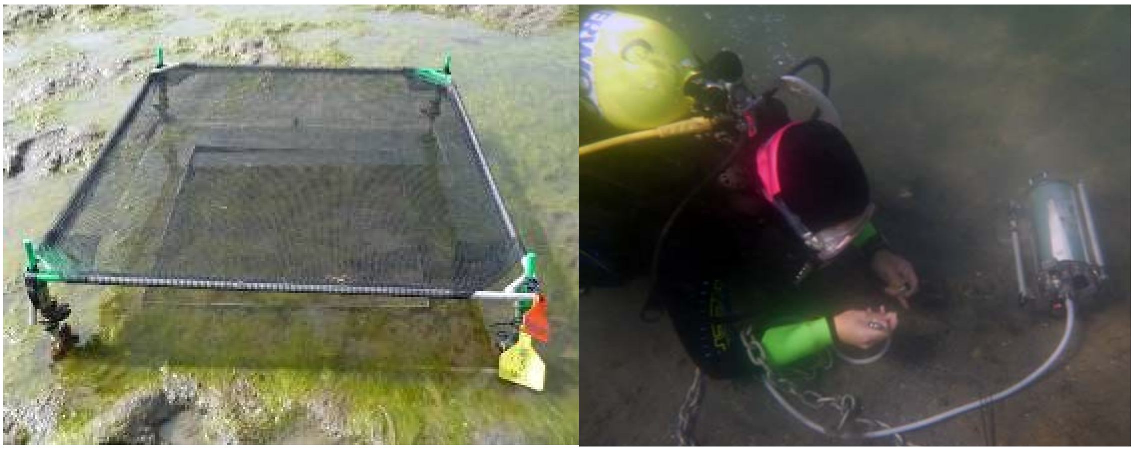
Department of Agriculture Fisheries and Forestry

Development of a Light-Based Seagrass Management Approach for the Gladstone Western Basin Dredging Program



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Executive Summary

This report presents a light-based management approach to protect Gladstone seagrasses from dredge plume impacts associated with the Western Basin dredging program. The management plan and trigger levels are based on findings from two years of seagrass research in Gladstone directed at establishing the required light levels for local seagrass survival.

The light trigger values and timelines for managing seagrass impacts are based on a multi-faceted approach including the ongoing Seagrass Health Study programs and a review of historical seagrass trends from permanent transect monitoring sites and associated benthic PAR recordings. This combined work has led to a working light trigger value of 6 mol m^{-2} d^{-1} over a rolling two week average, under which management actions and alerts are proposed to ensure appropriate steps are taken to mitigate seagrass declines.

There were four major studies that were used in developing the light trigger value:

1. In situ shading studies – completed seasonally to assess time-of-year effect on reduced light to seagrass meadows
2. Tidal exposure assessments – completed quarterly over spring tide cycles to determine the effect of exposure on seagrass photosynthetic condition
3. Spectral profiling – performed over spring and neap tide cycles and with and without dredging activity to measure spectrally available light through the water column
4. Laboratory manipulation experiments – refined field studies to assess detailed effects of shading and evaluate potential sub-lethal stress indicators

This report summarises the findings of the above studies, an evaluation of historical seagrass trends monitored in parallel with the actual benthic light climate (PAR), and how this information has led to the current working trigger value and draft management plan. The key findings are:

- *In situ* shading studies found Gladstone area seagrasses, specifically the dominant species *Zostera capricorni*, require between 4.5 and 12 mol quanta m^{-2} d^{-1} during the growing season over a minimum period of two weeks to survive.

- Seagrasses were not affected by a reduction in light during senescent season studies, therefore a light-based management approach is developed around the growing season only.

- Gladstone *Zostera capricorni* appears to cope with reductions in light over a two week period with no effect on seagrass health. Two weeks is used for a trigger value timeline as part of the working light management plan.

- In general, *Zostera capricorni* consistently received greater than 6 mol m^{-2} d^{-1} over a two week rolling average at most locations in the harbour during the growing season when seagrass remained stable or increased in abundance.

- Laboratory experiment results verified field study findings whereby significant changes in seagrass morphology were detected under high shading treatments.

- Gladstone intertidal seagrasses likely depend on the critical light window around low tide exposure for the greatest productivity. However, full tidal exposure does not provide a “window of opportunity” for relief from turbidity impacts as this period of immersion is stressful to plants.

While results have produced an initial set of management trigger values that can be trialled, ongoing work may lead to further refinements. This is in line with the adaptive management approach that has been set out in the Coordinator General’s conditions of approval for the project. The final results of ongoing experiments and light trials will include a definitive set of management trigger values following the completion of all studies.

Introduction

The Port Curtis Western Basin dredging program poses a high level of environmental risk to marine habitats in the Gladstone area, particularly to seagrass meadows. The process of marine dredging elevates suspended solids within the water column, reducing light availability to marine habitats, and can result in high levels of stress and mortality to benthic primary producers such as seagrasses (Erftemeijer and Lewis, 2006). Seagrass distribution is primarily driven by the availability of light or photosynthetic active radiation (PAR), which if changed, can lead to large scale loss of seagrasses over relatively short time scales.

To effectively manage and mitigate the environmental risk from Port Curtis dredging activities, it is critical to understand the background relationships that exist between seagrass and environmental variables on a local scale. Understanding the mechanistic response of seagrass to potential drivers such as light will allow better distinctions between natural climate-induced declines and dredge-related impacts.

Light is considered to be the primary environmental variable determining seagrass distribution, abundance and productivity (Duarte et al. 1997; Vermaat et al. 1997). Minimum light requirements for seagrass differ among species and seasons, yet it is well established that changes in the availability of light remains the primary factor affecting the distribution of seagrasses (Bjork et al. 1999; Hemminga and Duarte 2000; Erftemeijer and Lewis 2006; Ralph et al. 2007).

To minimise impacts associated with the port activities and infrastructure developments associated with the Western Basin developments, Gladstone Ports Corporation Limited (GPCL) commissioned Fisheries Queensland, Department of Agriculture Fisheries and Forestry (DAFF) to undertake a program to evaluate both the condition and major drivers of seagrass health in Port Curtis. The main objective of the program has been to establish the amount of light or photosynthetic active radiation (PAR) required for seagrasses to survive during a large scale dredging campaign.

The suite of seagrass studies outlined in this report were designed to develop a light-based management plan to assess the impacts associated with capital dredging for the Western Basin project in Gladstone. These studies have provided key information to aid in developing suitable management guidelines and monitoring programs to protect seagrasses as the development proceeds. The outcomes of each research program helped to develop the working set of light trigger values and draft light-based management plan.

While initial experiments assessed three of the species found in Port Curtis, efforts were focused on *Zostera capricorni*, the dominant species in distribution, biomass and density in the port and the species with the highest light requirement. Studies of seagrasses in tropical regions have indicated that genera such as *Zostera* have significantly greater light requirements (Grice et al 1996; Bach et al 1998; Collier et al. 2009) than other genera found in the Western Basin area, such as *Halophila* and *Halodule* (Freeman et al. 2008). Trigger values and management plans have therefore been built around the more sensitive and dominant species, *Zostera capricorni*, and only results from relevant studies are reported here.

Throughout this report, seasonal cycles are defined according to the climate-induced pattern of growth and senescence throughout the year on the east coast of Queensland (McKenzie 1994, Chartrand et al 2011). Two generalised seasons are distinguished: the growing season defined as July to January, typifies seagrasses natural increase in biomass and distribution as ideal growth conditions provide a period of opportunistic expansion; and the senescent season, February to June, is typically when seagrasses retract and rely on stores or seeds to get through wet season conditions, including flooding and poor water quality.

Shading Studies

Summary

Shading studies were conducted from November 2009 to December 2010 to simulate the effects of a dredge-related reduction in light from increased turbidity over a seagrass meadow and to establish an initial range of light required for local seagrass survival. Studies were carried out twice during the growing season (July to January) and once in the senescent season (February to June) to assess seasonal differences in seagrass response. Results of these studies established significant differences in light requirements between the seasons and that *Zostera capricorni* required between 4.5 and 12 mol quanta m^{-2} d^{-1} during the growing season over a minimum period of two weeks to survive. During the senescent season, seagrasses declined naturally without any further impact from experimental reductions in light.

Rationale & Approach

One of the key objectives to address seagrass light requirements and their tolerance to a reduced light environment in Port Curtis was to conduct *in situ* shading experiments on the major seagrass communities likely to be affected by the Western Basin dredging project. Shading studies help quantify the impact of prolonged incident light reduction and identify early warning signs of declines in seagrass health related to dredging induced light reduction.

Shading studies were carried out twice during summer peak seagrass abundance and once in winter following the late summer monsoon season (Rounds 1-3). Pelicans Banks was used as the principal study location due to the predominance of *Zostera capricorni* in the meadow. This location was also ideal due to its accessibility, semi-firm sediment composition for repeated measurements without compromising site integrity and continuous seagrass cover year-round to assess seasonal effects.

Experimental Design

Each study site covered approximately a 30 x 20m area with experimental plots randomly assigned to a shade treatment. In addition, some sites had a duplicate plot design set aside for destructive sampling which included coring for carbohydrates or leaf tissue/pigment analysis. 0.25m^2 quadrats with a 25cm depth were hammered into the sediment until flush with the surface to isolate plots where seagrass would be measured during shade experiments (i.e. isolation borders). This ensured that seagrass outside of the experiment could not translocate nutrients to seagrass within treatment plots. Plots were also “gardened” with a knife around the perimeter of all borders at the beginning of each sampling event. Aluminium frames were secured into the sediment with steel rio-bar from which 1m^2 shade screens were fixed 15cm above experimental plots.

Shade screens (i.e. treatments) were installed with varying intensity of shade cloth (high, medium, low and extra low) over the meadow and assigned to varying lengths of shading (1-3 months and/or fortnightly oscillations of shade/no shade) during each study. The four shade treatment intensities were used to assess varying levels of reduced light on seagrass health while control plots were established using quadrats without shade screens but with frames. Shade screens consisted of neutral-density light reducing high-density polyethelene shade cloth woven onto 1m^2 aluminium frames. Underwater light loggers were placed under each shade level over the course of the study to measure actual light reduction from shade treatments. Shade screens were changed and cleaned weekly during the summer and fortnightly in the winter months to reduce the effects of fouling on shade treatment levels.

Light & Morphometric Measurements

PAR levels for each shading treatment and key metrics on seagrass condition were recorded at regular intervals (i.e. weeks and/or months) in all treatments and control plots. PAR (μmol. m^{-2}.s^{-1}) was recorded at 15 or 20 minute intervals using a submersible Odyssey light logger (Dataflow Systems Pty Ltd, Christchurch, New Zealand) calibrated using a Li-Cor underwater sensor (Li-Cor Inc., Lincoln, Nebraska USA) and fitted to an underwater wiper unit. Submersible ib-tag temperature loggers were attached to light logger units and recorded at the same time intervals as PAR during each study.

Four key measurements of morphometrics were made in each treatment plot at each sampling event: shoot density, percent cover of seagrass, seagrass canopy height and above-ground biomass. Shoot density of each species present in the treatment was estimated by counting all shoots within a mini-quadrat (10 cm x 10cm) randomly placed three times in each measuring plot. Seagrass percent cover estimates of each measuring plot were made by an observer using a guide sheet to ensure best approximation while canopy height was measured from base to tip of three random shoots in each plot. Seagrass above ground biomass was determined using a "visual estimates of biomass" technique (see Kirkman 1978; Mellors 1991; Rasheed 1999). This technique involves an observer ranking seagrass biomass either directly in the field or from photographs taken of each measuring plot during sampling. Ranks were made in reference to a series of quadrat photographs of similar seagrass habitats for which the above ground biomass has previously been measured. Photograph biomass ranks were then converted into above ground biomass estimates in grams dry weight per square metre (g DW m^{-2}). At the completion of ranking, the observer ranked a series of photo calibration quadrats that represented the range of seagrass biomass in the survey for the ranking scale. The photographs of calibration quadrats were previously harvested and the actual biomass determined in the laboratory. A separate regression of ranks and biomass from these calibration quadrats was generated for the observer and applied to the measuring plot ranks to determine above ground biomass estimates. The best indicator of seagrass condition during all studies was above-ground biomass estimates and is the only metric presented as part of this summary report.

Following shading treatments, shade screens were removed and seagrass recovery was assessed over an additional one to two months, if significant declines had occurred due to shading.

Destructive Sampling – Below Ground Carbohydrates

Below-ground carbohydrate samples were taken from destructive plots during Round 1 and 2 sampling (the first growing season and senescent season respectively). During each sampling event, two cores were taken in each destructive sampling plot with care taken not to core in previously sampled areas. Cores were sieved to remove sediment and debris prior to freezing and transported back to the lab for further processing. In the lab, samples were defrosted, scraped clean and separated into below and above ground structures after which all root and rhizome material was dried and ground to a fine powder in a bead mill for wet lab analysis. Prepared samples were shipped to the University of Queensland where soluble and non-structural carbohydrates (i.e. starch) were extracted and quantified relative to total sample dry weight (Karakalas 1985, Weir et al 1977).

Data Analysis of Morphometrics

Differences in morphological responses of seagrass between treatments were assessed using a one-way analysis of variance (ANOVA) at $\alpha = 0.05$. All analyses were performed using Sigmastat statistical software (version 11.0 2010, USA). Data were first checked for homogeneity of variance using Shapiro-Wilk's. Treatment effects were interpreted using Tukey's post-hoc analysis.

Key Findings

During the growing season shading studies (Rounds 1 and 3), a clear negative effect on seagrass above-ground biomass was recorded in the high shaded treatments following 4 weeks in round 1 (Fig 1A), and 8 weeks of continuous shade in round 3 (Fig 1B). Further declines were also recorded in all other shading treatments from 8 to 12 weeks. There were signs of recovery in the lighter shaded plots after shades were removed at 12 weeks, while no recovery was found in high shade plots (Fig 1B).

The senescent season study (Round 2) found no significant change in any shading treatment compared to controls after four and even eight weeks (data not shown) of continuous shading (Fig 2).

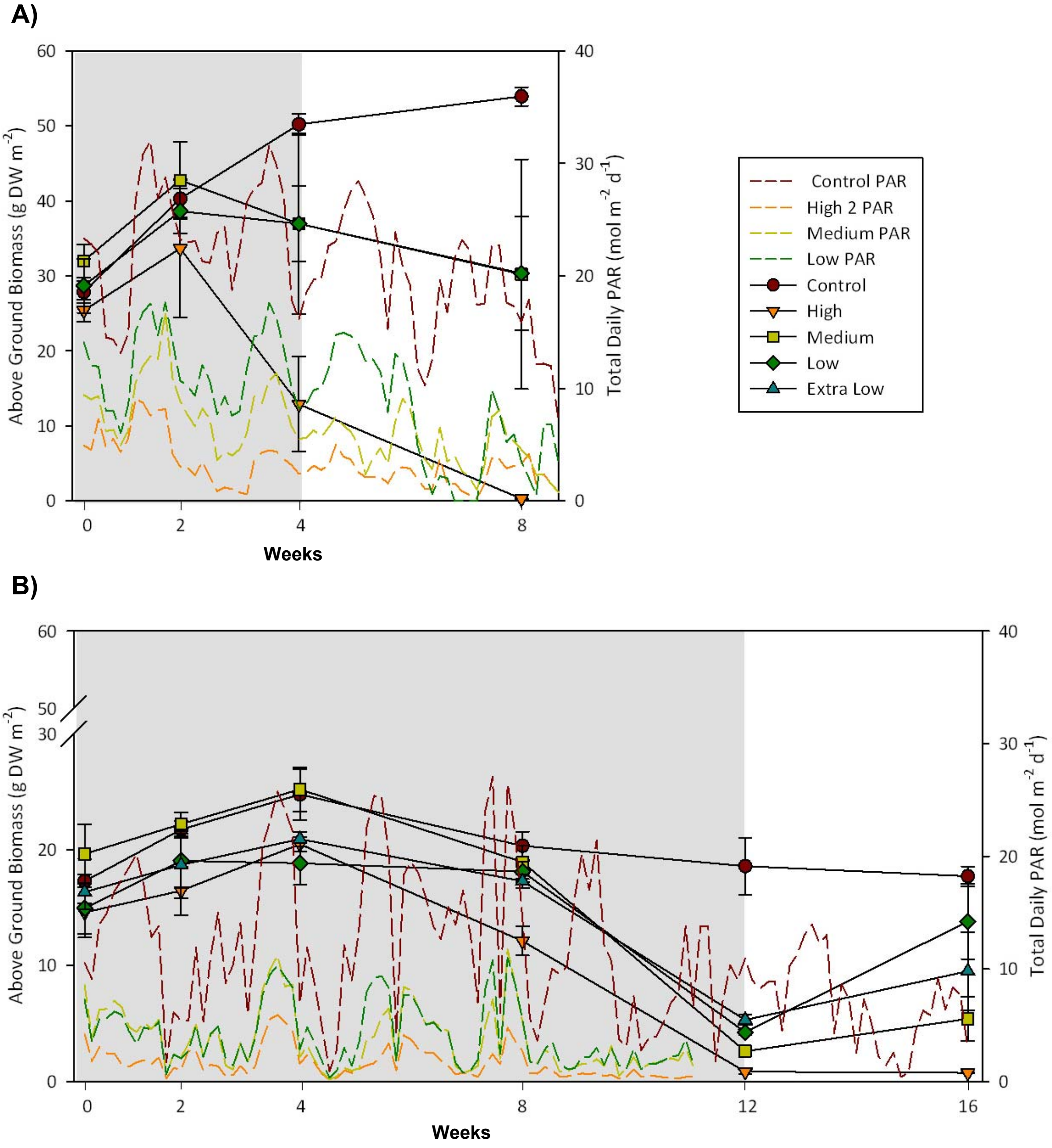


Figure 1. Estimates of above-ground biomass in growing season 1 (A) and growing season 2 (B) shading treatments. Data represent mean ± SEM (n=4). Total daily PAR is displayed on the right y-axis to show light availability under each shading treatment and controls. Gray shading indicates when shade screens were on.

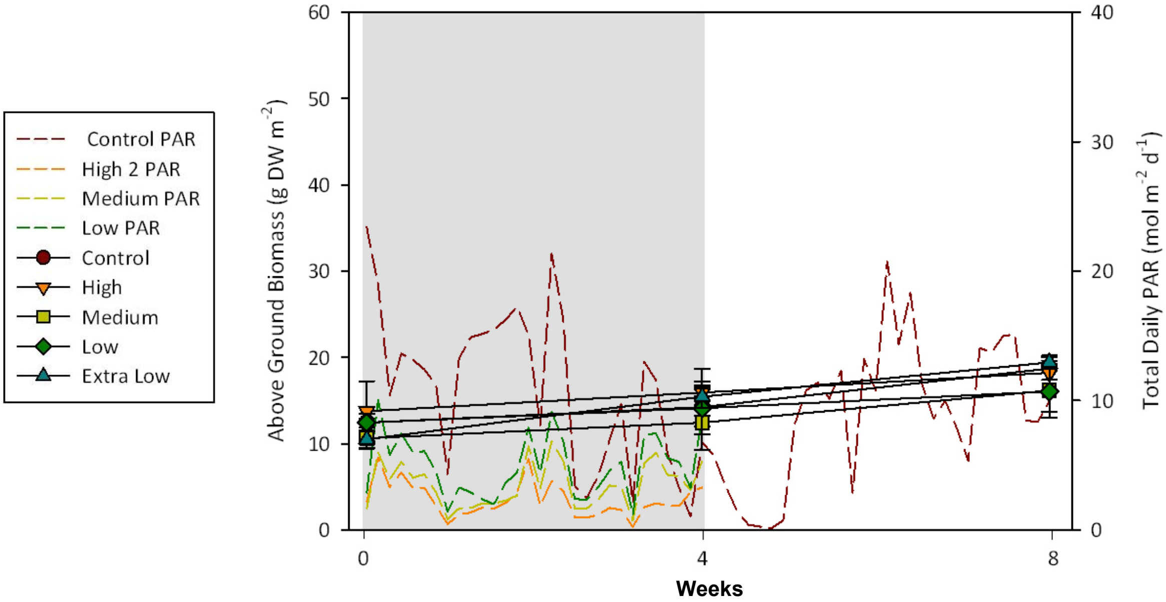


Figure 2. Estimates of above-ground biomass in senescent season shading treatments. Data represent mean ± SEM (n=4). Total daily PAR is displayed on the right y-axis for each shading treatment and controls. Gray shading indicates when shade screens were on.

No significant effects of shading were found during the senescent season and with strong effects of shading during the growing season, it was concluded that seagrasses in Gladstone Harbour do not rely heavily on light availability during the senescent season.

Further evidence from below ground energy stores sampled during shading studies reinforced these findings (Fig 3). While no differences were found among shading treatments, there was an overall decline in stores during the senescent months, whereas stores increased over the growing season study (Fig 3). This may indicate that seagrasses have shut down or reduced their requirements for light during the senescent months, as part of their inherent seasonal cycle and instead rely on energy stores built up during the productive growing season period (Collier 2006).

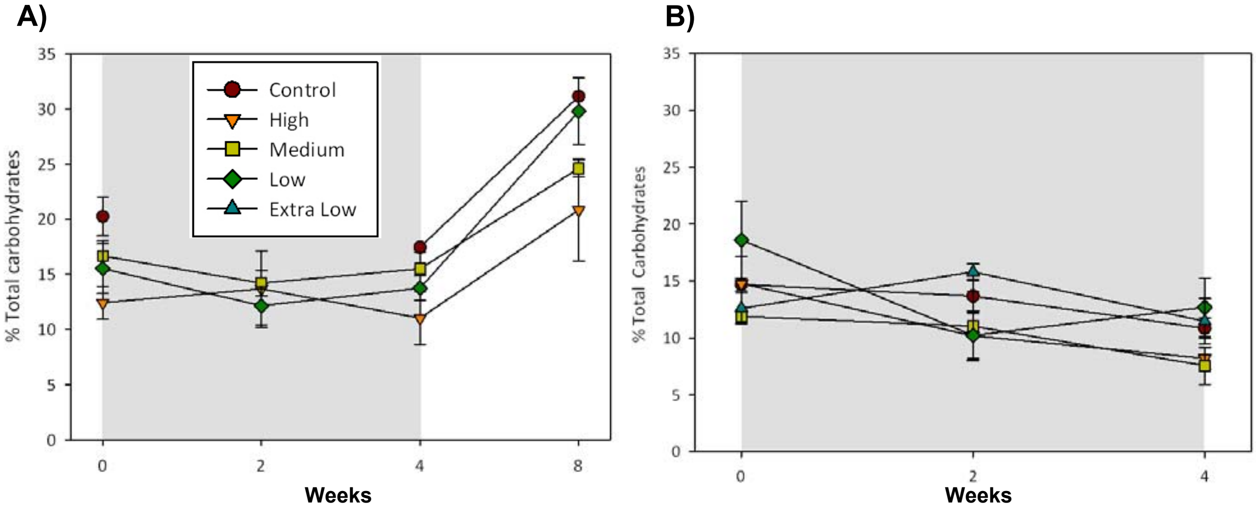


Figure 3. Total below-ground percent carbohydrates in the growing season (A) versus senescent season (B) shading studies. Data represent mean ± SEM (n=3).

An assessment of PAR levels recorded during the two growing season studies provided an initial range of light levels required for *Zostera capricorni* survival together with shading results (Fig 4). While ambient PAR available during each study was markedly different, the studies indicated that high shading or levels below an average of 4.5 mol m^{-2} d^{-1} is insufficient to sustain seagrass as demonstrated by seagrass morphometrics (see above). Alternatively, seagrasses in control plots appeared healthy for the time of year and location based on long-term monitoring data (see Chartrand et al 2011) indicating ambient PAR measured in control plots was sufficient. The lowest ambient PAR from the two studies was 12 mol m^{-2} d^{-}, which corresponds to the highest level of PAR that *Zostera capricorni* needs to survive.

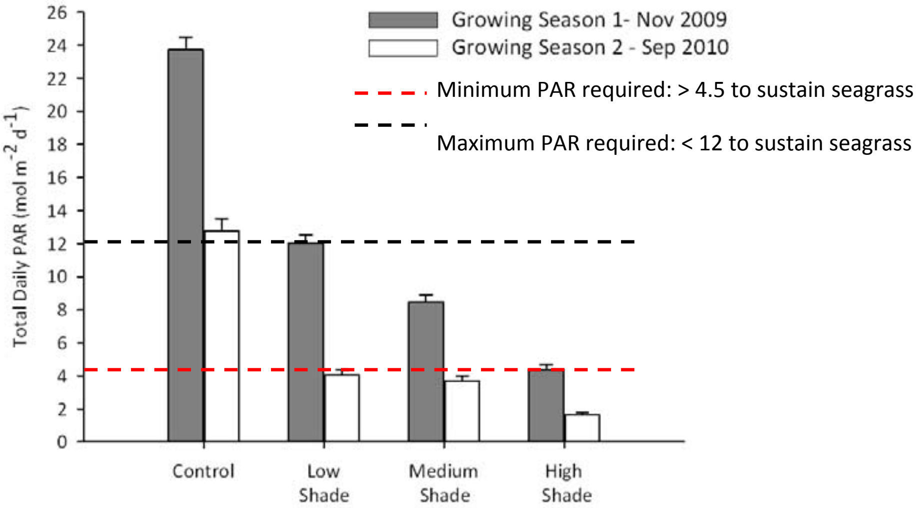


Figure 4. Mean total daily PAR recorded among shading treatments during the growing season studies. Data represent mean ± SEM over each shading period.

While the low and medium shade treatments showed significant declines during the second growing season study, these were only observed between 8 and 12 weeks of continuous shading, in excess of acceptable time frames for a dredging management strategy. Furthermore, the low and medium shade treatments showed signs of recovery only after 4 weeks once shades were removed, indicating the impacts were not irreparable (Fig 1B). Due to the ambiguous nature of the low and medium shade treatment findings, a further analysis of the 4.5-12 mol m^{-2} d^{-1} window of PAR required was assessed using other empirical studies which will be discussed in subsequent sections of this report.

The last major outcome of the *in situ* shading studies was derived from an assessment of shorter increments of shading. Shade screens were taken on and off of experimental plots at two week intervals, or fortnightly oscillations (Fig 5). This approach tested whether seagrasses could survive short, intense shading from turbidity plumes if given periods of “reprieve” from low light between shading events (i.e. turbidity plumes). No significant declines were observed in any shade treatments relative to the unshaded controls over 8 weeks. By 12 weeks, two weeks after shades were permanently removed from all treatments, seagrasses showed a downward trend in above-ground biomass across all treatments including the previously unshaded controls. These declines were largely due to extensive rainfall and river flows in the Gladstone region that resulted in widespread seagrass declines and a premature end to the growing season for seagrasses leading up to the week 12 (December 2010) and week 16 (January 2011) sampling (Fig 5). Although larger regional impacts were the likely cause of much of the declines, there were significant differences between the controls and shaded treatments (apart from extra low shade) at 12 weeks indicating that repeated cyclic shading over time (beyond two cycles) may have reduced the resilience of seagrasses to future large scale impacts. By 16 weeks (6 weeks after shades were removed) only the highest shade treatments were different to the controls and at the end of the experiment (12 weeks after shades were removed) the impacts of the flooding meant all treatments were similar and substantially reduced with seagrasses well into the expected senescent season decline (February 2011; Fig 5).

Cyclic shading results provide a defined interval that seagrasses are able to withstand low light with no observable effects on survivorship for at least a 2 month period. Seagrasses naturally integrate changes in their environment over time scales which can take days to weeks to observe physical changes at the canopy or meadow scale. In Gladstone, seagrasses appear to cope with reductions in light over a two week period with minimal effect on seagrass health. Two weeks is therefore used for a trigger value timeline as part of the working light management plan (further discussed below).

Overall, *in situ* shading studies found that Gladstone area seagrasses, specifically the dominant species *Zostera capricorni*, require between 4.5 and 12 mol quanta m^{-2} d^{-1} during the growing season over a minimum period of two weeks to survive.

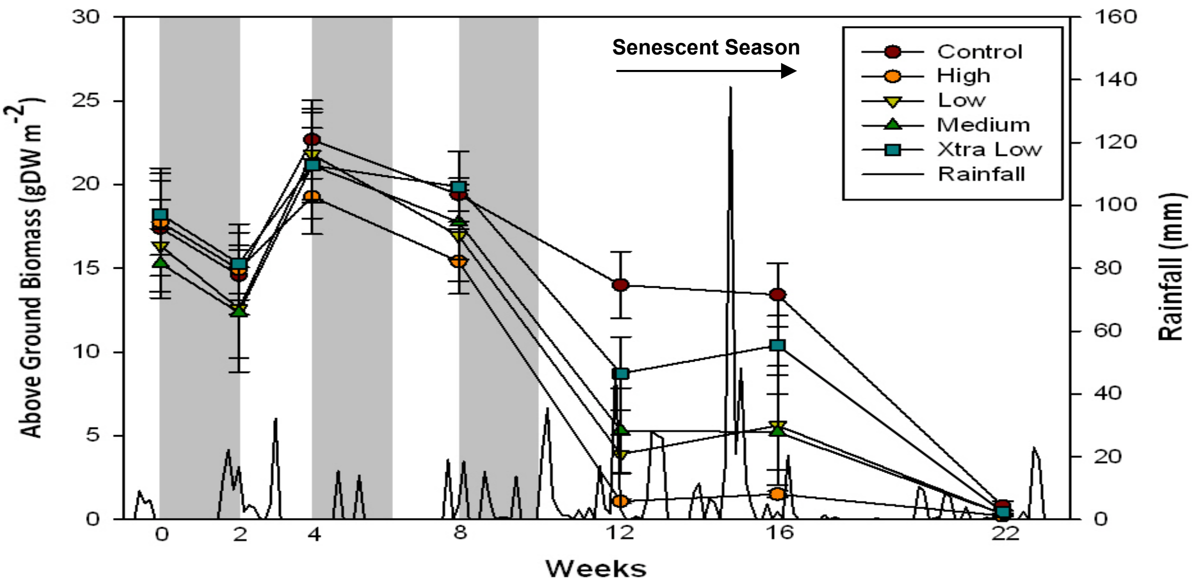


Figure 5. Estimates of above-ground biomass in growing season 2 shading treatments and rainfall during the experimental period. Gray shading indicates when shade screens were on. Data represent mean ± SEM (n=4).

Tidal Exposure Studies

Summary

Tidal exposure studies were conducted to determine how well seagrasses utilised light over various stages of the tidal cycle, and particularly to establish whether a “window of opportunity” occurred during low tide exposure to air in which seagrasses may be able to compensate for increased turbidity. Results of the study found that seagrasses likely depend on the critical light window around low tide exposure for the greatest productivity. However, full tidal exposure did not provide a “window of opportunity” for relief from turbidity impacts as this period of exposure was found to be stressful to the plants, producing a negative photosynthetic response.

Rationale & Approach

Gladstone Harbour has large tidal fluxes resulting in a highly variable light environment for intertidal seagrasses. This large tidal range means that the shallower seagrass meadows often become air-exposed during the day, potentially influencing photosynthetic performance and oxygen production. Exposure or near exposure at the lower tidal range may provide longer periods for positive net photosynthesis or result in photodamage due to high light stress on photosynthetic tissues. It is also possible that exposed seagrass blades exceed their thermal tolerance for photosynthesis or become limited in CO_2 supply when exposed for long periods at low tide which would also lead to a decline in net photosynthesis (Leuschner et al. 1998). Thus, it is important to understand whether exposure of intertidal meadows is beneficial or detrimental for seagrass photosynthesis. The outcome may affect how tidal exposure windows are treated as part of dredge mitigation strategies for seagrass management.

The study investigated how well seagrasses were able to use available light through a spring tidal cycle that included daytime exposure to air. To assess this, the photochemical efficiency, pigment composition and oxygen production of intertidal seagrass meadows were measured during different seasons during a spring tide cycle. Quarterly sampling provides a better understanding of the variability in the responses to air exposure in seagrasses over an annual cycle. Measurements on *Zostera capricorni* were made from before noon until sundown before, during and after air-exposure at near-hourly intervals starting three hours prior to the absolute low tide.

Key Findings

There was a significant and consistent decline in photosynthetic activity with air-exposure for *Z. capricorni* highlighting the sensitivity of this intertidal species to desiccation and/or temperature. Light had very little effect on photosynthetic activity until it was combined with air exposure (Fig 6). This has major implications with respect to their daily productivity, as during low tide, when sunlight exposure is maximal, photosynthetic activity declines in response to exposure-related stress and not high irradiance, thus limiting the 'window' for high rates of productivity to times of high irradiance whilst submerged.

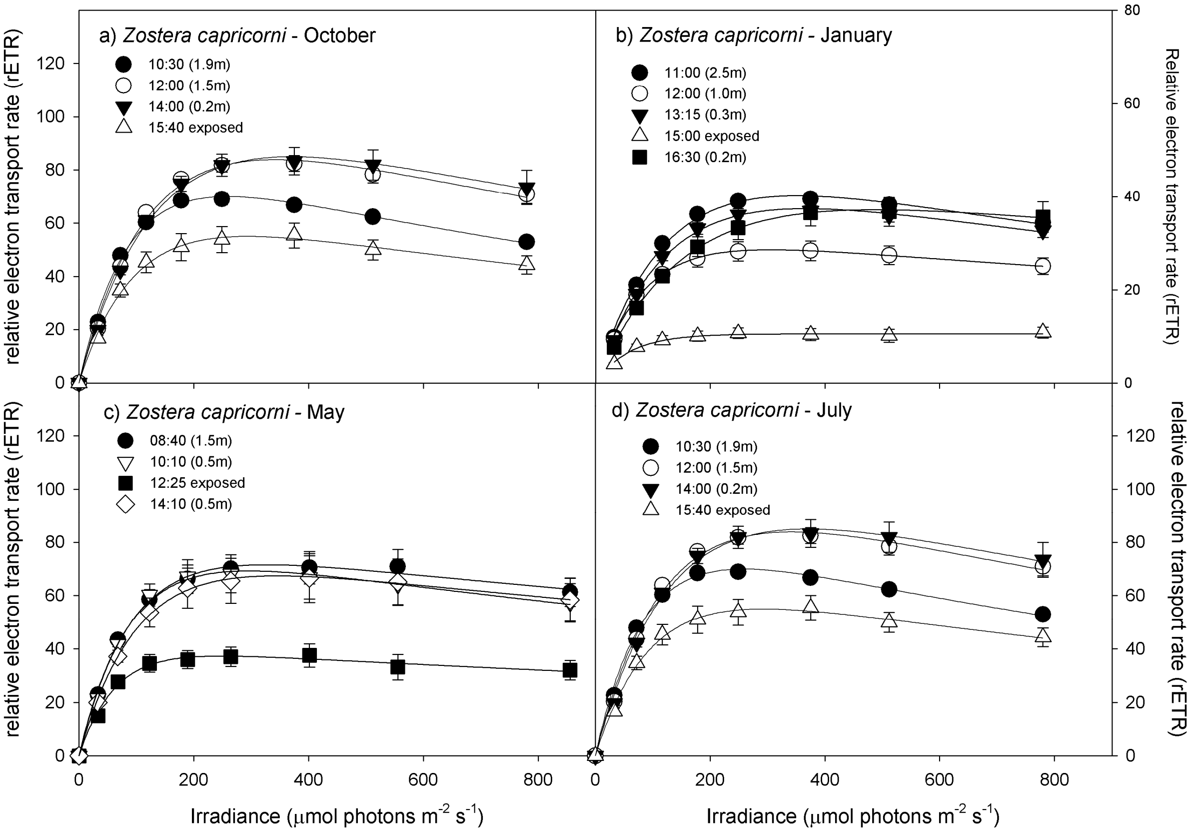


Figure 6. Relative electron transport rate (rETR) as a function of irradiance in the intertidal seagrass species *Zostera capricorni* over seasonal tidal cycles. Double exponential curve fit is shown (line). Time of day and depth of water at time of sampling in metres is given in legend. Data represent mean ± SEM (*n* = 6).

The increase in photosynthetic activity with increased irradiance in the October (growing season) study, suggests that this seagrass has evolved to take advantage of increased irradiance when tides recede (albeit without exposure). However, after May and July this response was no longer evident (see Appendix III for further detail). This trend matches their time of maximum growth and period for building a carbohydrate stock during the growing season, while suggesting a greater use of energy stores during autumn/winter (senescent season) months. When comparing the data between growing and senescent seasons, there was a reduction in the magnitude of the response to air-exposure in *Z. capricorni* during the autumn and winter studies – i.e. the senescent season. This difference in stress during air-exposure between the two seasons would suggest that temperature might play a significant role in the loss of photosynthetic activity in *Z. capricorni.*

There was an increase in oxygen evolution with increased irradiance as the tide recedes and a significant decline in oxygen production rates immediately after air-exposure during the growing season (Fig 7). This compliments the chl *a* fluorescence data (see Fig 6) and further suggests that during air-exposure, photosynthetic activity and oxygen evolution decline as a result of increased stress. The cause of this stress is still unknown; it could be the result of desiccation, CO_2 limitation, excess heat or a combination of all three. However, what is clear is air-exposure during a tidal cycle does not provide intertidal seagrass meadows with a 'window' of opportunity in which to enhance productivity. Instead, it leads to physiological stress that impedes photosynthetic activity and carbon fixation.

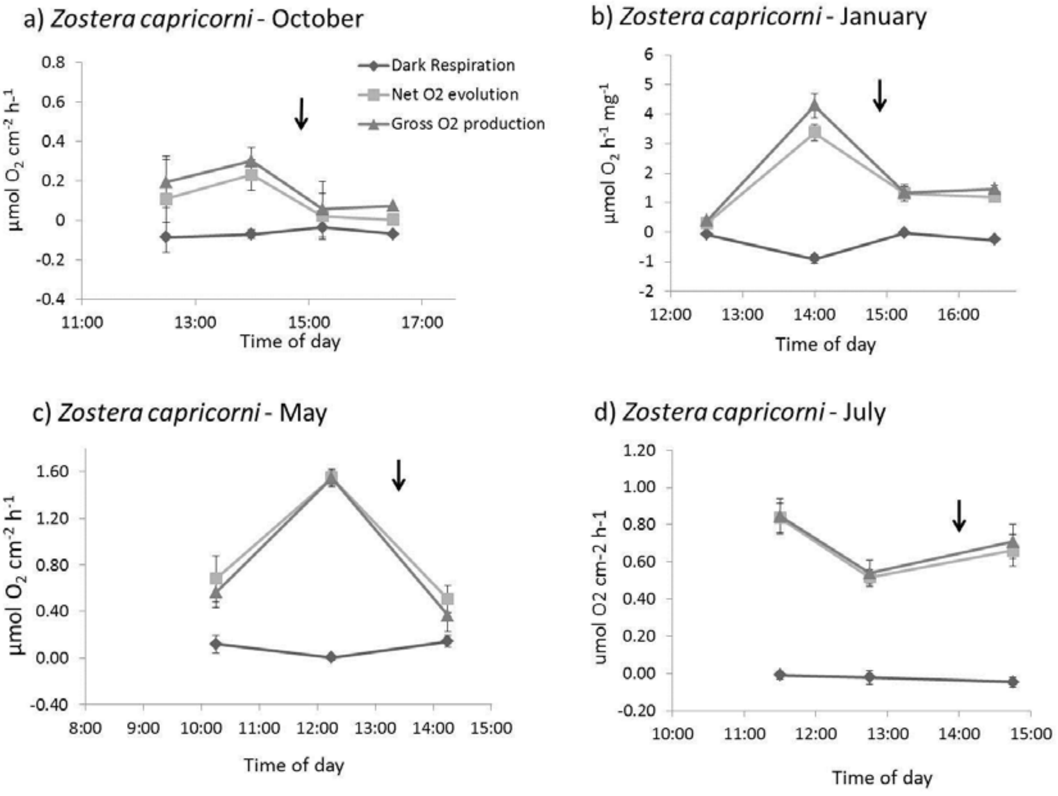


Figure 7. Rates of gross oxygenic photosynthesis (triangles) calculated from measurements of dark respiration (diamonds) and net photosynthesis (squares) *in situ* for allseasons during the tidal exposure study. Averages ± SE (*n*=6) are shown, and the arrow indicates the time of air exposure at low tide.

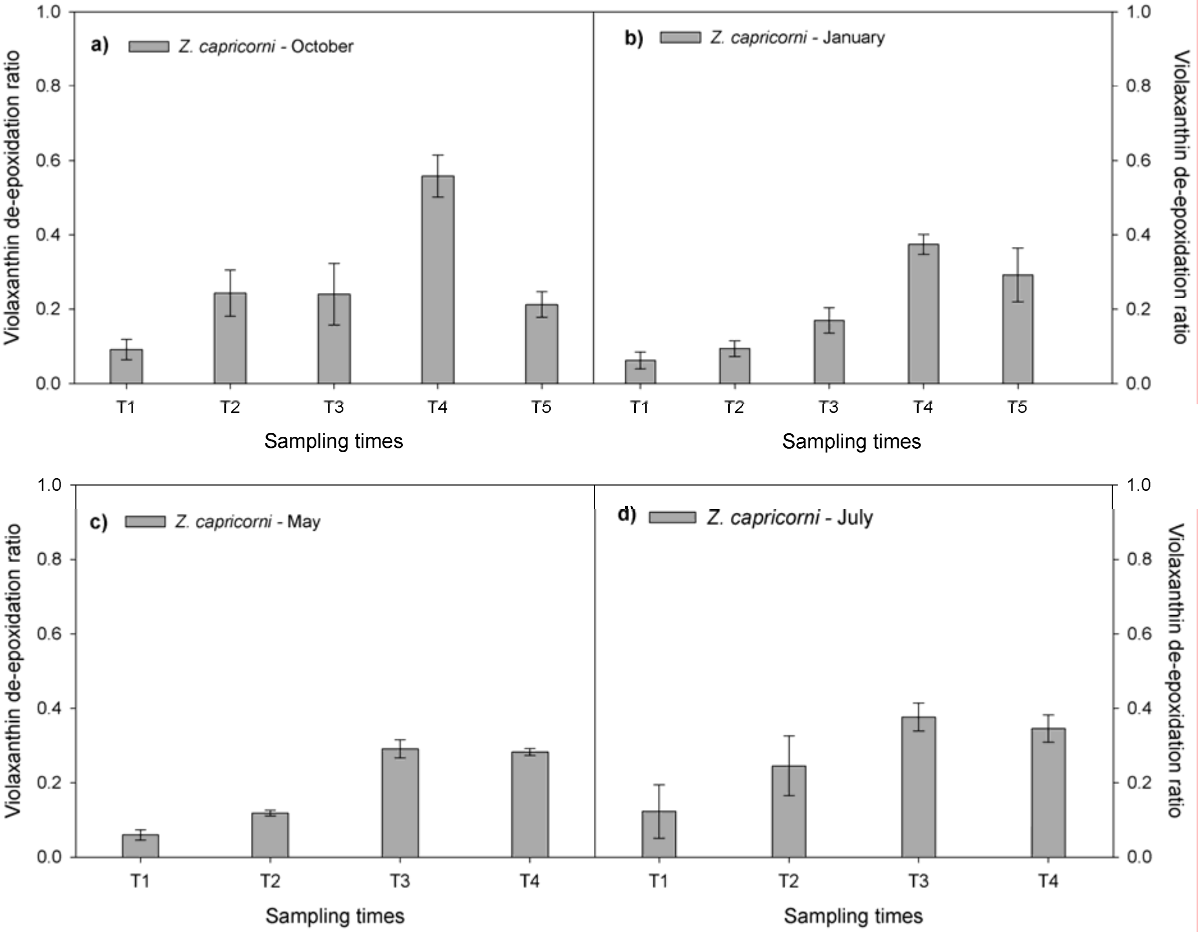


Figure 8. Violaxanthin de-epoxidation ratios for *Zostera capricorni* during tidal cycles for a) Spring b) Summer c) Autumn and d) Winter. For the October and January data, tidal exposure was at T4, in May and July tidal exposure was at T3. Data represent the mean ± SE (*n*=6).

