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Determining light stress bio-indicators and thresholds for a tropical multi-species seagrass assemblage

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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: Example of a fragment of *Cymodocea serrulata* used for planting within each plot. (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: Theme Leader Paul Lavery and Node Leader Science - Ross Jones examining experimental tanks used for the light reduction experiment.

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Executive Summary

Deterioration in water clarity by resuspension of dredged sediments is presumed to be a major mechanism by which dredging can impact seagrasses. This report presents findings from a controlled light experiment that aimed to determine the effects of low light stress on the growth of three 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-response relationships, sub-lethal and lethal bio-indicators and thresholds) to impact prediction, monitoring and/or management of dredging programs in northwest Western Australia.

To test the effects of intensity and duration of low light stress on co-occurring tropical seagrass species we established pots containing mixed assemblages of three seagrasses that commonly co-occur in NW WA, *Cymodocea serrulata, Halodule uninervis* and *Halophila ovalis.* Under climate-controlled mesocosm conditions, replicate pots were subjected to a gradient in light availability by shading (21.6, 13.1, 8.9, 5.0, 2.3, and 0.9 mol PAR m^{-2} day^{-1}) to simulate a range of light levels that are predicted to occur close to dredging operations (McMahon et al. 2016) as well as covering a range expected to elicit a mortality response in tropical seagrasses determined from published research (Collier et al. 2011). We ran the experiment for 12 weeks, harvesting and measuring plant condition at 3, 6, 9 and 12 weeks after light shading commenced.

Three 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 light reduction stress, we determined the cause-effect pathway from measurements of 18 response variables;
2. From (1), we identified response variables that changed in a consistent manner with increasing magnitude and duration of light reduction, to determine the most appropriate sub-lethal and lethal bio-indicators for use in monitoring programs; and
3. Using the variables identified in (2), we determined sub-lethal and lethal thresholds of tolerance to intensity and duration of light reduction.

The key findings were:

- Light reduction generally resulted in effects on plant physiology, productivity, morphology and biomass of all three species, though the magnitude of the effects depended on the intensity and duration of light reduction as well as the species of seagrass.
- While increased shading generally resulted in increased effects on all three species, the time-scales of response to light reduction were species specific. *H. uninervis* was more sensitive to low light than *C. serrulata*, possibly due to faster response rate and smaller carbohydrate storage reserves that act to buffer plants against low light stress. Results for *H. ovalis* were inconclusive due to difficulty in sustaining this species in culture for longer than 12 weeks, and we suggest a shorter acclimation period is well-justified for this species.
- We identified four robust bio-indicators that are appropriate for immediate incorporation into monitoring programs to identify impacts of reduced light availability on tropical seagrass assemblages: for individual species, ETR_{MAX}, total rhizome carbohydrate concentration, above-ground biomass; and for all species combined, total biomass. We identified three other variables that should be given consideration (leaf nitrogen, new shoot production/recruitment, leaf area). These seven variables were selected based on their sensitivity to stress (e.g. rapid response), applicability to at least 2 of the 3 species studied, and consistency in direction of response with increasing intensity and duration of light reductions.
- The recommended bio-indicators span different scales of plant response such that we can detect very early sub-lethal responses to light reduction (photosynthetic rate or ETR_{MAX} and rhizome

carbohydrates) as well as those that respond over longer time-scales and reflect lethal changes (e.g. above-ground biomass and total species abundance [biomass]).

- To develop thresholds for sub-lethal and lethal effects of light reduction, a variety of approaches were trialled. The most suitable approach followed the ANZECC and ARMCANZ guideline recommendations for biological indicators (ANZECC and ARMCANZ 2000), where impact conditions are compared to background or reference conditions. In our case, experimental treatments were compared to experimental controls. We used a relatively simple approach for determining a significant level of change in the bio-indicator. The 20th percentile of the controls was determined to be the most appropriate trigger value for all variables across all species. Using this approach, we could calculate the sub-lethal and lethal light thresholds that incorporate both the magnitude and duration of light reductions (Table ES 1). 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 compliance (e.g. dredging).

Table ES 1: Threshold values based on magnitude and duration of light reductions for Sub-lethal (ETR_{MAX} and Rhizome total carbohydrates) and Lethal (above-ground biomass for individual species or total biomass of all species pooled) indicators of light reduction stress in three NW Western Australian seagrass species.

Species	ETR_{MAX}		Total Rhizome		Above Ground		Total	
	PPFD		PPFD		PPFD		PPFD	
	mol m^{-2} d^{-1}	Wks	mol m^{-2} d^{-1}	Wks	mol m^{-2} d^{-1}	Wks	mol m^{-2} d^{-1}	Wks
Cymodocea serrulata								
	5	3	2.3	3	8.9	12	NA	
			8.9	9				
Halodule uninervis								
	5	3	5	3	0.9	6	NA	
	8.9	6	8.9	9	2.3	12	NA	
	13	12	13	12				
Halophila ovalis								
	0.9	3	NA		0.9	3	NA	
	2.3	9						
All species							5	6
							8.9	9

This work has led to the identification of strong bio-indicators of sub-lethal and lethal stress that would be appropriate for immediate incorporation into monitoring programs. The research has also identified species-specific threshold values for each bio-indicator that incorporate the magnitude and duration of light reduction and which can be applied as management action 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: 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 EAG7 framework.

Pre-development Surveys

Seagrass composition: Our findings suggest 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 (for either reference or impact sites) identify the species composition and relative abundances of all species within seagrass assemblages at survey sites, to improve the predictability of the mixed assemblage response to impacts and aid in monitoring design, bio-indicator choice and threshold development**.

S*urvey methods***: 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 4 robust bio-indicators that are appropriate for immediate incorporation into monitoring programs to identify light reduction impacts on a tropical seagrass assemblage: ETR_{MAX}, total rhizome carbohydrate concentrations, above-ground biomass and total biomass. It is important to note that there are other important considerations that all influence bio-indicator selection for monitoring programs and include the ease of collection, expertise to analyse and interpret results, and cost-effectiveness, which all need to align with management goals (see review by McMahon et al. 2013).

Timing of surveys and threshold development: Understanding the natural variability of field sites is integral to the development of impact thresholds when using the ANZECC & ARMCANZ (2000) guidelines to water quality monitoring and impact prediction. **We recommend that pre-development surveys should undertake bio-indicator assessment over time (inter- and intra-annual)** to determine the background natural variability of reference and impact sites with respect to the recommended and/or most practical and economically feasible bio-indicators. Failing to do this could increase the likelihood of reducing the precision of threshold predictions for these bio-indicators and lead to over- or under-prediction of impacts, which could result in a loss of species diversity and ecological function or an increase in time, effort and dollars to complete a dredging operation.

