Technical Report

Adelaide Coastal Waters Study

Stage 2 *Research Program 2003 - 2005*

Technical Report No. 9 August 2005

Responses to reduced salinities of the meadow forming seagrasses Amphibolis *and* Posidonia *from the Adelaide metropolitan coast*

Government of South Australia

WaterCare
It's in your hands

CSIRO

Responses to reduced salinities of the meadow forming seagrasses Amphibolis *and* Posidonia *from the Adelaide metropolitan coast*

Authors

Grant Westphalen, Emma O'Loughlin, Greg Collings, Jason Tanner, Yvette Eglinton, and Simon Bryars

South Australian Research and Development Institute
SARDI Aquatic Sciences
PO Box 120 Henley Beach
SA 5022

ISBN 1 876 562 95 1

16th August 2005

Reference

This report can be cited as:

Westphalen, G., O'Loughlin, E., Collings, G., Tanner, J., Eglinton, Y., and Bryars, S. (2005) "Responses to reduced salinities of the meadow forming seagrasses *Amphibolis* and *Posidonia* from the Adelaide metropolitan coast*".* ACWS Technical Report No. 9 prepared for the Adelaide Coastal Waters Study Steering Committee. South Australian Research and Development Institute (Aquatic Sciences) Publication No. RD01/020814, Adelaide.

Acknowledgement

This report is a product of the Adelaide Coastal Waters Study. In preparing this report, the authors acknowledge the financial and other support provided by the ACWS Steering Committee including the South Australian Environment Protection Authority, SA Water Corporation, the Torrens Patawalonga and Onkaparinga Catchment Water Management Boards, Department for Transport Energy and Infrastructure, Mobil Refining Australia Pty Ltd, TRUenergy, Coast Protection Board and PIRSA. Non-funding ACWS Steering Committee members include the Conservation Council of SA, SA Fishing Industry Council Inc, Local Government Association, Department of Water Land and Biodiversity Conservation and Planning SA.

SARDI Aquatic Sciences Disclaimer

The recommendations given in this report are based on the best available information at the time of writing. The South Australian Research and Development Institute (SARDI) makes no warranty of any kind expressed or implied concerning the use of the information contained in this report.

Authors: Dr Grant Westphalen, Emma O'Loughlin, Dr Greg Collings, Dr Jason Tanner, Yvette Eglinton, Dr Simon Bryars

Reviewers: Dr John Carragher, Prof. Peter Fairweather

Approved by: Dr John Carragher

Signed: 

Date: 16th August 2005

Distribution: ACWS Scientific Committee, ACWS Steering Committee, SARDI Aquatic Sciences Library

Circulation: Public Domain

Acknowledgements

The authors thank Mandee Theil, Dave Miller, Dr Sasi Nayar and Prof. Anthony Cheshire for their assistance in undertaking the experiments presented in this document. Suzanne Bennett is also thanked for assisting in the preparation of this document. Prof. Peter Fairweather from Flinders University and Dr John Carragher are thanked for their reviews of the draft document. This research has been funded as part of the Adelaide Coastal Waters Study.

Table of contents

Executive overview

Freshwater inputs (wastewater, stormwater and riverine) into the Adelaide near-shore appear to correlate with the area of seagrass loss within the metropolitan coast. The effect of reduced salinity on two meadow-forming species was investigated.

Adult plants of *Amphibolis antarctica* and *Posidonia sinuosa* were considered in terms of their response to short (72 hours) and long-term (seven weeks) exposure to salinities as low as 0 ppt. Both *A. antarctica* and *P. sinuosa* were highly tolerant to short-term reductions in salinity. Only after seven weeks of exposure to salinities of ~ 1 ppt were the plants essentially killed. Observations of freshwater inputs along Adelaide's metropolitan coast suggest that the likelihood of occurrence of salinity levels less than 10 ppt in the vicinity of seagrass beds is low except close to sources. As a factor determining the large-scale loss of adult seagrasses, in particular *A. antarctica* and *P. sinuosa*, reduced salinity is unlikely.

In addition to the study on adult seagrasses, the viviparous seedlings of *A. antarctica* and the fruits of *Posidonia angustifolia* were also considered in terms of their tolerance to reduced salinity. The floating seedlings and fruits of these species are the major transport mechanism outside established areas and more likely to be exposed to reduced salinities than the adults.

Photosynthetic efficiency of *A. antarctica* seedlings was substantially reduced after short-term (72 hours) exposure to salinities below ~ 5 ppt. Although none of the test specimens was killed by the salinity dilution, it may well be expected that survival after more prolonged exposure would be limited.

Posidonia angustifolia fruits suffered high levels of mortality when subjected to salinity levels of 10 ppt or less.

The capacity for either *P. angustifolia* or *A. antarctica* to successfully recruit into areas of reduced salinity is probably minimal. Although the generality of these results to other species of either genus is unknown, the expansion of populations of either *Amphibolis* or *Posidonia* into new areas, which would be the primary role of propagules, will be determined at least in part by the salinity regime (amongst other factors such as depth, substrate, wave action, etc).

It follows that, while freshwater may not have been a factor in historical seagrass losses, it may well play a role in determining the capacity for natural regeneration / recovery at sites close to terrigenous freshwater inputs.

1. Introduction

1.1. Background

Since the 1940's, about 5000 ha of seagrass meadows have been lost from the Adelaide metropolitan coast (Westphalen *et al.* 2004). In particular, major losses of nearshore seagrasses occurred in the region between Largs Bay and Holdfast Bay (Westphalen *et al.* 2004; Figure 1). Degradation and loss of seagrass meadows is a major cause of concern for coastal managers due to the importance of these systems to near-shore productivity, stability and biodiversity. Seagrass losses along the Adelaide coast were previously correlated with the construction of stormwater drains, sewage and sludge outfalls, and the re-channelling of the Torrens River to the sea (Westphalen *et al.* 2004). Nonetheless, primary causes of seagrass decline are poorly understood for the Adelaide metropolitan coast as seagrass loss mainly occurred in shallow water close to shore from where it has advanced to seaward. Potential causes of seagrass decline along the Adelaide coast include elevated nutrients, toxicants, increased turbidity, and decreased salinity (Westphalen *et al.* 2004).

Prior to European settlement, there was very little freshwater input along the Adelaide coast between Largs Bay and Holdfast Bay. The Patawalonga Creek and the Port River may have delivered some freshwater to the coast but, due to engineering works and urbanisation, inputs increased substantially during the 20th century. Major changes included the diversion of the Torrens River away from inland wetlands directly to the ocean at West Beach (Figure 2), and the construction of numerous stormwater drains and wastewater outfalls (Westphalen *et al.* 2004; Figure 1). The Adelaide metropolitan coastline is currently affected by many different sources of freshwater. For the region between Largs Bay and Holdfast Bay, average annual freshwater input is currently estimated at 44.8 GL from the Torrens River, Patawalonga, and Holdfast Drains catchments collectively (Wilkinson *et al.* 2004) and 18.4 GL from the Glenelg wastewater treatment plant (WWTP) outfall (Wilkinson *et al.*2003). Importantly, discharges to the sea are relatively constant year-round from the Glenelg WWTP outfall, but are pulsed and mainly occur from May to October in the catchments (Wilkinson *et al.* 2004). As the timing of seagrass recession along the Adelaide coast between Largs Bay and Holdfast Bay coincides with the completion of stormwater outlets in this region (Seddon 2002) and there is currently a significant annual input of freshwater (63.2 GL) to the region, it seems possible that decreases in nearshore salinity could be related to seagrass loss. Thus, further investigation is warranted.

Westphalen *et al*. (2004) reviewed the current state of knowledge with regard to the effects of changed salinity on seagrasses noting that, while numerous studies were conducted on the effects of reduced salinity on estuarine species (particularly from the Northern Hemisphere), very little work has occurred on more oceanic species from southern Australia. In the region of major nearshore seagrass loss between Largs Bay and Holdfast Bay, the spatially dominant meadow-forming seagrass species were believed to be *Posidonia sinuosa, Amphibolis antarctica* and to a lesser extent *Posidonia angustifolia* which is more common in deeper waters (Westphalen *et al.* 2004). The current nearshore margin is still dominated by those three species (Simon Bryars, unpublished data). Thus the present report documents investigations on the effects of reduced salinity on various life stages of *P. sinuosa, P. angustifolia* and *A. antarctica*.