The photoprotective pigment ratios determined by HPLC analysis show a strong and significant increase ($P < 0.001$) with increased irradiance, where the violaxanthing de-epoxidation state increased throughout the day, with the greatest violaxanthin de-epoxidation occurred during sample times of air-exposure (Fig 8).

The results from this study have successfully demonstrated that tidal exposure will not provide intertidal seagrass meadows with any respite from high turbidity that may result from dredging related activities. The differences between the growing and senescent seasons are more evident in the photosynthetic activity, primary production and pigment data, perhaps as a result of seasonal changes in internal stores of carbon or pigment proteins. This seasonal variability has provided a greater understanding of annual patterns in seagrass productivity over tidal cycles and has reinforced that seagrasses are more sensitive to high turbidity during the growing season when compared with the senescent season.

The tidal exposure study results reinforce the importance of the growing season period for Gladstone seagrasses in the development of a light-based management plan. Seagrasses are also likely to depend on the elevated irradiance around low tide exposure to enhance primary productivity, particularly during the growing season. However, full tidal exposure was found to be stressful producing a negative photosynthetic response indicating seagrasses do not compensate for otherwise low light conditions during these high light exposure periods. These findings build on the shading study experiments which also indicated the importance of adequate light during the growing period (July-January). The outcomes of this study have been incorporated into the light management plan and trigger values set out and discussed in the Light Management Plan section below.

Benthic PAR and Historic Seagrass Trends

Summary

A program of monitoring seagrass condition in conjunction with collection of light (PAR) at seven locations throughout Gladstone Harbour was used to test and refine seagrass light management approaches. Seagrass condition and light have been monitored directly since November 2009 providing a substantial dataset across several seasons and years to establish links between light and seagrass condition. Results from the field and laboratory experimental studies were used to develop and test various light triggers and management actions against this data set. This approach established how effective various triggers would have been in maintaining sufficient light for seagrass survival under natural conditions and their suitability as a management tool to protect seagrasses during dredging.

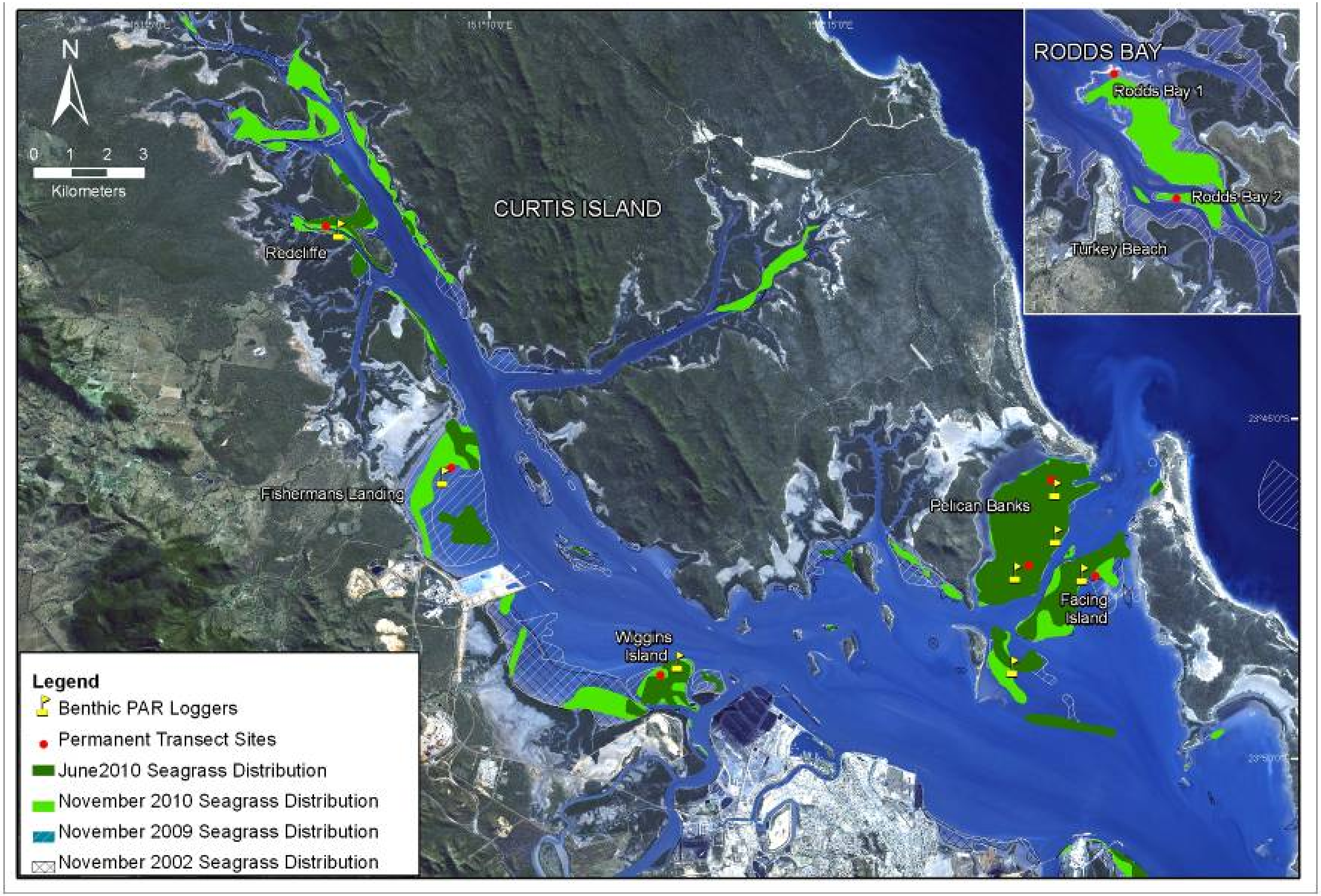
Rationale & Approach

Results from field shading studies indicated that the growing season (July – January) is the critical period for Gladstone seagrass productivity and expansion, as well as building up energy reserves for the senescent season. From the growing season studies, it was determined that Gladstone *Zostera capricorni* requires an average between 4.5 mol and 12 mol quanta m^{-2} d^{-1} over a two week period (see Shading Study section above). In order to refine this value further, trends in seagrass condition and ambient PAR within seagrass meadows were compared since detailed monitoring began in 2009. Data was collected quarterly from permanent transect sites located in key seagrass meadows in the harbour and were assessed against adjacent benthic PAR logger datasets. Trends between seagrass cover and availability of benthic PAR were used as a guide for when seagrasses were naturally receiving enough light to thrive over the remainder of the year. These trends were compared with experimental results from the shading studies and Seagrass Health Study programs.

Seven seagrass monitoring locations were established in Gladstone Harbour area to reflect a spread of the different seagrass meadow assemblage types present in the region (Chartrand et al. 2009). These are geographically distributed from inside the southern section of the 'The Narrows' (Redcliffe) to the southern end of the harbour at Rodds Bay, close to the town of Turkey Beach (Map 1). Benthic PAR loggers, deployed and maintained by Vision Environment, were positioned adjacent to each location to compare light with seagrass condition. A rolling mean total daily PAR at each location was calculated over a two week period, in line with an effective period of integrated light to potentially affect seagrass condition (see Shading Study section above). In other words, this two week period incorporates both spring and neap tide conditions as well as the variation in tide height and degree of exposure that affects the light conditions reaching the seagrass. Shading study results together with tidal exposure studies demonstrated the importance of incorporating all tidal conditions over an effective length of time to establish a seagrass response since seagrasses may senesce or rely on below-ground energy stores over shorter periods of inadequate light levels (see Shading Study and Tidal Exposure sections above for further details).

The total daily PAR for a given day and the previous 13 days were averaged and the calculation rolled forward each day respectively. The rolling mean PAR values were plotted against seagrass percent cover recorded at each of the monitoring locations quarterly until September 2011 and monthly thereafter.

Map 1. Gladstone Harbour permanent transect monitoring locations and adjacent benthic PAR loggers



Key Findings

Mean total daily PAR adjacent to seagrass monitoring locations was analysed to assess how often certain light levels coincided with a decline in seagrass percent cover. Light requirement values between 4.5 and 12 mol m^{-2} d^{-1} were assessed based on shading study and tidal exposure study results (see above). Only four of the seven sites had a complete enough light dataset to make an effective analysis, they were Fishermans Landing, Pelican Banks North, Wiggins Island and Redcliffe Island (Map 1; Fig 9).

During the growing season at sites where *Zostera capricorni* dominated throughout the study (Pelican Banks North & Redcliffe Island; Fig 10) it was found that when they received greater than 6 mol m^{-2} d^{-1} over a two week rolling average seagrass abundance (% cover and biomass) generally remained stable or increased. During the senescent season or when seagrass declined in response to major climate events, they received less than 6 mol m^{-2} d^{-1} (Fig. 9). Testing either side of this 6 mol m^{-2} d^{-1} was carried out, however other values did not provide a satisfactory light trigger point that fit the historical data. For example, a rolling two week average PAR of 10 mol m^{-2} d^{-1} was greater than the light required for *Zostera capricorni* in Gladstone based on the presence of seagrass at most sites when light rarely reached these levels (Fig. 9). Prior to November 2009, PAR was not monitored in Gladstone Harbour although seagrass monitoring has occurred annually since 2000. While seagrasses had reached peak abundance and distribution for the program in November 2009, no data is available to confirm the light environment which coincided with these trends (Thomas et al 2009).

During the assessment period there was a substantial rainfall and flood event in the 2010 wet season (and above average rainfall again in the 2011 wet season) which had a major impact on seagrasses. The impact was particularly severe in the inner harbour where many meadows suffered complete loss of above ground material. A result of this was that seagrass meadows at many inner harbour sites, including Fishermans Landing and Wiggins Island, also underwent a species change along with the significant reductions in seagrass cover, with *Halophila ovalis* dominating the sites post flood. *Halophila* has a much lower light requirement than *Zostera capricorni* (Grice et al 1996; Bach et al 1998; Collier et al. 2009; Freeman et al 2008). This combined with the extremely low seagrass cover explains why seagrass at the Fishermans Landing and Wiggins site did not respond particularly well to the *Zostera capricorni* light trigger. However at the sites where *Zostera capricorni* remained the dominant species the 6 mol m^{-2} d^{-1} over a two week rolling average threshold matched well with changes in seagrass abundance.

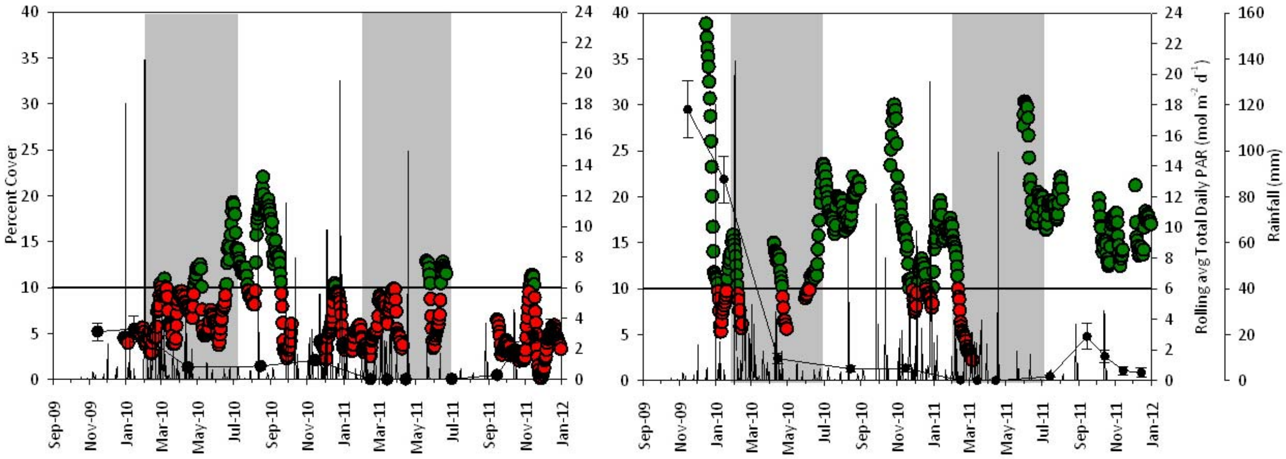
This rainfall event reduced light levels during the senescent season to well below 6 mol m^{-2} d^{-1}, corresponding with a significant decline in seagrass (Fig. 9). Seagrass did not recover to 2009 levels in the 2010 growing season despite PAR returning above 6 mol m^{-2} d^{-1}. This is not unexpected considering the scale and duration of the wet season flooding and the major loss of seagrasses that occurred. A subsequent above average wet season in late 2010/2011 put further pressure on seagrass condition and resilience, with PAR dropping well below 6 mol m^{-2} d^{-1} earlier than normal with heavy rainfalls beginning in October 2011 during the peak growing season period. Some locations had a complete loss of seagrass during this 2011 wet season period (Fig. 9A-C) while others had the greatest decline in seagrass cover in the history of the monitoring program (Fig. 9D).

In general, seagrass abundance and recovery following the senescent period and wet season rainfall events positively correlated with an increase in light above 6 mol m^{-2} d^{-1}, particularly for the sites dominated by *Zostera capricorni* in line with experimental results to determine seagrass light requirements in the Gladstone area (see sections above).

Analysis of benthic PAR data and seagrass trends at permanent transect monitoring sites is ongoing to validate a seasonal application of the light trigger value of 6 mol m^{-2} d^{-1}. These assessments together with the latest results from the laboratory studies confirm our current suggested light trigger value however modifications may be made as part of the adaptive management approach until the plan is formally adopted for the 2012 growing season.

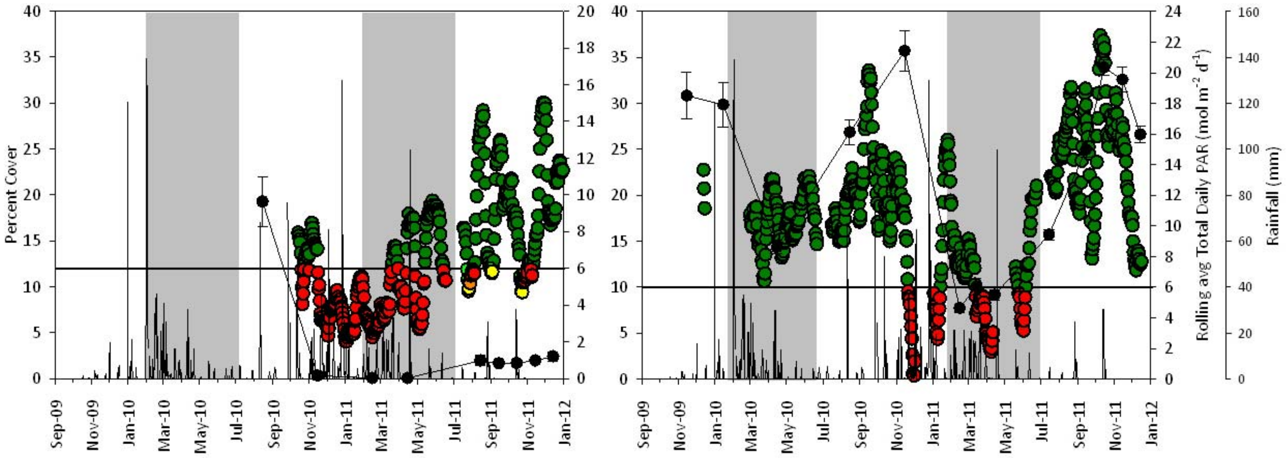
A) Fisherman’s Landing

C) Wiggins Island



B) Redcliffe Island*

D) Pelicans Bank North



* Monitoring location added in August 2010

Figure 9. Seagrass percent cover and the rolling two week average of total daily PAR recorded adjacent to seagrass monitoring locations. Black dots (± SE) indicate seagrass percent cover; Total Daily PAR is plotted on the y-axis with green dots indicate PAR greater than 6 mol m^{-2} d^{-1}; red dots indicate PAR less than 6 mol m^{-2} d^{-1}; grey shaded periods indicate senescent season and white shading indicates growing season periods. Total daily rainfall is presented on the far y-axis and as the black line behind all plots.

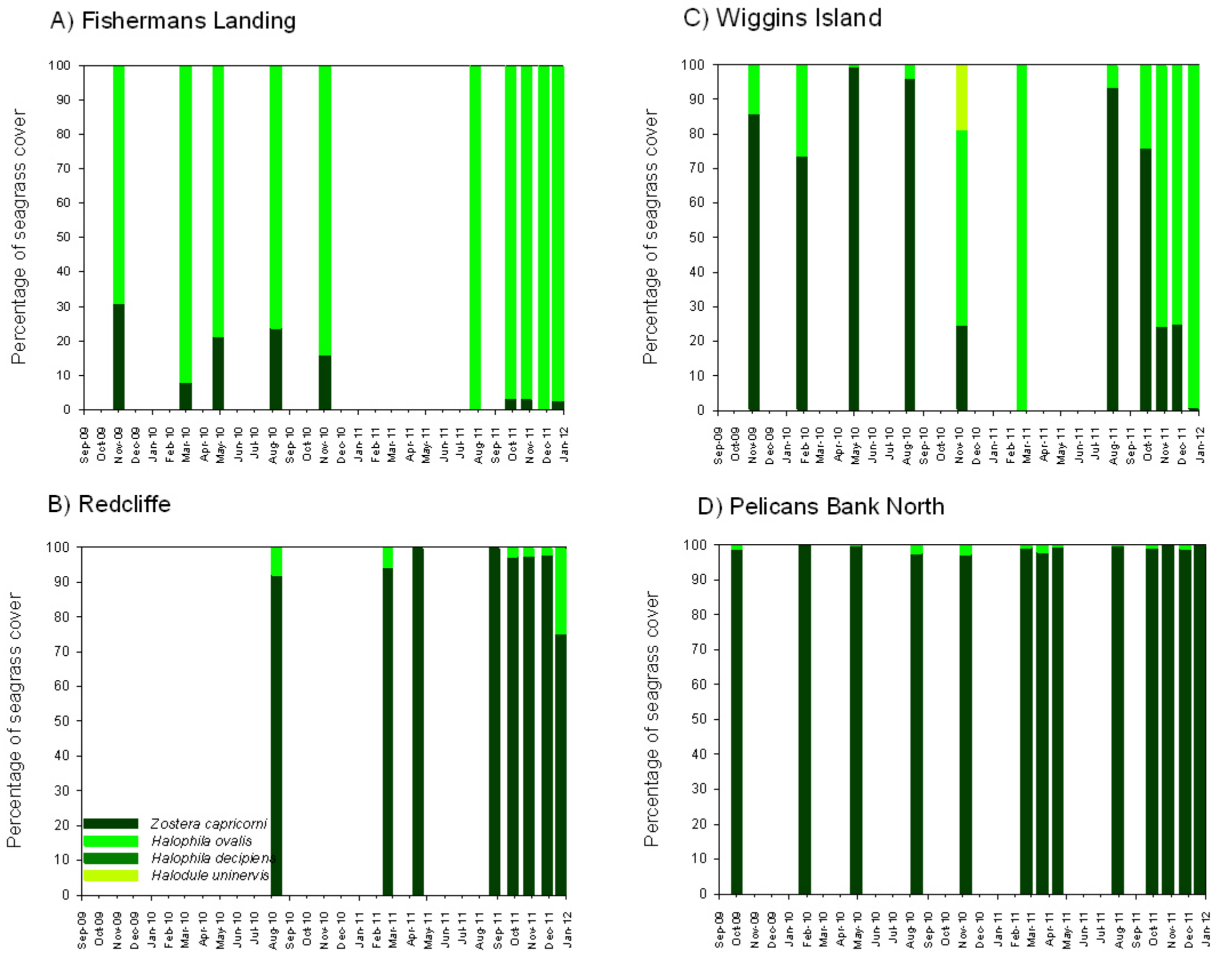


Figure 10. Seagrass species composition as % of total seagrass cover at permanent monitoring sites.

Laboratory Shading Experiments

Summary

Laboratory shading experiments and spectral profiling were conducted to further refine and test results from the field shading studies and to examine what changes may occur to the light requirements associated with a spectral shift in light (change in colour) due to dredging. In addition these studies looked to establish sub-lethal physiological indicators of light stress that could potentially be used as a tool to aid in management.

While a summary of the approach and findings are presented as part of this report, the detailed findings of this work can be found in the final laboratory experiment report (see Appendix I).

It is important to recognise the difference between field-based studies used to establish light trigger values and the lab-based studies used to validate field results and test photophysiological parameters. Laboratory studies were conducted in a controlled, artificial environment whereby the natural variability and effects from tides, sediment chemistry, temperature fluctuations and other environmental variables that can fluctuate on multiple time-scales (hours to days) in the seagrass meadow are removed in the laboratory. Laboratory conditions are tightly controlled to isolate effects caused by treatment conditions. An assessment of shading in the laboratory provides an assessment of true minimum light requirements for seagrasses which may be well below the environmental light requirement for the same seagrass due to external factors that synergistically affect the plants in nature in addition to the key driver of light.

A number of changes in morphological, bio-optical and physiological parameters were detected in seagrass acclimated to prolonged shaded conditions during the laboratory shading experiments. However, no strong evidence for an effective sub-lethal indicator of light limitation for *Zostera capricorni* was determined; a sub-lethal indicator would respond before the leaf dies. There is still some carbon incorporation (^{13}C) and real time PCR data to be processed which may confirm a decline in the plant resilience due to prolonged shading. We were looking for a sub-lethal indicator of extreme shade adaptation of the photosystems after which the plant can no longer maintain a positive carbon balance. We used a wide range of measures to monitor the process of acclimation in their metabolic pathways however, we have not been able to identify the threshold beyond which senescence occurs.

Laboratory experiment results verified field study findings whereby significant changes in seagrass morphology (both the number of leaves per shoot and total leaf volume) were detected after 57 days under the high shaded treatments.

Rationale & Approach

In addition to shading the seagrass, dredge-related turbidity can change the quality of light reaching the seagrasses at depth, where the light typically becomes enhanced in the yellow region of the spectrum. The dominant photosynthetic pigments in seagrasses are chlorophylls *a* and *b*, which absorb in the blue and red wavelengths (400-500 nm and 650-700 nm) of the light spectrum. Thus, yellow light enhanced conditions (i.e. a reduction in the blue and reds) have the potential to greatly reduce the effective light available for photosynthesis.

The laboratory study investigated the influence that shading and yellow enhanced shading have on the photosynthetic activity (pigment changes, oxygen evolution rates, fluorescence properties and respiration) of *Zostera capricorni* from Gladstone harbour. The study was designed to more precisely understand the relationship between seagrass absorptance and dredge-related reductions in the light environment and may potentially lead to further modifications of the light requirement model and triggers for seagrass management.

Laboratory studies were run initially in April (senescent season) and again in October 2011 (growing season). A number of changes in physiological parameters were detected in plants acclimated to shaded conditions in the senescent season study and a combination of these indicators would suggest imminent death of the plants. While the April study results provided some indications of overall seagrass response to shading, the growing season study fills the relevant gaps in our current understanding of the critical time period for seagrass growth and productivity and how dredging may affect seagrass health.

Key Findings

The light levels were not identical between the neutral density and yellow-enhanced treatments of low and high shading. Instead, the treatments provided a gradient of light levels (Fig. 11).

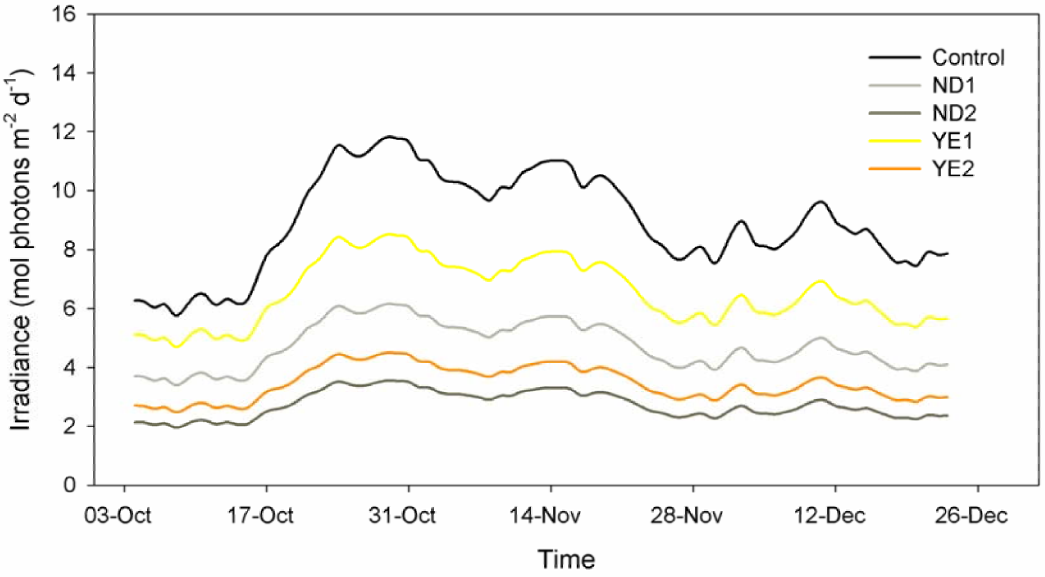


Figure 11. Glasshouse total daily irradiance (mol photons m^{-2} d^{-1}) received under control, neutral density and yellow enhanced light treatments. Lines represent 7-day rolling average. ND = neutral density shading, YE = yellow enhanced shading; 1 = low shading, 2 = high shading.

In the growing season study (October 2011), significant changes in seagrass morphology were detected after ~8 weeks (57 days) of shading, with a decline in the number of leaves per shoot and total leaf volume in the high shaded treatments (Figs. 12 and 13).

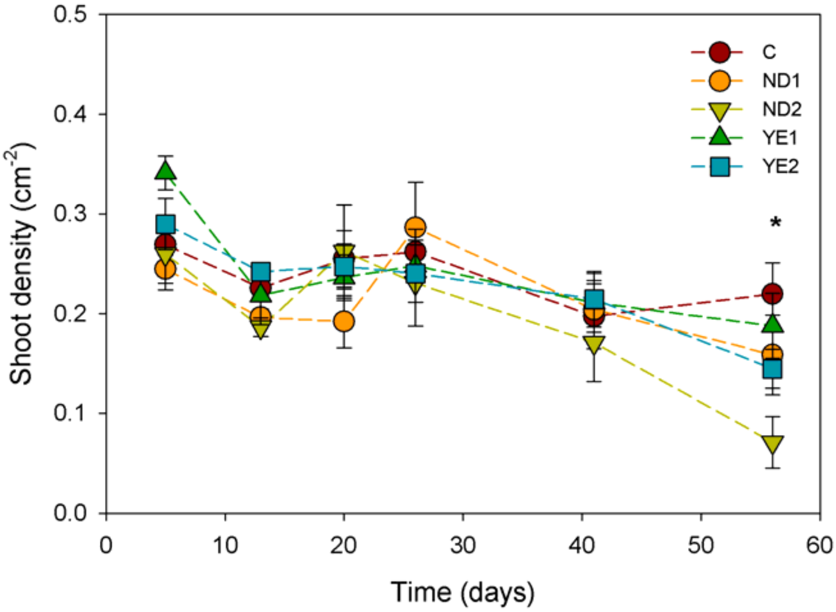


Figure 12. Seagrass shoot density (cm^{-2}) over entire study period (57 days) in *Z. capricorni* under control (C) and four light treatments, with the water maintained at 25°C. Data represent mean ± SEM (*n* = 6), *indicates significance at α = 0.05. ND = neutral density shading, YE = yellow enhanced shading; 1 = low shading, 2 = high shading.

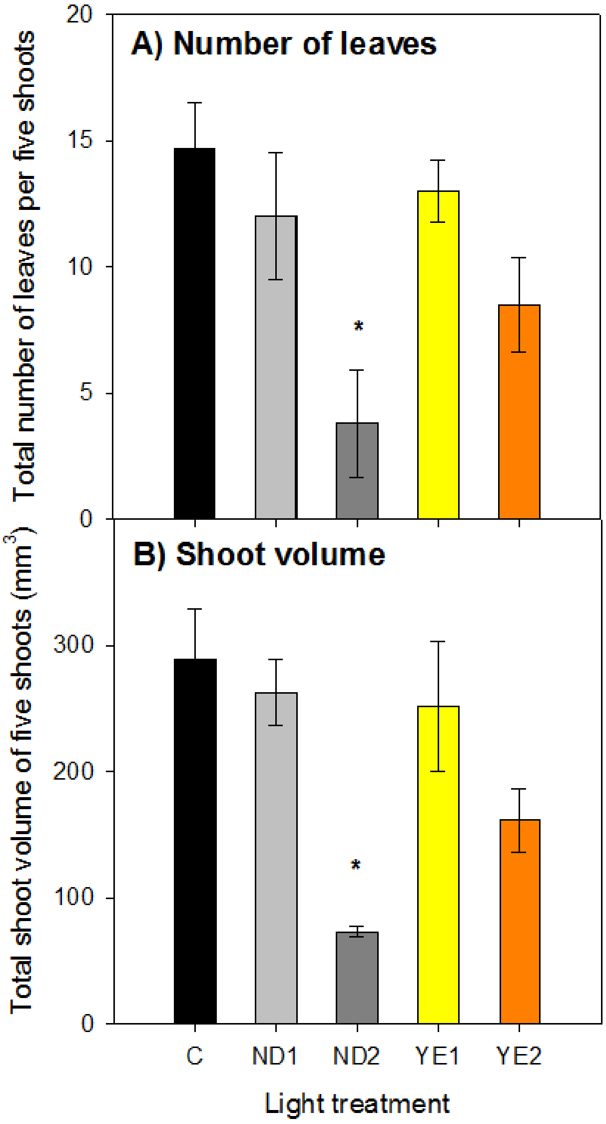


Figure 13. Seagrass total number of leaves per 5 shoots (A) and total shoot volume per 5 shoots in mm^3 (B) of *Z. capricorni* after 57 days of acclimation to different light conditions. Data represent the mean ± SEM (*n* = 6), *indicates significance at α=0.05. ND = neutral density shading, YE = yellow enhanced shading; 1 = low shading, 2 = high shading.

There were also significant shifts in leaf absorption properties of the highest shade treatment (Fig. 14). However, no differences were detected in the response to changes in light quality as a result of spectral shift ie: between the neutral density and yellow-enhanced treatments. A significant decline in the highest yellow enhanced shade treatment (YE2) after 10 weeks was detected in the leaf absorption at 600 nm, while leaf absorption at 680 nm was significantly lower in three of the shade treatments (ND1, ND2 & YE2) after 10 weeks (Fig 14) . Given the difference was only evident after 10 weeks, this parameter could potentially serve as a suitable sub-lethal indicator for seagrass management in cases of prolonged shading if morphological declines have not already been observed. Since the absorption peak at 680 nm is close to the chl *a* absorption peak (685 nm; Enriquez 2005), a reduction in absorption at this wavelength could indicate the beginning of the breakdown in the function of chl *a* to absorb useful wavelengths of light. Alternatively, leaf thickness strongly influences leaf absorptance, so as the shaded leaves are thinner, they will also have a reduced absorption; however if this were the case in this study, reduced absorption should occur across all wavelengths and not just 600 and 680 nm.

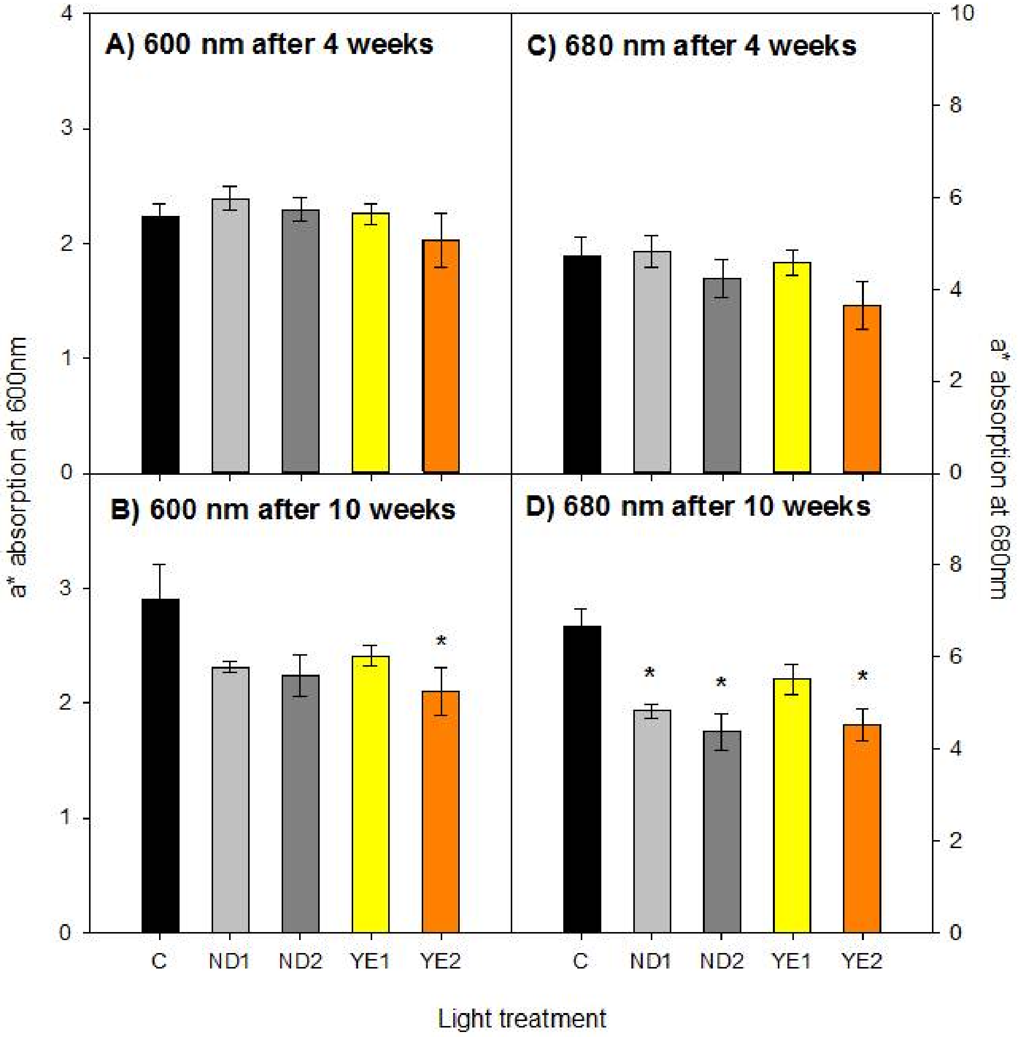


Figure 14. Leaf chlorophyll *a* specific optical cross section (*a**; m^2 mg chl a^{-1}) of *Z. capricorni* at 600 nm (A and B) and 680 nm (C and D) after 4 weeks (T1) and 10 weeks (T2) of acclimation to light treatments at 25°C. Data represent means ± SEM (*n* = 6), *indicates significance at α=0.05. ND = neutral density shading, YE = yellow enhanced shading; 1 = low shading, 2 = high shading.

No significant differences in photosynthetic characteristics were detected after 10 weeks of light limitation (low and high shading with and without yellow-enhanced spectral shift). Similarly, no differences in photosynthesis and respiration rates were detected between shade treatments. This lack of differences indicates that *Z. capricorni* was able to successfully photoacclimate to each of the shade treatments in the study, before the leaf finally senesces. Photoacclimation in *Z. capricorni* is supported by the RLC data, which shows significant differences in photosynthetic parameters α, $rETR_{max}$ and I_K after 4 weeks (T1) under the highest shade treatment (ND2), but that these differences were no longer evident after 10 weeks of acclimation when the plants have adapted their compensation irradiance (photosynthesis = respiration) (Fig. 15).

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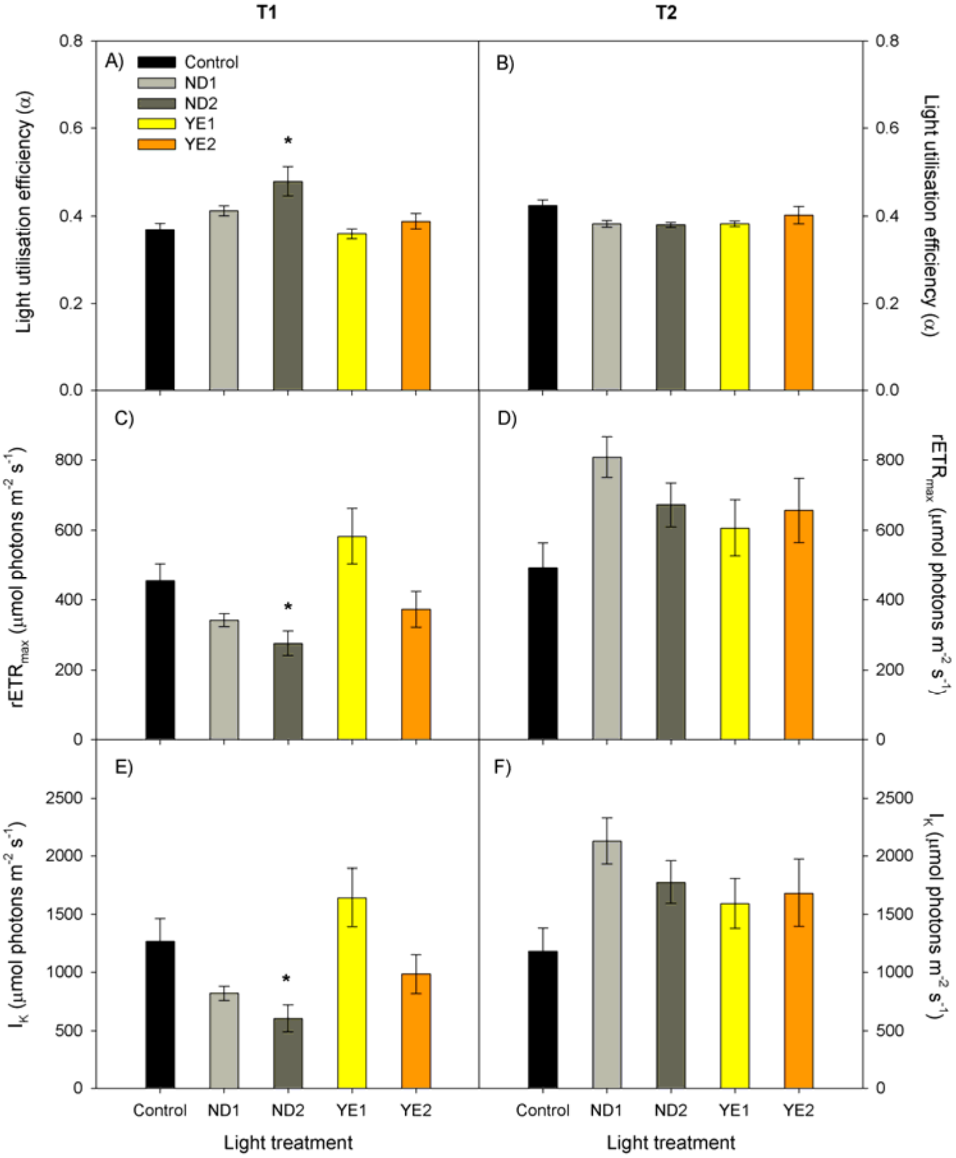


Figure 15. Photosynthetic parameters α (A-B), $rETR_{max}$ (C-D) and I_K (E-F) in *Z. capricorni* acclimated to 25°C under five different light treatments. Data represent mean ± SEM (*n* = 6). ND = neutral density shading, YE = yellow enhanced shading; 1 = low shading, 2 = high shading.

The pigment analyses (HPLC) showed clear increases in light harvesting pigment concentrations and chlorophyll *b:a* ratios with increased shading, consistent with shade acclimation, but no differences with yellow-enhanced treatment. No differences were detected in the relative proportion of photoprotective pigments; however, there was a decline in the violaxanthin de-epoxidation state (VDE) under the highest shade treatments.

This study included samples for carbon incorporation (^{13}C) and real-time PCR analyses. These data are yet to be processed and it is our hope that they will be able to confirm changes in plant resilience due to prolonged shading. We also hope that one if not both of these methods will succeed in providing us with a reliable sub-lethal indicator for seagrass health under changed light conditions.

In summary, the data have shown that under the highest shade treatment of 2.73 ± 0.46 mol photons m^{-2} d^{-1}, *Z. capricorni* exhibits signs of morphological and physiological stress and some minor effects when acclimated to 3.45 ± 0.58 mol photons m^{-2} d^{-1}, as was observed in the high-shade yellow-enhanced treatment.

This study was designed to investigate the impact that dredging could have on seagrass photosynthetic performance and health and did not reveal many significant morphological or physiological changes under shading treatments, providing very little information on light-induced changes to seagrass health. These data would suggest that a minimum light threshold above 3.45 mol photons m^{-2} d^{-1} would be sufficient to sustain seagrass health and viability at 25°C for up to 10-weeks. This value however, needs to be treated with caution, as many environmental variables were not accounted for in this laboratory study. In their natural meadow environment, air-exposure, temperature, sedimentation, nutrients and grazing all pose additional pressures on seagrass resilience (Erftemeijer and Lewis III, 2006) and therefore thresholds for a light-based management approach need to take these additional pressures into consideration. As the field studies have demonstrated, during the summer months, shading has a devastating impact on seagrass health and survival, so it would be advisable to work at a minimum light threshold that is higher than that determined in this study.

