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## Effects of Temporary PAR reduction on the seagrass *Amphibolis griffithii* (Black) den Hartog

Paul R. Mackey  
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**EFFECTS OF TEMPORARY PAR REDUCTION ON THE  
SEAGRASS *AMPHIBOLIS GRIFFITHII* (BLACK) den HARTOG.**

By

Paul R. MACKEY

Supervisors: A/Prof. Paul LAVERY & Ms Catherine COLLIER



A Thesis Submitted in Partial Fulfilment of the  
Requirements for the Award of  
Bachelor of Science (Environmental Management) Honours

At the School of Natural Sciences  
Faculty of Computing, Health and Science  
Edith Cowan University,  
Joondalup

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## USE OF THESIS

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## Abstract

Declines in seagrass health and distribution are commonly caused through human-induced reductions in the availability of photosynthetically active radiation (PAR). These reductions can result from a variety of human-induced perturbations, including channel dredging. The impetus for the research was driven by the broad-scale degradation of the ecologically important southern-Australian endemic seagrass *Amphibolis griffithii* (Black) den Hartog in Champion Bay, Geraldton, Western Australia. The study investigated the affects of reduced PAR on *A. griffithii* and identified responses that may be useful in developing management triggers to minimise the impact of PAR limitation events.

The study was carried out during late summer and winter at Jurien Bay on the mid-west coast of Western Australia. Replicate plots of *Amphibolis griffithii* meadow were subjected to 90% reduction in PAR availability for 106 days using shade screens suspended over the meadow. A variety of morphological and physiological variables were monitored in control and treatment plots at approximately monthly intervals during this time and after 42 days of recovery.

There was a noticeable meadow-scale response in *A. griffithii* with significant reductions in leaf biomass measurements, such that the number of leaves per stem (~12 leaves per stem) and total leaf biomass (<200g DWm<sup>-2</sup>) were approximately half that of ambient levels after 106 days of shading. This resulted in a dramatic change in the light attenuation coefficients between shaded (0.59 m<sup>-1</sup>) and control plots (2.38 m<sup>-1</sup>) allowing greater penetration of PAR through the canopy, effectively reducing self-shading in the lower canopy. These changes were paralleled by marked physiological responses with increases in chlorophyll and decreases in rhizome sugar concentrations in the shaded plants. Chlorophyll levels responded consistently in the upper canopy with highly significant increases after 106 days of treatment and a return to ambient levels after 42 days of recovery. Rhizome sugars depleted quickly and consistently with treatment, culminating in highly significant differences after 106 days of shading with concentrations at less than one third (<50.0 mg.gDW<sup>-1</sup>) when compared with ambient levels. The apparent reduction in

canopy self-shading was likely to have aided the considerable recovery of most variables, such as leaf extension which fully recovered after 42 days following shade removal.

This research identified a suite of specific responses to reduced PAR in *A. griffithii* and has assessed their inherent potential for future development of Environmental Quality Criteria (management trigger values) to high intensity, short duration impact events on the mid-west coast of Western Australia, including recommendations for further research. The study has highlighted the species specific nature of seagrass responses to reduced PAR climates; contributions to the broader ecological knowledge were made with specific reference to ecologically and morphologically unique species that do not necessarily conform to known responses in the blade-like species, such as *Posidonia*. The application of these research outcomes will ultimately help environmental managers minimise the impacts of broad-scale PAR induced degradation events like the dredging program at Champion Bay, Geraldton from re-occurring.

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To my mother Yvonne, my fiancée Rose and all my family and friends who so graciously and patiently endured my many misgivings over the past 5 years, thank you!

*Nellie's Decree*

*'Tis easy to be all happy and glee,  
When life goes along like a song.  
But the man worthwhile,  
Is the man that can smile;  
When everything goes dead wrong.*

This work is dedicated to the memory of my late father, Keith Alan Mackey and my late grandmother, Nellie May Mackey.

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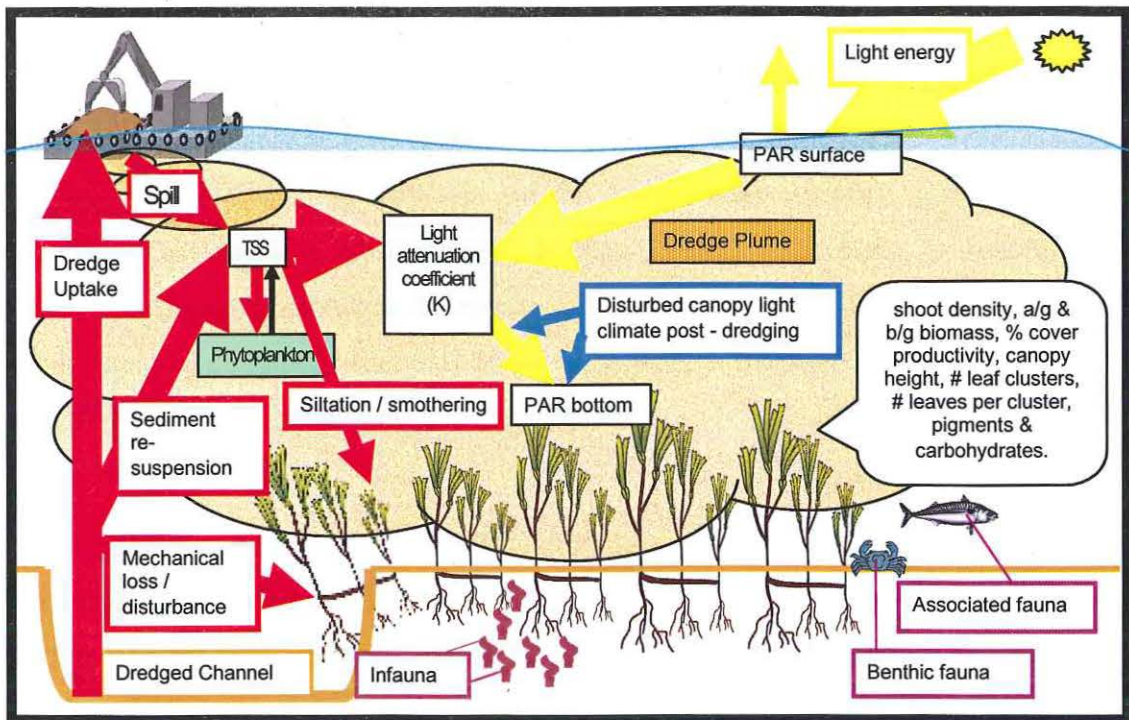
# 1 Introduction

*Amphibolis griffithii* is an ecologically important dominant meadow forming seagrass (Lavery & Vanderklift, 2002) that is commonly found off the central mid-west coast of Western Australia (DCLM, 1994). The genesis of the research was driven by the degradation and loss of the genus *Amphibolis* in Champion Bay as a result of the dredging program carried out under the auspices of the Geraldton Port Enhancement Project in 2002 and 2003. The Strategic Research Fund for the Marine Environment (SRFME a collaborative research and funding association between CSIRO and the Western Australian Government) and the Department of Environment (DoE) identified the need for the development of light-stress indicators in the morphologically and ecologically unique seagrass *A. griffithii*. The development of light stress indicators would allow proponents and management agencies to set quantitative guidelines to help avert the reoccurrence of seagrass degradation like those reported from the Champion Bay dredging program. This study forms the initial step in the identification of light stress responses in the seagrass *A. griffithii*.

Globally, the increasing knowledge of seagrass and their structural and functional importance has resulted in them being ranked among some of the world's most valuable ecological systems (Costanza *et al.* 1997). However, these important systems are becoming increasingly threatened and degraded by a suite of anthropogenic disturbances that can be summarised into the following broad categories: reductions in water quality; mechanical damage; and toxic pollution (Short & Wyllie-Echeverria, 1996; Hemminga & Duarte, 2000; Short *et al.* 2001). In Australian waters, losses of seagrass have been estimated between 45 000 to 150 000 hectares (Walker & McComb, 1992; Zann, 1995; Preen *et al.* 1995). The literature reveals that the major cause of degradation in seagrass systems appears to relate directly or indirectly to reductions in photosynthetically active radiation (PAR) (Bulthuis, 1983; Longstaff & Dennison, 1999; Walker, 2003). The intensity and duration of PAR availability has been identified as a primary environmental driver that can limit the survival, abundance and distribution of seagrasses (Hemminga & Duarte, 2000; Olesen *et al.* 2002). The importance of PAR as a key environmental driver in seagrass systems has been recognised for many years and the minimum requirement for survival is thought to be approximately 10-20% of surface irradiance

(Duarte, 1991). One of the major anthropogenic activities resulting in PAR reductions in seagrass ecosystems on a regional (Walker, 2003), national (Walker & McComb, 1992; Kirkman, 1997) and global scale (Short & Wyllie-Echeverria; 1996, Duarte, 2002) is channel dredging.

Direct effects of dredging on benthic ecosystems are mechanical loss (Sheridan, 2004) and a host of indirect effects that can be attributed to the resultant turbidity or suspended sediment and siltation / smothering caused by the disturbance (Hemminga & Duarte, 2000; Walker, 2003; Figure 1.1). Turbidity increases water column light attenuation, therefore reducing PAR received at the seagrass canopy. Similarly, siltation may bury or smother seagrasses, effectively limiting their ability to photosynthesise (Hemminga & Duarte, 2000; Sheridan, 2004). The direct mechanical losses of seagrass caused by dredging are relatively easy to estimate. However, predicting the indirect losses caused by dredging is difficult since they are highly variable depending on their specific habitat conditions (e.g. sediment characteristics and oceanographic influences), species, intensity, extent and duration of the perturbation (Raaymakers, 1996; Boyd *et al.* 2003; Ruiz & Romero, 2003; Sheridan, 2004). Morphological and physiological responses to channel dredging in Australian seagrass meadows have been identified as a gap in the scientific knowledge (Butler & Jernakoff, 1999) and targeted as a research priority by the Strategic Research Fund for the Marine Environment (SRFME: a collaborative research and funding arrangement between CSIRO and the Western Australian Government).



**Figure 1-1 Conceptual diagram outlining the impacts of dredging, with particular emphasis on the light climate in an *A. griffithii* meadow during and after a dredging program. The red arrows indicate the effect of the disturbance, the blue box depicts the focus of the research and the large text box on the right hand side provides a summary of correlated light reduction responses in other seagrass species (Gordon, *et al.* 1994; Peralta *et al.* 2002) that are being tested in this study.**

A hypothetical response pathway under a reduced light climate may include various physiological and morphological changes by seagrasses in order to cope with light stress. According to the literature, if the light stress exceeds the meadows threshold to overcome or at least maintain a neutral carbon budget (Touchette & Burkholder, 2000; Perez & Romero, 1992), then morphological thinning may occur that reduces the self-shading effect of the individual plant/stem (Carruthers & Walker, 1997; Gordon *et al.* 1994). A carbon budget may be broadly categorised as the light energy converted into carbohydrates and sugars (photosynthates) via photosynthesis within the leaves of seagrasses (Walker & McComb, 1988; Walker & McComb, 1990; Perez & Romero, 1992; Touchette & Burkholder, 2000). Storage of carbohydrate takes place predominantly within the rhizome (Grice *et al.* 1996) and primary production occurs as evident through an increase in plant biomass (Touchette & Burkholder, 2000). These carbohydrate stores may be drawn upon during times of light stress (Bulthuis, 1983). A canopy thinning response reduces the respiratory load of the plant (Peralta *et al.* 2002), maximises the radiation use efficiency (RUE)

(Carruthers & Walker, 1997) and together with other physiological responses e.g. concentrating chlorophyll pigments (Czerny & Dunton, 1997; Lee & Dunton, 1995) may allow the plant the best chance of overcoming the light stress. Furthermore, reductions in self shading may also aid the canopy recovery once light conditions improve (Carruthers & Walker, 1997; Bulthuis, 1983). Therefore, morphological and physiological responses to reduced PAR climates are adaptations to new environmental conditions that may allow seagrasses to overcome unfavourable light conditions (Hemminga & Duarte, 2000; Figure 1).

Light limitation, shading or PAR reductions in various species of seagrasses have shown varying effects in a suite of morphological and physiological responses. Morphological responses that may contribute to reduced self shading and reduced respiratory load (described above) include: reductions in above and below-ground biomass (Onuf, 1996), reduced shoot density and leaf length (Gordon *et al.* 1994; Longstaff & Dennison, 1996), changes in canopy height (Longstaff & Dennison, 1999), changes in leaf width (Lee & Dunton, 1997) and epiphyte losses (Moore & Wetzel, 2000). Rhizome and leaf carbohydrate (starch) (Lee & Dunton, 1997, Peralta *et al.* 2002) and sugar depletions (Longstaff & Dennison, 1999; Cabello-Pasini *et al.* 2002) often occur indicating a negative carbon balance, where the seagrass must draw on carbohydrate reserves (Peralta *et al.* 2002). Despite this suite of morphological and physiological adjustments, decreases in productivity (Czerny & Dunton, 1995; Fitzpatrick & Kirkman, 1995) and photosynthetic rates (Masini *et al.* 1995; Olesen *et al.* 2002) are frequently reported. These seagrass responses to reduced PAR appear to be highly species specific and therefore the transfer of responses from one species to another may not be appropriate e.g. *Posidonia* responses described by Gordon *et al.* (1994) may differ to *Amphibolis* responses. The morphology of the genus *Amphibolis* differs considerably to the other temperate, Western Australian meadow forming dominant genera *Posidonia* (Lavery & Vanderklift, 2002) and this may provide an explanation for the recent seagrass declines observed in Champion Bay, Geraldton.

The seagrass genus *Amphibolis* appears to have had very little research attention over time, compared with the other dominant meadow-forming, Western Australian genus

*Posidonia* (Duarte, 1999). The genus *Amphibolis* contains two species (*A. antarctica* and *A. griffithii*) that are both Australian temperate endemics (Ducker *et al.* 1977), whose distributions range from the temperate west coast through to the temperate southern coasts of Australia (Edgar, 2000). *A. griffithii* is distributed from Kalbarri in Western Australia to Victor Harbour in South Australia (Kirkman, 1997; Edgar, 2000) and has been identified as a dominant meadow-forming seagrass species in the Jurien Bay region (DCLM, 1994). The lack of specific research on *Amphibolis* is perplexing since its structure (den Hartog, 1970), morphology (Marba & Walker, 1999), rhizome and branching patterns (unpublished: Carruthers, 1994; unpublished: Coupland, 1997), reproductive methods (Dawes, 1998) and physiology (Paling & McComb, 1994) differ considerably from the strap-like genus of *Posidonia*. *A. griffithii* is a large seagrass with a canopy ranging from 30 to 100cm and is characterised by a number of leaf clusters usually containing between three to five leaves (Ducker *et al.* 1977). The leaf clusters are situated at terminating ends of the vertical branching stem that have been previously characterised as analogues of their vertical rhizomes (Marba & Walker, 1999). Therefore, transposing models and knowledge of environmental impact from the morphologically unique genus *Amphibolis* from *Posidonia* indicators of PAR stress, such as shoot declines (Gordon *et al.* 1994), may not be appropriate.