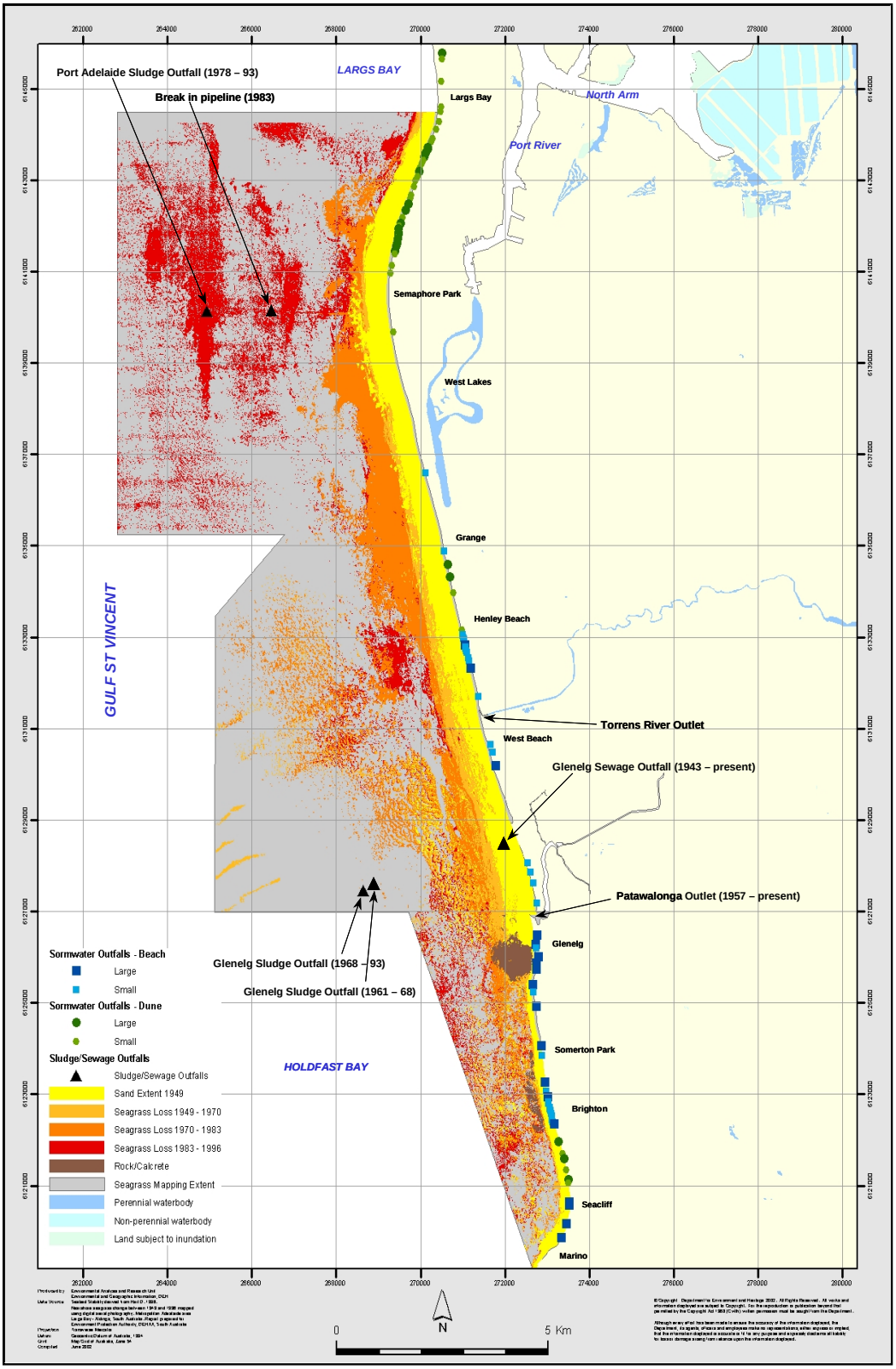

Figure 1 - Map of the Adelaide metropolitan coast showing the accumulated loss of seagrasses from 1949 to 1996 between Largs Bay and Marino. The location of input sources is also indicated. Image from Seddon (2002) constructed by Tim Noyce care of the South Australian Department of Environment and Heritage.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

Figure 2 - Torrens River discharging freshwater to Adelaide's coastal waters after heavy rains in June 2005. Mean annual flow from the Torrens River is currently estimated at 22.4 GL (Wilkinson *et al.* 2004).

1.2. Amphibolis Antarctica

Adult *Amphibolis antarctica* has been shown to tolerate high salinities in Shark Bay, Western Australia (58.5 and 70 ppt; Walker 1985, Kendrick *et al.* 1988). Walker and McComb (1990) observed that *A. antarctica* seedlings performed poorly at 65 ppt. However, for adults and seedlings, the large-scale occurrence of increased salinities of this magnitude (up to twice that of normal seawater) is highly unlikely on the relatively exposed coast of metropolitan Adelaide. As a mechanism for large-scale seagrass loss, reductions in salinity through the dilution of coastal waters with terrigenous inputs would appear to be more relevant in terms of their occurrence and spatial extent. Yet there is no information available on the effects of reduced salinity on *A. antarctica*.

Amphibolis antarctica is viviparous, meaning that seedlings germinate and grow while still attached to the parent plant (Kirkman 1998). *Amphibolis* seedlings are released from the adults from July to December, after they have developed 3 – 4 leaves and an attachment unit (Robertson 1984, Kirkman 1998; Figure 3). With a potentially protracted drifting phase, these seedlings have the capacity to disperse widely and assist the species in being an early coloniser (Clarke and Kirkman 1989), despite it having relatively slow horizontal growth (Marbà and Walker 1999). *Amphibolis* seedlings may have a different response to reduced salinity than do the adults.

Figure 3 - *Amphibolis antarctica* seedlings collected from the Adelaide metropolitan coast shortly after detaching from their parent.

1.3. *Posidonia* spp.

Tyerman *et al.* (1984) found that *Posidonia australis* was unaffected by salinities as low as 13 ppt, but there is little information on *P. sinuosa*, *P. angustifolia* or *P. coriacea*. *Posidonia australis* occurs in shallower areas (Shepherd and Robertson 1989), which may suggest a predisposition to salinity changes that may or may not translate to other species within the genus. In addition, all *Posidonia* species release mature seeds that float at the surface where they may come into contact with buoyant freshwater plumes. For example, *P. angustifolia* retains its seeds on parent plants until maturity, with release occurring from August to January (Robertson 1984, Kirkman 1998; Figure 4). The buoyant pericarp enables the seed to float and disperse substantial distances from the parent but the seed itself sinks after release from the split pericarp (Kirkman 1998).

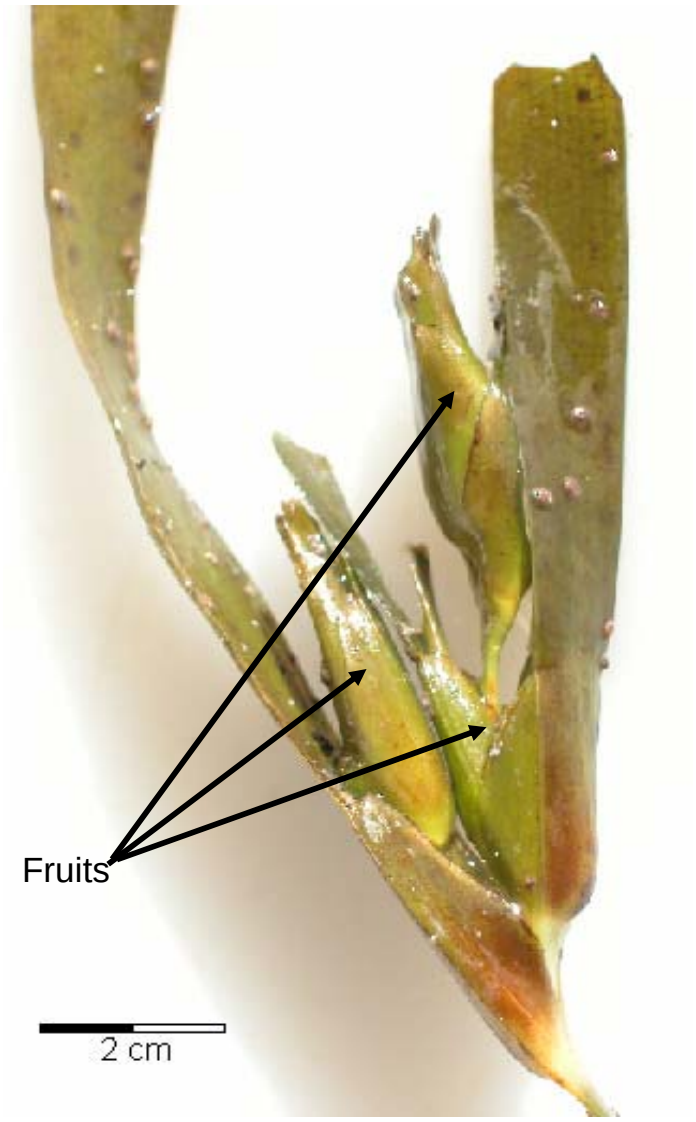

Figure 4 – Sprig of *Posidonia angustifolia* with mature fruits still attached.

1.4. Aims

In the first instance, the potential for reduced salinity to result in the loss of established adults of both *Amphibolis* and *Posidonia* was investigated. In addition, as seedlings of *Amphibolis* and fruits of *Posidonia* are the main propagules for establishment of new colonies (see above) (*sensu* Hemminga and Duarte 2000 in Sintes *et al.* 2005), the effect of reduced salinity on these propagules was also investigated. *Posidonia sinuosa* (rather than *P. angustifolia*) was used for the adult trials as it was assumed that there would be little difference in the physiological responses of the two morphologically similar and sympatric species. Likewise, as *P. sinuosa* fruits were unavailable at the time of the study, *Posidonia angustifolia* only was used in the response trial. Thus the present study had three main parts that relate to the lowered salinity tolerances of:

1. Adults of *P. sinuosa* and *A. antarctica*
2. Seedlings of *A. antarctica*, and
3. Fruit and seedlings of *P. angustifolia.*

2. Materials and Methods

Four separate salinity experiments were undertaken to examine the response of seagrass adults and seedlings or fruits. As an indicator of plant stress Chlorophyll *a* fluorescence of *A. antarctica* adults and seedlings and *P. sinuosa* adults was measured using a pulse amplitude modulated (PAM) fluorometer, (Diving-PAM, Walz, Germany 1998). Chlorophyll *a* fluorescence has been used to determine stress in seagrasses (Seddon and Cheshire 2001; Macinnis-Ng and Ralph 2003a; Macinnis-Ng and Ralph 2003b; Macinnis-Ng and Ralph 2004). Measurements of effective quantum yield (or EQY) and ambient fluorescence (Ft) were used in analyses. Neither in themselves is a definitive indicator of plant death, but a loss of photosynthetic operation indicates that key physiological processes within the plant have been disrupted (Cheshire *et al.* 2002). Both measures are useful in short-term experiments, particularly if photosynthesis remains non-functional after an appropriate period of recovery (Cheshire *et al.* 2002).

2.1. Short term – *A. antarctica* adults

Mature stems of *A. antarctica* without roots, were collected from 1.5 – 2 m depth from Kingston Park during April (autumn) 2004. Only the above-ground component of this seagrass was used, as *A. antarctica* makes little investment in below-ground biomass compared with other species of seagrass (Pedersen *et al.* 1997; Paling and McComb 1994; Paling and McComb 2000). Plants were kept overnight in a 500 L tank with flow-through sand filtered seawater, under 50% shade cloth.

Experimental units comprised of ten *A. antarctica* stems with between two and 10 leaf clusters per stem woven into 30 × 30 cm sheets of plastic mesh. Each unit was placed in one of twenty 68 L tubs (Nally Bins, Viscount Plastics, Australia) filled with seawater to a depth of ~40 cm (Figure 5). In each tub, a 600 L hr^{-1} aquarium pump with an air diffuser attached maintained water movement and oxygenation. To limit temperature differences between different salinity treatments, the tubs were placed in a flow-through seawater tank submerged to a depth of 36 – 44 cm. This large tank was covered with 50% shade cloth to give a nominal light regime equivalent to a depth of ~3 - 4 m.

Figure 5 - *Amphibolis antarctica* experimental unit in one of the experimental tubs. The aquarium pump and air diffuser can be seen on the lower left.