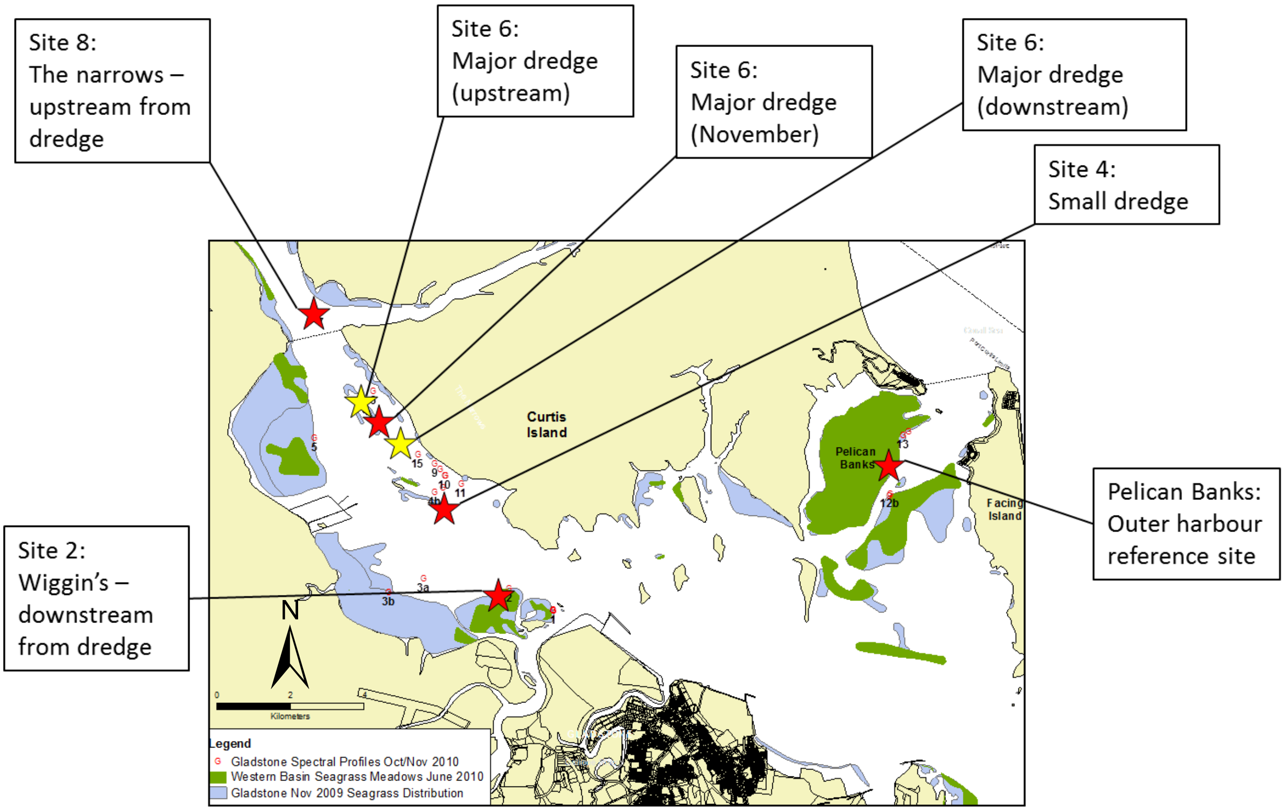
One possible reason for only being able to measure minimal morphological and physiological responses to the light treatments, when compared with field data, is the incubation temperature (25°C) that was used in this experiment. *Zostera capricorni*, as a predominantly temperate species, is known to exhibit stress under high temperatures (Collier et al 2011) and therefore, by growing them at a moderate temperature they were perhaps better able to withstand changes in their light climate. Indeed the previous laboratory experiment (July report) and field experiments (tidal and shading) both showed that higher temperature (summer seasonal data) showed greater responses to shading than in the winter or cooler temperatures. In addition, other variables, such as smothering from increased suspended particles (increasing the diffusion boundary layer for gas exchange), which were not considered in this study, but would provide an additional stress to the plants in their natural environment, potentially reducing their resilience to increased turbidity and low light. Therefore, lab-based results must be used collectively with field-based shading studies to shape light requirements for the Gladstone light-based management plan.

Spectral Profiling of Gladstone Harbour

Summary

The spectral profiling of Gladstone Harbour set out to describe the quality of light reaching seagrasses through the water column both under natural conditions and during dredging. Knowledge of the spectral requirements of seagrass and measurements of a change in the availability of the PAR spectrum during dredging may act as an indicator of seagrass health. This study measured the spectral transmittance and inherent optical properties of PAR to a maximum depth of 3.5 m in both the inner and outer harbour where seagrasses have previously been mapped during monitoring surveys and in varying proximity to current dredging activities (Map 2).

Map 2. Location of spectral profiling sites in relation to dredge activity.



This work provides an overview of the optical quality of the water and illustrates dredge-induced changes in the optical properties of the water column within the central harbour. There was a loss of light intensity reaching seagrass depths during dredging within the harbour, where the measured downwelling irradiance was below instrument detection limits beyond 0.5 m depth. Previous measurements made prior to the dredging activity recorded light penetration more than 3-fold greater than during the November dredge.

As not all absorbed photons result in photosynthesis, weighting the PAR with the seagrass absorption spectra of seagrass leaf blades, photosynthetically usable radiation (PUR) was modelled. PUR is a measure of what proportion of the available spectrum seagrasses actually take up and use in the photosynthetic process. While significant differences between irradiance as PAR and PUR were detected over different seasons in the absence of dredging in the harbour, the affect on seagrass condition from PAR recorded during shading studies and at permanent transect sites account for any overestimation of available light since studies took place under these naturally-shifted conditions.

While a summary of the approach and findings are presented as part of this report, the detailed findings of this work can be found in the final spectral profiling report (see Appendix II).

Rationale & Approach

The light environment of seagrasses growing adjacent to large port developments is commonly affected by regular dredging activities to create and maintain required draft depths for vessel movement. Dredging typically increases particulate matter in the water column which affects spectral quality. The size and type of particles re-suspended by dredging activity alter PAR transmission in a non-linear manner, with some wavelengths being more attenuated than others, resulting in a reduced light environment with a shift towards yellow wavelengths (Gallegos et al. 2009). Therefore, the minimum light requirement for seagrass health, as determined according to the full PAR spectrum available, may overestimate the actual light available for photosynthesis, as PAR measurements do not distinguish spectral shifts (Zimmerman 2003).

Describing the quality of light reaching seagrass in the Port of Gladstone under normal daily conditions (without dredging) and during dredging projects will enhance our knowledge of how increased turbidity due to dredging affects seagrass dynamics. Furthermore, knowledge of the spectral requirements of seagrass and measurements of a change in the availability of this spectrum during dredging may act as an indicator of seagrass health. This study measures the spectral transmittance and inherent optical properties of photosynthetically active radiation (PAR) to a maximum depth of 3.5 m which corresponds to <1% transmission of surface irradiance and therefore well below the theoretical compensation depth of the seagrass meadows (approx. 10%). It uses *in situ* data to create a radiative transfer light model to explore PAR and photosynthetically usable radiation (PUR) during an incoming and outgoing tide, during all seasons in the inner harbour.

Spectral transmittance measurements were collected at several different locations in Gladstone Harbour (Map 2). Measurements were made at each site during an incoming and outgoing tide at different times throughout the year. Four inner harbour sites were chosen, as well as one “outer” harbour reference site, at Pelican Banks. The boats position was recorded with a handheld GPS and the boat’s GPS. Water depth was measured with the boat’s echo sounder. The data presented in this report are taken from three sites within the harbour; one that is located downstream from the main dredge area (site 2), one within the small dock dredge area (site 4), one within the main dredging area (site 6), one located upstream in the Narrows (site 8), as well as one outer harbour site at Pelican Banks (reference site). These data help to characterise the inherent optical properties of the harbour under ’natural’ conditions and provide information on the extent of the dredge plume within the harbour.

In situ light attenuation and hyperspectral profiling:

The caged absorption/attenuation spectrophotometer (ACS; Wetlabs, USA) was lowered to the required depth in the water column using a winch. Care was taken to ensure that the instrument cage did not disturb the sediment surface resuspending fine sediments that could influence the data. Also, the angle of the boat with respect to the sun was taken into account to ensure that the measurements were not compromised by the boat’s shadow. Measurements were recorded continuously to permit detailed study of variation in the water’s optical properties with depth. Simultaneous measurements of downwelling irradiance were obtained using an integrated hyperspectral radiometer (RAMSES) that was attached to the ACS cage. The radiometer corrected for angle and inclination, providing fully calibrated radiometric data.

For the spectrally-integrated light profiles, PUR was calculated by weighting PAR with the absorption spectrum of *Z. capricorni* following the equation from Gallegos et al (2009):

$$PUR(z) = \int_{400}^{700} Q(\lambda, z)\, a_{Th}(\lambda) d\lambda$$

where, Q is the quantum flux, λ is the wavelength, (λ) is the absorption spectrum of *Z. capricorni* normalised to chl a. For comparison of attenuation rates, PAR and PUR were normalised to their surface values.

Key Findings

Comparing the November 2011 profiles at the cutter suction dredge site (site 6) with those measured earlier in the year (before dredging), the figures clearly show how the increase in suspended sediment load has had on the *in situ* light climate (Fig. 16). At site 6 (dredge site) irradiance reached a depth of 3.5 m during the incoming tide and 1.5 m during the outgoing tide in February 2011 (Fig. 16A and B). However, while the dredge was operating, downwelling irradiance was practically extinguished on both the incoming and outgoing tides (Fig. 16C and D), where light (>200 mW m^{-2} nm^{-1}) was unable to penetrate beyond the surface layer (<0.25m).

Additional profiles taken within the main cutter suction dredge site in November 2011 also provided greater spatial resolution of the plume dynamics both directly upstream and downstream of the dredge on ingoing and outgoing tides (see Appendix II for details).

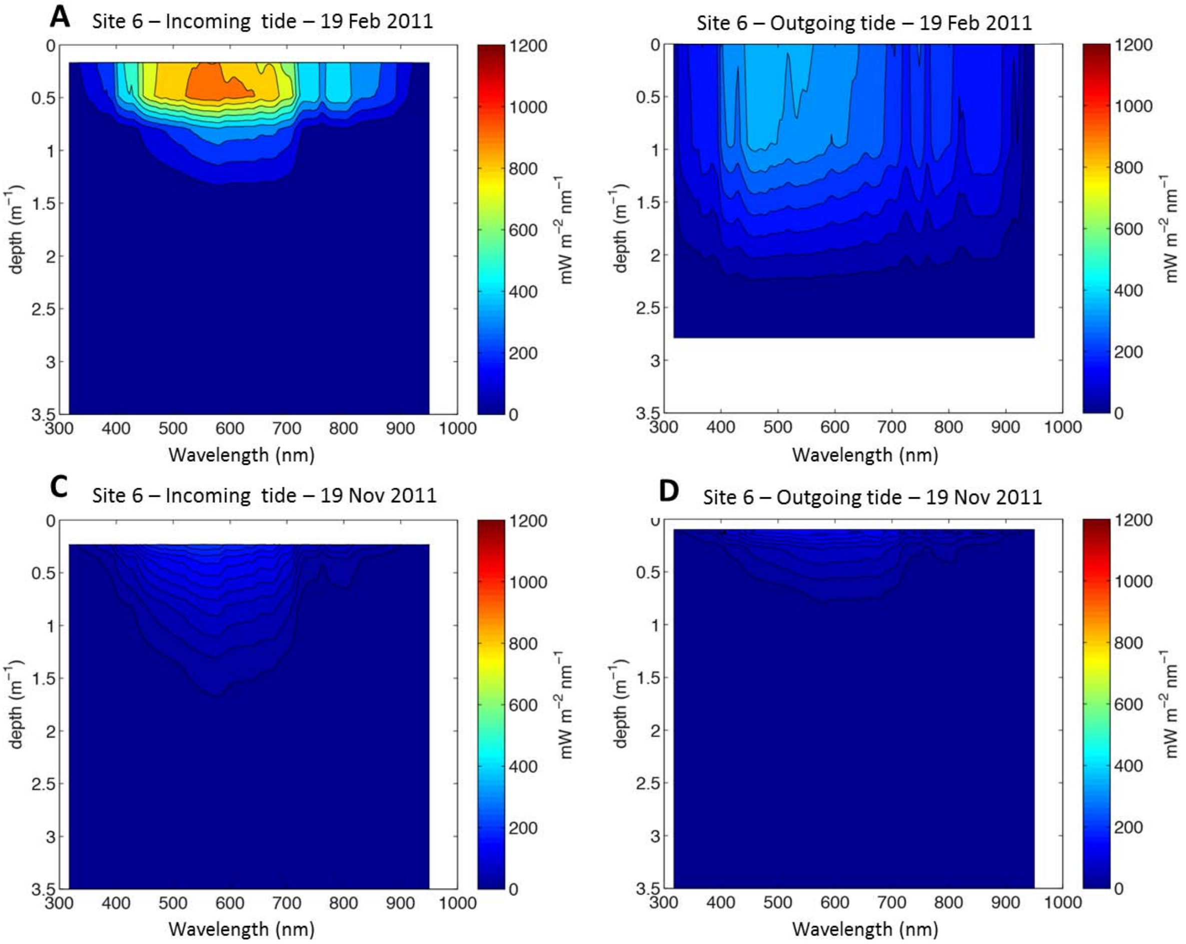


Figure 16. Hyperspectral depth profiles of downwelling irradiance at the main dredge site (site 6) over incoming and outgoing tides prior to dredging (A and B) and during the November dredge (C and D). Blank portions of the figures indicate absence of data or data trimming due to excess noise.

In the absence of dredging activity, the clearer waters around Pelican Banks showed maximum transmission in the green region of the spectrum (~550 nm), whereas at the dredge site (Site 6) maximum transmission was in the yellow region (~580 nm), demonstrating a spectral shift toward the less-photosynthetically available range of the spectrum. In other words, the spectral quality of the light differs between the inner and outer harbour sites, with a shift from yellow enhanced water within the harbour to waters outside the harbour at Pelican Banks, where greener wavelengths dominate the light climate at the depth of the seagrass meadows. Seagrass photosynthesis is sensitive to the spectral quality of light. They have high photosynthetic efficiency in the blue, poor efficiency in the green and yellow and maximum efficiency in the red regions—converse to the light transmission measured at the inner harbour sites. The turbid water removes most of the photosynthetically useful photons (i.e. blue and red) while transmitting the less photosynthetically active yellow photons. A clear picture of spectral characteristics could not be defined during the dredging activity due to the loss of light within the top centimetres of the water column hampering spectral resolution efforts.

PUR values (photosynthetically usable radiation) derived from the weighted absorption spectra of seagrass showed a reduction in irradiance during all tides and seasons (Table 1). This suggests an overestimation of PAR that is available for use by the seagrasses. In other words, seagrasses cannot utilise all wavelengths of light available to them due to the limitations of what portions of the spectra their pigments absorb and use in photosynthesis. Overestimation was on average 11 ± 1.14 % during an incoming tide and 4.6 ± 0.64 % during an outgoing tide. In winter, light for seagrasses was overestimated by 14.38%, which could prove quite significant for seagrass health under prolonged conditions.

Table 1. Maximum depth of scalar irradiance (I_{max}) and spectrally integrated light at 2.5 m depth for photosynthetically active radiation (PAR) and photosynthetically usable radiation (PUR) weighted by measured absorption spectrum of *Zostera capricorni* leaves. Percentage loss in irradiance when using PUR in replacement of PAR. Data are based on values derived from the radiative transfer model and are determined for inner harbour conditions in the absence of dredging.

Site	**Tide**	**I_{max} (m)**	**PAR ($\mu E\ m^{-2}\ s^{-1}$)**	**PUR ($\mu E\ m^{-2}\ s^{-1}$)**	**Overestimation (%)**
Autumn	**In**	3.2	86.60	78.15	9.75
	Out	2.6	40.40	39.08	3.24
Winter	**In**	4.1	126.00	107.80	14.38
	Out	2.9	40.20	38.02	5.42
Spring	**In**	3.8	190.00	171.87	9.99
	Out	3.0	77.20	74.25	3.81
Summer	**In**	3.3	108.00	97.38	9.83
	Out	2.9	66.00	62.07	5.95

The changes with tide and season were less defined when modelled for dredge conditions (see Appendix II for details). Overall, I_{max} was less than 2.0 m throughout the year and with tidal cycle and the light was approximately one-tenth of the irradiance modelled in the absence of dredging (Table 1). Therefore, the difference between PAR and PUR was negligible, resulting in no significant difference in light climate.

In summary, there are clear differences between the optical properties of the clear waters of Pelican Banks and the more turbid and highly attenuated waters within the harbour. The light profiles at Pelican Banks provide valuable information about *in situ* light conditions and optical properties that are able to sustain healthy seagrass meadows. Comparison of inner harbour sites have shown reduced light penetration with dredge activity both at the dredge site, as well as adjacent, upstream and downstream from the dredge site. The complete suite of sites within the harbour have provided greater resolution of inner harbour optical properties and resulting conditions from dredge activity. The calculated PUR values were as high as 14.9 % less than the PAR equivalent, highlighting the potential for a significant overestimation of light available for photosynthesis within the seagrass meadows from the telemeted PAR sensors. However, since the light trigger values developed for the light-based management approach have been developed from the shading studies and the assessment of seagrass condition and PAR at permanent transect sites throughout the harbour, any effect of PUR is built into the defined values presented in this report for management triggers.

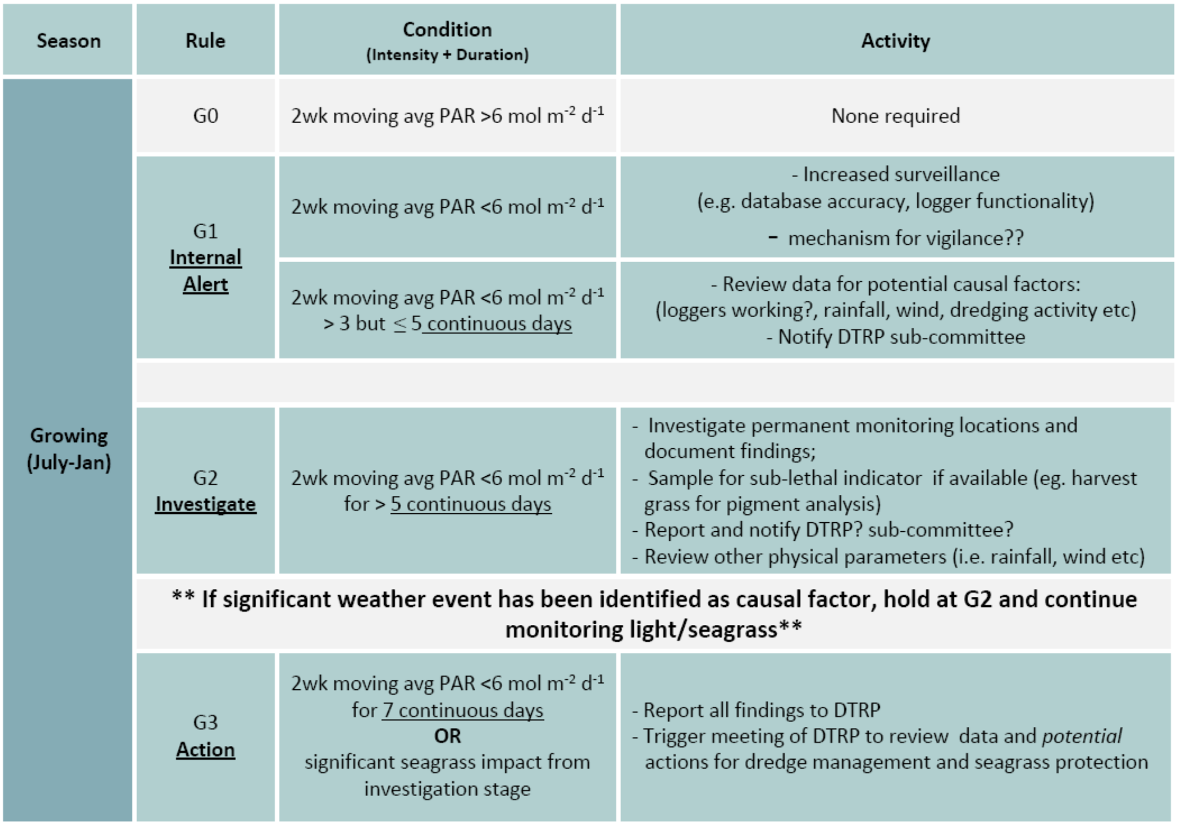
Draft Light Based Management Plan

The suite of seagrass studies outlined in the above report were designed to develop a light based management plan to assess the impacts associated with capital dredging for the Western Basin project in Gladstone. These studies provide key information to aid in developing suitable management guidelines, environmental trigger levels, and monitoring programs to protect seagrasses as the development proceeds.

To develop an effective predictive model of impact to seagrasses and appropriate dredge mitigation strategies to minimise the impacts, information on the distribution, light requirements and tolerances for Gladstone Harbour seagrass communities is required. A light based trigger value for impacts to *Zostera capricorni*, the dominant seagrass species in the harbour, and species with the greatest light requirement, was developed through research, monitoring and analyses of seagrass trends and available light conditions over the last two years. A rolling two week average of total daily PAR of 6 mol m^{-2} d^{-1} has been established as a working light trigger value, which if maintained should allow enough light to meet seagrass growth requirements and maintain seagrass health. It is important to note that this value is not the minimum light requirement (MLR) for effective seagrass photosynthesis. Rather, it is a value that incorporates other environmental drivers such as tidal cycles, seagrass meadow exposure events, temperature fluctuations and sediment chemistry dynamics that can influence seagrass condition together with light dynamics.

A draft outline for a working light management plan was established in parallel with the current turbidity based management plan and is not intended to replace existing dredge management strategies. It is intended to complement turbidity triggers by measuring a factor, PAR, that is ecologically relevant and directly effects the survivorship and health of seagrasses during dredging. In conjunction these two programs should work in concert such that the source of turbidity and the effect on seagrasses in the harbour can be better assessed for making management decisions. The light-based plan presented below is modelled from a draft schematic for the current turbidity management plan originally developed by Environmetrics Australia Pty Ltd (Table 2; Fox 2011).

Table 2. Draft light based management plan for the seagrass growing season (July-January)



Season	Rule	Condition (Intensity + Duration)	Activity
Growing (July-Jan)	G0	2wk moving avg PAR >6 mol m^{-2} d^{-1}	None required
	G1 Internal Alert	2wk moving avg PAR <6 mol m^{-2} d^{-1}	- Increased surveillance (e.g. database accuracy, logger functionality) - mechanism for vigilance??
		2wk moving avg PAR <6 mol m^{-2} d^{-1} > 3 but ≤ 5 continuous days	- Review data for potential causal factors: (loggers working?, rainfall, wind, dredging activity etc) - Notify DTRP sub-committee
	G2 Investigate	2wk moving avg PAR <6 mol m^{-2} d^{-1} for > 5 continuous days	- Investigate permanent monitoring locations and document findings; - Sample for sub-lethal indicator if available (eg. harvest grass for pigment analysis) - Report and notify DTRP? sub-committee? - Review other physical parameters (i.e. rainfall, wind etc)
	**** If significant weather event has been identified as causal factor, hold at G2 and continue monitoring light/seagrass****		
	G3 Action	2wk moving avg PAR <6 mol m^{-2} d^{-1} for 7 continuous days **OR** significant seagrass impact from investigation stage	- Report all findings to DTRP - Trigger meeting of DTRP to review data and *potential* actions for dredge management and seagrass protection

The draft plan assigns rules for potential conditions and associated actions that must be taken based on a decrease in the available light climate over a two week rolling average at one of the key receptor sites (i.e. permanent transect monitoring locations with associated PAR data). For instance, once the light climate at a given site drops below a mean of 6 mol m^{-2} d^{-1} for a two week period, an internal alert is triggered resulting in increased surveillance in data logger function, database accuracy and other potential mechanical factors in data collection. If the two-week rolling average of light remains below 6 mol m^{-2} d^{-1} for greater than three consecutive days, a further analysis of weather conditions, dredge activity or the like will be assessed. The Dredge Technical Reference Panel (DTRP) sub-committee established for the project may be notified at this stage if deemed necessary. The initial alert stage as well as additional alerts, investigations and actions presented in the above table are a suggested starting point for discussions by the DTRP committee members with all conditions and actions to be approved as part of an accepted working plan.

The number of consecutive days used for conditions and associated activities are currently based on *in situ* shading studies and trends in receptor site PAR and seagrass condition. For instance, no impact on seagrass above-ground biomass was recorded until at least 4 weeks of continuous shading of seagrass plots (see Shading Study section above). Therefore, using a two-week rolling average as a time scale for an initial alert trigger as well as up to 7 additional days below the trigger value for actions and investigations, ensures management action takes place within a conservative timeframe prior to seagrass loss (Table 1).

To test the sensitivity and effectiveness of the approach outlined in Table 1, the historical dataset on seagrass condition and light was examined under natural conditions prior to dredging commencing. For the management plan to be effective, the trigger needs to be sensitive enough to protect seagrass but not to result in continual major actions under natural conditions. To that end it would be expected that low level alerts would be reached occasionally, but major significant action (G3) should rarely be reached under natural conditions when seagrasses are healthy.

An example of how a particular receptor site would be triggered under natural conditions (prior to dredging commencing) is presented below for the Redcliffe Island site during the 2011 growing season period (Fig. 17). In general, the trigger value of less than 6 mol m^{-2} d^{-1} was reached on three occasions over the five month period. This is thought to be a reasonable number of action events for which management should be informed and activities critically reviewed in line with dredging activities and current turbidity values in the port in relation to seagrass health. At the outer harbour region around Pelican Banks where water quality is generally better light remained well above the 6 mol m^{-2} d^{-1} for the entire growing season (Fig 18).

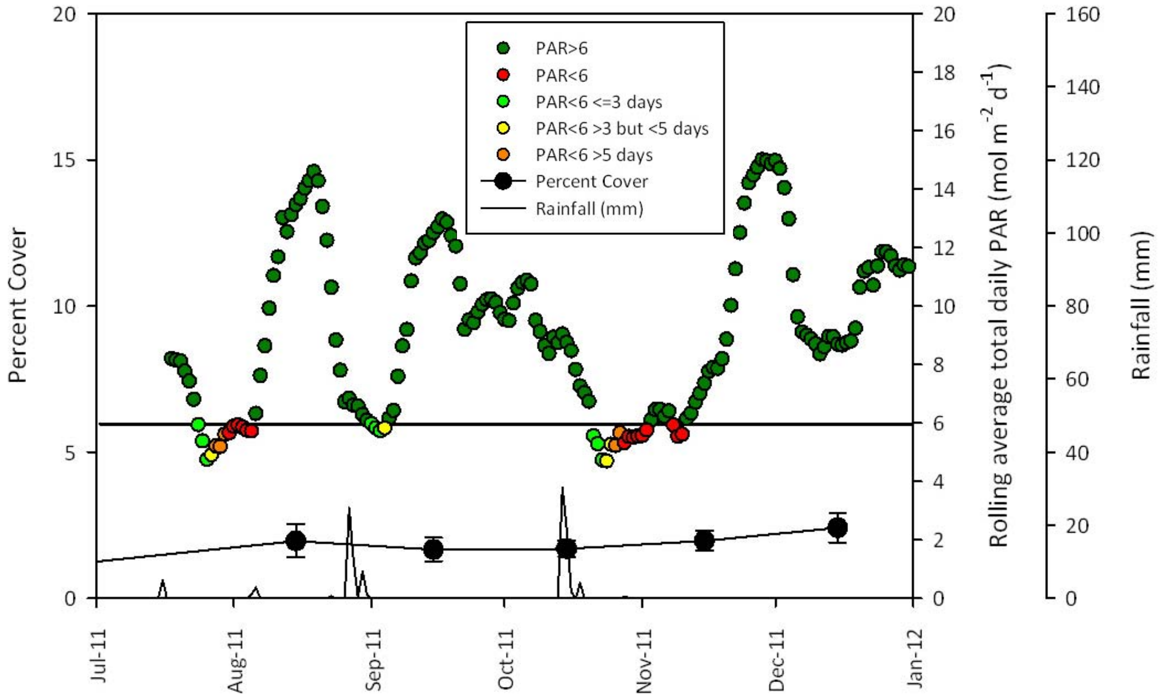


Figure 17. Seagrass percent cover and associated benthic PAR at Redcliffe Island permanent transect monitoring location for the 2011 growing season.

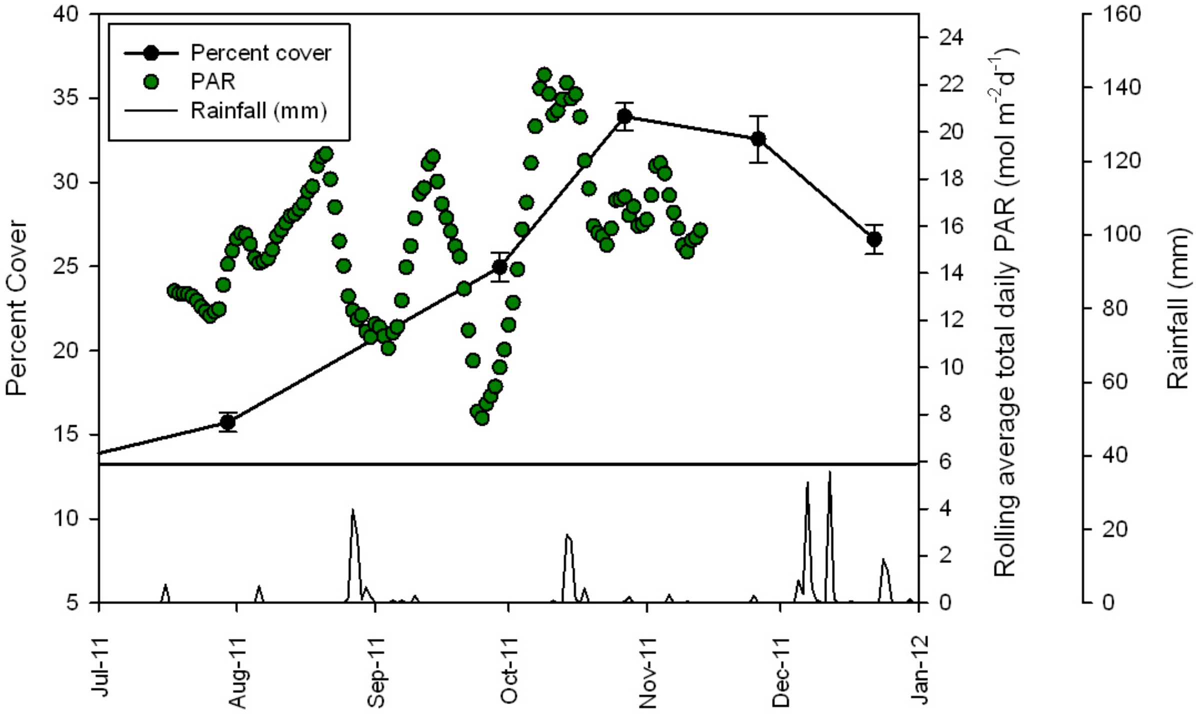


Figure 18. Seagrass percent cover and associated benthic PAR at Fishermans North permanent transect monitoring location for the 2011 growing season.

While results have produced an initial set of management triggers it is expected that ongoing work could lead to further improvements and modifications. This is in line with the adaptive management approach that has been set out in the Coordinator General's conditions of approval for the project. The final results of ongoing experiments and light trials will be reported in the coming months as well as a definitive set of management triggers following the completion of all studies and review of the light-based approach by the Dredge Technical Reference Panel.

References

Bach SS, Borum J, Fortes MD & Duarte CM (1998) Species composition and plant performance of mixed seagrass beds along a siltation gradient at Cape Bolinao, The Phillipines. *Marine Ecology Progress Series* 174:247-256

Björk M, Uka J, Weil A, Beer S (1999) Photosynthetic tolerances to desiccation of tropical intertidal seagrasses. *Marine Ecology Progress Series* 191:121-126

Chartrand KM, McCormack, CV & Rasheed MA (2011) Port Curtis and Rodds Bay seagrass monitoring program, November 2010. DEEDI Publication, Fisheries Queensland, Cairns, 57pp.

Chartrand KM, McKenna SA, Petrou K, Jimenez-Denness I, Franklin J, Sankey TL, Hedge SA, Rasheed MA and Ralph PJ (2010) Port Curtis Benthic PrimaryProducer Habitat Assessment and Health Studies Update: Interim Report December 2010. DEEDI Publication. Fisheries Queensland, Cairns, 128 pp.

Collier, CJ (2006) Thesis: Characterising response of the seagrass *Posidonia Sinuosa* to changes in light availability. Edith Cowan University, 201 pp.

Collier CJ, Lavery PS, Ralph PJ, and Masini RJ (2009) Shade-induced response and recovery of the seagrass Posidonia sinuosa. *Journal of Experimental Marine Biology and Ecology* 370**:** 89-103.

Collier, CJ, Uthicke, S and Waycott, M (2011) Thermal tolerance of two seagrass species at contrasting light levels: Implications for future distribution in the Great Barrier Reef. *Limnology and Oceanography* **56:** 2200-2210.

Duarte CM, Terrados J, Agawin NSR, Fortes MD, Bach S & Kenworthy WJ (1997) Response of a mixed Philippine seagrass meadow to experimental burial. *Marine Ecology Progress Series* 147:285-294

Erftemeijer PLA and Lewis III RRR (2006) Environmental impacts of dredging on seagrasses: A review. *Marine Pollution Bulletin* 52:1553-1572

Freeman AS, Short FT, Isnain I, Razak FA, Coles RG (2008) Seagrass on the edge: Land use practices threaten coastal seagrass communities in Sabah, Malaysia. *Biological Conservation* 141:2993-3005.

Fox D (2011) Interim Turbidity Triggers for the Western Basin Project – A Discussion Paper. Environmetrics Australia Pty Ltd, 19 pp.

Gallegos CL, Kenworthy WJ, Biber PD and Wolfe BS (2009) Underwater spectral energy distribution and seagrass depth limits along an optical water quality gradient. *Smithsonian contributions to the marine sciences.* 38: 359-367.

Grice AM, Loneragan NR, Dennison WC, (1996) Light intensity and the interactions between physiology, morphology and stable isotope ratios in five species of seagrass. *Journal of Experimental Marine Biology and Ecology* 195**:** 91-110.

Hemminga MA, Duarte CM (2000) Seagrass Ecology, Vol. Cambridge University Press

Kirkman H (1978) Decline of seagrass in northern areas of Moreton Bay, Queensland. Aquatic Botany 5: 63-76

Leuschner C, Landwehr S and Mehlig U (1998) Limitation of carbon assimilation of intertidal Zostera noltii and Z. marina by desiccation at low tide. *Aquatic Botany* 62:171-76.

McKenzie LJ (1994) Seasonal changes in biomass and shoot characteristics of a *Zostera capricorni* Aschers. dominant meadow in Cairns Harbour, northern Queensland. Mar *Freshwater Research* 45:1337-1352

Mellors JE (1991) An evaluation of a rapid visual technique for estimating seagrass biomass. Aquat Bot 42:67-73

Ralph PJ, Durako MJ, Enriquez S, Collier CJ, Doblin MA (2007) Impact of light limitation on seagrasses. *Journal of Experimental Marine Biology and Ecology* 350(1-2): 176-193.

Rasheed, M.A. (1999) Recovery of experimentally created gaps within a tropical Zostera capricorni (Aschers.) seagrass meadow, Queensland Australia. Journal Experimental Marine Biology and Ecology 235: 183-200

Thomas R, Unsworth RKF and Rasheed MA (2010) Seagrasses of Port Curtis and Rodds Bay and long term seagrass monitoring, November 2009. (DEEDI, Cairns).

Vermaat JE, Agawin NSR, Fortes MD, Uri JS, Duarte CM, Marba N, Enriquez S & van Vierssen W (1997) The capacity of seagrasses to survive increased turbidity and siltation; the significance of growth form and light use. *Ambio* 26: 499-504

Zimmerman RC (2003) A bio-optical model of irradiance distribution and photosynthesis in seagrass canopies. *Limnology and Oceanography*, 48: 568-585.

APPENDIX I

Leaf photoacclimatory responses of the seagrass *Zostera capricorni* to light attenuation and spectral shifts

Petrou K and Ralph PJ, and Chartrand KM,

SUMMARY

This study was conducted to investigate the impact of reduced light as well as changes in the spectral quality of light can have on seagrass photosynthetic performance and health. A number of changes in morphological, bio-optical and physiological parameters were detected in seagrass acclimated to prolonged shaded conditions. However, no strong evidence for an effective sub-lethal indicator of light limitation for *Z. capricorni* was determined; a sub-lethal indicator would respond before the leaf dies. There is still some carbon incorporation (^{13}C) and real time PCR data to be processed which may confirm a decline in the plant resilience due to prolonged shading. We were looking for a sub-lethal indicator of extreme shade adaptation of the photosystems after which the plant can no longer maintain a positive carbon balance. We have used a wide range of measures to monitor the process of acclimation in their metabolic pathways; however, we have not been able to identify the threshold beyond which senescence occurs.

This study commenced in October 2011 with preliminary findings reported in November 2011. Since then, additional physiological and morphological measurements were made after ten weeks of acclimation under the different light treatments and are reported here. The study showed:

- Significant changes in seagrass morphology were detected after approx. 8 weeks of shading, with a decline in the number of leaves per shoot and total leaf volume in the high shaded treatments. There were also significant shifts in leaf absorption properties of the highest shade treatment (ND2). However, no differences were detected in the response to changes in light quality as a result of spectral shift ie: between the neutral density and yellow-enhanced treatments.

- No significant differences in photosynthetic characteristics were detected after 10 weeks of light limitation (low and high shading with and without yellow-enhanced spectral shift). Similarly, no differences in photosynthesis and respiration rates were detected between shade treatments. This lack of differences indicates that *Z. capricorni* was able to successfully photoacclimate to each of the shade treatments in the study, before the leaf finally senesces.

- Photoacclimation in *Z. capricorni* is supported by the RLC data, which shows significant differences in photosynthetic parameters α, $rETR_{max}$ and I_K after 4 weeks (T1) under the highest shade treatment (ND2), but that these differences were no longer evident after 10

weeks of acclimation when the plants have adapted their compensation irradiance (photosynthesis = respiration) (Fig. 6).

- The pigment analyses (HPLC) showed clear increases in light harvesting pigment concentrations and chlorophyll *b:a* ratios with increased shading, consistent with shade acclimation, but no differences with yellow-enhanced treatment. No differences were detected in the relative proportion of photoprotective pigments; however, there was a decline in the violaxanthin de-epoxidation state (VDE) under the highest shade treatments.

Overall, these data indicate that there is very little change in the physiology and morphology of *Zostera capricorni* with changes in light quality and quantity after 10 weeks at 25°C.

ADDITIONAL NOTES:

1. The water temperature used in this study (25°C) was chosen to represent the mean annual temperature experienced by the seagrass in Gladstone harbour and it is considerably lower than the summer mean (29°C). It has been shown that *Z. capricorni* undergoes increased physiological stress at warmer temperatures. Thus, it is possible that by using a water temperature of only 25°C, the plants were more able to cope with a reduced light climate, whereas if this trial was performed at a higher water temperature, the seagrass would have been under greater physiological stress. This is supported by the field studies, which showed that greater photosynthetic stress responses were evident during the warmer months (growing season).

2. The light levels were not identical between the neutral density and yellow-enhanced treatments of low and high shading. Instead, the treatments provided a gradient of light levels. The data have shown that under the highest shade treatment (ND2) of 2.73 ± 0.46 mol photons m^{-2} d^{-1}, *Z. capricorni* exhibits signs of morphological and physiological stress and some minor effects when acclimated to 3.45 ± 0.58 mol photons m^{-2} d^{-1}, as was observed in the high-shade yellow-enhanced treatment.

MINIMUM LIGHT REQUIREMENT:

Based on the data presented in this study alone, an average light threshold above 3.45 mol photons m^{-2} d^{-1} for up to 10 weeks would be reasonable for maintaining seagrass health when plants are maintained at 25°C. However, this was a controlled laboratory study and in nature, there are many environmental variables at play (water temperature, tidal exposure, sediment and nutrient loading) that can greatly influence seagrass resilience. As such, to calculate a minimum light threshold, allowance for these significant factors needs to be applied. Therefore, a more conservative value needs to be considered, as field studies have shown a loss in seagrass extent and viability under prolonged low- light climates.

Background and Approach

The intertidal seagrass meadows of Gladstone Harbour are frequently subjected to lower than optimal light levels as a result of high turbidity from large tidal fluctuations, as well as the additional stress of dredging activity. Particles causing the turbidity reduce the light to the seagrass, but they also change the quality of light reaching the seagrasses (via scattering), where the light becomes enhanced in the yellow region of the spectrum and subsequently reduced in other regions of the spectra. The dominant photosynthetic pigments in seagrasses are chlorophylls *a* and *b*, which absorb in the blue and red wavelengths (400-500 nm and 650-700 nm) of the light spectrum. Thus, enhanced yellow light conditions have the potential to shift the light away from the wavelengths capable of supporting photosynthesis.

This study investigates the influence that shading and yellow-enhanced shading have on the photosynthetic activity (pigments, oxygen evolution rates, respiration and chl *a* fluorescence properties) of the intertidal seagrass species *Zostera capricorni* at 25°C. The study is designed to enable us to more precisely understand the relationship between seagrass light absorptance and dredge-related reductions in the light environment.

MATERIALS and METHODS

Sample collection

Zostera capricorni shoots were collected from an intertidal meadow at Pelican Banks, Port Curtis QLD. Square plots each containing 10 to 20 shoots (15.3 x 10 x 5 cm) were cut out of the sediment to obtain intact plants with roots and rhizomes, and placed in a tray with a small amount of water. The trays were stored in large nally bins overnight, packed into ziplock bags, and air freighted to the University of Technology, Sydney for a glasshouse laboratory experiment.

Design and setup

The sediment in the plastic trays was covered with a ~ 1 cm layer of washed river sand. A total of 90 trays were prepared, each containing 10 to 20 individual shoots. The replicate treatment units consisted of 30 white plastic tubs (30 x 21 x 18 cm) each filled with Sydney Harbour seawater (12 L) and each containing three plastic trays with seagrass shoots and maintained under continuous air bubbling.

The 30 white tubs were randomly distributed into four large temperature control tanks (7-8 white tubs per tank), each filled with freshwater and equipped with heater/chillers. After two weeks acclimation to the new growth conditions at ambient temperature (~ 25°C), separate light treatments were applied. Each white tub was randomly assigned one of five sets of light filters to modify light intensity, as well as spectral quality: clear (100% incident irradiance), 50% neutral density shading (low shade), 20% neutral density plus yellow enhanced (low yellow enhanced), 75% neutral density (high shade), 60% neutral density plus yellow enhanced (high yellow enhanced). Six replicate white tubs were allocated one of the five light treatments, and maintained for a total experimental period of 10 weeks. To minimise the effects of light gradient within the glasshouse, the white tubs were switched twice a week between the large holding tanks. Water changes (two-thirds fresh seawater to one third existing tank water) were performed twice a week.

Temperature in the white tubs were monitored by thermal sensors and irradiance was monitored by PAR loggers (Odyssey) equipped with each light treatment filter set and placed above the tanks. Spectral irradiance transmitted through each treatment filter set was measured from 400 to 700 nm at a 1 nm resolution using a spectrophotometer (USB2000 Ocean Optics) fitted with a 2 m optical fibre and a cosine correcting diffuser (CC-3 Ocean Optics) at the light receiving end. The fibre optic spectrometer was calibrated against a deuterium tungsten halogen calibration standard lamp (DH-2000-CAL Ocean Optics). The fibre tip was placed within an experimental tub, positioned vertically and facing upwards, and downwelling irradiance spectra were recorded under each filter set (clear, 50% neutral density, 20% neutral density plus yellow enhanced, 75% neutral density, 60% neutral density plus yellow enhanced).

Seagrass samples were harvested for i) morphological assessment over 57 days. Harvesting was also conducted for ii) measurement of productivity within the tubs ie*:* using *in situ* conditions iii) leaf pigment and light absorption properties and iv) photosynthetic response to increased irradiance at the start of the experiment (T0), at the end of the 4-week (T1) treatment period, and again after 10 weeks (T2).