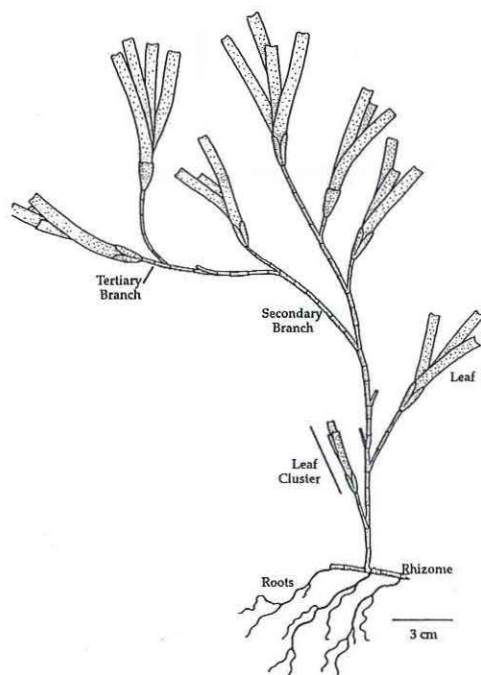


Figure 1-2 Sketch of *Amphibolis griffithii* morphology (after: Phillips & Meñez, 1988; Carruthers, 1994).

Walker *et al.* (1999), proposed a form and function model that classified all seagrass genera according to the size, structure and persistence of their rhizomes with *Halophila* being the smallest and *Posidonia* being the largest (Figure 1.3). The genus *Amphibolis* was classified as an intermediate genus leaning toward the larger side of the form and function model (Walker *et al.* 1999). From this model it would be fair to infer that *Amphibolis* would display a degree of resistance to a severely reduced light climate relative to smaller seagrass species, due mainly to an increased rhizome storage capacity. However, some literature (e.g. Carruthers & Walker, 1997) would indicate that *Amphibolis* may be susceptible to small shifts in light climate due largely to its heavy investment in above ground biomass allocation and limited capacity to store carbohydrate when compared with larger species, such as *Posidonia* (Walker *et al.* 1997). An above ground biomass allocation of approximately 80% indicates that *A. griffithii* would have a large respiratory load to maintain in the advent of a reduced light climate (Carruthers & Walker, 1997).

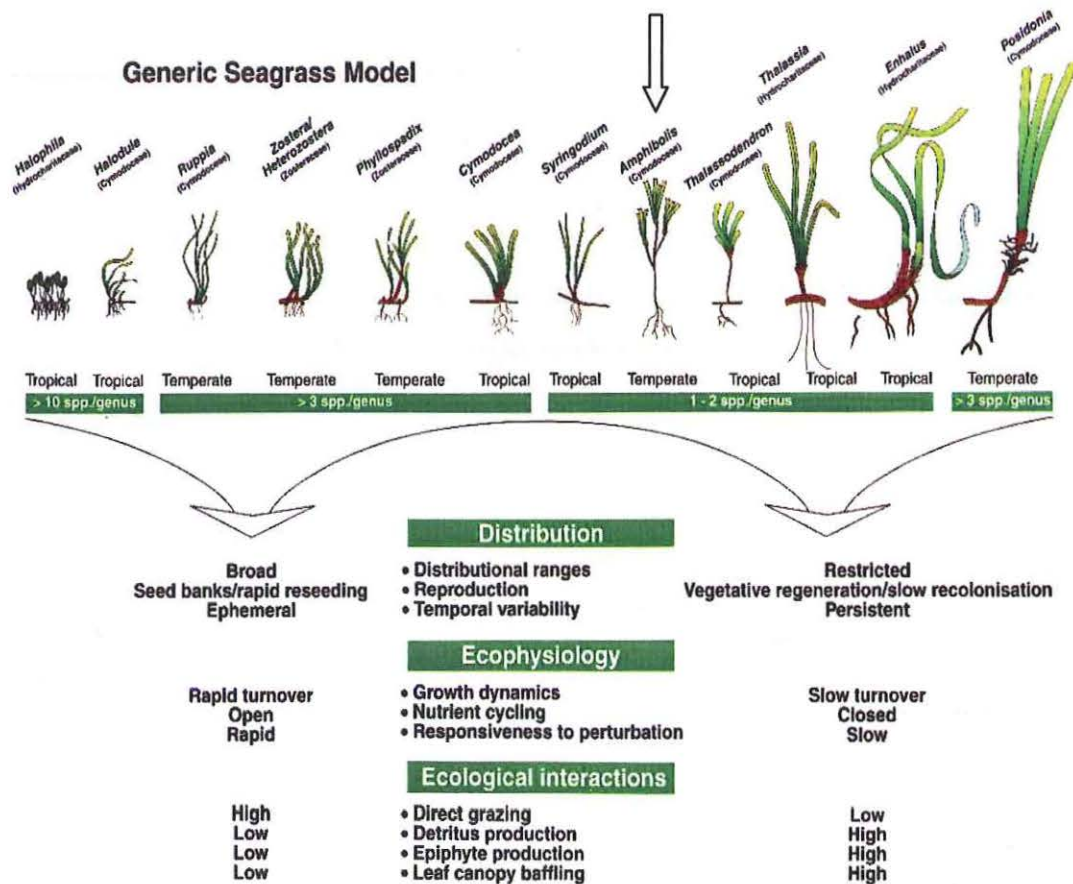


Figure 1-3 Generic functional form model with the genus *Amphibolis* denoted (model by: Walker *et al.* 1999).

The large-scale degradation events arising from the port enhancement project at Champion Bay, Geraldton illustrate the effects of channel-dredging on seagrass ecosystems (Figure 1; EPA, 2002; "Fears for seagrass", 2003). For approximately nine months the Geraldton Port Authority undertook an extensive dredging program to deepen and extend their main shipping channel in Champion Bay (unpublished: URS, 2003). The plume of re-suspended sediment was approximately 2 kilometres wide and extended northward for a distance of up to 70 kilometres (pers. comm. M. Mulligan, Geraldton Port Authority). *Amphibolis* species appeared to be dramatically affected with leaf and shoot losses in and around areas surrounding the dredge plume up to several kilometres away from the channel activities (pers. comm. Dr M. Westera, CSIRO; M. Mulligan, Geraldton Port Authority). The likely cause of the stress and degradation in seagrasses at Geraldton can be attributed to light limitation (Figure 1.1; Duarte, 1991). Another study on *Amphibolis griffithii* has shown that *Amphibolis* is particularly adept at overcoming burial and shifting sediment heights (unpublished: Coupland, 1997).

Traditional monitoring program variables, such as shoot density and percentage cover used to identify light stress in strap-like seagrass species, for example, *Posidonia*, appear to be inappropriate for indicators for *Amphibolis* species. The detectable *Amphibolis* responses reported by Geraldton Port Authority's consultant during the dredging program appeared to be leaf, epiphyte and leaf colour reductions (unpublished: URS, 2003). Leaf reductions were quantitatively sampled and the other reported responses were anecdotal observations. These observations together with a broader ranging suite of variables, that have shown correlations in other light stressed seagrass species, were used to investigate the problems associated with PAR reductions in *A. griffithii* meadows including some physiological characteristics.

In combination, the lack of understanding in regard to *Amphibolis* morphology and physiological responses and the extended temporal extensions granted to the dredging program are likely to have pushed these seagrass meadows past their light limitation thresholds that led to the wholesale losses described. The rationale for undertaking the research was largely tied to the degradation events that led to the losses at Champion Bay, Geraldton. The study was designed as a pilot or scoping

investigation to identify potential primary indicators of light stress in *A. griffithii* on the mid-west coast of Western Australia. Furthermore, given the coastal development pressures on these systems nationally (Walker & McComb, 1992; Kirkman, 1997) the results may be of use along the entire *A. griffithii* distribution and contribute to the global knowledge in the range of seagrass responses to environmental stress. Comparisons of the research outcomes against a form and function model (Walker *et al.* 1999) may provide impetus for further global research on seagrasses that do not conform to the dominant morphological forms e.g. the strap-like genus *Posidonia*.

The research will aid in the future development of Environmental Quality Criteria (EQC) that are used to quantitatively assess and monitor Environmental Values (EV) like ecosystem health (EPA, 2004). In Western Australia, the Environmental Quality Objectives (EQO) of the EV for seagrass systems are maintenance of ecosystem integrity and typically the level of ecological protection for these unique systems is high (EPA, 2002a). The development of specific quantitative EQC for light limitation in *A. griffithii* will allow dredging proponents and regulatory agencies to develop ecologically sound management proposals and management trigger values in regard to light limitation. The establishment of EQC with scientifically proven trigger values for allowable intensities and durations of light reduction in seagrass systems will help to avert the wholesale degradation events that occurred at Champion Bay from reoccurring. Essentially, the development of management trigger values and defined EQC would shift the development and environmental management practices in light impacted *A. griffithii* systems from a qualitative to quantitative framework.

The genesis of the project was driven by the current and likely, future port and marina development pressures placed on seagrass systems on the central mid-west coast of Western Australia. The importance of PAR to seagrass ecosystems coupled with the proliferation of port and marina developments involving channel dredging programs in Western Australia highlight the relevance and significance of the proposed project. Therefore, it is vital to understand the morphological and physiological responses of *A. griffithii* to reduced PAR in order to manage the specific threats facing these locally and regionally unique seagrass ecosystems.

The study will fill a variety of identified scientific gaps in local knowledge pertaining to light limitation on one of the dominant and ecologically unique, meadow-forming seagrasses on the temperate west coast of Australia, off Jurien Bay. Moreover, an increased understanding of the responses of *A. griffithii* to reduced PAR and the subsequent disturbances to the ecological pathway will contribute to the provision of quantitative data in the future development of Environmental Quality Criteria (EQC). Specifically, the study will assist in the identification of potential response indicators of *A. griffithii* to high intensity, short duration PAR reductions and their subsequent susceptibility to a disturbance during late summer through winter on the mid-west coast region. There are three main questions that will guide the study:

What effect does reduced PAR have on the morphological and physiological characteristics of *A. griffithii* over a moderate timescale of approximately 100 days?

If affected, do the reduced PAR responses in *A. griffithii* show any significant recovery within several weeks once the light impact has ceased?

Which morphological and physiological characteristics have the potential to serve as future indicators of reduced PAR stress and recovery in *A. griffithii*?

Therefore, the study aims to experimentally test and quantify the responses of PAR induced stress in *A. griffithii* subjected to high intensity, short duration light reductions via the following null hypothesis:

**Null Hypothesis ( $H_0$ ):** temporary imposed high intensity, moderate duration light limitation has no effect on the selected morphological and physiological attributes of the seagrass *Amphibolis griffithii*.

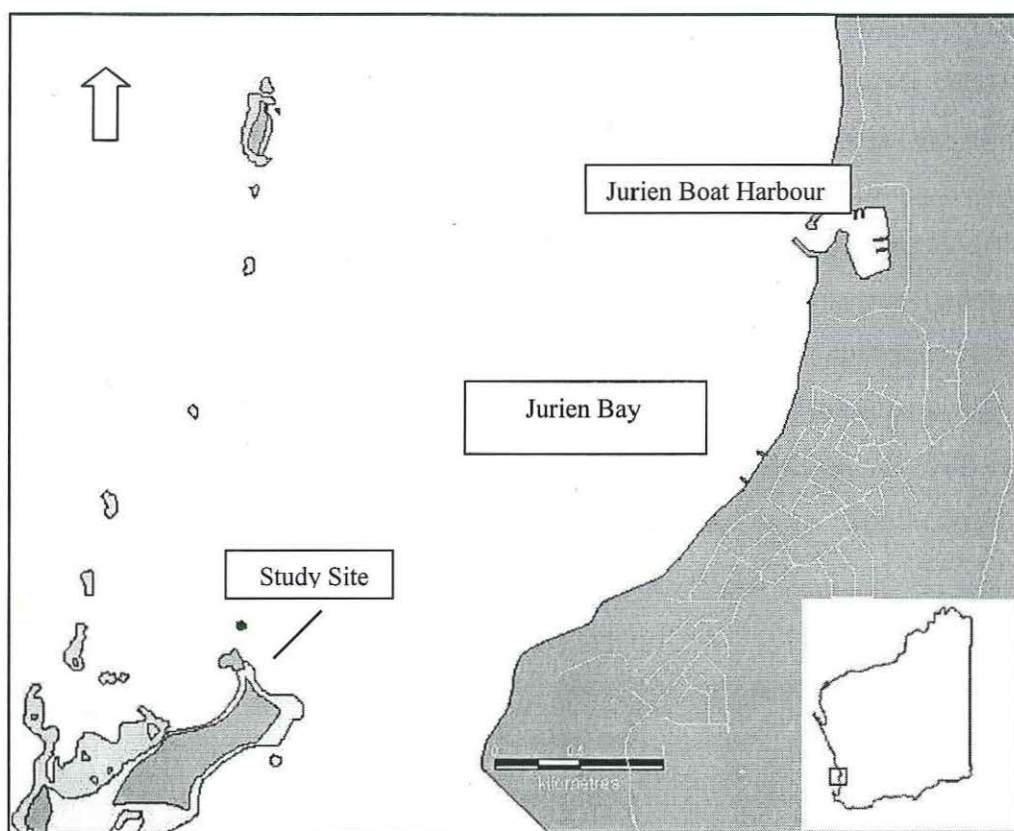
## 1.1 Thesis outline

Chapter 2 describes the methods, materials and techniques used in the study. Chapter 3 reports the results from the light measurements, the responsive characteristics and the other characteristics that displayed poor responses to reduced PAR levels. Chapter 4 provides a discussion and compares and contrasts the results of PAR reduced *Amphibolis griffithii* with other plants and seagrasses; provides a conceptualised response pathway and assesses the potential of the responses to act as future indicators for a high intensity, moderate duration PAR reduction event. Furthermore, recommendations are made for the planned and funded, future research and concludes by highlighting the management and broader implications of the study.

## 2 Research Plan, Methods and Techniques

### 2.1 Study Site: Jurien Bay

The experiment was conducted at Jurien Bay approximately 260 kilometres north of Perth. Jurien Bay was chosen as the study site as it was considered to represent a relatively pristine system on the central Western Australian coast (DCLM, 1994; EPA, 2001). Furthermore, Jurien Bay was the desired study area from the collaborative funding partner for the project, the Strategic Research Fund for the Marine Environment (SRFME). The offshore Jurien Bay region is dominated by seagrass, patchy sand and macro-algal reef habitats containing 'Tamala' limestone, reefs, platforms and islands that in some instances are overlaid by younger soft-sediments (DCLM, 1994). The study site was located on an expansive area of level bathymetry with an approximate depth of 4.0 – 4.5 metres located 200-300m north-east of Boullanger Island (308402E & 6645234N) (Figure 2.1).



**Figure 2-1:** Map showing Jurien Boat Harbour and the study site situated off north-east Boullanger Island, Jurien Bay (Adapted from: WALIS, 2004).