Natural light history and seagrass condition: Impact prediction will require an understanding of the background light environment at a site as well as the seagrass condition. We have identified a number of bio-indictors and appropriate thresholds for use with those indicators. This study has shown that these are appropriate indicators to use for the species we have studied, and then can be applied in dredging monitoring programs. However, it remains possible that while those indicators are the preferable ones to use, the environmental conditions at any given site may have resulted in plants having different baseline conditions than those used in our study- that is, at a particular site the 'control' condition could be different to that used in our study. For this reason, it would be appropriate to collect data on the background light and seagrass condition at any potential impact or monitoring sites since this would allow application of the percentile approach applied here on a site-specific basis. **We recommend pre-development surveys characterise the background natural light conditions, rhizome carbohydrate reserves and biomass to determine appropriate thresholds to apply in impact prediction**.

Impact Assessment

Appropriate bio-indicators of light stress: We identified four robust bio-indicators of seagrass plants being subjected to light-reduction stress. These indicators span different scales of plant response such that we can detect very early sub-lethal responses to light reduction (photosynthetic rate or ETR_{MAX} and rhizome carbohydrates) as well as those that respond over longer time-scales and reflect lethal changes (e.g. above-ground biomass and total species abundance [biomass]). **These bio-indicators are appropriate for application**

in dredging impact prediction, particularly the lethal indicators which reflect actual loss of seagrass biomass in response to light reduction. The indicators have additional applicability in distinguishing impacts related to light reduction or those related to sediment burial stress, another potential seagrass stressor introduced by dredging. Only one consistent bio-indicator relating to burial stress was determined in the WAMSI Dredging Science Node project 5.5.2 (Statton et al. 2016) and this differs to the four 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.

Which bio-indicators to use in impact prediction and monitoring? Of the four reliable indicators, three (total rhizome carbohydrate concentrations, above-ground biomass and total biomass) are likely to be less variable on short (i.e daily) timescales than photosynthetic characteristics (ETR_{MAX}).

- ETR_{MAX} will be most be useful in situations where short-term changes in seagrass condition need to be monitored or to define the Zone of Influence of a sediment plume over relatively short time periods. However, interpretation of changes in physiological data over weekly or longer time periods may be problematic, given the number of factors that can potentially influence photosynthesis. On the other hand, changes in ETR_{MAX} can be useful in interpreting changes in biomass, helping to confirm the cause-effect pathway of any loss of seagrass.
- Carbohydrate concentrations in the rhizome will be useful in integrating changes in light climate over a longer-period than ETR_{MAX}. Again, this could be applied to determine the boundary of the zone of influence, since change in carbohydrate concentrations with no associated loss of biomass would indicate an 'influence' of light reduction but not to the extent that this has translated into loss of biomass. Coupled with an observed response in ETR_{MAX}, carbohydrate data form an important link in confirming that any observed loss of seagrass at a site could be attributable to light reduction.
- Above-ground biomass and Total Biomass are useful indictors of lethal impacts on seagrass. As such, the threshold relating to these variables can be used in impact prediction to estimate the outer boundary of the Zone of Moderate Impact. It should be noted, however, that it remains unclear whether the seagrasses can recover from the sorts of impacts that we observed at the light thresholds used in this study. Because we cannot be certain that the seagrasses will not recover from stress that results in biomass loss, these 'lethal' thresholds should be considered as 'conservative', that is theya re likely to over-estimate the likelihood of a lethal impact.

It is apparent from the above that understanding the cause of any loss of seagrass requires information on variables that respond earlier in the cause-effect pathway. Therefore, **interpretation of bio-indicator data will be assisted by collecting information on all four variables or, at a minimum, biomass and carbohydrate concentrations**

Species-specific thresholds: The findings indicated that some species are more sensitive to reduced light conditions than others. We have shown that *H. uninervis* is more sensitive to low light than *C. serrulata*, possibly due to the differences in the faster rate of response and smaller size of carbohydrate storage reserves in *H. uninervis* that act to buffer plants against low light stress. Results for *H. ovalis* were inconclusive. Part of the reason these species were used in the experiments was that they can be considered representative of other seagrasses. *C. serrulata* is more representative of persistent seagrasses with larger rhizomes and storage reserves while *H. uninervis* and *H. ovalis* are more representative of colonising species with faster growth rates and smaller storage reserves. Given the species-dependent differences in response, **we recommend against extrapolating the findings for these two species to other species, or using one species as a surrogate for many (i.e. a mixed assemblage)**, 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.

Defining Impact Zones: The framework for managing dredging (EPA, 2016) requires spatially-explicit zones of different levels of impact to be predicted: the Zones of Influence, Moderate Impact and High Impact. The threshold values provided in this study define light conditions that will cause a measurable effect on seagrass, be this lethal or sub-lethal, and so can assist in determining whether a particular location is likely to be within or outside one the management zones defined in EPA (2016). Modelling of the light climate can indicate the magnitude and duration of light reduction at any given location. Where these light characteristics fail to trigger any of the bio-indicator thresholds the site could be classified as being within the Zone of Influence. Where the ETR_{MAX} and/or Total Rhizome Carbohydrate concentration thresholds are triggered without triggering the biomass threshold, the site could be classified as being within the Zone of Moderate Impact, and where the biomass threshold is triggered the site could be classified as being within the Zone of High Impact (Table 13) .

For example, for *Halodule uninervis* sub-lethal impacts were first observed when PPFD fell below 13 mol m^{-2} d^{-1} for 9 weeks (63 days) or below 8.9 mol m^{-2} d^{-1} for 6 weeks (42 days) or below 5 mol m^{-2} d^{-1} for 3 weeks (21 days). Therefore, sites where light is predicted to not fall below any these combinations of light intensity and durations would be within the ZoI. However, because we only sampled plants at 3 week intervals, we cannot be sure whether the onset of sub-lethal effects occurred exactly at the sampling period when it was first observed or immediately after the preceeding sampling period (i.e. almost 3 weeks earlier). Therefore, the most conservative approach to estimating the outer limit of the ZoI would be to use the duration prior that at which the effect was first observed. Using this approach, for *H. uninervi*s, the ZoI could be defined by those locations experiencing more than

Halodule uninervis	13 mol m^{-2} d^{-1} for 9 weeks (63 days)	(least conservative; i.e. less certainty)
	13 mol m^{-2} d^{-1} for 6 weeks (42 days)	(more conservative; ie. greater certainty); or
	8.9 mol m^{-2} d^{-1} for 6 weeks (42 days)	(least conservative)
	8.9 mol m^{-2} d^{-1} for 3 weeks (21 days)	(more conservative); or
	5 mol m^{-2} d^{-1} for 3 weeks (21 days)	(least conservative); or
	5 mol m^{-2} d^{-1} for <3 weeks (1 day)	(more conservative).

The thresholds can be applied in a similar manner for the other species.