Chlorophyll *a* fluorescence

Chlorophyll *a* fluorescence measurements were conducted using a Pulse Amplitude Modulated fluorometer (Diving-PAM; Walz GmbH, Effeltrich, Germany). Steady state light curves (SSLC) were measured by exposing the leaves to 4 different light levels ranging from 20 to 1500 µmol photons m^{-2} s^{-1} and saturation pulse after 35 min light exposure and after 35 min dark, respectively. All measurements were performed on the second youngest leaf blade to be comparable across all plants. The leaves were acclimated at 50 µmol photons m^{-2} s^{-1} for a minimum of 30 min prior to SSLC measurement to remove the influence of the time of day. Relative electron transport rate (rETR) was calculated as the product of effective quantum yield (Φ_{PSII}) and irradiance (µmol photons m^{-2} s^{-1}). Data were fitted according to the double exponential function as in Ralph and Gademann (2005) and three photosynthetic parameters; maximum rate of electron transport (rETR$_{MAX}$), light utilisation efficiency (α) and minimum saturating irradiance (I_K) were derived from these curves.

Oxygen evolution

Rates of photosynthesis for all light treatments were determined by measuring oxygen (O_2) evolution inside 5 ml air- and water-tight incubation bottles equipped with oxygen sensitive luminescent material (SDR SensorDish Reader Presens, Germany). Leaves were collected from each replicate tub, gently scraped clean of epiphytes, acclimatised at 50 µmol photons m^{-2} s^{-1} for a minimum of 30 min and placed into the incubation bottles filled with 0.2 µm filtered seawater (–1-2 leaves per bottle, n=4 bottles per light treatment). Oxygen concentrations within each bottle were first measured at the start (t0) and after 20 min (T1) and 35 min (T2) light (ranging from 20 to 1500 µmol photons m^{-2} s^{-1}) incubation period, and after the same time periods of dark incubation. Rates of gross oxygenic production within each bottle were determined as: $P = P_N - R_D$, where P_N and R_D are the net photosynthesis measured in the light and the respiration in the dark, respectively:

$$P_N = [C(t2) - C(t1)] / (t2-t1)$$

$$R_D = [C(t1) - C(t0)] / (t1-t0)$$

Where C(tx) is the oxygen concentration at time x. Bottle productivity was normalized to total photosynthetic surface area in the bottle and is reported as µmol O_2 cm^{-2} h^{-1}.

HPLC pigment analyses

Pigment concentrations were determined using high performance liquid chromatography (HPLC). One second youngest leaf blade from each treatment was selected and immediately frozen in liquid nitrogen. Pigments were extracted by grinding and analysed according to the methods of van

Heukelem and Thomas (2001) with the only modification being an extra filtration step through 0.2 µm PTFE 13 mm syringe filters (Micro-Analytix Pty Ltd). Clarified samples were stored in amber HPLC glass vials (Waters Australia Pty Ltd, Australia) and were stored at -80 °C overnight before analysis. The HPLC system included a pump, temperature controlled auto-injector (Waters Australia Pty Ltd, Australia), C8 column (150 x 4.6 mm; Eclipse XDB), and photodiode array detector (Waters Australia Pty Ltd, Australia). Pigments were identified by comparison of their retention times and spectra using calibration standards (DHI, Denmark) for each pigment. Peaks were integrated using graphical software (Empower Pro Waters Australia Pty Ltd, Australia) and checked manually to confirm the accuracy of the peak baselines and the similarity of the integrated peaks to that of the standard.

Total light harvesting pigment content (µg g^{-1} wet) was calculated as the sum of chlorophylls *a, b,* divinyl *a, b,* beta carotene and lutein, and the pigment ratio of Chl *b:a* was compared for all treatments. The proportion of photoprotective pigments (VAZ pool) to total pigment was calculated as the sum of antheraxanthin (A), zeaxanthin (Z) and violaxanthin (V), divided by the total pigment pool, while the de-epoxidation state of violaxanthin (a measure of violaxanthin conversion to the photoprotective zeaxanthin) was calculated as ((zeaxanthin) + (0.5*antheraxanthin)) / (V + A + Z).

Leaf spectral absorption

The leaf-specific absorptance (A(λ)) is a measure of the fraction of photosynthetically active radiation (PAR) captured by the leaf's photosynthetic pigments, and the chlorophyll a specific optical cross section (a^*, m^2 mg[chl $a]^{-1}$) is a measure of chlorophyll use efficiency. These parameters were measured on the same seagrass blade (the one incubated at 20 µmol photons m^{-2} s^{-1}) used in the O_2 measurements. After completion of the O_2 incubations, the seagrass blades were placed into 50 ml sample jars and left in each of their original light treatment tubs for 30 min prior to spectral absorption measurements.

Leaf spectral transmittance was measured from 400 to 750 nm at 1 nm resolution using a fibre optic spectrometer (USB2000+ Ocean Optics) interfaced with an integrating sphere (FOIS-1, Ocean Optics, USA). A tungsten halogen light source (Ocean Optics LS-1) was adjusted to completely irradiate the 9.5 mm diameter sample port of the integrating sphere. A seagrass leaf was placed on a clear microscope slide in front of the opening port of the sphere, and covered with a second slide obstructed with black tape except for a small ~ 2 mm^2 opening to mask the portion of the sample port not covered by leaf tissue. Leaf spectral transmittance (T(λ)) for each replicate was determined and referenced to the slide and tape without a leaf in place.

The leaves were then replaced into their sample jars and original light treatment tubs for a further 30 min prior to snap freezing in liquid nitrogen for subsequent determination of chlorophyll concentration. Leaf-specific absorptance A(λ) was calculated as (1 – T(λ)- A(750 nm)), where A(750 nm) = 1 – T(750 nm) is used as a correction for scattering and non-photosynthetic absorptance. Leaf-specific photosynthetic absorptance $A_{⍰}$(PAR) was calculated as the spectral average of A(λ) over the spectral range 400-700 nm. The chl *a*-specific optical cross-section a*(λ) was calculated from the absorptance A(λ) by normalizing a(λ) to the area specific Chl *a* concentration :

$$a^*(\lambda) = (-\ln [1-A(\lambda)]\ )/\ [\mathrm{Chl}\ a].$$

Chlorophyll concentration

Leaves were digitally photographed and their surface area was determined using image analysis software (ImageJ) prior to extraction in ice cold 80% acetone using a mortar and pestle with clean sand. Concentrations of chlorophyll *a* (Chl *a*) and chlorophyll *b* (Chl *b*) were determined spectrophotometrically using the equations and extinction coefficients of Jeffrey and Humphrey (1975).

IRGA measurements: CO_2 fluxes

Fluxes of CO_2 were measured using a newly developed metabolic chamber system. The setup included a water phase (245 ml), within a perspex cuvette (300 ml volume), through which a closed gas circuit passes (320 ml volume) connected to an infra-red gas analyser (IRGA; MGA3000, ADC Gas Analysis), thus allowing CO_2 analysis in the gas phase. The gas phase effervesces through the water phase at a rate of 500 ml min^{-1}. An LED-array fitted to the back of the cuvette is used to provide even illumination at increasing intensities, while a temperature sensor and 4 π-light sensor allows for temperature and light intensity reading on the surface of the seagrass blades. Carbon uptake and release rates are recorded using a custom LabView program (National Instruments) connected to a laptop.

Seagrass samples were processed over three days. Prior to measurement, 10 to 20 healthy leaves (i.e. green) were collected from one treatment unit (white tub) and incubated under controlled light (control: 50 µmol photons m^{-2} s^{-1}; shade: 25 µmol photons m^{-2} s^{-1} neutral density; yellow shade: 25 µmol photons m^{-2} s^{-1} yellow enhanced) and temperature 25˚C for 30 min. All harvested leaves were gently wiped clean of epiphytes and placed into the IRGA chamber. The chamber was filled with 245 ml of filtered seawater (0.25 µm filter pore size) from the tanks and the gas and water phases were allowed to equilibrate ~ 10 min prior to the first measurement. Concentration of CO_2 within the chamber was monitored until a constant CO_2 release or uptake rate was observed during exposure to 200 µmol photons m^{-2} s^{-1} and in the dark, respectively.

Data analysis

Differences in physiological and pigment responses of seagrass between light treatments were assessed using a one-way analysis of variance (ANOVA) at $\alpha = 0.05$. To ensure that the assumption of equal variances for all parametric tests was satisfied, a Levene's test for homogeneity of variance was applied to all data *a priori*. For data that failed to meet the assumptions of ANOVA, data were transformed. All analyses were performed using SPSS statistical software (version 17.0.0 2008, Pennsylvania, USA).

RESULTS

Light conditions:

Light reaching seagrass in the shade treatments was approximately 50% and 25% of controls in the low (ND1) and high (ND2) density treatments, respectively. While in the yellow-enhanced shade treatments, the light reaching the seagrass was approximately 80% and 40% of the control in YE1 and YE2, respectively (Fig. 1). Over the 10-week acclimation period, mean daily PAR was 8.93 ± 1.71 mol photons m^{-2} d^{-1} in the control treatment compared with 4.72 ± 0.80 and 2.73 ± 0.46 mol photons m^{-2} d^{-1} in the low (ND1) and high (ND2) shade treatments. Average PAR in the low (YE1) and high (YE2) shade yellow-enhanced treatments were 6.54 ± 1.10 and 3.45 ± 0.58 mol photons m^{-2} d^{-1}, respectively over the 10-weeks of acclimation (Fig. 1). These shade treatments form a light climate gradient from approximately 3-9 mol photons m^{-2} d^{-1}. They are designed to help determine a minimum light threshold for sustaining healthy populations of *Z. capricorni*.

Morphometrics:

Seagrass morphometrics showed a change in shoot density between the control and the ND2 treatment, with a significant decline in shoot density ($P = 0.05$) with time in the high shade treatment (Fig. 2). A similar trend was seen in the yellow enhanced high shade treatment (YE2), but was not significantly different from the control at 57 days (Fig. 2). In the highest shade treatment (ND2) many of the trays had no leaves left after 57 days (T2). Some of the loss was directly caused by the low light, but some was the result of grazing. To remove that grazing influence, the average leaves per shoot and average leaf volume were used. The number of leaves per shoot and shoot volume were significantly reduced in the high shade treatment by the final time point of sampling ($P = 0.014$; $P = 0.046$, respectively; F ig 3a and 3b, respectively).

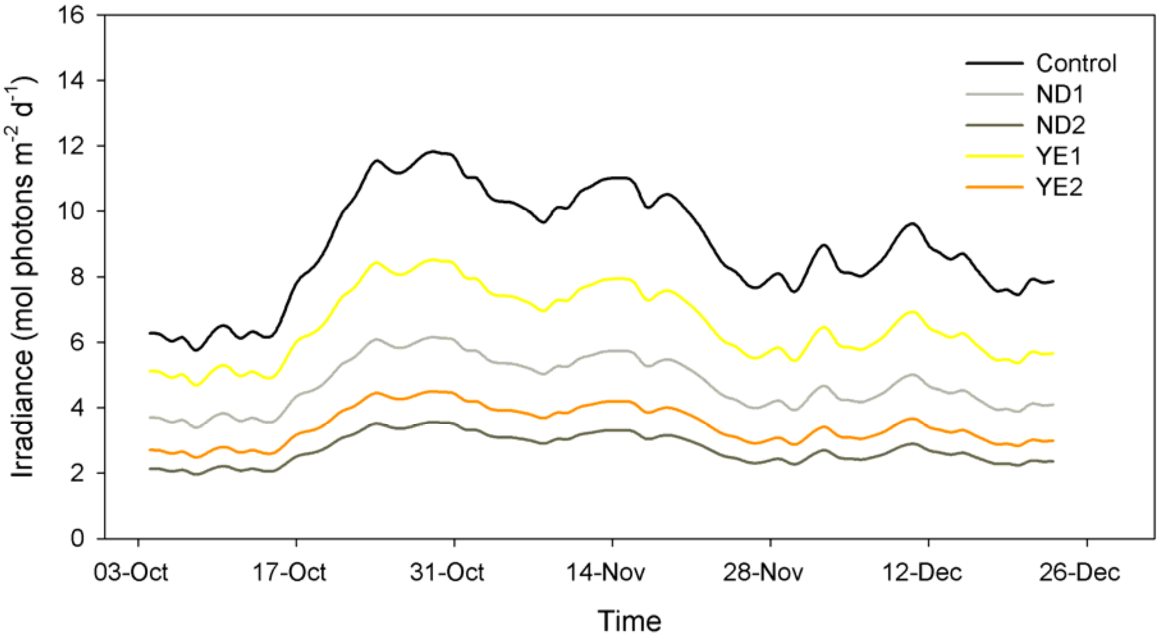


Figure 1: Glasshouse total daily irradiance (mol photons m^{-2} d^{-1}) received under control, neutral density and yellow enhanced light treatments. Lines represent 7-day rolling average. ND = neutral density shading, YE = yellow enhanced shading; 1 = low shading, 2 = high shading.

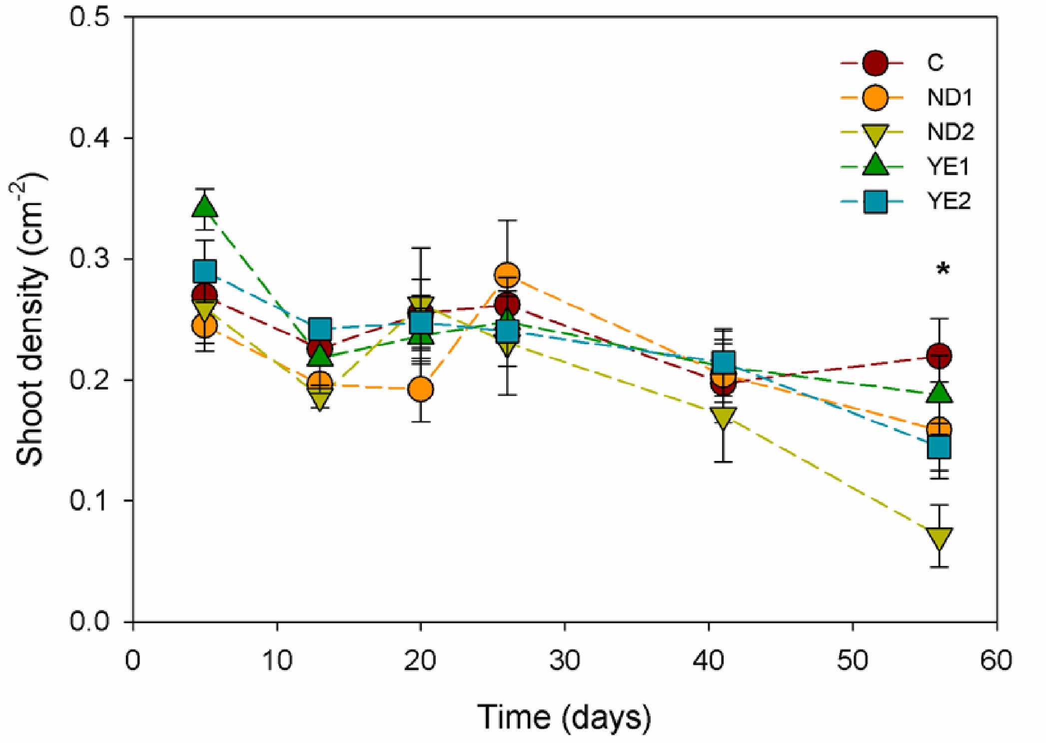


Figure 2: Seagrass shoot density (cm^{-2}) over entire study period (57 days) in *Z. capricorni* under control (C) and four light treatments, with the water maintained at 25°C. Data represent mean ± SEM (*n* = 6), *indicates significance at α = 0.05. ND = neutral density shading, YE = yellow enhanced shading; 1 = low shading, 2 = high shading.

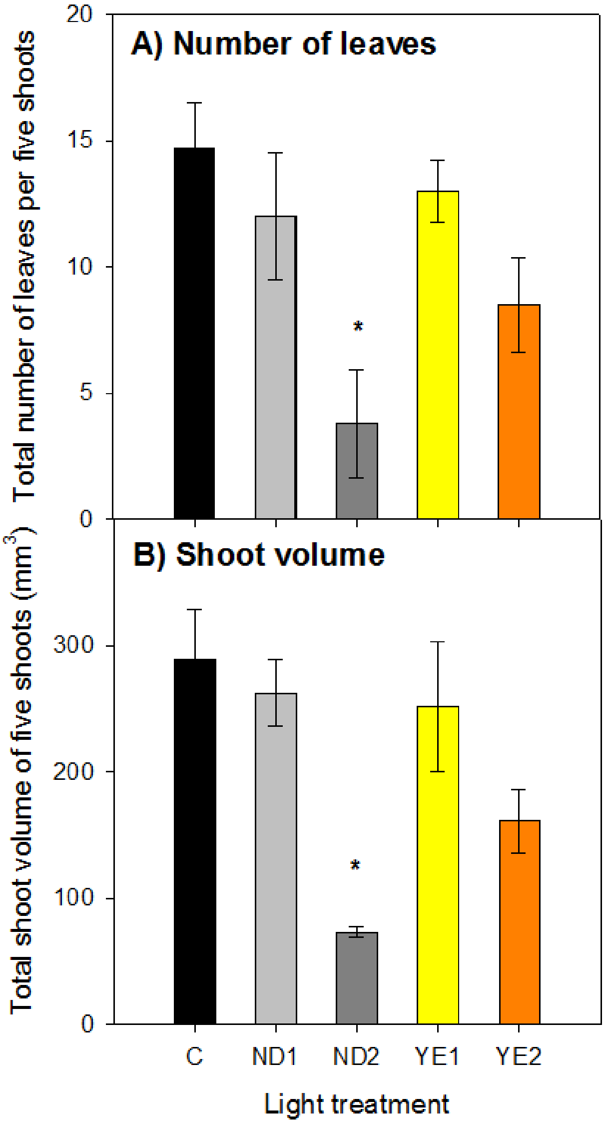


Figure 3: Seagrass total number of leaves per 5 shoots (A) and total shoot volume per 5 shoots in mm^3 (B) of *Z. capricorni* after 57 days of acclimation to different light conditions. Data represent the mean ± SEM (*n* = 6), *indicates significance at α=0.05. ND = neutral density shading, YE = yellow enhanced shading; 1 = low shading, 2 = high shading.

Bio-optical properties, chlorophyll *a* fluorescence and HPLC pigments:

After the first 4 weeks, no changes in chlorophyll *a* specific optical cross section a* (chlorophyll use efficiency) were observed in *Z. capricorni* between shade treatments (Fig. 4a and c). However, after 10 weeks, shade treatments showed a significant decline in the chlorophyll *a* specific optical cross section (a*; chlorophyll use efficiency) at 600 (Fig. 4cb) and 680 nm (Fig. 4d) wavelengths in the high neutral density (ND2) and yellow enhanced (YE2) treatments.

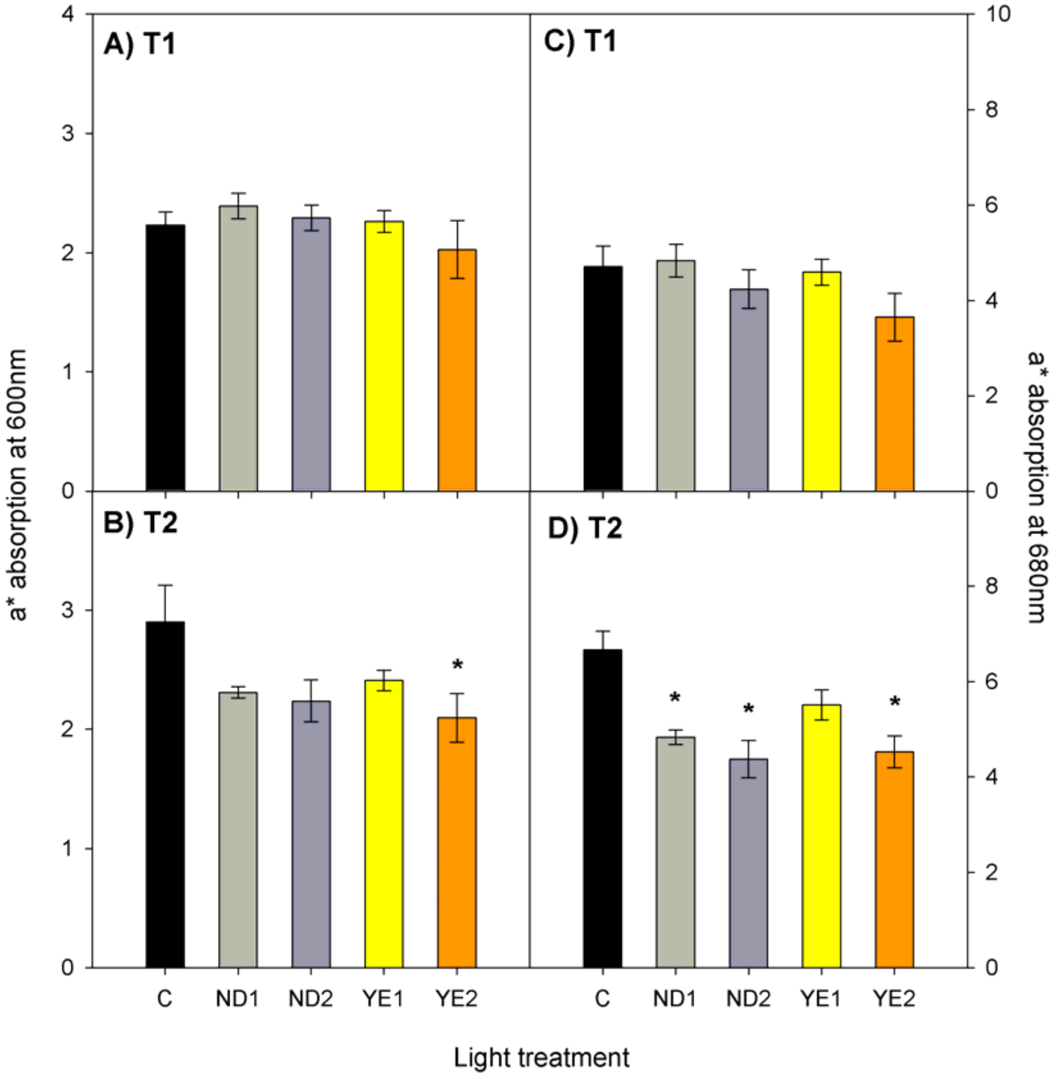


Figure 4: Leaf chlorophyll *a* specific optical cross section (*a**; m^2 mg chl a^{-1}) of *Z. capricorni* at 600 nm (A and B) and 680 nm (C and D) after 4 weeks (T1) and 10 weeks (T2) of acclimation to light treatments at 25°C. Data represent means ± SEM (*n* = 6), *indicates significance at α=0.05. ND = neutral density shading, YE = yellow enhanced shading; 1 = low shading, 2 = high shading.

Effective quantum yield as a function of irradiance did not vary between shade treatments throughout the study (Fig. 5a and b). Similarly, there was no change in relative electron transport rates (rETR) measured as a function of irradiance between light treatments in *Z. capricorni* over the course of the study (Fig. 5c and d).

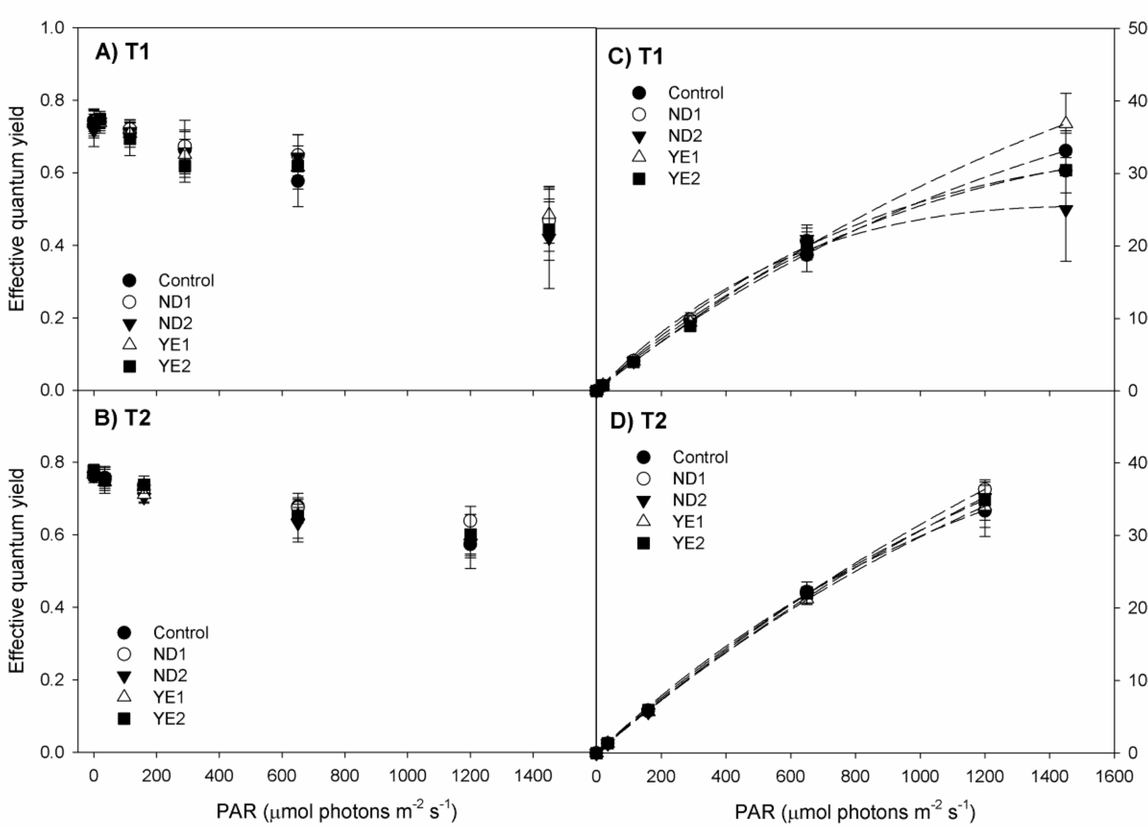


Figure 5: Effective quantum yield after 4 (A) and 10 weeks (B) and relative electron transport rates (rETR) after 4 (C) and 10 weeks (D) of acclimation light treatments at 25°C. Data represent mean ± SEM (*n* = 6). ND = neutral density shading, YE = yellow enhanced shading; 1 = low shading, 2 = high shading.

A significant increase (*P* = 0.010) in the light utilisation efficiency (α) of *Z. capricorni* was detected after 4 weeks under the highest neutral density (ND2) shade treatment (Fig. 6a). However, after 10 weeks of acclimation this difference was no longer detectable (Fig. 6b). There was a significant decline in maximum relative electron transport rate ($rETR_{max}$) under the high shade treatment after 4 weeks of acclimation (*P* = 0.015; Fig 6c), which again, was no longer evident after 10 weeks (Fig. 6d). Minimum saturating irradiance (I_K) was significantly lower in the high shade treatment (ND2) after 4 weeks (Fig. 6e), but no differences were detected after 10 weeks (Fig. 6f). Interestingly, there seemed to be a period of physiological adjustment, where at 4 weeks (T1) differences in photosynthetic parameters were evident in response to shading, but after 10 weeks the seagrass had completely photo-acclimated to their new light conditions.

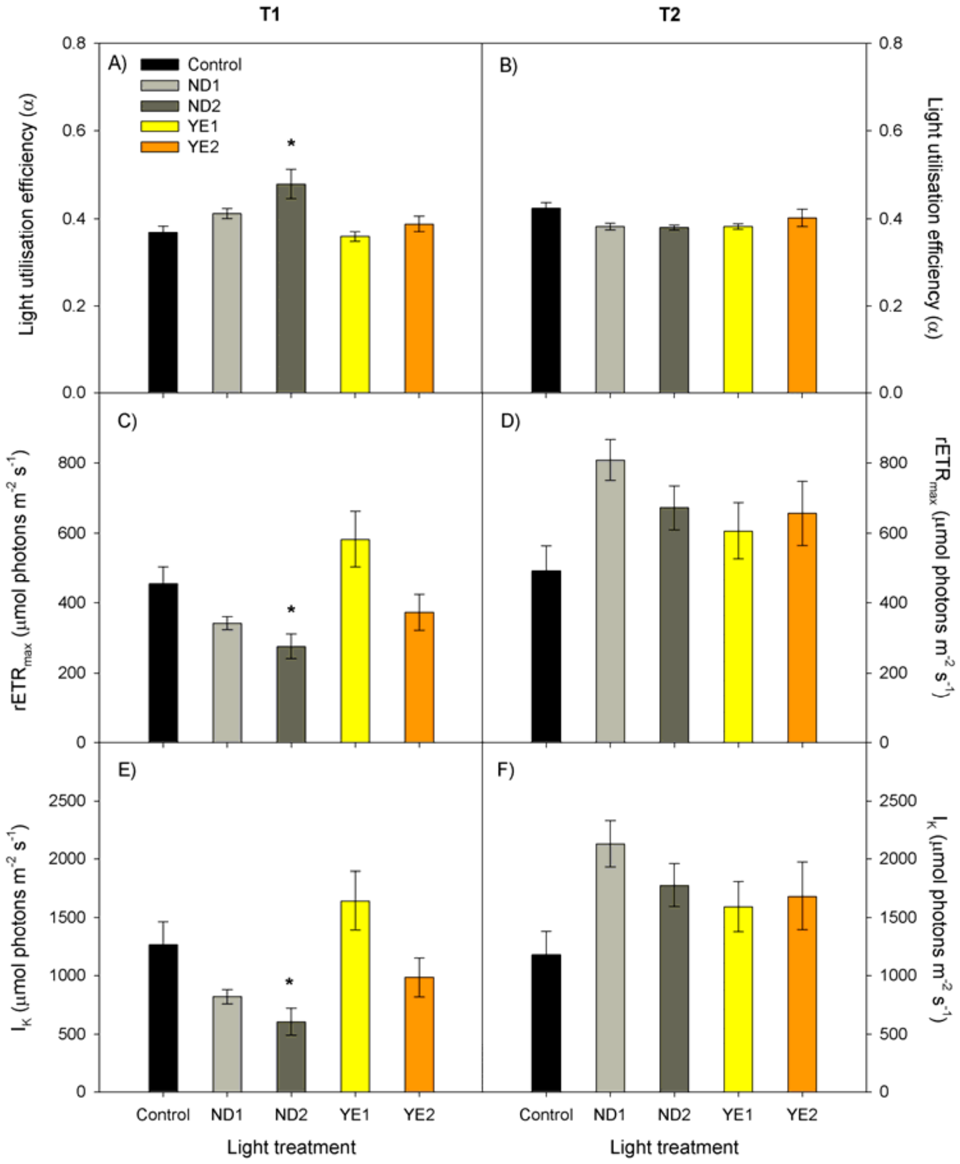


Figure 6: Photosynthetic parameters α (A-B), rETR$_{max}$ (C-D) and I$_K$ (E-F) in *Z. capricorni* acclimated to 25°C under five different light treatments. Data represent mean ± SEM (*n* = 6). ND = neutral density shading, YE = yellow enhanced shading; 1 = low shading, 2 = high shading.

The pool size of light harvesting pigments showed a significant decline ($P < 0.001$) in the control plants over the 10-week study (Fig. 7a). Leaves collected after the first four weeks of acclimation (T1) showed a significant difference in LHP pool size ($P < 0.001$), where the pool size increased with increased shading in both the neutral density and yellow enhanced treatments (Fig. 7a). This same pattern was seen after 10 weeks of acclimation (T2), although the overall LHP pool size was smaller in all light treatments ($P < 0.001$; Fig. 7a). Differences in the relative proportion of photoprotective pigments were detected between the control treatment over time ($P = 0.002$), with a slight increase in relative size with time (Fig. 7b). There were no differences between shade treatments. There was no change in the chlorophyll *b:a* ratio over time, but a significant increase ($P < 0.001$) was detected in the chlorophyll *b:a* ratio of the highest shade (ND2) treatment (Fig. 7c). The violaxanthin de-epoxidation state (VDE) showed a significant increase in the control over the initial four weeks of the study (*P*<0.001) and a significant decline in de-epoxidation ratio at the two highest shade treatments and the controls after both four and ten weeks (*P* <0.014; Fig. 7d).

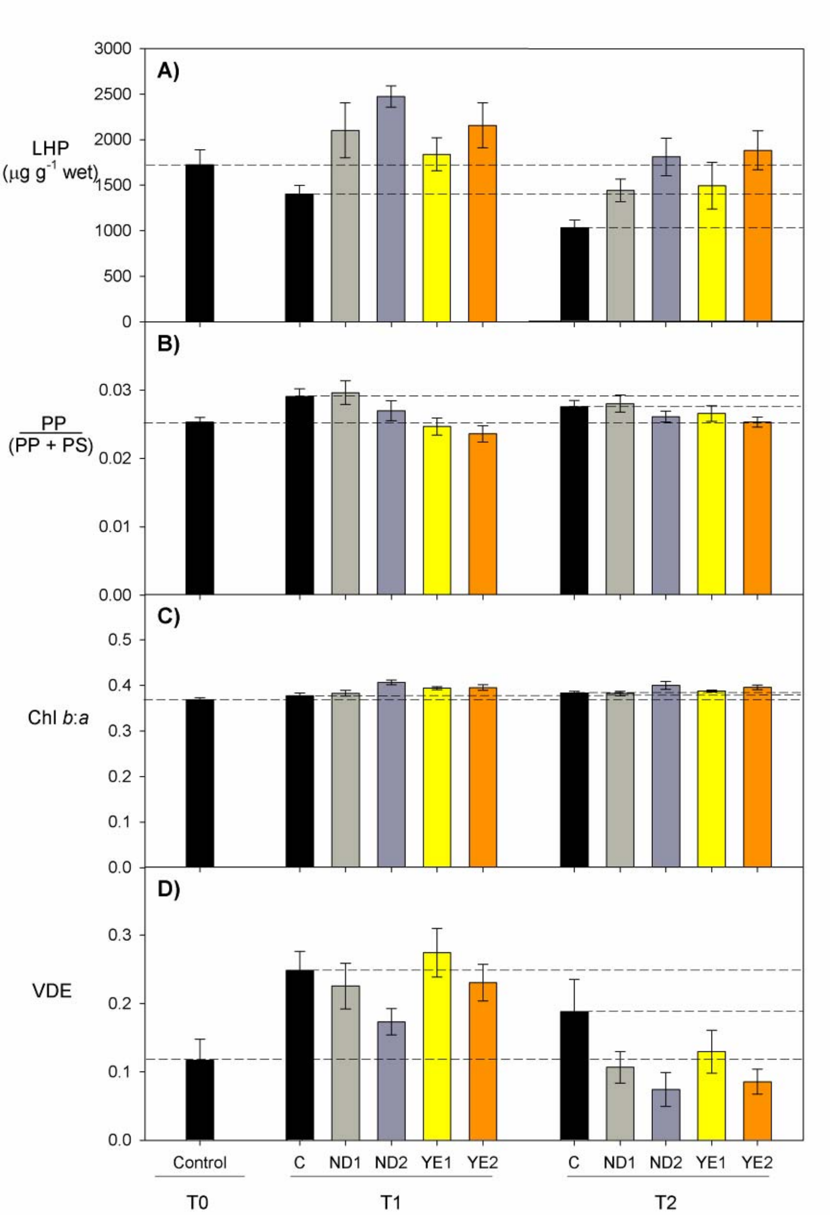


Figure 7: LHP: Light harvesting pigment pool (µg g^{-1} wet) (A), PP: photoprotective pigment (V + A + Z); PP + PS: photosynthetic pigment pool ratio (B), chlorophyll *b:a* ratio (C) and VDE: violaxanthin de-epoxidation state (D) using the ratio [(Z+ 0.5An)/(V+An+Z)] in *Z. capricorni* at the beginning of the experiment (T0), after 4 weeks (T1) and after 10 weeks (T2) of acclimation to 25°C under different light treatments. Z=zeaxanthin, An=Antheraxanthin, V=violaxanthin. Data represent means ± SEM (*n* = 6), *indicates significance at α=0.05. ND = neutral density shading, YE = yellow enhanced shading; 1 = low shading, 2 = high shading.

Photosynthesis and respiration rates:

No shading effect was apparent with the oxygenic production rates of *Z. capricorni* after 10 weeks of acclimation to the three light treatments (Fig. 8a). Similarly, CO_2 production and consumption rates measured by infrared gas analyser (IRGA) were largely unaffected by the light treatments. However, the rate of CO_2 uptake (respiration) by *Z. capricorni* was several fold greater than CO_2 released in all treatments (Fig.8b).

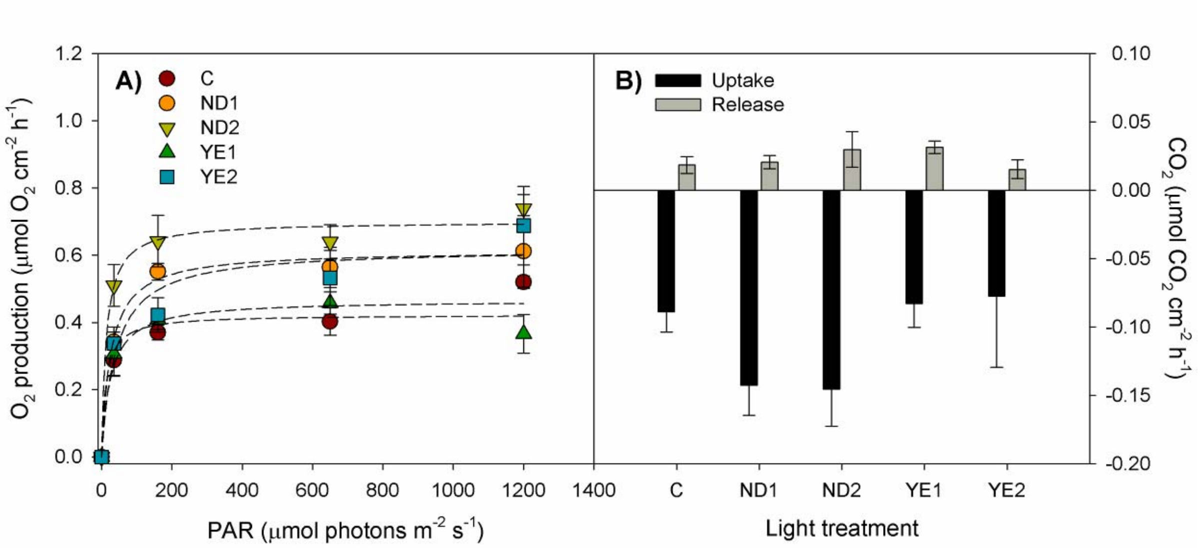


Figure 8: Rates of gross oxygenic photosynthesis calculated from measurements of dark respiration and net photosynthesis in the greenhouse acclimation aquaria as a function of irradiance (A) and CO_2 uptake (black bars) and release (grey bars) measurements (B) for *Z. capricorni* acclimated to 25˚C under different light treatments. Data represent means ± SEM (*n*=6). ND = neutral density shading, YE = yellow enhanced shading; 1 = low shading, 2 = high shading.

DISCUSSION

This study found differences in seagrass morphology, pigments and bio-optical characteristics after 8-10 weeks of acclimation to shaded conditions at 25°C. Morphologically, the highest shade treated plants (>75% light attenuation) showed a loss in leaf density and volume, however, this difference was not evident until 10 weeks of acclimation had passed. The HPLC pigment data were consistent with shade acclimation across the five light treatments. Increased light harvesting pigments measured in the neutral density and yellow-enhanced high-shade treatments is a photoacclimation response to increased shading. Similarly, the increase in chlorophyll *b:a* in the highest shade treatment is consistent with previous studies, where the chlorophyll *b:a* ratio is often indicative of light acclimation properties in plants (Falkowski and Owens 1980). Although not significant, there was a reduction in the proportion of photoprotective pigments under increased shading, which would support shade acclimation. The significant decline in VDE (violaxanthin de-epoxidation state) measured in the high shade treatments is an expected response to more shaded conditions, as the need for photoprotective pigments and thus the proportion of de-epoxidised violaxanthin is reduced.

Significant changes were detected in the leaf absorption at 600 and 680 nm in the highest shade treatments (ND2 and YE2) after 10 weeks. Given the difference was only evident after 10 weeks, this parameter could potentially serve as a suitable sub-lethal indicator for seagrass management in cases of prolonged shading. Since the absorption peak at 680 nm is close to the chl *a* absorption peak (685 nm; Enriquez 2005), a reduction in absorption at this wavelength could indicate the beginning of the breakdown in the function of chl *a* to absorb useful wavelengths of light. Alternatively, leaf thickness strongly influences leaf absorptance, so as the shaded leaves are thinner, they will also have a reduced absorption; however if this were the case in this study, reduced absorption should occur across all wavelengths and not just 600 and 680 nm.

No clear differences in photosynthetic parameters were detected between the control and shaded treatments after 10 weeks of acclimation, although differences were detected after 4 weeks in the highest shade treatment (ND2). Ten weeks of shading caused no changes in photosynthetic electron transport rates and no change in oxygen evolution under prolonged shading. Similarly, the data from the IRGA showed no significant differences in CO_2 fluxes with light treatment. The inability to detect differences in the photophysiology and gas exchange measurements between light treatments suggests that *Z. capricorni* is highly proficient at acclimating and adjusting its physiology, in particularly its photosynthetic processes to *in situ* light conditions over the long term. The adjustment to a consistent light climate—whether neutral low light or yellow enhanced—appears to be achieved through changes in pigment concentrations, with some slight adjustment of optical cross sectional area. It is possible that the ability for *Z. capricorni* to adapt successfully to the low light conditions in this study was aided by the absence of any additional stressors, such as elevated temperature.

This study included samples for carbon incorporation (^{13}C) and real-time PCR analyses. These data are yet to be processed and it is our hope that they will be able to confirm changes in plant resilience due to prolonged shading. We also hope that one if not both of these methods will succeed in providing us with a reliable sub-lethal indicator for seagrass health under changed light conditions.

This study would appear to suggest that the long-term consequences of prolonged shading have a minimal impact on the health and photosynthetic activity of *Z. capricorni.* Although none of the plants showed signs of mortality by the completion of the study, the plants in the two highest shade treatments (ND2 and YE2) had greatly reduced leaf density and much shorter leaves than those in the low-shade treatments and even less when compared with the controls (Fig. 9; Appendix A). Furthermore, very few green and new shoots were seen in the tubs under these two treatments by the end of the study. These observations match those from the field studies that indicate prolonged shading as impacting negatively on seagrass health and results in loss in seagrass coverage (Appendix A).

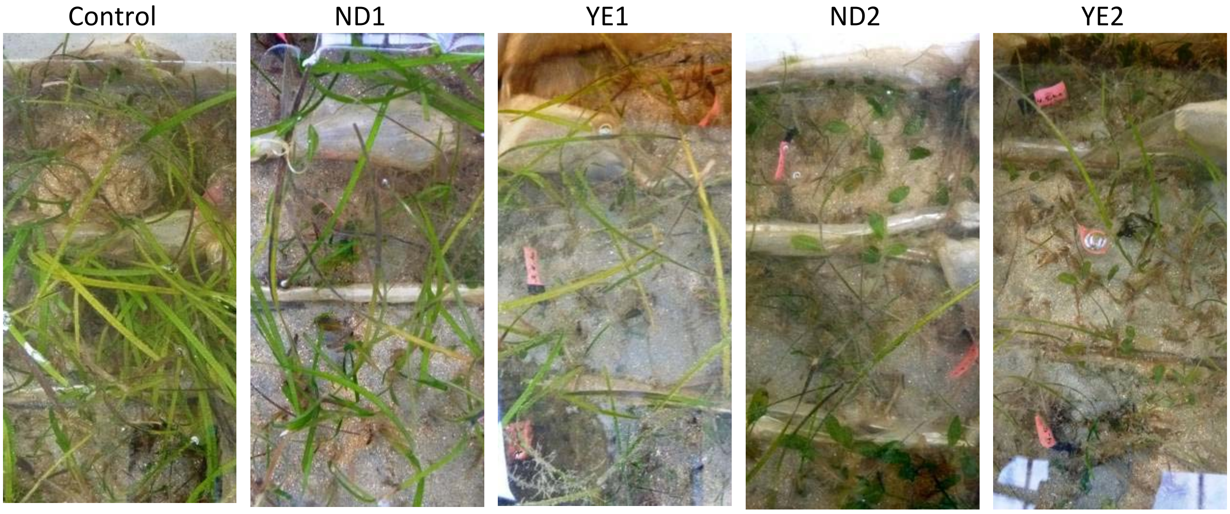


Figure 9: Photographs of seagrass coverage in control, low shade neutral density (ND1), low shade yellow-enhanced (YE1), high shade neutral density (ND2) and high shade yellow-enhanced (YE2) tubs after 10 weeks acclimation.