## 2.2 Design and Establishing Experimental Units

The study aims have been achieved by implementing a single intensity (80% PAR attenuating shade-cloth that fouled to ~90% PAR attenuating) shading experiment imposed over an *Amphibolis griffithii* meadow. The shade cloth was rapidly colonised by epiphytic algae. Various measurements were taken to calculate the percentage of light reduction in the shaded plots relative to the control plots. Eighty percent attenuating shade cloth was chosen as it matched the aim of the study in trying to ascertain the tolerance of *A. griffithii* under an intensely light reduced canopy climate. This intense light reduction in the water column is commonly found in the immediate surrounds of a working dredge e.g. Geraldton Port Enhancement project where available PAR was reduced to comparable experimental levels up to an approximate distance of 3kms away (Pers. comm. Mike Mulligan; unpublished data, Geraldton Port Authority).

A total of 12 experimental units were installed, 6 replicate shade screens and 6 replicate unshaded controls. Unfortunately, two treatment plots were lost during the shading phase and this is likely to be attributed to boat anchors and / or crayfish pot retrieval. The ideal replication size was estimated by inputting *A. griffithii* data (courtesy of M. Mulligan, Geraldton Port Authority) into a model described by Bros & Cowell (1987), who resolve relative effort and sample size through manipulating multiple estimates of variation around the mean. The outputs revealed that four replicates were sufficiently powerful and two extra replications per treatment were added as an inbuilt safety buffer, in case of unforeseeable circumstances e.g. boat anchor damage. The experiment commenced in late March (late summer) to capture the *A. griffithii* meadow at its peak carbohydrate store (Carruthers & Walker, 1997). Therefore, the treatment was applied at a time when it is hypothesised that *A. griffithii* was best equipped to cope with the shading stress.

Major sampling events took place on five occasions, one event just prior to treatment implementation, three during shading (a total of 106 days impact) and one post shade removal (42 days recovery). The impact phase of the experiment mimics a typical maintenance dredging program, these rarely continue beyond a temporal scale of 2-3 months on any one occasion (pers. comm. M. Mulligan, Geraldton Port Authority).

The planned duration between sampling events was at least 28 days and the maximum duration between events was 42 days. The timing of the sampling regime was deemed necessary as seagrass systems typically respond in a non – linear manner, wherein they display a degree of tolerance and responsive plasticity before impacts become evident (Hemminga & Duarte, 2000). The extent of the 42 day recovery period was limited due to temporal constraints of the Honours program. Therefore, recovery is used throughout as relative term and may be indicated by a partial or total reversal in the effects of the impact. The experiment can be best described as a Before After Control Impact Repeated measures (BACIR) design and a suite of relevant morphological and physiological variables (Table 2-1) were measured and the results were used to interpret the differences between treatments over time (Osenberg & Schmitt, 1996). The BACIR design has been successfully implemented by other researchers (e.g. Long *et al.* 1996) investigating the effects of dredging in seagrass systems.

The study area perimeter was set out in a manageable 50 x 50m (250m<sup>2</sup>) work area. Placement of the experimental units (both shaded and control plots) was determined by the use of randomly generated numbers. Each experimental unit measured 4.5m x 3.0m providing a total area of 13.5m<sup>2</sup>. To confirm that the area receiving the intended shade intensity a light profile was measured under the shade screens using a Li-Cor meter. The final workable central area used for sampling was approximately 4.5m<sup>2</sup> and the remainder of the shaded area was deemed unusable due to the lateral intrusion of incident light. The total destructive sampling area throughout the life of the study was calculated at approximately 0.4m<sup>2</sup>, which minimised the overall disturbance in the useable area to less than nine percent.

The shade screens comprised of eight metal star pickets and the shade cloths were attached using stainless steel wire rope, shackles, turnbuckles and cable ties (Figure 2.2). The control plots comprised of the structural star pickets only. Experimental/procedural controls have been deemed unrealistic as fouling of the procedural control material would alter or filter the incident PAR to some degree and confound the results. Fiscal constraints (e.g. accommodation, vehicle & boat costs) precluded the daily maintenance of procedural controls due to the study sites locality in a regional area. Previous attempts at manipulating and maintaining shading

procedural controls have appeared futile (Bulthuis, 1983; pers. comm. Catherine Collier, Edith Cowan University).



**Figure 2-2** Photograph in the corner of an *Amphibolis griffithii* treatment plot with shade cloth attached by stainless steel wire rope, shackles, turnbuckles and cable ties (Photo by author).

A single permanent  $0.04\text{m}^2$  quadrat was established in the centre of all experimental plots at the commencement of the study in order to measure the non-destructive variables repeatedly over time and to minimise disturbance within the plots. Stainless steel pins were driven into the sediment and remained in the sediment throughout the duration in order to maintain the permanent quadrats. The quadrats were designed to slide into the pins, in order to remove them after every sampling event and therefore, minimise any disturbance from the quadrat frames.

### **2.3 Sampling Variables**

The destructive sampling was minimised by harvesting above ground biomass and below ground core samples in a single randomly chosen  $0.04\text{m}^2$  quadrat. Pigment and carbohydrate samples were picked immediately adjacent to the harvested destructive quadrat and the disturbance sites were marked with a steel peg and

floating cork to ensure the same area was not sampled again. A synthesis of all the sampling and measurement variables carried out during the course of the experiment are found in Table 2.1. The rationale for choosing the suite of variables tested during the study were determined by reviewing the literature and identifying potential correlate responses in other seagrasses.

**Table 2-1: Synthesis table highlighting the category, number of sampling events, procedure and reference for all variables measured during the *A. griffithii* study in Jurien Bay, 2004.**

Category	Variable	Number of sampling events or duration	Procedure overview & reference for detailed method
<b>Morphological</b>	Shoot density	5	Manual count <i>in situ</i> (Duarte & Kirkman, 2001)
	Percentage cover	5	<i>In situ</i> (Duarte & Kirkman, 2001)
	Max. canopy height	5	<i>In situ</i> (Duarte & Kirkman, 2001)
	Avg. canopy height	5	<i>In situ</i> (Duarte & Kirkman, 2001)
	Leaf extension	4	<i>In situ</i> marking & lab count (Short & Duarte, 2001; pers. comm. Dr G. A. Kendrick)
	Above ground biomass	5	Sort, dry & weigh (Duarte & Kirkman, 2001)
	Below ground biomass	5	Sort, dry & weigh (Duarte & Kirkman, 2001)
	Number of leaves per stem	5	Lab count (Duarte & Kirkman, 2001)
	Number of leaves per cluster	5	Lab count (Duarte & Kirkman, 2001)
	Number of clusters	5	Lab count (Duarte & Kirkman, 2001)
<b>Physiological</b>	Pigment analysis (chlorophyll)	5	Lab extraction (Granger & Lizumi, 2001; Longstaff & Dennison, 1999)
	Sugar and starch analysis	5	Lab extraction (Dubois & Gilles, 1956)
<b>Light</b>	Light reduction	5 weeks (2 x loggers)	<i>In situ</i> measurements download data in lab (Canuthers <i>et al.</i> 2001)
	Light attenuation coefficient (canopy)	2 sampling events (1 x during & 1 x post shading)	<i>In situ</i> with Li-Cor 2π meter, 2 plots per treatment & 3 reps per plot (Canuthers <i>et al.</i> 2001)

Shoot density, percentage cover and maximum and average heights were all measured non-destructively in the permanent quadrats. These four variables were counted and their scores recorded according to the procedures outlined in detail by

Duarte & Kirkman (2001). Leaf extension measurements followed the Zieman (1974) procedure refined by Short & Duarte (2001) with *Amphibolis* specific advice regarding the placement of the punch hole method from Dr G. A. Kendrick (pers. comm. University of Western Australia). The placement of the punch hole was critical as it must mark the innermost or newest leaf sheath, as *A. griffithii* grows from the inside of the cluster, with the oldest leaves and sheaths being located on the outer and lowest positions in the cluster and the youngest leaves and sheaths being located in the centre of the terminal cluster (Ducker *et al.* 1977). Hole punching was carried out *in situ* using a standard leather punch. The incubation time prior to harvest and measurement in the lab was typically around 14 days (Figure 2-3). All leaf clusters were marked on approximately 12 stems per plot, per sampling event, thus including all age structures within the canopy. The method was developed during the experiment and some early attempts were unsuccessful and therefore, account for missing data for this variable in the early stages of the study.



**Figure 2-3** Photograph depicting the placement of the leaf extension hole punch and subsequent growth post incubation in *Amphibolis griffithii* (photo by author).

Above ground biomass was collected in a single, randomly placed 20x20cm (0.04m<sup>2</sup>) quadrat. All stems in the quadrat were cut off at sediment level and placed immediately in a bag and stored at -18<sup>0</sup>C prior to analysis. Analysis of the above ground biomass followed Duarte & Kirkman (2001) and incorporated all leaf, leaf cluster and epiphyte parameters. The above ground material was separated into leaf, stem and epiphyte components. These were further separated into 10cm canopy layers for each component, similar to the method outlined by Carruthers & Walker (1997) and Carruthers (1999) (Figure 2-4) who have previously described *A. griffithii* as having a conspicuous canopy distribution. The separate components were labelled and weighed after 48 hours in a drying oven at 60<sup>0</sup>C. The below ground biomass was collected using an 11cm inside diameter, or 0.0095m<sup>2</sup> stainless steel corer. Below ground biomass was separated into three main components: root, rhizome and dead material and dried in an oven at 60<sup>0</sup>C for 48 hours.



**Figure 2-4** Photograph of a lower through to mid canopy leaf, stem and epiphyte biomass breakdown in 10 cm intervals (from left to right: 0-10, 10-20, 20-30 & 30-40cm) for *Amphibolis griffithii* (photo by author).

Samples collected for chlorophyll analysis were collected adjacent to the biomass cores. These samples were immediately wrapped in aluminium foil, stored on ice during transport and stored in darkness at  $-18^{\circ}\text{C}$  prior to analysis. Details of the spectrophotometric pigment (chlorophyll) analysis are found in Granger & Lizumi (2001) and Longstaff & Dennison (1999). A 30mm section from the youngest mature leaf, both at the top and bottom of the canopy in *A. griffithii* was consistently chosen for analysis based on the assumptions that the older leaves may be senescent and / or necrotic and mature leaves have fully developed pigment characteristics compared with immature leaves (Hemminga & Duarte, 2000). In a darkened room, fresh leaf material was finely chopped with a razor blade then ground in a cold mortar and pestle and combined in a centrifuge tube with 10ml of chilled 90% acetone. The material was extracted on ice in the dark for two hours prior to centrifuging at 4000rpm for two minutes. Spectrophotometric absorbance was measured at 750, 663 and 643nm with the first reading (750nm) deducted from the others as a turbidity calibration. The total chlorophyll and chlorophyll a/b ratio were determined.

Leaf and rhizome sugar and starch analysis followed the method outlined by Dubois and Gilles (1956) using oven dried material. Leaf material was consistently taken from the uppermost canopy layer from the above ground biomass samples and rhizome samples were bulked together from the below ground rhizome biomass. Leaf and rhizome material were ground in a 'Retsch MM200 mixer ball mill' and twice extracted in hot 80% ethanol. Spectrophotometric concentrations were determined using the 'phenol – sulphuric acid' method described in Dubois & Gilles (1956).

Light availability was collected at fifteen minute intervals from 'Odyssey Dataflow' submersible incident light sensors and automated brush sensor cleaners (Figure 2.5) for a period of five weeks. A single light logger was attached to a star picket just above canopy height, in both the treatment and control plots following the instructions described by Carruthers *et al.* (2001). The data was downloaded and a percentage value for mean light reduction in the shaded plots over the five week period was derived.



**Figure 2-5** Photograph of a light logger and automated wiper assembly installed in an *Amphibolis griffithii* meadow (photo by author).

PAR was measured during and after the shading phase of the experiment using a 'Li-Cor' meter at the water surface, at the level of the shade screens and at the top and bottom of the seagrass canopy. Canopy Light Attenuation Coefficients (LAC) were calculated from the differences between light availability at the top and bottom of the canopy in both shaded and control plots was inferred (Carruthers *et al.* 2001).

## **2.4 Statistical Analysis**

Differences within and between treatments over time were investigated by a repeated measures 2-way ANOVA in the statistical package SPSS (v.11.5). The repeated measures analysis was used to combat the issue of sample independence, due to repeatedly measuring the same plots over time. The repeated measures analysis aims to capture the effects treatment and automatically decreases the available degrees of freedom in order to adjust for the lack of sample independence (Dytham, 2003). Therefore, this reduction in degrees of freedom reduces the risk of committing a Type I error. Any significant effects between treatments along with any interactions were analysed using a *t*-test (Coakes & Steed, 2001).

The hypotheses were initially trialled with hypothetical dummy data to ensure that the experimental design was appropriate, as suggested by Dytham (2003). Prior to running any repeated measures ANOVAs all data were tested for compliance with parametric rules of homogeneity and normalised distribution. Any data that did not

comply with these assumptions were transformed and normalised using a natural log (Ln), a log ten (log) or a square root (sqrt) and the best fit transformation was used.

Treatment was considered a fixed factor as it was a predetermined intense level of shading that closely mimics the maximum PAR reductions caused by dredging (unpublished data, Geraldton Port Authority). Time was also considered a fixed factor because the design matches the temporal scale (usually short term) of typical dredging programs. Furthermore, time is required as a fixed factor in order to tease out any differences in *A. griffithii* to reduced PAR to aid the development of EQCs (Environmental Quality Criteria). If the 'null hypothesis' is rejected, then this may be considered as reasonable justification for inferring effects on the seagrass *A. griffithii* as a result of temporary light limitation.