Amphibolis antarctica experimental units were acclimatised in their respective tubs for two days prior to salinity dilution treatment. Each 68 L tub was randomly assigned a salinity of 0, 5, 10, 15, 20 (n = 3) or 35 ppt (control, n=5). Additional controls gave a better measure of differences resulting from light variation across the larger tank. Water was changed twice during the experiment, once to lower salinity and again, 72 hours later, to return to ambient levels, with 100% of the water replaced each time. Experimental units were moved into a 35 ppt salinity tank during the period of salinity adjustment, ensuring adequate mixing in the tubs prior to exposure. Controls were moved amongst themselves.

PAM measurements were taken during three timephases – the 72 hour period of exposure to treatment salinity levels (hereafter referred to as "treatment period"); the 48 hour period prior to this ("pre-treatment period"); and the 48 hours succeeding the treatment period ("post-treatment period"). All PAM measurements were taken before 11:00 am. Fluorescence measurements were collected from ten different *A. antarctica* leaves haphazardly selected in each container. Temperature and salinity were also measured daily using a hand-held Eco Scan Salt 6 salinity meter (Eutech Instruments Pty Ltd, Singapore).

2.2. *A. antarctica* and *P. sinuosa* adults

Based on results of the *Amphibolis* trial, a more complicated experiment was conducted in eighteen large (2,300 L) outdoor tanks. Using larger tanks allowed the addition of *Posidonia sinuosa*, the size of which would not permit inclusion in the previous experiment. Two experiments were run simultaneously: a short-term experiment for seven days (similar to the previous trial) conducted against *P. sinuosa* and a long-term experiment, which lasted for seven weeks employing both species.

Mature *P. sinuosa* plants were collected from the southern margin of Section Bank, in about 3 m depth in May (autumn) 2004. Plants were kept in flow-through seawater for two days under 50% shade cloth. Between six and 20 shoots were potted in washed beach sand in plastic cubes (36.5 x 33.5 x 29 cm; n = 18; Figure 6) and kept in flow-through seawater under 50% shade cloth until required. Adult *A. antarctica* plants were collected with roots attached from the same location as the first trial, in June (winter) 2004. Plants were then returned to the lab and kept in flow-through seawater under 50% shade cloth. Between 10 and 15 stems were potted in washed beach sand (n = 6) similar to *P. sinuosa*.

Figure 6 - Image of *A. antarctica* and *P. sinuosa* planted in the larger tanks.

A. antarctica and *P. sinuosa* cubes were randomly assigned across eighteen 2,300 L tanks covered with 50% shade cloth (Figure 6). All tanks had a 20 mm air injection pipe for aeration and water movement. In one tank, two LI-COR quantum sensors and a data logger recorded photosynthetically active radiation (PAR), averaged over a one-minute period each hour for the duration of the experiment, excluding a four day period in July. PAR was measured just under the water surface and at the same level as the seagrass (~1 m deep – with shade cloth this light environment equated to a depth of ~ 4 – 7 m).

Three replicate tanks were randomly assigned to six salinity levels: 0, 5, 10, 15, 20, 35 ppt (control). Before the treatment period, PAM measurements were taken once between 9:00 and 11:00 am. Movement of all plants out of experimental tanks and into one of three 9,000 L flow-through tanks (also under 50% shade cloth) was required during salinity adjustment which took ten days to allow water mixing and temperature equilibration in each tank.

2.2.1. Short-term salinity trial with *P. sinuosa*

A short-term experiment was used to determine if *P. sinuosa* was affected by a short-term (72 hours) decrease in salinity. PAM measurements were taken just prior to plants being put back into assigned experimental tanks and then at 24, 48, and 72 hourly intervals. Five fluorescence measurements were obtained from each experimental unit every 24 hours for two days after plants were returned to ambient seawater. All PAM measurements were taken between 9:00 and 11:00 am. On each occasion that PAM measurements were taken, temperature and salinity were also measured as per the previous experiment.

2.2.2. Long-term salinity trial with *P. sinuosa* and *A. antarctica*

The 0 ppt treatment was not returned to ambient seawater, but run out for seven weeks to determine if prolonged exposure could ultimately kill *A. antarctica* or *P. sinuosa*. 50% water changes were completed once a week. PAM measurements (5 replicate measurements per species in each tank) were taken every 24 hours, between 9:00 and 10:00 am for the first week and then taken three times per week for the duration of the experiment. Temperature and salinity were measured at the same time as PAM observations.

2.3. *Amphibolis* seedlings

Twenty 68 L tanks were filled with seawater in a constant environment room maintained at 16ºC. Water movement in each tank was facilitated by two submersible aquarium pumps (600 L h^{-1}) connected to a timer allowing alternate pumping on a 10-minute cycle. A venturi air diffuser was attached to one pump in each tank. Light was provided by eight fluorescent lights (6,700 - 18,000 K; 12:12 light: dark cycle), two 400 W metal halide lamps (Phillips SON-T ARGO; 8:16 light: dark) and one 1000 W metal halide lamp (Sylvania M47-R; 4:20 light: dark). Light diffusers were placed in front of the 400 W and 1000 W lights to provide a more even light field. The light regime closely followed that employed by Seddon (2000), and was designed to replicate natural conditions with high irradiance in the middle of the day.

Unattached (newly released) *A. antarctica* seedlings were collected during October 2004 and planted in small pots containing washed beach sand. Ten of these units were randomly allocated to each tank and acclimated for one week, with 50% of the water replaced after three days. The plants were then removed to one of the four randomly selected control tanks. The remaining sixteen tubs were then randomly assigned a salinity of either 0, 5, 10 or 20 ppt with four replicates per salinity level. After ensuring each experimental tank was adequately mixed, plants were returned to their appropriate tank (controls were moved between themselves). The treatment period lasted for 72 hours then the plants were moved back to control tanks and all tub salinities were returned to ambient levels. The plants were

then replaced and left in their respective tanks at 35 ppt for a further 72 hours to monitor recovery.

Before the treatment period, PAM readings were obtained to assess the health of the plants. Immediately prior to the treatment period, a final pre-treatment reading was obtained (time = 0), with subsequent readings taken at 1, 3, 6, 12, 24, 30, 48 and 72 hours after the treatment period began. On return to ambient seawater, the same protocol was followed over the following 72 hours (i.e. measurements at 1, 3, 6, 24, 30, 48 and 72 hours). Measures of the ambient fluorescence (Ft) and effective quantum yield (EQY) were obtained.

Salinity adjustment and daily water quality were assessed across the course of the experiment with a Horiba W22XD water quality meter (pH, conductivity, temperature, dissolved oxygen, turbidity, salinity and redox).

2.4. *P. angustifolia fruits*

Posidonia angustifolia fruits were collected from Adelaide's metropolitan beaches as drift between West Beach and Somerton Park in December 2004. Fruits were kept under 50% shade cloth in flow-through sand-filtered seawater for three days prior to use in experiments.

Trials were conducted in sixteen large (2,300 L) tanks, containing flow-through seawater to a depth of 30 cm within which one 68 L container sat three quarters submerged. The large tank thus acted as a water bath that maintained a stable temperature in each of the smaller tubs within which the *Posidonia* fruits could be readily monitored. Each 68 L container had a 5 cm layer of washed beach sand and a 600 L h^{-1} aquarium pump facilitating water movement and aeration. A cover of 50% shade cloth was used over each large tank to replicate a light intensity at a depth of ~ 3 - 5 m. This approach accepted that while floating on the surface the fruit had a less-than-natural light environment. Conversely, it also meant that the settled seedlings in the bottom of the tubs could germinate in a more natural light regime.

Each tub was randomly assigned a salinity of 0, 10, 20 or 35 ppt (control), with four replicates of each level. A total of 100 randomly selected fruits, all of which were floating, green and unsplit, were incubated in each tub for 72 hours after which all fruits were categorised according to four factors:

1. Floating or sunk
2. Dehisced (split) or whole
3. Seedlings released (note that split fruit may still hold their seedlings)
4. Seedling viability (signs of growth or decay)

Fruit pericarps that no longer contained seedlings were removed from the container, thus simplifying assessment of the remainder. Fruits with or without seedlings could float or sink (as opposed to seedlings, which always sink). All seedlings that were firm and/or had produced a cotyledon were counted as living, whereas those with signs of decay were assumed to be dead. A few fruits rotted away completely and were considered as missing/dead.

After 72 hours exposure, the tubs were returned to ambient salinity levels (35 ppt) and left for a further five days when fruit and seedlings were again assessed (5 days ambient). The large holding tanks were then filled to capacity (2,300 L) with sand-filtered seawater and left for a further 11 days, when an assessment was undertaken for the final time (16 days ambient).

3. Data analysis

3.1. Adult seagrass

To determine how important changes in salinity were to plant health, measured as effective quantum yield, a series of statistical models were developed and compared using Akaike's Information Criteria (AIC; Burnham and Anderson 2002). For this purpose, Generalised Additive Models (GAM; Hastie and Tibshirani 1990) were fit to the data, allowing time responses to be fit as smooth non-linear terms, rather than assuming a pre-defined parametric fit. All non-linear terms were fit using cubic spline smoothers with four degrees of freedom, and all models assumed a normal distribution of the data. In all cases, the importance of salinity changes was assessed by comparing a model with an interaction between time and salinity to a model without this interaction, as salinity was changed part-way through the experimental period. This model formulation allowed for an initial chance difference between treatments to persist, but determined whether treatment groups responded differently over time. To determine if there was a non-linear response over time, linear versions of both models were also included in the model selection process. Finally, a model without any treatment effects was also included, allowing for the situation that there were no differences between treatments at the start of the experiment, and no differences in response of different treatments over time.

3.1.1. Short term *A. antarctica* trial

For the short term *A. antarctica* experiment, where PAR was also measured, the full model was:

LnYield ~ s(PAR)+Salinity*s(Time)+Salinity/Bin*s(Time)

where s(Time) indicates a non-linear smooth fit for Time, and Salinity/Bin indicates that Bin is nested in Salinity.

For this experiment, a series of models without PAR were also fitted. Finally, a series of models with PAR entered as a linear term was also analysed. For all of these models, data from day 1 was not used as PAR was not measured due to instrument error. In addition, a second series of models without PAR was also included in the analysis, allowing the first day of data to be used. As this last series used a different data set (ie included data from day 1 that was not included in the other analyses), it could not be compared to the other models developed for this data set, but instead was used to further inform model choice for the first set.