One possible reason for only being able to measure minimal morphological and physiological responses to the light treatments, when compared with field data, is the incubation temperature (25°C) that was used in this experiment. *Zostera capricorni*, as a predominantly temperate species, is known to exhibit stress under high temperatures (Collier et al 2011) and therefore, by growing them at a moderate temperature they were perhaps better able to withstand changes in their light climate. Indeed the previous laboratory experiment (July report) and field experiments (tidal and shading) both showed that higher temperature (summer seasonal data) showed greater responses to shading than in the winter or cooler temperatures. In addition, other variables, such as smothering from increased suspended particles (increasing the diffusion boundary layer for gas exchange), which were not considered in this study, but would provide an additional stress to the plants in their natural environment, potentially reducing their resilience to increased turbidity and low light.

Designed to investigate the impact that dredging could have on seagrass photosynthetic performance and health, this study has not revealed many significant morphological or physiological changes under shading treatments, providing very little information on light-induced changes to seagrass health. These data would suggest that a minimum light threshold above 3.45 mol photons m^{-2} d^{-1} would be sufficient to sustain seagrass health and viability at 25°C for up to 10-weeks. This value however, needs to be treated with caution, as many environmental variables were not accounted for in this laboratory study. In their natural meadow environment, air-exposure, temperature, sedimentation, nutrients and grazing all pose additional pressures on seagrass resilience (Erftemeijer and Lewis III, 2006) and therefore a more conservative threshold needs to be considered. As the field studies have demonstrated, during the summer months, shading has a devastating impact on seagrass health and survival, so it would be advisable to work at a minimum light threshold that is higher than that determined in this study.

REFERENCES

Collier, C. J., Uthicke, S. & Waycott, M (2011). Thermal tolerance of two seagrass species at contrasting light levels: Implications for future distribution in the Great Barrier Reef. *Limnology and Oceanography* **56:** 2200-2210.

Enriquez S (2005) Light absorption efficiency and the package effect in the leaves of the seagrass *Thalassia testudinum MEPS* 289 141-150.

Erftemeijer, P. L. A. & Lewis III, R. R. R. (2006). Environmental impacts of dredging on seagrasses: A review. *Marine Pollution Bulletin.* **52**: 1553-1572.

Falkowski, P. G. & Owens, T. G. 1980. Light--Shade Adaptation : Two strategies in marine phytoplankton. *Plant Physiol.* **66**:592-95.

Jeffrey, S. W. & Humphrey, G. F. 1975. New spectrophotometric equations for determining chlorophyll *a, b, c1,* and *c2* in higher plants and natural phytoplankton. . *Biochem. Physiol. Pfl.* **165**:191-94.

Johnsen, G. & Sakshaug, E. 2007. Biooptical characteristics of PSII and PSI in 33 species (13 pigment groups) of marine phytoplankton, and the relevance for pulse-amplitude-modulated and fast-repitition-rate fluorometry. *Journal of Phycology* **43**:1236-51.

Ralph, P. J. & Gademann, R. 2005. Rapid light curves: A powerful tool to assess photosynthetic activity. *Aquatic Botany* **82**:222-37.

van Heukelem, L. & Thomas, C. 2001. Computer-assisted high-performance liquid chromatography method development with applications to the isolation and analysis of phytoplankton pigments. *Journal of Chromatography A* **910**:31-49.

Appendix A:

Control	YE1	ND1	YE2	ND2
9 mol photons d^{-1}	6.5 mol photons d^{-1}	5 mol photons d^{-1}	3.5 mol photons d^{-1}	2.7 mol photons d^{-1}
				
				
				
				

APPENDIX II

Shifts in the spectral quality of light: the effects of dredging on the optical properties of Gladstone Harbour

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PLANT FUNCTIONAL BIOLOGY & CLIMATE CHANGE CLUSTER

Queensland Government

SUMMARY

This report contains the optical data that has been collected in Gladstone Harbour over the last year and output obtained from the light model that was developed from the data collected. This report provides a good overview of the optical quality of the water and clearly illustrates dredge-induced changes in the optical properties of the water column within the central harbour. The data presented are from a number of key sites within the harbour—including the site of the current dredge operation—as well as an outer harbour site at Pelican Banks. Data were collected between February and November 2011.

KEY FINDINGS:

1. There was a loss of light intensity reaching seagrass depths during dredging within the harbour, where the measured downwelling irradiance was below instrument detection limits beyond 0.5 m depth. Previous measurements made prior to the dredging activity recorded light penetration more than 3-fold greater than during the November dredge.
2. There were major differences in the intensity and attenuation of light with depth between the inner and outer harbour sites.
3. There was evidence of the localised and widespread effect of the dredge plume within the inner harbour – the upstream and downstream sites showed high attenuation and reduced light transmission during the dredge operation in November.
4. Pre-dredge measurements showed a clear shift towards the yellow region of the spectrum at all the sites within the inner harbour, whereas wavelengths were shifted more towards the blue in waters around Pelican Banks.
5. The PAR output from the light model show large differences in light vertical profiles from Pelican Banks to the main dredge site. There was a total loss of PAR beyond 2.0 m depth at the dredge site, where as 2.0 m at Pelican Banks there was more than 500 µmol photons m^{-2} s^{-1} of available irradiance.
6. Modelled simulations of the light field and spectrally-integrated irradiance showed tidal and seasonal patterns for un-dredged conditions. However, due to the high scattering from particles associated with dredge activity, the model was unable to compute similar changes for dredge conditions.
7. By weighting the PAR with the absorption spectra of seagrass leaf blades, we were able to model photosynthetically usable radiation (PUR). Significant differences between irradiance as PAR and PUR were detectable, with a maximum 14.9 % overestimation of PAR under winter conditions.

BACKGROUND

Seagrasses are very sensitive to the degree of attenuation of light before it reaches the bottom where seagrass grows. Light transmitted through the water column is essential for photosynthesis, and therefore growth and health (Dennison et al 1993). The quality of the light environment reaching seagrasses is as important as the quantity of light received. Seagrass capacity to harvest light varies across the PAR spectrum with greater absorption in the blue and red region (400-500 nm and 650-700 nm) than in the green to yellow region (500-650 nm). As light passes through the water column, spectral quality is altered not only by depth, but also by the optical property of particles in the water column.

The light environment of seagrasses growing adjacent to large port developments is commonly affected by regular dredging activities to create and maintain required draft depths for vessel movement. Dredging typically increases particulate matter in the water column which affects spectral quality. The size and type of particles re-suspended by dredging activity alter PAR transmission in a non-linear manner, with some wavelength being more attenuated than others, resulting in a reduced light environment with a shift towards yellow wavelengths (Gallegos et al. 2009). Therefore, the minimum light requirement for seagrass health, as determined according to the full PAR spectrum available, may overestimate the actual light available for photosynthesis, as PAR measurements do not distinguish spectral shifts (Zimmerman 2003).

Describing the quality of light reaching seagrass in the Port of Gladstone under normal daily conditions (without dredging) and during dredging projects will enhance our knowledge of how increased turbidity due to dredging affects seagrass dynamics. Furthermore, knowledge of the spectral requirements of seagrass and measurements of a change in the availability of this spectrum during dredging may act as an indicator of seagrass health. This study measures the spectral transmittance and inherent optical properties of photosynthetically active radiation (PAR) to a maximum depth of 3.5 m. It uses *in situ* data to create a radiative transfer light model to explore PAR and photosynthetically usable radiation (PUR) during an incoming and outgoing tide, during all seasons at the dredge site and an inner harbour reference site.

METHODS

Site location and water depth:

Water depth varies greatly within Gladstone Harbour (2 - 20 m); however to describe the changes in optical properties across a range of sites with varied depths it was decided to use a standard optical depth of 3.5 m which corresponds to <1% transmission of surface irradiance and therefore well below the theoretical compensation depth of the seagrass meadows (approx. 10%). Spectral transmittance measurements were collected at several different locations in Gladstone Harbour (Map 1). Measurements were made at each site during an incoming and outgoing tide at different times throughout the year. Four inner harbour sites were chosen, as well as one “outer” harbour reference site, at Pelican Banks. The boats position was recorded with a handheld GPS and the boat’s GPS. Water depth was measured with the boat’s echo sounder. The data presented in this report are taken from three sites within the harbour; one that is located downstream from the main dredge area (site 2), one within the small dock dredge area (site 4), one within the main dredging area (site 6), one located upstream in the Narrows (site 8), as well as one outer harbour site at Pelican Banks (reference site). These data help to characterise the inherent optical properties of the harbour under ’natural’ conditions and provide information on the extent of the dredge plume within the harbour.

In situ light attenuation and hyperspectral profiling:

The caged absorption/attenuation spectrophotometer (ACS; Wetlabs, USA) was lowered to the required depth in the water column using a winch. Care was taken to ensure that the instrument cage did not disturb the sediment surface resuspending fine sediments that could influence the data. Also, the angle of the boat with respect to the sun was taken into account to ensure that the measurements were not compromised by the boat’s shadow. Measurements were recorded continuously to permit detailed study of variation in the water’s optical properties with depth. Simultaneous measurements of downwelling irradiance were obtained using an integrated hyperspectral radiometer (RAMSES) that was attached to the ACS cage. The radiometer corrected for angle and inclination, providing fully calibrated radiometric data.

Radiative transfer model:

To compute the radiative transfer function, a commercially available radiative transfer model Ecolight (Sequoia Scientific Inc.) was used. The model was then refined using the source code FORTRAN and set to compute the radiative transfer function of light under different scenarios. User defined inputs included specific inherent optical properties (IOPs), such as absorption and attenuation, from which scattering values could be derived. The *in situ* measurements of attenuation and absorption provided on a continuous scale were used to supply the model parameters. From the simulations of spectral scalar irradiance, PAR was calculated for Pelican Banks, Wiggins (site 2) and the dredge site (site 6). The model was used to derive simulated seasonal depth profiles of PAR and PUR (Photosynthetically Usable Radiation) for incoming and outgoing tides in the presence and absence of dredging. For modelling scalar irradiance, solar intensity (mW) was converted into PAR (μE m^{-2} s^{-1}) using the conversion equation:

$$PAR = \frac{1e^{6}\,(I * \lambda)}{h * \mathrm{N_A} * C}$$

where I is solar intensity, λ is the wavelength, h is Plank's constant, $\mathrm{N_A}$ is Avogadro's number and C is the speed of light in a vacuum. For the spectrally-integrated light profiles, PUR was calculated by weighting PAR with the absorption spectrum of *Z. capricorni* (Fig. 1) following the equation from Gallegos et al (2009):

$$PUR(z) = \int_{400}^{700} Q(\lambda, z)\, \bar{a}_{Th}(\lambda)\, d\lambda$$

where, Q is the quantum flux, λ is the wavelength, $\bar{a}_{Th}$(λ) is the absorption spectrum of *Z. capricorni* normalised to chl *a*. For comparison of attenuation rates, PAR and PUR were normalised to their surface values.

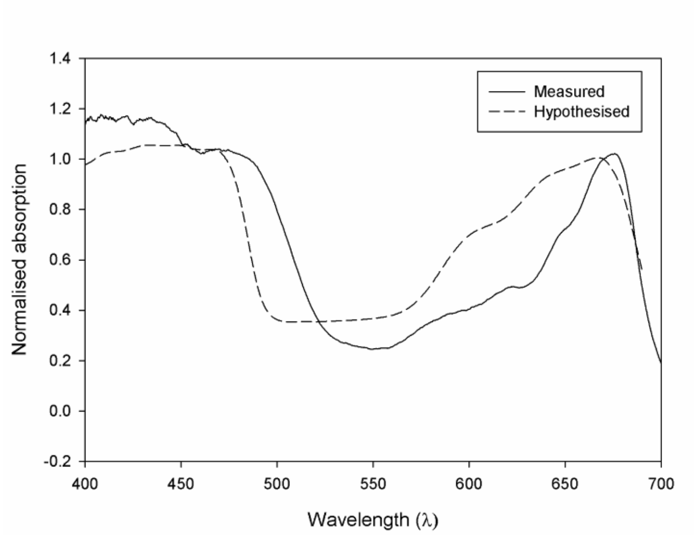
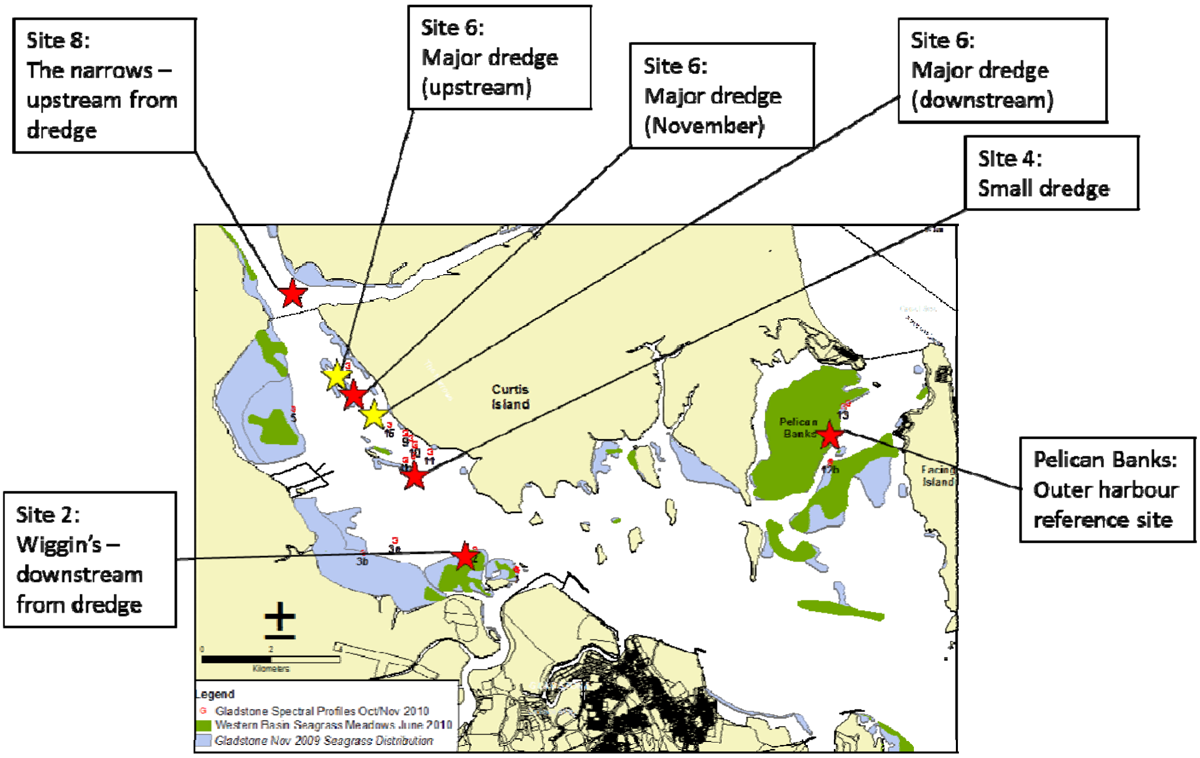


Figure 1. Normalized absorption spectra used for calculating photosynthetically usable radiation (PUR), based on absorption spectrum measured on *Zostera capricorni* leaves (solid line), and a hypothetical action spectrum derived by assuming only light absorbed by chlorophylls *a* and *b* (dashed line).

Leaf absorbance spectra

Leaf spectral transmittance was measured from 400 to 750 nm at 1 nm resolution using a fibre optic spectrometer (USB2000+ Ocean Optics) interfaced with an integrating sphere (FOIS-1, Ocean Optics, USA). A tungsten halogen light source (Ocean Optics LS-1) was adjusted to completely irradiate the 9.5 mm diameter sample port of the integrating sphere. A seagrass leaf was placed on a clear microscope slide in front of the opening port of the sphere, and covered with a second slide obstructed with black tape except for a small ~ 2 mm^2 opening to mask the portion of the sample port not covered by leaf tissue. Leaf spectral transmittance (T(ë)) for each replicate leaf blade (n= 4) was determined and referenced to the slide and tape without a leaf in place. Leaf-specific absorptance A(ë) was calculated as (1 – T(ë)- A(750 nm)), where A(750 nm) = 1 – T(750 nm) is used as a correction for scattering and non-photosynthetic absorptance.



Map 1: Map of Gladstone Harbour showing the location of the four inner harbour sites and outer reference site characterised in this report (denoted by the red star). The yellow stars indicate additional sites within the main cutter suction dredge site (Site 6: upstream and downstream). G = sites of spectral profiles conducted in 2010 using Ocean Optics and are not part of the present data reported here.

RESULTS

HYPERSPECTRAL RADIOMETRY:

The hyperspectral depth profiles derived from the radiometer (RAMSES) show clear differences between sites, seasons and over tidal cycles. The hyperspectral profiles from site 2 illustrate seasonal and tidal variability in light climate within the harbour in the absence of dredge activity (Fig. 2). With the exception of April, the outgoing tide allowed for greater light penetration than the more turbid incoming tide. During the outgoing tide, downwelling irradiance <200 mW m^{-2} nm^{-1} reach depths between 1-2 m (Fig. 1B, D and F), compared with the incoming tide, where irradiances < 200 mW m^{-2} nm^{-1} failed to penetrate to 1.0 m (Fig.2A and C).

Profiles from site 4, during the small con dock dredge (Map 1) showed substantial reduction in light penetration (Fig. 3A and B). Light failed to reach 1.0 m depth, compared with the profiles taken in April after dredging had ceased, where light penetrated well beyond 2.0 m (Fig. 3C and D).

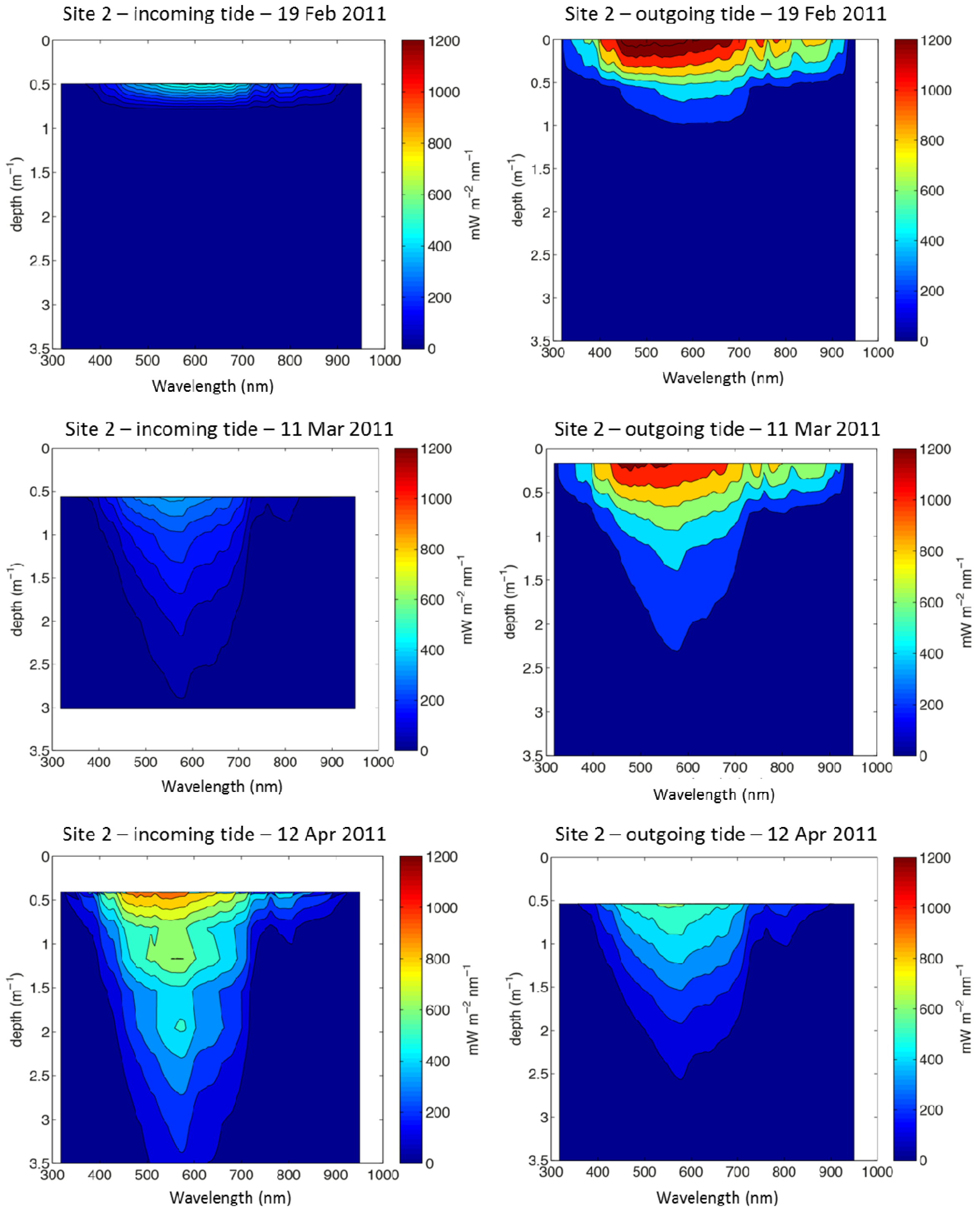


Figure 2. Hyperspectral depth profiles of downwelling irradiance in the water column at Wiggins (Site 2) during incoming and outgoing tides in the months of February (A and B), March (C and D) and April (E and F) 2011. Blank portions of the figures indicate absence of data or data trimming due to noise.

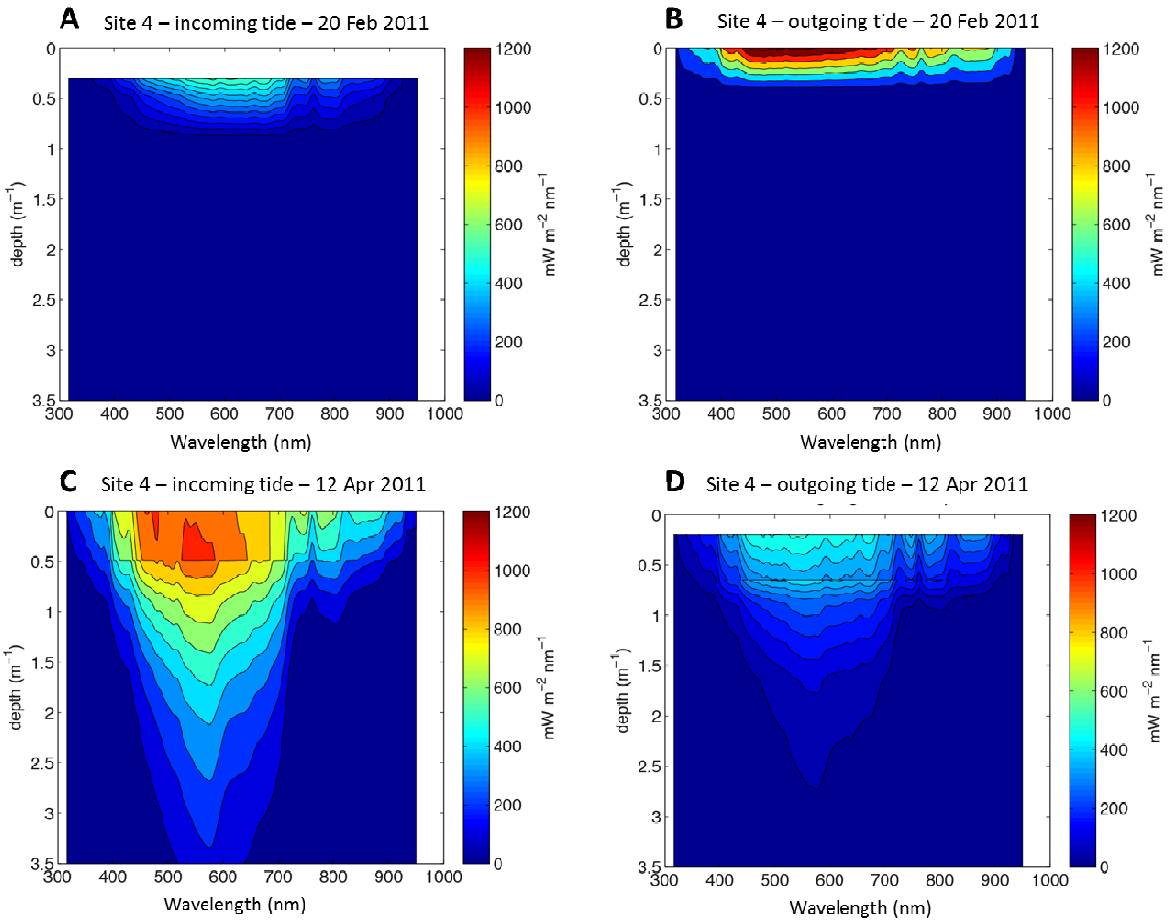


Figure 3. Hyperspectral depth profiles of downwelling irradiance at site 4 (the location of the con dock dredge) within the inner harbour over incoming and outgoing tides during dredge activity in February 2011 (A and B), and after dredging ceased in April 2011 (C and D). Blank portions of the figures indicate absence of data or data trimming due to excess noise.

The *in situ* light conditions of the outer harbour contrast greatly to those within the harbour. The hyperspectral profiles measured at Pelican Banks show the maximum depth transmission occurred at > 3.0 m depth on both incoming and outgoing tides in April (Fig. 4). This did not change during the cutter suction dredge activity in November 2011 (Fig. 4C and D), indicating that the light intensity and depth at Pelican Banks remained unaffected. The lower irradiance during the incoming tide at Pelican Banks in November (Fig 4C), was due to the lower solar irradiance of early morning sampling and not turbidity, as the depth of light penetration was well over three metres (Fig 4C).

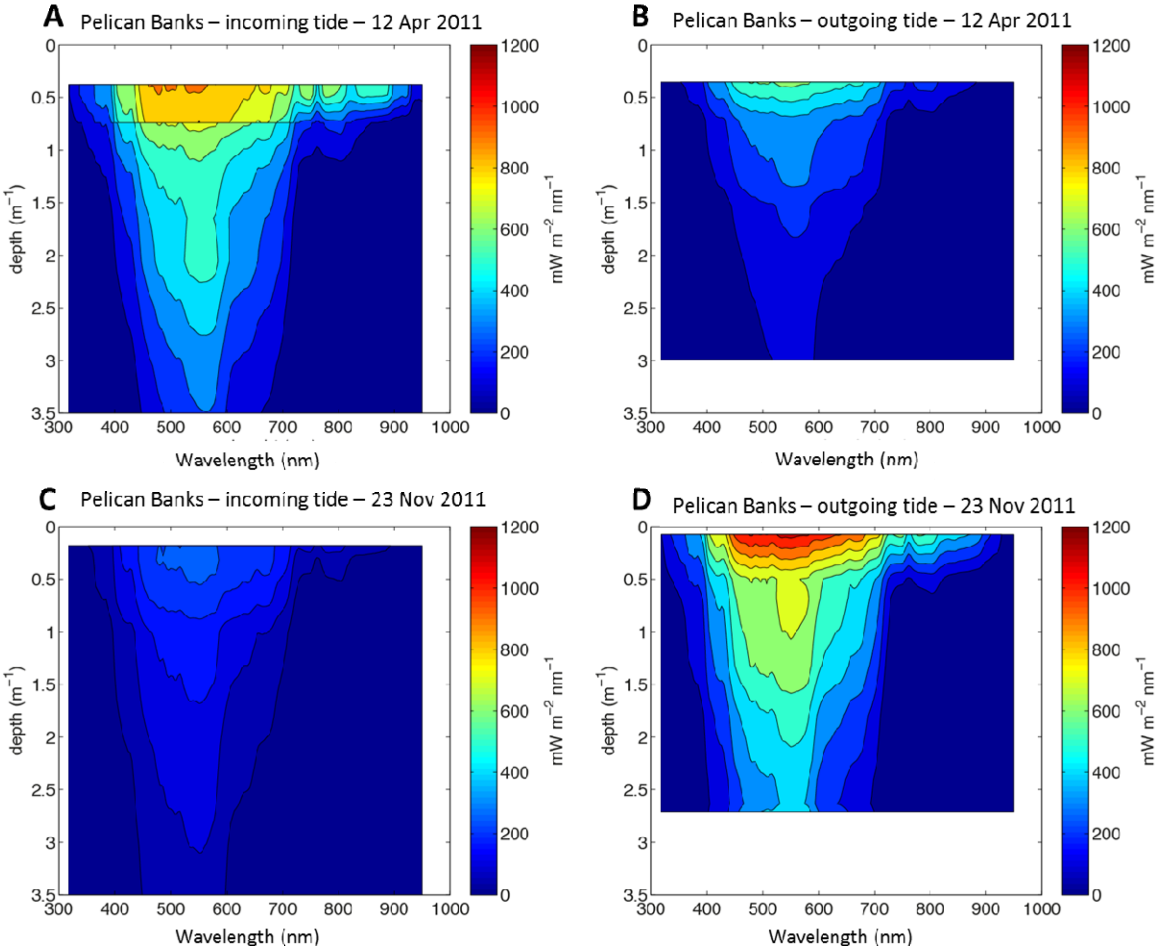


Figure 4. Hyperspectral depth profiles of downwelling irradiance at Pelican Banks (outer harbour reference site) over incoming and outgoing tides prior to dredging (A and B) and during the November dredge (C and D). Blank portions of the figures indicate absence of data or data trimming due to excess noise.

Comparing the November 2011 profiles at the cutter suction dredge site (site 6) with those measured earlier in the year (before dredging), the figures clearly show how the increase in suspended sediment load has had on the *in situ* light climate (Fig. 5). At site 6 (dredge site) irradiance reached a depth of 3.5 m during the incoming tide and 1.5 m during the outgoing tide in February 2011 (Fig. 5A and B). However, while the dredge was operating, downwelling irradiance was practically extinguished on both the incoming and outgoing tides (Fig. 5C and D), where light (>200 mW m^{-2} nm^{-1}) was unable to penetrate beyond the surface layer (<0.25m).

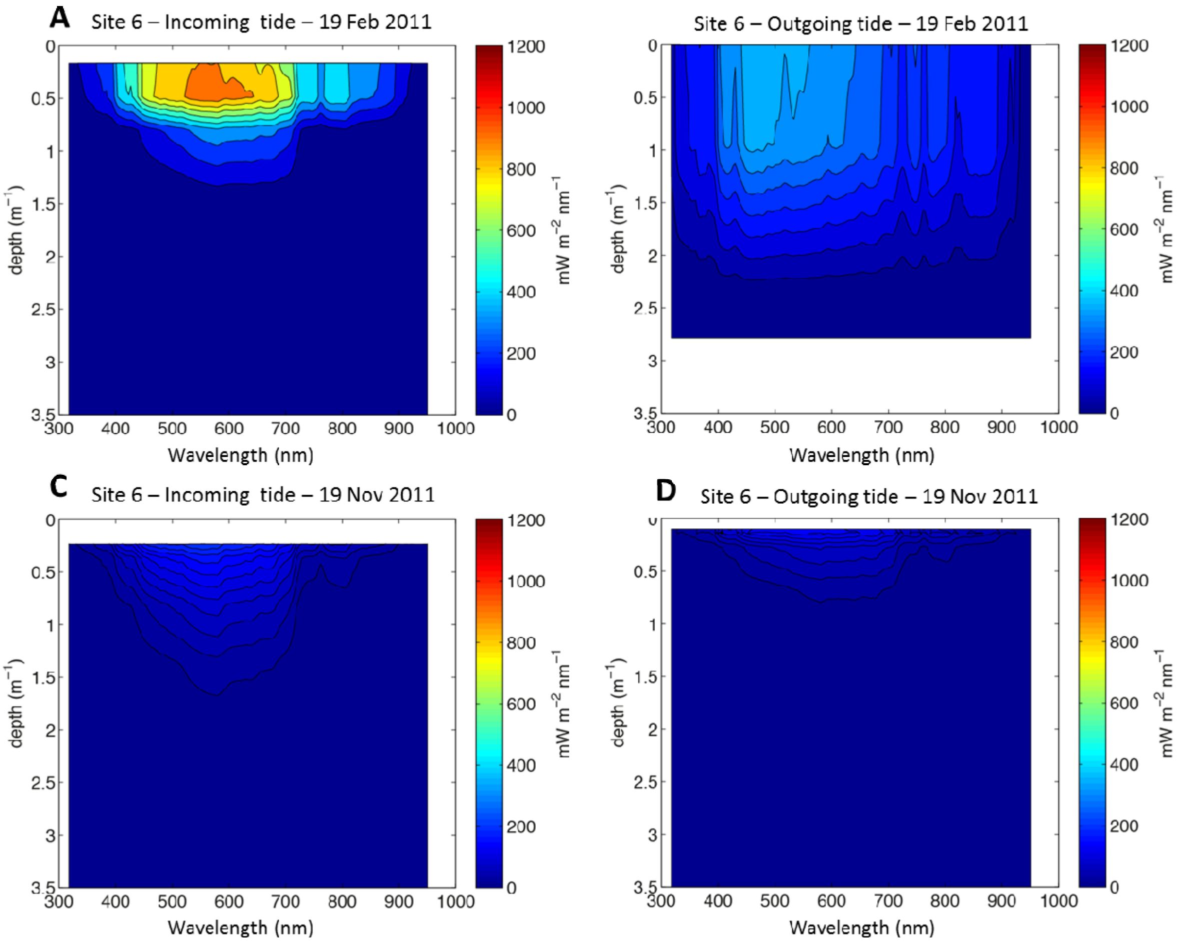


Figure 5. Hyperspectral depth profiles of downwelling irradiance at the main dredge site (site 6) over incoming and outgoing tides prior to dredging (A and B) and during the November dredge (C and D). Blank portions of the figures indicate absence of data or data trimming due to excess noise.

The additional profiles taken within the main cutter suction dredge site in November provide greater spatial resolution of the plume dynamics (Fig. 6). There are some differences in light attenuation at the different points around the dredge, with slightly lower turbidity south of the dredge on the outgoing tide (Fig. 6B). However, when compared with values obtained in February for the same site (Fig. 5), it is still highly attenuated. Overall, the light intensity is greatly attenuated in all instances, showing the localised impact of suspended sediments on the light climate within the harbour dredge site.

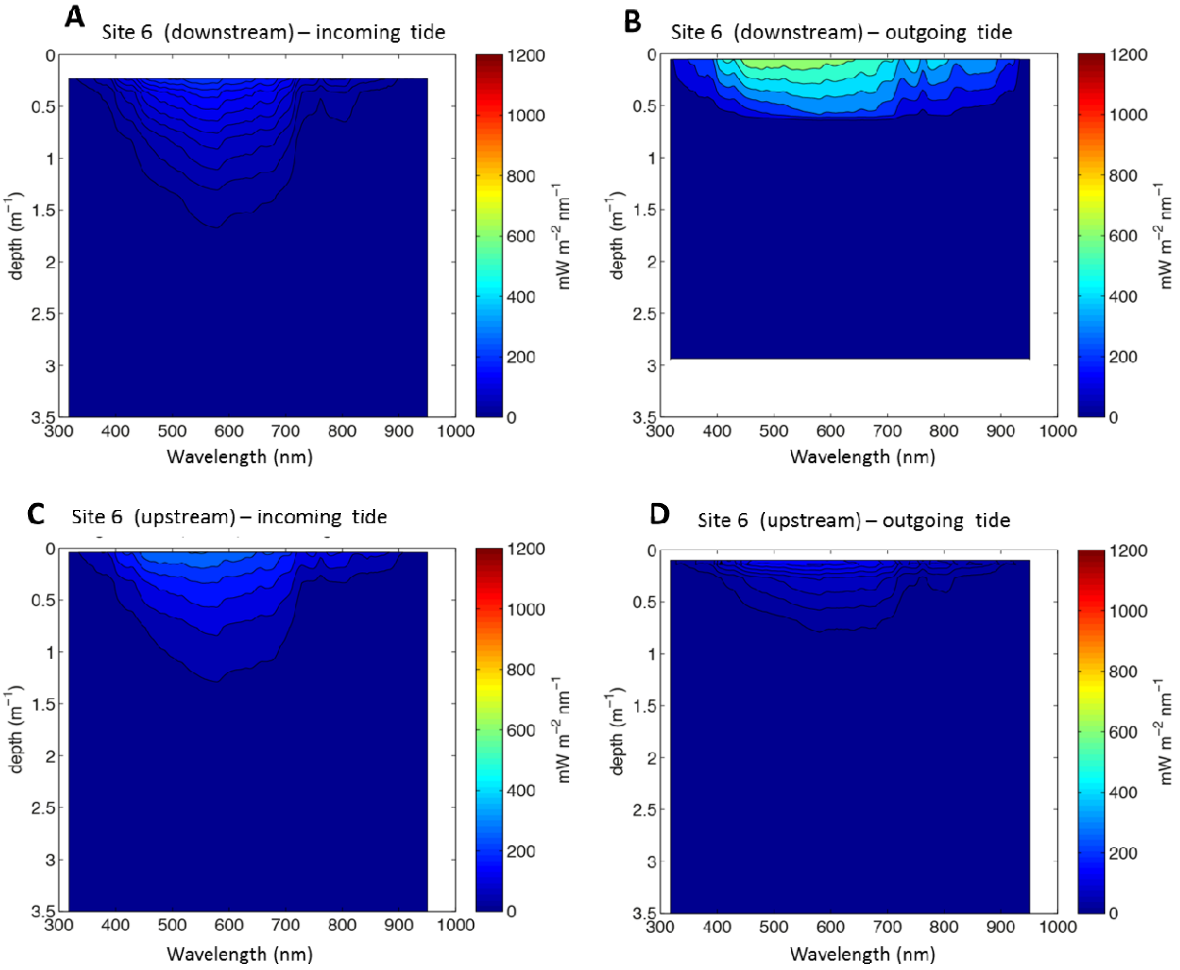


Figure 6. Hyperspectral depth profiles of downwelling irradiance upstream and downstream of dredge activity at the cutter suction dredge site (site 6) over incoming and outgoing tides during the November dredge. Blank portions of the figures indicate absence of data or data trimming due to excess noise.

The data indicate a wide spread of the plume based on the measured changes in the light properties of the upper and lower harbour sites during the November cutter suction dredge (CSD) activity. The inner harbour sites (upstream and downstream from the dredge) showed large differences in water clarity as a results of dredging compared with the data collected in the absence of dredging. The light profiles at site 2 (downstream of the dredge) collected prior to dredging showed light intensities of 200 mW at depths between 1.0 m and 3.0 m (Fig. 7A and B). In November, however, there was substantial light attenuation during the incoming tide; with maximum spectral intensities restricted to the surface centimetres only (Fig. 7C). Light penetration did increase during the outgoing tide, where light was able to penetrate to a maximum depth of 1.2 m (Fig. 7D). The additional profiles taken at site 6 (CSD) in November 2011 show high attenuation, although in some instances downwelling irradiance increases, providing a gradient of light penetration from the central dredge activity to the outer edge and in the greater harbour.

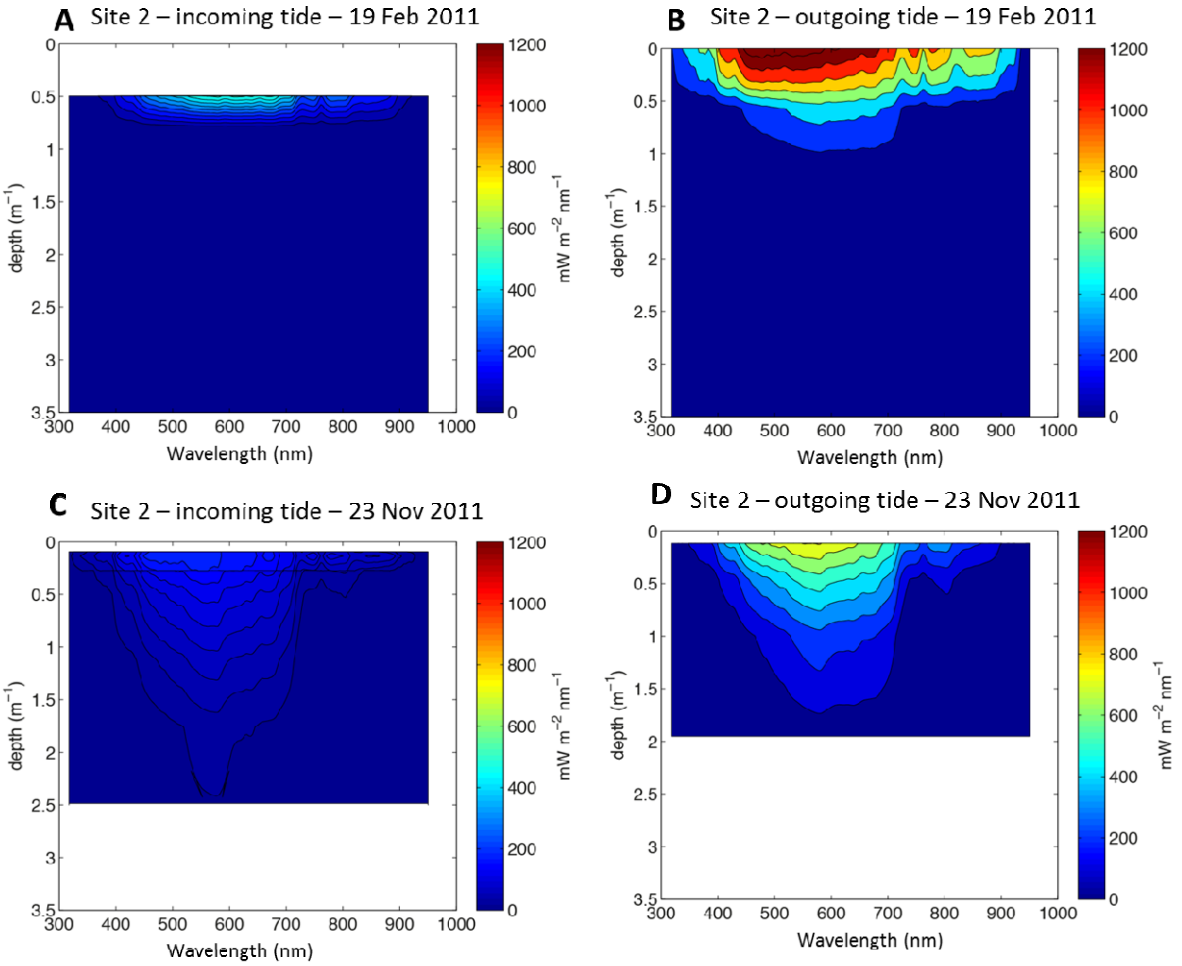


Figure 7. Hyperspectral depth profiles of downwelling irradiance downstream from the dredge at Wiggins (site 2) over incoming and outgoing tides in February, prior to the dredge (A and B) and during the November dredge (C and D). Blank portions of the figures indicate absence of data or data trimming due to excess noise.

