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Response and recovery of a mixed tropical seagrass assemblage to variation in the frequency and magnitude of light deprivation. Report of Theme 5 - Project 5.5.3 prepared for the Dredging Science Node

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WAMSI Dredging Science Node

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WAMSI Dredging Science Node

The WAMSI Dredging Science Node is a strategic research initiative that evolved in response to uncertainties in the environmental impact assessment and management of large-scale dredging operations and coastal infrastructure developments. Its goal is to enhance capacity within government and the private sector to predict and manage the environmental impacts of dredging in Western Australia, delivered through a combination of reviews, field studies, laboratory experimentation, relationship testing and development of standardised protocols and guidance for impact prediction, monitoring and management.

Funding Sources

The $20million Dredging Science Node is delivering one of the largest single issue environmental research programs in Australia. This applied research is funded by **Woodside Energy, Chevron Australia, BHP Billiton and the WAMSI Partners** and designed to provide a significant and meaningful improvement in the certainty around the effects, and management, of dredging operations in Western Australia. Although focussed on port and coastal development in Western Australia, the outputs will also be broadly applicable across Australia and globally.

This remarkable **collaboration between industry, government and research** extends beyond the classical funder-provider model. End-users of science in regulator and conservation agencies, and consultant and industry groups are actively involved in the governance of the node, to ensure ongoing focus on applicable science and converting the outputs into fit-for-purpose and usable products. The governance structure includes clear delineation between end-user focussed scoping and the arms-length research activity to ensure it is independent, unbiased and defensible.

And critically, the trusted across-sector collaboration developed through the WAMSI model has allowed the sharing of hundreds of millions of dollars worth of environmental monitoring data, much of it collected by environmental consultants on behalf of industry. By providing access to this usually **confidential data,** the **Industry Partners** are substantially enhancing WAMSI researchers' ability to determine the real-world impacts of dredging projects, and how they can best be managed. Rio Tinto's voluntary data contribution is particularly noteworthy, as it is not one of the funding contributors to the Node.

Funding and critical data **Critical data**

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Front cover images (L-R)

Image 1: Trailing Suction Hopper Dredge *Gateway* in operation during the Fremantle Port Inner Harbour and Channel Deepening Project. (Source: OEPA)

Image 2: Light logger positioned within the middle of an experimental tank at seagrass canopy height. (Source: John Statton).

Image 3: Dredge Plume at Barrow Island. Image produced with data from the Japan Aerospace Exploration Agency (JAXA) Advanced Land Observing Satellite (ALOS) taken on 29 August 2010.

Image 4: Pot with mixed seagrass species in the UWA Seagrass Facility at commencement of the Light-Frequency experiment. (Source: John Statton).

Contents

Executive Summary

The capacity of seagrasses to cope with episodes of light deprivation from overlying turbid waters may not only depend on the absolute quantity of light they receive during that episode, but also on how the light deprivation varies through time. For example, turbidity and therefore light reduction may be relatively constant over the episode or it may fluctuate depending on the frequency of pulsed turbidity events. This report presents findings from a controlled mesocosm experiment that aimed to determine the responses of seagrasses to, and recovery from, differences in the pattern of the delivery of light. The study focussed on two seagrass species found in the northwest of Western Australia (NW WA). The report provides guidance and protocols for the application of the research outputs (e.g. light stress frequency and response relationships, recovery potential, sub-lethal and lethal bio-indicators and thresholds) to impact prediction, monitoring and/or management of dredging programs in NW WA.

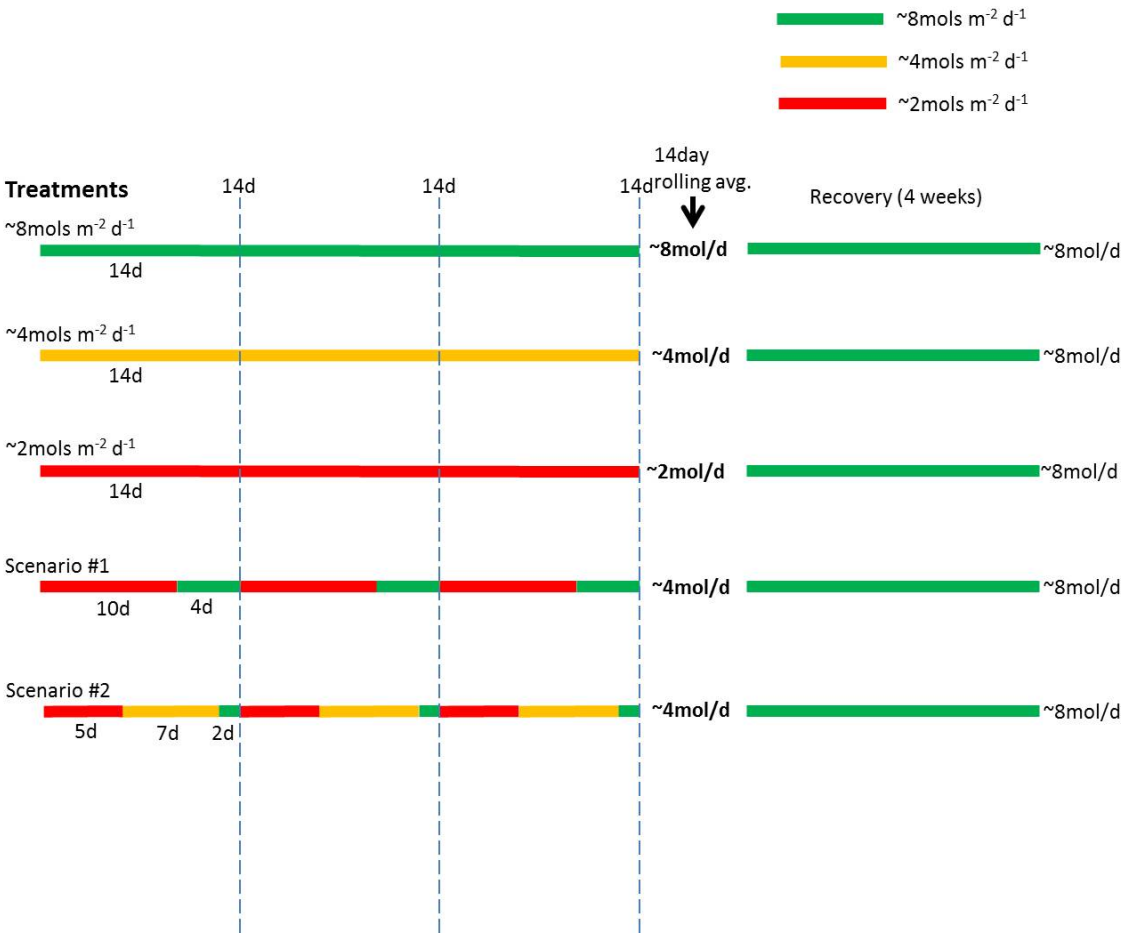
To test the responses to and recovery from changes in the pattern of the delivery of light on co-occurring tropical seagrass species we established pots containing mixed assemblages of two seagrasses that commonly co-occur in NW WA, *Cymodocea serrulata* and *Halodule uninervis.* Under climate-controlled mesocosm conditions, we manipulated the amount, duration and frequency of light reduction seagrasses received to simulate adverse pulsed conditions of extreme light-limitation due to reduced water clarity (e.g. turbidity plume) followed by periods of adequate light for survival.

We achieved this by alternating delivery of light in two scenarios, so that over a 14 d period seagrasses receive on average ~ 4 mol photons m^{-2} d^{-1} of light; Scenario 1 had 10 days of Low light followed by 4 d of High light (yielding a fortnightly average light of 3.8 mol photons m^{-2} d^{-1}) and Scenario 2 had 5 d of Low light followed by 7 d of Moderate light and then 2 d of High light (yielding an average of 4.4 mol photons m^{-2} d^{-1}, Table 3, Figure ES1). In addition we had 3 procedural controls (High, Moderate and Low light) delivering a continuous amount of light; (fortnightly average of 8.8, 3.9 and 2.2 mol photons m^{-2} d^{-1} respectively - Table 3). Based on the results of the WAMSI DSN Project 5.5.1 (Statton et al. 2017a), this range of control light intensities encompasses those known to enable seagrass survival (High light; 8 mol photons m^{-2} d^{-1}), produce some declines but still allow seagrass to survive (Moderate light; 4 mol photons m^{-2} d^{-1}) and levels which result in seagrass mortality (Low light; <2 mol photons m^{-2} d^{-1}). Because over a 14 d period the absolute quantity of light delivered to seagrasses was the same in the continuous Moderate light delivery treatment and the two alternating light delivery scenarios, we were able to explore how the delivery of light impacts plant growth and survival. We ran the experiment for 10 weeks. In the first 6 weeks plants cycled through the experimental treatments every 14 d (i.e. 3 times). Plants were then given a 4 week recovery period where they received High light. Harvesting and measuring of plant condition was undertaken at 2, 4 and 6 weeks during the treatment period and at the end of the 4 week recovery period.

Four separate but linked components of the study were used in developing sub-lethal and lethal bio-indicators and light reduction threshold values:

1. Under the imposed continuous and alternating light reduction stress, we determined the cause-effect pathway from measurements of 16 response variables;
2. After the imposed continuous and alternating light reduction stress was removed, we determined the recovery potential from measurements of the same 16 response variables;
3. While it was not a main aim to assess bio-indicators in this study, because similar treatments were tested in WAMSI DSN Project 5.5.1 (Statton et al. 2017a) we identified bio-indicators and compared them to see if they were consistent with that earlier study. To do this, we identified response variables from 1 and 2 (above) that changed in a consistent manner with increasing magnitude and duration of light reduction; and

4. Using the variables identified in (3), together with the findings from WAMSI DSN Project 5.5.1 (Statton et al. 2017a), we determined impact thresholds that take into account the magniture and duration of light reduction as well as the pattern in which that light is delivered (i.e. number of consecutive low light days).



ES Figure 1: Diagram illustrating the experimental design: continuous light shading treatments (top three treatments) and light delivery scenarios (bottom two treatments) over three sets of 14 days followed by a recovery period of 4 weeks.

The key findings were:

- The pattern in the delivery of light does impact seagrasses.
    - When plants received a continuous supply of low light (average of 2.2 mol photons m^{-2} d^{-1}) they were impacted.
    - Similar impacts were observed when plants received an average of 3.8 mol photons m^{-2} d^{-1} over 14 d (as 10 d of Low light followed by 4 d of High light - Scenario 1).
    - However, if plants received only 5 d of Low light, followed by 7 d of Moderate light and 2 d of High light (scenario 2; average of 4.4 mol photons m^{-2} d^{-1} over 14d), the impacts were not as severe, and were similar to a continuous Moderate supply of light.
- Previous light history had an impact on both species ability to recover.
    - Plants that previously experienced the Low and Scenario 1 light treatments showed no signs of recovery in biomass. Therefore, recovery potential from extended low light periods is not compensated by short periods of high light (e.g. Scenario 1) even if the average over a 2 week period is the same.

    - However, if the frequency of Moderate/High light is longer (e.g. Scenario 2 and the Moderate light treatments), the impact on their potential for recovery is not as great.

- We identified 2 response variables (rhizome carbohydrates and biomass) which clearly and consistently responded to light reduction for both species and should be relatively robust bio-indicators, as well as several other variables for consideration that showed a response in at least one species and were identified as a robust bio-indicator in the WAMSI DSN Project 5.5.1 (Statton et al. 2017a).
- In the light reduction experiments conducted for the WAMSI DSN Projects 5.5.1 (Statton et al. 2017a) and 5.5.3 (reported here), and for both species, the timing of responses differed. For example, in WAMSI DSN Project 5.5.1, *C. serrulata* showed a decrease in biomass after 9 weeks and at light intensities lower than 2.3 mols photons m^{-2} d^{-1}, whereas in this study plants responded within 2 weeks at ~4 mols photons m^{-2} d^{-1}. The differences in timing and magnitude of response may be a result of the way light was delivered. In the earlier study, plants received a constant intensity of light over a 12 hour photo-period. Plants also showed a strong ability to photo-adapt to each light intensity such that even the lowest light levels were saturating to photosynthesis. On the other hand, the study reported here tested the light response under ambient light conditions, where plants were responding to the average light received each day, which also fluctuated due to cloud-cover, and over a much shorter day length (< 8 hours, winter). Consequently, conditions plants were exposed to in this study better reflect natural conditions and, therefore, provide more realistic light-related thresholds. Based on these findings, light-related thresholds and plant responses in 5.5.1 may overestimate species resilience.
- This experiment has demonstrated that the number of consecutive days of low light does influence the response of plants, and should be considered in threshold development. Continuous low light for 10 d was more detrimental than 5 d of continuous low light, even if over a two week period plants received the same total amount of light.
- Using the same percentiles approach as that applied in WAMSI DSN Project 5.5.1 (Statton et al. 2017a; ANZECC 2000) we found that we could calculate sub-lethal and lethal impact thresholds. These threshold values and response times were consistent (within the range) with those reported in WAMSI DSN Project 5.5.1.
- In treatments with the longest duration of low light intensity (10 d or more; Low light and Scenario 1 treatments), plants showed no signs of recovery in biomass during the recovery period. This indicates that the previous light history was still causing an impact. However, rhizome carbohydrate concentrations had increased during the recovery period such that plants no longer breached the P_{20} for this indicator. This suggests that although plants in the Low light and Scenario 1 treatments were potentially on a trajectory for recovery, it required longer than 4 weeks to regain similar biomass to the control plants.

This experiment has determined that the pattern in the delivery of light does impact seagrasses and that previous light history impacts the ability of both species to recover. The research has allowed species-specific and mixed-species meadow impact thresholds that incorporate the magnitude, duration and pattern of light reduction and which can be applied to predict impacts or as management actions alerts during dredging to mitigate seagrass declines.

Considerations for predicting and managing the impacts of dredging

In Western Australia, predicting and managing the impacts of dredging is guided significantly by the Environmental Protection Authority's Technical Guidance on Environmental Impact Assessment of Marine Dredging Proposals (EPA 2016). The same framework is applied, in modified forms, elsewhere in Australia. The framework has three phases which can benefit from the input of new information on biological components of marine ecosystems: the Pre-development phase, which includes surveys and investigations to define the system

in which dredging might occur; the Impact Assessment phase, in which the potential dredging-generated pressure fields and the spatial extent, severity and duration of any effects on sensitive components of the environment need to be predicted, and monitoring and management plans developed; and finally Post-approval phase where the approved monitoring programs are implemented at impact assessment and reference sites to inform adaptive management and demonstrate compliance with conditions of approval. Below, we consider the implications of the findings of this project in the context of the various phases of the impact assessment framework. Please note that many of the implications for management that have emerged from this study are similar to those arising from WAMSI DSN Project 5.5.1 (Statton et al. 2017a). We have repeated the relevant implications here for completeness.

Pre-development Surveys

Seagrass composition

Our findings are consistent with the earlier findings (WAMSI DSN Project 5.5.1; Statton et al. 2017a) that a single species approach to threshold development is not appropriate for a diverse seagrass assemblage, typical of NW W.A. Different species display different sensitivities to light reduction and applying thresholds relevant to one species may under- or over-estimate the potential for impact on other species and the meadow as a whole (see impact prediction section below). **We recommend pre-development surveys identify the species composition of a diverse seagrass assemblage to improve the predictability of the mixed assemblage response to impacts and aid in monitoring design, bio-indicator choice and threshold development**.

Survey variables and threshold development

We recommend that **pre-development surveys obtain baseline information on the bio-indicators identified in this study as well as determine the feasibility of undertaking assessments on these bio-indicators**. We identified 2 robust bio-indicators that were previously identified in WAMSI DSN Project 5.5.1 (Statton et al. 2017), that are appropriate for immediate incorporation into monitoring programs to identify light reduction impacts on a tropical seagrass assemblage, and 3 others that should be given consideration. Other considerations that influence bio-indicator selection for monitoring programs include the ease of collection, the availability of expertise to analyse and interpret results, and cost-effectiveness, which all need to align with management goals (see review by McMahon et al. 2013).

WAMSI DSN Projects 5.1.1 (Statton et al. 2017) and 5.5.3 (this study) have identified a number of bio-indictors and appropriate thresholds for use with those indicators. However, this study has also shown that previous light history could result in plants having different baseline conditions, which could alter their sensitivity to light reduction and, therefore, their thresholds of tolerance. So although the generic thresholds are useful, reliance on them carries some risk. The use of generic thresholds may lead to over- or under-prediction of impacts on species diversity and ecological function, or an increase in time, effort and cost to complete a dredging operation. For these reasons, developing and applying site-specific thresholds, as is recommended by ANZECC/ARMCANZ (2000), would improve confidence by factoring in much of the inherent resilience/susceptibility of the seagrasses at that site. To do this, we recommend that **pre-development surveys collect data on the previous light history, seagrass condition and the variability in the metrics of the potential bio-indictors on which thresholds are based at any potential impact or monitoring sites.** Bio-indicator assessment over time (inter- and intra-annual) will better characterise the natural variability of reference and impact sites with respect to the recommended and/or most practical and economically feasible bio-indicators.

Impact Prediction and Assessment

Appropriate bio-indicators of light stress

We identified two robust bio-indicators of seagrass plants being subjected to light-reduction stress. In this study, under natural light conditions, these indicators (rhizome carbohydrates, biomass) responded early (2 weeks) to light reduction and also showed a recovery response over short time-scales when light conditions improved. **These bio-indicators are appropriate for application in dredging impact prediction.** The indicators have additional applicability in distinguishing impacts related to light reduction or those related to sediment burial stress introduced by dredging. Only one consistent bio-indicator relating to burial stress was determined in the WAMSI Dredging Science Node project 5.5.3 (Statton et al. 2016b) and this differs to the bio-indicators for low-light stress. This is an important distinction since dredging operators may need to adjust their operations according to light reduction or sedimentation impacts which may differ depending on location or distance from the dredge.

Species-specific thresholds

In both projects 5.5.1 (Statton et al 2017a Statton et al 2017a) and 5.5.3 (this report), we have shown that *H. uninervis* is more sensitive to low light than *C. serrulata*, possibly due to the faster rate of response and smaller size of carbohydrate storage reserves in *H. uninervis* that act to buffer plants against low light stress. *C. serrulata* is representative of more persistent seagrasses with larger rhizomes and storage reserves while *H. uninervis* is more representative of colonising species with faster growth rates and smaller storage reserves. Given the species-dependent differences in response, **extrapolating the findings for these two species to other species, or using one species as a surrogate for many (i.e. a mixed assemblage) is not advised**, since it may lead to erroneous conclusions. However, in the absence of any other data, a less conservative approach would be to apply the tolerance threshold of *C. serrulata* to larger seagrasses that are similar to *C. serrulata* in the seagrass functional-form model (Walker et al. 1999), and apply the *H. uninervis* threshold to smaller, colonising species. We stress, however, that **the validity of impact predictions for a site will be improved by basing them on the species that have been observed at the site in previous studies or in pre-development surveys.**

Using a combination of data from WAMSI DSN Projects 5.5.1 (Statton et al. 2017a) and 5.5.3 (this project), we developed sets of thresholds for impact prediction. These thresholds extend on those presented in the earlier WAMSI DSN Projects 5.5.1 (Statton et al. 2017a) because they incorporate consideration of the duration of light reduction, the light intensity and the frequency (or delivery pattern) of that light reduction stress. We present two sets of thresholds:

- 'More conservative' thresholds, which provide the greatest level of confidence that seagrasses will not be negatively affected; and
- 'Less conservative thresholds', which factor in some of the variability in the findings and are thresholds which are likely to result in no significant impact on seagrasses but in which we have less confidence because of the variability in the findings.

Patterns of dredging activity

The findings of this study indicate that any efforts to predict the impacts of dredging need to take into account not only the magnitude and duration of light reduction, but also the pattern of delivery of that light reduction. There is a growing move to define seagrass tolerance to light reduction in terms of a total amount of light reaching seagrasses over an averaging period (e.g. Chartrand et al. 2012). While that remains a fundamentally sound approach, this study clearly indicates that the pattern in which that amount of light is delivered is also critical. In particular, as the pressure approaches the critical threshold, the frequency and duration of low light periods appears to be increasingly important, with this study showing that 10 consecutive days of low light

produces greater impacts than periods of 5 consecutive days even if the plants receive the same total light over 14 days. As a guiding principle, designing dredging programs to minimise the number of consecutive days of low light would likely result in lower impacts than using continuous or prolonged periods of dredging that induce persistent or long periods of low light. Impact prediction should, therefore, explicitly consider not only the average light climate that seagrasses will experience, but also whether the dynamics of plume movement would result in pattern of light delivery pattern likely to increase or decrease the impact relative to the same light delivered in a continuous rather than pulsed nature.