3.1.2. Short term *P. sinuosa* trial

The full model used for the short-term *P. sinuosa* experiment was:

LnYield ~ Salinity*s(Time)+Salinity/Bin*s(Time)

3.1.3. Long term *A. antarctica* and *P. sinuosa* trial

The long-term experiment was analysed for the first 600 hrs only with LnYield as the response variable, because effective quantum yield values become less reliable at low levels of ambient fluorescence (Ft; Walz 1998). In addition, Ft was analysed for the full length of the experiment, using the same model as for LnYield.

For the long-term experiment, the full model was:

LnYield ~ Salinity*s(Time)*Plant+Salinity/Bin*s(Time)*Plant

where Plant distinguishes between *Posidonia* and *Amphibolis*.

LnYield was calculated as:

LnYield = ln (1 – Yield)

Yield was transformed to homogenise the variance associated with the means (Anthony Cheshire; SARDI pers comm.).

For each model series, models were compared on the basis of Akaike weights and evidence ratios (Burnham & Anderson 2002). Akaike weights give the weight of evidence in favour of each model being the best model, given that one of the models in the set being compared is the best model. Evidence ratios are simply the ratio of the Akaike weights for any 2 models, in this case we used the ratio of the best model (lowest AIC) to each other model, and indicate how much more likely one model in the pair is compared to the other. Small evidence ratios (say<5) indicate that there is not much evidence for one model over the other, whereas large evidence ratios indicate that one model is much more likely than the other.

3.2. *Amphibolis* seedlings

The objective of the analysis was to examine the effect of salinity on yield and to determine whether this effect was different at three critical points in time, these being before the reduced salinity exposure was applied (where there was not expected to be a difference), after 72 hours of exposure (the immediate response) and after the subsequent 120 hour recovery period in ambient seawater (to determine whether any effects noted at 72 hours were transitory or more permanent in nature). A two-way repeated measure ANOVA was performed, using Plant (a random factor nested within Salinity) as the appropriate error term to test the effects of Salinity, Time and the Salinity × Time interaction. Having demonstrated this interaction, separate one way ANOVAs on the effect of salinity were undertaken for each of the three points in time. Subsequently, a Dunnett's test was used to localize differences between each of the lowered salinity treatments and the control. In this instance a one-tailed test was used, as we were interested only in a negative effect of reduced salinity. Effective quantum yield data were transformed (as X' = ln(1-X)). This transformation was undertaken to minimise the tendency of the variances to vary with the inverse of the mean (Anthony Cheshire, SARDI pers. comm.).

3.3. *P. angustifolia* fruits

The objective of this analysis was to identify whether a pulse of reduced salinity caused an increase in the mortality of *Posidonia* seedlings. With this end in mind, a one-way ANOVA was applied to determine whether there was a significant difference in the number of dead seedlings at the end of the trial, i.e. after the 16 day "recovery" period. In two cases a small proportion of the fruits (3 and 10 of 100) went missing, so mortality was calculated on a proportional basis for these two. All data were transformed (X'=arcsin ($\sqrt{X}$) as befits proportional type data (Zar 1984).

4. Results

4.1. Short term *A. antarctica* experiment

The short term *A. antarctica* experiment showed no evidence of a reduction in yield due to changes in salinity (evidence ratio for reduced model = 1.15, AIC=46), although the full model did have the lowest AIC (45.8). The effect of time was non-linear in both the full (evidence ratio = 13, AIC=50.9) and reduced (evidence ratio = 16.5, AIC=51.4) models. These conclusions did not change when the full data set including day 1, without light, was analysed (results not shown). The amount of photosynthetically active radiation was important in determining yield (evidence ratio = 116 & 157, AIC=55.3 & 55.9 for the full and reduced models respectively), and this effect was non-linear (evidence ratio = 11.8 & 15.5, AIC=50.7 & 51.2 for the full and reduced models respectively). Most of the variation in the yield is clearly related to light (Figure 7), with high yields occurring in low light. At the end of the experiment plants in the 0 ppt and control treatments had virtually identical yields, indicating no biologically significant effect of a reduction in salinity.

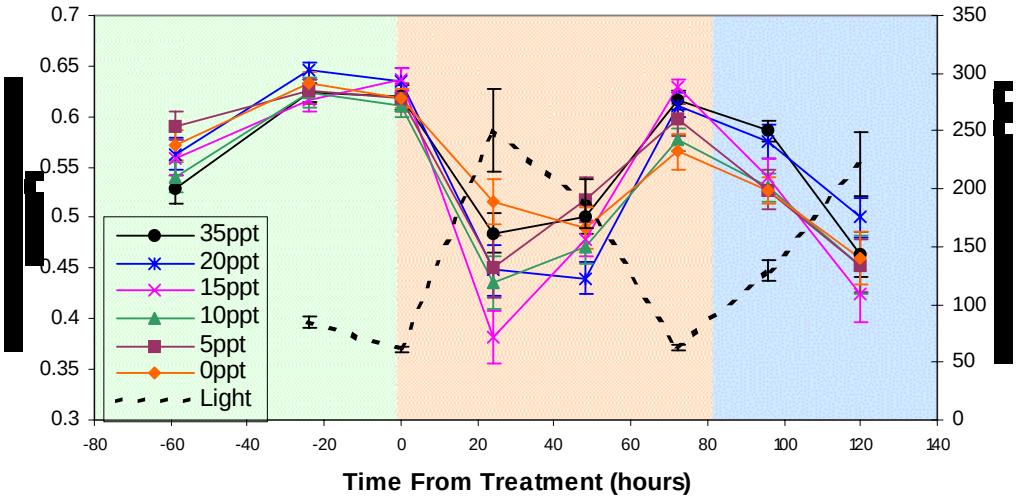

Figure 7 - Average effective quantum yield response of *A. antarctica* to reduced salinity under natural light: pre-treatment period (green), treatment period (orange) and post-treatment period (blue). Bars = standard error. Black dashed line represents light on the secondary (right hand) Y axis.

4.2. Short-term *P. sinuosa* experiment

Similarly to the *A. antarctica* experiment, the short-term *P. sinuosa* trial indicated no evidence to suggest that the change in salinity had an effect on seagrass health. While the full model incorporating an interaction between time and salinity did have the lowest AIC (34.6), and therefore showed the best fit to the data, the evidence ratio for it in comparison to the reduced model with no interaction between time and salinity was only 1.4 (AIC=35.3). The next best model was that which only included a time effect, although the evidence ratio for it was 19.2 (AIC=40.5), indicating that it is very unlikely to be the correct model. For both the full and reduced models, there was no evidence of time being linear, as the linear models had evidence ratios of 625 and 904 respectively. While there was clearly a decline in yield for all lowered salinity treatments after salinity was changed, this decline also occurred for the control plants (Figure 8), suggesting that some environmental factor other than decreased salinity was the cause. Interestingly, when plants were placed back into full-strength seawater, yield increased, including the unmanipulated controls.

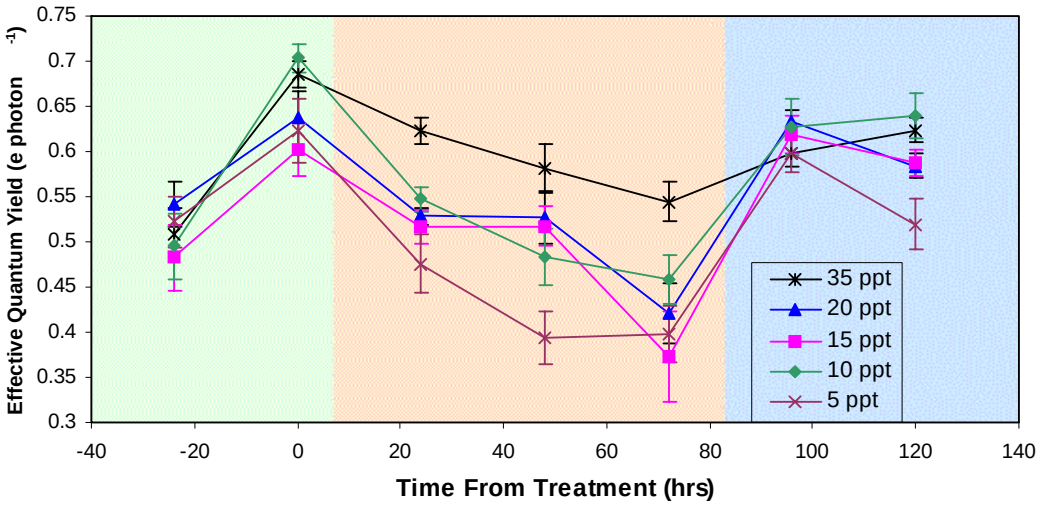

Figure 8 - Average effective quantum yield response of *P. sinuosa*, pre-treatment (green), treatment (orange) and post-treatment (blue). Bars = standard error.

4.3. Long-term *A. antarctica* and *P. sinuosa* experiment

After about 250 hours (~ 10 days), the yield in low salinity treatments declined in comparison to controls (Figure 9), and despite initial differences in yield, both *Amphibolis* and *Posidonia* responded similarly. However, there was no statistical indication that LnYield was influenced by salinity treatment, with the evidence ratio for the reduced model being 2.4 (AIC for full and reduced models = 41 & 42.7 respectively). There was also little evidence for a difference between seagrass species, or for a non-linear effect of time (evidence ratios for non-linear and linear models without species = 1.5 & 3.4 respectively, AIC = 41.8 & 43.4).