On the other hand, lethal effects (biomass loss) was first observed when light fell to 8.9 mol m^{-2} d^{-1} light reduction for 3 weeks and at 2.3 mol m^{-2} d^{-1} light reduction for 12 weeks. Sites with light values above these would therefore be expected to show sub-lethal impacts but not necessarily lethal (biomass loss) impacts. Following the approach above, the ZoMI could be defined by locations where light was greater than:

Halodule uninervis	8.9 mol m^{-2} d^{-1} for 3 weeks (21 days)	(least conservative; i.e. less certainty)
	8.9 mol m^{-2} d^{-1} for <3 weeks (1 day)	(more conservative; ie. greater certainty); or
	2.3 mol m^{-2} d^{-1} for 12 weeks (84 days)	(least conservative)
	2.3 mol m^{-2} d^{-1} for 9 weeks (63 days)	(more conservative).

Again, the thresholds can be applied in a similar manner for the other species.

Loss of ecological function: Our study point to possible consequences of sub-lethal and lethal pressures for the ecological function of seagrass meadows:

1. **Low-light stress could drive a change in seagrass diversity**. Our findings suggest that *Halodule uninervis*, with its rapid growth rates, is able to adjust quickly to low light stress and is likely to cope with dredging-induced low-light pressures. That is, *H. uninervis* above-ground biomass was only impacted after 12 weeks and at 2.3 mol PAR m^{-2} s^{-1} or lower. *Cymodocea serrulata* on the other

hand, is representative of slower growing species that cannot respond as quickly and are likely to be impacted at slightly higher light levels, earlier. If sub-lethal burial occurs during dredging, which is very likely, we might expect to see a shift in species assemblage away from slow-growing species such as *C. serrulata* to species like *H. uninervis* that can cope with increased levels of disturbance as a result of their superior rate of response;

2. **Low light stress may have implications for the forage value of seagrasses**, especially *H. uninervis*, which is an import food source for dugongs. For *H. uninervis*, starch concentrations accounted for up to 18% of rhizome dry weight, but were significantly lower in 5 mol PAR m^{-2} s^{-1}or lower light treatments after 3 – 6 weeks. This suggests that the forage value of *H. uninervis* may be reduced during periods of low light while the plant is responding to sub-lethal light pressures.
3. **Low-light stress could drive over-grazing of seagrass leaves,** all species leaves are important food source for herbivorous fish and green turtles. For all species, leaf N concentration increased significantly with sub-lethal low light stress. The forage value of seagrass leaves for herbivorous fish generally increases with an increase in leaf N (Goecker et al. 2005). This suggests that the forage value may be increased during periods of low light stress which could result in a feedback loop that causes over-grazing.

Post-Approval

Compliance monitoring and dredging management programs: The seagrass indicators identified in this study are appropriate for application in compliance monitoring programs and in monitoring undertaken to guide the timing of monitoring. Of the four reliable indicators, total rhizome carbohydrate concentrations, above-ground biomass and total biomass are likely to be the least variable on short (i.e. daily) timescales and may prove easier to implement than photosynthetic characteristics which could be highly variable and require careful interpretation. The advice provided in Section 2.2 is also relevant here.

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.

Halophila ovalis *bio-indicators and thresholds of tolerance*: Many of the results for *H. ovalis* were inconclusive due to difficulty in sustaining this species in culture for longer than 12 weeks. The plant appeared to grow exceptionally well for the first 12 weeks in culture (6 weeks acclimation plus 6 weeks under experimental treatments) and it appears that it does not require the length of acclimation to laboratory conditions that the other species do. Additional work is required to produce a rigorous data set for this species, and this could be accomplished in mesocosm facilities using shorter acclimation and experimental periods.

Determining the recovery potential of each species after light reduction: In this study we did not monitor the recovery potential of seagrasses after removal of light stress. Therefore we are unable to determine which of the combinations of light intensity and duration that resulted in an impact a species could recover from. This is important since our thresholds identify impact only and there is uncertainty whether species could recover after such impacts. For example, despite no observable effects on shoot density for *C. serrulata* at the end of the light reduction phase, there can be subsequent declines even after the stress has been removed (McMahon et al. 2011; see also WAMSI subproject 5.5.3 (Statton et al. 2017). Consequently, it is imperative to monitor plant responses once the stress is removed to ascertain if there have been any ongoing impacts. As explained in the previous section, this also means that while we can use the thresholds developed here to estimate the boundary between the Zone of Influence and the Zone of Moderate Impact, we are currently unable to estimate the boundary between the Zones of Moderate and High Impact. While we anticipate the biological indicators of impact to also be useful as bio-indicators of recovery, this has not been rigorously tested for these tropical

species. The lack of definitive thresholds and clear indicators of recovery/non-recovery supports the argument for taking a conservative approach in applying these thresholds.

Transferability to in-situ conditions: Our experiments were conducted in mesocosm facilities, which allowed control of a number of factors that would normally be highly variable in field conditions. In this case, the experimental light levels that were used ranged from 0.9 to 21.6 and bracketed the range of daily light intensities that have been recorded at sites around commercial dredging operations in NW WA (McMahon et al. 2017a). The threshold values that are derived from the research and which are based on changes in biomass, cover a number of combinations of light intensity and duration conditions, ranging from 0.9 µmol m^{-2} d^{-1} for 3 weeks (*H. ovalis*) to 8.9 µmol m^{-2} d^{-1} for 12 weeks (*C. serrulata*). Based on data from the Gorgon Dredging Project near Barrow Island for 2009-10 presented by McMahon et al (2017a), the reference monitoring sites and the Zone of Influence sites rarely experienced light intensities as low as 0.9 µmol m^{-2} d^{-1} and not for 3 consecutive weeks. On the other hand, some of these sites did regularly experience light intensities of less than 8.9 µmol m^{-2} d^{-1} and, in some cases, for period of up to 12 weeks. That analysis by McMahon et al. (2017a) also showed that mean light intensity was reduced during periods of dredging and, therefore, it is likely that the above thresholds would be exceeded more frequently during periods of dredging.

In real dredging conditions, and even in field conditions without dredging, the daily light delivered to seagrasses will fluctuate in time and space. The transferability of the threshold values developed here to *in situ* conditions is likely to depend on how each species responds to light reduction within a backdrop of fluctuating light conditions. This remains a knowledge gap for the majority of seagrass species including those examined in this experiment, and is being explored in WAMSI subproject 5.5.3 (Statton et al. 2017). Furthermore, this study examined the impact of light reduction stress, in isolation. However, in order to incorporate these variables into a monitoring context, consideration needs to be given to a suite of other environmental factors, such as the added stress of sedimentation from turbid plumes. It is likely that impacts observed in this study would be more severe with the added stress of sedimentation, and as a consequence the impacts we observed here may occur over shorter timescales when light reduction and sediment deposition are occurring concurrently.

