepede Bay	*P. australis*	flowering	Rachel Wear (SARDI)
14 Nov 2004	Brighton	*Posidonia*	fruiting	Anthony Fowler (SARDI)
17 Nov 2004	Semaphore	*Posidonia*	fruiting	Patricia Irvine (DEH)
04 Dec 2004	Second Valley	*P. coriacea*	fruiting	David Cowan (Reef Watch)
07 Dec 2004	Henley Beach - Brighton (beach)	*P. sinuosa & P. angustifolia*	fruiting	Sonja Venema & Bruce Miller Smith (SARDI)
08 Dec 2004	Henley Beach (beach)	*Posidonia*	fruiting	Emma O'Loughlin (SARDI)
12 Dec 2004	Blow hole creek (beach)	*Posidonia*	fruiting	Shirley Sorokin (SARDI)
23 Dec 2004	Miranda (south of Port Augusta)	*Posidonia*	fruiting	Bryan McDonald (DEH)
27 Dec 2004	Flat mouth beach, Kangaroo Is.	*Posidonia*	fruiting	Sandra Leigh (SARDI)
27 Dec 2004	Yatala	*Posidonia*	fruiting	Ali Bloomfield (DEH)
28 Dec 2004	Carraickalinga (15 m)	*Posidonia*	fruiting	James Brooke (Reef Watch)
28 Dec 2004	Rapid Bay (diver observations off the jetty)	*Posidonia*	fruiting	Steve Cally & Ali Bloomfield (DEH)
28 Dec 2004	Second Valley (15 m)	*Posidonia*	fruiting	James Brooke (Reef Watch)
31 Dec 2004	Emu Bay, Kangaroo Island (beach)	*P. sinuosa & P. angustifolia*	fruiting	Rachel Wear (SARDI)
03 Jan 2005	Rapid Bay	*P. angustifolia*	fruiting	Yvette Eglinton (SARDI)
03 Jan 2005	West Beach (beach)	*P. sinuosa & P. angustifolia*	fruiting	Rachel Wear (SARDI)
04 Jan 2005	West Beach (beach)	*P. sinuosa & P. angustifolia*	fruiting	Rachel Wear & Greg Collings (SARDI)
07 Jan 2005	Henley Beach (beach)	*P. sinuosa & P. angustifolia*	fruiting	Rachel Wear & Mandee Theil (SARDI)
07 Jan 2005	West Beach (beach)	*P. sinuosa & P. angustifolia*	fruiting	Rachel Wear & Mandee Theil (SARDI)
12 Jan 2005	West Beach (beach)	*P. sinuosa*	fruiting	Rachel Wear (SARDI)
17 Jan 2005	Stuart Bay (Yorke Peninsula)	*P. kirkmanii*	fruiting	Hugh Kirkman
? Jan 2005	Blow hole creek (beach)	*Posidonia*	fruiting	James Brooke (Reef Watch)