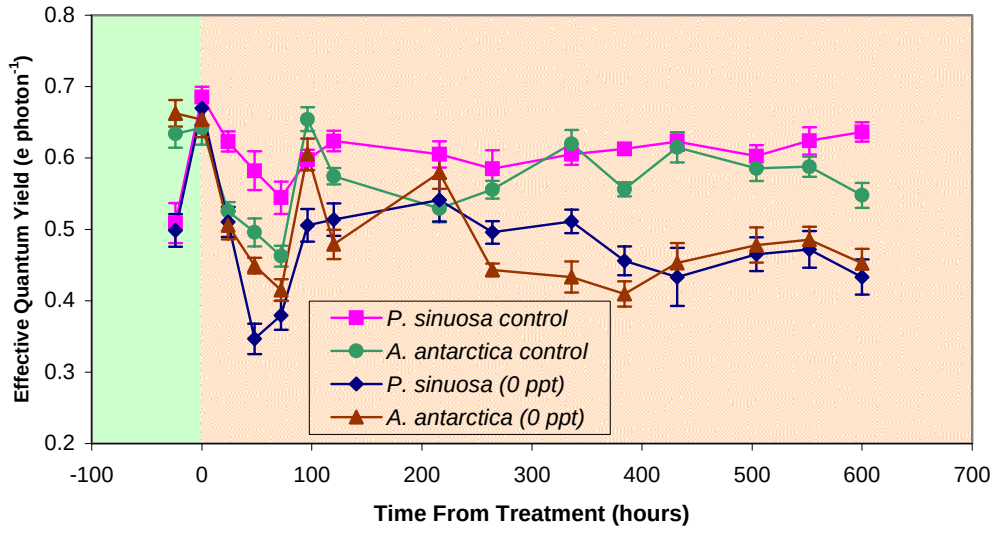

Figure 9 - Average effective quantum yield response of *A. antarctica* and *P. sinuosa* to reduced salinity of 0 ppt for 600 hours (~ four weeks). Time zero represents the beginning of the treatment with freshwater. Bars = standard error.

In contrast to LnYield in the first 600 hours, when Ft was considered as the response variable, there was a clear effect of salinity reductions over the full course of the experiment (Figure 10). Again, the full model had the lowest AIC (13,175,531), but in this case no other model had an AIC value within 5 orders of magnitude of this, meaning that evidence ratios were undefined. This situation indicates that salinity reductions had an effect, the response to time was non-linear and there was a substantial difference between seagrass species.

By the end of the experiment (1100 hours), the ambient fluorescence (Ft) values of the controls of both species were similar whilst the low salinity treatments were substantially lower, particularly for *A. antarctica*. *P. sinuosa* exposed to reduced salinity did not seem to be affected until after 600 hours (~ four weeks), while *A. Antarctica* appeared to show differences relative to the controls at approximately 300 hours (~ two weeks; Figure 10).

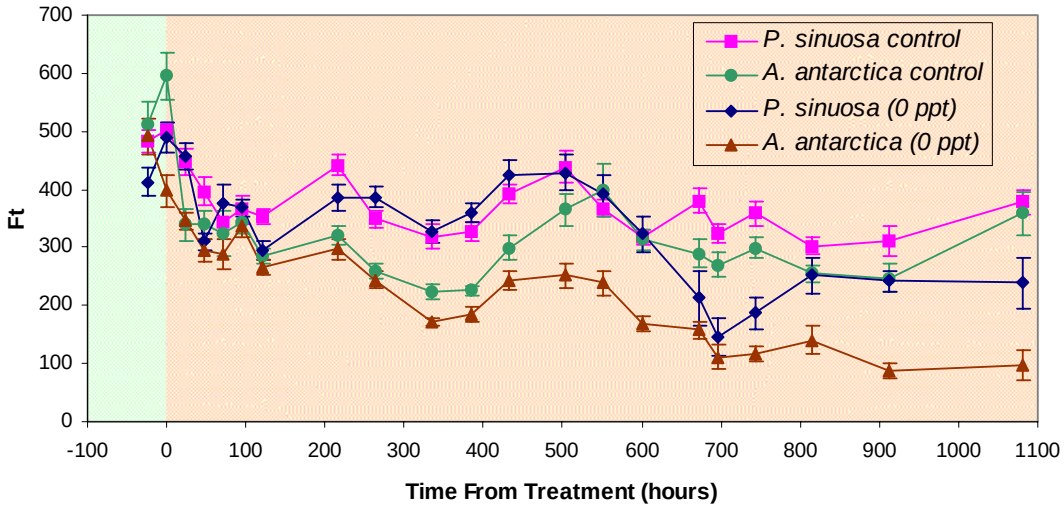

Figure 10 - Average ambient fluorescence (Ft) of *A. antarctica* and *P. sinuosa* within each measurement time over the seven week experimental period. Bars = standard error.

4.4. *Amphibolis* seedlings

Low salinity treatments had a negative effect on the effective quantum yield of *A. antarctica* seedlings (Figure 11). Analysis of transformed data before, immediately after the 72 hour treatment phase, and after a 120 hour recovery phase in ambient seawater indicated an effect of salinity that differed with time (time x salinity interaction, P=0.0008; repeated measure two-way ANOVA; Table 1). No significant difference in yield was found amongst the different salinities before the exposure (P > 0.05, one-way ANOVA; Table 2), but a significant effect of salinity was detected both immediately after the exposure period and after 120 hours of recovery (one-way ANOVAs P = 0.0088 and P = 0.0053 respectively; Table 2). At both times, it was the 0 and 5 ppt treatments which were significantly lower than the control, whilst exposure to 10 ppt and 20 ppt failed to demonstrate a statistically significant difference (Dunnett's test; 3).

While the experiment failed to demonstrate any statistically significant effect at a salinity level of 10 ppt, a smaller, non-significant effect cannot be discounted. Some evidence of this possibility is provided by calculating the loss in yield of plants in each of the different salinity treatments (Figure 12) as a function of their initial pre-treatment value (i.e. $(EQY_{initial}-EQY_{final})/EQY_{initial}$). It appears that, at least up to a salinity of 10 ppt, that the loss in yield measured across the period of exposure (i.e. up to 72 hours) is positively related to the

salinity. The smaller apparent effect at 10 ppt makes it more difficult to detect statistically, while at 20 ppt, there is clearly no difference relative to the control. Thus, 0 ppt and 5 ppt salinities have an obvious effect on yield which persists even after 120 hours recovery, and it is possible (but not statistically demonstrated) that 10 ppt may have an effect immediately following the treatment period, but after 120 hours of recovery this effect is no longer evident (the yield is actually higher than prior to the treatment period (Figure 12)).

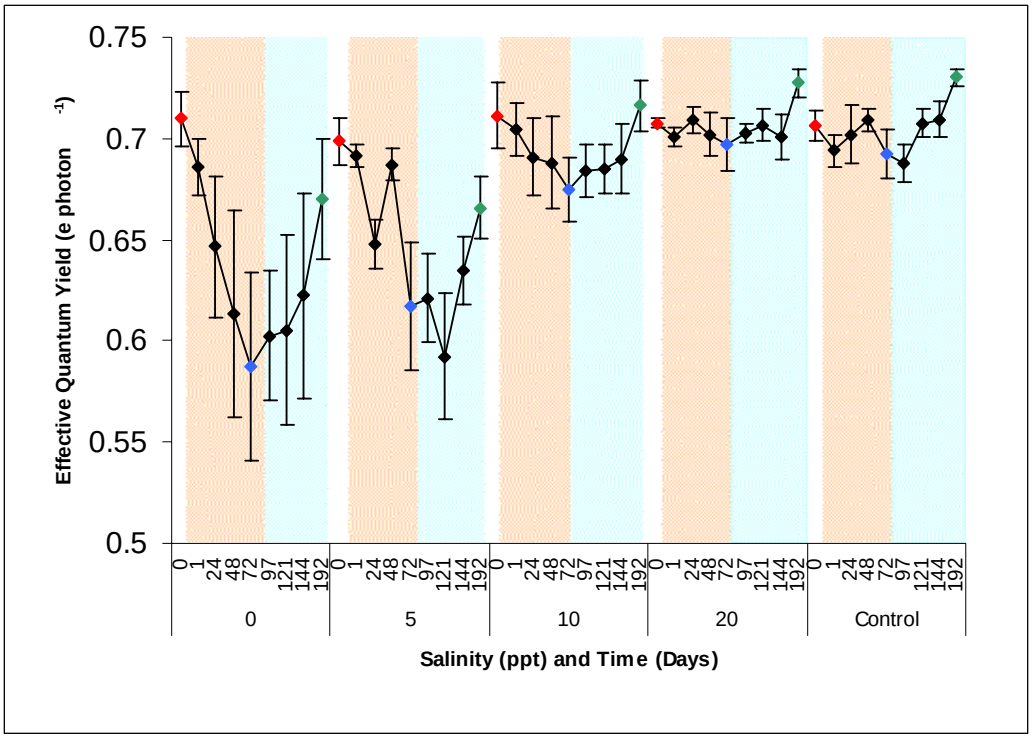

Figure 11 - Effective Quantum Yield across time in each of five different salinity treatments. Treatment at each salinity level was applied immediately after the time zero reading and ceased after the 72-hour reading (indicated by the orange band). Subsequent to this, a 120-hour recovery period in ambient salinity seawater occurred. Error bars are standard error, n=4 in all cases. Statistical analysis was applied for times 0, 72 and 192 hours. After identification of a time × salinity interaction, one-way ANOVAs were carried out at each of these times. Comparison was made between points indicated by the same colour (red = T_0, blue = T_{72}, green =T_{192}).

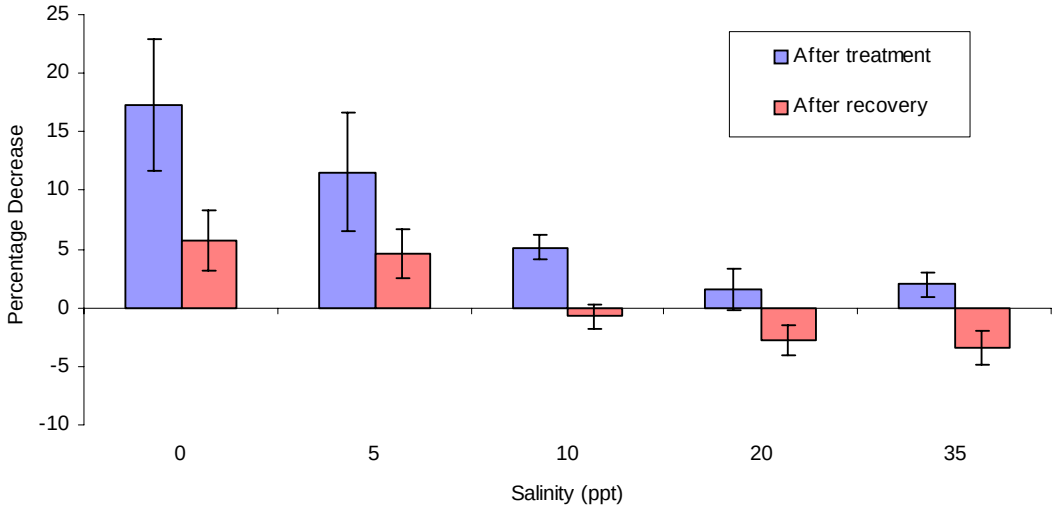

Figure 12 - Decrease in yield immediately after exposure to altered salinity level (blue) and after the combined exposure and recovery phases (red) as a percentage of the original, pre-treatment yield value. Error bars represent standard error, n=4.