Based on the above approach, a set of 'effects' criteria are presented (Table ES1) for the tropical seagrasses (*Cycmodocea serrulata* and *Halodule uninervis)* and also for what could be termed a generic 'mixed or multi-species meadow'. These criteria indicate the light conditions that, if maintained or exceeded, are expected to result in no detectable effects on these species of seagrass, and incorporate consideration of the duration of light reduction, the light intensity and the frequency (or delivery pattern) of that light reduction stress. As such they can be used to interrogate the outputs of dredge plume models to identify the transition between the zone of moderate impact (where effects are allowed) and the zone of influence (where no effects are allowed). Importantly, by taking into account the respective levels of confidence in the criteria, it is possible to establish management targets that proponents should aim to meet through adaptive management and also the compliance limits that they must meet to comply with the conditions of approval (see EPA 2016 for further explanation).

We present two sets of thresholds, 'Most' and 'Less' conservative. The more conservative criteria afford a high level of confidence that if these conditions can be maintained there will be no measurable impact on seagrass (as measured by the relevant biomass parameters). As such they provide a rational basis to establish the location of the 'compliance' line. The less conservative criteria are still reasonably robust and afford a level of confidence that seagrasses will not be measurably affected – albeit with lower confidence than that of the conservative criteria. As such they provide an objective basis to determine the location of the 'management target' line, which in turn represents the most likely best-case scenario for the outer limit of measurable effects on seagrass communities.

It is important to note that the thresholds in Table 17 are based on the data from laboratory experiments. As such, they are presented as recommended default thresholds. Pre-development surveys and dredging-period monitoring data on light and seagrass condition could be used to increase confidence in these default thresholds.

Post-Approval

Compliance monitoring and dredging management programs

The advice provided in the preceding point on impact assessment applies equally to the planning and operation of dredging programs. Where possible, designing dredging operations to minimise the number of consecutive low light days will provide the best opportunity to minimise impacts on seagrasses.

Table ES1 Recommended impact thresholds for *Cymodocea serrulata* and *Halodule uninervis*. These thresholds are based on the combined findings of this project and WAMSI DSN Project 5.5.1. The Most and Less Conservative thresholds can be applied to predict the outer and inner limits of the Zone of Moderate Impact, respectively. Thresholds are not provided for *Halophila ovalis* as there were no significant difference among treatments prior to the point where the control plants began to show stress, making the responses unreliable.

	Two week averaging period		**Permissible low light periods within 2 wk averaging period**	
	Duration	Mean Light intensity mol photons m^{-2} d^{-1}	Duration	Mean Light Intensity mol photons m^{-2} d^{-1}
Cymodocea serrulata (based on Aboveground biomass)				
Most conservative*	12 wk	8.9	5 d	2 to ≤ 4
	9 wk	2.3	5 d	2 to ≤ 4
Less conservative#	12 wk	2.3	10 d	2 to ≤ 4
Halodule uninervis (based on Aboveground biomass)				
Most conservative*	12 wk	13.1	5 d	2 to ≤ 4
	6 wk	13.1	5 d	2 to ≤ 4
Less conservative#	12 wk	2.3	10 d	2 to ≤ 4
Mixed Meadow (based on total biomass of all species in a multi-species meadow)				
Most conservative*	12 wks	13.1	5 d	2 to ≤ 4
Less conservative#	12 wks	8.9	10 d	2 to ≤ 4
	6 wks	5	10 d	2 to ≤ 4

* *Most conservative reflects higher confidence of no impact to seagrass.*

\# *Where the thresholds were the same for multiple durations, the longer duration is presented as the recommended threshold. For example, for H. uninervis there was no difference in the 'Less conservative' thresholds for 6 weeks and 12 weeks data – for both, the 2-weekly running average was 2.3 mol m^{-2} d^{-1}. In this case, it is recommended that for any given period of 12 successive weeks, this average must be maintained in successive two week periods.*

Residual Knowledge Gaps

A number of significant knowledge gaps remain in relation to predicting and managing the impacts of dredging-induced light reduction on seagrasses.

Determining the long-term recovery potential of each species after light reduction

In this study we monitored the recovery potential of seagrasses after removal of light stress, however, this was only monitored for four weeks and maybe too short in duration to accurately determine each species recovery potential. Therefore we are unable to forecast long-term recovery potential for the most severe light reduction treatments.

Acceptable length of time under severe light reduction

In this limited study it was not possible to test every conceivable combination of light delivery for a given total light over set averaging periods. The study has shown that the number of consecutive low light days does affect seagrass responses to a given total light over set periods of time. More extensive work is required to refine these

findings and determine the sensitivity of seagrasses to a wider range of light delivery patterns and to further understand the effect of past light history on recovery potential.

1 Introduction

We know that light reduction is a key threat to the survival of seagrasses and has been identified as a major cause of seagrass loss globally (Green and Short 2003). In many cases, the reduction in light availability that leads to seagrass loss is caused through increases in turbidity. Tropical marine systems are highly dynamic environments that experience transient periods of turbidity from factors such as sediment re-suspension caused by storms, prevailing winds and tides, and riverine inputs (Petus et al. 2014; Collier et al. 2012a). Subsequently, seagrass meadows are frequently exposed to turbid conditions and as such the seagrass species living there are able to respond swiftly during short-term perturbations in order to survive (Ralph et al. 2007). However, their location in nearshore coastal waters exposes them to additional, human-induced episodes of turbidity, such as those arising from coastal development activities (e.g. port and channel dredging). Human-mediated episodes of turbidity may be prolonged or occur in pulses, punctuated by periods of lower natural turbidity which may repeat due to dredging operations, or may fluctuate due to the resuspension of dredged sediments driven by weather patterns, tides, or currents (Erftemejier and Lewis III 2006). Under dredging conditions, the light available to benthic organisms can be highly variable with periods of low light punctuated by higher light due to the movement of turbid dredge plumes (Jones et al. 2015; McMahon et al. 2016). The capacity of seagrasses to cope with episodes of light deprivation from overlying turbid waters may not only depend on the absolute quantity of light they receive and the duration over which it is reduced, but also on how the light deprivation varies through time, for example, the temporal separation (frequency) of pulsed turbidity events (Biber et al. 2009).

Our understanding of plant responses to light reduction and thresholds of response have been developed generally from experimental studies where plants have been subjected to consistent light treatments (e.g. mol photons d^{-1}, percent of surface irradiance, or a certain level of reduction relative to ambient conditions; Lavery et al. 2009; Collier et al. 2012a). Continuous light deprivation studies may not necessarily reflect what a seagrass experiences under field conditions of fluctuating light deprivation such as during a dredging operation when repeated periods of stress may be followed by intervals of recovery. While both continuous and fluctuating light regimes may deliver the same amount of light averaged over a period of time, the periods of higher light in a fluctuating regime may affect the long-term viability of seagrasses. We do not have a good understanding of how delivery of light affects seagrass resilience to light deprivation. Yet the significance of the frequency or timing of delivery of other environmental variables such as water and rainfall has been demonstrated for terrestrial plants. For example, plants respond to the quantity of water available within a particular period of time (Cherwin and Knapp 2012) and to the time between succeeding rain events (Snyder and Tartowski 2006) rather than to total annual rainfall. Therefore the timing of delivery of light is also likely to be important for marine plants. To our knowledge, only one study to date has examined this for seagrasses (Biber et al. 2009). For most seagrass species, the capacity to withstand and recover from such events are not well known, which places a constraint on coastal resource managers to correctly identify impacts and predict future responses within such dynamic environments.

The aim of this study was to investigate the responses to, and recovery from, changes in the pattern of the delivery of light, for two tropical seagrasses with different life-history strategies (Kilminster et al. 2015). The amount, duration and frequency of light was manipulated. Alternating light deprivation was used to simulate adverse pulsed conditions of extreme light-limitation due to reduced water clarity (e.g. turbidity plume) followed by periods of adequate light for survival. This was achieved by alternating delivery of light in two scenarios, so that over a 14 d period seagrasses receive ~4 mol photons m^{-2} d^{-1} of light. Three procedural controls delivered continuous amounts of light (8.8, 3.9 and 2.2 mol photons m^{-2} d^{-1}) were also examined, a range of light supply that we know from previous studies (WAMSI DSN project 5.5.1; Statton et al. 2017a) will allow seagrass survival (8 mol photons m^{-2} d^{-1}), some declines but seagrass survival (4 mol photons m^{-2} d^{-1}) and seagrass mortality (< 2 mol photons m^{-2} d^{-1}). Because the absolute quantity of light delivered to seagrasses over 14 d was the essentially the same in the continuous light delivery treatments compared to the two alternating light delivery scenarios, across a 14 d period, we could explore how changing the delivery of light impacts plant growth and survival.

2 Materials and Methods

2.1 Experimental design

The light exposure experiments in WAMSI DSN Project 5.5.1 (Statton et al. 2017a) showed that continuous application of 41–100% SI (> 8 mol photons m^{-2} d^{-1}) allowed shoot production over 12 weeks for 3 different seagrass species (*Cymodocea serrulata* (R. Brown) Ascherson and Magnus, *Halodule uninervis* (Forsskål) Ascherson (1882), and *Halophila ovalis* R. Brown J.D. Hooker (1858). However, for all species, when they were grown at 23% SI (~4 mol photons m^{-2} d^{-1}) there was a 50% reduction in the shoot production rate, indicating that this level of light impacts plants but they persist, albeit with lower growth rates. In contrast, plants exposed to 4–11% SI (<2 mol photons m^{-2} d^{-1}) had negligible new shoot production and death of plant material occurred over time, with the complete loss of one species (*H. ovalis*).

Therefore, we set the delivery treatments to an average of about 4 mol photons m^{-2} d^{-1}, which would result in detectable light reduction impacts but survival with reduced growth and other modifications (WAMSI DSN Project 5.5.1; Statton et al. 2017a). The ~4 mol photons m^{-2} d^{-1} was delivered in three different temporal patterns; continuous delivery (Moderate light treatment, which averaged 3.9 mol photons m^{-2} d^{-1}) and two 'scenarios'. Scenario 1 had 10 d of low light followed by 4 d of high light (yielding an average over 14 d of 3.8 mol photons m^{-2} d^{-1}), while Scenario 2 had 5 d of low light followed by 7 d of moderate light and then 2 d of high light (yielding an average over 14 d of 4.4 mol photons m^{-2} d^{-1}; see Figure 1 and Table 3).

Two other continuous light delivery treatments were also applied: Low light (2.2 mol photons m^{-2} d^{-1}), which we predicted would have a very negative impact with negligible shoot production leading to death of seagrass; and a High light treatment (8.8 mol photons m^{-2} d^{-1}), which we predicted would not negatively impact plants. If the delivery of light did not affect plant responses then we would expect that there would be no significant difference between the Moderate light treatment and the two delivery scenarios.

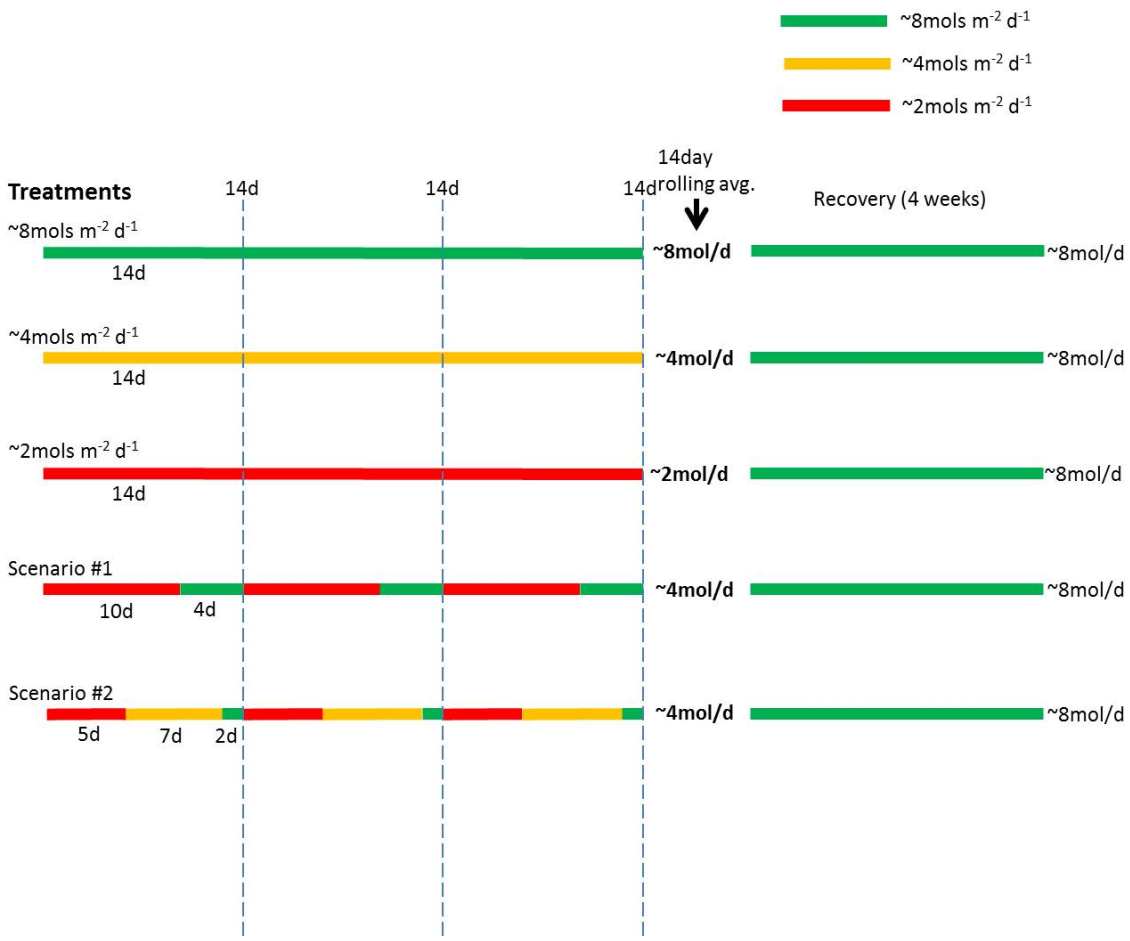


Figure 1: Diagram illustrating the experimental design to test the effect of continuous versus pulsed light reduction on seagrasses. The design consisted of three continuous light shading treatments (top three treatments) and two pulsed light delivery scenarios (bottom two treatments) over three sets of 14 days followed by a recovery period of 4 weeks.

2.2 Plant collection

On the 21st of May 2015, ~ 1000 ramets each of the seagrasses *Cymodocea serrulata* and *Halodule uninervis* were collected by gently excavating by hand, from Useless Loop, Shark Bay, Western Australia (49 J 742027E 7108005S) eight weeks prior to the beginning of the experiments. Ramets were then placed in aerated and insulated containers filled with seawater for transport to University of Western Australia's seagrass growth facility, Perth, Western Australia (1 000 km or 12 h travel time). At the seagrass growth facility, ramets were prepared for planting. They consisted of one or more intact apical shoots and with at least three and up to six mature shoots. When a ramet had more than six mature shoots, additional shoots were removed using a sharp blade. If the apical shoot was damaged or missing, the ramet was discarded. On 23rd of May 2015, three ramets of each species were planted into a single square pot (280 mm sides x 300 mm deep), and a total of 280 pots were planted.

2.3 Tank system

Experiments were conducted in10 × 1800 L rectangle plastic, fibreglass reinforced tanks, with each receiving ambient light. Each 1800 L tank was a closed, recirculating system, with seawater recirculating from a 600 L reservoir beneath each tank. Natural seawater from a nearby unpolluted area was used to fill each mesocosm system, with 25% water exchanges every 14 d throughout the experimental period. Seawater was circulated

using an 8000 L h^{-1} submersible pump, allowing complete replacement of water in the system 80× per day. Within each tank, incoming seawater was spread through a diffuser (T–bar) in order to create a homogenous movement of water. The seagrass research facility is temperature controlled, with temperature set at 27 ^{o}C. Seawater quality was controlled through continuous chemical and mechanical filtration. Salinity levels were monitored daily and adjusted via addition of deionised water.

2.4 Experimental design and setup

To test the effect of light frequency treatments and subsequent recovery on co-occurring tropical seagrass species we used a two factor randomized block design, where light treatment and time were fixed factors and tanks were the blocks. We installed 28 pots in each tank and plants were acclimated for approximately 56 d, at a temperature of 27 ^{o}C (salinity of 37-38 ppt), and ambient light measured 5 cm from the pot sediment surface (HOBO PAR light loggers, Bourne, MA, US).

After the acclimation period, the five light treatments were applied on 23rd July 2015 (see Figure 1). High light was delivered by ambient light, and the light treatments were created by applying shading using neutral density shade cloth. For continuous light shading treatments (Low and Moderate) we applied shade cloth in layers until the desired light levels for each treatment were reached at the level of the plant canopy. For the light scenario treatments, the same shading was applied (Low and Moderate) but was removed or added depending on the duration of each shading intensity (Figure 1).

For the recovery period, all shading was removed and plants were re-exposed to ambient (High) light. Two replicate tanks were randomly assigned to each light treatment (Table 1). Plant measurements (shoot density, growth and morphology, 6 pots) were conducted at 4 time periods; 2 weeks (6th Aug), 4 weeks (20th Aug) and 6 weeks (3rd Sept) after light treatments were installed, then after plants went through a 4 week recovery phase (1st Oct, Table 1). Plant physiology (rhizome carbohydrates, leaf nutrients and isotopes) and biomass measurements were conducted at 2 and 6 weeks, then at the end of the recovery period, and 4 pots (containing all species) were harvested for biomass from each tank, but plant physiology was measured on only 3 pots due to costs. Each species was then placed in a separate labelled ziplock bag (i.e. all ramets from one species in the same bag), snap-frozen with dry ice, then stored in a -20°C freezer for later processing.

Table 1: Model of experimental design. n indicates number of replicate pots per treatment, time and tank, but this number changed depending on the variable being measured (see indicators measured). Each replicate pot contains both seagrass species (2 levels). R4 indicates recovery. Note that not all variables were measured at Week 4.

Light treatment (5 levels)		**High (8.8 mol/d)**		**Moderate (3.9 mol/d)**		**Low (2.2 mol/d)**		**Scenario 1 (3.8 mol/d)**		**Scenario 2 (4.4 mol/d)**	
	Tank (2 levels)	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**
Monitoring Time (weeks, 4 levels)	**2**	*n*	*n*	*n*	*n*	*n*	*n*	*n*	*n*	*n*	*n*
	4	*n*	*n*	*n*	*n*	*n*	*n*	*n*	*n*	*n*	*n*
	6	*n*	*n*	*n*	*n*	*n*	*n*	*n*	*n*	*n*	*n*
	R4	*n*	*n*	*n*	*n*	*n*	*n*	*n*	*n*	*n*	*n*

2.5 Indicators measured

Indicators of seagrass status were tested throughout this experiment and these indicators ranged from sub-lethal physiological through to population level indicators (Table 2). The number of pots measured for biomass was 4,

at 2 and 6 weeks during the light treatment period, and at the end of the recovery period. For shoot density, growth and morphology measures, 6 randomly selected pots were measured *in situ* (i.e. plants were not harvested) at 2, 4 and 6 weeks during the light treatment period, and at the end of the recovery period. Some variables (carbohydrates, leaf nutrients and isotopes), only 3 replicates were processed (due to costs) at 2 and 6 weeks during the light treatment period, and at the end of the recovery period.

Table 2: Summary of indicators tested for each species, light intensity and duration

Level	Indicator Grouping	Indicator	Replication
Physiological (sub-lethal)	Carbohydrate reserves	Rhizome soluble carbohydrates	3
		Rhizome starch	3
	Nutrients	Leaf Carbon	3
		Leaf Nitrogen	3
		Leaf CN ratio	3
		δ^{13}Carbon	3
		δ^{15}Nitrogen	3
Plant-scale (state change)	Growth & biomass	Shoot density	6
		Shoot production rate	6
		Total biomass	4
		Above-ground biomass	4
		Below-ground biomass	4
		Leaf productivity	6
	Morphology	Number of leaves per shoot	6
		Leaf area	6
Meadow-scale (pot level)	Abundance	Biomass	4

2.6 Physiological indicators

Rhizome material (horizontal and vertical) was oven-dried and ground (ball – mill grinder). Soluble sugars and starch were then extracted using 80 % (v/v) ethanol (Quarmby & Allen 1989). Soluble sugars (% DW) and starch (% DW) were analysed by colorimetric determination (420 nm) with an amylase pre-digest to convert the starch to glucose (Yemm & Willis 1954).