Generally turbid, the Narrows (site 8), located upstream from the dredge site, show high light attenuation with a spectral intensity barely penetrating the uppermost surface layer (0.2 – 0.5 m) during the incoming and outgoing tides prior to dredging in February 2011 (Figs. 8A and B, respectively). However, during CSD activity light was further reduced, with almost no measureable irradiance beyond the very surface layer (Fig. 8C and D). These data indicate the effect of the dredge plume is widespread in the inner harbour area.

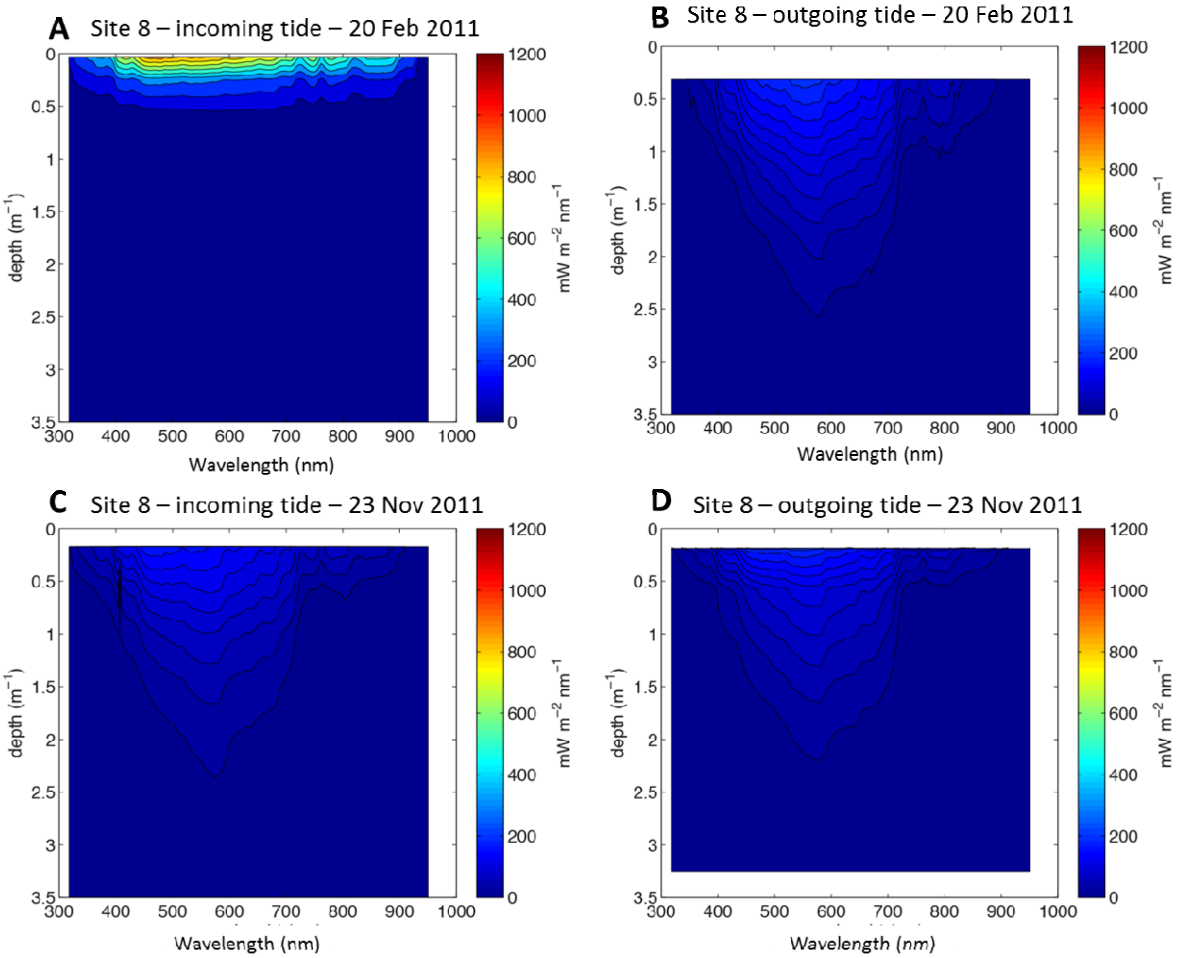


Figure 8. Hyperspectral depth profiles of downwelling irradiance upstream from the dredge site in the Narrows (site 8) over incoming and outgoing tides in February, prior to the dredge (A and B) and during the November cutter suction dredge (C and D). Blank portions of the figures indicate absence of data or data trimming due to excess noise.

SPECTRAL SHIFT:

In the absence of dredging activity, the clearer waters around Pelican Banks showed maximum transmission in the green region of the spectrum (~550 nm), whereas at the dredge site (Site 6) maximum transmission was in the yellow region (~580 nm), demonstrating a spectral shift toward the less-photosynthetically available range of the spectrum. In other words, the spectral quality of the light differs between the inner and outer harbour sites, with a shift from yellow enhanced water within the harbour to waters outside the harbour at Pelican Banks, where greener wavelengths dominate the light climate at the depth of the seagrass meadows (Fig. 4). A clear picture of spectral characteristics could not be defined during the dredging activity due to the loss of light within the top centimetres of the water column hampering spectral resolution efforts.

AC-S:

The AC-S deployments during the November 2011 sampling (while the CSD was active at Site 6) have provided a precise measure of the inherent optical properties (absorption and attenuation) of the water column at each of the four chosen sites (Map 1; Fig. 9). As expected, the absorption coefficients calculated from the AC-S data increase with increased turbidity, with a maximum value of 0.7 at Pelican Banks, 3.5 at both upstream and downstream inner harbour sites and >5.0 at the dredge site (Fig. 9A, C, E and G, respectively). Absorption was greatest in the blue range (~400-475nm) of the electromagnetic spectrum, and didn’t change with depth during the incoming tide.

These data also show large differences in attenuation among the four sites (Fig. 9B, D, F and H). The highest attenuation coefficient for Pelican Banks was 1.8 (Fig. 9B), while the dredge site (Site 6) had a maximum value >21 (Fig. 9F), more than 10 times greater. The other two inner harbour sites had intermediate values of 11 (Fig. 9D and H), clearly demonstrating a pattern of turbidity which was greatest at the dredge site and dissipated with distance from the dredge. At Pelican Banks, the attenuation coefficient was consistent with depth, corresponding with the hyperspectral data that showed light penetration beyond 5.0 m. In contrast, the inner harbour sites all showed an increase in attenuation with depth, closely matching the hyperspectral data that showed downwelling irradiance to disappear with depth.

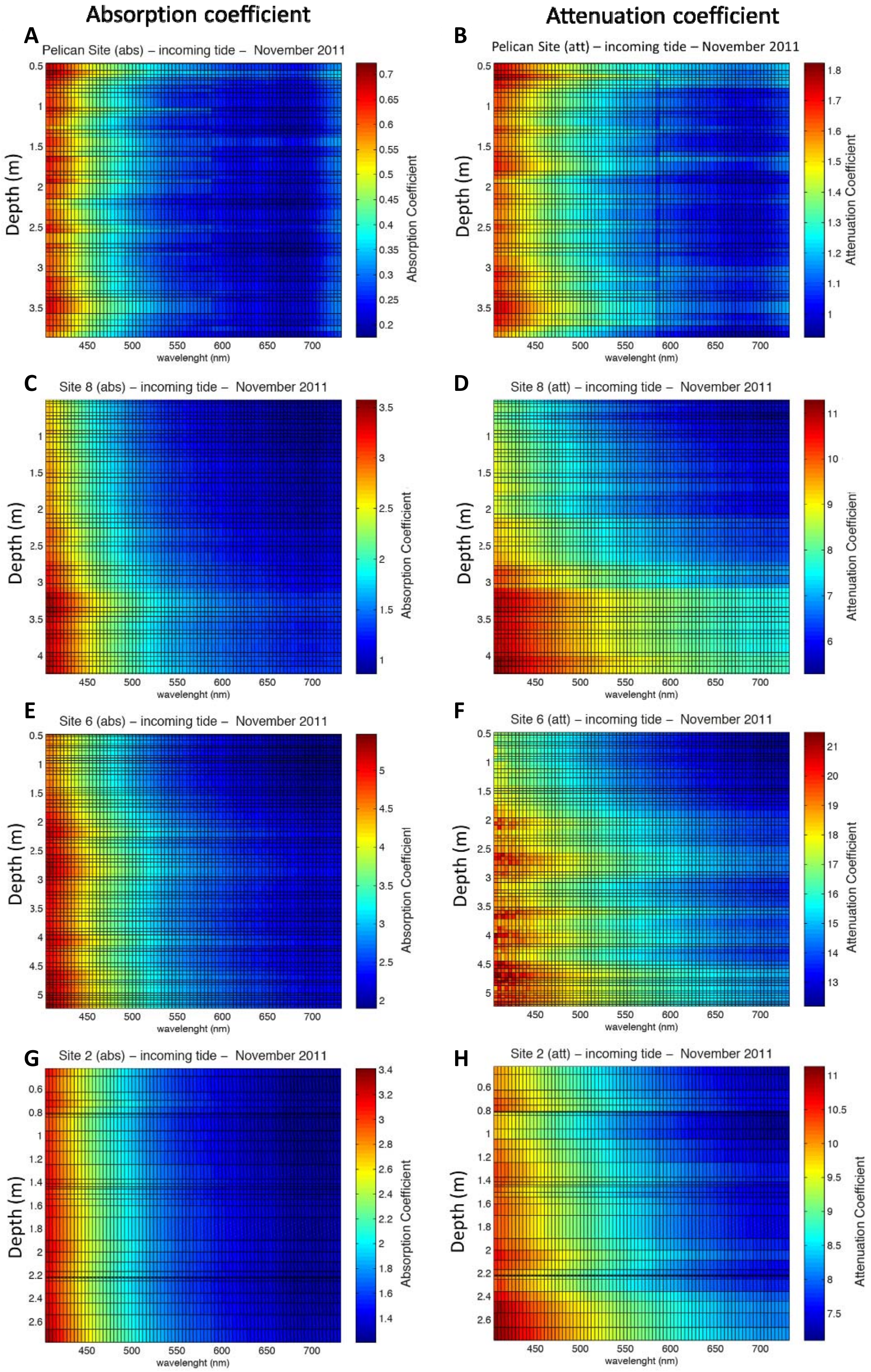


Figure 9. *In situ* measurements of light absorption and attenuation at Pelican Banks, Site 8 (Narrows: upstream), Site 6 (dredge) and Site 2 (Wiggins: downstream) during the CSD in November 2011. Note: difference in y axes (depth) and colour legend.

LIGHT MODEL:

The light property data obtained from the AC-S for three of the sites was used to parameterise the radiative transfer model (Ecolight). The results from the light model are expressed as PAR at all three study sites during the dredging activity in November 2011. The data show a rapid loss of PAR with depth at the dredge site (Site 6) with a total loss of light at 2.0 m (Fig. 10). In contrast, the modelled *in situ* PAR at Pelican Banks shows light penetrating beyond 5.0 m and more than 500 µmol photons m^{-2} s^{-1} at a depth of 2.0 m. Site 2 (Wiggins; downstream of dredge) shows equally rapid loss of light in the top 1.0 m as that modelled for the dredge site, but low light levels are seen to penetrate beyond 3.0 m.

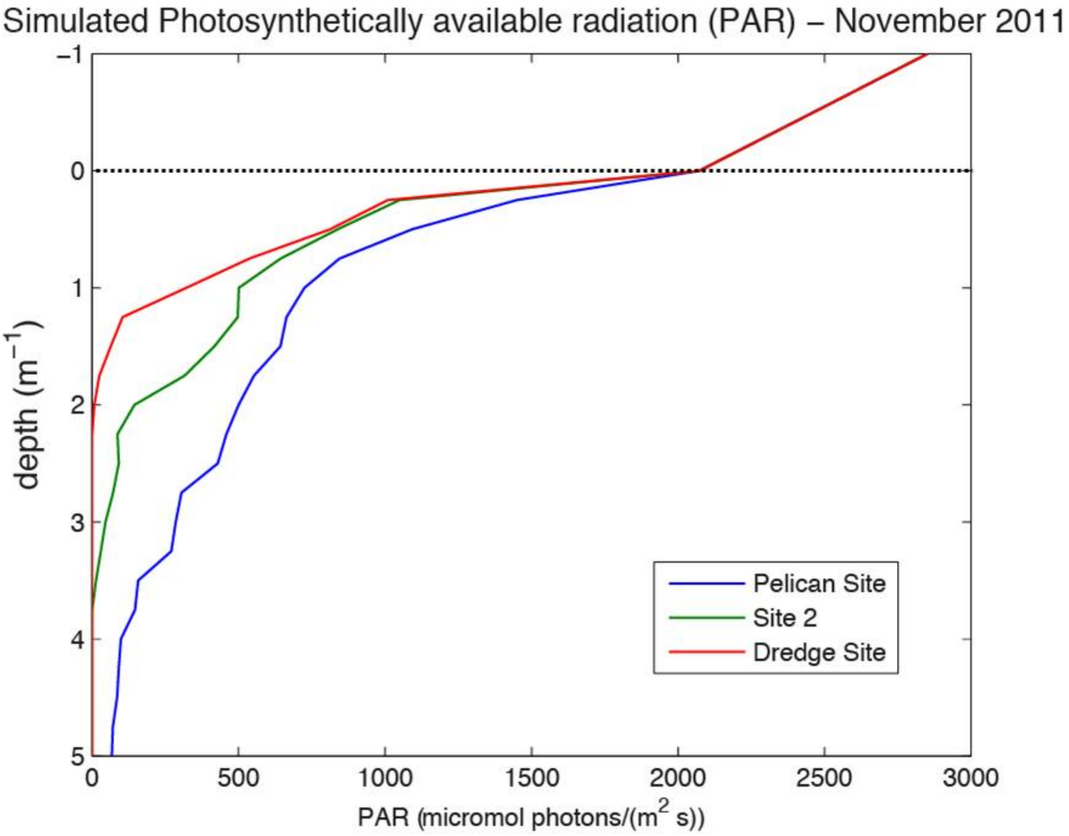


Figure 10. Modelled photosynthetically active radiation (PAR) depth profile for Pelican Banks (blue), Site 2 (green) and dredge site (red) using assimilated data from the AC-S collected during the November dredge.

There are notable differences in the scalar irradiance when modelled in the presence or absence of dredging, as well as measureable differences during tides and across all seasons. (Scalar irradiance takes into account light coming in from all directions and therefore incorporates both downwelling and upwelling light from reflection, refraction and scattering.) The scalar irradiance during dredging does not penetrate more than 1.5 m depth throughout the year, whereas for the non-dredged water column, scalar irradiance reaches between 3 and 4 m in the simulations for Autumn (based on Mar/Apr 2011 data) and Winter (based on July 2011 data) seasons (Fig. 11 and 12, respectively) and slightly deeper in the Spring (November) and Summer (February; Fig. 13 and 14). Aside from the overall greater attenuation of light during dredging, spectrally, the data show that the greatest irradiance to reach at depth in the inner harbour was in the yellow region of the light spectrum. This indicates that the light is strongly yellow-enhanced by the particles and sediments suspended in the water column both in the presence and absence of dredge activity.

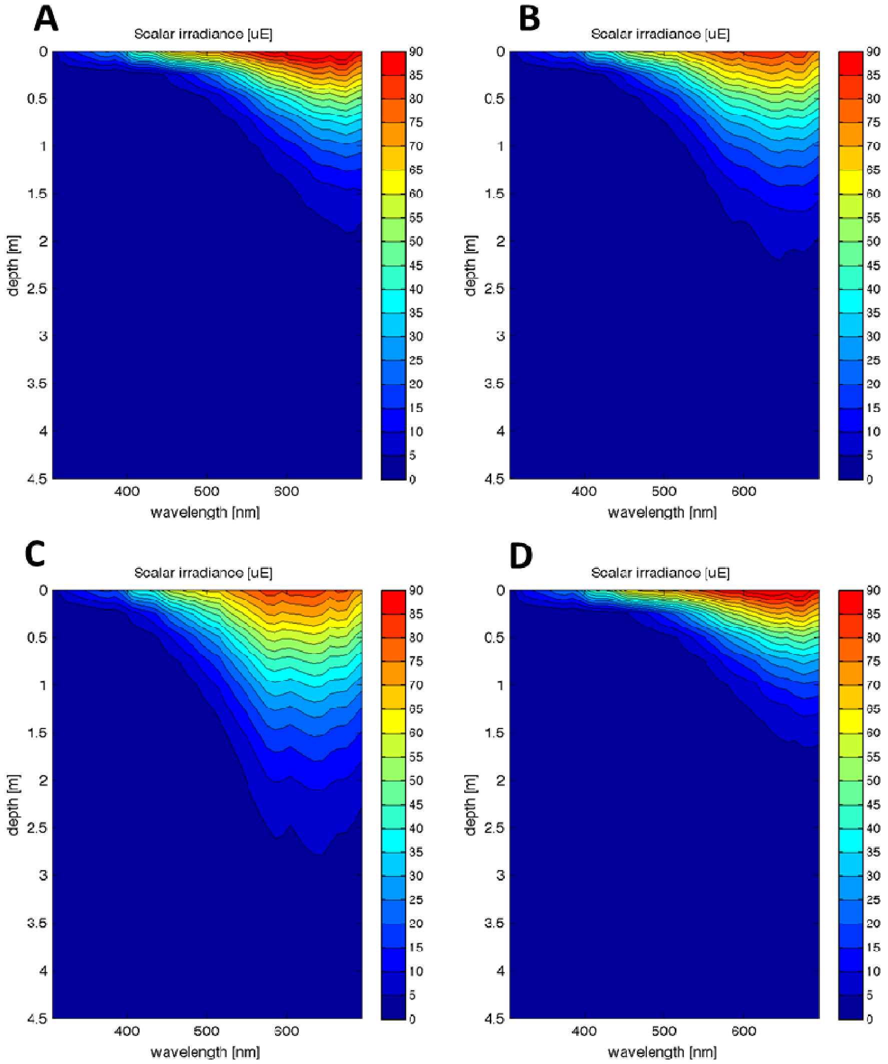


Figure 11. Modelled scalar radiation of the inner harbour water column as a function of depth for an A) incoming tide in the presence of dredging , B) outgoing tide in the presence of dredging, C) incoming tide in the absence of dredging and D) outgoing tide in the absence of dredging during Autumn.

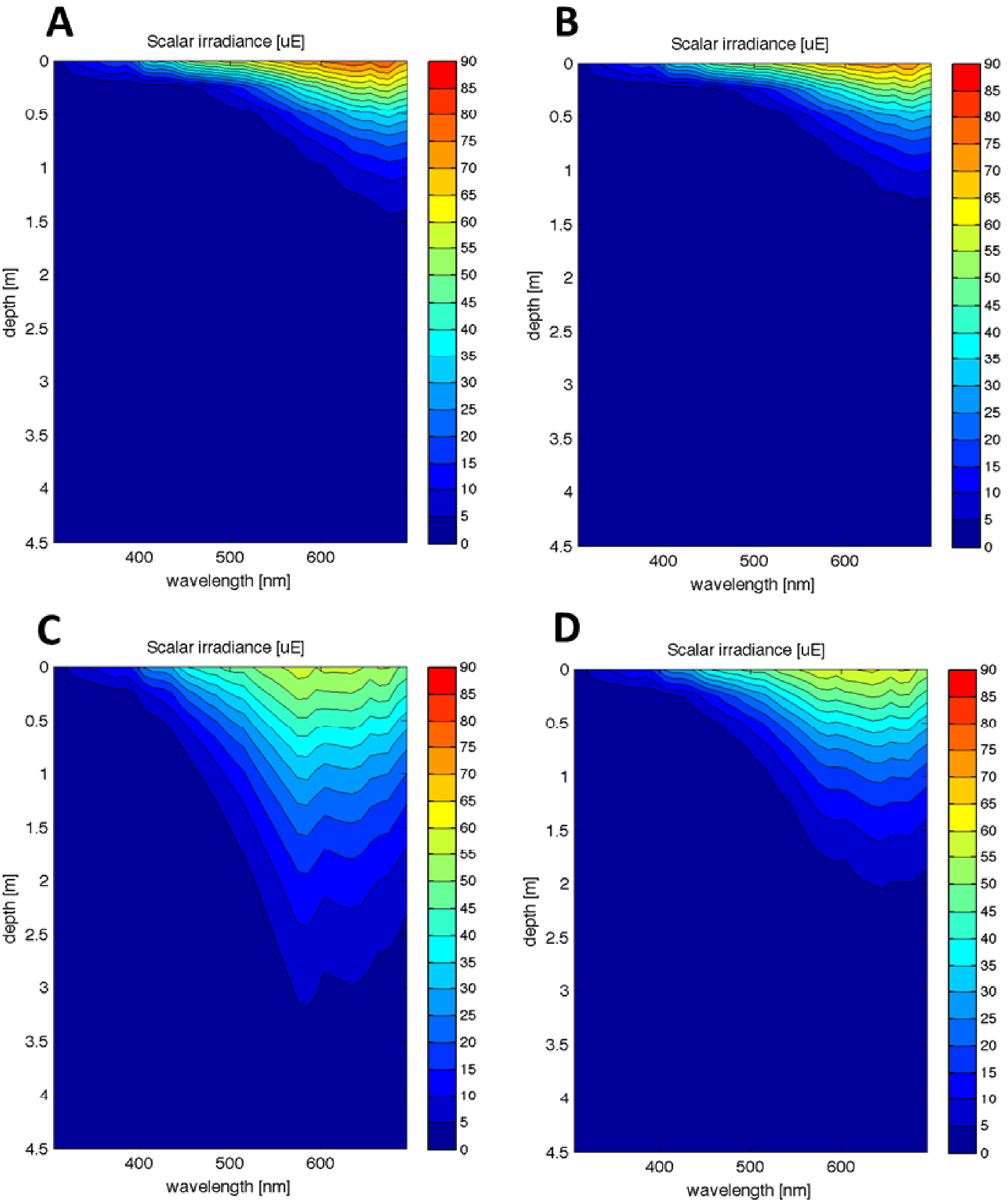


Figure 12. Modelled scalar radiation of the inner harbour water column as a function of depth for an A) incoming tide in the presence of dredging , B) outgoing tide in the presence of dredging, C) incoming tide in the absence of dredging and D) outgoing tide in the absence of dredging during Winter.

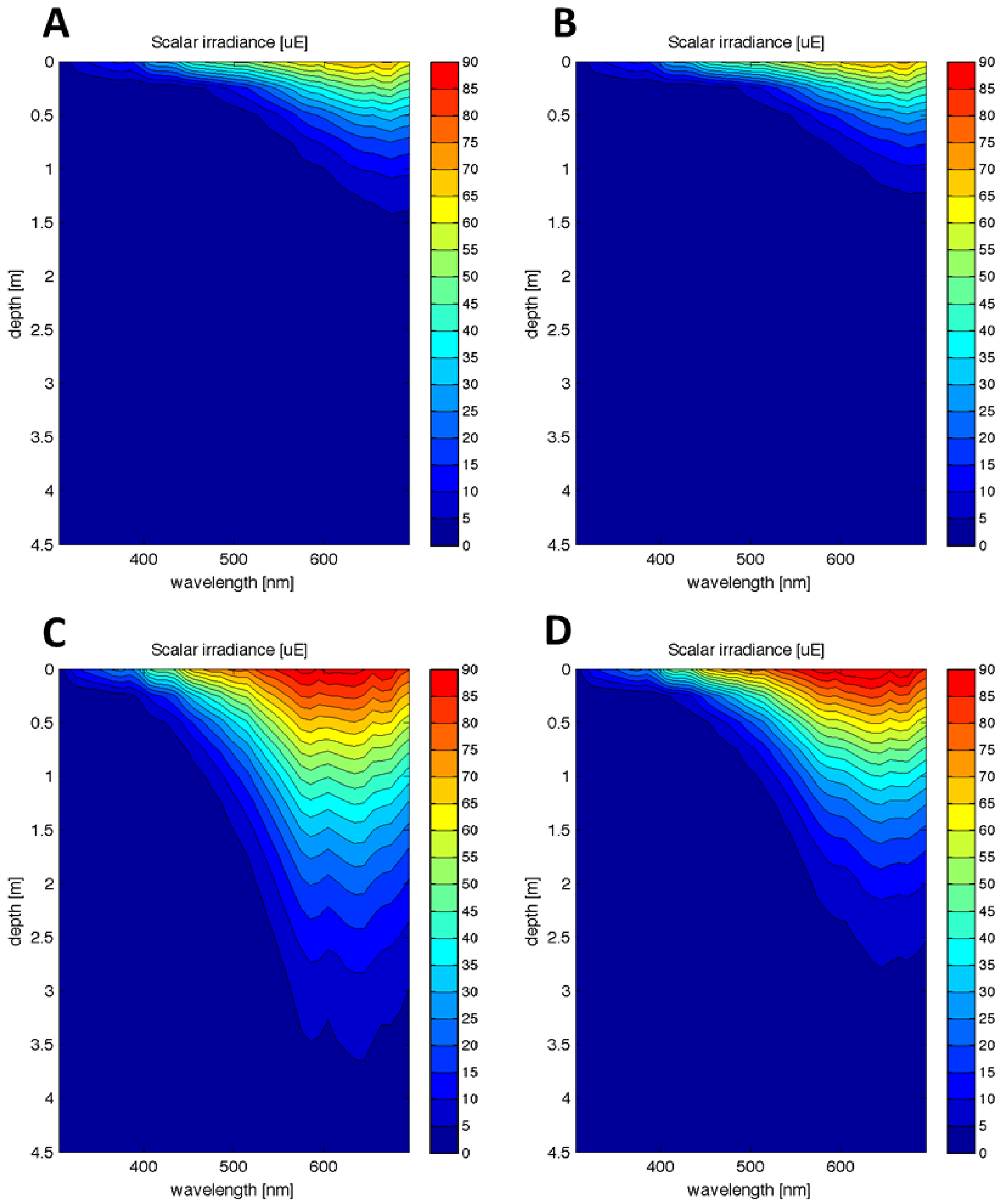


Figure 13. Modelled scalar radiation of the inner harbour water column as a function of depth for an A) incoming tide in the presence of dredging , B) outgoing tide in the presence of dredging, C) incoming tide in the absence of dredging and D) outgoing tide in the absence of dredging during Spring.

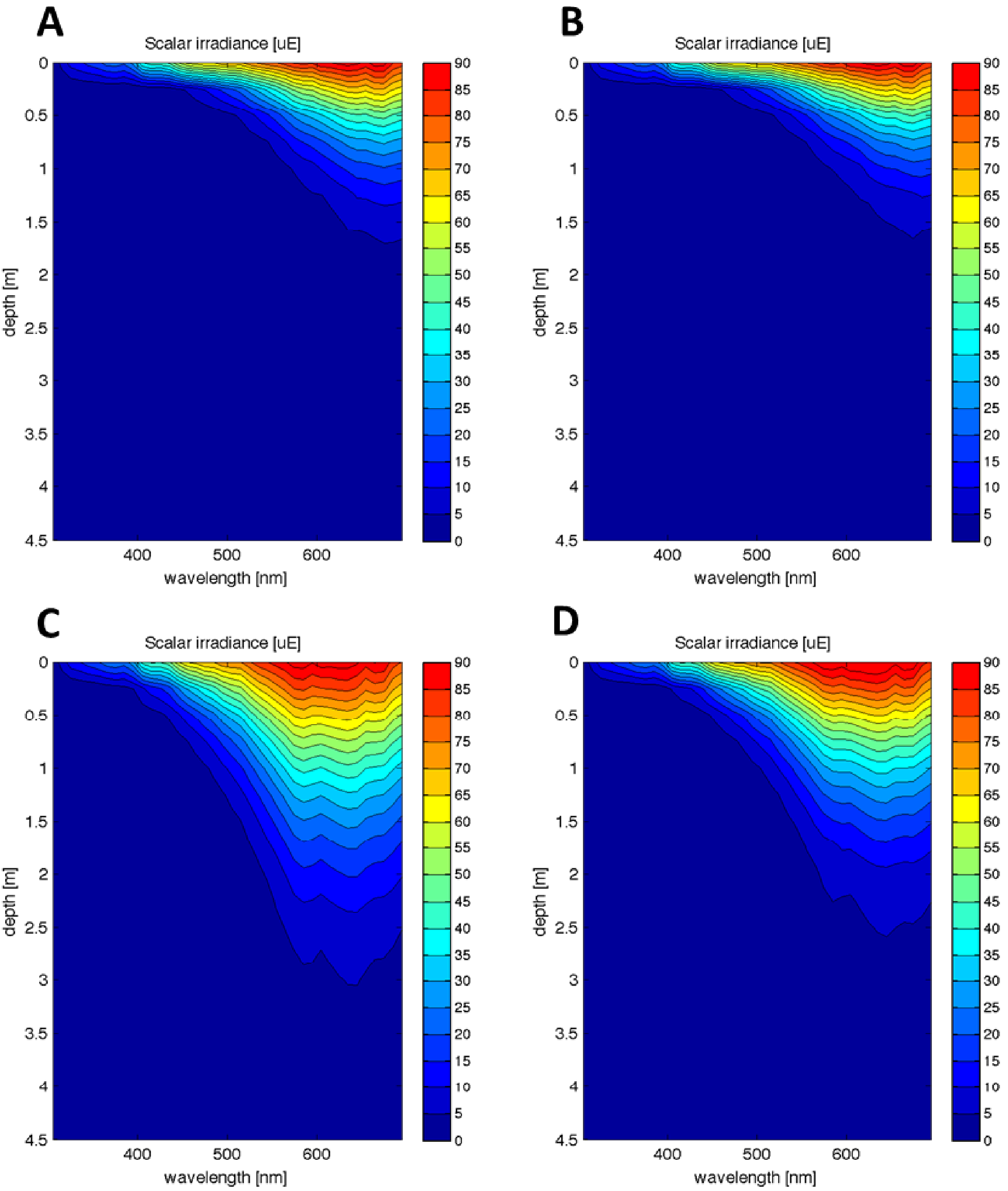


Figure 14. Modelled scalar radiation of the inner harbour water column as a function of depth for an A) incoming tide in the presence of dredging , B) outgoing tide in the presence of dredging, C) incoming tide in the absence of dredging and D) outgoing tide in the absence of dredging during Spring.

The model was able to simulate a natural tidal effect of light penetration within the inner harbour during all seasons, with a change from maximum light penetration (I_{max}) depth during the incoming tide to a decline in I_{max} of 0.5 -1.2 m during the outgoing tide (Table 1). In the absence of dredging, irradiance penetration reached an average (±SE) depth of 3.6 ± 0.21 m on an incoming tide and 2.85 ± 0.08 m on the outgoing tide (Table 1). PAR values at a 2.5 m depth varied greatly with both tide and season (Table 1). The average (±SE) annual PAR at this depth was 127 ± 22.3 on the incoming tide and only 56 ± 9.3 µE m^{-2} s^{-1} on the outgoing tide; there was also a seasonal effect, with greatest light values occurring during the winter and spring.

PUR values (photosynthetically usable radiation) derived from the weighted absorption spectra of seagrass showed a reduction in irradiance during all tides and seasons (Table 1). This suggests an overestimation of PAR that is available for use by the seagrasses. In other words, seagrasses cannot utilise all wavelengths of light available to them due to the limitations of what portions of the spectra their pigments absorb and use in photosynthesis. Overestimation was on average 11 ± 1.14 % during an incoming tide and 4.6 ± 0.64 % during an outgoing tide. In winter, light for seagrasses was overestimated by 14.38%, which could prove quite significant for seagrass health under prolonged conditions.

Table 1. Maximum depth of scalar irradiance (I_{max}) and spectrally integrated light at 2.5 m depth for photosynthetically active radiation (PAR) and photosynthetically usable radiation (PUR) weighted by measured absorption spectrum of *Zostera capricorni* leaves. Percentage loss in irradiance when using PUR in replacement of PAR. Data are based on values derived from the radiative transfer model and are determined for inner harbour conditions in the absence of dredging.

Site	Tide	I_{max} (m)	PAR ($\mu E\ m^{-2}\ s^{-1}$)	PUR ($\mu E\ m^{-2}\ s^{-1}$)	Overestimation (%)
Autumn	In	3.2	86.60	78.15	9.75
	Out	2.6	40.40	39.08	3.24
Winter	In	4.1	126.00	107.80	14.38
	Out	2.9	40.20	38.02	5.42
Spring	In	3.8	190.00	171.87	9.99
	Out	3.0	77.20	74.25	3.81
Summer	In	3.3	108.00	97.38	9.83
	Out	2.9	66.00	62.07	5.95

Table 2. Maximum depth of scalar irradiance (I_{max}) and spectrally integrated light at 2.5 m depth for photosynthetically active radiation (PAR) and photosynthetically usable radiation (PUR) weighted by measured absorption spectrum of *Zostera capricorni* leaves. Percentage loss in irradiance when using PUR in replacement of PAR. Data are based on values derived from the radiative transfer model and are determined for inner harbour conditions in the presence of dredging.

Site	Tide	I_{max} (m)	PAR ($\mu Em^{-2}\ s^{-1}$)	PUR ($\mu Em^{-2}\ s^{-1}$)	Overestimation (%)
Autumn	In	1.9	18.30	18.72	-2.32
	Out	1.6	11.40	11.55	-1.33

Winter	In	1.5	6.91	7.01	-1.53
	Out	1.3	4.85	4.85	-1.42
Spring	In	1.4	8.12	8.34	-2.82
	Out	1.3	4.35	4.58	-0.04
Summer	In	1.7	13.80	13.95	-1.12
	Out	1.6	10.70	10.98	-2.68

The changes with tide and season were less defined when modelled for dredge conditions (Table 2). The difference between PAR and PUR was negligible, resulting in no significant difference in light climate. Overall, I_{max} was less than 2.0 m throughout the year and with tidal cycle and the light was approximately one-tenth of the irradiance modelled in the absence of dredging (Table 1). The model was unable to differentiate scalar irradiance and the spectrally-integrated light between seasons and tides during the dredge scenario. This was due to the measured high refraction from the high concentration of suspended particles, which exceeded the computational capabilities of the model.

By plotting the logarithmic of scalar irradiance (log PAR) at a 2.5 m depth against the % overestimation of light for seagrass usability that was calculated from the model output, we were able to obtain a linear relationship. In the presence of dredging, this relationship was non-significant and weak (R^2 = 0.1953), data not shown. However, in the absence of dredging, a positive and highly significant relationship was determined (Fig. 15).

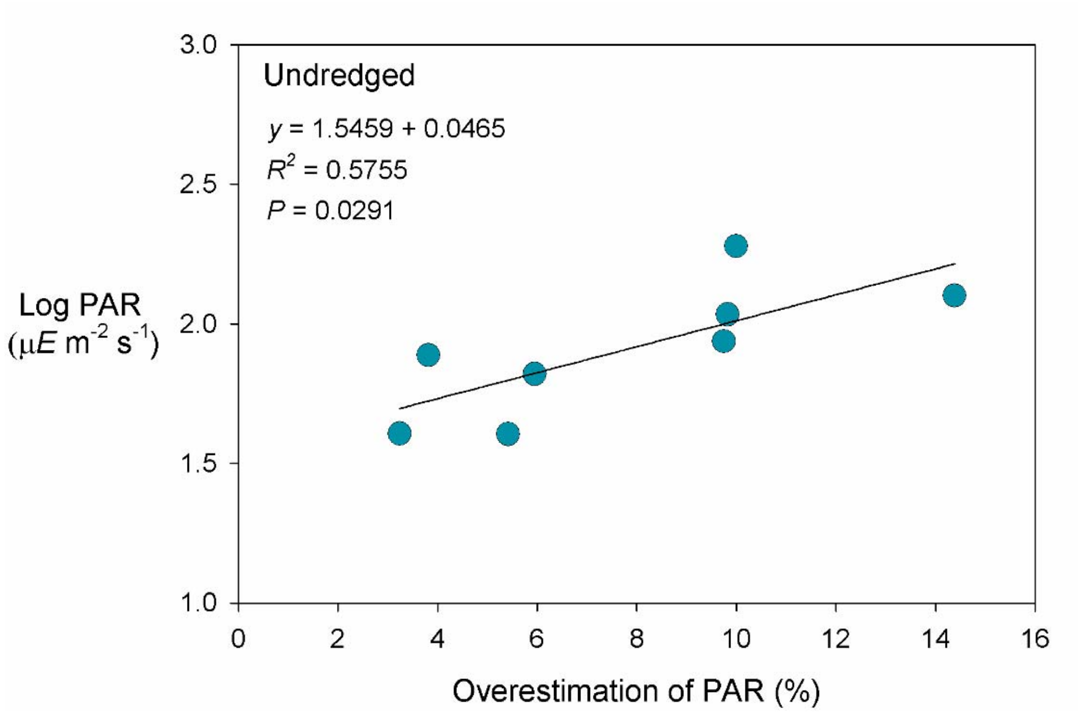


Figure 15. Log PAR taken from each tide and season plotted as a function of % overestimation of PAR in modelled un-dredged waters. Line represents linear regression; the slope and intercept, R^2 and statistical significance are given.

Model capabilities and simulations:

Using the inherent optical properties for the non-dredged conditions, we are able to simulate the light field of the water column under different scenarios; such as changes in sediment type, mineral type, as well as various concentrations of chlorophyll *a* and colour dissolved organic matter (CDOM). The model can be used to test the effects these variables have on water optical properties and light field, some examples are presented below.

The first simulation shows a dramatic change in the light field with the addition or removal of chlorophyll *a* and CDOM (Fig. 16). In the bloom scenario (increased chlorophyll *a* and CDOM concentrations), the light is heavily attenuated and the spectral properties of the light clearly show absorption of wavelengths by photoautotrophs (Fig. 16A). In contrast, when absorption by chlorophyll *a* and CDOM are removed completely, *in situ* irradiance increases and there is no spectrally weighted absorption, but rather an even spread of wavelengths across the spectrum (Fig. 16B).

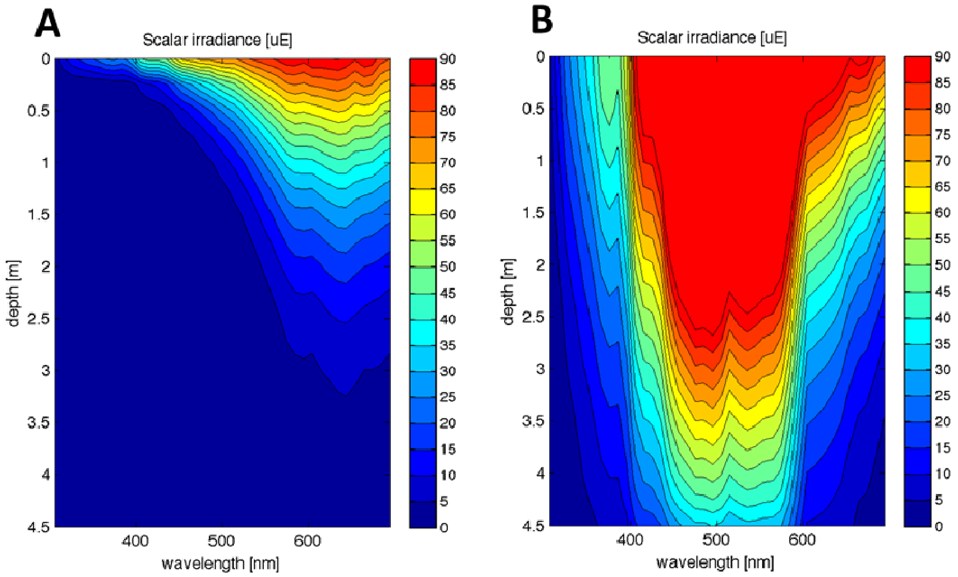


Figure 16. Simulation of light field model to test for the effect of chlorophyll *a* and CDOM in the water column during A) a bloom scenario and B) in the absence of chlorophyll *a* and CDOM.

The second simulation tests the effects of sediment type and water column properties on *in situ* light field. No major differences in light field are visible when the sediment type is changed from dark sediments to red clay sediments (Fig. 17A and B, respectively). However, by increasing the mineral concentration in the water column there is a notable increase in attenuation, due to the high scattering properties of the minerals (Fig. 17C). There are more than ten possible parameters that can induce non-linear changes in light field and those presented in this report illustrate the potential for the model to generate different environmental scenarios and predict the changes in the *in situ* light field.

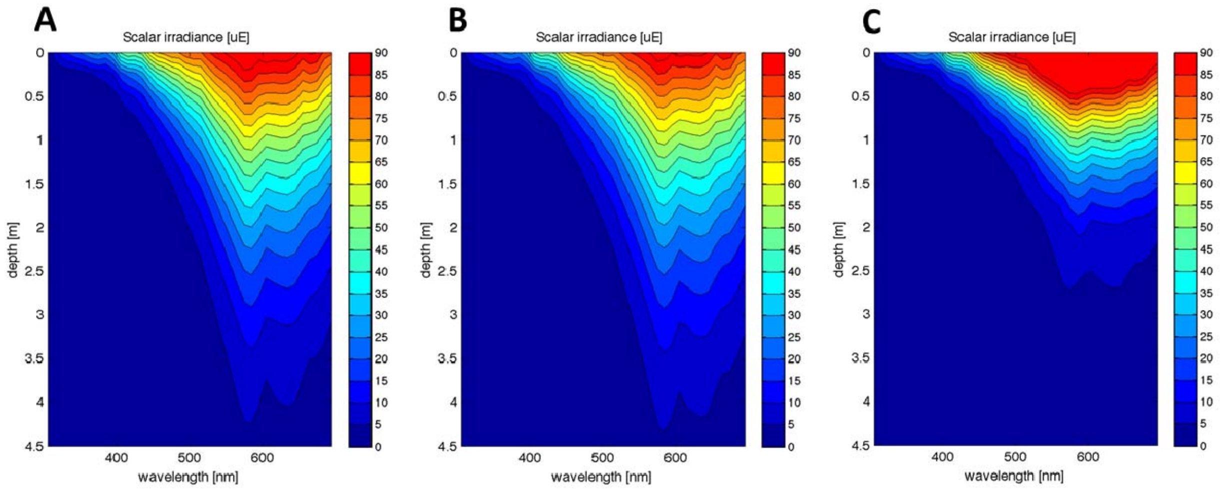


Figure 17. Simulation of light field model to test for effect of sediment type on the optical properties of the water column using A) dark sediments B) red clay, and C) mineral in the water column.

CONCLUSION

This report characterises the *in situ* optical properties of Gladstone Harbour and how they change in relation to dredge activity. The naturally turbid waters of the inner harbour vary greatly from the clearer waters outside the main harbour at Pelican Banks. There is high natural variability (from runoff, weather and tidal effects) in the transmission of light through the water column for the inner harbour, but the report has clearly demonstrated that dredging activity greatly reduces light penetration causing substantial changes in light conditions around the dredging activity, potentially impacting on seagrass growth and photosynthesis.