## 2.5 Assumptions and Limitations

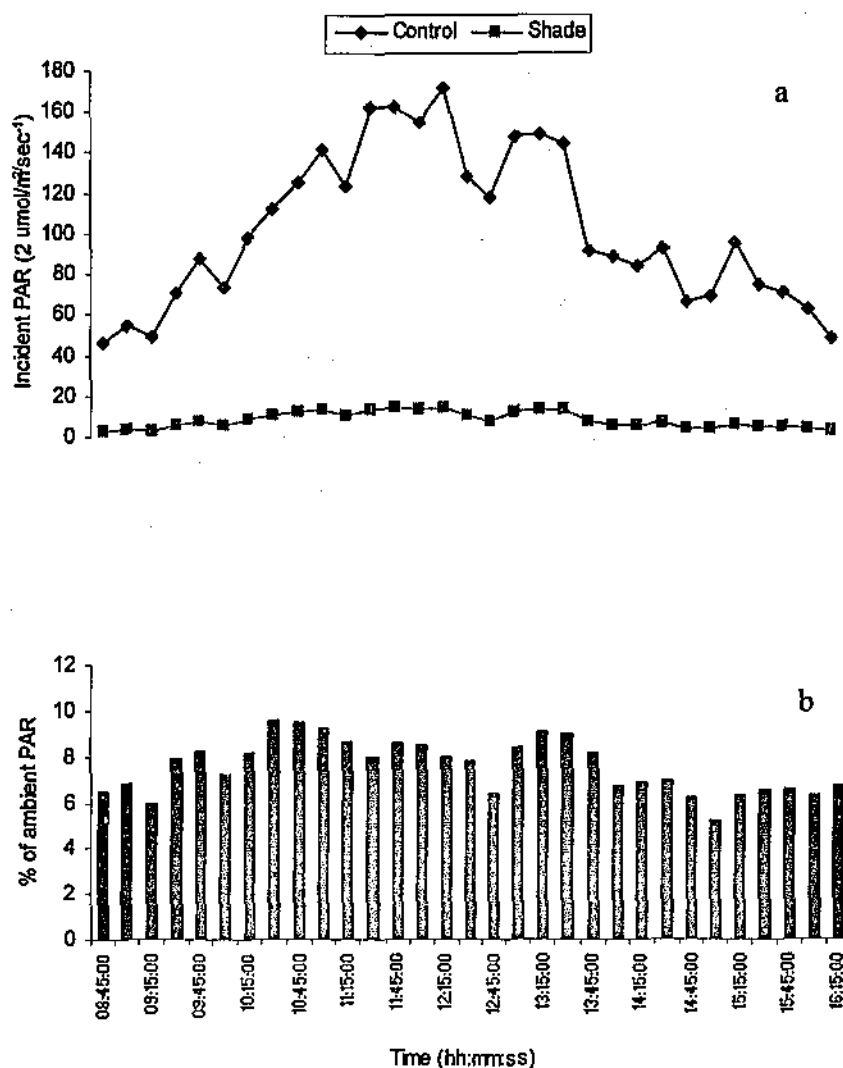
The most obvious limitation in the study is the limited temporal scope constrained by the Honours program (36 weeks). This precludes any study of seasonal variation in the response to *Amphibolis griffithii* to shading. However, any statistical significance in the overarching hypothesis would allow for direct quantitatively backed statements pertaining to the impact of light limitation on *A. griffithii* during late summer and autumn months. Seasonality will be addressed in a related project by an Edith Cowan University, Master of Science student, Michael Mulligan. Incorporating the related studies into a collaborative framework, as advocated by Butler & Jernakoff (1999) and Duarte (2002) including sedimentation/siltation analysis (experimentally de-confounded from this study) coupled with light limitation will enhance the utility of the proposed study. The study is limited to a single study area and depth, due to temporal and fiscal constraints. However, this does not detract from the value of the investigation, as the impacts and responses of light limitation (Butler & Jernakoff, 1999) on *A. griffithii* are poorly understood. Furthermore, the results may be relatively transferable in and around the central west coast of Western Australia from Port Denison to Whitfords (a region that has been classified as analogous in terms of its inshore benthic environment) (DCLM, 1994). However, the author stresses caution with the application or transfer of these results

elsewhere, as seagrass systems are notoriously dynamic and responsive to localised influences (Hemminga & Duarte, 2000). Seagrasses are clonal and there was a risk of only capturing a limited range of genetic variability of the entire species. However, the genus *Amphibolis* have displayed very low rates of genetic variability throughout their entire distribution (Waycott *et al.* 1996). An experimental/procedural control was deemed to be unrealistic for this experiment as any solid material mimicking the shade cloth would alter (filter, refract, deflect) the light incidence reaching the canopy. Moreover, besides the filtering effect this procedural control material would also foul up rapidly, further confounding the results. This anomaly could realistically be overcome however, given the distance, logistics, equipment and labour involved in doing so would prove cost inhibitive and unrealistic given the budget and time allowed for the project.

### 3 Results

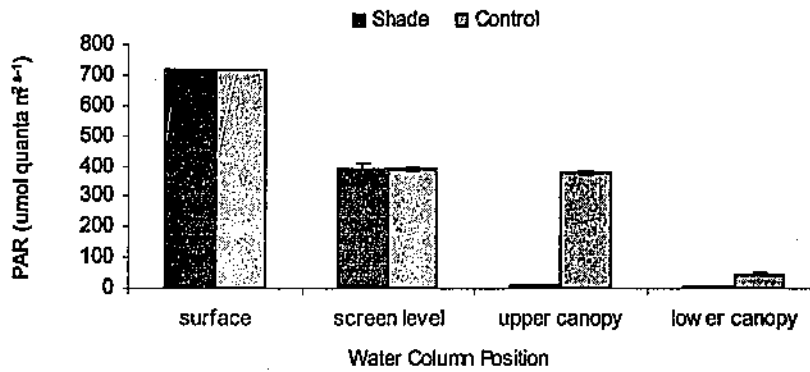
#### 3.1 Light (Photosynthetically Active Radiation)

On average, 10.20% of control or ambient photosynthetically active radiation (PAR) was reaching the top of the seagrass canopy in the shaded treatments (i.e. available light or PAR was reduced by 89.8%). A typical daily light distribution was calculated for PAR climates under control and treatment conditions (Figure 3.1a). Further calculations of the daily light distribution revealed that between the hours of 08:45 and 16:15 the percentage of control light reaching the shaded canopy were less than 10% of ambient or control percentages (Figure 3.1b). There were almost two orders of magnitude difference in PAR availability between shaded (Light Attenuation Coefficient =  $0.59\text{ m}^{-1}$ ) and control plots (LAC =  $2.38\text{ m}^{-1}$ ). Therefore, the control canopies were effectively extinguishing almost two orders of magnitude more PAR relative to the shaded plots.



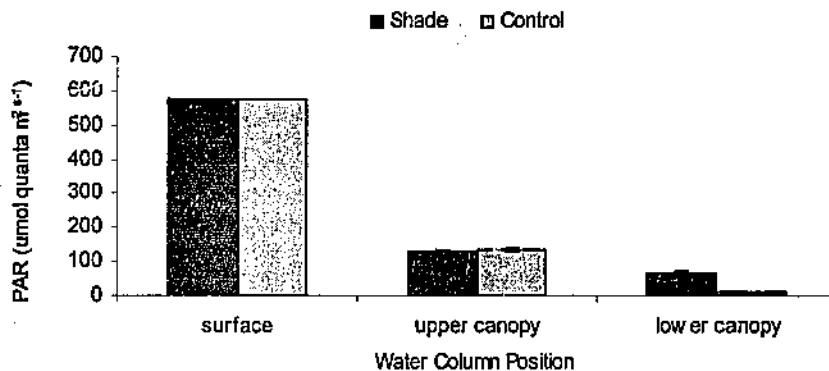
**Figure 3-1 : a) Typical daily incident PAR distribution for shaded and control plots between 08:45 and 16:15 hours b) Typical daily percentage of control ambient PAR reaching the upper canopy of the shaded meadow between 08:45 and 16:15 hours.**

Absolute PAR levels, at the surface of the seagrass canopy received on average  $377$  and  $12 \mu\text{mol m}^2 \text{s}^{-1}$  in the control and treatment plots, respectively (Figure 3.2). Similarly, the lower canopy readings also differed considerably in the control and shaded plots receiving values of approximately  $46$  and  $4 \mu\text{mol m}^2 \text{s}^{-1}$  each. Therefore, shaded plots received approximately 3 and 9% of relative control PAR in the upper and lower canopies, respectively (Figure 3.2).



**Figure 3-2 : Mean water column and canopy PAR climate just below the surface, at the screen level, upper canopy and lower canopy levels during the shading phase of the study ( $n=2 \pm SE$ ).**

During the recovery phase (shade cloth removed), the PAR values at the upper canopy were similar for both the shaded and control plots (Figure 3.3). However, the lower canopy values differed dramatically between treatment and control with light values of approximately 68 and 9  $\mu\text{mol m}^2 \text{s}^{-1}$ , respectively. Therefore, shaded plots received in excess of 700% more light at the bottom of the canopy relative to control plots following shade removal.



**Figure 3-3 : Mean water column and canopy PAR climate just below the surface, upper canopy and lower canopy levels during the recovery phase of the study ( $n=2 \pm SE$ ).**

### 3.2 Morphological and Physiological Results in manipulated *Amphibolis griffithii*.

Among the range of variables measured during the experiment, several displayed very little response, others showed limited response and many displayed a significant response to shading or light limitation (Table 3.1). The suite of variables that displayed a significant response to imposed shading (treatment) over the course of the experiment included both morphological and physiological responses: mean leaves per stem, mean leaves per cluster, leaf cluster density, mean total leaf biomass, mean epiphyte biomass, mean leaf extension, estimated mean areal leaf extension, mean rhizome sugar, mean leaf sugar and mean rhizome starches and upper canopy total chlorophyll concentrations (Figures 3.4 – 3.7).

The majority of variables that responded with a significant effect of treatment, or significant interaction between treatment and time were found in the above ground portion of *A. griffithii* and typically related to the growth, biomass or productivity of the above ground standing crop. Generally, these variables displayed a quantitative reduction in shaded plots, whilst control plots remained relatively constant over time.

**Table 3-1: Results of two-way repeated measures ANOVA (RM ANOVA) testing the effects between treatments and for any interactions between treatment and time in the responsive morphological and physiological characteristics of the seagrass *Amphibolis griffithii*.**

Characteristic	Variable	ss	d.f.	ms	f	p
No. of leaves per stem	time	286.124	4	71.531	3.596	0.02
	trt	429.413	1	429.413	13.582	0.01
	time * trt	113.204	4	28.301	1.423	0.257
	err	189.695	6	31.616		
No. of leaves per cluster	time	1.362	1.684	0.809	11.46	0.003
	trt	4.788	1	4.788	34.634	0.001
	time * trt	1.575	1.684	0.935	13.245	0.002
	err	0.713	10.103	0.071		
Clusters density m <sup>2</sup>	time	2000284	4	500071	0.347	0.843
	trt	7272299	1	7272299	10.254	0.019
	time * trt	8063971	4	2015992	1.399	0.264
	err	4255185.2	6	709197.5		
total leaf bio	time	126.758	4	31.69	1.979	0.13
	trt	390.71	1	390.71	8.491	0.027
	time * trt	155.14	4	38.785	2.422	0.076
	err	172551.92	6	28758.65		
epi biomass (sqrt trans.)	time	2.728	4	0.682	0.951	0.452
	trt	20.058	1	20.058	6.833	0.04
	time * trt	5.728	4	1.432	1.996	0.127
	err	440.318	6	73.386		
leaf extension	time	0.22	3	0.073	14.821	0.00

	trt	0.378	1	0.378	80.351	0.00
	time * trt	0.156	3	0.052	10.563	0.00
	err	0.089	18	0.005		
	time	3189684	2	1594842	5.803	0.017
	trt	6802590	1	6802590	24.122	0.003
areal leaf	time * trt	1571754	2	785877	2.859	0.096
extensn	err	1692034.3	6	282005.7		
	time	74.426	4	19.606	5.94	0.002
	trt	123.297	1	123.297	32.811	0.001
rhizome	time * trt	54.617	4	13.654	4.137	0.011
sugar	err	79.217	24	3.301		
	time	69402	4	17350	3.319	0.027
	trt	15440	1	15440	8.049	0.03
	time * trt	44663	4	11165	2.136	0.107
leaf sugar	err	11510.407	6	1918.401		
	time	1.622	4	0.405	4.477	0.008
	trt	1.397	1	1.397	15.718	0.007
rhizome	time * trt	0.808	4	0.202	2.229	0.096
starches	err	0.533	6	0.089		
(Ln trans.)	time	139006784	4	34751696	17.749	0.00
	trt	41154751	1	41154751	13.952	0.01
upper canopy	time * trt	55096586	4	13774146	7.035	0.001
tot. chl	err	46992083	24	1958003		

### 3.3 Morphological Parameters

Mean leaves per stem remained constant throughout the experiment in the control plots with approximately 25 leaves per stem (Figure 3.4a). In treatment plots there was a trend in declining numbers of leaves per stem and a highly significant difference between treatments and controls ( $p < 0.01$ ) (Table 3.1). Post hoc analysis revealed a significant difference after 38 days of shading and a highly significant difference after 106 days and considerable recovery back within control levels by 42 days, following shade removal (Figure 3.4a). The trend in the number of leaves per stem closely followed those in mean leaves per cluster with RM ANOVA revealing a highly significant interaction between treatment and time ( $p < 0.01$ ) (Figure 3.4b & Table 3.1). Post hoc analysis revealed increasingly significant differences during the shading phase by 38 days of shading that culminated in highly significant differences by Times 66 & 106 days of treatment (Figure 3.4b). Mean leaves per cluster displayed a sharp recovery post shade removal, however, post hoc analysis showed that significant differences still remained between treatment and control after 148 days of experiment (Figure 3.4b). Values ranged from approximately 1.8 leaves per cluster in the shaded plots after 106 days of shading to approximately 3.4 leaves per cluster after 148 days in the control plots (Figure 3.4b).

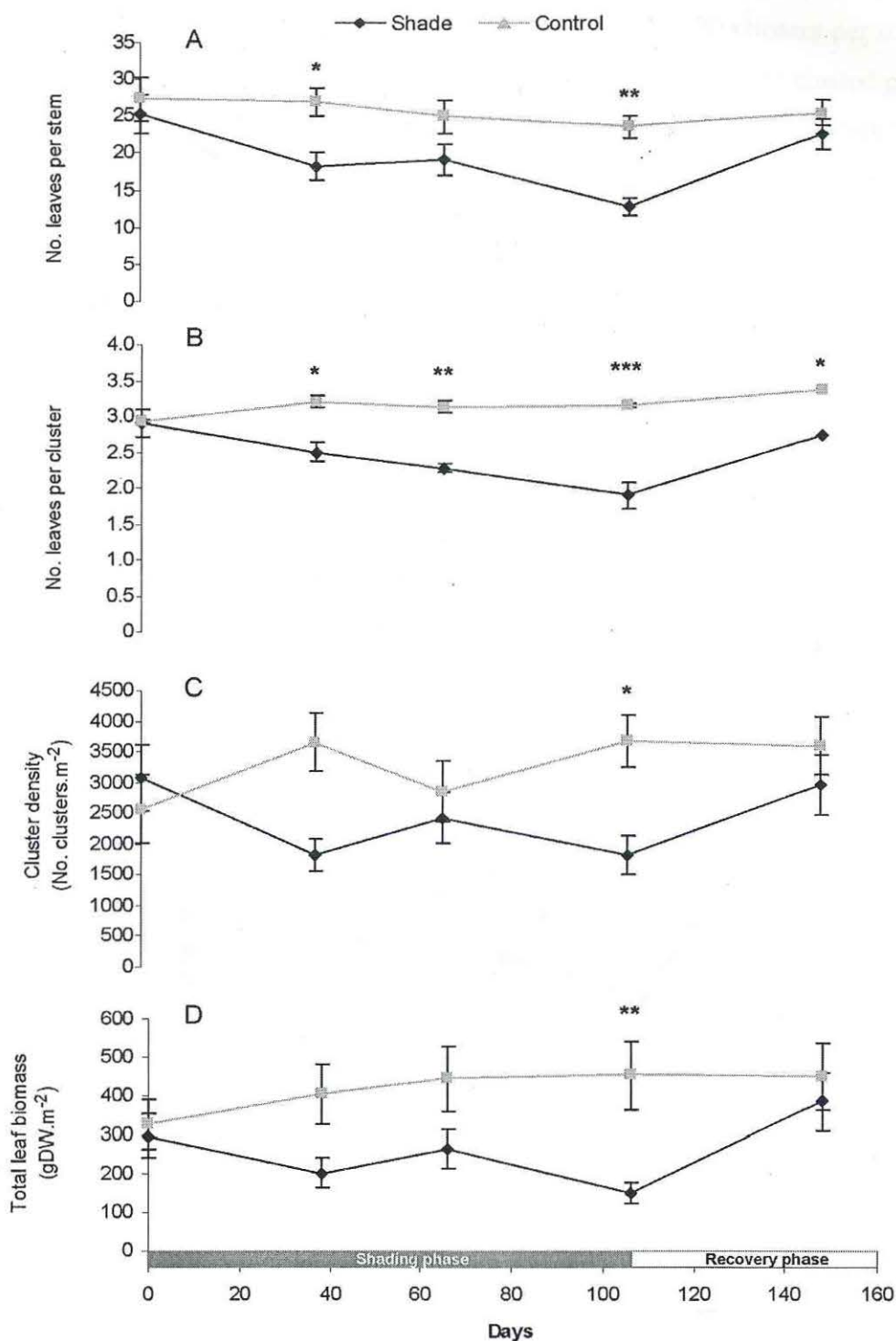


Figure 3-4: Leaf and leaf cluster responses in the seagrass *Amphibolis griffithii* during both the shading and recovery phases of the study A) number of leaves per stem, B) number of leaves per cluster, C) areal leaf cluster density, D) total leaf biomass ( $n=4 \pm SE$ ). Values significantly different between control and treatment at each time indicated by \* ( $p<0.05$ ), \*\* ( $p<0.01$ ) and \*\*\* ( $p<0.001$ ).