Seagrass leaves were dried and ground to a fine powder using a steel ball-mill. Carbon (C) and Nitrogen (N) concentrations, and δ^{13}C and δ^{15}N values were determined using an Automated C/N Analyser-Mass Spectrometer consisting of a 20/22 mass spectrometer connected to an ANCA–S1 preparation system (Sercon, Crewe, UK) at the Western Australian Biogeochemistry Centre at the University of Western Australia. All samples were standardized using multi-point normalization against a secondary reference of Radish collegate (3.167% N, δ^{15}N 5.71‰, 41.51% C, δ^{13}C 28.61‰), which was in turn standardized against primary analytical standards (International Atomic Energy Agency, Vienna). The external error of analyses (one standard deviation) was no more than 0.1 for C:N ratio, 0.15‰ for δ^{13}C, and 0.3‰ for δ^{15}N. Elemental contents of seagrass leaf samples were calculated as a percentage of dry weight, and elemental ratios were calculated on a mol:mol basis.

2.7 Plant- and meadow-scale indicators

In situ measurements consisted of counting the number of shoots for each species on six randomly selected pots. Apical shoots were tagged at time 0, then at two-weekly intervals and the number of new shoots produced was counted. Counting the total shoot number and new shoot recruitment at each sampling period allowed accurate determination of shoot density during that time.

Leaf productivity was measured on 6 randomly selected mature shoots for *C. serrulata* and *H. uninervis*. Leaves were hole-punched at the base of the leaf sheath 7 days prior to each sampling occasion (i.e. 7 d before time 0, then at two-weekly intervals). During sampling, leaf growth was estimated from the leaf area produced relative to where leaves were initially hole-punched by measuring the length and width of the new leaf. Additionally, leaf morphometrics (length, width, area) were measured on one fully expanded mature leaf in six randomly selected mature shoots by measuring leaf length (from just above the base of the sheath to the leaf apex) and width (middle of the leaf).

At each harvest (2 and 6 weeks and 4 weeks recovery), plant samples were separated into above-ground (leaves) and below-ground (rhizome and roots) material. Above- and below-ground material and biomass (dry weight) estimates were determined from oven dried samples dried for 72 h at 60°C.

2.8 Statistical analysis

For the impact period, a four-way nested ANOVA (R package 'agricolae'; Felipe de Mendiburu, 2009) was used to test direct and interactive effects of light Treatment (fixed factor; High, Moderate, Low, Scenario 1, Scenario 2), Time (fixed factor; 2, 4, and 6 weeks), Species (fixed factor; *Cymodocea serrulata* and *Halodule uninervis*), and Tanks (1 and 2 (blocks)) nested within Treatment on physiology, plant growth, biomass and morphology variables. Note the 4 weeks measurement was not included for biomass and physiology analysis since these variables were only measured at each harvest (2 and 6 weeks). Similarly, for the recovery period a four-way nested ANOVA (R package 'agricolae') was used to test direct and interactive effects of light Treatment (fixed factor; High, Moderate, Low, Scenario 1, Scenario 2), Time (fixed factor; 0 and 4 weeks), Species (fixed factor; *Cymodocea serrulata* and *Halodule uninervis*), and Tanks (1 and 2 (blocks)) nested within Treatment on physiology, plant growth, biomass and morphology variables. Because total pot biomass summed the biomass of all species within a pot, this was also analysed using a three-way nested ANOVA (Treatment, Time, Tank(Treatment)) for both impact and recovery periods. Following a significant main effect or interaction, a Tukey's post hoc test was used to test for significant differences in treatment means. Prior to analysis, data were tested for normality using the Shapiro-Wilk test and homogeneity of variance using a Bartlett test (R core team), and transformed where appropriate.

2.9 Bio-indicators assessment

It was not a main aim to assess bio-indicators in this study. However, as similar treatments were tested in WAMSI DSN Project 5.5.1 (Statton et al. 2017a) we identified bio-indicators here and compared for consistency. To identify the most appropriate bio-indicators of response to light reduction, the variables that showed a significant effect of light reduction either as a single factor or as part of an interaction were examined further. Each species was assessed separately as there was usually a significant species effect or interaction with another variable. For each species at each time step the significance and direction of response relative to controls was determined and categorized as not significantly different to the controls (green symbol), intermediate between controls and treatments (orange symbol), and significantly different to the control (red symbol). The direction of response was defined as either higher than the controls (upward arrow) or less than the controls (downward arrow). For each variable these responses were plotted in a matrix to show the pattern of response with increasing duration and magnitude of light reduction.

To be useful, a bio-indicator should show a consistent direction and magnitude of response with increasing duration and intensity of light reduction. Controls (High light treatment) were compared against each light

reduction treatment level to determine if there was a consistent direction and magnitude of response with increasing magnitude and duration of light reduction. The identified bio-indicators were then compared to those in WAMSI DSN project 5.5.1 (Statton et al. 2017a).

2.10 Threshold development

For consistency with the earlier WAMSI DSN Project 5.5.1 (Statton et al. 2017a), we initially developed a set of impact threshold based on the ANZECC/ARCANZ (2000) guidelines. ANZECC (2000) recommend that the 20th percentile (P_{20}) value of the relevant control data be used as a threshold or trigger value, indicating that the treatments have deviated from the controls. In our case, we compared the median of treatments at each light intensity by duration level against the 20th percentile of all control data pooled across the four durations, giving a total of 24 values from which to derive percentile values. This analysis was performed separately for a set of three bio-indicators considered potentially useful for monitoring and impact prediction: one species-specific sub-lethal indicator of light stress, total rhizome carbohydrates (combining soluble sugars plus starch); and two lethal indicators, total biomass of all species pooled and the above-ground biomass of each species.

The above thresholds indicate when a dredging-related stress at a site is likely to result in a specific magnitude of effect, in this case a shift to a value at or below the 20th percentile of plants in an unimpacted site. This is consistent with recommendations in the ANZECC/ARCANZ (2000) guidelines. However, in the context of the EPA (2016) guidance on assessing impacts of dredging, it is necessary to predict when detectable change to seagrasses will occur as a result of dredging-induced stress,. This can then be used to delineate the boundary of the Zone of Moderate Impact/Zone of Influence. To do this, we developed a second set of thresholds, which integrate the findings of this study with those of the earlier WAMSI DSN Project 5.5.1 (Statton et al. 2017a). These thresholds extend on those presented in the earlier WAMSI DSN Projects 5.5.1 (Statton et al. 2017a) because they incorporate consideration of the duration of light reduction, the light intensity and the frequency (or delivery pattern) of that light reduction stress. We present two sets of thresholds:

- 'More conservative' thresholds, which provide the greatest level of confidence that seagrasses will not be negatively affected); and
- 'Less conservative thresholds', which factor in some of the variability in the findings and are thresholds which are likely to result in no significant impact on seagrasses but in which we have less confidence because of the variability in the findings.

The development of these thresholds is explained in the Discussion of this report following presentation of the findings on effects of different frequencies of light reduction on the seagrasses.

3 Results

3.1 Light conditions

In general, there was an increase in light over the duration of the experiment reflecting the change in season from winter to spring. Total light received was highest in the control and lowest in the Low light treatment, as expected (Table 3). The Moderate, Scenario 1 and Scenario 2 treatments were similar to each other within each fortnightly interval over the impact period (6 weeks) and intermediate between the High and Low light treatments (Table 3). However, during each fortnightly interval the quantity of light plants received on a daily basis differed between both scenarios and Moderate light treatments. On average, the Moderate light treatment received a relatively constant and 'moderate' quantity of light (3.91 mol photons m^{-2} d^{-1}), Scenario 1 received 10 d of 'low' light followed by 4 d of 'high' light (3.84 mol photons m^{-2} d^{-1}), whereas Scenario 2 plants were exposed to half the number of days of 'low' light (5 d) as Scenario 1, then 7 d of 'moderate' light followed by 2 d of 'high' light (4.41 mol photons m^{-2} d^{-1}). Scenario 2 was exposed to, on average, ~0.5 mol photons m^{-2} d^{-1} more light than

both Moderate and Scenario 1 light treatments, though this difference was variable within each fortnight. During the recovery period (4 weeks), controls and all plants previously exposed to light stress received between 13.3–15.6 mol photons m^{-2} d^{-1}.

Table 3: Summary of the quantity of light received in each light treatment during the 6 week light deprivation period and recovery period following re-exposure to ambient light. Sum values are total light received over 14 days, whereas average is mean mol photons m^{-2} d^{-1} (± 1SE).

		TREATMENT				
		High	**Moderate**	**Low**	**Scenario 1**	**Scenario 2**
Sampling time						
Fortnight 1	Sum	101.60	41.25	19.16	43.90	46.01
	Avg	7.26 (0.62)	2.95 (0.26)	1.37 (0.22)	3.14 (0.92)	3.29 (0.79)
Fortnight 2	Sum	115.46	55.88	30.67	47.87	57.48
	Avg	8.25 (0.57)	3.99 (0.40)	2.19 (0.54)	3.42 (1.07)	4.11 (0.99)
Fortnight 3	Sum	150.95	67.18	43.99	69.61	81.79
	Avg	10.78 (0.63)	4.80 (0.43)	3.14 (0.46)	4.97 (1.18)	5.84 (1.03)
Total	Sum	368	164.3	93.8	161.4	185.3
	Avg	8.76 (0.41)	3.91 (0.24)	2.23 (0.27)	3.84 (0.61)	4.41 (0.56)
Recovery						
Fortnight 1	Sum	188.98	198.84		186.68	205.05
	Avg	13.50 (0.84)	14.20 (0.86)		13.33 (0.82)	14.65 (0.89)
Fortnight 2	Sum	203.41	217.39		196.35	218.67
	Avg	14.53 (1.02)	15.53 (1.07)		14.02 (1.04)	15.62 (0.91)
Total	Sum	392.4	416.2		383.0	423.7
	Avg	14.01 (0.66)	14.87 (0.69)		13.68 (0.65)	15.13 (0.63)

3.2 Impact period

3.2.1 Physiological scale

The proportions of different rhizome carbohydrates (soluble sugars and starch) differed in *Cymodocea serrulata* and *Halodule uninervis*; *C. serrulata* had >25% soluble sugars compared to <0.2% starch whereas *H. uninervis* had 8% soluble sugars and 8% starch. Because soluble sugars so dominate the carbohydrates in *C. serrulata*, starch is not considered in the following discussion for this species. In *C. serrulata* and *H. uninervis,* rhizome carbohydrate concentrations decreased with reduced light availability but this changed over time (Treatment × Species × Time, MS = 40, p = 0.016, Table 4). For C. *serrulata*, soluble sugar concentration significantly reduced, and this was most obvious at 2 weeks where all light shading treatments were significantly less than the continuous high light. Following 6 weeks of light reduction, the concentrations increased in all except the Low treatment, probably due to the seasonal increase in the amount of light received over this period (Table 3), but the Low light and both scenarios remained significantly lower than the High light treatment, and the Moderate light was intermediate between these two groups (Figure 2 i). For *H. uninervis*, starch showed the stronger response with significant reductions in concentration at 2 weeks for all light shading treatments (Figure 2 iv). As occurred with *C. serrulata*,

the concentrations for most treatments increased over 6 weeks, but the Low and Scenario 1 light treatments remained significantly less than the controls, whereas Moderate and Scenario 2 light treatments were intermediate between the two groups. For soluble rhizome carbohydrates, at 2 weeks, only the Low light treatment was significantly lower than the controls, with the other treatments intermediate between the two groups. In contrast, by 6 weeks, Scenario 1 showed an increase in soluble sugars above the controls (Figure 2 ii).

Table 4: Results of four-way nested ANOVA testing for the effects of treatment, species, time and treatment nested within tank on rhizome carbohydrates during the light impact period. Bold text denotes significant differences.

		Rhizome Soluble Carbohydrates		**Rhizome Starch**	
	df	MS	p	MS	P
Species	1	511	**<0.001**	999	**<0.001**
Treatment	4	228	**<0.001**	49	**<0.001**
Time	1	463	**<0.001**	158	**<0.001**
Tank (Treatment)	5	28	0.443	5	0.909
Species x Treatment	4	174	**<0.001**	48	**<0.001**
Species x Time	1	145	**<0.001**	157	**<0.001**
Treatment x Time	4	30	0.055	8	**0.0494**
Species x Treatment x Time	4	40	**0.016**	7	0.0641

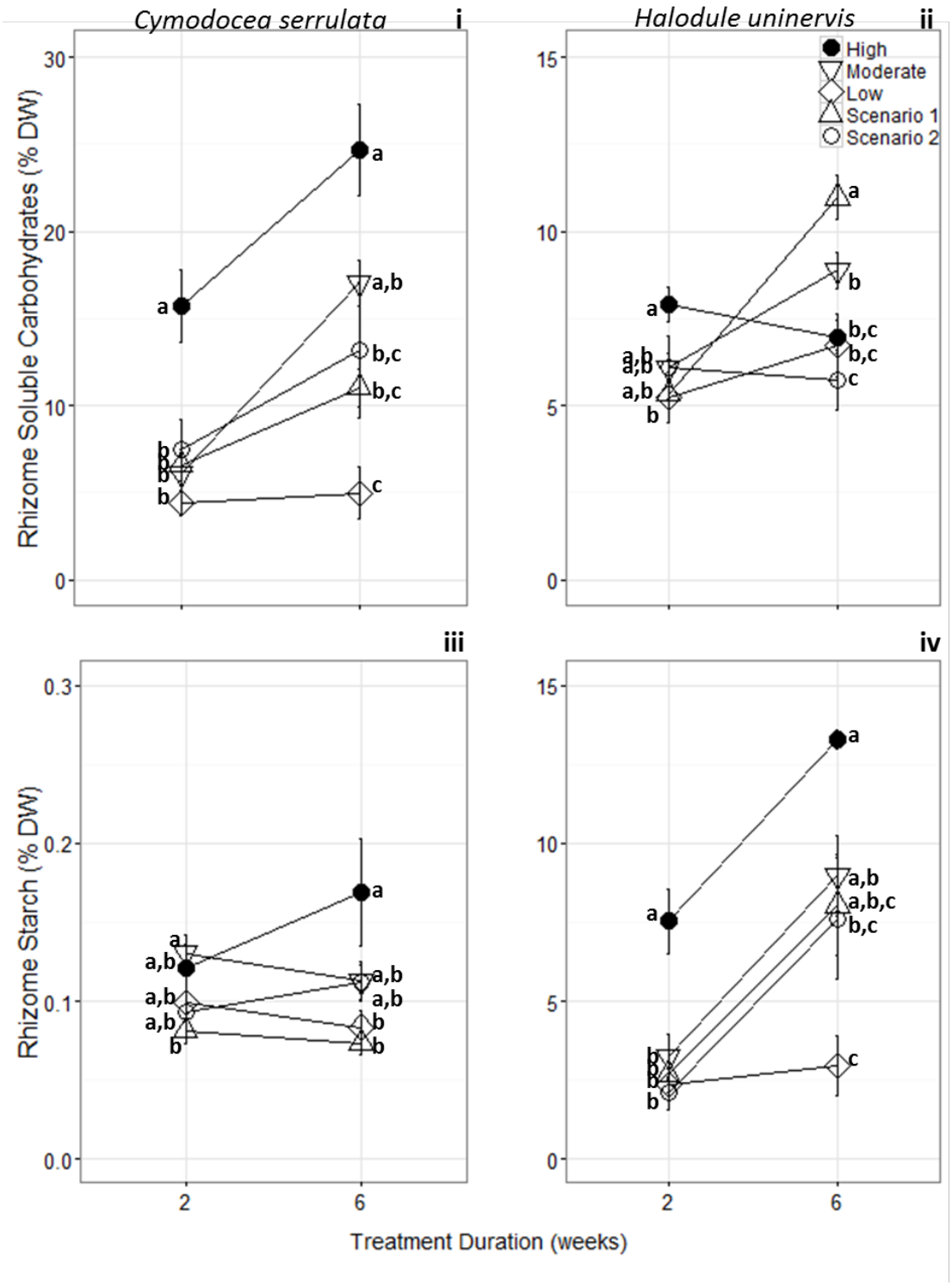


Figure 2: Effect of light reduction on seagrass rhizome carbohydrates. Rhizome soluble carbohydrates, % DW (i – ii) and rhizome starch, % DW (iii – iv) for *Cymodocea serrulata* (left), *Halodule uninervis* (right) under each of the five light treatments at 2 and 6 weeks. Symbols represent means pooled across treatment tanks (n = 8 for 2 and 6 weeks) ±1SE. Different letters denote significant differences.

Leaf carbon (%C) was not affected by light treatment in *C. serrulata*, but was for *H. uninervis* (Treatment × Species × Time, MS = 17.25, p = 0.022, Table 5). In *H. uninervis*, leaf carbon was unaltered at 2 weeks but by 6 weeks it was significantly greater in Scenario 2 than the High light and Scenario 1 treatments, whereas the Low and Moderate light treatments showed an intermediate increase (Figure 3 i, ii). Leaf nitrogen (%N) concentration was significantly affected by light treatment with time, but the response differed between species (Treatment × Species × Time, MS=0.067, p=0.005, Table 5). For *C. serrulata* at 2 weeks, both Moderate and Low light treatments had higher leaf N than the High light treatment, whereas both scenarios were intermediate between these two groups. However, by 6 weeks all light treatments had higher leaf N than the High light treatments (Figure 3 iii). For *H. uninervis*, Low light and Scenario 1 and 2 light treatments had greater leaf N concentrations than the High light treatment. Scenario 1 was also significantly higher than the Moderate light treatment, and these patterns persisted at 6 weeks (Figure 3 iv). Leaf C:N ratio decreased with light reduction treatments, but the response differed over time for each species (Treatment × Species × Time, MS = 30.4, p=0.024, Table 5). For both species, in general, the C:N ratio increased over time in the High light treatment. For *C. serrulata* at 2 weeks, only the Moderate and Low light treatments were reduced relative to the High light treatment but by 6 weeks, all light reduction treatments were significantly less than the High light treatment (Figure 3 v). For *H. uninervis,* at 2 weeks, all light reduction treatments had reduced C:N ratios relative to the High light treatment but by 6 weeks the Moderate light treatment was not significantly different to the High light treatment but was significantly greater than Scenario 1 (Figure 3 vi).

Leaf δ^{13}C showed a general decrease with light reduction but the magnitude of response was dependent on species and time (Treatment × Species × Time, MS = 0.95, p = 0.019, Table 5). *C. serrulata* had a δ^{13}C signature between -12 to -13‰ in the High light treatment over the impact duration. At 2 weeks, δ^{13}C was reduced in the Moderate light treatment relative to the High light treatment, whereas all other treatments were intermediate between the two groups (Figure 3 vii). By six weeks, the Low, Moderate and Scenario 1 light treatments were significantly lower than the High light treatment. On the other hand, δ^{13}C of the Highlight treatment for *H. uninervis* remained relatively stable over the experiment, at -15 to -15.5 and was not affected by light treatment at 2 weeks. However, by six weeks, all light reduction treatments were less than the High light treatment (Figure 3 viii), but Scenario 1 and 2 and the Moderate light treatment were not significantly different to each other. Both species showed an early response in δ^{15}N (Species × Time, MS = 11.7, p<0.001, Table 5), although the magnitude of response differed between species (Treatment × Species, MS = 4.9, p<0.001, Table 5). *C. serrulata* had a δ^{15}N signature of ~4‰ in the High light treatment during the impact period, and was significantly reduced in the Low light treatment at 2 weeks only. By 6 weeks, there was no difference in δ^{15}N across light treatments (Figure 3 ix). In contrast, *H. uninervis* had a δ^{15}N signature of -0.3‰ (at 2 weeks) to 1.3‰ (at 6 weeks) in the High light treatment. At two weeks, only Scenario 2 showed a significantly greater δ^{15}N value (1.3‰) compared to the High light treatment, whereas all other light treatments showed an intermediate response between the two groups (Figure 3 x). At six weeks there was no effect of light treatment on δ^{15}N.

Table 5: Results of four-way ANOVA testing for the effects of treatment, species, time and treatment nested within tank on leaf C and N concentrations, leaf CN ratios, δ^{13}C and δ^{15}N during the light impact period. Bold text denotes significant differences.