1 Introduction

Deterioration in water clarity associated with dredging for industrialized ports can have profound impacts on seagrass distribution, health and abundance, often resulting in the absence or near absence of seagrasses (Short & Wyllie-Echeverria 1996, Erftemeijer & Lewis 2006, Orth et al. 2006). Concerns about intensive use of coastal areas for commercial activities and deteriorating water clarity have become central issues in marine policy decision-making, and as a result much research in recent years has focused on trying to minimize light reduction to seagrass habitats during commercial dredging operations (Erftemeijer & Lewis 2006). This focus has, in part, stemmed from an increasing awareness among coastal industry and regulatory bodies of the ecological and economic value of seagrass beds, reflected in many parts of the world in tighter control measures including appropriate impact assessments, strict water quality regulations, and proper enforcement and monitoring, and mitigation strategies (Erftemeijer & Lewis 2006). In many cases, loss of seagrass is avoidable if early detection and immediate remediation processes are in place.

In Western Australia (WA), dredging is a critical component of most major marine infrastructure developments, with large developments occurring throughout north-western WA. As part of the process of gaining approvals, most large-scale dredging proposals are subject to strict control measures to prevent or minimize adverse impact on adjacent seagrass meadows (EPA 2016). However, for most tropical seagrass species in the northwest of Western Australia, there is almost no knowledge on light thresholds from which predictions and management of dredging impacts can be based (McMahon et al. 2015). This has led to uncertainty in the prediction of impacts and the appropriateness of proposed management frameworks.

To predict seagrass response to changing light conditions, it is important to consider how seagrasses respond to changing light availability. A crucial factor in the long-term survival and growth of plants to altered light conditions is the balance between photosynthetic carbon fixation and carbon consumption during respiration (Hemminga & Duarte 2000). Optimisation of growth and prolonged survival under reduced light conditions can be achieved through a number of physiological and morphological adjustments. However, under severe or prolonged light reductions, modifying physiology and morphology may not be enough to maintain the carbon balance of the plant and meadow-scale losses can result (Lee & Dunton 1997; Longstaff et al. 1999). Seagrasses can thus display a diversity of responses to reduced light availability (Ralph et al. 2007; McMahon et al. 2013), and those that are fundamental response mechanisms are likely to be appropriate indicators to predict or reflect changes in seagrass condition.

Recent models have described in detail the diversity of responses to reduced light availability, ranging from physiological and plant-scale (sub-lethal) changes, through to longer term meadow-scale (lethal) losses (Ralph et al. 2007; Waycott et al. 2005; McMahon et al. 2013). The timescales at which these indicators respond can range from seconds to months for physiological and morphological changes, and weeks to months for meadow-scale impacts (Figure 1). To accurately predict thresholds of tolerance to episodes of severe light reduction, indicators must respond in a predictable manner and indicate timescales and levels of pressure. The thresholds of tolerance, from sub-lethal (early warning) through to lethal (plant loss) are not yet quantified for most species of seagrass and the appropriateness of the potential indicators of stress (McMahon et al. 2013) remain, largely, untested for most species.

In subtropical and tropical systems, seagrass meadows tend to form multi-species communities. North west Western Australia (NWWA) is within the Indo-West Pacific bioregion, which is arguably the most seagrass species-rich region globally ((24 species total, Short et al. (2007)), and contains 11 species across 5-6 genera). Three of these seagrass species (*Cymodocea serrulata, Halodule uninervis* and *Halophila ovalis)* have been identified as being widespread and commonly occurring across 9 sub-regions within north-western WA. Furthermore, in most instances, these species co-occur, forming multi-specific meadows, though the relative abundance of each species may vary throughout the year or at longer-timescales (see WAMSI DSN Project 5.3; Vanderklift et al. 2017). Although thresholds of tolerance to low light stress are unclear for many seagrass species, species-specific differences are evident. Resilience to low light stress is size-dependent, with large

species more resilient than small species (Longstaff et al. 1999; Collier et al. 2009; Lavery et al. 2009; Collier et al. 2012). Large species tend to have an enhanced capacity to store carbohydrates in belowground storage organs (Czerny & Dunton, 1995; Longstaff et al. 1999), moderating the effects of light limitation. Such small differences in the way species in a community respond to low light stress may lead to abrupt changes in species composition. A better understanding of this will improve predictions of changing species abundance as a result of light limitation.

For many sub-tropical and tropical seagrasses, there is limited knowledge on light reduction effects that can be applied in an impact prediction or management framework. Examples of tropical literature are almost exclusively single species studies (e.g. Collier et al. 2012). While single-species studies have progressed our understanding of light thresholds for some tropical species, the strict focus on individual species outcomes means these insights may not be representative of how a mixed species seagrass community may respond. The existing approaches aimed at understanding mixed-seagrass species responses to perturbations (e.g. dredging-related light reduction) are based on the temperate zone seagrass species model (single species). The advantage of using mixed-species experiments is that this more closely represents the *in-situ* assemblages. Clearly, to improve our understanding of impact predictions on tropical seagrasses, a multi-species approach is necessary.

The objective of this study was to determine the response to different magnitudes and durations of light reduction for a mixed tropical seagrass assemblage. Then from this, identify appropriate bio-indicators to use for monitoring, and develop thresholds of tolerance to the intensity and duration of light reduction. Here, we examine the light stress–response pathway for three common and co-occurring north-west Australian tropical seagrasses, C. serrulata, H. uninervis and H. ovalis grown sympatrically in large aquaria. We hypothesize that there will be a threshold intensity and duration of light reduction indicating sub-lethal and lethal effects, and that the magnitude and time required for the response to occur will depend upon species.