Leaf cluster density revealed a significant difference ( $p<0.05$ ) between treatment and control (Table 3.1). Leaf cluster density remained constant in the control plots (approximately 2600–3600 clusters per m<sup>2</sup>), but declined in shaded plots,

culminating in a significant difference between treatments and controls after 106 days of the experiment with an approximate density of 1800 clusters per m<sup>2</sup> (Figure 3.4c). A sharp recovery in leaf cluster density was evident in the shaded plots back to comparable control levels after 42 days following shade removal (Figure 3.4c).

Mean total leaf and total epiphyte dry weights followed similar trajectories wherein control plots remained relatively constant with marginal increases over time (Figures 3.4d & 3.5a), but both variables were significantly lower in the treatment ( $p<0.05$  &  $p<0.05$ ), compared to the controls (Table 3.1). Total leaf and total epiphyte dry weights diminished steadily and post hoc analysis reported highly significant differences after 106 days of shading and sharp recoveries back within control levels by 148 days (Figures 3.4d & 3.5a).

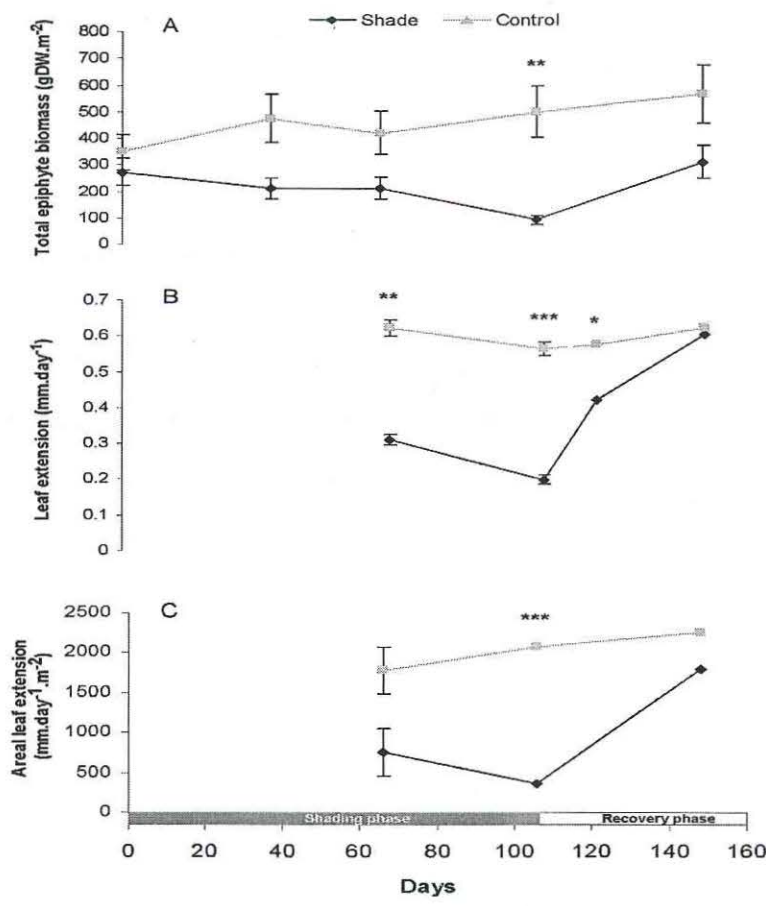


Figure 3-5: Epiphyte biomass and leaf extension responses in the seagrass *Amphibolis griffithii* during both the shading and recovery phases of the experiment A) total epiphyte biomass, B) daily leaf extension, C) estimated areal leaf extension ( $n=4 \pm SE$ ). Values significantly different between control and treatment at each time indicated by \* ( $p<0.05$ ), \*\* ( $p<0.01$ ) and \*\*\* ( $p<0.001$ ).

Mean leaf extension displayed a highly significant interaction in the differences between treatments and controls ( $p<0.001$ ); control leaf extensions remained constant at approximately  $0.6\text{mm/leaf extension/day}^{-1}$ , whilst leaf extension during the shading phase in the treatment plots decreased to approximately one half (at 66 days of shading  $=0.3\text{mm/leaf extension/day}^{-1}$ ) to one third (106 days of shading  $=0.2\text{mm/leaf extension/day}^{-1}$ ) of control extension levels (Table 1 & Figure 3.5b). Post hoc analysis revealed highly significant differences in leaf extension rate after 66 and 106 days of shading and a significant difference persisting after 12 days recovery wherein a sharp recovery was evident in the shaded plots following shade removal, but significant differences to control plants still persisted (Figure 3.5b). The sharp recovery rate continued in the shaded plants culminating in a leaf extension rate comparable to control levels after 42 days following shade removal (Figure 3.5b).

Estimated areal leaf production remained relatively constant in the control plots with a slight increase evident over time (Figure 3.5c). There was a highly significant effect between treatments ( $p<0.01$ ) and post hoc analysis displayed a highly significant difference between shaded and control plots after 106 days of shading (Figure 3.5c & Table 3.1). The difference between estimated areal leaf production in shaded and control plants was no longer apparent 42 days after shade removal (Figure 3.5c).

Notable shifts in the overall biomass and specific canopy distributions of this biomass were discovered (Figures 3.4d, 3.5a & 3.6). Both variables display reductions of biomass in the shade treatment over time and some recovery following shade removal. The largest differences were evident in the canopy intervals that lay between 20 – 40cm of canopy height range (Figure 3.6 a&b).

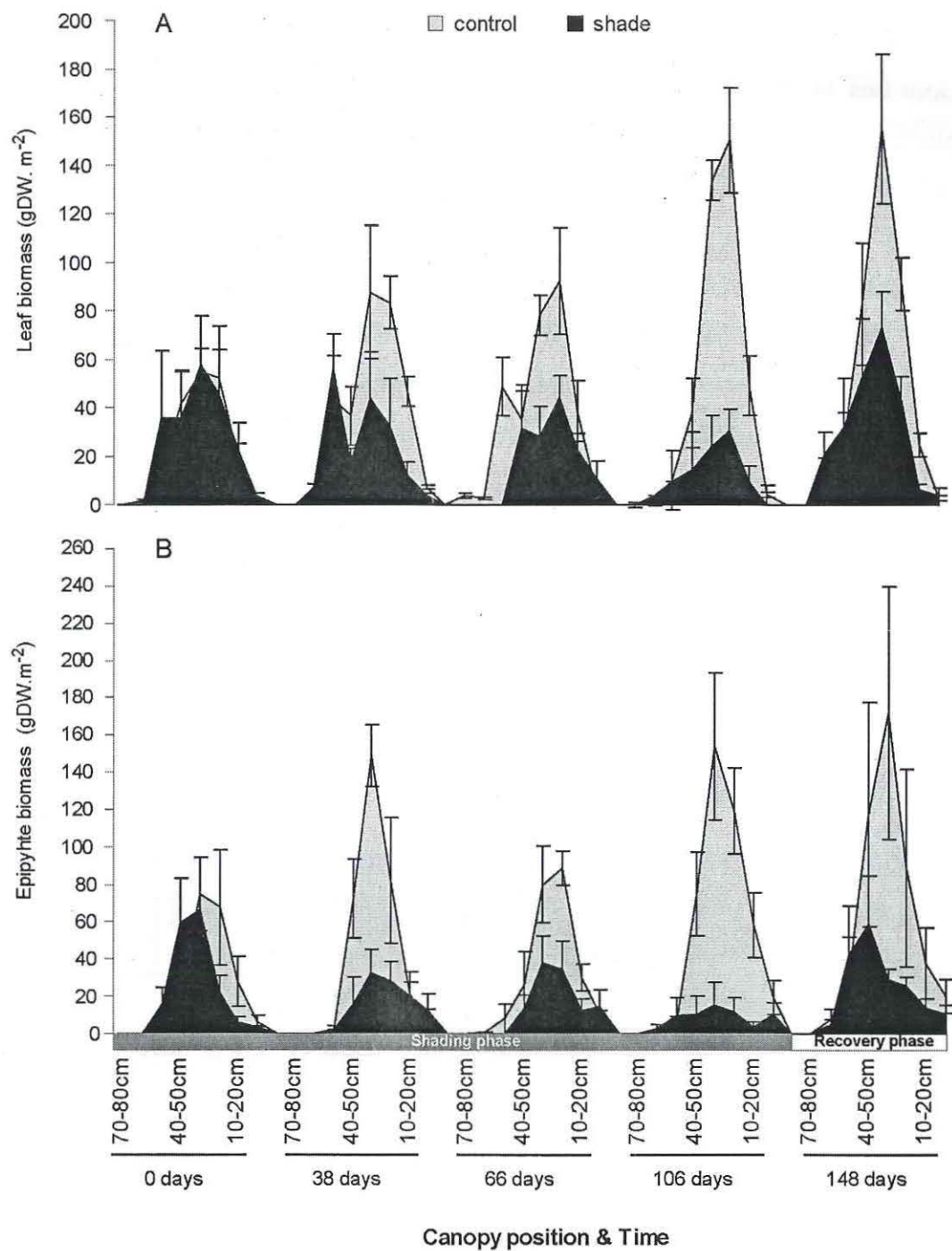


Figure 3-6 Leaf and epiphyte biomass canopy distribution for *Amphibolis griffithii* separated down in to 10cm canopy strata during both the shading and recovery phases of the study A) areal leaf biomass distribution, B) areal epiphyte biomass distributions ( $n=4 \pm SE$ ).

### 3.4 Physiological Parameters

A significant interaction ( $p < 0.05$ ) was highlighted between treatment and time in total soluble rhizome sugars (Table 3.1). In the controls, rhizome sugars remained relatively constant over time, averaging around 150 mg/g (Figure 3.7b). Shaded plants displayed a sharp decrease in rhizome sugars during the shading phase, followed by an equally sharp rate of recovery during the recovery phase of the experiment (Figure 3.7b). Post hoc analysis revealed significant differences after 38 & 66 days of shading, highly significant differences after 106 days of shading (where sugar concentrations were approximately 30 mg/g) and continued with a significant difference after 42 days of recovery (Figure 3.7b).

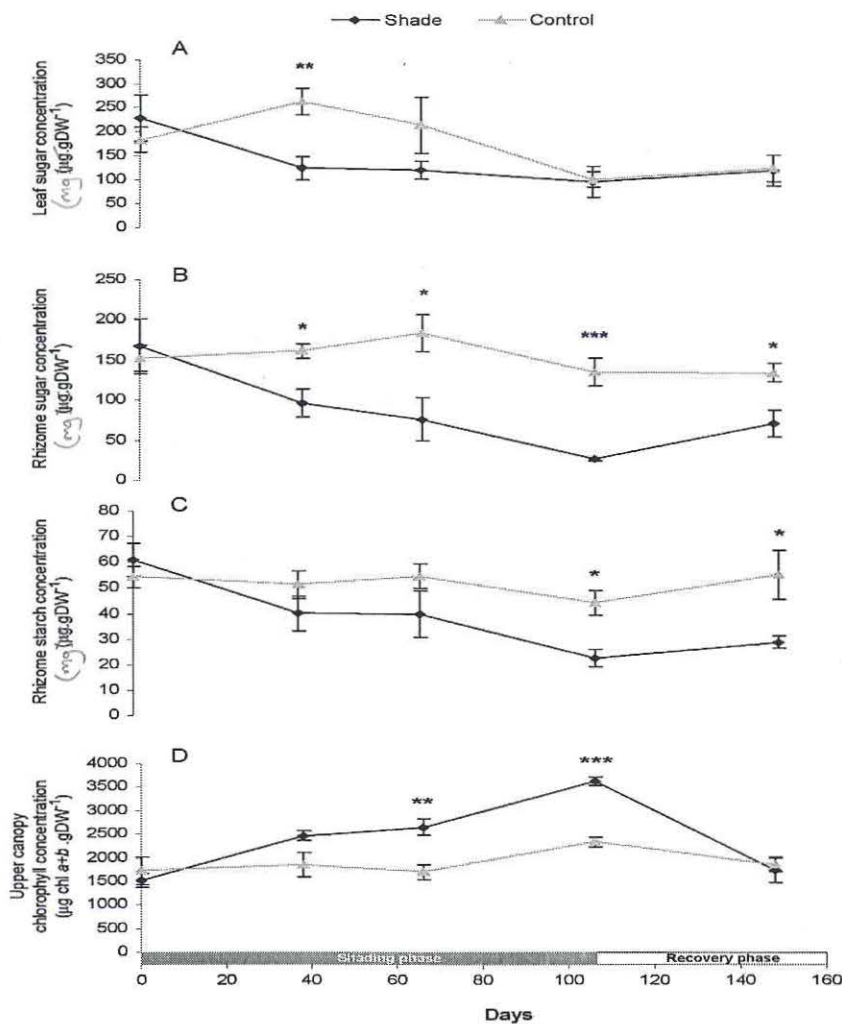


Figure 3-7 Carbohydrate and pigment characteristics in the seagrass *Amphibolis griffithii* during both the shading and recovery phases of the experiment A) leaf sugar concentration, B) rhizome sugar concentration C) rhizome starch concentration, D) chlorophyll concentration in the upper canopy ( $n=4 \pm \text{SE}$ ). Values significantly different between control and treatment at each time indicated by \* ( $p < 0.05$ ), \*\* ( $p < 0.01$ ) and \*\*\* ( $p < 0.001$ ).

Interestingly, rhizome starches appeared to respond to shading at a slower and less dramatic rate than rhizome sugars (Figures 3.7b & 3.7c) although similar overall trends were observed. Rhizome starch concentrations remained relatively constant in the control plants at approximately 50 mg/g (Figure 3.7c) and steadily declined in the shaded plants, revealing a highly significant difference between treatments ( $p < 0.01$ ) (Figure 3.7c & Table 1). Post hoc analysis showed a significant difference after 106 days of shading (where rhizome starch concentrations were approximately 23 mg/g) and a significant difference remained after 42 days recovery despite the relatively sharp rate of recovery following shade removal (Figure 3.7c).