		Leaf Carbon		**Leaf Nitrogen**		**Leaf CN ratio**		**δ^{13}C**		**δ^{15}N**	
	df	MS	p	MS	p	MS	p	MS	p	MS	p
Species	1	6.15	0.041	1.045	**<0.001**	489.9	**<0.001**	145.4	**<0.001**	142.2	**<0.001**
Treatment	4	2.40	0.164	0.812	**<0.001**	550.6	**<0.001**	4.1	**<0.001**	2.1	0.049
Time	1	74.4	**>0.001**	3.182	**<0.001**	1451	**<0.001**	45.6	**<0.001**	8.4	0.002
Tank (Treatment)	5	2.60	0.374	0.038	0.667	23.5	0.569	1.06	0.777	1.58	0.665
Species x Treatment	4	1.23	0.494	0.056	**0.014**	28.6	**0.031**	0.53	0.148	4.9	**<0.001**
Species x Time	1	3.93	0.101	0.175	**0.002**	6.3	0.437	0.31	0.317	11.7	**<0.001**
Treatment x Time	4	26.87	**0.002**	0.036	0.082	58.4	**<0.001**	2.78	**<0.001**	1.6	0.121
Species x Treatment x Time	4	17.25	**0.022**	0.067	**0.005**	30.4	**0.024**	0.95	**0.019**	1.0	0.322

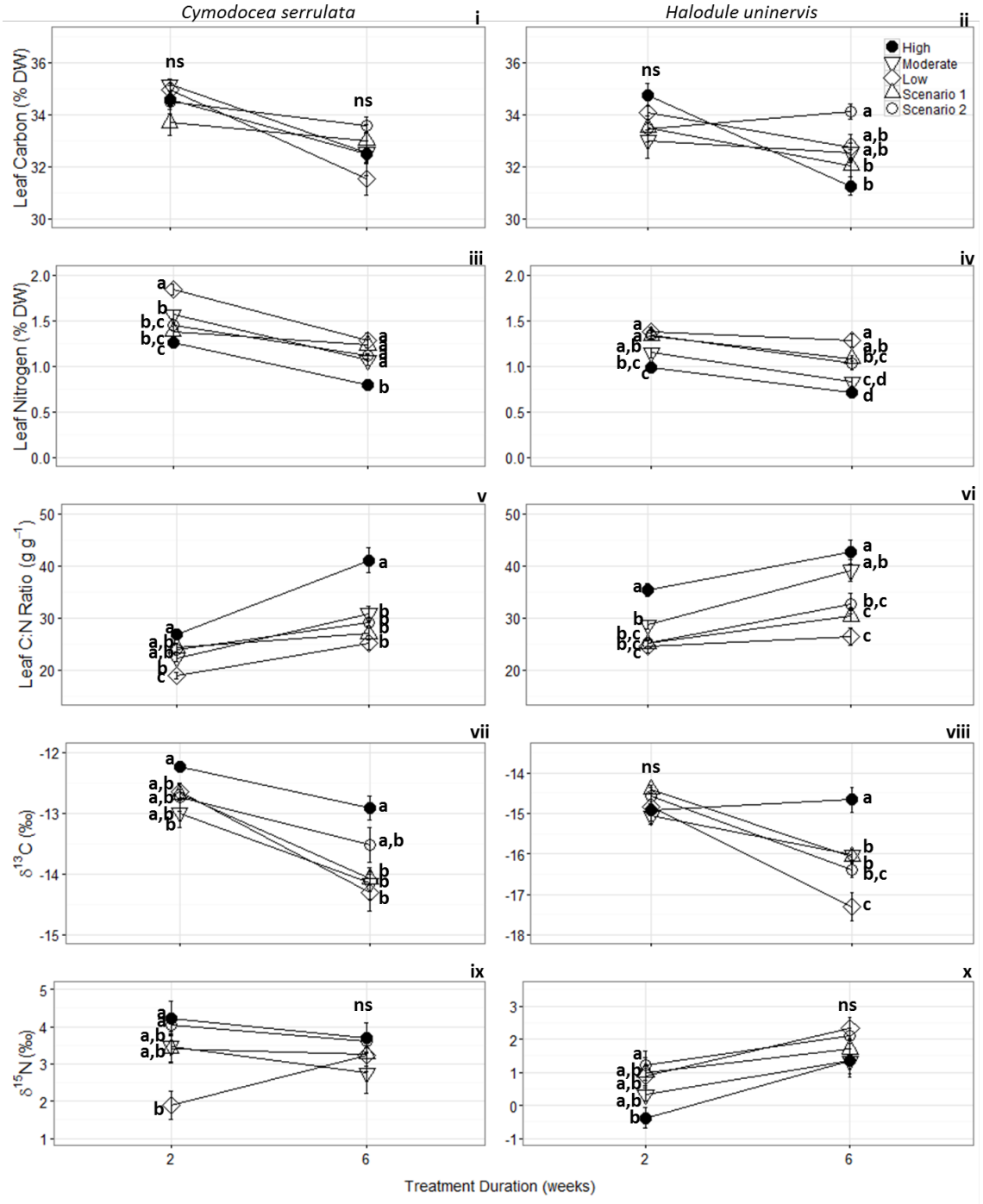


Figure 3: Effect of light reduction on seagrass nutrient characteristics. Leaf carbon, % DW (i–ii), leaf nitrogen, % DW (iii–iv), leaf C:N ratio, g g^{-1}, (v–vi), δ13C (vii–viii), and $\delta^{15}N$ (ix–x) for *Cymodocea serrulata* (left), *Halodule uninervis* (right) under each of the 5 light treatments at 2 and 6 weeks. Symbols represent means pooled across treatment tanks (n = 8 for 2 and 6 weeks) ± 1SE. Different letters denote significant differences.

3.2.2 Plant scale

For *C. serrulata*, there was no significant change in leaf area amongst light treatments for the first 4 weeks, but by 6 weeks, plants in Scenario 1 had a reduced leaf area compared to all other light treatments (Figure 4 i). Leaves of *H. uninervis* responded faster but not in a consistent manner, by 4 weeks leaf area in Scenario 2 was greater than the High light, and the remaining treatments were intermediate between the two, but by 6 weeks there was no significant effect of light treatment on leaf area (Figure 4 ii). The number of leaves per shoot was affected by light treatment, but only for *C. serrulata* (Treatment × Species, MS = 0.252, p = 0.026, Table 6). The number of leaves per shoot reduced in Scenario 1 compared to the High light treatment after 2 weeks. This was maintained at 4 and 6 weeks, and the Low light treatment also reduced relative to the High light treatment (Figure 4iii). Scenario 2 always had a greater number of leaves per shoot compared to Scenario 1, but not relative to the Moderate light treatment for the duration of the impact period.

Although both species were planted with a similar rhizome length and the same number of ramets within each pot, there were 50 % less shoots for *C. serrulata* (7–9) compared to *H. uninervis* (16–18) in controls at any time, highlighting the higher productivity of *H. uninervis*. Light treatment affected shoot productivity (the number of shoots produced) of *H. uninervis* but not *C. serrulata* (Treatment × Species, MS = 0.007, p = 0.006, Table 7) and this was weakly significant over time (Treatment × Time, MS = 0.003, p = 0.053, Table 7). At 2 weeks, the Low, Moderate and Scenario 1 treatments had significantly reduced rates of shoot production compared to High light, whereas Scenario 2 was intermediate between the two groups. There was no significant difference between Moderate and both Scenarios 1 and 2. By 4 weeks, Scenario 1 and 2 were significantly lower than High and Moderate light treatments, with the Low light treatment intermediate between the two groups. By 6 weeks, the Low and Moderate light treatments significantly reduced relative to the High light treatment (Figure 5 v, vi). On the other hand, leaf productivity, the area of leaf produced declined in response to light treatment but only for *C. serrulata* (Treatment × Species, MS = 0.100, p<0.001, Table 7). At 2 weeks, the Scenario 1 and Scenario 2 light treatments showed the greatest decline, but the Low treatment was also significantly reduced compared to High light treatment, and the Moderate light treatment was intermediate between the two groups (Figure 5 iii). The light treatments had no clear effect on shoot density for *C. serrulata* during the impact period but *H. uninervis* was affected (Treatment × Species, MS = 21, p = 0.043, Table 7). These effects were due to differences between the scenarios and the continuously reduced light treatments. At 2 weeks, shoot densities in the Moderate and Low light treatments were significantly reduced relative to Scenarios 1 and 2, and at 4 and 6 weeks, only the Low light treatment was reduced relative to the scenarios (Figure 5 ii).

Cymodocea serrulata had considerably more plant biomass (60–70 %) than *H. uninervis* (Species, MS = 16.37, p<0.001, Table 8), but both showed similar responses to light treatment (Treatment, MS = 0.208, p = 0.017, Table 8). Plants exposed to the Low light treatment had significantly less total biomass than the High light treatment and this was evident after 2 weeks (Figure 6 i, ii), all other light treatments were intermediate between these two. Above-ground biomass was impacted by light treatment, but responses differed for each species (Treatment × Species, MS = 0.008, p = 0.017, Table 8). *C. serrulata* had a reduced above-ground biomass in the Low and Scenario 1 light treatments relative to High light treatment, whereas the Moderate and Scenario 2 treatments showed intermediate responses (Figure 6 iii, iv). These differences were evident after 2 weeks and persisted through to 6 weeks. For *H. uninervis*, the Low light treatment significantly reduced aboveground biomass compared to all other treatments. Although there were clear differences in below-ground biomass between species (Species, MS = 9.89, p <0.001, Table 8) with *C. serrulata* 60 % larger than *H. uninervis*, light treatment influenced both species similarly (Treatment, MS = 0.208, p = 0.017, Table 8). Plants exposed to the Low light treatments had significantly less below-ground biomass than the High light treatment and this was evident after 2 weeks (Figure 6 v, vi). All other light treatments were intermediate between the two groups. At each sampling interval (2 and 6 weeks), the total biomass of both species combined within a pot was affected by light treatment (Treatment, MS = 0.416, p = 0.018, Table 8), where only the Low light treatment had significantly reduced pot biomass relative to the controls (Figure 7). All other treatments showed intermediate responses between the two groups.

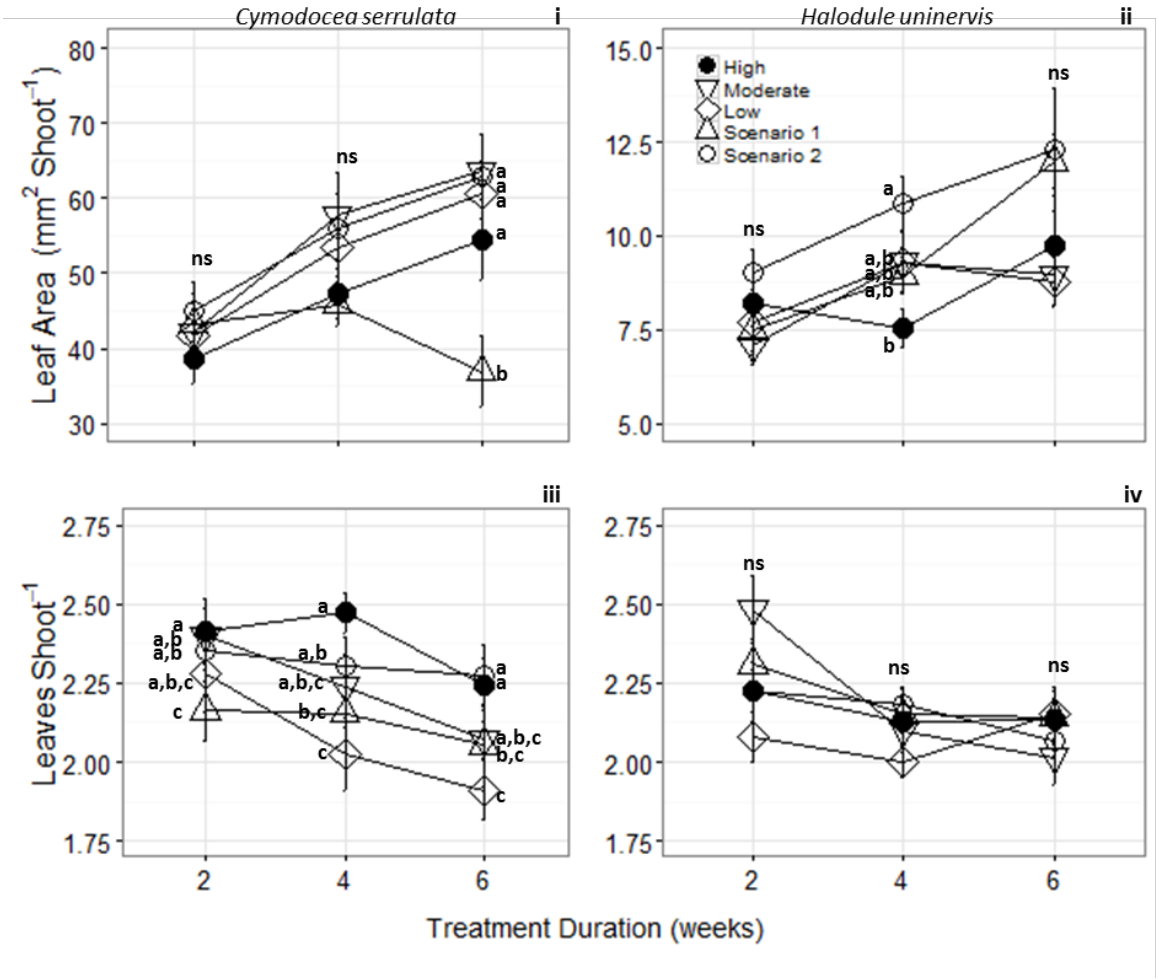


Figure 4. Effect of light reduction on seagrass morphology. Leaf length, mm (i–ii), leaf width, mm (iii–iv), mean leaf area per shoot, cm2, (v–vi), and leaves per shoot (vii–viii) for *Cymodocea serrulata* (left), *Halodule uninervis* (right) under each of the 5 light treatments at 2, 4 and 6 weeks. Symbols represent means pooled across treatment tanks (n = 12 for 2, 4 and 6 weeks) ± 1 SE. Different letters denote significant differences.

Table 6: Results of a four-way nested ANOVA testing for the effects of treatment species, time and treatment nested within tank on leaf morphology during the impact period. Bold text denotes significant differences

		Leaf Area		**Leaves per shoot**	
	df	MS	P	MS	p
Species	1	149443	**<0.001**	0.372	**0.043**
Treatment	4	591	**<0.001**	0.422	**0.001**
Time	2	1972	**<0.001**	1.067	**<0.001**
Tank (Treatment)	5	152	0.928	0.335	**0.041**
Species x Treatment	4	536	**<0.001**	0.252	**0.026**
Species x Time	2	978	**<0.001**	0.102	0.323
Treatment x Time	8	172	0.103	0.112	0.269
Species x Treatment x Time	8	230	**0.020**	0.099	0.356

Table 7: Results of a four-way nested ANOVA testing for the effects of treatment species, time and treatment nested within tank on plant growth during the impact period. Bold text denotes significant differences

		Shoot density		**Leaf Productivity**		**Shoot Productivity**	
	df	MS	p	MS	p	MS	p
Species	1	6537	**<0.001**	503	**<0.001**	0.088	**<0.001**
Treatment	4	30	**0.0101**	20.6	**<0.001**	0.007	**0.003**
Time	2	139	**<0.001**	0.1	0.927	0.012	**<0.001**
Tank (Treatment)	5	56.31	0.074	4.47	0.277	0.004	0.072
Species x Treatment	4	21	**0.043**	0.1	**<0.001**	0.007	**0.006**
Species x Time	2	22	0.092	1.6	0.938	0.007	**0.017**
Treatment x Time	8	4	0.088	1.5	0.592	0.003	0.053
Species x Treatment x Time	8	5	0.85	2	0.625	0.003	0.165

Table 8: Results of a four-way nested ANOVA testing for the effects of treatment, species, time and treatment nested within tank on plant biomass; and results of three-way nested ANOVA testing for the effects of treatment and time on total pot biomass during the light impact period. Bold text denotes significant differences

		Total Biomass		**Above-ground Biomass**		**Below-ground Biomass**		**Total (Pot) Biomass**	
	df	MS	p	MS	p	MS	p	MS	p
Species	1	16.37	**<0.001**	0.812	**<0.001**	9.89	**<0.001**	NA	**NA**
Treatment	4	0.208	**0.017**	0.014	**<0.001**	0.115	**0.046**	0.416	**0.018**
Time	1	0.199	0.086	0.000	**0.925**	0.203	**0.037**	0.398	0.085
Tank (Treatment)	5	0.072	0.843	0.002	0.964	0.056	0.774	0.143	0.410
Species x Treatment	4	0.071	0.377	0.008	0.017	0.035	0.550	NA	NA
Species x Time	1	0.078	0.279	0.001	0.692	0.067	0.228	NA	NA
Treatment x Time	4	0.494	0.121	0.003	0.339	0.089	0.106	0.247	0.121
Species x Treatment x Time	4	0.209	0.535	0.001	0.744	0.038	0.509	NA	NA

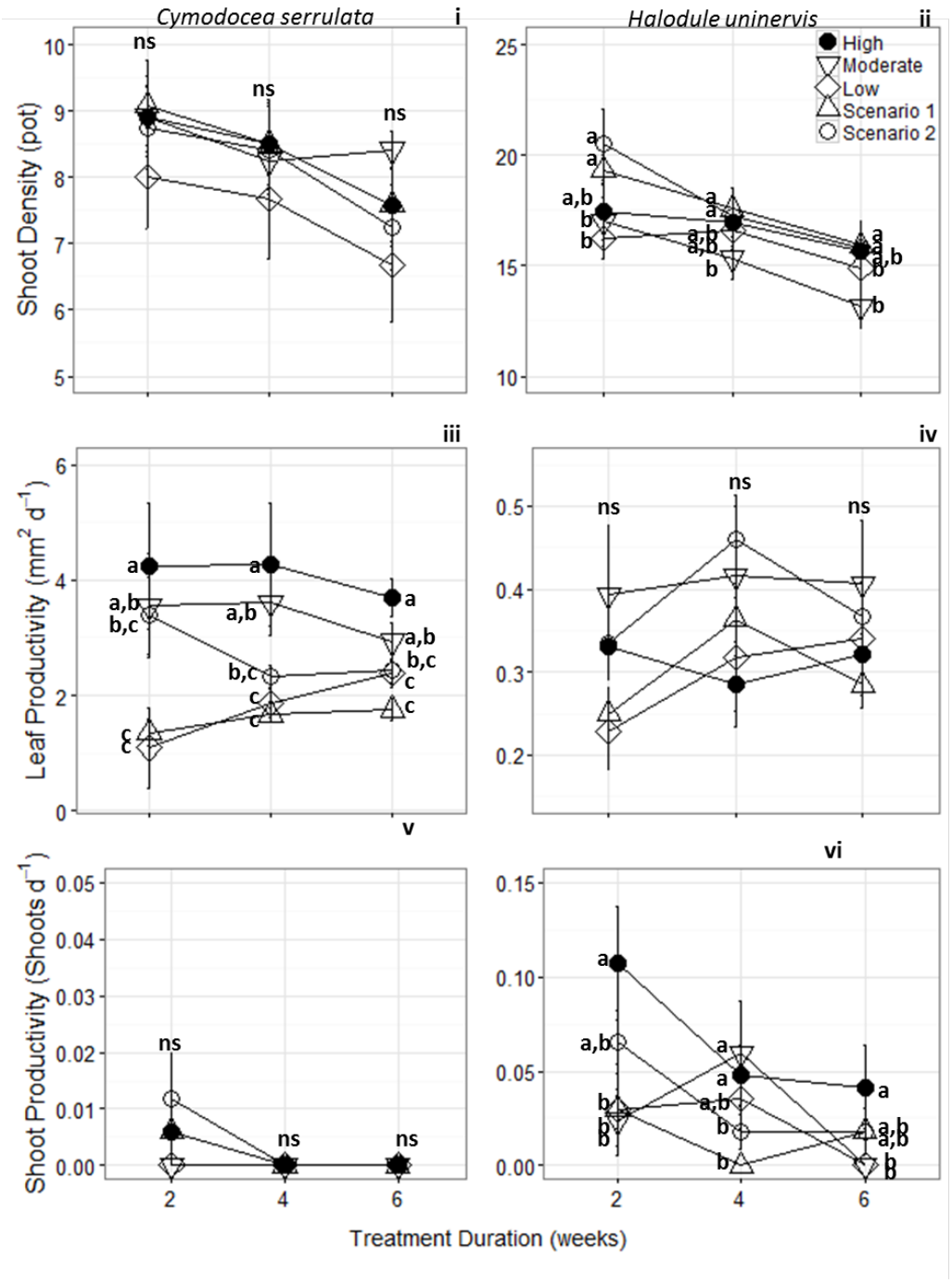


Figure 5. Effect of light reduction on seagrass shoot density and growth. Shoot density (i–ii), leaf productivity, $mm^2\ d^{-1}$ (iii–iv), and shoot production, shoots d^{-1}, (v–vi) for *Cymodocea serrulata* (left), *Halodule uninervis* (right) under each of the 5 light treatments at 2, 4 and 6 weeks. Symbols represent means pooled across treatment tanks (n = 12 for 2, 4 and 6 weeks) ± 1 SE. Different letters denote significant differences.