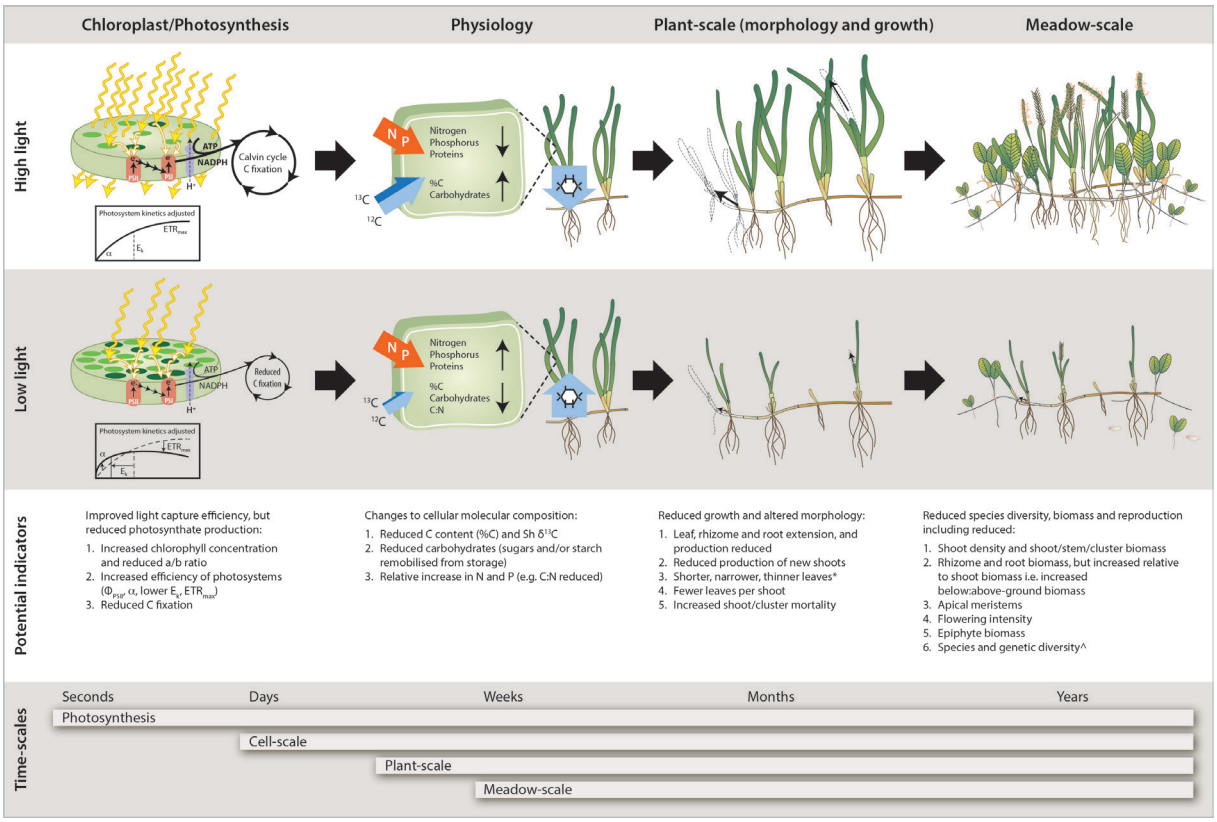


Figure 1: Conceptual diagram of the current understanding of the seagrass response pathway under low light conditions separated into photosynthetic, plant-scale (growth and morphology) and meadow-scale variables. The timescales at which the responses to light reduction generally occur are indicated at the base of the diagram. Potential bio-indicators are highlighted (from McMahon et al. 2013). The author retains the right to use this published figure. The original is available at doi:10.1016/j.ecolind.2013.01.030.

2 Materials and Methods

2.1 Plant Collection

On the 17th of April 2014, the seagrasses *Cymodocea serrulata* (R. Brown) Ascherson and Magnus, *Halodule uninervis* (Forsskål) Ascherson (1882), and *Halophila ovalis* R. Brown J.D. Hooker (1858) were collected from Useless Loop (26° 6'59.12"S, 113°24'39.81"E), Shark Bay, WA six and a half weeks prior to the beginning of the experiment. All species were collected by excavating fragments, herein referred to as ramets. Ramets were then placed in aerated and insulated containers filled with seawater for transport to Fremantle, Perth, Western Australia (1000 km or 12 h travel time). At the Fremantle facility, ramets were prepared for planting, which consisted of identifying ramets with at least one intact apical shoot and with at least 3 and up to 6 mature shoots. When a ramet had more than 6 mature shoots, additional shoots were removed using a sharp cutter, and if the apical shoot was damaged or missing, the ramet was discarded. On 20th April 2014, ramets were planted into circular pots (0.25 m diameter × 0.25 m deep). Because of the size differences (biomass and internode distance) between each species (*C. serrulata* >*H. ovalis* >*H. uninervis*) the number of ramets planted of each species in each pot was based on plant biomass (wet weight). Subsequently, we planted one ramet of *C. serrulata*, two ramets of *H. ovalis* and three ramets of *H. uninervis*.

2.2 Experimental tests

Experiments were conducted in large 12 × 1800 L rectangular plastic, fibreglass reinforced tanks. Each with their own illumination source provided by 2 × 720 W High Intensity Discharge (HID) lamps mounted 0.9 m above the water surface. Each HID lamp contained one metal halide globe (blue spectrum) and one high pressure sodium

globe (red spectrum). This light source created a highly homogenous field of irradiance across the cross section of each aquarium (~500 µmol quanta m^{-2} s^{-1}). Each 1800 L aquarium was a closed, recirculating system, with seawater recirculating from a 600 L reservoir beneath each aquarium. Natural seawater from a nearby unpolluted area was used to fill each mesocosm system, with one quarter exchanged 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 aquarium, incoming seawater was spread through a diffuser (T-bar) in order to create a homogenous movement of water. Each aquarium system had independent temperature control from a heater/chiller unit, which allowed water temperature to be controlled within ±0.5°C from the digital setting. Seawater quality was controlled through continuous chemical and mechanical filtration. Salinity levels were monitored daily and adjusted via addition of, deionized freshwater.

2.3 Experimental design and setup

Each experimental tank was assigned a light treatment as per the experimental design in Table 1 and 25 pots were added to each tank. Each pot was a single Easi-lift® grow-bags (UV stable; 0.28 m Diameter x 0.20 m Height). Pots contained marine sediments (20 kg) from a dredge material placement (land reclamation) site stockpile (Cockburn Sound, Perth, WA) with added organic matter (0.5% sediment dry weight or 10 g per pot) in the form of dried and ground (<2 mm) *Posidonia* seagrass leaves (Statton et al. 2013; Fraser et al. 2016). Organic matter was added to the pot sediments and mixed homogenously to provide a natural, nutrient supplement that can sustain seagrass growth (see Statton et al. 2013) without causing nuisance algal blooms, for example, when slow-release inorganic nutrients are used (Statton et al 2014). Pots were then added to seawater-filled tanks for 1 week to allow sediments to settle. Prior to applying the experimental treatments, the plants were acclimated for 47 d, at a temperature of 27°C, salinity of 37–38 ppt, and an irradiance of ~500 µmol quanta m^{-2} s^{-1} measured 5 cm from the sediment surface in the pot (LiCor LI 1400 datalogger with a LI-192 underwater quantum sensor) on a 12h:12h light:dark cycle (i.e. 21.6 mol photons m^{-2} day^{-1}). Plants were deemed acclimated to our aquarium conditions when we observed each species had produced at least one new shoot in the majority of pots. After the acclimation period, six light shading treatments were applied on 6th June 2014 by placing neutral density shade screens (100% monofilament, Jaylon Industries Pty Ltd) over the aquaria: the screens were supported by the rim of the aquaria and tensioned so as not to touch the water surface (to prevent any influence of algal attachment on light availability). Light treatments were pre-determined to cover a wide range of light availabilities, with greater resolution at lower light intensities, and to reflect light intensities reported near dredging operations (McMahon et al. 2015). Our shading levels consisted of controls or 100% Incident Photosynthetically Active Radiation, I_{PAR} (21.6 mol PAR m^{-2} day^{-1}), 60% I_{PAR} (13.0. mol PAR m^{-2} day^{-1}), 41% I_{PAR} (8.9 mol PAR m^{-2} day^{-1}), 23% I_{PAR} (5.0 mol PAR m^{-2} day^{-1}), 11% I_{PAR} (2.3 mol PAR m^{-2} day^{-1}) and 4% I_{PAR} (0.9 mol PAR m^{-2} day^{-1}). Two replicate aquaria were randomly assigned to each light treatment (Table 1).