Total soluble sugar concentration in the leaves was significantly affected by treatment ( $p < 0.05$ ) (Table 3.1). After 38 days, sugar concentration was significantly lower in the shaded plants compared with control plants according to post hoc analysis. Following this, sugars in the shaded leaves remained steady throughout the experiment at approximately 100mg/g, as the control concentrations fell to comparable levels (Figure 3.7a).

A general linear increase was observed in the upper canopy total chlorophyll concentrations in the shaded plants resulting in a highly significant interaction between treatment and time ( $p < 0.01$ ) (Table 3.1 & Figure 3.7d). Post hoc analysis revealed highly significant differences between treatments after 66 and 106 days of shading and a sharp recovery back within control levels 42 days following shade removal (Figure 3.7d). Total chlorophyll concentrations in the upper canopy shaded plots peaked at a concentration of approximately 3500 ug/chl/g<sup>-1</sup> at 106 days of shading, whereas control concentrations remained within the 1500 – 2500 ug/chl/g<sup>-1</sup> range throughout the study (Figure 3.7d).

### 3.5 Other Variables

The remaining variables displayed no clear interactions or effects of treatment apart from the results for leaf length and percentage cover. Leaf length results showed a significant interaction ( $p < 0.05$ ) and post hoc analysis revealed a significant difference after 66 days of shading, but data from 38 days of shading were missing (Table 3.2 Figure 3.10a). Percentage cover revealed a significant interaction

( $p < 0.01$ ) and post hoc analysis revealed a significant difference remained after 42 days of recovery (Table 3.2 & Figure 3.8d).

**Table 3-2: Results of two-way repeated measures ANOVA (RM ANOVA) testing the effects between treatments and for any interactions between treatment and time in the less responsive morphological and physiological characteristics of the seagrass *Amphibolis griffithii*.**

Characteristic	Variable	ss	df	ms	f	p
mean leaf length (sqrt trans.)	time	0.144	3	0.048	7.201	0.002
	trt	0.01	1	0.01	0.966	0.364
	time * trt	0.071	3	0.024	3.54	0.036
	err	0.12	18	0.007		
mean stem density m2 (Ln trans.)	time	1.014	4	0.254	1.659	0.192
	trt	0.518	1	0.518	0.488	0.511
	time * trt	0.34	4	0.085	0.556	0.696
	err	3.668	24	0.153		
mean cnpy. % cover (sqrt trans.)	time	59.061	4	14.765	23.828	0.00
	trt	21.673	1	21.673	3.477	0.112
	time * trt	13.581	4	3.395	5.479	0.003
	err	14.872	24	0.62		
maximum cnpy. height (Ln trans.)	time	0.952	1.152	0.826	2.466	0.161
	trt	0.047	1	0.047	0.513	0.501
	time * trt	0.334	1.152	0.29	0.866	0.4
	err	2.316	6.912	0.335		
average cnpy. height	time	1308	1.6	817.606	6.18	0.023
	trt	176.4	1	176.4	2.176	0.191
	time * trt	236.6	1.6	147.849	1.118	0.352
	err	1270.2	9.602	132.289		
mean stem biomass	time	70.61	4	17.653	1.332	0.287
	trt	14.073	1	14.073	0.768	0.415
	time * trt	80.502	4	20.125	1.518	0.228
	err	198826.8	24	8284.45		
mean root biomass	time	0.089	4	0.022	0.261	0.9
	trt	0.137	1	0.137	10.033	0.019
	time * trt	0.25	4	0.063	0.732	0.579
	err	0.082	6	0.014		
mean rhizome biomass	time	2.666	4	0.666	1.292	0.301
	trt	1.024	1	1.024	3.514	0.11
	time * trt	0.919	4	0.23	0.446	0.775
	err	12.379	24	0.516		
mean detrital biomass	time	125.969	4	31.492	2.682	0.056
	trt	15.265	1	15.265	0.456	0.524
	time * trt	14.473	4	3.618	0.308	0.87
	err	281.801	24	11.742		
mean leaf starches	time	32345	4	8086	1.437	0.252
	trt	464.203	1	464.203	0.171	0.694
	time * trt	28167	4	7041	1.252	0.316
	err	135034.1	24	5626.42		
lower cnpy. total chl	time	48323064	4	12080766	3.576	0.02
	trt	655671	1	655671	0.194	0.675
	time * trt	6447291	4	1611822	0.477	0.752
	err	81073202	24	3378050		
upper cnpy. chl a/b ratio	time	2.051	4	0.513	16.44	0.00
	trt	0.159	1	0.159	3.365	0.116
	time * trt	0.221	4	0.055	1.774	0.167
	err	0.749	24	0.031		
lower cnpy. chl a/b ratio	time	0.259	1.518	0.17	6.261	0.025
	trt	0.073	1	0.073	5.034	0.066
	time * trt	0.01	1.518	0.007	0.25	0.725
	err	0.248	24	0.01		

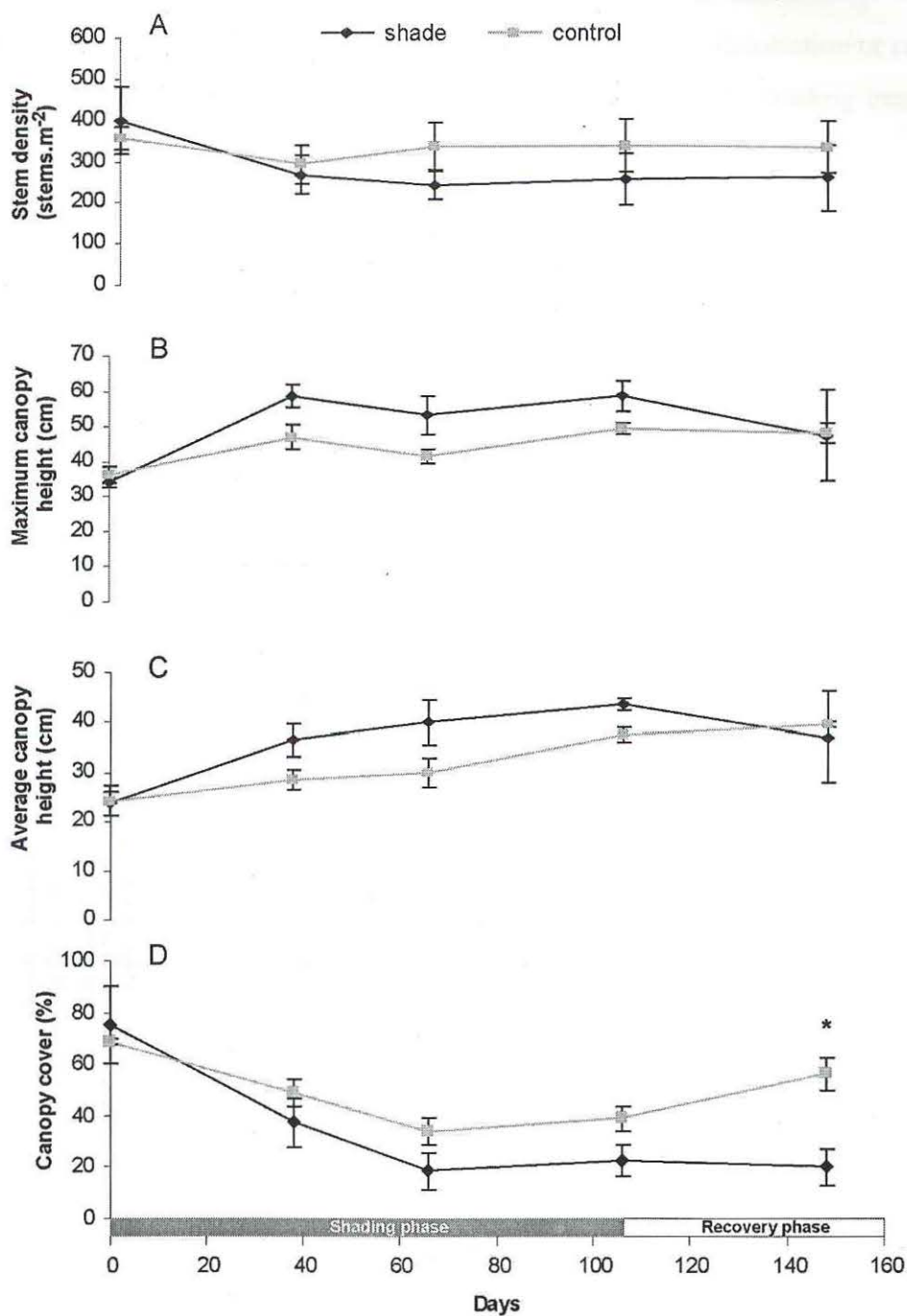


Figure 3-8 Canopy characteristics of the seagrass *Amphibolis griffithii* measured in fixed quadrats during the shading and recovery phases of the experiment A) stem density, B) maximum canopy height, C) average canopy height, D) canopy cover ( $n=4 \pm \text{SE}$ ). Values significantly different between control and treatment at each time indicated by \* ( $p < 0.05$ ).

Stem biomass and stem density in both treatment and control plots remained relatively stable and ranged from approximately 250 – 400 g DW.m<sup>2</sup> and 250 – 400 stems per m<sup>2</sup> throughout, respectively (Figures 3.9a & 3.8a). Maximum and average

canopy heights also remained relatively stable over time with maximum heights ranging from approximately 30 – 58 centimetres (Figure 3.8b) and average heights ranging from 25 – 38 centimetres (Figure 3.8c). Stem biomass distribution or canopy breakdown by 10cm intervals displayed very little affect from the shading treatment (Figure 3.11).

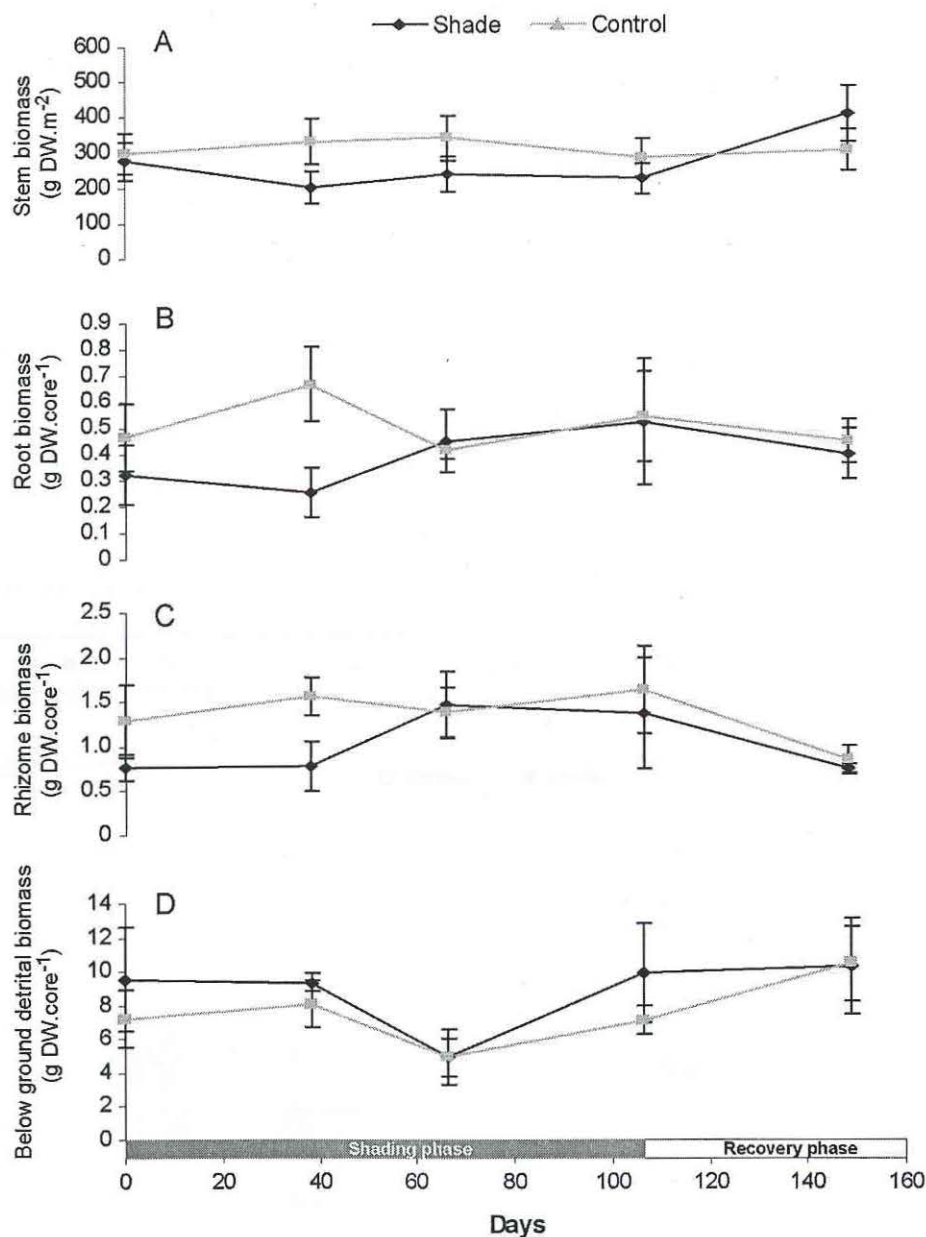
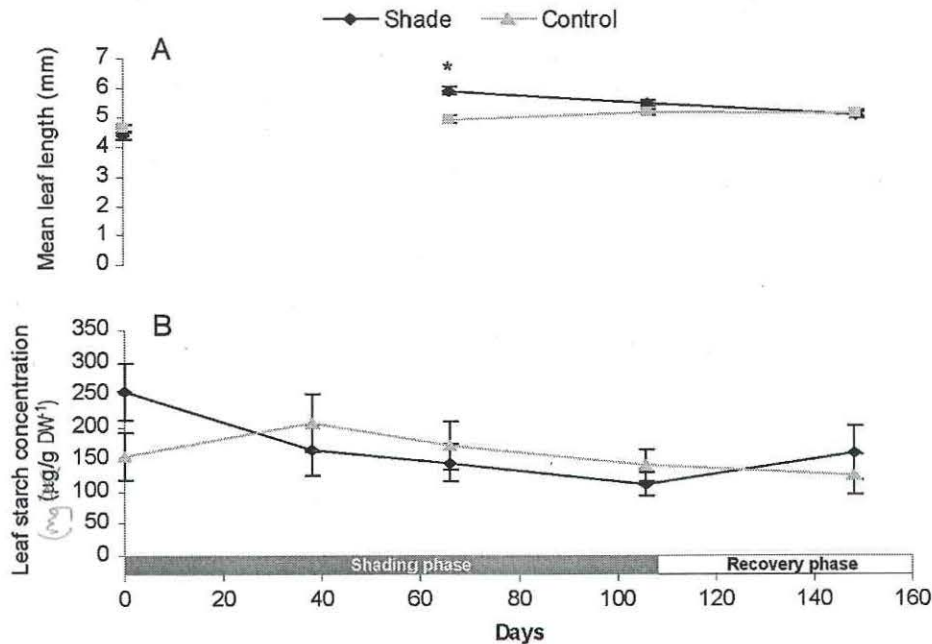