There are clear differences between the optical properties of the clear waters of Pelican Banks and the more turbid and highly attenuated waters within the harbour. While Pelican Banks is not appropriate as a reference site for the inner harbour, the light profiles at Pelican Banks do provide valuable information about *in situ* light conditions and optical properties that are able to sustain healthy seagrass meadows. More appropriate reference conditions for the inner harbour would be to compare between the inner harbour sites and making direct comparisons with measurements taken prior to dredge operations with those measured during the dredge activity (November 2011). Comparison of inner harbour sites have shown reduced light penetration with dredge activity both at the dredge site, as well as adjacent, upstream and downstream from the dredge site. The complete suite of sites within the harbour have provided greater resolution of inner harbour optical properties and resulting conditions from dredge activity.

For all light profiles in the harbour there was a low transmission in the blue (450-475 nm), moderate transmission in the green (495-570 nm), highest transmission in the yellow (580-600) and low transmission in the red (620-750 nm) parts of the spectrum. Seagrass photosynthesis is sensitive to the spectral quality of light, with high photosynthetic efficiency in the blue, poor sensitivity in the green and yellow and maximum efficiency in the red regions of the electromagnetic spectrum. This pattern is the converse of the light transmission measured at the inner harbour sites. The turbid water removes most of the photosynthetically useful photons (i.e. blue and red) while transmitting the less photosynthetically active yellow photons.

The output from the light model has shown the models ability to clearly distinguish between dredge and non-dredged sites. The high scattering from the increased suspended particles, as a result of the dredge activity, limits the models' capabilities to clearly differentiate between tides, seasons and compare PAR with PUR. However, the modelled output from the non-dredge conditions show clear differences with tides, seasons and spectrally-integrated light climate. The calculated PUR values were as high as 14.9 % less than the PAR equivalent, highlighting the potential for a significant overestimation of light available for photosynthesis within the seagrass meadows from the telemetered PAR sensors. The simulations showcase the capabilities and applicability of the light model for the harbour under non-dredge conditions. Based on the overall light field of the sites in the absence of dredging—ranging from 50-200 μ*E* m^{-2} s^{-1} at a depth of 2.5 m —, we can expect that the percentage of overestimation of PAR to lie somewhere in the range of 5-15%. By taking the log of *in situ* PAR and using the relationship determined in figure 14, we can estimate a percentage of light that would be approximately equivalent to PUR.

This study has provided evidence of changes in optical properties and light conditions within the inner harbour as a result of dredging activities. The hyperspectral and attenuation depth profiles clearly show the effect of the dredging activity on the transmissivity of the water within and around site 6, with a total loss of light penetration beyond 1.0 m depth. The large attenuation of light adjacent, upstream and downstream of the dredge site during the November cutter suction dredge activity suggest that the plume from the dredge site could be spreading throughout the harbour.

The new parameters derived from the model, include scalar irradiance during an incoming or outgoing tide in a particular season, as well as spectrally weighted PAR to determine PUR (photosynthetically useable radiation). Since not all absorbed photons result in photosynthesis, weighting the PAR with the seagrass absorption spectra, we were able to model PUR, which provides an excellent comparison for the relative amounts of useable irradiance that the seagrass receive within and outside of the harbour in the absence of dredge activity. During the dredging activity within the dredge site, the scattering is unfortunately too high and attenuation too great for any detectable differences in spectral radiation to be derived by the model. However, it is of vital importance to understand the potential over-estimation of PAR from the sensors on seagrass banks adjacent or downstream from the dredge. In obtaining a calculable percentage of loss in light, we are better equipped to construct a minimum light requirement. PUR gives us the ability to understand the light the plants are receiving for photosynthesis and therefore better manage the impact on seagrass health and survival. Therefore, any PAR-based minimum light requirement should be lowered by an appropriate percentage to ensure that the PUR is enough to sustain a healthy seagrass meadow.

REFERENCES

Dennison, W. C., Orth, R. J., Moore, K. A., Stevenson, J. C., Cater, V., Kollar, S., Bergstrom, P. W. & Batiuk, A. (1993). Assessing water quality with submerged aquatic vegetation. *BioScience* **43**: 86-94.

Gallegos, C. L., Kenworthy, W. J., Biber, P. D. & Wolfe, B. S. (2009). Underwater spectral energy distribution and seagrass depth limits along an optical water quality gradient. *Smithsonian contributions to the marine sciences.* **38**: 359-367.

Zimmerman, R. C. (2003). A biooptical model of irradiance distribution and photosynthesis in seagrass canopies. *Limnology and Oceanography,* **48**: 568-585.

APPENDIX III

Seagrass tidal study: Temporal changes in seagrass production of two tropical intertidal seagrass meadows (*Zostera capricorni* and *Halophila ovalis*) of Gladstone Harbour

Petrou K and Ralph PJ, and Chartrand KM

Introduction

Gladstone harbour is subject to large tidal fluxes resulting in a highly variable light environment for intertidal seagrasses. This large tidal range means that the shallower seagrass meadows often become air exposed during the day, potentially influencing photosynthetic performance and oxygen production. Exposure or near exposure at the lower tidal range may provide longer periods for positive net photosynthesis or result in photodamage due to high light stress on photosynthetic tissues. It is also possible that exposed seagrass blades exceed their thermal tolerance for photosynthesis or become limited in CO_2 supply when exposed for long periods at low tide which would also lead to a decline in net photosynthesis (Leuschner et al. 1998). Thus, it is important to understand how exposure of intertidal meadows affects photosynthesis, which may influence dredge mitigation strategies by potentially providing a “window” of relief from turbidity plumes at low tide.

This study investigates the effect of tidal exposure on the photochemical efficiency, pigment properties and oxygen production of intertidal seagrass meadows from Gladstone Harbour over a tidal cycle during different seasons. The quarterly sampling provides a better understanding of the variability in the responses to air exposure in seagrasses over an annual cycle, and helps to differentiate between temperature stress and desiccation or CO_2 limitation during air-exposure.

Materials and Methods

Sampling protocol

Field measurements were made over two separate days at Pelican Banks, Gladstone on four separate field trips, each during a different season –Spring (22nd & 24th October, 2010), Summer (19th and 20th January, 2011), Autumn (May, 2011) and Winter (July, 2011). Measurements were made from before noon until sundown at near-hourly intervals starting three hours prior to the absolute low tide. This sampling protocol was used to ensure that before, during and after air-exposure photosynthetic activity was captured in the sampling program on two common seagrass species *Zostera capricorni* and *Halophila ovalis*. Fluorescence measurements were performed using scuba to capture *in situ* photosynthetic activity and 2nd leaf blade samples were collected by the divers for measuring oxygen production, bio-optical properties and pigment determinations.

Chlorophyll *a* fluorescence

Chlorophyll *a* fluorescence measurements were conducted using a Pulse Amplitude Modulated fluorometer (Diving-PAM; Walz GmbH, Effeltrich, Germany). Rapid light curves (RLCs) were measured using the in-built software routine of nine incrementing actinic illumination steps (0, 33, 72, 117, 178, 249, 375, 512, 780 µmol photons m^{-2} s^{-1}) at 10 s intervals. A specialised leaf clip was used to position the fibre optic probe at a fixed distance from the leaf blade for each measurement. All measurements were performed on the second youngest leaf blade to be comparable across all plants. Six independent leaf blades were measured every 1–2 h on the outgoing tide.

Relative electron transport rate (rETR) was calculated as the product of effective quantum yield (Φ_{PSII}) and irradiance (µmol photons m^{-2} s^{-1}). Data were fitted according to the double exponential function as in Ralph and Gademann (2005) and three photosynthetic parameters; maximum rate of oxygen evolution (ETR_{MAX}), light utilisation efficiency (α) and minimum saturating irradiance (I_K) were derived from these curves. Effective quantum yield as a function of irradiance was plotted for *H. ovalis* and *Z. capricorni* to determine a photosynthetically dependent light response. Data were plotted and a linear regression analysis of the data applied.

Direct O_2 measurements

Rates of photosynthesis were determined before, during and after exposure at low tide by measuring oxygen (O_2) evolution inside 5 ml air- and water-tight incubation bottles equipped with oxygen sensitive luminescent material and read by an optical sensor (SDR SensorDish Reader, Presens, Germany). Rank 2 leaves were collected at 1-2 h intervals between 11:00 and 16:00 h on 22nd, 23rd and 24th Oct 2010 and processed on board within 1-2 h. Leaves were cleaned of epiphytes and placed into the incubation bottles filled with filtered seawater (3 to 5 leaves per bottle, n= 6 bottles). Oxygen concentrations within each bottle were measured at the start (t0) and end (t1) of a 20 min dark incubation period within a constant temperature seawater bath. After respiration measurements (R_D), the bottles were then placed into a transparent chamber that was returned to the seabed for 30 min *in situ* incubation, and recovered for measurement of the final O_2 concentration (t2). Rates of gross oxygenic production within each bottle were determined as: $P = P_N - R_D$, where P_N and R_D are the net photosynthesis measured in the light and the respiration in the dark, respectively:

$$P_N = [C(t2) - C(t1)] / (t2-t1)$$

$$R_D = [C(t1) - C(t0)] / (t1-t0)$$

Bottle productivity was normalized to total photosynthetic biomass in the bottle and is reported as mg O_2 g^{-1} h^{-1}.

Underwater light climate

Downwelling Photosynthetically Active Radiation (PAR) was determined at each the seabed using an underwater quantum sensor (LI-COR LI192SA) attached to a lowering frame and a photometer (LI-1400).

Leaf-specific absortpance A(λ)

Leaf-specific absorptance is a measure of the fraction of photosynthetically active radiation (PAR) captured by the leaf's photosynthetic pigments. Leaf spectral transmittance and reflectance were measured from 400 to 750 nm at 1 nm resolution using two fibre optic spectrometers (Ocean Optics USB2000+ and USB2000) interfaced with two integrating spheres (FOIS-1 and ISP-REF, respectively; Ocean Optics, USA). Leaves collected from each plot were placed in numbered plastic containers and kept moist and in the dark until optical properties were measured (within ~1 hour). Leaves were gently scraped clean of epiphytes and placed between two microscope slides. Black tape was used to obscure the portion of the sample port not covered by leaf tissue.

For transmittance measurements, a tungsten halogen light source (Ocean Optics LS-1) was adjusted to completely irradiate the 9.5 mm diameter sample port of the integrating sphere (FOIS-1). Leaf spectral transmittance (T(λ)) was calculated referenced to the slide and tape without a leaf in place. For reflectance measurements, the sample was placed over the port of the second integrating sphere (ISP-REF) so that the same side faced the light source (internal to the sphere in the case of the ISP-REF). Leaf spectral reflectance (R(λ)) was calculated referenced to the slide and tape with a diffusive reflectance standard (Spectralon 98%). Leaf-specific absorptance A(λ) was then calculated as:

$$A(\lambda) = 1 - T(\lambda) - R(\lambda) - A(750\text{ nm})$$

Where A(750 nm) is a correction for non-photosynthetic absorptance:

$$A(750\text{ nm}) = 1 - T(750\text{ nm}) - R(750\text{ nm})$$

Leaf-specific photosynthetic absorptance A_{ϕ}(PAR) was calculated as the spectral average of A(λ) over the spectral range 400-700 nm.

Leaf optical cross section a*(λ)

Leaf optical cross section (*a**) is a measure of chlorophyll use efficiency. The collected leaves were photographed and their surface area was determined digitally using image analysis software (ImageJ). Pigments were then extracted by grinding weighted leaf samples in ice cold 80% acetone using a mortar and pestle with clean sand. Concentrations of chlorophyll *a* (Chl *a*), chlorophyll b (Chl *b*) were determined spectrophotometrically using the equations and extinction coefficients of Jeffrey and Humphrey (1975). The optical cross-section of each leaf, a*(λ), was calculated by normalizing a(λ) to the area-specific Chl *a* concentration of each leaf. The leaf-specific absorption coefficient a(λ) was calculated from the absorptance A(λ) as -ln [1-A(λ)] and the optical cross-section a*(λ) was calculated by normalizing a(λ) to the area specific Chl *a* concentration :

$$a^*(\lambda) = a(\lambda) / [\text{Chl } a]$$

HPLC Pigments

Pigment concentrations were determined using high performance liquid chromatography (HPLC). Three leaf blades from each treatment plot were collected from underneath the shade and then immediately frozen in liquid nitrogen. Pigments were extracted by grinding and analysed according to the methods of van Heukelem and Thomas (2001) with the only modification being an extra filtration step through 0.2 µm PTFE 13 mm syringe filters (Micro-Analytix Pty Ltd). Clarified samples were stored in amber HPLC glass vials (Waters Australia Pty Ltd, Australia) and were stored at -80 °C overnight before analysis. The HPLC system included a pump, temperature controlled auto-injector (Waters Australia Pty Ltd, Australia), C8 column (150 x 4.6 mm; Eclipse XDB), and photodiode array detector (Waters Australia Pty Ltd, Australia). Pigments were identified by comparison of their retention times and spectra using calibration standards (DHI, Denmark) for each pigment. Peaks were integrated using Empower Pro software (Waters Australia Pty Ltd, Australia) and checked manually to confirm the accuracy of the peak baselines and the similarity of the integrated peaks to that of the standard. The pigment data were used to investigate rapid photoprotective responses over a tidal cycle by measuring the de-epoxidation state of violaxanthin (a measure of violaxanthin conversion to the photoprotective zeaxanthin). This was calculated as: ((zeaxanthin) + (0.5*antheraxanthin)) / (Violaxanthin + Antheraxanthin + Zeaxanthin).

Data analysis

To test for significant differences in the photosynthetic parameters and oxygen production throughout the day a one-way analysis of variance (ANOVA) was used at $\alpha = 0.05$. To ensure that the assumption of equal variances for all parametric tests was satisfied, a Levene's test for homogeneity of variance was applied to all data *a priori*. In cases where the assumption of homoscedasticity was not met, data were log transformed before analysis or a non-parametric Kruskal-Wallis test was used instead. All analyses were performed using Minitab statistical software (version 15.1.0.0 2006, Pennsylvania, USA).

Results

Spring (growing season):

Chlorophyll *a* fluorescence and pigments

Relative electron transport rate (rETR) varied over the course of the day. The increase in rETRs was concomitant with increased irradiance just after solar noon on both days for *Z. capricorni*, showing a decline in the late afternoon (Fig 1a and b). *Halophila ovalis* also showed a light-dependent response, where with increased irradiance there was an increase in the rETR (Fig. 1c). This would suggest that these species are acclimated to a high light environment and have a high-light requirement for growth and photosynthesis. The earlier decline in rETR at 14:15 on the 22nd for *Z. capricorni* (Fig. 1a) coincided with air exposure at low tide, indicating a negative response to desiccation. On the 24th however, the seagrass did not become exposed until the final measurement at 15:30 which coincided with lower irradiance levels (Fig. 1b and c).

Table 1: Photosynthetic parameters maximum electron transport rate (ETR$_{MAX}$), minimum saturating irradiance (I$_K$) and light utilisation efficiency (α) calculated from the light response curves of *Zostera capricorni* (22nd October, 2010) according to Ralph and Gademann (2005). Data represent means ± SD (*n* = 6). * indicates statistical significance at α < 0.05.

Time	12:20	13:30	14:15	16:45
PAR	199	360	1097	53
Water depth	1.5m	0.5m	Exposed	1m
EQY	0.762 ± 0.014	0.747 ± 0.020	0.505 ± 0.173*	0.757 ± 0.014
rETR$_{max}$	73.8 ± 7.6	74.2 ± 11.8	66.30 ± 9.70	53.20 ± 5.60*
I$_k$	75.48 ± 6.7	77.8 ± 14.15	106.10 ± 40.60*	53.70 ± 7.50*
α	0.97 ± 0.02	0.95 ± 0.04	0.64 ± 0.22*	0.99 ± 0.06

Effective quantum yield declined with increased irradiance in *Z. capricorni* on both days, with the minimum occurring during air exposure (Tables 1 and 2). The same pattern was seen in *H. ovalis* with a consistent and significant decline in EQY with increasing *in situ* irradiance and a minimum during air exposure. However, there was no significant difference in EQY between the highest irradiance at 0.2m and the slightly lower irradiance during air-exposure (Table 3), suggesting that unlike *Z. capricorni,* light plays more of a significant role in down regulation of photosynthetic efficiency in *H. ovalis* than air-exposure.

Table 2: Photosynthetic parameters maximum electron transport rate (rETR$_{MAX}$), minimum saturating irradiance (I$_K$) and light utilisation efficiency (α) calculated from the light response curves of *Zostera capricorni* (24th October, 2010) according to Ralph and Gademann (2005). Data represent means ± SD (*n* = 6). * indicates statistical significance at α < 0.05.

Time	10:30	12:00	14:00	15:40
PAR	240-320	358-510	792-774	733-543
Water depth	1.9m	1.5m	0.2m	Exposed
EQY	0.726 ± 0.01	0.702 ± 0.02	0.647 ± 0.026*	0.598 ± 0.036*
rETR$_{MAX}$	69.90 ± 4.80	84.04 ± 7.33	84.90 ± 12.20*	55.05 ± 11.9*
I$_k$	70.90 ± 5.40	97.70 ± 9.02	108.30 ± 27.40*	79.90 ± 7.88
α	0.98 ± 0.02	0.86 ± 0.02	0.82 ± 0.11	0.68 ± 0.13*

Effective quantum yield declined significantly ($P < 0.001$) with air-exposure in *Z. capricorni* (Tables 1 and 2) and *H. ovalis* (Table 3). The data from the 22nd of October showed a significant lowering of ETR$_{MAX}$ when plants became air-exposed under maximum irradiance ($P = 0.002$; Table 1). Similarly, maximum ETR (rETR$_{MAX}$) and minimum saturating irradiance (I$_K$) values showed a light-dependent response in both species measured on the 24th October (Tables 2 & 3). There was a significant increase in rETR$_{MAX}$ with increased irradiance followed by a significant decline in the afternoon once air-exposed in both *Z. capricorni* (*P* < 0.000; Table 2) and *H. ovalis* (*P* = 0.011; Table 3). The changes in I_K were also significant and followed the same pattern as ETR_{MAX} for both *Z. capricorni* (*P* = 0.001; Table 2) and *H. ovalis* (*P* = 0.003; Table 3). This further supports the idea of additional stress to the plants when exposed through desiccation, even when irradiance is optimal for greater rates of electron transport. Light utilisation efficiency (α) decreased throughout the day in *H. ovalis*, declining with increased irradiance and then declining further as the plants became air exposed (*P* <0.001; Table 3). This same pattern was seen in *Z. capricorni* on the 24th (*P* = 0.001; Table 2), however, on the 22nd, the lowest α value were measured during air exposure (*P* = 0.004), recovering to initial values when re-submerged (Table 1). This would suggest that the plant's ability to efficiently utilise the light available is greatly diminished under air-exposed conditions and that this parameter is more sensitive to desiccation than it is to increased irradiance in these seagrass species.

Table 3: Photosynthetic parameters maximum electron transport rate (ETR_{MAX}), minimum saturating irradiance (I_K) and light utilisation efficiency (α) calculated from the rapid light curves of *Halophila ovalis* (24th October, 2010) according to Ralph and Gademann (2005). Data corresponds to data in figure 2 and represent means ± SD (*n* = 6). * indicates statistical significance at α < 0.05.

Time PAR Water depth	10:30 240-320 1.9m	12:00 358-510 1.5m	14:00 792-774 0.2m	15:40 733-543 Exposed
EQY	0.737 ± 0.020	0.673 ± 0.014	0.657 ± 0.081	0.549 ± 0.102*
$rETR_{max}$	69.6 ± 13.2	74.8 ± 12.75	91.1 ± 16.8*	64.1 ± 7.5
I_k	69.0 ± 15.4	79.4 ± 18.6	113.0 ± 21.5*	84.5 ± 15.6
α	1.0 ± 0.04	0.96 ± 0.09	0.81 ± 0.028*	0.77 ± 0.08*

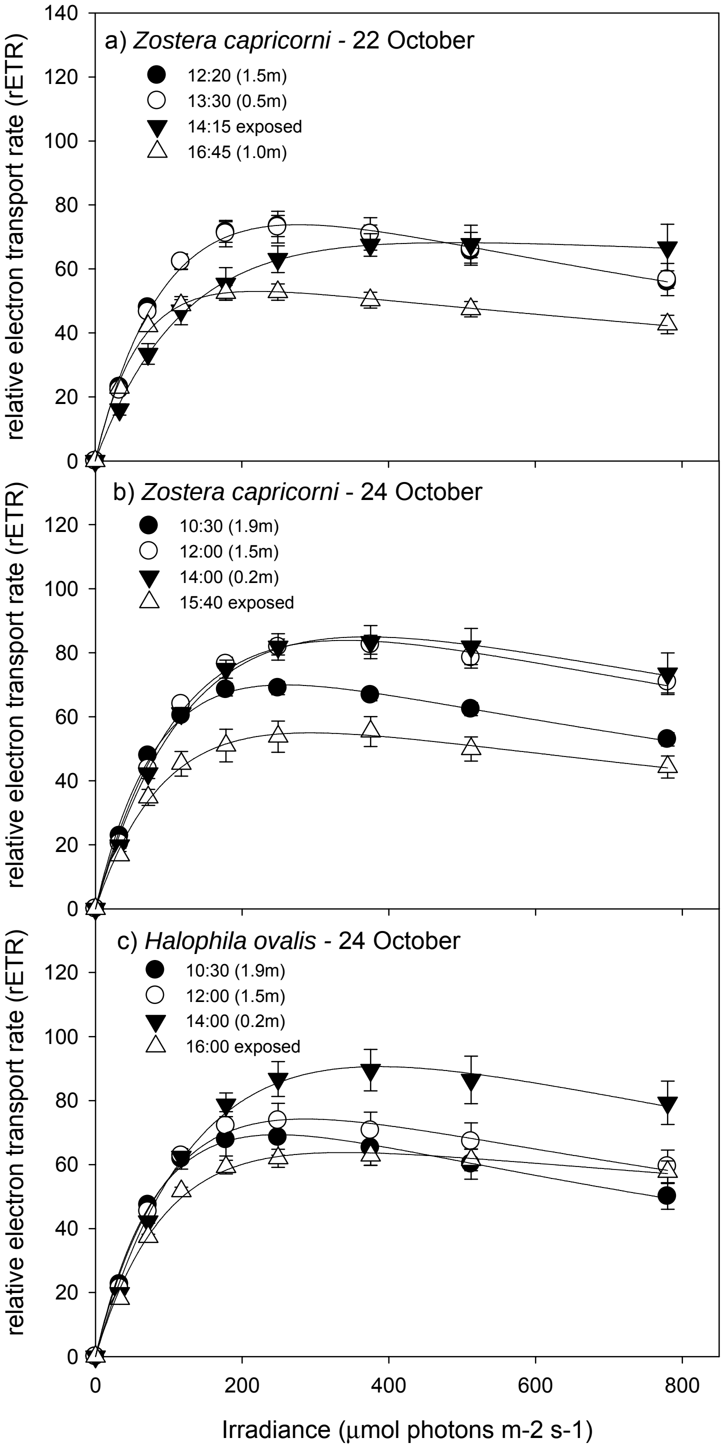


Figure 1: Relative electron transport rate (rETR) as a function of irradiance in the intertidal seagrass species *Zostera capricorni* and *Halophila ovalis* over a tidal cycle on two different days. Double exponential curve fit is shown (line). Time of day and water depth of seagrass at time of sampling in metres is given in legend. Data represent mean ± SEM (*n* = 6).

The photoprotective pigment ratios determined by HPLC analysis show a strong and significant increase ($P < 0.001$) with increased irradiance, where the violaxanthing de-epoxidation state increased throughout the day (Fig. 2). On all three days, the greatest violaxanthin de-epoxidation occurred during sample times of air-exposure (black, red and green arrows) in both *Z. capricorni* and *H. ovalis*.

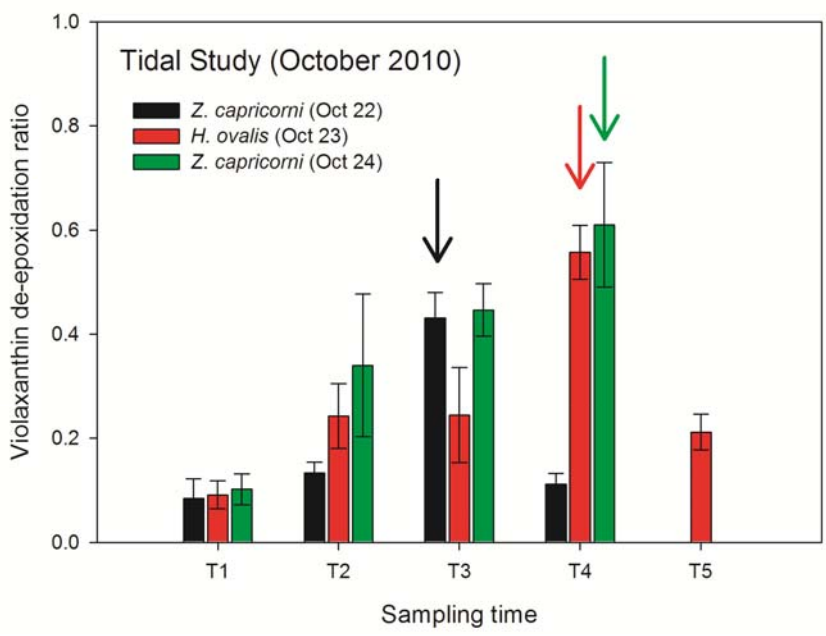


Figure 2: Violaxanthin de-epoxidation ratio for *Z. capricorni* (black and green bars) and *H. ovalis* (red bars) during a tidal cycle on the 22nd, 23rd and 24th of October 2010, respectively. Down arrows indicate time of air-exposure on each day. Time of day and depth in metres are the same as shown in Figure 3, for each species and indicated here as sample times. Data represent mean ± SD (n = 6).

O_2 evolution (incubation chambers)

Oxygen production by *Z. capricorni* showed different responses to tidal exposure on the 22nd and 23rd of October (Fig. 3). While on the 22nd O_2 production did not vary significantly ($P > 0.05$) between immersed and exposed time points, but on the following day there was a significant decrease in O_2 production after exposure ($P < 0.05$). On the 22nd, strong winds pushed water further up the bank, resulting in fewer patches of exposed bank. Consequently the samples used for incubation in air had to be collected from an exposed bank adjacent to the submerged area, and may have exhibited differences in photophysiology. However, on the 23rd, all samples were collected within the same area. *Halophila ovalis* exhibited a similar behaviour on the 24th, whereby O_2 production during air exposure was significantly lower than during the immersed periods ($P < 0.05$). Reduced light levels at the end of the day may have contributed to the decline in photosynthetic rate after immersion for *Z. capricorni* on the 23rd of October, and in the case of *H.*

ovalis on the 24th during air exposure. These patterns need to be further examined in relation to the weather conditions and irradiance levels on each day.

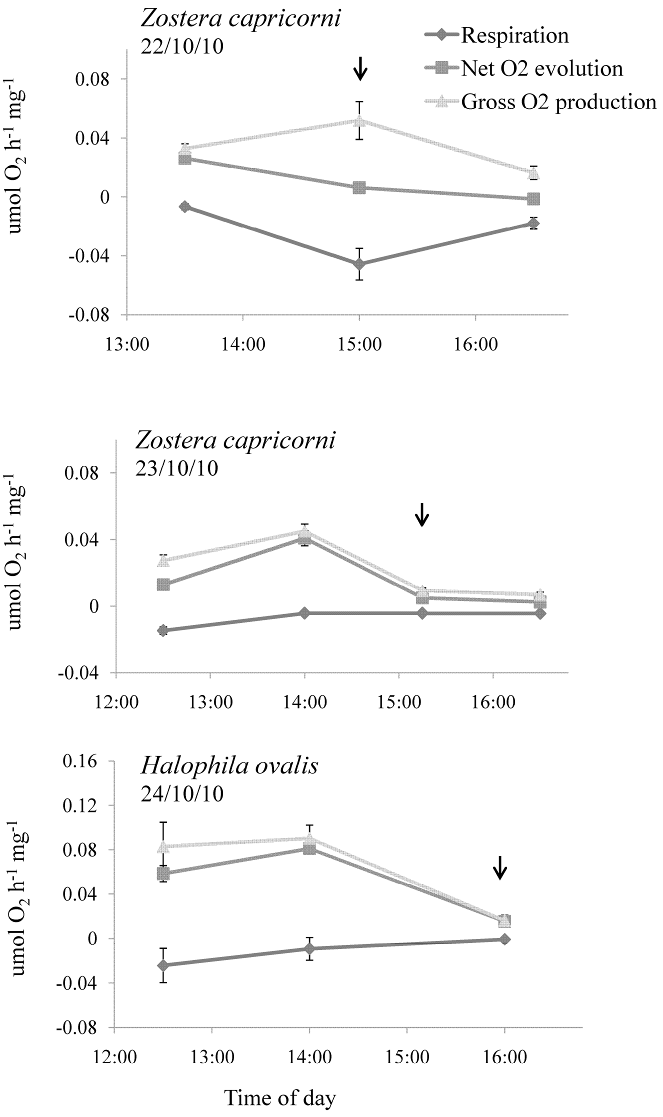


Figure 3: Rates of gross oxygenic photosynthesis (triangles) calculated from measurements of dark respiration (diamonds) and net photosynthesis (squares) *in situ* for the three days of the tidal exposure study. Averages ± SE (*n*=6) are shown, and the arrow indicates the time of air exposure at low tide.

Summary (growing season):

Chlorophyll *a* fluorescence and pigments

In both *Z. capricorni* and *H. ovalis* there was a light-dependent response, where with increased irradiance there was an increase in the rETR, with the exception of rETRs during air exposure (Fig. 4). Again, this suggests that these species both have a relatively high-light requirement for growth and photosynthesis. The less consistent patterns of rETR in *Z. capricorni* measured on the 19th of January compared to the data collected on the 20th is due to intermittent cloud cover throughout the day, which affected the synchronisation of the rapid light curve measurements with *in situ* PAR. There was a significant decline in rETRs at 15:00 h, which coincided with air exposure at low tide, irrespective of the high irradiance levels, demonstrating a negative response to air-exposure (Fig. 4a). Similarly, on the 20th, the air-exposed seagrass at 16:00 h showed rETRs equivalent to those at 2.0 m depth earlier that day (Fig. 4b). These data are consistent with the tidal exposure results from the October 2010 study, where both *Z. capricorni* and *H. ovalis* increased rETR as irradiance increased and displayed a clear negative response to air-exposure.

Table 4: Photosynthetic parameters effective quantum yield of photosystem II (EQY), maximum electron transport rate (ETR_{MAX}), minimum saturating irradiance (I_K) and light utilisation efficiency (α) calculated from the rapid light curves of *Zostera capricorni* (19th January, 2011) according to Ralph and Gademann (2005). Data corresponds to data in figure 2 and represent means ± SD (*n* = 6). * indicates statistical significance at α < 0.05.

Time	11:00	12:00	13:30	15:00	16:30
PAR	400	86	1550	1692	320
Water depth	2.5m	1.0m	0.3m	Exposed	0.2m
EQY	0.773 ± 0.010	0.737 ± 0.042	0.730 ± 0.030	0.346 ± 0.081	0.607 ± 0.080
ETR_{max}	40.27 ± 3.16	28.62 ± 5.30*	37.48 ± 3.51	10.65 ± 3.03*	37.41 ± 7.75
I_k	99.49 ± 8.36	74.06 ± 12.39	102.68 ± 9.13	60.52 ± 13.58*	130.83 ± 23.82
α	0.41 ± 0.01	0.38 ± 0.03	0.36 ± 0.03	0.17 ± 0.03*	0.28 ± 0.04*

There was a significant decline ($P < 0.005$) in effective quantum yield (EQY) during air-exposure in *Z. capricorni* (Table 4) and *H. ovalis* (Table 5). A slight decline in EQY was evident with increased irradiance, however the increase in irradiance actually lead to greater photosynthetic activity (as seen with the RLC data), which is consistent with the October 2010 findings as well as previous studies (Beer and Björk 2000).

Table 5: Photosynthetic parameters effective quantum yield of photosystem II (EQY), maximum electron transport rate (rETR$_{MAX}$), minimum saturating irradiance (I$_K$) and light utilisation efficiency (α) calculated from the light response curves of *Halophila ovalis* (20th January, 2011) according to Ralph and Gademann (2005). Data represent means ± SD (n = 6). * indicates statistical significance at $\alpha < 0.05$.

Time	11:30	12:30	13:45	15:00	16:00
PAR	153	741	591	1200	1053
Water depth	2.0m	1.0m	0.6m	0.05m	Exposed
EQY	0.759 ± 0.022	0.741 ± 0.018	0.734 ± 0.017	0.614 ± 0.076	0.436 ± 0.053*
ETR$_{max}$	15.64 ± 2.59*	26.82 ± 4.96	28.17 ± 8.17	42.20 ± 13.95	14.74 ± 5.91*
I$_k$	36.70 ± 6.19	61.86 ± 13.52	69.22 ± 23.03	145.07 ± 46.89	69.34 ± 31.91
α	0.43 ± 0.06	0.44 ± 0.02	0.41 ± 0.03	0.31 ± 0.03*	0.22 ± 0.03*

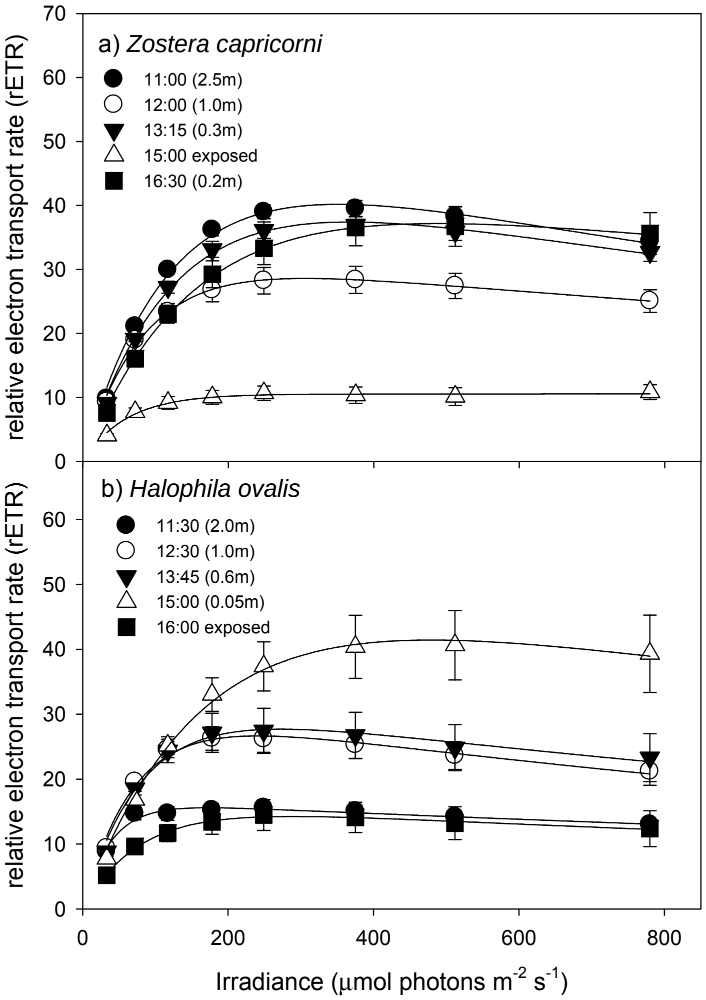


Figure 4: Relative electron transport rate (rETR) as a function of irradiance in the intertidal seagrass species *Zostera capricorni* and *Halophila ovalis* over a tidal cycle. Double exponential curve fit is shown (line). Time of day and depth in metres is given in legend. Data represent mean ± SEM (n = 6).

Maximum rETR ($rETR_{MAX}$) and minimum saturating irradiance (I_K) values showed a light-dependent response in both species (Tables 4 and 5), although with some inconsistencies for *Z. capricorni*. These were likely due to the intermittent cloud cover during sampling which may have affected some of the fluorescence and PAR values. In both species there was a significant decline in $rETR_{MAX}$ during air-exposure ($P < 0.001$; Tables 4 and 5). In both cases irradiance was very high during air-exposure, further supporting the suggestion of additional stress to the plants when exposed through desiccation, even when irradiance is optimal for greater rates of electron transport. Light utilisation efficiency (α) decreased throughout the day in both *Z. capricorni* and *H. ovalis*, declining with increased irradiance and then declining further as the plants became air exposed ($P < 0.001$; Table 4 and 5). The significant recovery in α following exposure (Table 4) emphasises the impact that air-exposure has on photosynthetic efficiency of *Z. capricorni*.

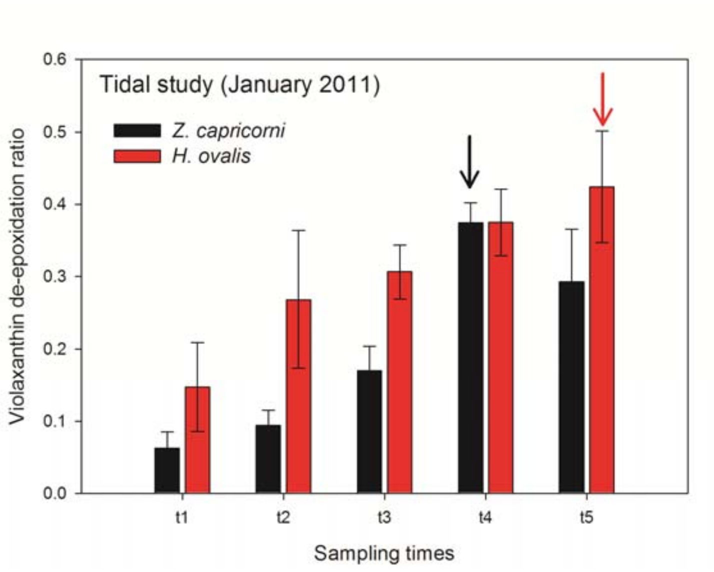


Figure 5. Violaxanthin de-epoxidation ratio for *Z. capricorni* (black bars) and *H. ovalis* (red bars) during a tidal cycle on the 19th and 20th of January 2011, respectively. Down arrows indicate time of air-exposure. Time of day and depth in metres are the same as in Tables 4 and 5 for each species and indicated here as sample times. Data represent mean ± SD ($n = 6$).

There was a significant increase ($P < 0.001$) in the violaxanthing de-epoxidation state with increased irradiance (Fig. 5). In both *Z. capricorni* and *H. ovalis* greatest violaxanthin de-epoxidation occurred during sample times of air-exposure (black and red arrows). This is consistent with the trend in photoprotective pigment ratios seen during the October tidal study (Fig. 2).

O_2 evolution (incubation chambers)

Oxygen production in *Z. capricorni* (Fig. 6) and *H. ovalis* (Fig. 7) followed a similar trend during tidal exposure, whereby O_2 production rate considerably increased with increasing irradiance as the tide withdrew. Net O_2 evolution of *Z. capricorni* and *H. ovalis* just prior to air exposure were 2-3 times greater than the noon values measured at ~ 1m depth. This is consistent with the results from the October tidal study, and shows that seagrass photosynthetic production closely tracks variations in irradiance.

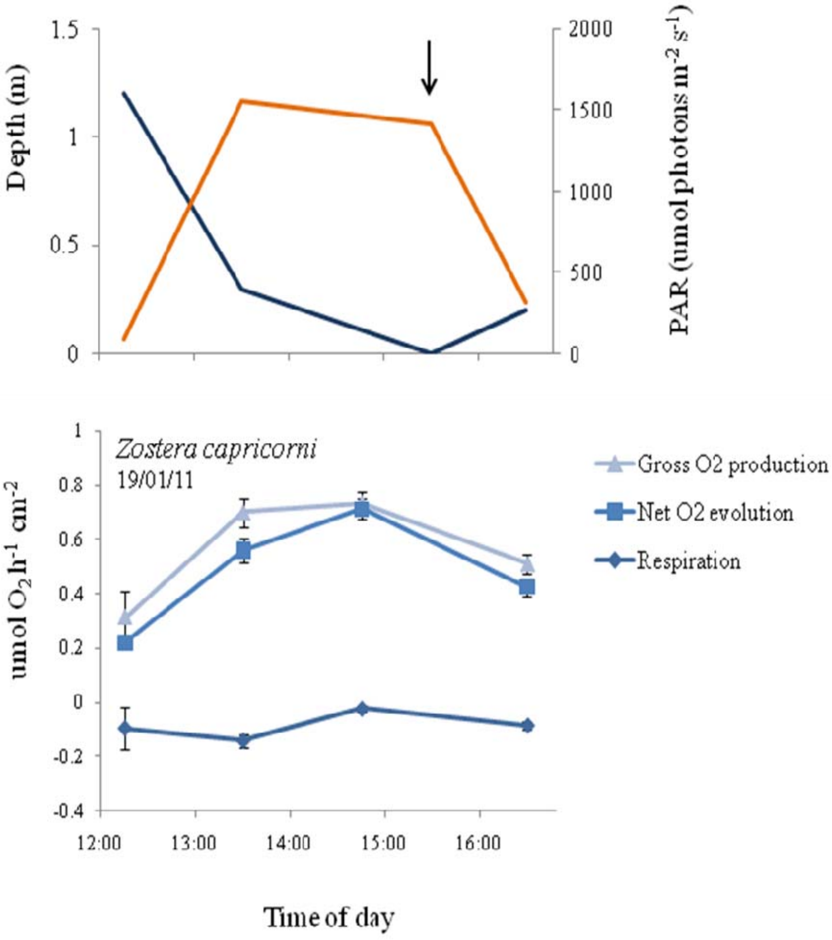


Figure 6. Rates of gross oxygenic photosynthesis (triangles) calculated from measurements of dark respiration (diamonds) and net photosynthesis (squares) *in situ* for *Zostera capricorni* at Pelican Banks. Averages ± SE (*n*=6) are shown, and the arrow indicates the time of air exposure at low tide.