Table 1: Model of experimental design. Italicized number indicates number of replicate pots per treatment (% Incident PAR (I_{PAR})), tank and time. Each replicate pot contains all three seagrass species (3 levels).

Light treatment (mol m^{-2} d^{-1}; 6 levels)		21.6		13.0		8.9		5.0		2.3		0.9	
Tank (2 levels)		1	2	1	2	1	2	1	2	1	2	1	2
Monitoring Time (weeks, 4 levels)	3	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*
	6	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*
	9	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*
	12	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*

Physiological through to population level indicator measurements of seagrass status were tested throughout the experimental period (Table 2). Plant harvesting was conducted at four times, at 3 (27th June), 6 (18th July), 9 (7th Aug) and 12 (27th Aug) weeks after applying light treatments. At each harvest time, four pots (containing all species) were removed from each tank, and all the plants harvested from each pot (Table 1). 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 2: Summary of indicators tested for each species, light intensity and duration

Level	Indicator Grouping	Indicator	Replication
Physiological (sub-lethal)	Photophysiology (PAM fluorometry)	Maximum Electron Transport Rate (ETR_{MAX})	6
		Photochemical efficiency ($\propto$)	6
		Half-saturating irradiance (E_k)	6
		Rhizome soluble carbohydrates	3
		Rhizome starch	3
Plant-scale (state change)	Physiology (Carbohydrates, Nutrients)	Leaf Carbon and Nitrogen concentration	3
		Leaf Carbon and Nitrogen isotopes	3
	Growth/Biomass	Shoot density	4
		Shoot production rate	4
		Total biomass	4
		Above-ground biomass	4
		Below-ground biomass	4
	Morphology	Number of leaves per shoot	4
		Leaf area	4
Meadow-scale (pot level)	Abundance	Total biomass	4

2.4 Physiological indicators

2.4.1 Photo-physiology

Photosynthetic characteristics were measured using a pulse-amplitude modulated fluorometer (Diving-PAM fluorometer, Walz GmbH, Effeltrich, Germany). This technique provides estimates of the maximum photosynthetic yield of photosystem II (PS II maximum quantum yield, dark-adapted yield or F_v/F_m), effective photosynthetic yield of photosystem II (PS II effective quantum yield, light-adapted yield or F_v'/F_m') (Genty et al. 1989) as well as the maximum rate at which electrons are transported through PS II (Electron Transport Rate, ETT) and used for photochemistry (ETR_{MAX}), the efficiency of electron transport ($\propto$), and the half-saturating irradiance (E_k). ETR_{MAX}, $\propto$ and E_k are determined from rapid light curves (RLCs), which measure the effective quantum yield as a function of irradiance using the pre-installed software routine, where photosynthetic yield was measured through nine pre-determined steps of increasing light intensity (0, 11, 26, 77, 115, 206, 317, 443 and 555 µmol quantam^{-2} s^{-1} on average) with a fiber optic cable. The fiber optic probe was placed 3 mm away from the leaf using a spacer and used for all measurements to ensure identical readings for each replicate. RLCs were performed on one fully expanded leaf from a mature shoot for each species contained within a pot and replicated on six randomly assigned pots for each replicate treatment (two tanks per treatment) on each sampling occasion (3, 6, 9 and 12 weeks) prior to plant harvesting. However, problems with PAM fluorometer at 6 weeks meant that we were unable to utilize this time period.

To measure the photosynthetic rate of seagrasses using a Diving-PAM, it is first necessary to determine how much of the light reaching the leaves is absorbed and used in photosynthesis. The absorbance factors (AF) of each seagrass species are then used to determine the ETR and subsequently the photosynthetic rate. To measure AF, we measured light transmitted through one seagrass leaf (replicated five times) using the quantum sensor on the PAM and compared this to ambient light (light intensity for each treatment). AF was calculated as:

$$AF = (incident_{PAR} - transmitted_{PAR})/incident_{PAR} \quad \text{Equation 1}$$

Because absorbance is likely to change with different light availability (i.e. change with changing leaf chlorophyll content as a response to light availability) we measured leaf absorbance for each species in each light treatment at each of the three time periods instead of the Diving-PAM's default value of 0.84.

The RLCs were analyzed by non-linear regression to obtain estimates of the maximum relative electron transfer rate (ETR_{MAX}) and the sub-saturation irradiance (irradiance level at which photosynthesis starts to become saturated, E_k). To do so, the quantum yield was multiplied by the light intensity increments to convert quantum yield into a measure of relative electron transfer rate (ETR) (Ralph & Gademann 2005). These non-regressions were performed in R using the 'nls' routine (R Development Core Team, 2008) by fitting the following photosynthesis/irradiance equation to the data:

$$ETR = ETR_{max}\left(1 - e^{-(\propto \mathrm{E_d}/\, ETR_{max})}\right)e^{-(\beta \mathrm{E_d}/ETR_{max})} \qquad \text{Equation 2}$$

Where:

ETR_{MAX} is the maximum ETR rate;

E_d is the PAR light intensity (400-700 nm)

$\propto$ is the initial slope of the curve (representing photosynthetic efficiency);

β is the final slope of the curve (representing photoinhibition through damage to the PSII)

$$Ek = (ETR_{max} | \propto) \qquad \text{Equation 3}$$

Where:

ETR_{MAX} is the maximum ETR rate;

$\propto$ is the initial slope of the curve (representing photosynthetic efficiency).

2.4.2 Rhizome Carbohydrates

Storage carbohydrates in seagrass rhizomes for each species and light treatment were assessed at 3, 6, 9, and 12 weeks after light treatments were installed. Rhizome material was snap oven-dried and ground (ball-mill grinder). Soluble sugars and starch were then extracted from rhizomes using 80% (v/v) ethanol (Quarmby & Allen 1989). Soluble sugars and starch were analysed by colorimetric determination (420 nm) with an amylase pre-digest to convert the starch to glucose (Yemm & Willis 1954; Abal et al. 1994), and calculated as a percentage of dry weight.