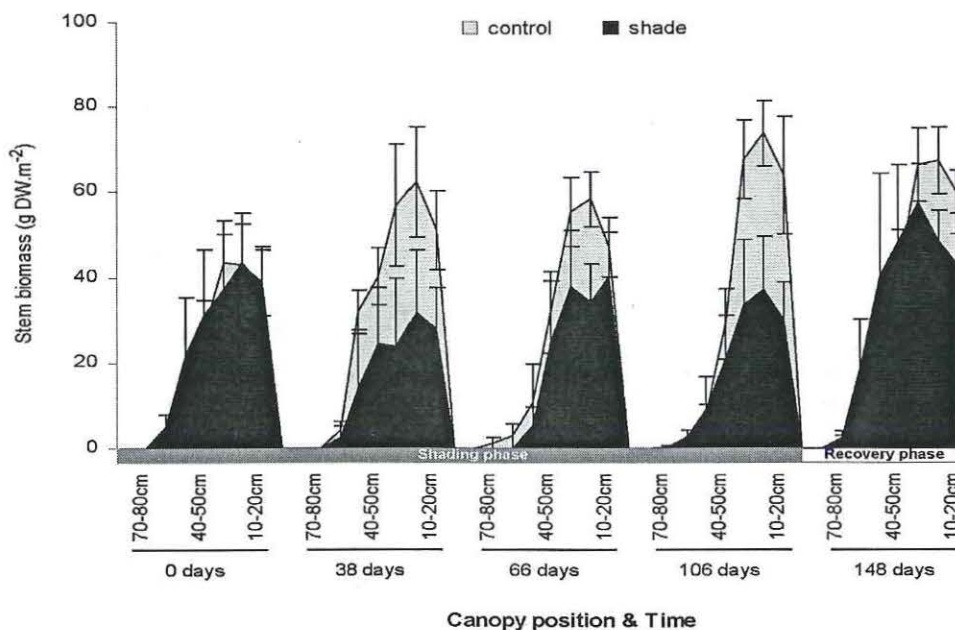
Figure 3-9 Stem and below ground biomass variables for the seagrass *Amphibolis griffithii* during the shading and recovery phases of the study A) stem biomass, B) root biomass, C) rhizome biomass, D) below ground detrital biomass (n=4 ±SE).

Below ground biomass parameters including root biomass, rhizome biomass and detrital biomass values remained relatively stable throughout the study with little

difference between treatments and control (Figures 3.9b, c&d). Root biomass revealed a significant treatment affect according to RM ANOVA ( $p=0.019$ ) however, post hoc analysis showed no differences (Table 3.2 & Figure 3.9b).



**Figure 3-10** Leaf length and starch concentration for the seagrass *Amphibolis griffithii* during the shading and recovery phases of the experiment A) mean leaf length, B) leaf starch concentration ( $n=4 \pm SE$ ). Values significantly different between control and treatment at each time indicated by \* ( $p<0.05$ ).



**Figure 3-11** Stem biomass canopy distribution for *Amphibolis griffithii* separated down in to 10cm canopy strata during both the shading and recovery phases of the study ( $n=4 \pm SE$ ).

Leaf starch concentrations remained comparable between treatment and control and maintained relatively constant levels throughout the study (Figure 3.10b).

The upper canopy chlorophyll *a/b* ratio remained relatively comparable over time between treatment and control plots with no significant difference ( $p=0.116$ ) between treatment and control (Table 3.2). Lower canopy chlorophyll *a/b* ratios remained constant throughout treatments and control over time, ranging from approximately 1.2 – 1.5 (3.12b). Lower canopy total chlorophyll concentrations also varied little between treatment and control with values ranging from 1750 – 2500  $\mu\text{g chl } a+b \cdot \text{g DW}^{-1}$  (Figure 3.12c).

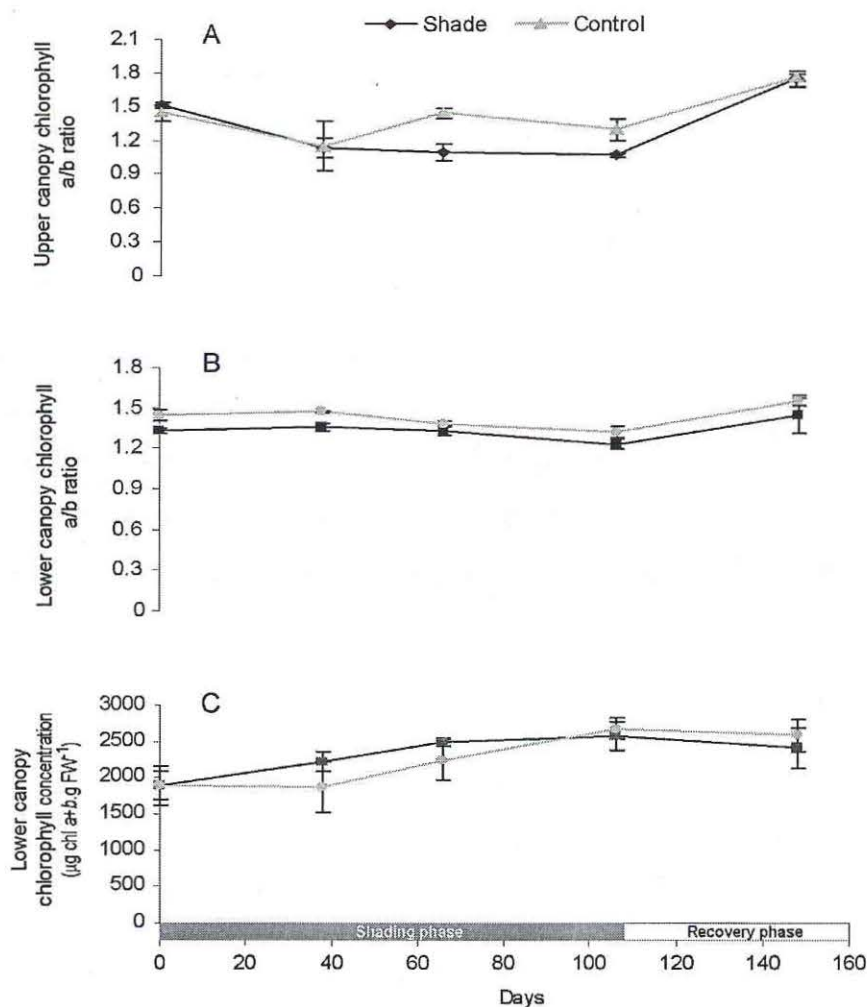


Figure 3-12 Chlorophyll variables for the seagrass *Amphibolis griffithii* during both the shading and recovery phases of the study A) *a/b* chlorophyll ratio in the upper canopy, B) chlorophyll *a/b* ratio in the lower canopy, C) total chlorophyll concentration in the lower canopy ( $n=4 \pm \text{SE}$ ).

## 4 Discussion

Experimental reduction of PAR reaching *Amphibolis griffithii* significantly affected a range of morphological and physiological characteristics in the seagrass. The strongest morphological responses included reductions in the number of leaves per stem, number of leaves per cluster, total leaf biomass, density of leaf clusters and total epiphyte biomass. Decreased leaf extension per cluster and estimated areal leaf extension per cluster demonstrated the effects of reduced PAR on growth. Physiological responses were noted in the reductions of total sugar and starch concentrations in the rhizomes and sugars in the leaves, and increased total chlorophyll concentrations in the upper seagrass canopy. The results clearly require that the null hypothesis ( $H_0$ ) be rejected; high intensity PAR reductions do have an affect on a variety of morphological and physiological characteristics in the seagrass *A. griffithii*.

Many of the morphological and physiological attributes of *A. griffithii* that were affected by reductions in PAR showed considerable recovery following the removal of the shade treatment (e.g. number of leaves per stem, mean leaf extension and upper canopy total chlorophyll). Furthermore, a variety of morphological and physiological characteristics of *A. griffithii* displayed potential as indicators of shading induced stress in these ecologically important systems. The study revealed that many of the morphological and physiological responses in PAR limited *A. griffithii* have been identified in other seagrasses.

While *Amphibolis griffithii* displayed reductions in a suite of above ground measures (e.g. number of leaves per stem and number of leaves per cluster) there were few discernable differences in below ground characteristics (root, rhizome and detritus). This contrasts other seagrasses that have shown reductions in above and below ground biomass in response to reduced light availability including *Halodule wrightii* (Onuf, 1996), *Cymodacea nodosa* and *Posidonia oceanica* (Olesen *et al.* 2002). With respect to above ground responses, those noted in *A. griffithii* were comparable to those reported in other species. Bulthuis (1983) noted various rates of leaf cluster reductions in *Heterozostera tasmanica* as a response to reduced PAR although the morphology of the species is markedly different from that of *A. griffithii*.

Shoot, or in this case stem density values did not appear to differentiate between shaded and control treatments in *A. griffithii* whereas changes in shoot density were reported in light limited *Posidonia sinuosa* (Gordon *et al.* 1994). The fact that *A. griffithii* did not show shoot density responses to reduced PAR similar to those found in *Posidonia* (Gordon *et al.* 1994) is not surprising and can be accounted by the differences in morphology (Lavery & Vanderklift, 2002) and above ground biomass allocations (Paling & McComb, 2000). *P. sinuosa* has a much lower below to above ground biomass ratio than *A. griffithii* (Paling & McComb, 2000) and these above ground components differ considerably in their constituents. *A. griffithii* has branching structural stems analogous to its below ground rhizome (Marba & Walker, 1999) complete with terminal leaf clusters comprising of 3-5 leaves (Ducker *et al.* 1977), whereas *P. sinuosa* has an above ground biomass that consists of leaf sheathes and leaves with very little structural material (Paling & McComb, 2000). The respiratory energy demands of both species have been investigated and the leaves of both plants have comparable demands (Masini *et al.* 1995). However, *A. griffithii* has an above ground stem that has a relatively low respiratory demand. Therefore, under a reduced PAR climate, the *Posidonia* plant gains efficiency by dropping energy demanding shoots (leaves), whereas *A. griffithii* would not make considerable gains by thinning stems (shoots); instead it sheds the comparably high respiratory demanding components, leaves and leaf clusters.

In the current study, the rates of leaf extension per leaf cluster in *A. griffithii* declined from approximately half to a third of control levels by 66 and 106 days of shading, respectively. Analogous leaf responses have been reported in other light limited species, such as *Heterozostera tasmanica* (Bulthuis, 1983), *Thalassia testudinum* (Lee & Dunton, 1997) and *Zostera marina* (Moore & Wetzel, 2000). The leaf growth similarities between *T. testudinum* and *A. griffithii* are understandable as they are relatively comparable in terms of their form and function (Walker *et al.* 1999). *Z. marina* leaf growth rates (Moore & Wetzel, 2000) were also comparable yet the morphologically and functionally similar (Walker *et al.* 1999) *H. tasmanica* leaf growth rates did not show any leaf growth response to decreased PAR (Bulthuis, 1983). These differences in adaptation to reduced PAR levels outline the highly specific responses of seagrasses and also indicate that the form and function model of

Walker *et al.* (1999) should be used with care when employed as a predictive tool in response to light reduction.

*A. griffithii* displayed a marked total chlorophyll response in the upper canopy of the treatment plots over time and this response has been reported in other species of seagrasses, such as *Thalassia testudinum* (Lee & Dunton, 1997), *Halodule pinifolia* and *Halophila ovalis* (Longstaff & Dennison, 1999). This indicates that *A. griffithii* physiologically responds in order to maximise energy efficiency under reduced PAR levels, an inference that has been noted for the species previously (Caruthers, 1999).

Other studies investigating leaf and rhizome sugars and starches in seagrasses have been inconclusive in their responses to reduced PAR levels, with some finding responses, such as *Zostera marina* (Cabello-Pasini *et al.* 2002) and *Zostera noltii* (Peralta *et al.* 2002) and others finding no relationship, for example *Halodule pinifolia* (Longstaff & Dennison, 1999). *A. griffithii* sugar and starch concentrations showed marked responses to light limitation in leaf and rhizome sugars and rhizome starches. Leaf sugars initially responded rapidly to reductions in PAR as did rhizome sugars that continued to deplete over time under unfavourable light conditions. Rhizome starch responses declined steadily with continued PAR treatment and the rate of decline was considerably lower than that of the rhizome sugars. The apparent lag in the rate of rhizome starch decline relative to rhizome sugars is indicative of a physiological reallocation of resource response (Touchette & Burkholder, 2000) found in other seagrass species such as those in the genus *Zostera* (Cabello-Pasini *et al.* 2002; Peralta *et al.* 2002). The seagrasses that have shown comparable starch and sugar responses to those of *A. griffithii* are more closely related in terms of their form and function (Walker *et al.* 1999) than *H. pinifolia* that did not respond (Longstaff & Dennison, 1999). This is likely to be attributed to the differences in rhizome persistence and above and below-ground biomass allocations (Walker *et al.* 1999).

#### **4.1 Identifying the likely factors affecting the physiological and morphological characteristics in light limited *Amphibolis griffithii***

Environmental stressors like PAR reductions can interrupt the ability of seagrasses to photosynthesise and maintain a positive carbon budget (Forqurean & Zieman, 1991). The carbon budget within a plant can be summarised as the net difference between production in photosynthesis and respiration associated with growth, maintenance and reproduction (Forqurean & Zieman, 1991; Lambers *et al.* 1998). It would be fair to assume that the responses identified in this study were attempts to balance the plant's carbon budget under the new reduced PAR climate and that both changes in carbohydrate stores and canopy morphology are consistent with this suggestion. A positive carbon budget is a state where an individual plant or seagrass meadow produces a net gain in photosynthates (energy in the form of carbohydrates) through photosynthetic production and a neutral carbon budget is where total net respiration equals total net production (Lambers *et al.* 1998). Simply stated, if an individual plant or seagrass meadow cannot produce, at the minimum, a neutral carbon budget then its health and ongoing viability are declining (Forqurean & Zieman, 1991; Lambers *et al.* 1998). Seagrasses typically store excess photosynthates produced in the above ground canopy to the below ground rhizome in order to overcome periods of unfavourable environmental conditions, for example light limitation or stress (Hemminga & Duarte, 2000; Peralta *et al.* 2002) and for maintenance, growth and reproduction (Lambers *et al.* 1998; Hemminga & Duarte, 2000). Seagrasses are accustomed to diurnal and seasonal patterns of light stress (Hemminga & Duarte, 2000) and are physiologically and morphologically equipped to cope with these natural occurrences. However, under prolonged light stress energy from carbon sinks (rhizomes) can be remobilised and translocated to the above ground biomass in order to maintain those parts of the plant that photosynthesise (Masini *et al.* 1995), a response that has been noted in *Zostera noltii* (Peralta *et al.* 2002). It would appear that *A. griffithii* responded in a similar manner to *Z. noltii* by tapping into the below ground sugar and starch storage in an effort to maintain the above ground biomass. In doing so, the plants would likely be experiencing a carbon deficit, where respiration exceeded photosynthetic production, as reported in *Zostera marina* (Cabello-Pasini *et al.* 2002).