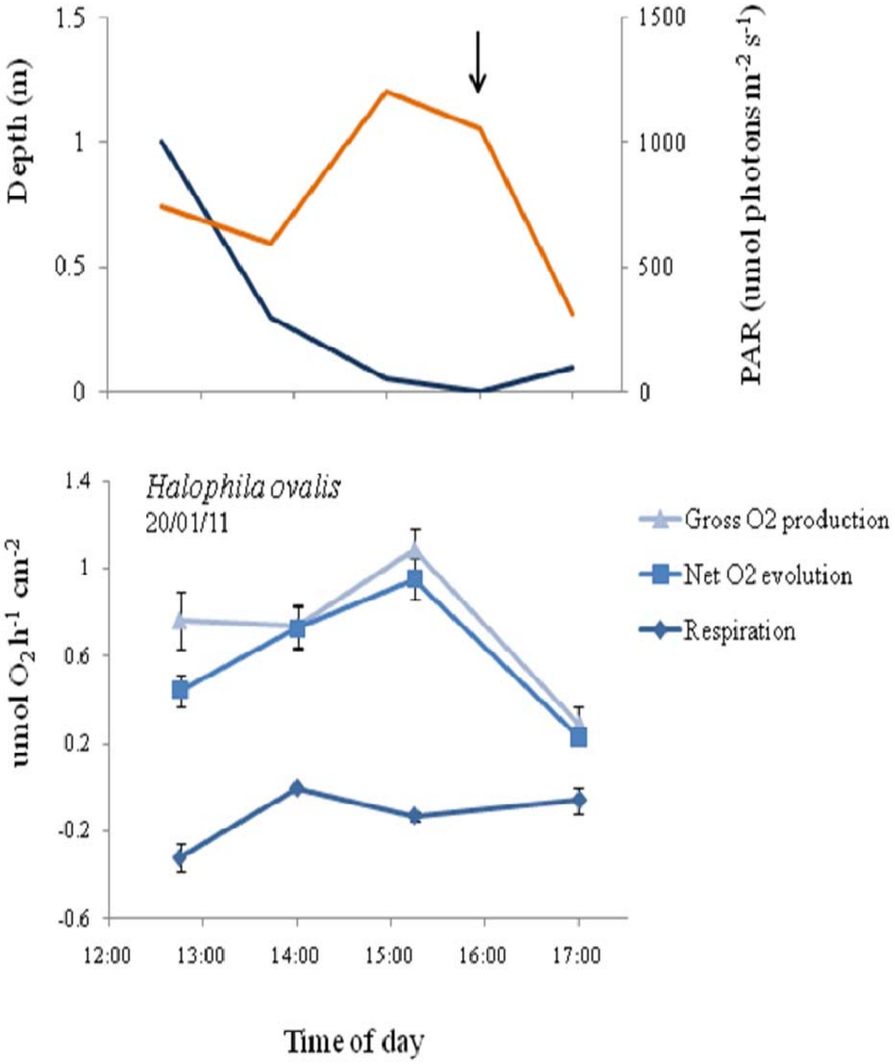


Figure 7. Rates of gross oxygenic photosynthesis (triangles) calculated from measurements of dark respiration (diamonds) and net photosynthesis (squares) *in situ* for *Halophila ovalis* at Pelican Banks. Averages ± SE (*n*=6) are shown, and the arrow indicates the time of air exposure at low tide.

Bio-optical characteristics - photosynthetic absorptance A_{Φ}(PAR):

Seagrass photosynthetic light absorption capacity remained largely constant throughout the tidal exposure event (Fig. 8). Only a slight and non-significant decline in A_{Φ}(PAR) was observed for *H. ovalis* during air exposure. Therefore these seagrass do not regulate their light capturing efficiency under rapidly changing light intensities.

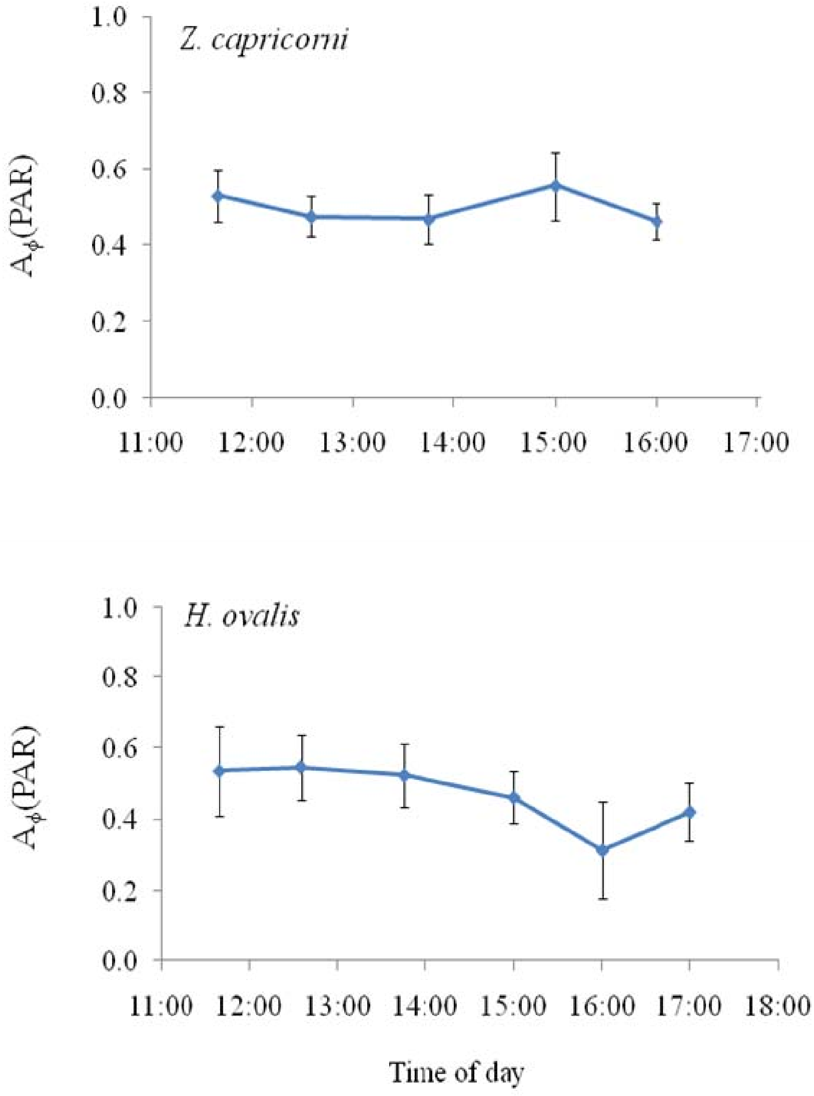


Figure 8. Photosynthetic absorptance A_{Φ}(PAR) of *Z. capricorni* and *H. ovalis* during a tidal exposure event. Averages ± standard deviation (n=10)

The oxygen and fluorescence data are consistent with respect to the seagrass physiological response over a tidal cycle and match well the October (Spring) tidal exposure data. The increase in photosynthetic activity with increased irradiance, suggests that these seagrass are evolved to take advantage of increased irradiance when the tide recedes. However, the consistent decline in photosynthetic activity with air-exposure for both species highlights the sensitivity of these intertidal species to the impacts of desiccation, CO_2 limitation or thermal stress.

Autumn (senescent season):

Chlorophyll *a* fluorescence and pigments

Relative electron transport rates (rETR) in *Z. capricorni* showed a clear decline of around 50% during air-exposure (Fig. 9a). No such clear response was evident in *H. ovalis,* instead all shallow or exposed rETRs declined significantly when compared with the first measurement at 1.5m depth (Fig. 9b). This is consistent with the finding during the other seasons, where *H. ovalis* shows greater sensitivity to increased irradiance than air exposure. An opposite response to that observed in *Z. capricorni,* which shows a much stronger response to air-exposure than to increased irradiance.

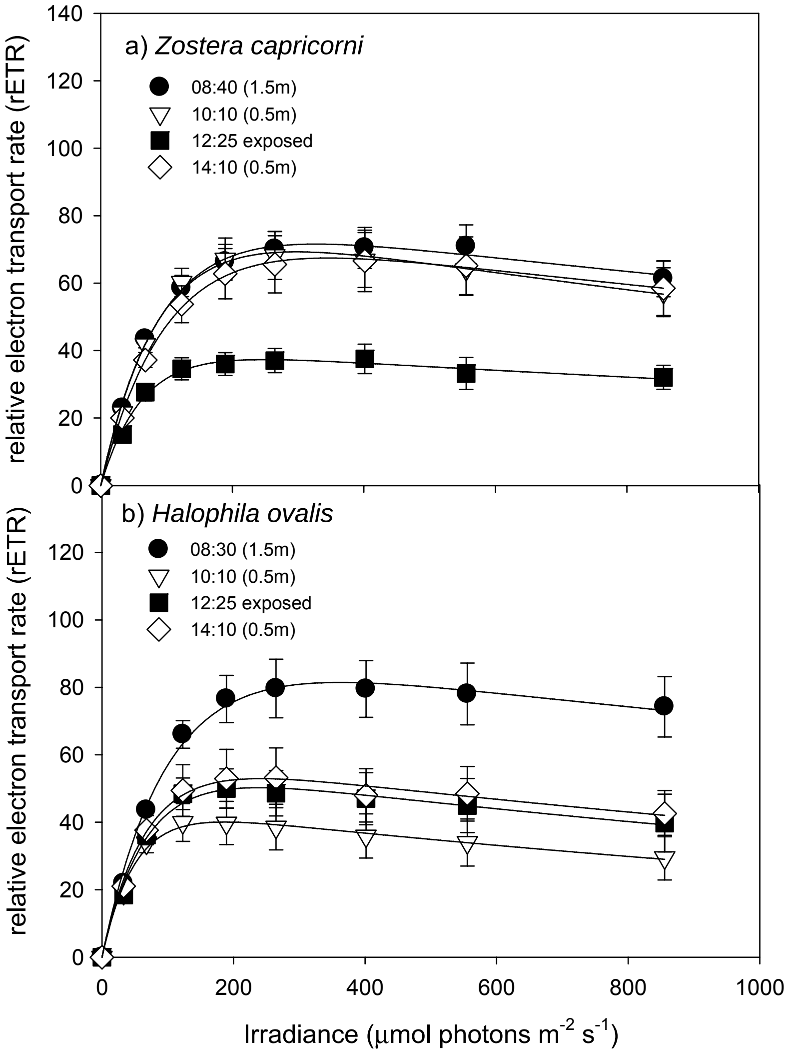


Figure 9: Relative electron transport rate (rETR) as a function of irradiance in the intertidal seagrass species (a) *Zostera capricorni* and (b) *Halophila ovalis* over a tidal cycle. Double exponential curve fit is shown (line). Time of day and depth in metres is given in legend. Data represent mean ± SD (*Z. capricorni n* = 6; *H. ovalis n* = 4).

Effective quantum yield (EQY) showed a light-dependent response with a significant decline ($P < 0.001$) during air exposure and greatest irradiance in both species (Tables 6 and 7). A similar pattern was observed for maximum rETR and α in *Z. capricorni* declining significantly during air exposure ($P = 0.012$ & $P = 0.001$; Table 6). *H. ovalis* did not show the same trend in photosynthetic parameters, instead, significant differences were only detected for $ETR_{MAX,}$ due to the high values measured at the lowest irradiance levels and deepest depth (Table 7). No differences were detected between sampling times for *H. ovalis.*

Table 6: Photosynthetic parameters effective quantum yield of photosystem II (EQY), maximum electron transport rate (ETR_{MAX}), minimum saturating irradiance (I_K) and light utilisation efficiency (α) calculated from the rapid light curves of *Z. capricorni* (May, 2011) according to Ralph and Gademann (2005). Data corresponds to data in figure 1a and represent means ± SD ($n = 6$). * indicates statistical significance at $\alpha < 0.05$.

Time	08:30	10:10	12:30	14:10
PAR	350	750	1800	750
Water depth	1.5m	0.5m	Exposed	0.5m
EQY	0.749 ± 0.019	0.732 ± 0.034	0.535 ± 0.070*	0.719 ± 0.050
$rETR_{MAX}$	71.84 ± 15.23	67.79 ± 21.24	37.69 ± 9.798*	67.74 ± 22.23
I_k	78.79 ± 18.61	73.04 ± 25.86	58.63 ± 17.16	83.23 ± 26.48
α	0.916 ± 0.041	0.937 ± 0.040	0.674 ± 0.194*	0.812 ± 0.067

Table 7: Photosynthetic parameters effective quantum yield of photosystem II (EQY), maximum electron transport rate ($rETR_{MAX}$), minimum saturating irradiance (I_K) and light utilisation efficiency (α) calculated from the light response curves of *H. ovalis* (May, 2011) according to Ralph and Gademann (2005). Data corresponds to data in figure 1b and represent means ± SD ($n = 4$). * indicates statistical significance at $\alpha < 0.05$.

Time	08:30	10:10	12:30	14:10
PAR	350	750	1800	750
Water depth	1.5m	0.5m	Exposed	0.5m
EQY	0.723 ± 0.022	0.638 ± 0.100	0.609 ± 0.072*	0.733 ± 0.033
$rETR_{MAX}$	82.24 ± 21.30*	40.82 ± 15.54*	51.92 ± 15.21	53.15 ± 21.90
I_k	86.61 ± 23.21	44.41 ± 15.13	60.56 ± 24.51	54.04 ± 22.75
α	0.954 ± 0.062	0.921 ± 0.619	0.888 ± 0.112	0.978 ± 0.114

Consistent with the previous two tidal studies, the violaxanthin de-epoxidation state showed a significant increase in de-epoxidation ratio with increased irradiance ($P < 0.05$) and maximum de-epoxidation occurring during air-exposure (Fig. 10).

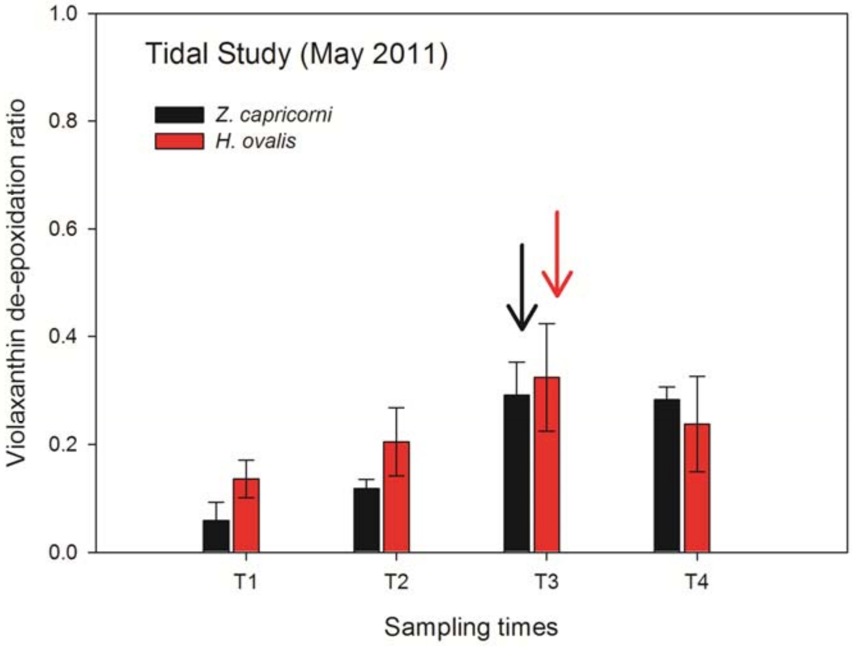


Figure 10. Violaxanthin de-epoxidation ratio for *Z. capricorni* (black bars) and *H. ovalis* (red bars) during a tidal cycle on the 14th of May 2011, respectively. Down arrows indicate time of air-exposure. Time of day and depth in metres are the same as in Tables 6 and 7 for each species and indicated here as sample times. Data represent mean ± SD (n = 6).

O_2 evolution (incubation chambers)

Consistent with the previous tidal exposure studies, rates of net and gross O_2 production in both species were significantly greater ($P < 0.01$) at 12:30 (just prior to exposure) than at 10:10 and 14:10 (Fig. 11). No change in dark respiration rate was found throughout the day in either species. In this sampling round (Autumn), *Z. capricorni* and *H. ovalis* were incubated simultaneously and thus exposed to identical light and temperature conditions, yet O_2 production rate appears more sensitive to irradiance variations and air exposure in *Z. capricorni* than *H. ovalis*, closely matching the fluorescence data.

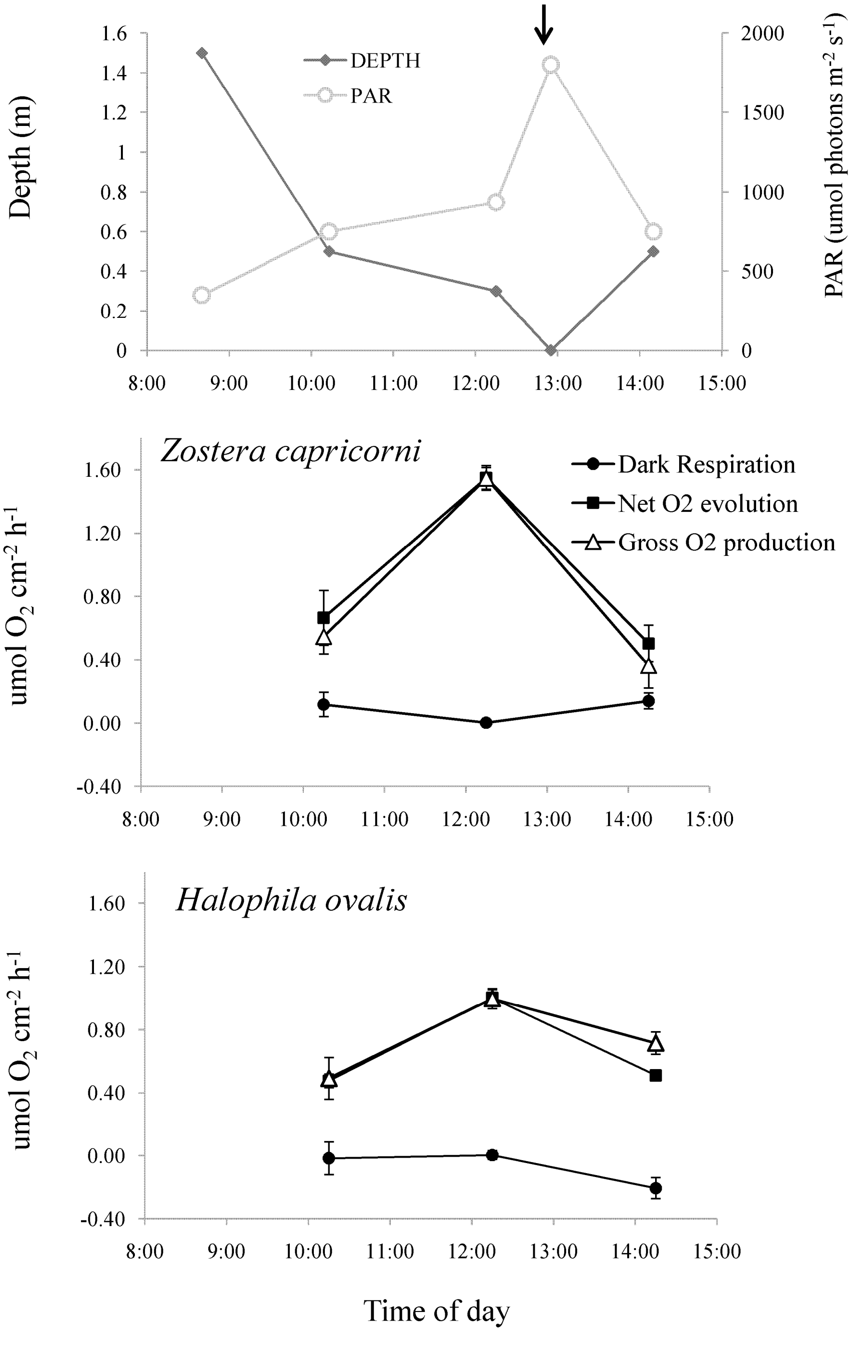


Figure 11: Rates of gross oxygenic photosynthesis (triangles) calculated from measurements of dark respiration (circles) and net photosynthesis (squares) *in situ* for *Zostera capricorni* and *Halophila ovalis* at Pelican Banks on 14th May 2011. Averages ± SE (*n*=6) are shown, and the arrow indicates the time of air exposure at low tide.

Bio-optical characteristics - photosynthetic absorptance A_{Φ}(PAR):

Consistent with the previous tidal exposure studies, the fraction of light absorbed by seagrass leaves remained constant throughout the tidal cycle (Fig. 12), thus further confirming that these seagrass do not regulate their light capturing efficiency under rapidly changing light intensities.

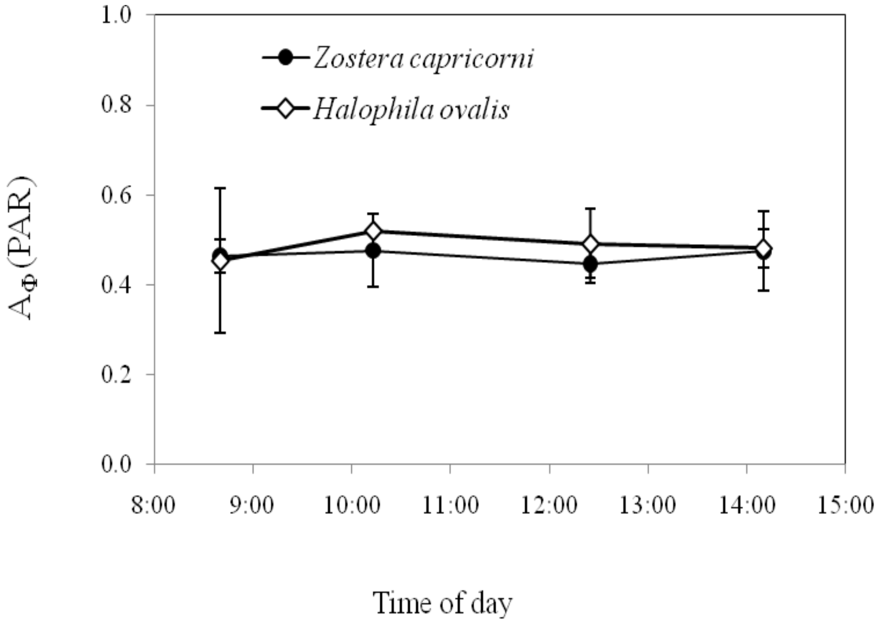


Figure 12: Photosynthetic absorptance A_{Φ}(PAR) of *Z. capricorni* and *H. ovalis* during a tidal exposure event in Autumn (14th May 2011). Data represent mean ± SD (*n*=6).

Winter (senescent season):
Chlorophyll *a* fluorescence and pigments

Due to poor weather conditions, only one day of sampling was possible. Therefore data was only collected for *Z. capricorni* for the winter period. Consistent with the other seasons, photosynthetic rETR was lowest during air-exposure (Fig. 13). Photosynthetic parameters effective quantum yield of PSII (Φ_{PSII}), maximum relative electron transport rate ($rETR_{max}$) and light utilisation efficiency (α) all declined significantly ($P < 0.05$) during air-exposure (Table 8), but did not differ during the other parts of the day. There was no significant change in minimum saturating irradiance (I_K) between times of day.

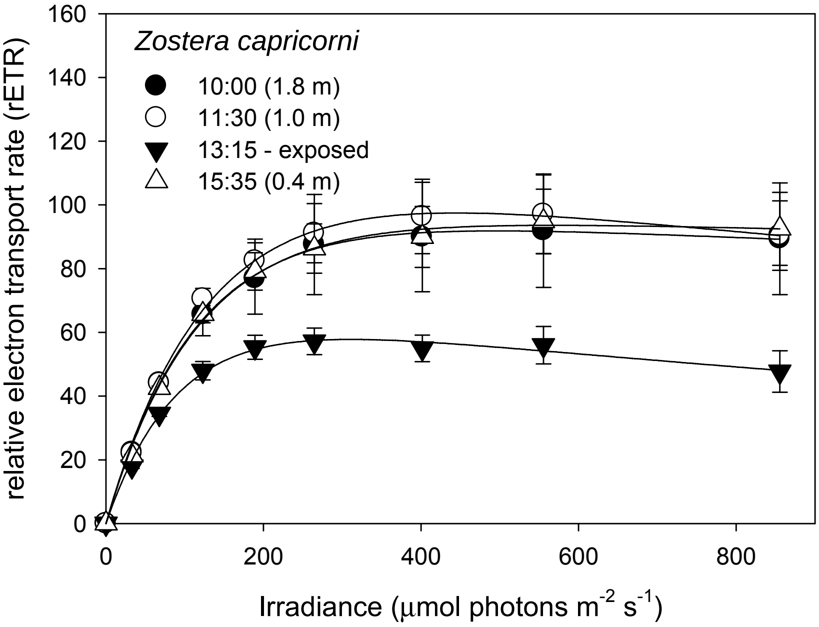


Figure 13: Relative electron transport rate (rETR) as a function of irradiance in the intertidal seagrass species *Zostera capricorni*. Double exponential curve fit is shown (line). Time of day and depth in metres is given in legend. Data represent mean ± SD (n = 6).

Table 8: Photosynthetic parameters effective quantum yield of photosystem II (EQY), maximum electron transport rate ($rETR_{MAX}$), minimum saturating irradiance (I_K) and light utilisation efficiency (α) calculated from the light response curves of *Z. capricorni* (July, 2011) according to Ralph and Gademann (2005). Data corresponds to data in figure 13 and represent means ± SD (n = 6). * indicates statistical significance at α < 0.05.

Time	10:00	11:30	13:15	15:35
PAR	400	800	1800	350
Water depth	1.8m	1.0m	Exposed	0.4m
EQY	0.736 ± 0.017	0.712 ± 0.023	0.558 ± 0.049*	0.694 ± 0.036
$rETR_{MAX}$	92.77 ± 45.17	105.6 ± 27.56	57.40 ± 12.39*	92.64 ± 23.72
I_k	101.3 ± 57.37	121.0 ± 41.12	75.15 ± 18.77	107.3 ± 30.89
α	0.939 ± 0.086	0.894 ± 0.082	0.776 ± 0.095*	0.871 ± 0.055

The violaxanthin de-epoxidation state was consistent with the previous tidal studies, in that it showed a significant increase in de-epoxidation ratio with increased irradiance ($P < 0.05$) and maximum de-epoxidation occurring during air-exposure (Fig. 14).

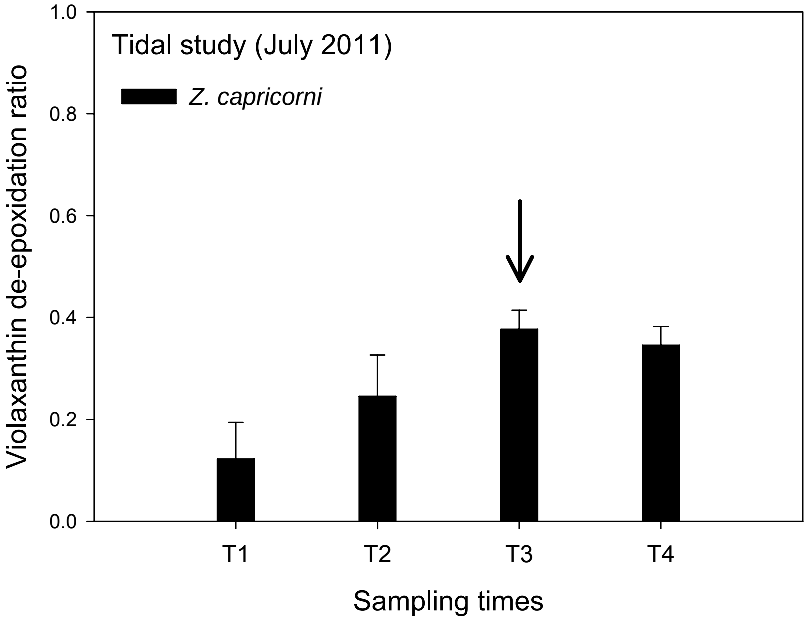


Figure 14. Violaxanthin de-epoxidation ratio for *Z. capricorni* during a tidal cycle on the 14th of June 2011. Down arrow indicates time of air-exposure. Time of day and depth in metres are the same as in Tables 8 and indicated here as sample times. Data represent mean ± SD ($n = 6$).

The highly dynamic nature of this parameter makes it a perfect indicator for light exposure. However, given the fast rate at which it is able to change, using it as a sub-lethal indicator for shading on seagrasses could prove problematic. While it woul definitely decrease under shaded conditions and be greater under high light, the actual ratio could alter depending on the time of day the sample is taken, potentially altering with respect to water depth or cloud cover, as well as longer-term light climate.

O_2 evolution (incubation chambers)

Tidal and PAR data showed an inverse pattern, consistent with previous seasons, of high irradiance during low tide (Fig. 15a). Unlike previous seasons however, oxygen production declined significantly ($P < 0.05$) just before air-exposure and then stayed low following exposure (Fig. 15b). This would suggest that the decline in photosynthesis was due to high irradiance and not necessarily air exposure, as was seen in the previous three seasons.

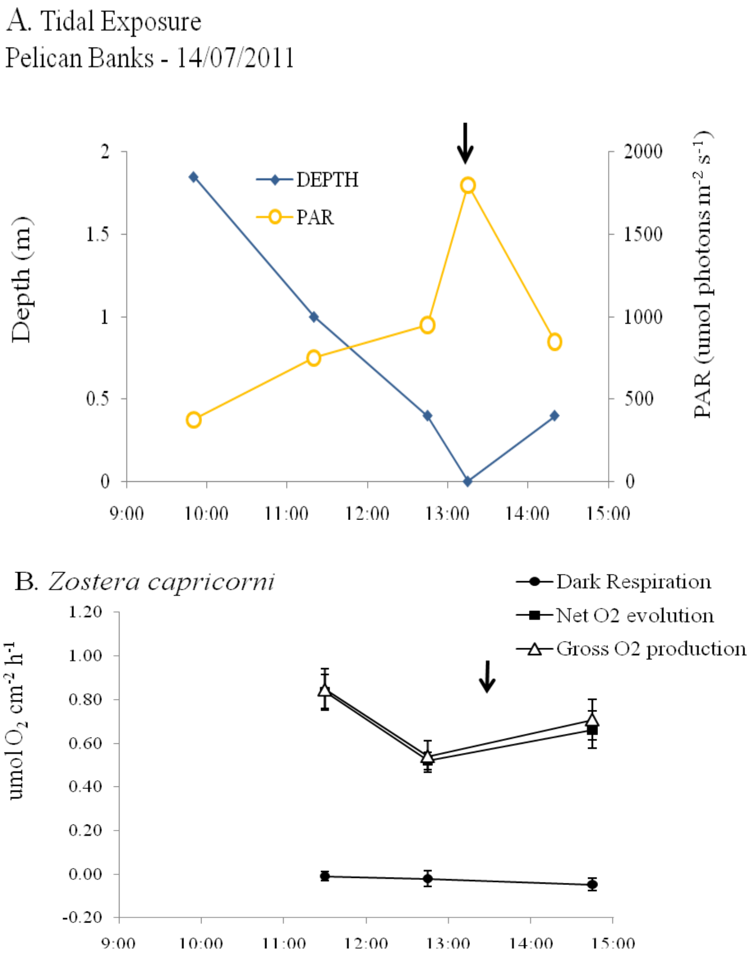


Figure 15: A) Water depth and benthic photosynthetically active radiation (PAR) over the tidal cycle on the 14th of June 2011 B)Rates of gross oxygenic photosynthesis (triangles) calculated from measurements of dark respiration (circles) and net photosynthesis (squares) *in situ* for *Zostera capricorni* at Pelican Banks on 14th June 2011. Averages ± SE (*n*=6) are shown, and the arrow indicates the time of air exposure at low tide.

Bio-optical characteristics - photosynthetic absorptance A_Φ(PAR):

Seagrass photosynthetic light absorption capacity remained largely constant throughout the tidal exposure event (Fig. 16). Consistent with previous tidal studies, the seagrass did not regulate their light capturing efficiency under rapidly changing light intensities.

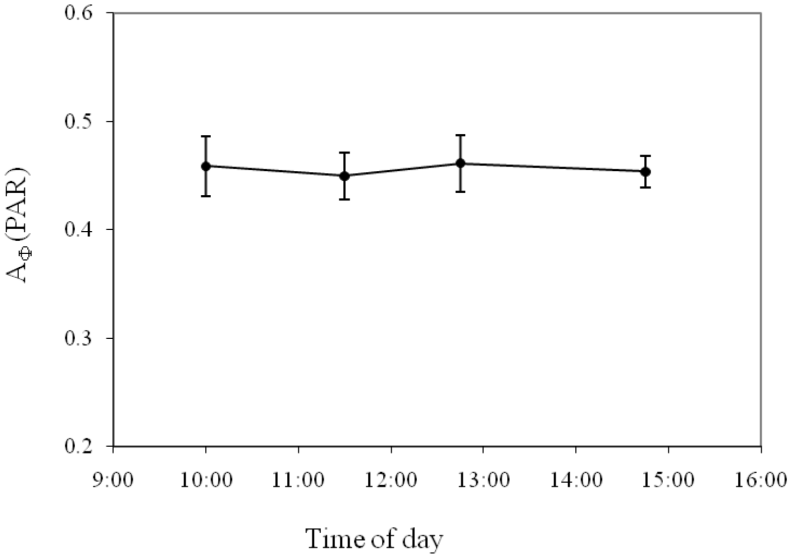


Figure 16: Photosynthetic absorptance A_Φ(PAR) of *Z. capricorni* during a tidal exposure event in Autumn (14th July 2011). Data represent mean ± SD (*n*=6).

To help differentiate a light-dependent response from the effects of desiccation, effective quantum yield as a function of *in situ* irradiance was plotted for *Z. capricorni* and *H. ovalis.* Regression analyses for all data and for the submerged or exposed data only were conducted to ascertain the strongest effect. Effective quantum yield as a function of irradiance for *Z. capricorni* showed no correlation (R^2 = 0.0103) when only the submerged data were used (Fig. 16a). However, when regression was applied to all the data (including the air exposed data) there was a significant although weak negative correlation in EQY with increased irradiance (R^2 = 0.3752; $P < 0.0001$; Fig. 16a). The increase in irradiance actually led to greater photosynthetic activity in Spring and Summer (as seen with the RLC data; Figs. 1 and 4), which is consistent with previous studies that have found photoinhibition to be primarily absent in intertidal seagrasses (Beer and Björk 2000). Furthermore, it confirms that photosynthetic activity in *Z. capricorni* is more sensitive to air exposure than high irradiances. In contrast, an inverse relationship was detected in *H. ovalis* (Fig. 16b), where air exposed data showed no correlation with irradiance, instead a relationship was detected when the submerged data were included in the regression (R^2 = 0.3267; $P < 0.0001$; Fig. 16b).

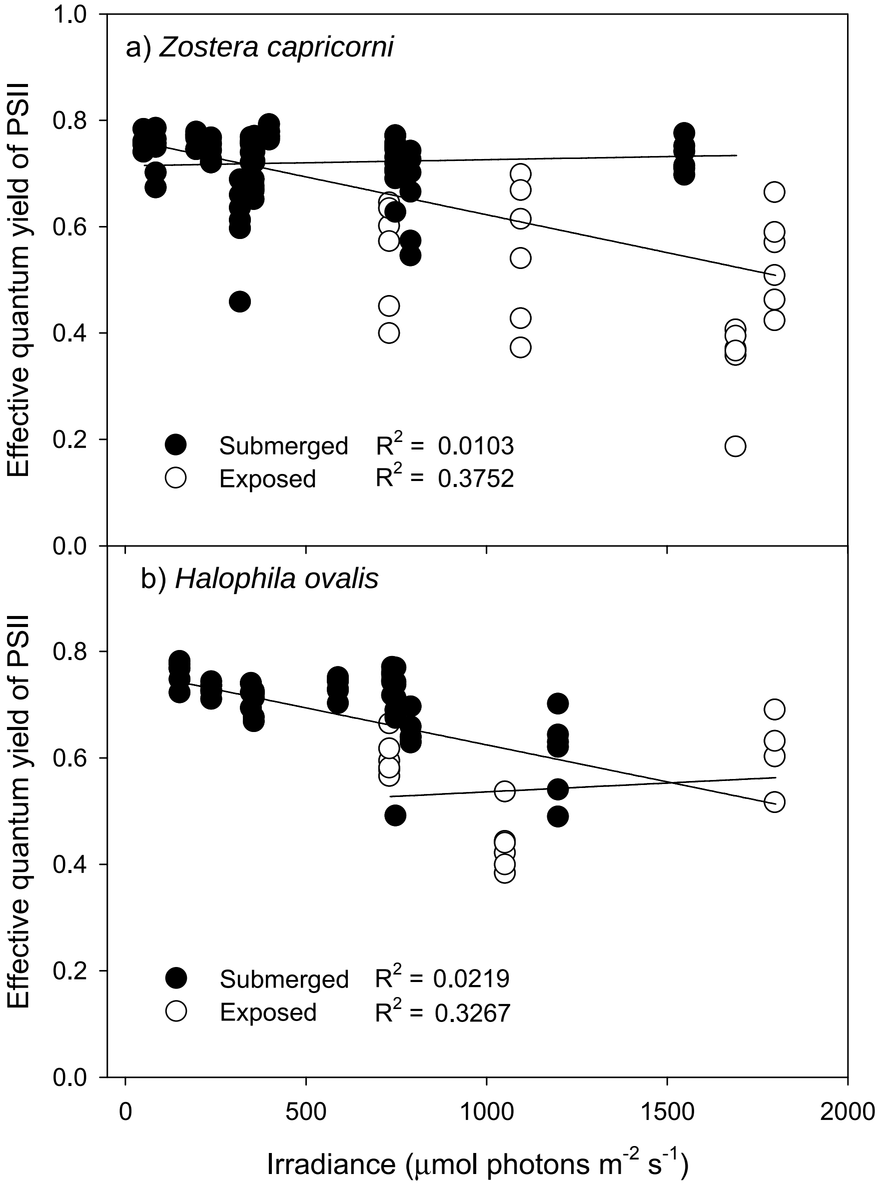


Figure 16: Effective quantum yield as a function of irradiance in (a) *Z. capricorni* and (b) *H. ovalis* for all data collected (black and white circles) and for EQY during air exposure (white dots only). Dots represent all data collected for the initial yield measurement at *in situ* irradiance from each field study. The relationships between EQY and irradiance are fitted with a linear regression (solid line).

Discussion

Physiological response to air-exposure and seasonal differences

The oxygen and fluorescence data are consistent with respect to the seagrass physiological response over a tidal cycle. The rapid light curves (RLCs) provided a quick assessment of the photosynthetic activity under natural conditions where the light field rapidly fluctuates due to tidal movement. For each season, measurements were taken during midday low tide windows when light conditions were strongest. The increase in photosynthetic activity with increased irradiance in the spring study, suggests that these seagrass are evolved to take advantage of increased irradiance when tides recede. However, once seagrass enter autumn and winter this response is no longer evident. This matches their time of maximum growth (growth season), suggesting a greater use of winter energy stores and therefore greater sink capacity for photosynthetic energy and similarly, the loss of this response coincides with the senescent season (autumn and winter). The significant and consistent decline in photosynthetic activity with air-exposure for *Z. capricorni* highlights the sensitivity of this intertidal species to desiccation. Light had very little effect on photosynthetic activity until it was combined with air exposure in all seasons. This has major implications with respect to their daily productivity, as during low tide, when sunlight exposure is maximal, photosynthetic activity declines in response to desiccation stress and not high irradiance, thus limiting the 'window' for high rates of productivity to times of high irradiance with complete submergence. Although this response was also seen in *H. ovalis,* it was only observed when corresponding with high irradiance and was rarely significantly different from the high irradiance response. Indeed, *H. ovalis* shows a much greater sensitivity to light condition than to desiccation effects.

Comparison of the data between growing and senescent seasons, there was a reduction in the magnitude of the response to air exposure in *Z. capricorni* during the autumn and winter studies – ie the senescent season. This difference in stress during air-exposure between the two seasons would suggest that temperature might play a significant role in the loss of photosynthetic activity in *Z. capricorni.* This temperature sensitivity is consistent with Gladstone being close to the most northern geographical range of the typically temperate seagrass *Z. capricorni*. Similarly, *H. ovalis*, a typically tropical seagrass, showed higher irradiance lead to a higher ETR, except during exposure where they are equal to ETR at depth (2.0 m). However, this was only evident during the growth season (October and January). In the senescent season, *H. ovalis* showed the inverse response, with greater sensitivity to high light. Electron transport rates were greatest at depth and slowed with increased irradiance. This could be a seasonal adaptation to optimised photosystems for maximal light capture under minimal light conditions, inevitably resulting in greater photoinhibition during periods of higher light. Overall, the data would suggest that *H. ovalis* shows much less sensitivity to air-exposure than *Z. capricorni* and predominantly only sensitivity to changes in irradiance.

Due to the nature of the measurements, oxygen data could only be collected during submersion. Therefore, samples were taken immediately prior to exposure and after 1 h of complete air exposure. It is clear from the data that there is an increase in oxygen evolution with increased irradiance as the tide recedes and a significant decline in oxygen production rates immediately after air-exposure during the growing season. This compliments the fluorescence data and further suggests that during air-exposure, photosynthetic activity and thus oxygen evolution decline as a result of increased stress. The cause of this stress is still unknown; it could be the result of desiccation, CO_2 limitation, excess heat or a combination of all three. However, what is clear is air exposure during a tidal cycle does not provide intertidal seagrass meadows with a 'window' of opportunity in which to maximise productivity. Instead, it leads to physiological stress that impedes photosynthetic activity and carbon fixation. The same pattern was also measured in May, where oxygen production rates were the highest of all seasons and were strongly impacted by air exposure. This contrasts strongly to the late senescent season (winter) where production rates declined and there was no change in response to air exposure. Instead, production declined with increased irradiance prior to exposure and remained low following exposure. This seasonal shift in sensitivity to high light could be the result of changes in pigment concentrations. *Zostera capricorni* showed no change in the pigment de-epoxidation ratio between seasons. In contrast, *Halophila ovalis* shoed maximum de-epoxidation ratios during the growth season, which declined steadily at the end of the growth season and into the senescent season. Seagrass photosynthetic light absorption capacity remained largely constant throughout the tidal exposure event in all seasons, confirming that these seagrass species do not regulate their light capturing efficiency under rapidly changing light intensities. Similarly, photosynthetic light absorption capacity did not change throughout the year with values of approx. 0.5 during growth and senescent seasons.

In nature, intertidal habitats exert many environmental stresses simultaneously on its inhabitants, some of which can work synergistically or antagonistically. Consequently, trying to ascertain the effect of a single environmental stress, such as air-exposure, is difficult in isolation of other environmental factors (ie: irradiance or temperature). Given this complex interaction of environmental factors in the intertidal environment, determining definite seasonal trends is difficult. However, some differences in physiological responses were detectable in both seagrass species between the growing and senescent seasons.

The results from this study have successfully demonstrated that tidal exposure will not provide intertidal seagrass meadows with any respite from high turbidity that may result from dredging related activities. Furthermore, the increased understanding that has been attained of *in situ* photosynthetic activity and measured changes in oxygen evolution and physiology as a result of changes in irradiance over a tidal cycle has provided critical data to develop the overall light requirement model describing the relationship between the efficiency of the photosynthetic pathway (as measured during tidal exposure), absorptance and total productivity. The two species investigated in this study differ in their photosynthetic acclimation strategies that are able to adjust at very quick time scales over a tidal cycle. The differences between the growing and senescent seasons are more evident in the production, pigment and optical data, perhaps as a result of seasonal changes in internal stores of C or pigment proteins. This seasonal variability has provided a greater understanding of annual patterns in seagrass productivity over a tidal cycle and has shown that seagrasses are more sensitive to high turbidity during the growing season when compared with the senescent.

References

Beer, S. & Björk, M. 2000. Measuring rates of photosynthesis of two tropical seagrasses by pulse amplitude modulated (PAM) fluorometry. *Aquatic Botany* **66**:69-76.

Jeffrey, S. W. & Humphrey, G. F. 1975. New spectrophotometric equations for determining chlorophyll *a, b, c1,* and *c2* in higher plants and natural phytoplankton. . *Biochem. Physiol. Pfl.* **165**:191-94.

Leuschner, C., Landwehr, S. & Mehlig, U. 1998. Limitation of carbon assimilation of intertidal Zostera noltii and Z. marina by desiccation at low tide. *Aquatic Botany* **62**:171-76.

Ralph, P. J. & Gademann, R. 2005. Rapid light curves: A powerful tool to assess photosynthetic activity. *Aquatic Botany* **82**:222-37.

van Heukelem, L. & Thomas, C. 2001. Computer-assisted high-performance liquid chromatography method development with applications to the isolation and analysis of phytoplankton pigments. *Journal of Chromatography A* **910**:31-49.