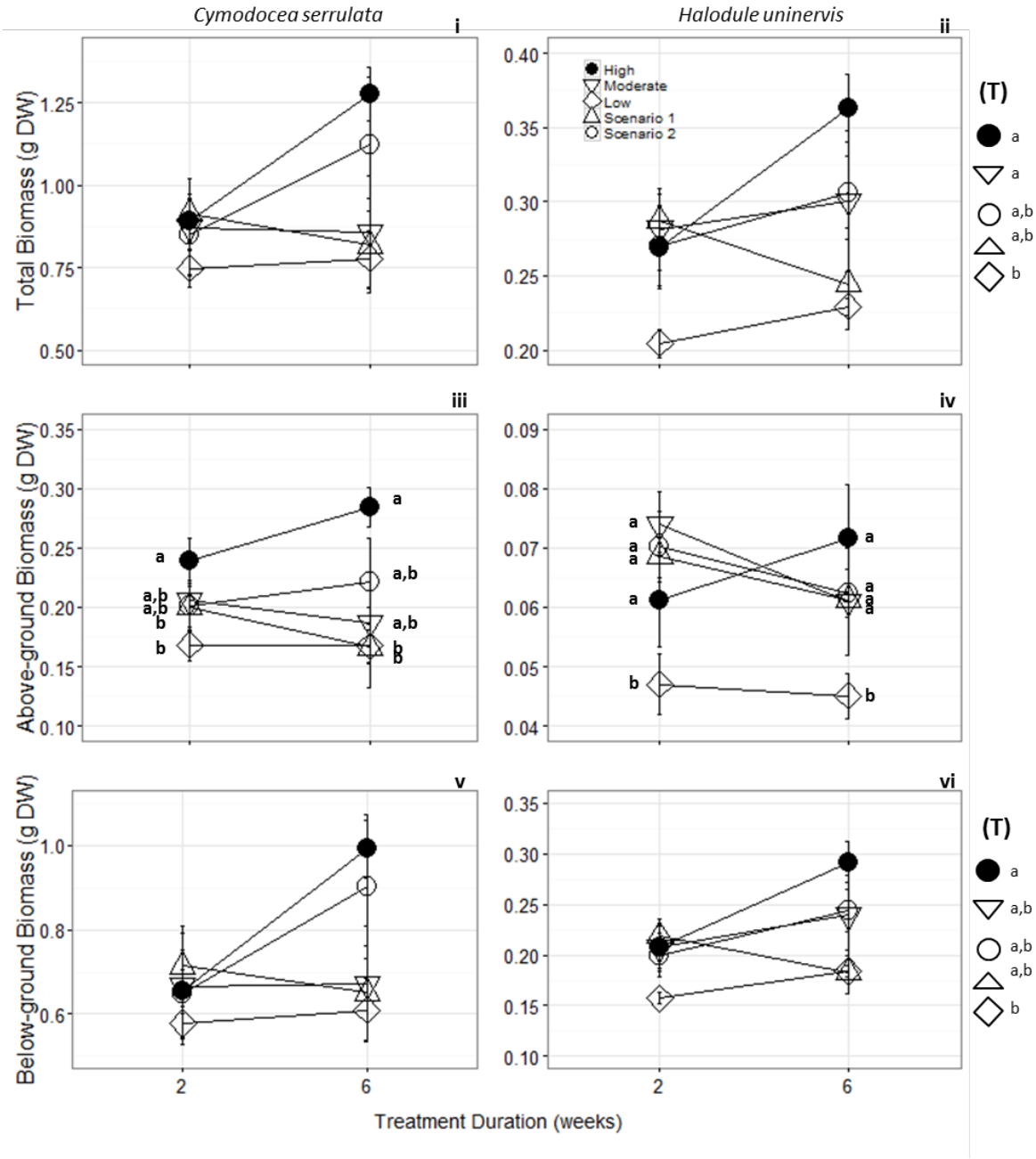


Figure 6. Effect of light reduction on seagrass biomass. Total plant biomass, g DW (i–ii), above-ground biomass, g DW (iii–iv), and below-ground biomass, g DW (v–vi) for *Cymodocea serrulata* (left), *Halodule uninervis* (right) under each of the 5 light treatments at 2 and 6 weeks. Symbols represent means pooled across treatment tanks (n = 8 for 2 and 6 weeks) ± 1 SE. Different letters denote significant differences. (T) represents a Treatment main effect, symbols beneath represent each light treatment

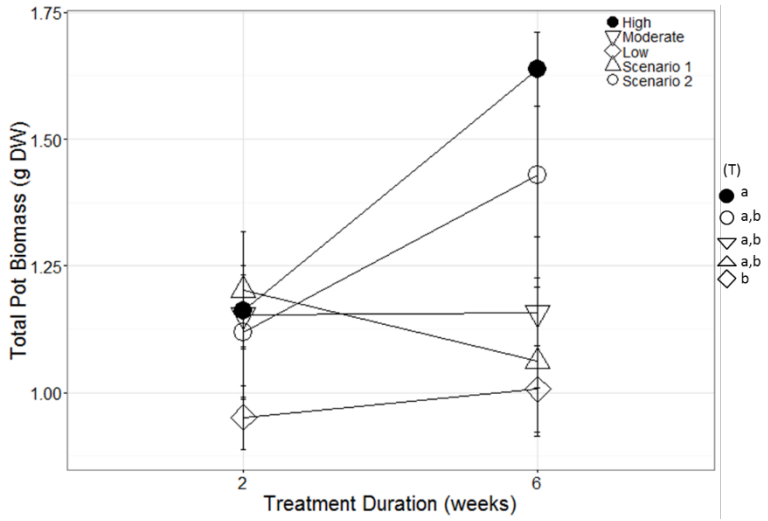


Figure 7. Total (pot) biomass (g DW) for both species (*Cymodocea serrulata* and *Halodule uninervis*) under each of the 5 light treatments at 2 and 6 weeks. Symbols represent means pooled across treatment tanks (n = 8 for 2 and 6 weeks) ± 1 SE. Different letters denote significant differences. (T) represents a Treatment main effect, symbols beneath represent each light treatment.

3.3 Recovery period

3.3.1 Physiological scale

At the end of the recovery period there were differences in rhizome carbohydrate concentrations but it was clear that all treatments were on a trajectory for recovery of carbohydrate concentrations towards controls levels (Table 9, Figure 8). In *C. serrulata,* rhizome soluble sugars had increased in all treatments, but the Low and Scenario 1 light treatments were still significantly lower than the High Light (control) treatment (Figure 8 i). By the end of the recovery period there were no detectable differences in starch, indicating this variable had recovered to control conditions. For *H. uninervis*, rhizome starch showed a significant increase in all light treatments such that there were no significant differences between controls and treatments by the end of the recovery period (Figure 8 iv). But for rhizome soluble sugars, not all treatments increased, and only the Scenario 1 light treatment was significantly less than controls by the end of the recovery period (Figure 8 ii).

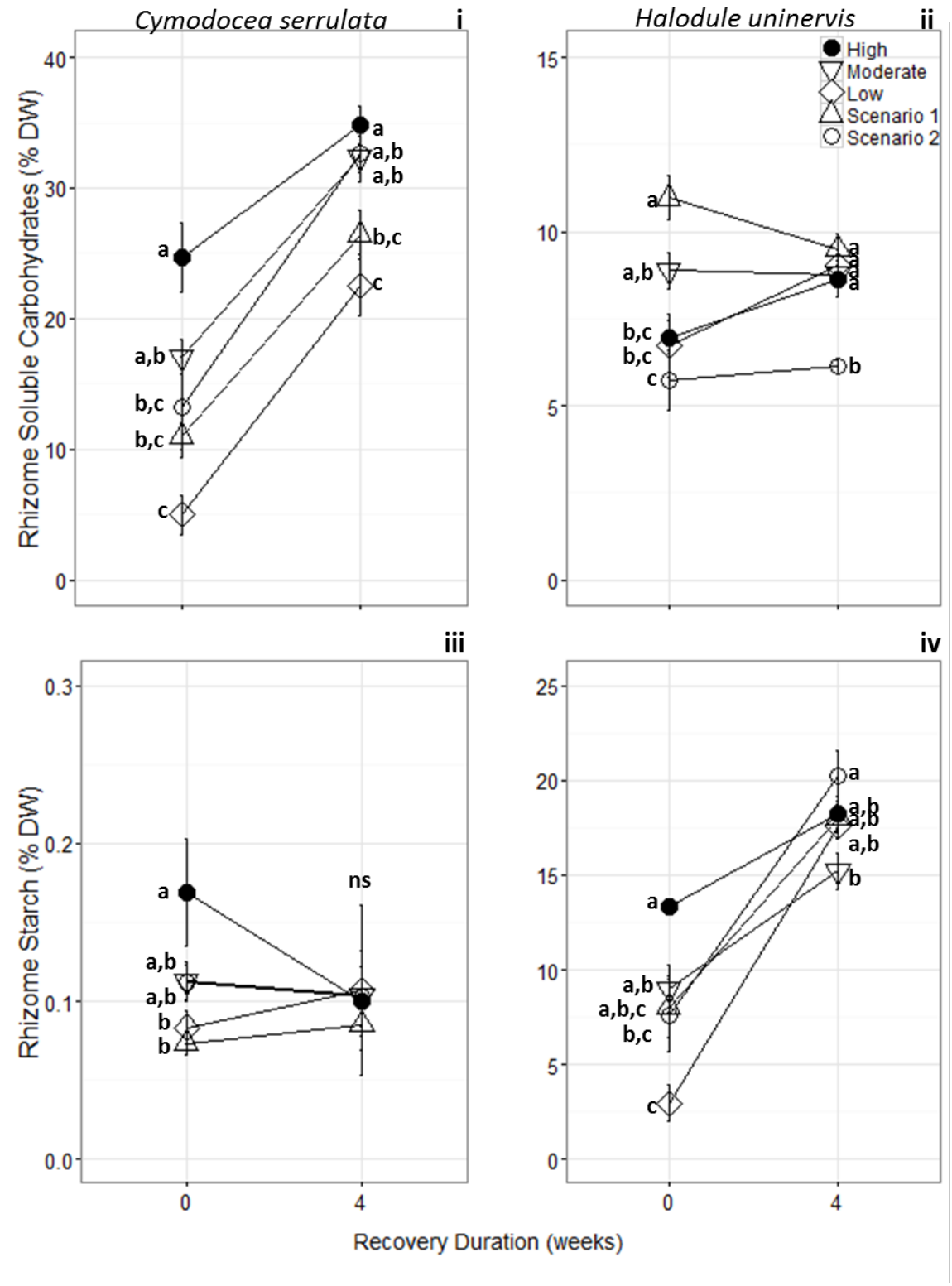


Figure 8. Effect of light reduction on seagrass rhizome carbohydrates during the recovery period. Rhizome soluble carbohydrates, % DW (i–ii) and rhizome starch, % DW (iii–iv) for *Cymodocea serrulata* (left), *Halodule uninervis* (right) under each of the 5 light treatments at 0 and 4 weeks. Symbols represent means pooled across treatment tanks (n = 8 for 0 and 4 weeks) ± 1 SE. Different letters denote significant differences.

Table 9 Results of four-way nested ANOVA testing for the effects of treatment, species, time and treatment nested within tank on rhizome carbohydrates during the light recovery period. Bold text denotes significant differences.

		Rhizome Soluble Carbohydrates		Rhizome Starch	
	df	MS	p	MS	P
Species	1	5742	**<0.001**	4998	**<0.001**
Treatment	4	213	**<0.001**	25	**<0.001**
Time	1	1952	**<0.001**	701	**<0.001**
Tank (Treatment)	5	22	0.959	3	0.998
Species x Treatment	4	258	**<0.001**	25	**<0.001**
Species x Time	1	1690	**<0.001**	704	**<0.001**
Treatment x Time	4	19	**0.0247**	26	**<0.001**
Species x Treatment x Time	4	23	**0.0164**	25	**<0.001**

The leaf physiology variables showed a variety of patterns with re-exposure to ambient light, with some clearly showing recovery. Leaf carbon (%C) was not impacted for *C. serrulata* during the light reduction period and this was maintained when plants were re-exposed to high light; however, this was not the case for *H. uninervis* (Treatment × Species, MS = 11.4, p<0.001, Table 10). Leaf carbon in the Scenario 2 treatment was elevated at the end of the impact phase and was maintained during the recovery period (Figure 9 i, ii). The response of leaf nitrogen to re-exposure to ambient light varied depending on the previous light reduction treatment and species (Treatment × Species × Time, MS = 0.411, p = 0.049, Table 10). For *C. serrulata*, all treatments showed a general decline in leaf N by the end of the shading period, and did not recover to the High light treatment concentrations after 4 weeks (Figure 9 iii). In contrast, *H. uninervis* showed recovery of leaf N concentrations for all treatments except for the Low light treatment (Figure 9 iv). Combining these two variables into the C:N ratio showed that for *C. serrulata* there was a trajectory of recovery but all treatments remained significantly lower than the High light treatment. Whereas *H. uninervis* showed a mixed response (Treatment × Species × Time, MS = 167, p<0.001, Table 10). Plants that previously experienced the Moderate light treatment showed an elevated C:N ratio relative to the High light treatment, whereas plants in the Low light treatment were significantly reduced (Figure 9 v, vi). In addition, the Scenario 1 treatment was significantly reduced relative to the Moderate light treatment, whereas the Scenario 2 treatment was not significantly different. The process of carbon uptake clearly recovered with re-exposure to ambient light levels since the δ^{13}C signature for both species recovered to control levels after 4 weeks (Treatment × Time, MS = 3.14, p<0.001; Species × Time, MS = 2.39, p = 0.014, Table 10). Leaf δ^{15}N was not affected by light reduction treatments at the end of the 6 week light deprivation period for *H. uninervis*, but there were subtle differences for *C. serrulata*, but at the end of the recovery period there were no significant differences (Figure 9 ix, x).

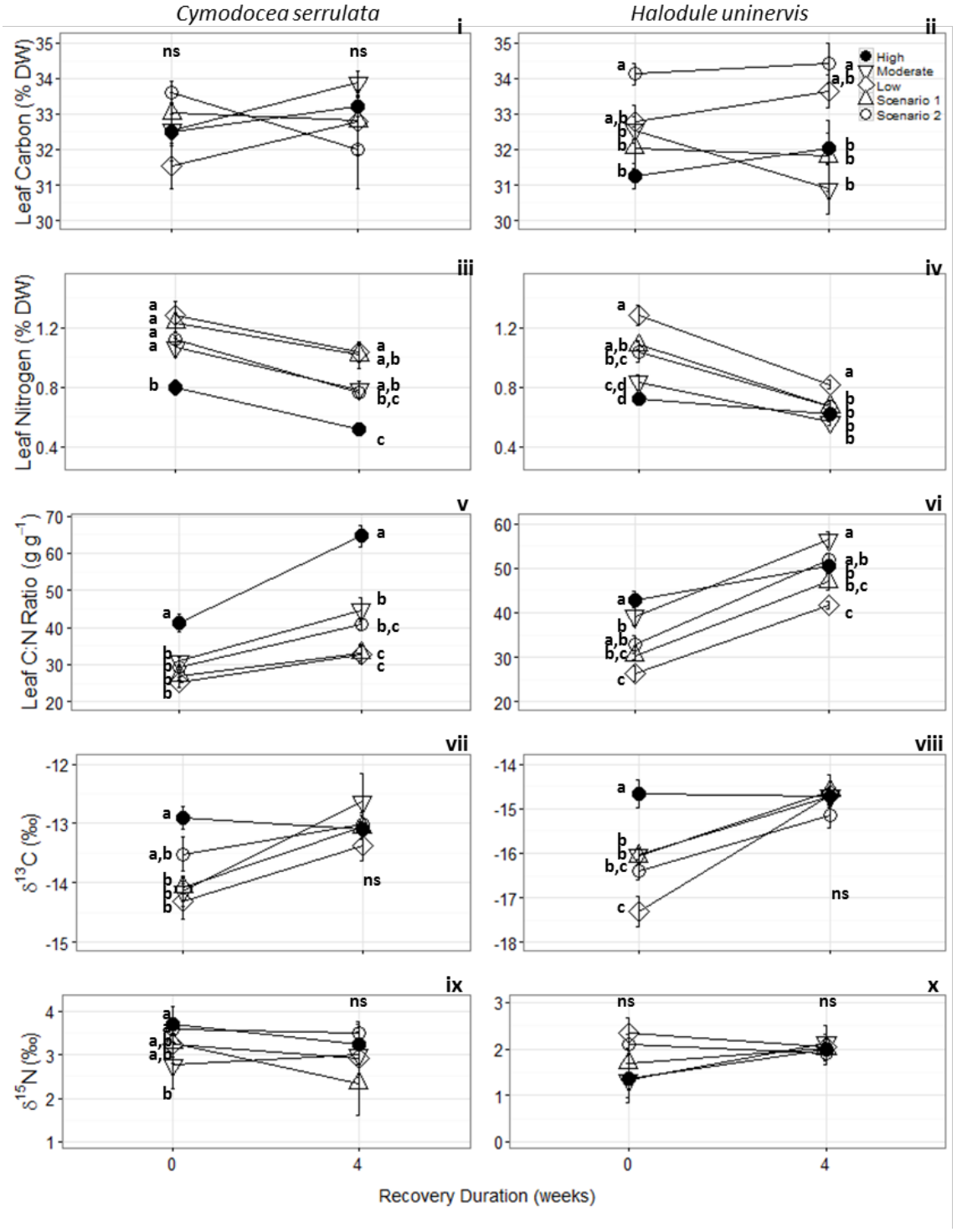


Figure 9. Effect of light reduction on seagrass nutrient characteristics during the recovery period. Leaf carbon, %DW (i–ii), leaf nitrogen, % DW (iii–iv), leaf C:N ratio, g g^{-1}, (v–vi), $\delta^{13}C$ (vii–viii), and $\delta^{15}N$ (ix–x) for *Cymodocea serrulata* (left), *Halodule uninervis* (right) under each of the 5 light treatments at 0 and 4 weeks. Symbols represent means pooled across treatment tanks (n = 8 for 0 and 4 weeks) ±1SE. Different letters denote significant differences.

Table 10: Results of four-way ANOVA testing for the effects of treatment, species, time and treatment nested within tank on leaf C and N concentrations, leaf C:N ratios, $\delta^{13}C$ and $\delta^{15}N$ during the light recovery period. Bold text denotes significant differences.

		Leaf Carbon		**Leaf Nitrogen**		**Leaf C:N ratio**		**$\delta^{13}C$**		**$\delta^{15}N$**	
	df	MS	p	MS	p	MS	p	MS	P	MS	p
Species	1	1.59	0.403	0.535	**<0.001**	734	**<0.001**	122.5	**<0.001**	48.0	**<0.001**
Treatment	4	6.08	0.035	0.695	**<0.001**	1245	**<0.001**	3.57	**<0.001**	0.97	0.439
Time	1	0.61	**0.603**	2.703	**<0.001**	5774	**<0.001**	32.0	**<0.001**	0.02	0.882
Tank (Treatment)	5	2.54	0.448	0.028	0.712	28	0.912	0.711	0.867	1.44	0.403
Species x Treatment	4	11.4	<0.001	0.066	**0.005**	254	**<0.001**	0.64	0.164	3.76	0.454
Species x Time	1	0.59	0.611	0.016	**0.328**	56	0.099	2.39	**0.014**	2.47	0.123
Treatment x Time	4	3.09	**0.249**	0.029	0.143	32	**0.181**	3.14	**<0.001**	2.92	0.582
Species x Treatment x Time	4	4.60	**0.095**	0.411	**0.049**	167	**<0.001**	0.78	0.098	2.15	0.716

3.3.2 Plant scale

Leaf area was also affected by previous light treatment after re-exposure to high light, though species differences were evident (Treatment × Species, MS = 5981, p<0.001, Table 11). For *C. serrulata*, Moderate light showed a greater leaf area than controls (Figure 10 i). Low and Scenario 2 light treatments showed an intermediate response between the Moderate light and High Light treatments. For *H. uninervis*, leaf area was not affected after re-exposure to ambient light (Figure 10 ii). Although there was a significant effect of previous light history on the number of leaves per shoot for *C. serrulata* (Treatment × Species, MS = 0.979, p<0.037, Table 11),there was no significant change from the controls. The Moderate, Scenario 1 and Low light treatments remained lower than controls (High light treatment) after re-exposure to ambient light, but this difference was not significant (Figure 10 iii). Only the Scenario 1 light treatment maintained significantly more leaves per shoot than the low light treatment.