2.4.3 Leaf nutrient and isotopic analysis

Nutrient and stable isotope analysis in seagrass leaves for each species and light treatment were assessed at 3, 6, 9, and 12 weeks after light treatments were installed. Seagrass leaves were dried and ground to a fine powder using a steel ball-mill grinder, ready for nutrient and stable isotope analysis. Carbon (C) and Nitrogen (N) concentrations, and $\delta^{13}C$ and $\delta^{15}N$ isotope signatures 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, $\delta^{15}N$ 5.71‰, 41.51% C, $\delta^{13}C$, 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 $\delta^{13}C$, and 0.3 ‰ for $\delta^{15}N$. Elemental contents of seagrass leaf samples were calculated as a percentage of dry weight, and elemental ratios were calculated on a mol: mol basis.

2.5 Plant- and meadow-scale indicators

2.5.1 Growth, biomass and morphology

Growth, biomass, and morphology were measured on harvested plants. To assess shoot production rate (shoots ramet^{-1} day^{-1}) one ramet per species was tagged (behind the apical shoot) in each pot 14 d prior to each harvest time then new shoots produced after the apical tag were counted. Laboratory processing of harvested plant samples consisted of initially counting the total number of shoots for each species (shoot density) and number of leaves per mature shoot (leaves shoot^{-1}).

Plant samples were then separated into leaves, roots and rhizome and leaves were placed flat onto a scaled (1 cm^2) waterproof paper-backed processing station and photographed. A scale was imperative for later image analysis. Plant images were analysed using WinRhizo^{TM} 4.0 (Arsenault et al. 1995) to accurately measures lengths, widths, and area of objects within an image. We determined leaf area (cm^2 shoot^{-1}) using this method. Post-photographing, leaves, roots and rhizome material was then placed into foil pouches for drying (60^{o}C for 48 h) and later measures of dry weight (biomass), soluble rhizome carbohydrates and starch, and leaf nutrients and isotopes (physiology).

2.6 Statistical analysis

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; 100, 60, 41, 23, 11, 4% I_{PAR}), Time (fixed factor; 3, 6, 9, and 12 weeks), Species (fixed factor; *Cymodocea serrulata*, *Halodule uninervis* and *Halophila ovalis*), and Tanks (1 and 2 (blocks)) nested within Treatment on photophysiology, plant growth, biomass and morphology variables. Note the 6 weeks measurement was removed from photophysiology analysis due to technical difficulties with the PAM fluorometer (see methodology). Also, the tank term was removed from analyses of absorbance factor since only one tank was assessed per treatment and only 5 replicates were sampled at each time. A three-way nested ANOVA (R package '*agricolae*') was used to test direct and interactive effects of light Treatment (fixed factor; 100, 60, 41, 23, 11, 4% I_{PAR}), Time (fixed factor; 3, 6, 9, and 12 weeks), and Tanks (1 and 2 (blocks)) nested within Treatment on rhizome carbohydrates, leaf nutrient and isotopic data. There was no species term due to insufficient samples of *H. ovalis* for several light treatments and time periods. 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)). Following a significant main effect or interaction, a Tukey's *post hoc* test was used to test for significant differences in treatment means (R package *agricolae*). Prior to analysis, data were tested for normality using the Shapiro-Wilk test and homogeneity of variance using a Bartlett test, and transformed where appropriate.

2.7 Bio-indicators assessment

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 always 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. A variable was considered to show a consistent response) for each light reduction treatment level (i.e. 13.1, 8.9, 5, 2.3, 0.9 mol photons m^{-2} d^{-1}) there was a consistent direction and magnitude of response, for each duration (i.e. 3, 6, 9, 12 weeks) there was a consistent direction and magnitude of response.

2.8 Threshold development

To develop thresholds for sub-lethal and lethal effects of light reduction, a variety of approaches were trialled. As our statistics showed that the effect of light reduction was dependent on the interaction of the duration and magnitude of light reduction, we decided not to pursue a simple single value approach such as a minimum daily light threshold. Instead we investigated approaches to develop thresholds which incorporated the duration and magnitude of light reduction. For each treatment (magnitude × duration) we summed the total amount of light received, as well as the sum of the light deficit relative to controls. Therefore, we had a different value for each intensity (n = 6) by duration (n = 4) treatments. We then plotted this cumulative light received against the average above-ground biomass per treatment, or the cumulative deficit, against the percent loss in biomass relative to controls. In all cases there was no clear relationship and thresholds were not able to be developed.

We attempted to follow the approach of Lavery et al. (2009), where the deficit in the number of hours of saturating irradiance relative to the controls was plotted against the percent loss in biomass relative to controls. However, in many cases, under the different light reduction treatments and durations there were significant differences in E_k (the half-saturating light intensity) the value required to calculate the hours of saturating irradiance. Therefore this approach could not be employed due to the large variation in this variable.

The final approach followed the ANZECC & ARMCANZ (2000) guideline recommendations for bio-indicators where impact sites or monitoring sites are compared to background or reference sites. In our case, experimental treatments were compared to experimental controls using a range of percentile values derived from the control data. This analysis was performed separately for each of the bio-indicators considered potentially useful for monitoring and impact prediction (see previous section). To establish the control percentile values, we pooled the data from all control across the four durations, giving a total of 32 values from which to derive percentile values. The 20th percentile (P_{20}) value of the relevant control data was used as a threshold or trigger value that represents a significant change in the bio-indicator, indicating that the treatments have exceeded the controls. This is consistent with the approach recommended in ANZECC & ARMCANZ (2000) for determining a significant change in physical and chemical stressors that can also vary from location to location. The approach has also been used by the Western Australian Environmental Protection Authority for determining when an unacceptable reduction in seagrass (*Posidonia sinuosa*) shoot density has occurred in Cockburn Sound as a result of poor water quality.

ANZECC & ARMCANZ (2000) also recommends that study-specific data should be used to confirm if this is an appropriate trigger value to apply. In our case, the control data were quite variable over time and so to ensure the variation of our control values at each duration did not breach this trigger value we assessed the median of the controls from each duration (n = 8) against the pooled control percentile values (P_{75}, P_{50}, P_{20}, P_{10}, P_5 and P_1). The lowest percentile value from these analyses was used as the threshold value for that variable, thereby ensuring that the threshold value was above the variation that could reasonably be expected within the controls. Once the threshold value had been established, we compared the median of each experimental treatment (i.e. each light intensity × duration level) against the control data to determine whether it exceeded the lowest threshold value.

3 Results

3.1 Physiological responses

3.1.1 Photophysiology

All species showed a strong photophysiological response to light reduction, with a general decrease in ETR_{MAX}, E_k, and an increase in $\propto$. This adaptive response subsequently influenced the leaf light absorption factor (AF) used to calculate these three photosynthetic parameters. However there were species-specific differences in response to light reduction and these changed over time (Treatment × Species × Time, p = <0.01, Table 3) so, in

all cases, we further analysed the Treatment and Time effects separately for each species.