Table 1 - Repeated measures ANOVA of the effects of TIME (fixed) and SALINITY (fixed) on transformed YIELD, with PLANT (nested within SALINITY) as the appropriate error term.

Source	SS	DF	MS	F Ratio	P
Salinity	0.29402	4	0.07351	4.2192	0.0174
Plant [Salinity]	0.26133	15	0.01742	4.4489	0.0002
Time	0.29033	2	0.14517	37.07	< 0.0001
Salinity × Time	0.149	8	0.01863	4.7562	0.0008

Table 2 - Separate one-way ANOVAs analyzing the effect of SALINITY (fixed) on transformed YIELD before the treatment (top), immediately after the treatment (middle) and after the recovery phase (bottom).

Before Treatment Period (0 hrs)

Source	SS	DF	MS	F Ratio	P
Salinity	0.004369	4	0.001092	0.2433	0.9093
Error	0.067346	15	0.00449		

Immediately After Treatment Period(72 hrs)

Source	SS	DF	MS	F Ratio	P
Salinity	0.278755	4	0.069689	5.0599	0.0088
Error	0.20659	15	0.013773		

Following Recovery Phase (192 hrs)

Source	SS	DF	MS	F Ratio	P
Salinity	0.159902	4	0.039975	5.7178	0.0053
Error	0.104871	15	0.006991		

Table 3 – Results of Dunnett's test to determine which salinities caused yields significantly lower than the control at T72 and T19. Tests not applied for 0 hours as ANOVA detected no difference.

	Salinity (ppt.)			
	0	**5**	**10**	**20**
At 72 hours	**P<0.01**	**P<0.05**	**P>0.05**	**P>0.05**
At 192 hours	**P<0.01**	**P<0.01**	**P>0.05**	**P>0.05**

4.5. *P. angustifolia* fruits

Exposure to salinities of 20 ppt or less had a profound effect on the survival of *Posidonia angustifolia* seedlings (Figure 13). Mortality was significantly higher in all reduced salinities than in the control (Table 4). Furthermore, mortality was inversely related to salinity (Figure 13). The control suffered only 24% mortality, whilst the 0 ppt treatment suffered 100% mortality. In all treatments, the remaining fruits had released their seedlings.

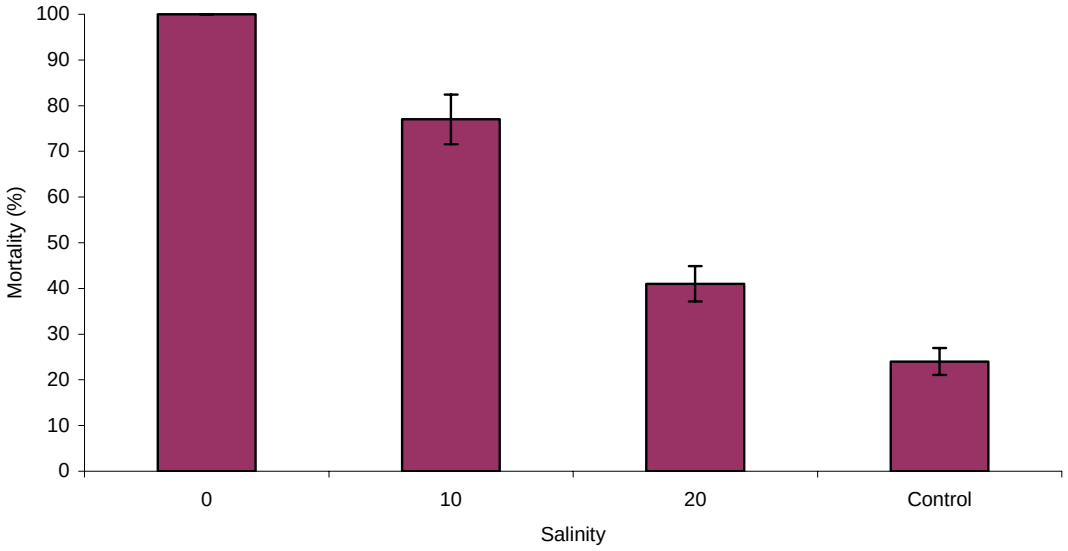

Figure 13 - Mortality (expressed as a percentage) of *Posidonia angustifolia* fruits exposed for 72 hours at a range of salinities. Error bars represent standard error, n=4. At 0 ppt salinity, all replicates exhibited 100% mortality and so no error bars are shown.

Table 4 - ANOVA table for the effect of salinity on *Posidonia angustifolia* seedling mortality transformed as arc sin (square root (mortality)).

Source	SS	DF	MS	F Ratio	P
Salinity	5.929609	3	1.97654	142.9115	<0.0001
Error	0.165966	12	0.01383		

Dunnett's test (one tailed) identified significant differences in yield between all lowered salinity treatments and the control ($P < 0.01$ in all cases).

5. Discussion

Reductions in salinity are unlikely to be the cause of seagrass loss on the Adelaide coast at anything other than small spatial scales. *Amphibolis antarctica* and *Posidonia sinuosa* adults are highly resilient to reduced salinity, even as low as 0 – 1 ppt. It is only after prolonged exposure (i.e. in the order of weeks) to low salinity that adult *A. antarctica,* and to a lesser degree *P. sinuosa*, exhibit signs of stress in the form of photosynthetic decline followed by leaf decay and eventually death. Furthermore, observed changes in salinity on Adelaide's coast due to stormwater and waste water treatment plant outputs are small in magnitude, extent and longevity (SARDI unpublished data; Kaempf 2004). Seagrass losses due to reduced salinity are thus possible only very close (within 100 m) to freshwater sources such as waste and stormwater outfalls and riverine inputs.

While short-term exposure to lower salinities is not toxic to adults, the same may not be said for propagules. The results of this study suggest that 72 hours exposure to lowered salinity is enough to inhibit the seedlings of *Amphibolis* and to kill the fruit from *Posidonia*. Recruitment of either species into areas subject to major salinity declines is thus unlikely.

5.1. *A. antarctica* and *P. sinuosa* adults

The resilience to lowered salinities observed in adult *P. sinuosa* and *A. antarctica* is in keeping with what is known about some other marine angiosperms e.g. *Posidonia australis*, Tyerman *et al.* 1984; *Ruppia maritima,* Bird *et al.* 1993; *Zostera marina,* Hellblom and Björk 1999; *Posidonia oceanica*, Fernández-Torquemada and Sánchez-Lizaso 2005; and *Zostera tasmanica*, personal observation Grant Westphalen. *Posidonia australis* can tolerate prolonged periods of reduced salinity (Tyerman *et al.* 1984), although it never occurs in persistently brackish conditions (Larkum 1977). Recent work on *P. oceanica* by Fernández-Torquemada and Sánchez-Lizaso (2005) suggests that this species is more tolerant of lowered rather than raised salinities, although the lowest salinity tested in that study was 25 ppt, which was substantially higher than levels considered here (0-1 ppt). Seagrasses cope with salinity through anatomical and physiological strategies (Jagels 1983, Tyerman 1989, Arai *et al.* 1991, Pak *et al.* 1995, Fukuhara *et al.* 1996). However, while it is apparent that seagrasses can adjust to moderate changes in salinity (Tyerman 1989), the responses to extremes are poorly known. Changes in salinity have been suggested as determinants of seagrass distributions, although other factors associated with freshwater inputs, such as turbidity, could also be influential (Tyerman 1989).

Probably the best understood species in terms of salinity tolerances is *Zostera marina*. Tutin (1938) suggested that *Z. marina* grows across a salinity range of 5 – 42 ppt, while den Hartog (1970) defined a slightly narrower range (5 – 35 ppt). However, there are substantial differences in tolerance related to the conditions in which the plant has grown. Both Kammermans *et al.* (1999) and van Katwijk *et al.* (1999) found that estuarine forms of *Z. marina* performed worse at increased salinity than marine stock. Biebl and McRoy (1971) found that *Z. marina* could survive short-term (24 hour) exposure to salinity extremes from 0 ppt (distilled water) to ~ 3 × seawater, although 4 × seawater was almost certainly fatal.

In a comparative study of the response of *Zostera capensis* and *Ruppia cirrhosa* to salinity level, Adams and Bate (1994) found a narrower maximal growth range for the former (15 – 35 ppt relative to 0 – 75 ppt). Osmotic balance in many halophytic plant tissues is assisted by the manufacture of proline (Stewart and Lee 1974, Brock 1981). For *R. cirrhosa* the levels of proline increased with salinity, but while a similar response may have occurred in *Z. capensis*, the plant died at salinity extremes. Proline levels in both plants tended to be

higher in leaves than in roots, a feature also observed in other halophytes (Stewart and Lee 1974).

In the present study, *A. antarctica* responded initially to changes in salinity (a few hours), but recovered within the first day of the experiment. This period is comparable with Tyerman (1989) who observed that turgor adjustment to reduced salinity in *Halophila ovalis, Zostera capricorni* and *Posidonia australis* required around 24 hours.

In our study, longer-term exposure to 0–1 ppt was ultimately detrimental to both *A. antarctica* and *P. sinuosa* adults, with a loss of photosynthetic capability, browning of leafage and the loss of leaves from *A. antarctica*. Similar results were recorded for marine populations of *Z. marina* after five weeks exposure to reduced salinity (van Katwijk *et al.* 1999).

Death of both seagrass species in our study may be due to dilution of the water column salts responsible for maintenance of turgor (see Tyerman 1989). However, van Katwijk *et al.* (1999) found that *Zostera* plants at sightly reduced salinity were more tolerant to increased nutrient loads. This may be supported by the observations of Hellblom and Björk (1999), who found that it was the dilution of inorganic carbon in the water column rather than loss of "osmolality" that resulted in a loss of photosynthesis in *Z. marina*.