Shoot density was impacted by light treatments for *H. uninervis* only, and no recovery was detected, and no differences were detected at the end of the recovery period for *C. serrulata* (Species × Treatment, MS = 80.7, p = 0.019, Table 12). In fact, the shoot density declined over the recovery period, and this was most obvious in the Low and Moderate light treatments (Figure 11 i, ii). Similar patterns were observed for leaf productivity (Species × Treatment, MS = 20.7, p<0.001, Table 12), but in this variable, differences were detected in *C. serrulata* with no changes over time (Figure 11 iii), and no differences were detected at any time period for *H. uninervis* (Figure 11 iv). Shoot production rate showed some differences between species and over time but there was no significant effect of treatment or interactions with treatment and time (Table 12, Figure 11 v, vi).

Table 11: Results of a four-way nested ANOVA testing for the effects of treatment species, time and treatment nested within tank on leaf morphology during the light recovery period. Bold text denotes significant differences

		Leaf Area		**Leaves per Shoot**	
	df	MS	p	MS	p
Species	1	133314	**<0.001**	0.406	**0.039**
Treatment	4	7170	**<0.001**	0.793	0.081
Time	1	7	0.800	0.821	**0.003**
Tank (Treatment)	5	998	0.923	1.94	**0.001**
Species x Treatment	4	5981	**<0.001**	0.979	**0.037**
Species x Time	1	220	0.150	0.316	0.068
Treatment x Time	4	449	0.376	0.092	0.913
Species x Treatment x Time	4	420	0.411	0.091	0.914

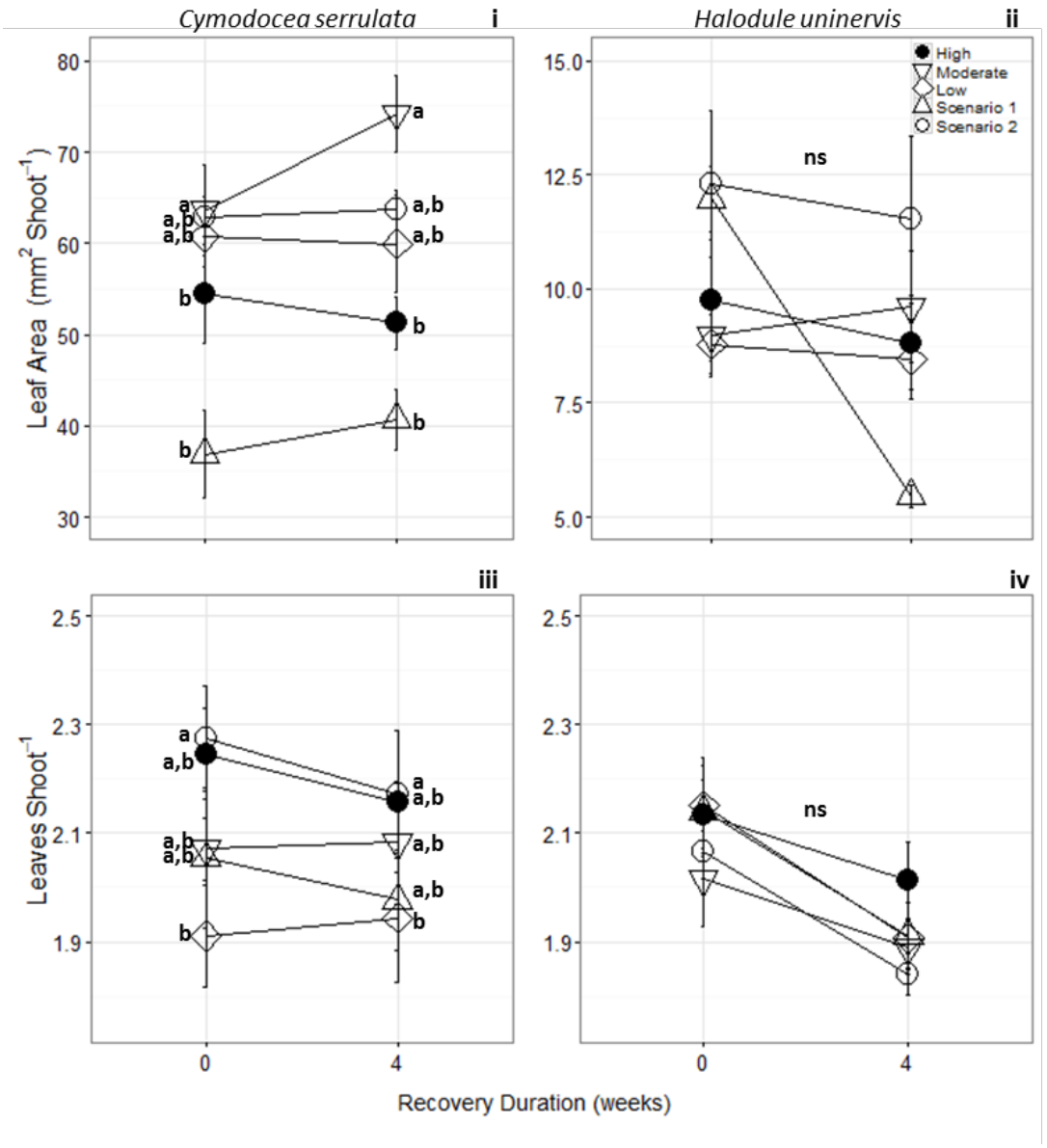


Figure 10. Effect of light reduction on seagrass morphology during the recovery period. Leaf length, mm (i–ii), leaf width, mm (iii–iv), mean leaf area per shoot, cm^2, (v–vi), and leaves per shoot (vii–viii) for *Cymodocea serrulata* (left), *Halodule uninervis* (right) under each of the 5 light treatments at 0 and 4 weeks. Symbols represent means pooled across treatment tanks (n = 8 for 0 and 4 weeks) ± 1SE. Different letters denote significant differences.

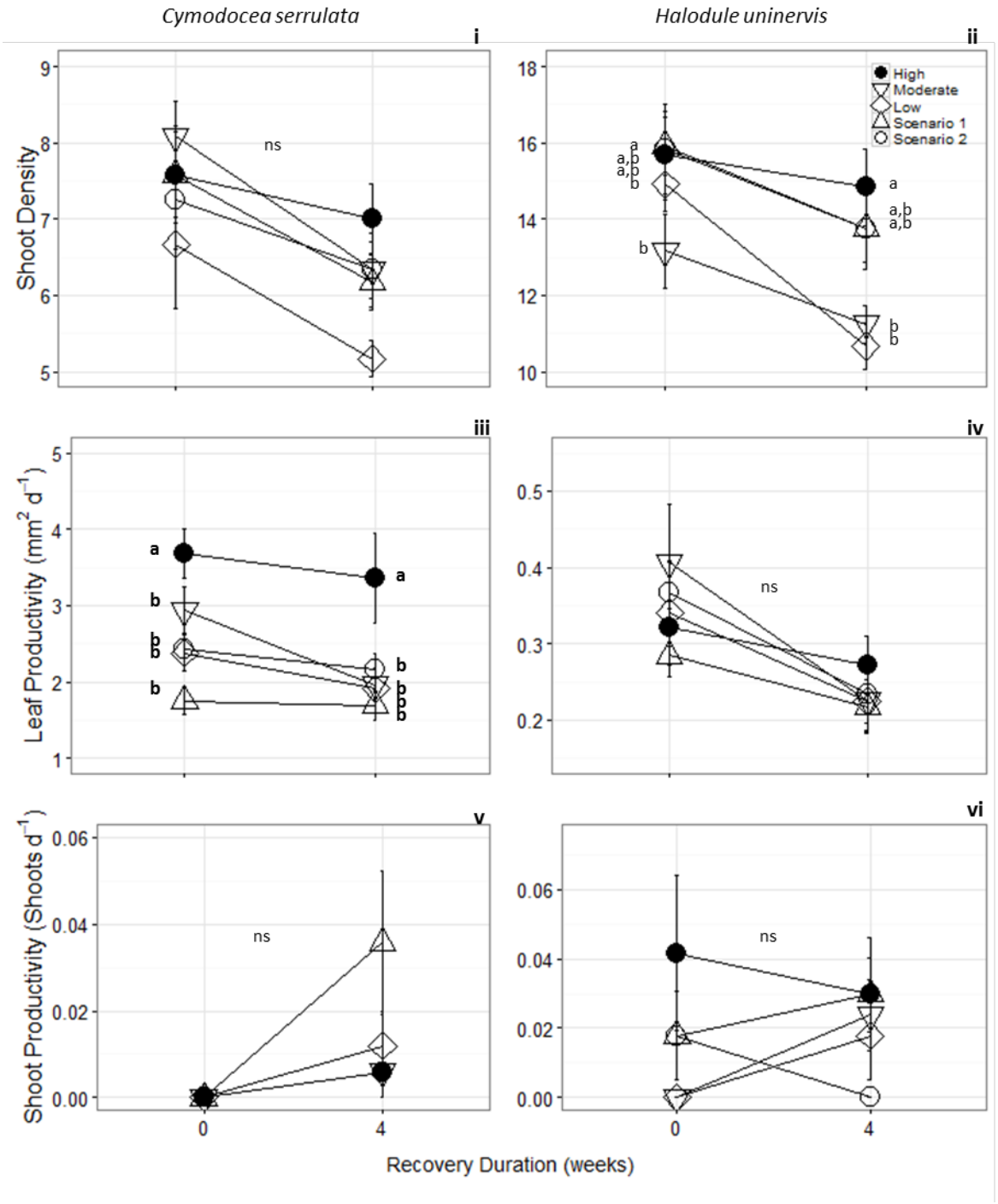


Figure 11. Effect of light reduction on seagrass shoot density and growth during the recovery period. Shoot density (i–ii), leaf productivity, $mm^2\ d^{-1}$ (iii–iv), and shoot production, shoots d^{-1}, (–vi) for *Cymodocea serrulata* (left), *Halodule uninervis* (right) under each of the 5 light treatments at 0 and 4 weeks. Symbols represent means pooled across treatment tanks (n = 8 for 0 and 4 weeks) ± 1SE. Different letters denote significant differences.

Table 12: Results of a four-way nested ANOVA testing for the effects of treatment species, time and treatment nested within tank on plant growth during the light recovery period. Bold text denotes significant differences

		Shoot density		**Leaf Productivity**		**Shoot Productivity**	
	df	MS	p	MS	p	MS	p
Species	1	3075	**<0.001**	274	**<0.001**	0.008	**0.010**
Treatment	4	129	**<0.001**	22.4	**<0.001**	0.010	0.071
Time	2	182	**<0.001**	4.18	**0.005**	0.005	**0.043**
Tank (Treatment)	5	94	0.479	1.57	0.972	0.011	0.094
Species x Treatment	4	80.7	**0.019**	20.7	**<0.001**	0.007	0.191
Species x Time	2	15.5	0.130	1.43	0.097	0.001	0.342
Treatment x Time	8	29.1	0.366	1.81	0.476	0.008	0.149
Species x Treatment x Time	8	13.22	0.741	1.12	0.701	0.004	0.434

Table 13: Results of a four-way nested ANOVA testing for the effects of treatment, species, time and treatment nested within tank on plant biomass; and results of three-way nested ANOVA testing for the effects of treatment and time on total pot biomass during the light recovery period. Bold text denotes significant differences

		Total Biomass		**Above-ground Biomass**		**Below-ground Biomass**		**Total (Pot) Biomass**	
	df	MS	p	MS	p	MS	p	MS	p
Species	1	33.6	**<0.001**	1.38	**<0.001**	21.3	**<0.001**	NA	**NA**
Treatment	4	2.27	**<0.001**	0.111	**<0.001**	1.39	**<0.001**	4.54	**<0.001**
Time	1	1.17	**<0.001**	0.004	0.279	1.04	**<0.001**	2.34	**<0.001**
Tank (Treatment)	5	0.374	0.927	0.017	0.907	0.268	0.914	0.748	0.581
Species x Treatment	4	0.511	0.223	0.041	**0.015**	0.269	0.364	NA	NA
Species x Time	1	0.636	**0.008**	0.015	**0.034**	0.456	**0.007**	NA	NA
Treatment x Time	4	0.176	0.738	0.012	0.469	0.099	0.808	0.353	0.734
Species x Treatment x Time	4	0.275	0.543	0.012	0.459	0.184	0.564	NA	NA

For both species, biomass variables that were affected by light reduction treatments by the end of the impact period (Low and Scenario 1) generally did not recover (within 4 weeks) after re-exposure to ambient light (Table 13, Figure 12). For above-ground biomass, although the differences between treatments were maintained, *C. serrulata* increased its biomass whereas *H. uninervis* decreased. For below-ground biomass, *C. serrulata* showed a greater increase (within 4 weeks) than *H. uninervis* (Species × Time, MS = 0.456, p = 0.007, Table 13). Similarly, total (pot) biomass did not recover, and the differences among treatments were maintained, but despite, this there was an increase in biomass over time across all treatments (Figure 13).

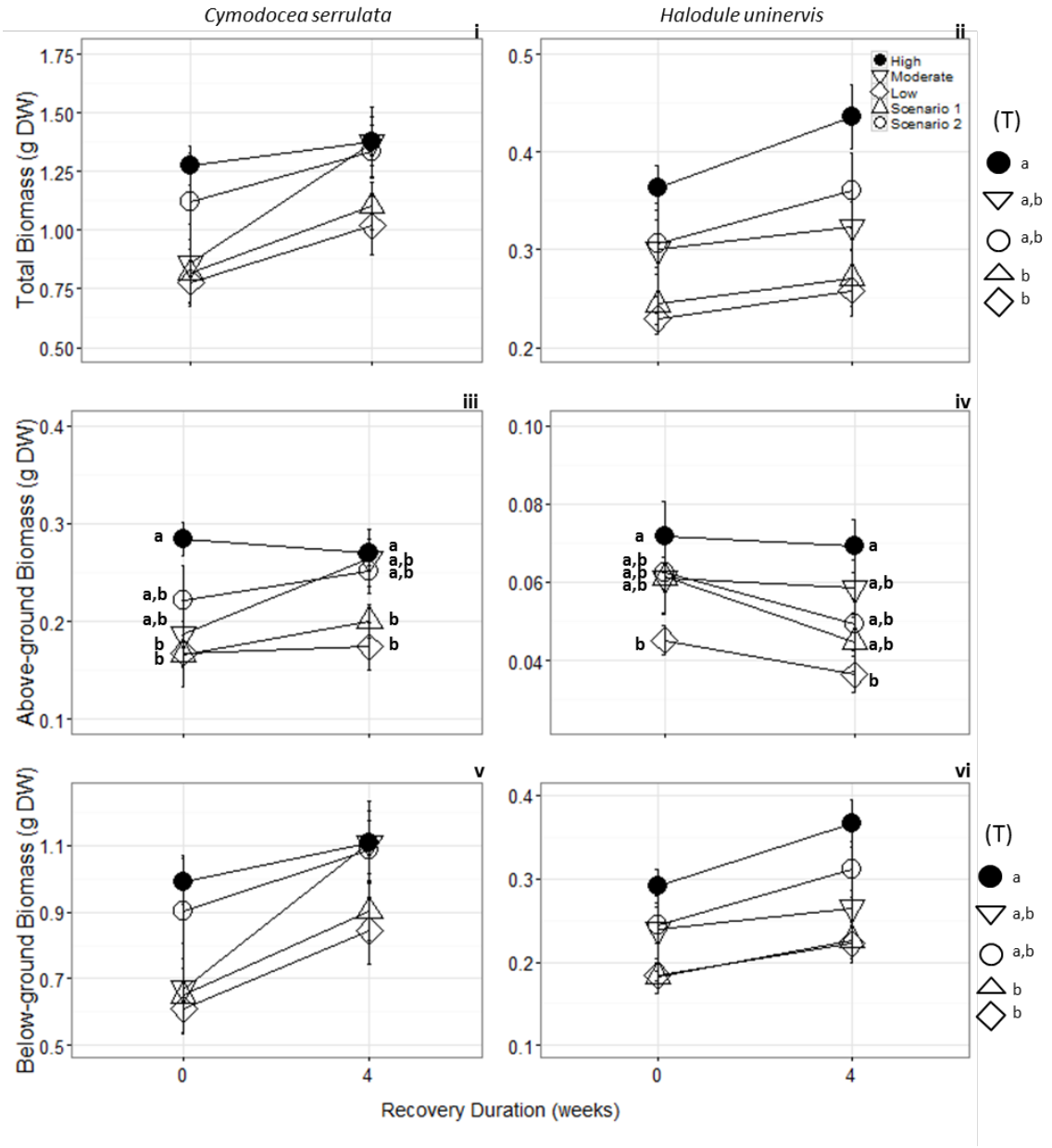


Figure 12. Effect of light reduction on seagrass biomass during the recovery period. Total plant biomass, g DW (i–ii), above-ground biomass, g DW (iii–iv), and below-ground biomass, g DW (v–vi) for *Cymodocea serrulata* (left), *Halodule uninervis* (right) under each of the 5 light treatments at 0 and 4 weeks. Symbols represent means pooled across treatment tanks (n = 8 for 0 and 4 weeks) ± 1 SE. Different letters denote significant differences

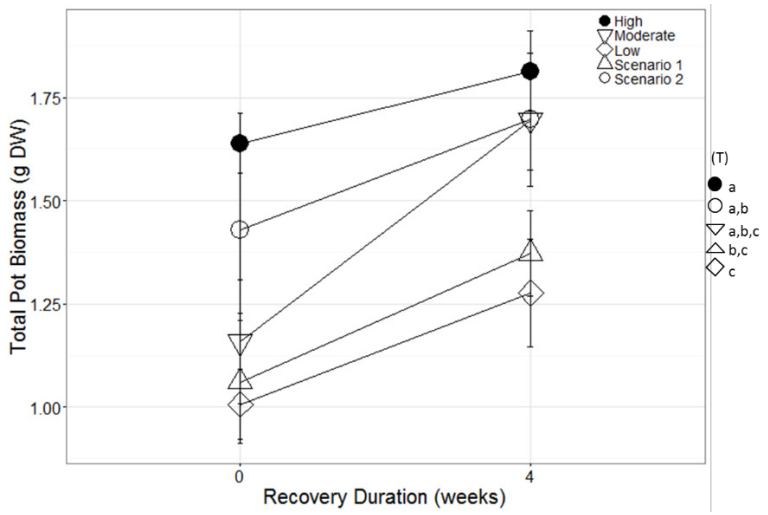


Figure 13. Total (pot) biomass (g DW) for both species (*Cymodocea serrulata* and *Halodule uninervis*) under each of the 5 light treatments at 0 and 4 weeks. Symbols represent means pooled across treatment tanks (n = 8 for 0 and 4 weeks) ± 1 SE. Different letters denote significant differences. (T) represents a Treatment main effect, symbols beneath represent each light treatment.

3.4 Bioindicators

It was not the aim of this experiment to assess bio-indicators of light reduction stress. However, as some treatments were similar to those used in WAMSI DSN Project 5.5.1 (Statton et al. 2016a) we assessed for bio-indicators to confirm consistency. In this assessment we did not consider the frequency of light delivery (i.e. Scenario 1 and 2 treatments), but only the High, Moderate and Low light treatments. We compared the responses of the Moderate and Low treatments to the High (control) treatment.