It is assumed that the mobilisation of energy from the rhizome to the high energy demanding above ground tissue (Masini *et al.* 1995) could only be maintained for a limited period of time, as the rhizome storage systems in seagrasses are finite. If the unfavourable PAR conditions continue, as during an extensive dredging program, then the plant has to respond in a way that reduces its respiratory demand in order to balance the prolonged carbon deficit. The reduction in leaf biomass in *A. griffithii* would have this end result. Different types of seagrass tissue have different energy requirements. Masini *et al.* (1995) demonstrated that seagrass leaves have a respiratory energy demand approximately six times that of below ground components. The below ground to above ground biomass ratio in the genus *Amphibolis* has been reported at approximately 1:6 (Paling & McComb, 2000); most of the plant's total biomass in the above ground proportions. In a continually reduced PAR climate a threshold must be realised wherein rhizome stores are depleted and the plant responds by thinning the energy-sapping above ground tissue, such as leaves (Masini *et al.* 1995) to minimise the total plant respiratory load of these energy demanding tissues (Peralta *et al.* 2002). Furthermore, the reduction in above ground biomass or canopy thinning simultaneously reduces the degree of canopy self-shading (Peralta *et al.* 2002) and may assist in increased radiation use efficiency and recovery of the remaining leaves once PAR conditions improve (Carruthers & Walker, 1997).

Reductions in canopy self shading have been identified as a typical response of terrestrial plants (Lambers *et al.* 1998) and seagrasses (Via *et al.* 1998; Olesen *et al.* 2002) to light limitation. Self shading can be reduced through a number of mechanisms and they are: through daily leaf and stem rearrangement, which is common in shade adapted terrestrial plants (Lambers *et al.* 1998); through leaf and stem arrangement. For example, *A. griffithii* has demonstrated a stem and leaf arrangement that maximises radiation use efficiency dependant on seasonal energy dynamics (Carruthers & Walker, 1997; Carruthers, 1999); reductions in above ground morphological biomass have been widely reported in a variety of seagrasses (Via *et al.* 1998; Peralta *et al.* 2002; Cabello-Pasini *et al.* 2002). These canopy self shading responses may aid the plant in several ways: firstly, by reducing the respiratory load on the PAR limited plant; secondly, by maximising the light available to the remaining canopy in order for it to maximise the photosynthetic

potential or radiation use efficiency that may allow maximal carbon fixation in the given PAR climate (Carruthers & Walker, 1997); finally, the reduction in canopy self shading may aid the recovery of the plant once PAR conditions improve through increased radiation use efficiency and reduced within canopy competition for light (Via et al. 1998; Carruthers & Walker, 1997).

## 4.2 Conceptualising the response to reduced PAR

Through a combination of the results of this study, the responses of other seagrasses and an understanding of the concepts of carbon budgets and self shading, a likely pathway can be postulated to conceptualise the responses of *Amphibolis griffithii* to reduced PAR. Although the likely response pathway cannot be categorically proven from the results in the current study, it does provide a plausible explanation of the observations and may guide future indicators of light stress in *A. griffithii*. Therefore, the development of a likely chronology of PAR limited responses in *A. griffithii* will be outlined.

The mean PAR reduction in the shaded *A. griffithii* treatments was approximately 90% of ambient control levels. It appeared that the shade treatments had a rapid and dramatic effect on *A. griffithii* as depicted in the leaf and rhizome sugars. Initially, leaf sugars responded dramatically and the response appeared to level out over time. Rhizome total sugar and total starch concentrations in the shaded plots consistently declined during the shading phase of the experiment with the rate of rhizome sugar depletion occurring more rapidly than the rhizome starch. It would appear from the responses in these physiological variables that leaf sugar concentrations in *A. griffithii* are extremely responsive to changes in environmental conditions as demonstrated in other seagrasses (e.g. *Zostera* species: Cabello-Pasini et al. 2002; Peralta et al. 2002). As a response to rapid and continued declines in leaf sugars *A. griffithii* appears to translocate and temporarily subsidise the leaf sugar deficiencies through the use and mobilisation of rhizome sugars and starches. Evidence to support this explanation are found in the rate and continued depletion of these rhizome sugars and starches during the shading phase along with supporting evidence from other seagrass species e.g. *Zostera marina* (Cabello-Pasini et al. 2002), *Zostera noltii* (Peralta et al. 2002) and *Thalassia testudinum* (Lee & Dunton,

1997). The shaded plants continue to utilise the more readily available or soluble rhizome sugars in preference to the more persistent starches (Touchette & Burkholder, 2000; Peralta *et al.* 2002). The rhizome sugars are depleting rapidly and are subsidised by the rhizome starches that are also gradually depleted. This series of events helps to explain the rates of depletion quantified in the leaf and rhizome sugars and rhizome starches.

Along with the energy reallocation described above there appears to be some simultaneous responses transpiring particularly in regard to the reductions in the leaf growth rate and number of leaves per stem and leaves per cluster. As mentioned previously, Masini *et al.* (1995) demonstrated that above ground biomass constituents in three species of seagrass, including *Amphibolis griffithii*, have a high respiratory demand, up to six times more demanding than the structural and storage material found below ground. It would appear that under prolonged reductions in PAR, *A. griffithii* sheds its respiratory demanding leaf tissue and in order to maintain the reduced above ground canopy through a reallocation of energy resources from the rhizome. This, in turn, reduces the self shading effect of the canopy (Vermaat & Verhagen, 1996; Holmer & Laursen, 2002) maximising the chance of capturing the limited light available and decreases the rate of rhizome energy depletion (Peralta *et al.* 2002) due to the reductions in respiratory demanding above ground components. The two orders of magnitude difference in the amount of light penetrating through the shaded canopies demonstrates this reduction in self shading. This potentially provides the plant with a new light climate while stabilising the declining carbon deficit through reductions in above ground biomass. In addition to the changes in leaf growth and biomass (including leaf extension, total leaf biomass, number of leaves per cluster and leaves per stem), *A. griffithii* displayed a reduction in the density of leaf clusters and total epiphyte biomass over time, both of which would reduce self shading.

Coincident with the canopy response, a reduced investment in leaves and an increased investment in total chlorophyll in the remaining leaves (especially those in the upper canopy that had not previously been subjected to self-shading) had a greater concentration of leaf chlorophyll. The differences in chlorophyll concentrations according to canopy position may be accounted by the canopy light

climate data that indicate the lower canopies in both treatment and control plots had comparable light levels during the shading phase. Therefore, the lower canopies were likely to be adapted to low light conditions due to canopy self shading and the imposed treatment did not alter the light climate dramatically. The shaded upper canopies were accustomed to a high PAR climate and responded when light levels were dramatically reduced by treatment. These statements are consistent with reports of increased chlorophyll in other PAR limited seagrasses (e.g. *Thalassia testudinum* Lee & Dunton, 1997) and the responses may increase the radiation use efficiency (Carruthers & Walker, 1997) of the canopy. Presumably then this would have the effect of increasing the overall PAR harvesting efficiency while simultaneously minimising the respiratory drain on storage reserves until such a time as the PAR climate improves.

### 4.3 Recovery

The majority of variables that responded to PAR reductions showed substantial recovery within the study timeframe. The number of leaves per stem, leaf extension, leaf cluster density, total leaf and total epiphyte biomass and the upper canopy total leaf chlorophyll all displayed full recoveries after 42 days. Leaf extension showed considerable recovery 12 days after shade removal and a full recovery by 42 days once PAR treatments were removed. As described earlier, reductions in canopy self shading allow increased PAR to penetrate throughout the canopy. This probably provides the impetus for the apparent high rate of recovery displayed within most of the responsive variables. The extent and rate of the recoveries in these variables indicates *Amphibolis griffithii* is largely able to withstand a single high intensity moderate duration PAR reduction.

The mean number of leaves per cluster and the concentration of sugars in the rhizomes and leaves showed moderate recovery. The mean number of leaves per cluster displayed an increased rate of recovery compared with the rate of impact, however, significant differences between impact and control still remained after 42 days of recovery. Thus, indicating that a high intensity moderate duration PAR reduction of this magnitude may not considerably impact the long-term survival of the seagrass *A. griffithii*. Rhizome starch concentrations displayed poor recovery

after the treatment was removed. This may be attributed to the fact that any excess energy the plants were producing after PAR levels normalised was allocated to the energy producing photosynthetic material like leaves, leaf clusters and total leaf biomass, maximising the plant's radiation use efficiency (Carruthers & Walker, 1997) and providing the best chance of continued survival; plants are known to allocate excess energy into growth, maintenance and reproduction (Lambers *et al*, 1998; Hemming & Duarte, 2000; Fitter & Hay, 2002).

#### 4.4 Potential indicators of reduced PAR-induced stress

In order to assess the potential of responsive variables in *Amphibolis griffithii* subjected to reduced intensity PAR a characterisation of indicator performance has been devised (Table 4.1). The potential indicator performance ratings for individual responses were inferred from the data collected and the specific characteristics assessed against four categories: consistent response over time; speed of response; speed of recovery and degree of recovery. Responses were assigned a qualitative rating of high (H), moderate (M) or low (L). The development of the potential indicator performance rating was designed to assist future research into PAR reductions on *A. griffithii*. This synthesis will provide future researchers with an indication of *A. griffithii* specific responses and aid the assessment of variable selection in order to answer questions regarding responses in a range of intensities and timing and their subsequent effects to PAR reductions.

**Table 4-1: Potential indicator performance table for measurable characteristics of *Amphibolis griffithii* subjected to moderate duration, intensely reduced PAR climates in Jurien Bay, Western Australia.**

Variable	Consistent response over time	Speed of response	Speed of recovery	Degree of recovery
No. leaves per stem	M/H	H	H	H
No. leaves per cluster	H	H	M/H	M
Cluster density	M	M	H	M/H
Total leaf biomass	M	M	H	M/H
Total epiphyte biomass	H	M/H	M/H	M/H
Leaf extension per cluster	H#	H	H	H
Estimated areal leaf extension	H#	M/H	H	M/H
Leaf canopy	M/H	H	M/H	M/H

distribution				
Epiphyte canopy distribution	H	H	M/H	M
Leaf sugars	M	H	M	H
Rhizome sugars	H#	H	M/H	M
Rhizome starches	H#	M	L/M	L
Upper canopy chlorophyll	H#	M/H	H	H
Leaf length	Shows potential, further investigation required			

Table legend: H – high indicator potential, M – moderate indicator potential, L – low indicator potential, # - further research is needed into the response of this variable to reduced PAR in the very short-term (e.g. less than 1 month timescale).

The *A. griffithii* characteristics that displayed the best potential as indicators of response to reduced PAR were the number of leaves per stem, the number of leaves per cluster, leaf extension, total chlorophyll and sugar concentration in both the leaves and rhizomes (Table 4.1).

The potential indicator performance table suggests that particular emphasis should be placed on trying to capture the initial responses of several parameters including the physiological responses of sugars, starches and chlorophyll content. Sugar and starch analysis deserve more detailed research attention considering that the results from this study were all bulked samples (i.e. a canopy stratification approach to analysis e.g. lower, mid and upper canopies). Total chlorophyll responses were investigated in the upper and lower canopies, with only the upper canopy displaying a response. Further research should investigate the mid-canopy level for chlorophyll responses as the majority of *A. griffithii* biomass was found in this stratum, as proven by the canopy breakdown intervals. The responses of the mid-canopy stratum have management implications when considering the allocation and distribution of *A. griffithii* above ground biomass.

Leaf extension displayed rapid and dramatic responses to reduced PAR and was equally responsive during recovery. However, the study did not capture the initial impact response due to the ongoing development of the method and it is highly recommended that future research incorporate these responses into their data for use as potential indicators of PAR reduction. Furthermore, a suite of other leaf morphometric responses (e.g. width, thickness and leaf area index) have been identified as responsive to reduced PAR in other seagrasses e.g. *Zostera marina*

(Cabello-Pasini *et al.* 2002) and *Thalassia testudinum* (Lee & Dunton, 1997), but were not measured in this study. In the shaded plots, there were several anecdotal observations of leaf characteristic differences in the latter stages of the experiment, such as leaf width and thickness and combining these with the reported correlated responses in other species justifies further investigation.

#### 4.5 Management implications

As discussed earlier, a reduction in PAR has an effect on the morphologically and ecologically unique seagrass *Amphibolis griffithii* and these effects are likely to affect other components of the ecosystem, such as epiflora and epifauna (Jernakoff & Nielsen, 1998). The dominant meadow-forming capabilities and structural persistence of the seagrass (Walker *et al.* 1999) are likely to account for the considerable biodiversity associated with these systems (Jernakoff & Nielsen, 1998; Lavery & Vanderklift, 2002). The flow-on implications of continual PAR reductions, which this study has shown to affect the morphological attributes of *A. griffithii* meadows, are likely, therefore, to have significant, or even catastrophic, ramifications for biodiversity. The high intensity, moderate duration PAR treatment clearly had a range of effects on the seagrass, including reductions in associated total epiphyte biomass. This, in turn, may have affected habitat and food availability for higher order organisms (Jernakoff & Nielsen, 1998). These scenarios are clearly unacceptable given the ecological importance of these productive and unique ecosystems, not only regionally and nationally, but throughout the world.

Considering the timing of the study, it has been assumed that *A. griffithii* was manipulated at a time of peak carbohydrate store, a time when we could expect the greatest resilience to reductions in PAR. Therefore, the likely responses to reduced PAR at a time of year when *A. griffithii* has lower carbohydrate stores may be amplified. Extrapolation of the responses in many of the variables measured in this study to longer timescales suggests considerable habitat damage would occur if pressed with continually reduced PAR levels. Epiphyte biomass, rhizome sugars and starches were severely depleted in the shaded plots during the impact phase and exhaustion in these variables could have been realised within weeks if the impact was extended. Extrapolation of leaf and cluster variables inferred that total reduction

in these responses would have been realised within nine months, which coincides with the Champion Bay dredging program timeframe. These inferences were confirmed by the limited quantitative data (e.g. URS, 2003) and anecdotal reports from various sources (e.g. pers. comm. M. Mulligan, Geraldton Port Authority).

The study revealed that while high intensity, moderate duration PAR reductions do significantly affect *A. griffithii* meadows during the late summer through to winter period, the meadows also display the potential for significant recovery within a relatively short time. The study also demonstrates that *A. griffithii* displays the potential to significantly recover within a relatively short temporal scale after a single, short duration PAR limiting event. The above ground components of the seagrass recovered relatively quickly, presumably due to the reduction in canopy self-shading. However, the carbohydrate stores of the plant displayed poor recovery rates, suggesting that any excess energy produced during the recovery phase were allocated to the above ground components. Such a scenario is likely to leave the plant vulnerable to repeated high intensity or prolonged duration impacts for some time after the initial stress. Therefore, extreme caution must be applied when interpreting the rates of impact and recovery in *A. griffithii* with particular reference to anything other than a single, high intensity, moderate duration PAR reduction commencing during late summer in the Jurien Bay region. The preceding scenario provides a likely explanation for the wholesale degradation that took place in Geraldton after prolonged extensions to the initial dredging program were granted by regulatory authorities.

## 5 References

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