Nutrient up-take for *A. antarctica* is via the leaves (Paling and McComb 1994), rather than through the roots as in *P. sinuosa* (Maier and Pregnall 1990; Paling and McComb 1994; Duarte and Chiscano 1999; Lee and Dunton 1999). *Amphibolis antarctica* may thus be more sensitive to salinity stress due to greater leakage from the plant via higher numbers of nutrient / carbon channels in the above-ground biomass relative to *P. sinuosa* as well as the relative lack of below-ground storage (Paling and McComb 2000). Given that *P. sinuosa* also ultimately dies after prolonged exposure to low salinity, it may well be that carbon depletion rather than dilution of nutrients or osmolites is the primary cause. Carbon dilution might also explain the greater effect of short-term reduced salinity on *Posidonia* fruit and *Amphibolis* seedlings relative to adults (see below).

Lirman and Cropper (2003) considered three tropical species, *Thalassia testudinum, Syringodium filiforme* and *Halodule wrightii*. Their study found marked differences in the tolerance between species with *S. filiforme* the most sensitive, then *T. testudinum* while *H. wrightii* tolerated the full range of salinities considered (5 – 45 ppt for 14 days). In placing these results in context with field observations, Lirman and Cropper (2003) considered that freshwater inputs might affect density and spread in single species stands, but that in multi-species complexes, competitive interactions may be altered resulting in exclusion/replacement. Given that *A. antarctica* appears to be more sensitive to reduced salinity than *P. sinuosa*, it may be anticipated that the latter would dominate in areas prone to salinity decline. However, given the slow horizontal growth rate of *Posidonia* (Kirkman 1998, Meehan and West 2000), the ability to exclude or replace other species would likely require a more prolonged and consistent change to prevailing conditions than might be expected, even close to sources of freshwater.

5.2. *A. antarctica* seedlings

Amphibolis antarctica seedlings were significantly affected by short-term (72 hours) exposure to salinities of 5 ppt or less. At low salinities, effective quantum yield was positively related to salinity, which suggests that the 10 ppt treatment, while not significantly different to the control, may well have resulted in a more pronounced effect at longer exposure times (> 72 hours). Walker and McComb (1990) found that *A. antarctica* seedlings were affected by increased salinity (65 ppt), but the influence of reduced salinity levels on seagrass seedlings of any species seems to be largely unknown.

Seedlings of other meadow-forming seagrasses from the Adelaide coast may also be sensitive to reduced salinity, but as with adults, the possibility of even short-term exposure to harmful doses is remote. Other than *A. antarctica* and *A. griffithii*, no other seagrass on the Adelaide metropolitan coast is viviparous (Robertson 1984), and there is thus a greater possibility of exposure to variable salinities during their seedlings drifting phase. *A. griffithii* is a not as large a component of Adelaide's seagrass population as *A. antarctica*, tending to occur in deeper water to the south of Adelaide (Robertson 1984, Shepherd and Robertson 1989) where there are relatively fewer terrigenous inputs (Steffensen *et al.* 1989). Given the prevailing south to north current (Townsend 2002), the likelihood of reduced salinity impacts on *A. griffithii* (either seedlings or adults) is probably less than that of *A. antarctica*.

It must be acknowledged that exposure of *Amphibolis* seedlings in the drift phase is still relatively unlikely as they move along the bottom rather than the surface as apposed to *Posidonia* fruits that float (see below). Rather it is more likely that the salinity regime at the settlement location of the seedlings is the more influential factor.

5.3. *P. angustifolia* fruits

Short-term (72 hour) exposure to reduced salinity had profound effects on the survival of *P. angustifolia* fruits and seedlings, with survival positively related to salinity level at levels of 35 ppt and below. At 0 ppt salinity, this was evidenced by complete mortality of all seedlings.

Seedlings of seagrasses may be differently affected by reduced salinity than adults (Tyerman 1989). Phillips *et al.* (1983) found that seed set and germination in *Z. marina* was increased at reduced salinity, although this depended on the prevailing environmental conditions in which the plant grew, with estuarine plants more accommodating than those that were purely marine. In contrast, our study found seedling survival in *P. angustifolia* to be severely inhibited by reduced salinity. Depending on its habitat, *Z. marina* will tend to act as a ruderal species, capable of high levels of sexual reproduction within a short individual lifespan (Phillips *et al.* 1983). In contrast, *P. angustifolia* is most certainly at the opposite end of this range, with slow growth and relatively low levels of genet production (Clarke and Kirkman 1989, Kirkman 1998, Marbà and Walker 1999, Meehan and West 2000). Seagrass meadows are thought to be maintained primarily through clonal rather than seedling growth (Marbá and Walker 1999, Kendrick *et al.* 2005, Sintes *et al.* 2005). However, floating fruits are the only means of long-range dispersal available to *Posidonia* spp. and, given the very slow recovery of even small patches by clonal growth (Kirkman 1998, Meehan and West 2000), the value of seedlings should not be discounted, particularly for recovery of seagrass meadows over large areas.

Relative to other components of the seagrass community on the Adelaide coast, the floating fruits of *Posidonia* spp. are at greatest risk of exposure to reduced salinity levels as rain events may affect surface salinities, and freshwater inputs will tend to float over the denser seawater if mixing is poor.

The fruits used in our study were collected from the beach and thus there is no way to know how long these had floated. Sand abrasion and/or desiccation of the fruit may have influenced fruit / seedling phenology relative to floating fruit, possibly making them more susceptible to low salinity. The phenology of *P. sinuosa, P. coriacea* and *P. australis* fruits was investigated by Kirkman (1998), the results of which may be extended to *P. angustifolia,* although there are differences between species. After removal from the parent plant, Kirkman (1998) found that dehiscence of *Posidonia* varies between species; 90% in 7 days for *P. sinuosa*, 11 days for *P. australis* and 16 days for *P. coriacea*. *P. angustifolia* is ecologically and morphologically most similar to *P. sinuosa* and the timeframe for dehiscence observed in this species appears to be the same (probably ~ 7 days to complete), which

combined with the high level of viability observed in the resultant seedlings (~ 76 % at 16 days ambient) suggests that their exposure on the beach had no detrimental effect. All fruits were collected after a storm event and while desiccation would have been minimal, exposure to abrasion from the action of the storm could not be avoided. The degree to which abrasion and bruising might predispose the fruit to reduced salinity cannot be directly determined with the current experimental design but it might well be estimated that this type of disturbance would increase the variability of the response to salinity. Given the consistency of results within salinity levels and the fact that there was a gradient of increasing response in line with salinity level, it would appear that the effect of abrasion or bruising would be minimal.

The degree to which the results of this study may apply to other species of *Posidonia* are unknown, although *P. sinuosa* and *P. angustifolia* are both considered to rely on ramet (i.e. clonal) rather than genet (sexual reproduction) for maintenance of meadows (Marbá and Walker 1999). The investment that these species make in seedlings may be less than that of other species of *Posidonia* (in particular *P. australis*). *P. sinuosa* and *P. australis* are the other species in the genus that have suffered major losses on the Adelaide coast (Shepherd *et al.* 1989) and factors that limit *P. angustifolia* survival, be they salinity or otherwise, are likely to be influential on the genus as a whole.

5.4. Conclusions

It is apparent that a salinity change to 0 ppt would require several weeks to influence adult seagrass photosynthetic efficiency. At higher salinities, the period of exposure would need to be progressively longer, assuming that it has any effect at all. Observations of stormwater input at the River Torrens outlet show very limited salinity changes both spatially and temporally for an average storm event (20 mm rainfall within the preceding 24 hours) (Simon Bryars, unpublished data). Such small changes, support the notions that stormwater-induced reductions in salinity are not a cause for large-scale nearshore seagrass loss.

For seedlings, the situation may be rather different and the capacity for either *P. angustifolia* or *A. antarctica* to successfully recruit into areas of lowered salinity is probably minimal. This may be extended to suggest that the expansion of populations of either genus into new areas, which would be the primary role of seedlings, will be determined at least in part by the salinity regime (amongst other factors such as depth, substrate, wave action, etc). It follows that, while freshwater may not have been a factor in historical seagrass losses, it may well play a role in determining the capacity for natural regeneration / recovery at sites very close (within a few hundred metres or so) to terrigenous freshwater inputs.

The previous suggestion that seagrass loss on the Adelaide coast is due to stormwater inputs (EPA 1998; Seddon 2002) is based on correlation rather than demonstrated causes, which is one of the ongoing problems with seagrass loss research (Ralph *et al.* in review). Having said this, it remains probable that seagrass decline from the shallow nearshore region off the Adelaide metropolitan coast is due to factors specific to that zone, the most obvious of which would be a factor related to stormwater, given that it is not the stormwater itself. Hence, while salinity reductions are apparently not the cause for seagrass loss, it remains possible that other factors in stormwater (nutrients, toxicants, turbidity/ sedimentation) might be responsible (see review by Westphalen *et al.* 2004).

6. References

Adams J.B. and Bate G.C. 1994. The ecological implications of tolerance to salinity by *Ruppia cirrhosa* Petagna) grande and *Zostera capensis* Setchell. *Botanica Marina* 37: 449-456.

Arai M., Pak J.Y., Nomura J.Y. and Nitta T. 1991. Seawater-resistant non-sperical protoplasts from seagrass leaves. *Physiologia Plantarum* 83: 551-559.

Biebl R. and McRoy C.P. 1971. Plasmatic resistance and rate of respiration and photosynthesis of *Zostera marina* at different salinities and temperatures. *Marine Biology* 8: 48-56.

Bird K.T., Cody B.R., Jewett-Smith J. and Kane M.E. 1993. Salinity effects of *Ruppia maritima* L. cultured *in vitro*. *Botanica Marina* 36: 23-28.

Brock M.A. 1981. Accumulation of proline in a submerged aquatic halophyte, *Ruppia* L. *Oecologia* 51: 217-219.

Burnham K.P. and Anderson D.R. 2002. *Model selection and multi-model inference: a practical information theoretic approach.* Springer, New York. 488 pp.

Cheshire A.C., Westphalen G., Boxall V., Marsh R., Gilliland J., Collings G., Seddon S. and Loo M. 2002. *Caulerpa taxifolia in West Lakes and the Port River, South Australia: distribution, eradication options and consequences.* South Australian Research and Development Institute, Publication Number RD02/0161 (Adelaide: Australia).

Clarke S.M. and Kirkman H. 1989. Seagrass dynamics. *In:* Larkum A.W.D., McComb A.J. and Shepherd S.A. Eds. *Biology of seagrasses.* Elsevier, Amsterdam, pp. 304-345.

den Hartog C. 1970. *Seagrasses of the world.* Elsevier North Holland, Amsterdam. 275 pp.

Duarte C.M. and Chiscano C.L. 1999. Seagrass biomass and production: a reassessment. *Aquatic Botany* 65: 159-174.