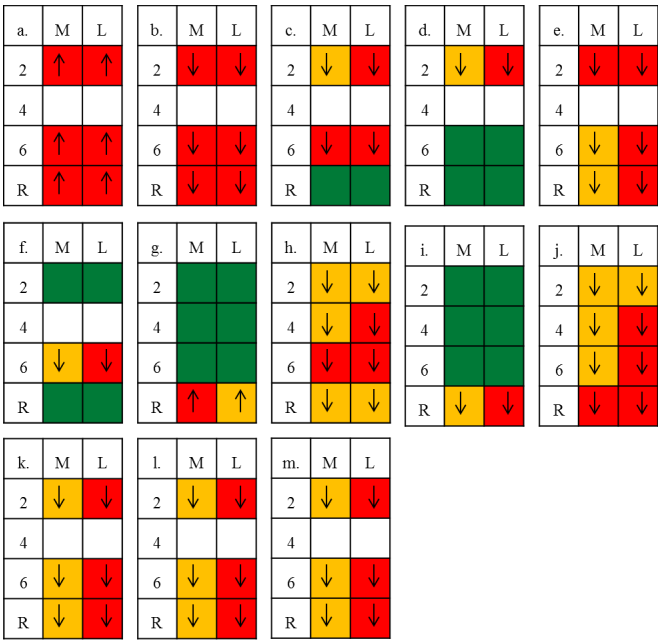
3.4.1 Cymodocea serrulata

The variables that responded most consistently to light reduction for C. serrulata were leaf nitrogen, leaf C:N ratio, leaf carbon isotope ratio, rhizome starch, leaves per shoot, leaf productivity, total biomass, above-ground and below-ground biomass (Figure 14, Table 14). They all reduced with increased magnitude and duration of light reduction. Only leaf nitrogen content, rhizome soluble sugars and above-ground biomass were identified as bio-indicators in both WAMSI DSN Projects 5.5.1 and this study (5.5.3).

3.4.2 Halodule uninervis

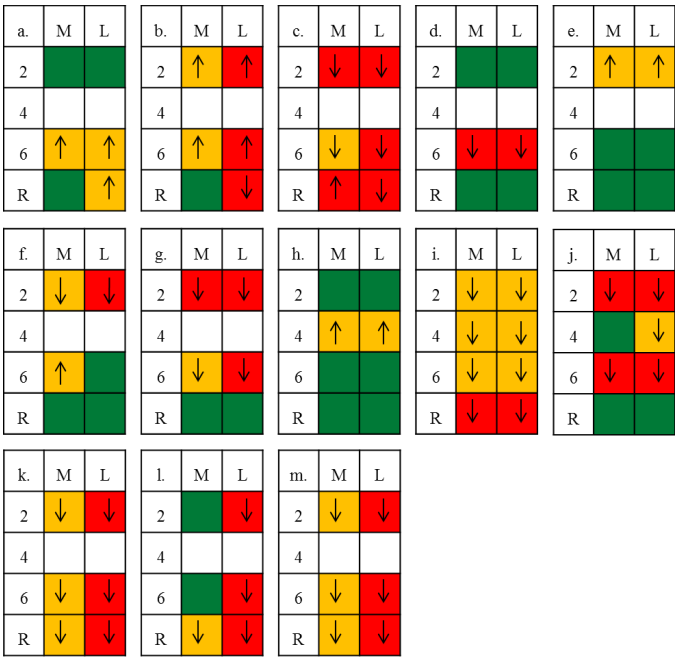
The variables that responded most consistently were carbon content, nitrogen content, carbon isotope ratio, shoot density, total biomass and above and below-ground biomass (Figure 14). But only total biomass and below-ground biomass were consistent with increasing magnitude and duration of light reduction (Table 14). Bio-indicators identified in WAMSI DSN Project 5.5.2 were not observed in WAMSI DSN Project 5.5.1.

Cymodocea serrulata



a. Nitrogen concentration (%DW),
b. Leaf C:N ratio (g g^{-1}),
c. Carbon isotope ratio,
d. Nitrogen isotope ratio,
e. Rhizome soluble carbohydrates (% DW),
f. Rhizome starch (%DW),
g. Mean leaf area per shoot (cm^2),
h. Leaves per shoot,
i. Shoot density,
j. Leaf productivity (cm^2 d^{-1}),
k. Total biomass (g DW),
l. Above-ground biomass (g DW),
m. Below-ground biomass.

Halodule uninervis



a. Carbon concentration (%DW)
b. Nitrogen concentration (%DW)
c. Leaf C:N ratio (g g-1)
d. Carbon isotope ratio
e. Nitrogen isotope ratio
f. Rhizome soluble carbohydrates (%DW)
g. Rhizome starch (% DW)
h. Mean leaf area per shoot (cm2)
i. Shoot density
j. Shoot production (shoots d-1),
k. Total biomass (g DW)
l. Above-ground biomass (g DW)
m. Below-ground biomass.

Figure 14. Summary of direction of responses for all variables that showed a significant effect or interacting effect of light treatments for *Cymodocea serrulata* (Top) and *Halodule uninervis* (Bottom). Within each large box the magnitude of light reduction increases on the top axis from a total daily average light of ~4 mol photons m^{-2} d^{-1} (Moderate light, M) down to 2.2 mol photons m^{-2} d^{-1} (Low light, L). The duration of light reduction increases down the left axis, from 2 to 6 weeks, then a recovery period (R) where plants received ambient light (8.8 mol photons m^{-2} d^{-1}) for 4 weeks. Within each coloured box the arrow indicates the direction of response, either increasing or decreasing relative to the control. A white box indicates no samples were collected at that time period.

Table 14: Potential bio-indicators of light reduction stress in the seagrass *Cymodocea serrulata (Top) and Halodule uninervis (Bottom)*. Bold variables responded most consistently and in the same direction to increasing durations and magnitudes of light reduction.

Variable	Consistent direction of response with duration	Consistent direction of response with magnitude	Identified in WAMSI DSN Project 5.5.1 (Statton et al. 2017a)
Cymodocea serrulata			
Nitrogen concentration	**Y**	**Y**	**Y**
Leaf CN ratio	**Y**	**Y**	
Carbon isotope ratio	**Y**	**Y**	
Nitrogen isotope ratio	N	N	
Rhizome soluble carbohydrates	**Y**	**Y**	**Y**
Rhizome starch	**Y**	**Y**	
Leaf length	N	N	
Leaf width	N	N	
Mean leaf area per shoot	N	N	**Y**
Leaves per shoot	**Y**	**Y**	
Shoot density	N	N	
Leaf productivity	**Y**	**Y**	
Total biomass	**Y**	**Y**	
Above-ground biomass	**Y**	**Y**	**Y**
Below-ground biomass	**Y**	**Y**	
Halodule uninervis			
Carbon concentration	N	N	
Nitrogen concentration	N	N	
Leaf CN ratio	N	N	
Carbon isotope ratio	N	N	
Nitrogen isotope ratio	N	N	
Rhizome soluble carbohydrates	N	N	
Rhizome starch	N	Y	
Mean leaf area per shoot (cm2)	N	N	**Y**
Shoot density	N	N	
Shoot production	N	N	**Y**
Total biomass	**Y**	**Y**	
Above-ground biomass	N	N	**Y**
Below-ground biomass	**Y**	**Y**	

3.5 Thresholds

3.5.1 Total biomass within a pot

For total biomass (within a pot) a light reduction impact can be identified when the median of a treatment falls below the 20th percentile (P_{20}) of the controls (Figure 15). When plants receive 4 mols photons m^{-2} d^{-1}, the median of the treatments dropped below the P_{20} level at 2 and 6 weeks for all treatments except the Scenario 2 treatment at 6 weeks which showed an increase in biomass above the P_{20} (Figure 16). Four weeks after removing light

reduction treatments, this P_{20} trigger value was not breached for any light reduction (pre)treatment, suggesting the biomass had recovered (Figure 15).

Table 15: Analysis of median values of seagrass variables in control samples at each duration of light reduction against the total control data set of all durations pooled. For each variable and species, the values indicate the percentile value of the pooled control data below which the control data for any single duration did not fall. In some cases the values fell below the 50th percentile but not the 20th percentile. On this basis, the 20th percentile was used as the nominal 'threshold value'.

	All species			***Cymodocea***			***Halodule***		
Variable	**2 wks**	**6 wks**	**Recovery**	**2 wks**	**6 wks**	**Recovery**	**2**	**6**	**Recovery**
Rhizome total carbohydrates				20	20	50	20	20	20
Above-ground biomass				20	20	20	20	20	50
Total biomass	20	20	20						

Cymodocea serrulata

For *Cymodocea* above-ground biomass, the lethal threshold for a light reduction impact was triggered within 2 weeks of light reduction for all treatments, whereas at 6 weeks the Scenario 2 light treatment showed an increase in above-ground biomass above the P_{20} trigger (Figure 16). By the end of the recovery period, plants that had received the Low and Scenario 1 light treatments previously, remained impacted, whereas all other treatments were above the P_{20}.

For the sub-lethal indicator, rhizome total carbohydrates, the P_{20} was triggered within 2 weeks of light reduction for all treatments, whereas at 6 weeks, moderate light treatments showed an increase in total rhizome carbohydrates above the P_{20} (Figure 17). During the recovery period, plants in all light treatments did not breach the P_{20} suggesting rhizome carbohydrates recovered after light levels increased to ambient levels.

Halodule uninervis

For *Halodule uninervis* the lethal threshold for a light reduction impact based on above-ground biomass was triggered within 2 weeks of light reduction but only for the Low light treatments, whereas at 6 weeks, the Scenario 1 light treatment decreased below the P_{20} (Figure 16). During the recovery period, plants that had previously received the Low, Scenario 1 and Scenario 2 light treatments were impacted by the previous light history, even Scenario 2, which had not shown an effect during the impact phase.

For the sub-lethal indicator, rhizome total carbohydrates, the P_{20} was triggered within 2 weeks of light reduction for all treatments and this was maintained into 6 weeks (Figure 17). During the recovery period, plants in all light treatments did not breach the P_{20} suggesting rhizome carbohydrates recovered after light levels increased to ambient levels.

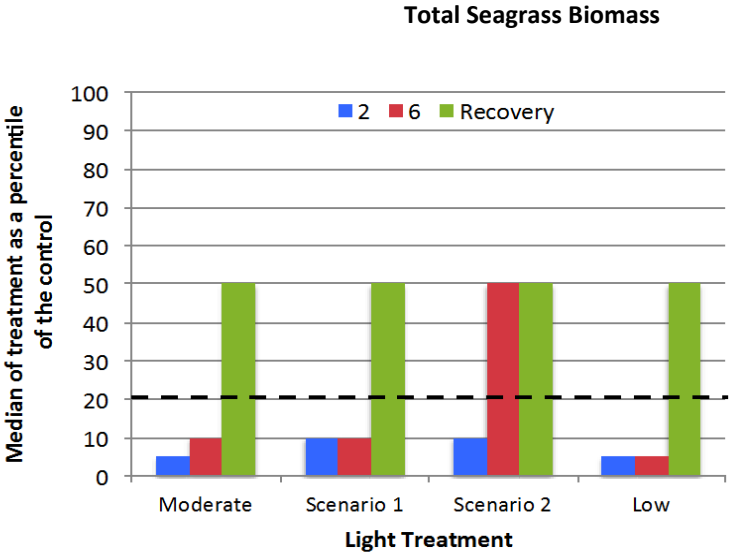


Figure 15: Light reduction threshold values for total seagrass biomass. The figure shows the total pot biomass (all species pooled) at different intensities and durations of light reduction and recovery relative to controls. The dashed line denotes the 20th percentile impact trigger value. For each magnitude of light reduction, the earliest duration at which the P_{20} is breached is the threshold value.

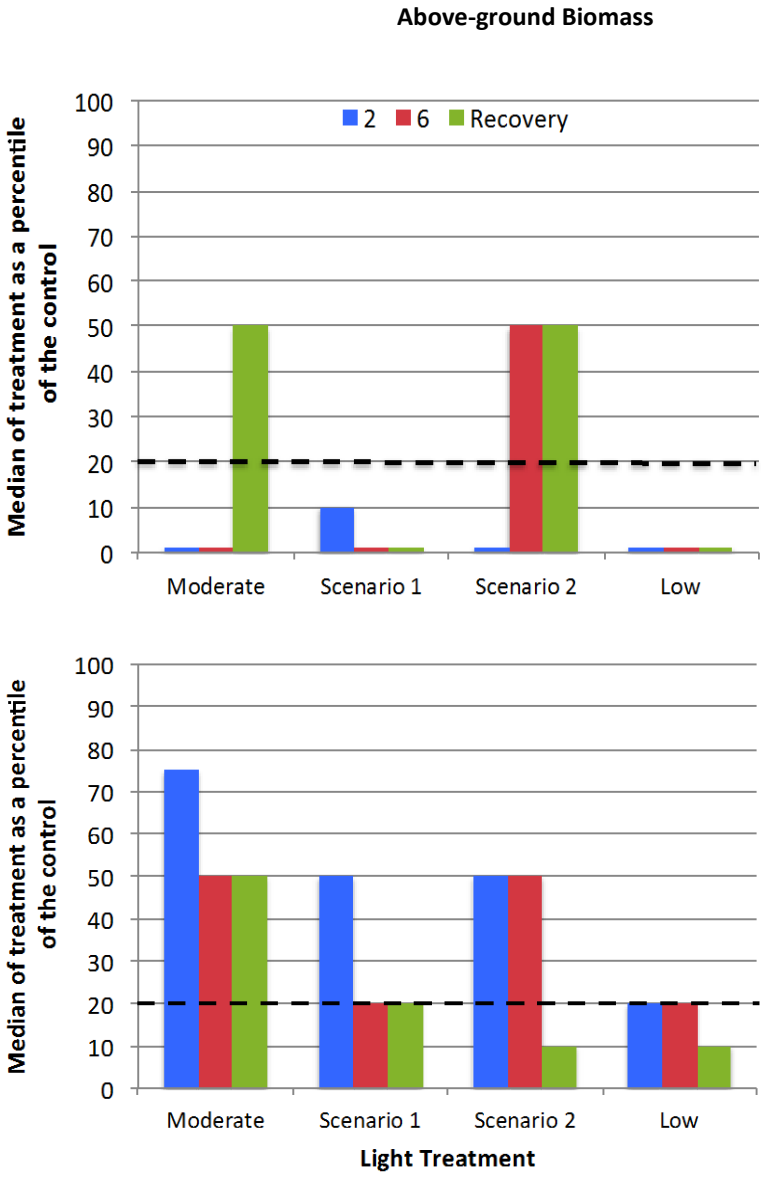


Figure 16 Light reduction threshold values for total above-ground biomass at different light treatments and durations of light reduction and recovery for *Cymodocea serrulata* (top) and *Halodule uninervis* (bottom) relative to the percentiles of the controls. The dashed line denotes the P_{20} impact trigger value. For each light reduction treatment, the earliest duration at which the P_{20} is breached is the threshold value

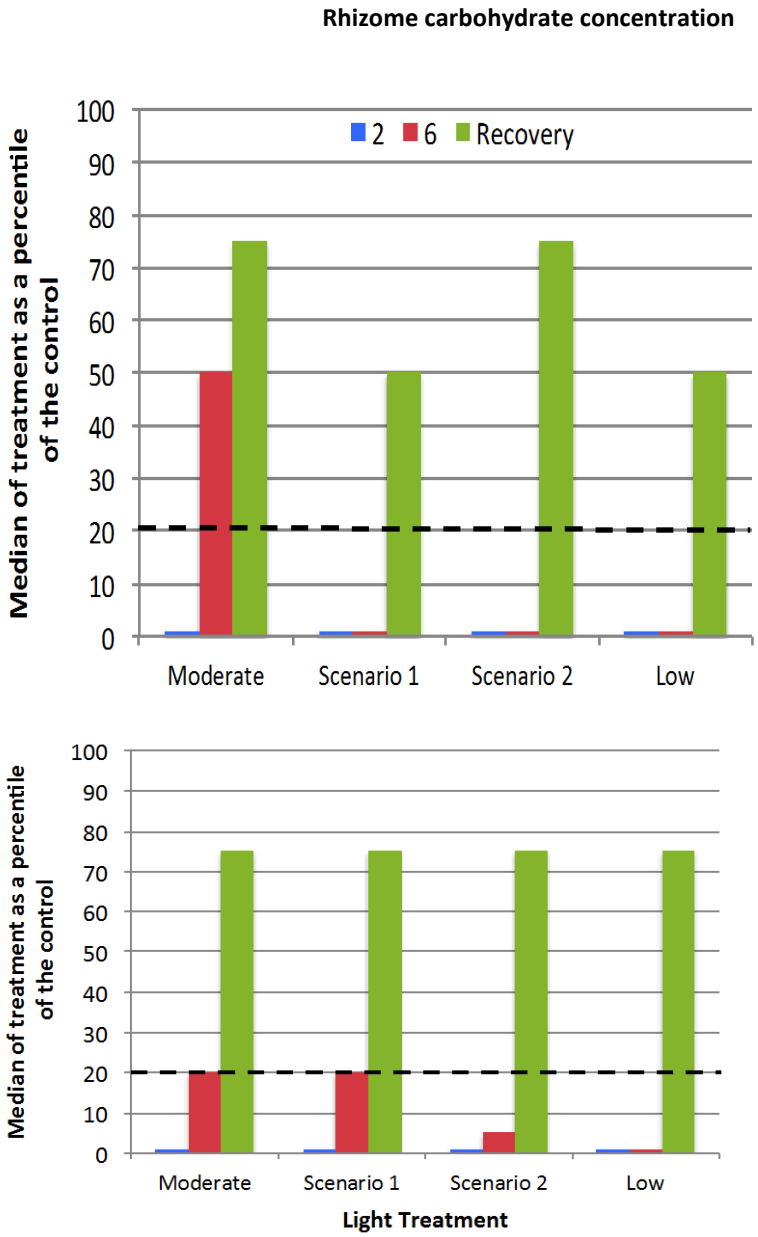


Figure 17. Light reduction threshold values for total rhizome carbohydrate concentration. The figure shows the concentrations at different intensities and durations of light reduction and recovery for *Cymodocea serrulata* (top) and *Halodule uninervis* (bottom) relative to the percentiles of the controls. The dashed line denotes the P_{20} impact trigger value. For each light reduction treatment, the earliest duration at which the P_{20} is breached is the threshold value

4 Discussion

4.1 Key *Findings*

The north west of Western Australia is characterised as a highly dynamic and variable environment, and as such, seagrass species living there should respond swiftly to short-term perturbations in order to survive. Dredging can introduce a pressure to seagrasses in the form of light reduction, but the magnitude of this pressure can change over time due to the activity of the dredge and the movement of the turbid plume. We specifically set out to test whether the variability in the delivery of light affects seagrass health and survival. We did this by keeping the amount of light seagrasses received over a two week period constant, but modified the delivery pattern of this light using pulses of Low, Moderate and High light of different durations. Although seagrasses in the Scenario 2 treatment received slightly more light than those in either the Moderate or Scenario 1 treatments (Table 3), because the Scenario 1 and Moderate treatments had such similar light (i.e. the Scenario 1 treatment was 98 % of the Moderate light treatment), and these differed from each other in terms of impact, and because Moderate treatment did not differ from the Scenario 2 treatment, we are confident that the effect was due to the pattern in the delivery rather that the total light received. When plants received a continuous supply of Low light (<2 mol photons m^{-2} d^{-1}) they were impacted in a manner consistent with previous studies (see WAMSI DSN Project 5.5.1).Similar impacts were also observed when plants received on average 4 mol photons m^{-2} d^{-1} delivered as 10 d of Low light (<2 mol photons m^{-2} d^{-1}) followed by 4 d of High light (~8 mol photons m^{-2} d^{-1}). However, if this same amount of light (4 mol photons m^{-2} d^{-1}) was delivered with less severe periods of Low light (i.e. 5 d of Low light, followed by 7 d of Moderate light and 2 d of High light), the impacts were not as severe, and were similar to a continuous supply of 4 mol photons m^{-2} d^{-1}. This indicates that during the impact phase, short periods of very high light intensity are not sufficient to prevent the impact of lengthy low light periods.

The previous light history also had an impact on both species' ability to recover following removal of the light reduction pressure. Plants that received moderately reduced light levels or short periods of severe light reduction (the Scenario 2 treatment), tended to show an increase in biomass over the 4 week recovery period, suggesting plants were on a trajectory for recovery. Whereas after prolonged, severe light deprivation episodes (i.e. the Low and Scenario 1 treatments), there was no recovery of biomass and shoot density continued to decline for both species. Other studies have made similar observations. The recovery period of *H. ovalis* biomass after severe light reduction was longer (18 d) than the time it took for the biomass to decline (15 d, Longstaff et al. 1999). *Amphibolis griffithii* required 10 months to recover after 3 months of light deprivation (5–18 % of ambient light), but longer durations of light stress (6–9 months at 6–19 % of ambient light) resulted in no recovery after 15–23 months (McMahon et al. 2011). In contrast, *Zostera marina* recovered within 30 d after 60 d of severe light reduction (Backman and Barilotti 1976). For *C. serrulata* and *H. uninervis*, loss of biomass during light deprivation, in conjunction with the ongoing decline in shoot density during recovery periods, would imply that the resilience of both species would be greatly affected by light deprivation events recurring in short succession. Consequently, the light history (frequency and duration of light deprivation events) before light deprivation appear to be just as important as the duration of light deprivation itself in affecting the resilience of seagrasses in dynamic environments that are subject to transient light deprivation events. The findings suggest that the temporal separation (frequency) of pulsed turbidity events is important, and that an over-simplification in characterizing the light environment could have consequences for the health and longevity of a tropical seagrass assemblage.

Shoot density continued to decline in the light deprivation treatments for both species following removal of shading, and could possibly be a consequence of a legacy effect from depleted storage reserves during light deprivation (see Fraser et al. 2014). As a result, plants would need to replenish depleted carbohydrate reserves as well as balance respiratory load, in this instance by reducing the number of shoots, to regain a positive carbon balance. In other light-reduction studies, leaf biomass has continued to decline following removal of shading stress (Malta et al. 2006; McMahon et al. 2011). McMahon et al. (2011) offered another explanation whereby the sudden increase in light following removal of shading may cause photo-oxidative damage to previously dark-

adapted plant tissue. Regardless of the mechanisms, results from these studies suggest that recovery from light deprivation events should not be presumed when light conditions improve, even if plants have shown no morphological response during the shading period (McMahon et al. 2011).

4.2 Bioindicators

This experiment identified 2 response variables which clearly and consistently responded to light reduction for both species and should be relatively robust bio-indicators, as well as several other variables for consideration that showed a response in at least one species and were identified as a robust bio-indicator in the WAMSI DSN Project 5.5.1 report. Combined, these included indicators on plant physiology (rhizome carbohydrate concentration, leaf N), morphology (leaf area), growth/biomass (shoot production, above-ground biomass) and abundance (biomass, pot level for all species combined). Although, abundance did not show a consistent direction of response in the magnitude and duration of light reduction, this is likely because of the observed variation in response of biomass between species.

While the above variables have potential usefulness as bio-indicators, in the following we provide a comparison across the two light experiments (WAMSI DSN Project 5.5.1 and this study) for some of these bio-indicators and other variables that show potential as bio-indicators. In both experiments, and for both species, plants clearly responded by reducing plant biomass (above- and below-ground and total biomass) with light reduction. This is a good indicator of lethal low light stress. However, the timing of response differed between the two experiments. For example, in WAMSI DSN Project 5.5.1, *C. serrulata* showed a decrease in biomass after 9 weeks and at light intensities lower than 2.3 mol photons m^{-2} d^{-1}, whereas in this study it responded within 2 weeks at 4 mol photons m^{-2} d^{-1}. The differences in timing and magnitude of response may be a result of the way light was delivered. In WAMSI DSN Project 5.5.1, plants received a constant intensity of light over a 12 h period. Plants also showed a strong ability to photo-adapt to each light intensity, such that even the lowest light levels were saturating to photosynthesis. On the other hand, in this study we tested the light response under ambient light conditions where plants were responding to the average light received each day, which also fluctuated due to cloud-cover, and over a much shorter day length (<8 h, winter). Consequently, the conditions plants were exposed to in this study better reflected natural conditions and are, therefore, more likely to yield realistic light thresholds. Light thresholds and plant responses in WAMSI DSN Project 5.5.1 may overestimate species resilience.

Rhizome carbohydrates are typically a good and early indicator of sub-lethal light-reduction stress. In WAMSI DSN Project 5.5.1, rhizome carbohydrates showed a response at 3 weeks even under low levels of shading, whereas in this study the temporal sampling resolution was greater and we found that plants responded within 2 weeks. This confirms that rhizome carbohydrate concentration is a good early-warning indicator of low light stress, and considering there was reduction in biomass at 2 weeks, rhizome carbohydrates may respond even earlier.

Starch, rather than soluble carbohydrates, was the most abundant energy storage compound in rhizomes of *H. uninervis* in both experiments. So starch may be a useful bio-indicator for this species. However, under severe light reduction that coincides with anoxia starch utilisation may be inhibited (Longstaff et al. 1999), potentially causing the inconsistency observed in the direction of response over time. It is recommended that use of starch as a bio-indicator requires further validation. However, when there is uncertainty as to the main carbohydrate source utilized by plants during periods of light deprivation, one approach would be to pool both soluble sugars and starch (total carbohydrates) which we've shown, in both the WAMSI DSN Project 5.5.1 and this study, can indicate the plant response regardless of carbohydrate source.

In some variables, there was either a different direction of response than anticipated from the WAMSI DSN Project 5.5.1 or there was no response where one would be expected. This included several growth and morphological variables (shoot production, mean leaf area per shoot, leaves per shoot). For example, shoot

production decreased for both species in the WAMSI DSN Project 5.5.1 but was non-responsive in *C. serrulata* and highly variable in *H. uninervis* in this study, despite reasonable theoretical expectation it should decline (Collier et al. 2012; McMahon et al. 2013). These differences may be related to the differences in the range of light treatments tested across both experiments. In the earlier WAMSI DSN Project 5.5.1, the control light level was about 21 mol photons m^{-2} d^{-1}, whereas in the current study the control was 8.8 mol photons m^{-2} d^{-1}. Consequently, the lack of a clear separation in growth and morphological variables with light reduction treatments in the current study probably reflects the lower magnitude of light reduction relative to the controls compared to WAMSI DSN Project 5.5.1.

4.3 Thresholds

This experiment has demonstrated that it is not only the amount of light that seagrasses receive over a given time that affects their response to light reduction, but it is also the pattern of delivery of that light. In particular, the number of continuous days of low light are important, and should be considered for threshold development. Continuous low light for 10 d was more detrimental to plants than 5 d of continuous low light, even if over a two week period they received the same total amount of light.

Using the ANZECC/ARMCANZ (2000) percentiles-based approach it was possible to calculate the thresholds that incorporate both the magnitude and duration of light reduction (Figure 16–18). Importantly, these thresholds relate to a defined level of impact (i.e. a shift to in the median of the test population to the 20th percentile of the control/unimpacted population) and do not factor in recovery potential. These threshold values can contribute to the development of water quality guidelines for the species of tropical seagrasses studied here, in particular, in relation to short-term water quality management (e.g. dredging). The threshold values and response times are within the range reported in the WAMSI DSN project 5.5.1. For early warning indicators (e.g. rhizome carbohydrates) we suggest species-specific trigger values are appropriate since *C. serrulata* (soluble sugars) and *H. uninervis* (starch) utilised different forms of rhizome carbohydrates as an energy source to cope with light reduction (similar to WAMSI DSN project 5.5.1). In WAMSI DSN Project 5.5.1, the rate at which each form was utilized differed between species (3 weeks for soluble sugars in *C. serrulata* and 6 weeks for starch in *H. uninervis*), but this was not the case in this experiment. However, in this experiment we tested light levels that were on the lower end of the range tested in WAMSI DSN Project 5.5.1, and therefore, we defer to the WAMSI DSN Project 5.5.1 with regards to each species magnitude and timing of response. Trigger values for lethal impacts (biomass) are best viewed at the level of the seagrass assemblage rather than assigned to individual species. We also found that a single light threshold developed for one species could over- or under-estimate the amount of light needed for protection of a mixed seagrass assemblage. In light of this, an adaptable management approach to light thresholds could be more appropriate, considering strong evidence for species differences in light thresholds magnitude and duration (WAMSI DSN Project 5.5.1). Under circumstances where there are mixed species seagrass assemblages, and where these species have vastly different threshold values, to determine appropriate light thresholds, it may also be necessary and more practically feasible to assess the dominance, ecological- and economic-value of each species, along with each species capacity and timeframe to recover if they were to be impacted, when deciding which trigger value to apply.

By monitoring the recovery of plants after 4 weeks re-exposure to ambient light conditions, we found that in the Low light and Scenario 1 treatments, with the longest duration of low light intensity (10 d or more), plants showed no signs of recovery in biomass. Therefore, these plants continued to breach the P_{20} trigger value, suggesting the previous light history was still causing an impact. However, rhizome carbohydrate concentrations had increased during the recovery period such that those plants no longer breached the P_{20}. This suggests that although plants in the Low light and Scenario 1 treatments were potentially on a trajectory for recovery, they required longer than 4 weeks to regain similar biomass to control plants.

The above thresholds indicate when a dredging-related stress at a site is likely to result in a specific magnitude of effect, in this case a shift to a value at or below the 20th percentile of plants in an unimpacted site. While this is consistent with recommendations in the ANZECC/ARCANZ (2000) guidelines it has limited application in the context of the EPA (2016) guidance on assessing impacts of dredging. Following EPA (2016) it is necessary to predict when detectable change to seagrasses will occur as a result of dredging-induced stress,. This can be used to delineate the boundary of the Zone of Moderate Impact/Zone of Influence. To do this, we developed a second set of thresholds, which integrate the findings of this study with those of the earlier WAMSI DSN Project 5.5.1 (Statton et al. 2017a). These thresholds extend on those presented in the earlier WAMSI DSN Projects 5.5.1 (Statton et al. 2017a) because they incorporate consideration of the duration of light reduction, the light intensity and the frequency (or delivery pattern) of that light reduction stress. We present two sets of thresholds:

- 'More conservative' thresholds, which provide the greatest level of confidence that seagrasses will not be negatively affected); and
- 'Less conservative thresholds', which factor in some of the variability in the findings and are thresholds which are likely to result in no significant impact on seagrasses but in which we have less confidence because of the variability in the findings.

The thresholds were derived using a two-step approach:

1. Above ground biomass (AGB) data or total pot plant biomass data from WAMSI DSN Project 5.5.1 (Figs 19 & 20) were used to identify initial effects thresholds. AGB was used as the indicator variable for individual species while total pot plant biomass was used as the indicator for mixed meadow responses. These variables were used as they had been identified as appropriate indicators by Statton et al. (2017a). Within each duration of light reduction treatment (3, 6, 9 and 12 wks) we identified the lowest light intensity that had resulted in AGB not significantly lower than the controls and which was also significantly different to lower light intensities that had produced a significantly negative effect. For example, in Figure 19 at 12 weeks for *C. serrulata* this would be 41% of incident PAR (I_{PAR}) as this was not different to the control but was different to 4% I_{PAR} treatment, which produced negative effects. The 23 and 11% I_{PAR} treatments were not different to the controls but were also not different to the 4% I_{PAR} treatment). For the 'Most conservative' thresholds, we then used that light intensity as the value that needs to be maintained over any given two week averaging period. This reflects the averaging light period applied in WAMSI DSN Project 5.5.3. Where there was an inconsistent effect of decreasing light intensity on AGB (for example, 41% I_{PAR} had no significant negative effect but 60% I_{PAR} did, then we used the highest light level that had produced a significant difference (in this example, 60%), on the basis that this was more conservative (i.e. provided more confidence that seagrasses will be protected. For the 'Less conservative' thresholds we used lowest light level that had not resulted in a statistically significantly lower AGB than the controls, irrespective of whether it was also not different to a lower light level that had produced a statistically significant negative effect.

2. We then applied the findings of WAMSI DSN Project 5.5.3 (this report) regarding the effects of prolonged periods of low light. Our findings indicated that when plants experienced a average of 4 mol photons m^{-2} d^{-1} over a two weeks period, the effect depended on the pattern of light delivery; if the two weeks included a period of 10 days at low light (~2 mol photons m^{-2} d^{-1}) they were negatively affected relative to the controls, but if the low light periods only extended for 5 days then there was no negative effect. We therefore qualified the initial thresholds by specifying the maximum number of low light days that plants could experience within the two weeks averaging period. Taking a conservative approach, the no-effects threshold requires that plants do not experience more than 5 consecutive days of low light within a two week period and the average daily light intensity for that two week period must be equal to or greater than the stated light intensity. In this case, 'low light' is defined as between 2 and 4 mol m^{-2} d^{-1}, since we know the effect

occurred at some point less than 4 but more than 2 mol m^{-2} d^{-1}. The less conservative approach allows plants to experience up to 10 consecutive days of low light, but still meeting the two weekly average light intensity requirement.

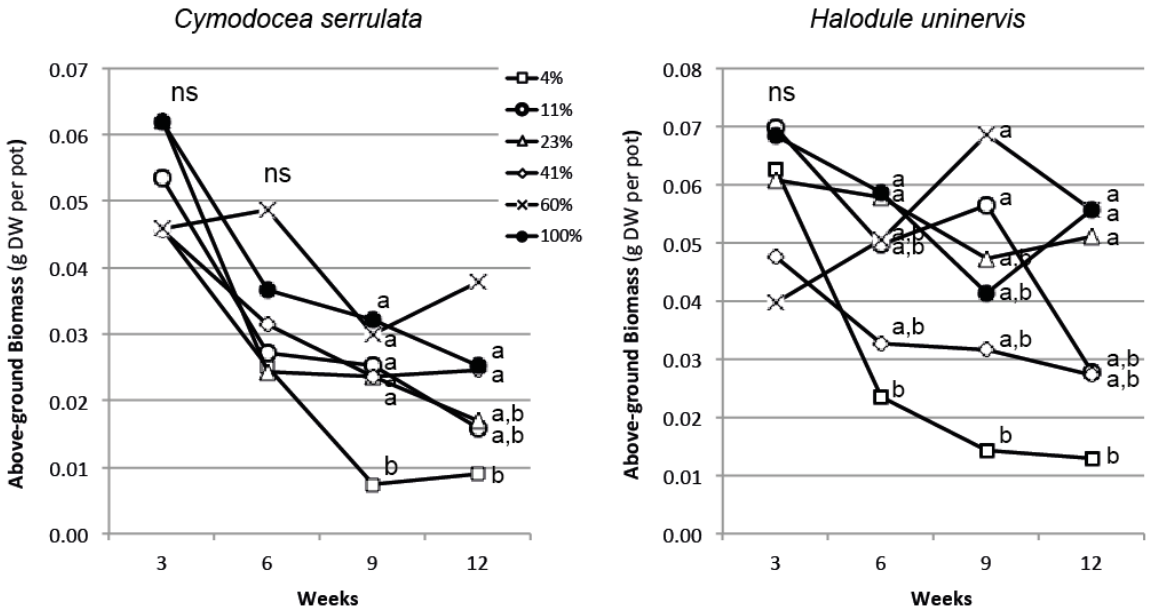


Figure 18: Effect of light reduction treatments on above-ground biomass of *Cymodocea serrulata* and *Halodule uninervis*. Data reproduced from WAMSI DSN Project 5.5.1 report (Statton et al. 2017a). Figures show biomass at 3, 6, 9 and 12 weeks after shading; 100% I_{PAR} (21.6 mol quanta m^{-2} day^{-1}), 60% I_{PAR} (13.1 mol quanta m^{-2} day^{-1}), 41% I_{PAR} (8.9 mol quanta m^{-2} day^{-1}), 23% I_{PAR} (5 mol quanta m^{-2} day^{-1}), 11% I_{PAR} (2.3 mol quanta m^{-2} day^{-1}) and 4% I_{PAR} (0.9 mol quanta m^{-2} day^{-1}). Values are means (n = 8) ± SE. Letters within figure indicate significant differences between treatments for each species and at each time.

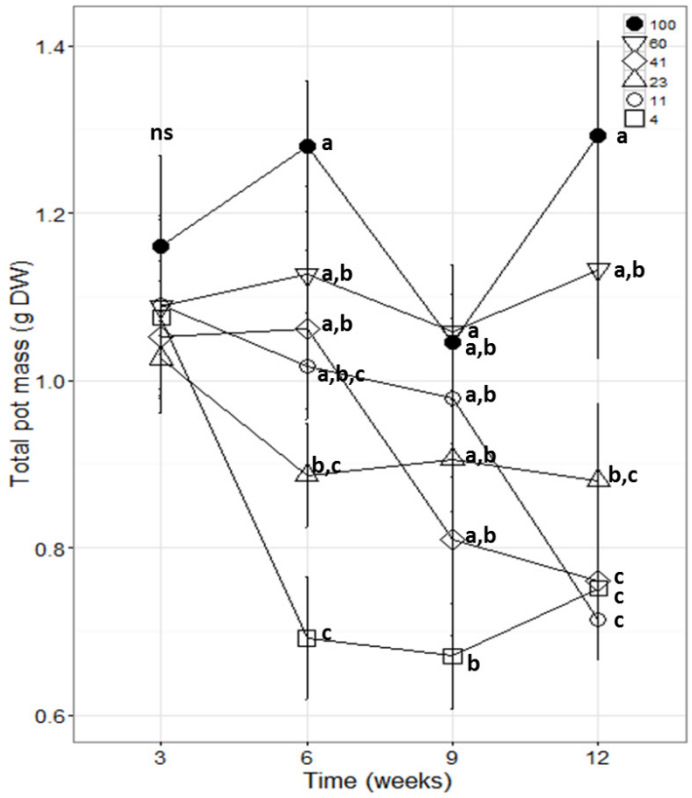


Figure 19: Effect of light reduction on the total biomass of seagrass in mixed-species pots. (From WAMSI DSN Project 5.5.1 report; Statton et al. 2017a). Graph shows total biomass within experimental pot at 3, 6, 9 and 12 weeks after shading; 100% I_{PAR} (21.6 mol quanta m^{-2} day^{-1}), 60% I_{PAR} (13.1 mol quanta m^{-2} day^{-1}), 41% I_{PAR} (8.9 mol quanta m^{-2} day^{-1}), 23% I_{PAR} (5.0 mol quanta m^{-2} day^{-1}), 11% I_{PAR} (2.3 mol quanta m^{-2} day^{-1}) and 4% I_{PAR} (0.9 mol quanta m^{-2} day^{-1}). Values are means (n = 8) ± S.E. Letters indicate significant differences between treatments for each species and at each time.

Based on the above approach, a set of 'effects' criteria are presented (Table 17) for the tropical seagrasses (*Cycmodocea serrulata* and *Halodule uninervis)* and also for what could be termed a generic 'mixed meadow'. These criteria indicate the light conditions that if maintained or exceeded are expected to result in no detectable effects on these species of seagrass. As such they can be used to interrogate the outputs of dredge plume models to identify the transition between the zone of moderate impact (where effects are allowed) and the zone of influence (where no effects are allowed). Importantly, by taking into account the respective levels of confidence in the criteria, it is possible to establish management targets that proponents should aim to meet through adaptive management and also the compliance limits that they must meet to comply with the conditions of approval (see EPA 2016 for further explanation).

The more conservative criteria afford a high level of confidence that if these conditions can be maintained there will be no measurable impact on seagrass (as measured by the relevant biomass parameters). As such they provide a rational basis to establish the location of the 'compliance' line. The less conservative criteria are still reasonably robust and afford a level of confidence that seagrasses will not be measurably affected – albeit with lower confidence than that of the conservative criteria. As such they provide an objective basis to determine the location of the 'management target' line, which in turn represents the most likely best-case scenario for the outer limit of measurable effects on seagrass communities.

Table 17: Recommended impact thresholds for *Cymodocea serrulata* and *Halodule uninervis*. These thresholds are based on the combined findings of this project and WAMSI DSN Project 5.5.1. The Most and Less Conservative thresholds can be applied to predict the outer and inner limits of the Zone of Moderate Impact, respectively. Thresholds are not provided for *Halophila ovalis* as there were no significant difference among treatments prior to the point where the control plants began to show stress, making the responses unreliable.

	Two week averaging period		**Permissible low light periods within 2 wk averaging period**	
	Duration	Mean Light intensity mol photons m^{-2} d^{-1}	Duration	Mean Light Intensity mol photons m^{-2} d^{-1}
Cymodocea serrulata (based on Aboveground biomass)				
Most conservative*	12 wk	8.9	5 d	2 to ≤ 4
	9 wk	2.3	5 d	2 to ≤ 4
Less conservative#	12 wk	2.3	10 d	2 to ≤ 4
Halodule uninervis (based on Aboveground biomass)				
Most conservative*	12 wk	13.1	5 d	2 to ≤ 4
	6 wk	13.1	5 d	2 to ≤ 4
Less conservative#	12 wk	2.3	10 d	2 to ≤ 4
Mixed Meadow (based on total biomass of all species in a multi-species meadow)				
Most conservative*	12 wks	13.1	5 d	2 to ≤ 4
Less conservative#	12 wks	8.9	10 d	2 to ≤ 4
	6 wks	5	10 d	2 to ≤ 4

* *Most conservative reflects higher confidence of no impact to seagrass.*

\# *Where the thresholds were the same for multiple durations, the longer duration is presented as the recommended threshold. For example, for H. uninervis there was no difference in the 'Less conservative' thresholds for 6 weeks and 12 weeks data – for both, the 2-weekly running average was 2.3 mol m^{-2} d^{-1}. In this case, it is recommended that for any given period of 12 successive weeks, this average must be maintained in successive two week periods.*

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