EPA 1998. *Changes in seagrass coverage.* Adelaide, Environment Protection Agency (SA).

Fernández-Torquemada, Y. and Sánchez-Lizaso, J.L. 2005. Effects of salinity on leaf growth and survival of the Mediterranean seagrass *Posidonia oceanica* (L.) Delile. *Journal of Experimental Marine Biology and Ecology* 320: 57-63.

Fukuhara T., Pak J.Y., Ohwaki Y., Tsujimura H. and Nitta T. 1996. tissue-specific expression of the gene for putative plasma membrane H^+-ATPase in a seagrass. *Plant Physiology* 110: 34-42.

Hastie T.J. and Tibshirani R.J. 1990. *Generalized Additive Models.* Chapman and Hall, London.

Hellblom F. and Björk M. 1999. Photosynthetic responses in *Zostera marina* to decreasing salinity, inorganic carbon content and osmolality. *Aquatic Botany* 65: 97-104.

Jagels R. 1983. Further evidence for osmoregulation in epidermal leaf cells of seagrasses. *American Journal of Botany* 70(3): 327-333.

Kaempf, J., Edwards, P. and Sandery, P. 2004. Technical Report #4: Cruise Report - R/L HERO cruise ACWS #21-23 prepared for the Adelaide Coastal Waters Study as part of sub-task 1; In-situ field measurements. July 2 2004.

Kammermans P., Hemminga, M.A. and de Jong D.J. 1999. Significance of salinity and silicon levels for growth of a formerly estuarine eelgrass (*Zostera marina*) population (Lake Grevelingen, The Netherlands). *Marine Biology* 133: 527-539.

Kendrick G.A., Duarte C.M. and Marbá N. 2005. Clonality in seagrasses, emergent properties and seagrass landscapes. *Marine Ecology Progress Series* 290: 291-296.

Kendrick G.A., Walker D.I., McComb A.J. 1988. Changes in distribution of macro-algal epiphytes on stems of the seagrass *Amphibolis antarctica* along a salinity gradient in Shark Bay, Western Australia. *Phycologia* 27: 201-208.

Kirkman H. 1998. Pilot experiments on planting seedlings and small seagrass propagules in Western Australia. *Marine Pollution Bulletin* 37: 460-467.

Larkum, A.W.D. 1977. Recent research on seagrass communities in Australia. *In* McRoy, C.P. and Helfferich, C. Eds. *Seagrass Ecosystems. A scientific perspective.* M. Dekker, New York pp 247-262.

Lee, K.S. and Dunton, K.H. 1999. Inorganic nitrogen acquisition in the seagrass *Thalassia testudinum*: development of a whole-plant nitrogen budget. *Limnology and Oceanography* 44, 1204-1215.

Lirman D. and Cropper W.P.Jr. 2003. The influence of salinity on seagrass growth survivorship, and distribution within Biscayne Bay, Florida: Field, experimental, and modelling studies. *Estuaries* 26(1): 131-141.

Macinnis-Ng C.M.O. and Ralph P.J. 2003a. *In situ* impact of petrochemicals on the photosynthesis of the seagrass *Zostera capricorni*. *Marine Pollution Bulletin* 46: 1395-1407.

Macinnis-Ng C.M.O. and Ralph P.J. 2003b. Short-term exposure response and recovery of *Zostera capricorni* photosynthesis after herbicide exposure. *Aquatic Botany* 76: 1-15.

Macinnis-Ng C.M.O. and Ralph P.J. 2004. In situ impact of multiple pulses of metal herbicide on the seagrass, *Zostera capricorni*. *Aquatic Toxicology* 67: 227-237.

Maier, C. and Pregnall, A. 1990. Increased microphyte nitrate reductase activity as a consequence of groundwater input of nitrate through sandy beaches. *Marine Biology*. 107, 263-271.

Marbá N. and Walker D.I. 1999. Growth, flowering and population dynamics of temperate Western Australian seagrasses. *Marine Ecology Progress Series* 184: 105-118.

Meehan A.J. and West R.J. 2000. Recovery times for a damaged *Posidonia australis* bed in south eastern Australia. *Aquatic Botany* 67: 161-167.

O'Loughlin E. 2004. *Stress response of Amphibolis antarctica and Posidonia sinuosa to freshwater inputs.* Honours Thesis, Department of Environmental Biology, The University of Adelaide. 54 pp.

Pak J.Y., Fukuhara T. and Nitta T. 1995. Discrete subcellular localization of membrane-bound ATPase activity in marine angiosperms and marine algae. *Planta* 196: 15-22.

Paling E.I. and McComb A.J. 1994. Nitrogen and phosphorus uptake in seedlings of the seagrass *Amphibolis antarctica* in Western Australia. *Hydrobiologia* 294: 1-4.

Paling E.I. and McComb A.J. 2000. Autumn biomass, below-ground productivity, rhizome growth at the bed edge and nitrogen content in seagrasses from Western Australia. *Aquatic Botany* 67: 207–219.

Pedersen M.F., Paling E.I. and Walker D.I. 1997. Nitrogen uptake and allocation in the seagrass *Amphibolis antarctica*. *Aquatic Botany* 56: 105-117.

Phillips R.C, Grant W.S. and McRoy C.D. 1983. Reproductive strategies of eelgrass (*Zostera marina* L.). *Aquatic Botany* 16: 1-20.

Ralph, P.J., Tomasko, D., Moore, K., Seddon, S. and Macinnis-Ng, C.M.O. In review. Human impacts on seagrass: contamination and eutrophication. In: Larkum, A.W.D., Orth, R. and Duarte, C. (Eds.) *Treatise on Seagrass Biology*.

Robertson E.L. 1984. Seagrasses. *In:* Womersley H.B.S. (ed.). *The Marine Benthic Flora of Southern Australia - Part 1.* Adelaide, S.A. Government Printer**.**

Seddon S. 2002. Issues for seagrass rehabilitation along the Adelaide Metropolitan coast: An overview. *In* Seddon S, and Murray-Jones S. (Eds). *Proceedings of the Seagrass Restoration Workshop for Gulf St Vincent 15 - 16 May 2001*. Department for Environment and Heritage and SARDI Aquatic Sciences: Adelaide. pp. 1-8.

Seddon S. and Cheshire A.C. 2001. Photosynthetic response of *Amphibolis antarctica* and *Posidonia australis* to temperature and desiccation using chlorophyll fluorescence. *Marine Ecology Progress Series* 220: 119-130.

Shepherd S.A. and Robertson E.L. 1989. Regional studies - seagrasses of South Australia, Victoria and Bass Strait. *In:* Larkum A.W.D., McComb A.J. and Shepherd S.A. Eds. *Biology of seagrasses.* Elsevier, Amsterdam, pp. 211-229.

Shepherd S.A., McComb A.J., Bulthuis D.A., Neverauskas V.P., Steffensen D.A. and West R. 1989. Decline of seagrasses. *In:* Larkum A.W.D., McComb A.J. and Shepherd S.A. Eds. *Biology of seagrasses.* Elsevier, Amsterdam, pp. 346-388.

Sintes T., Marbá N., Duarte C.M. and Kendrick G.A. 2005. Nonlinear processes in seagrass colonization explained by simple growth rules. *Oikos* 108: 165-175.

Steffensen D.A., Kirkegaard I. and Johnson J. 1989. *Position and background papers on man-made changes to Gulf St. Vincent. Adelaide.* South Australian Committee on Pollution of Gulf St Vincent. Government of South Australia, Adelaide. 27 pp.

Stewart G.R. and Lee J.A. 1974. The role of proline accumulation in halophytes. *Planta (Berl.)* 120: 279-289.

Townsend M. 2002. The exposure of Adelaide's seagrasses to waves and currents. *In:* Seddon S. and Murray-Jones S. Eds. *Proceedings of the seagrass restoration workshop for Gulf St Vincent 15-16 May 2001.* Department of Environment and Heritage and SARDI Aquatic Sciences, Adelaide.

Tutin T.G. 1938. The autecology of *Zostera marina* in relation to its wasting disease. *New Phytologist* 37(1): 50-71.

Tyerman S.D. 1989. Solute and water relations in seagrass. *In:* Larkum A.W.D., McComb A.J. and Shepherd S.A. Eds. *Biology of seagrasses.* Elsevier, Amsterdam, pp. 723-759.

Tyerman S.D., Hatcher A.I., West R.J. and Larkum A.W.D. 1984. *Posidonia australis* growing in altered salinities: leaf growth, regulation of turgor and the development of osmotic gradients. *Australian Journal of Plant Physiology* 11: 35-47.

van Katwijk M.M., Schmitz G.H.W., Gassling A.P. and van Avesaagth P.A. 1999. Effects of salinity and nutrient load and their interaction on *Zostera marina. Marine Ecology Progress Series* 190: 155-165.

Walker D.I. 1985. Correlations between salinity and growth of the seagrass *Amphibolis antarctica* (Labill.) Sonder & Aschers., in Shark Bay, Western Australia, using a new method for measuring production rate. *Aquatic Botany* 23: 13-26.

Walker D.I. and McComb A.J. 1990. Salinity responses of the seagrass *Amphibolis antarctica* (Labill.) Sonder et Aschers.: Experimental validation of field results. *Aquatic Botany* 36: 359-366.

Westphalen G., Collings G., Wear R., Fernandes M., Bryars S. and Cheshire A. 2004. *Adelaide Coastal Waters Study: A review of seagrass loss on the Adelaide metropolitan coastline.* South Australian Research and Development Institute Aquatic Sciences Publication Number RD04/0073. 72 pp.

Wilkinson J., Hutson, J., Bestland E. and Fallowfield H. 2004. A*udit of contemporary and historical quality and quantity data of stormwater discharging into the marine environment, and field work programme.* ACWS Technical Report No. 3 prepared for the Adelaide Coastal Waters Study Steering Committee, June 2004. Department of Environmental Health, Flinders University of South Australia.

Wilkinson J., Pearce M., Cromar, N. and Fallowfield, H. 2003. *Audit of the quality and quantity of effluent discharging from wastewater treatment plants (WwTPs) discharging into the marine environment.* ACWS Technical Report No. 1 prepared for the Adelaide Coastal Waters Study Steering Committee, November 2003. Department of Environmental Health, Flinders University of South Australia.

Zar, J.H. 1984. *Biostatistical Analysis Second Edition.* Prentice-Hall International, Inc. New Jersey. 718 pp.