The ETR_{MAX} for *C. serrulata* generally reduced with light reduction treatments, and those treatments that were significantly different to the controls changed over time. The controls (100% I_{PAR}) remained relatively stable over the 12 week experiment (Figure 2i). ETR_{MAX} was lowest in the 4% I_{PAR} treatment, and was significantly different to the controls and all other treatments in all time periods however the rate increased over time. At 3 weeks, all treatments were significantly different to the controls, but by 9 weeks, the 60% I_{PAR} treatment was not significantly different to the controls and by 12 weeks both the 60% I_{PAR} and 41% I_{PAR} treatments were not different to the controls. For *H. uninervis*, in general, light reduction reduced ETR_{MAX} and the 4–23% I_{PAR} treatments had significantly reduced ETR_{MAX} relative to controls over the 12 week experiment (Figure 2ii). This was not the case for moderate light reduction treatments (60 and 41% I_{PAR}). At 3 weeks, the 60% I_{PAR} treatment had a greater ETR_{MAX} than controls. At 9 and 12 weeks the 41% I_{PAR} treatment showed a significant reduction in ETR_{MAX} for the remainder of the experiment. By 12 weeks, ETR_{MAX} in the 60% I_{PAR} treatment also decreased relative to the controls. For *H. ovalis*, ETR_{MAX} showed a general decline with light reduction but this changed with time. ETR_{MAX} for controls varied over time, increasing between 3 and 9 weeks but remaining relatively stable between 9 and 12 weeks. Subsequently, at 3 weeks only the 23 and 4% I_{PAR} treatments were reduced relative to controls, and 60 and 40% treatments had higher ETR_{MAX} relative to controls. At 9 and 12 weeks ETR_{MAX} was reduced in all light treatments relative to controls (Figure 2iii).

E_k for *C. serrulata* was significantly reduced in all light reduction treatments relative to controls, with the exception of the 11% treatment at 12 weeks (Figure 2iv). For *H. uninervis*, light reduction reduced E_k but not in all treatments. The controls (100% I_{PAR}) remained relatively stable over the 12 week experiment (Fig 2v). Ek was lowest in the 4–23% I_{PAR} treatment, and were significantly lower than controls in all time periods. At 3 weeks, 41–60% treatments were not significantly different from controls, but were significantly reduced by nine weeks for 41% I_{PAR} treatment and 12 weeks for the 60% I_{PAR} treatment. For *H. ovalis* E_k remained stable in controls for the 12 week experiment whereas light reduction treatments changed with time. At 3 weeks E_k was significantly higher in the 60% treatment only, but at 9 and 12 weeks E_k was significantly reduced in all light reduction treatments relative to controls (Figure 2vi).

Photosynthetic efficiency of *C. serrulata* remained relatively stable in the controls over the 12 week experiment while light reduction treatments varied relative to controls over time (Figure 2vii). At 3 weeks, $\propto$ in the 4% I_{PAR} treatment was lower than controls, while 11, 41 and 60% I_{PAR}, were higher than controls. By 9 weeks, α in all light reduction treatments, were significantly higher than controls. At 12 weeks, 23–60% I_{PAR} treatments remained elevated relative to controls whereas 4–11% I_{PAR} treatments showed a significant decrease in $\propto$. For *H. uninervis*, $\propto$ varied initially relative to controls but then became relatively stable over time (Fig 2viii). At 3 weeks, $\propto$ in 4 and 23% I_{PAR} treatments was significantly lower than controls but were elevated for 11 and 60% I_{PAR}. At 9 weeks light reduction had no significant effect on α relative to controls. At 12 weeks, 4–11% treatments showed a significant but small decline. For *H. ovalis,* $\propto$ in controls varied over time, increasing between 3 and 9 weeks but remaining relatively stable between 9 and 12 weeks. Subsequently, at 3 weeks only 23 and 4% I_{PAR} treatments were reduced relative to controls, and 60 and 40% I_{PAR} treatments had higher $\propto$ relative to controls. At 9 weeks, $\propto$ was reduced 4 and 60% I_{PAR} treatments and by 12 weeks in the 4% I_{PAR} treatment only (Figure 2ix).

Leaf light absorption (absorbance factor, AF) showed a general increase with a decrease in light availability but the response depended on both Species and Time (Treatment × Species × Time, MS=0.008, p = 0.011, Table 3). For *C. serrulata*, light absorption was not significantly different from controls at 3 weeks, but at 9 weeks the lowest light treatments, 4–23% I_{PAR}, had significantly higher (40% increase) light absorption than controls (Figure 3i). At 12 weeks, leaf absorbance factors for light intensities below 41% I_{PAR} were significantly higher than controls. Similarly, light absorption for *H. uninervis* at 3 weeks was not significantly different from the control, but by 9 weeks 11 and 23% I_{PAR} light treatments had significantly higher light absorption than controls with all other treatments intermediate between these two groups (Figure 3ii). At 12 weeks, only plants grown at 23% I_{PAR} showed significantly higher light absorption than controls with all other treatments intermediate between these two groups. For *H. ovalis* at 3 weeks, there was a significantly higher light absorption when plants were grown at

41% I_{PAR} (Figure 3iii). At 9 weeks light absorption was significantly higher for 4% I_{PAR}, but at 12 weeks, there was no difference among treatments.

Table 3: Results of four-way nested ANOVA testing for the effects of treatment (integrated daily irradiance mol PAR m^{-2} d^{-1}), species, time and treatment nested within tank on photophysiology. Note absorbance factor was tested in only one tank per treatment therefore no nesting term is applied. Bold text denotes significant differences.

		Maximum Electron Transport Rate (ETR_{MAX})		Photosynthetic Efficiency (α)		Half-Saturating Irradiance (E_k)		Absorbance Factor (AF)	
	df	MS	p	MS	p	MS	p	MS	p
Species	2	112	**<0.001**	0.064	**<0.001**	531	**<0.001**	0.166	**<0.001**
Treatment	5	2025	**<0.001**	0.454	**<0.001**	13380	**<0.001**	0.260	**<0.001**
Time	2	92.7	**<0.001**	0.207	**<0.001**	3919	**<0.001**	0.604	**<0.001**
Tank (Treatment)	6	4.9	0.841	0.004	0.956	121	0.590	NA	NA
Species × Treatment	10	63.6	**<0.001**	0.075	**<0.001**	1088	**<0.001**	0.019	**0.012**
Species × Time	4	112	**<0.001**	0.381	**<0.001**	6124	**<0.001**	0.025	**0.005**
Treatment × Time	10	128	**<0.001**	0.228	**<0.001**	813	**<0.001**	0.016	**0.001**
Species × Treatment × Time	20	65	**<0.001**	0.093	**<0.001**	811	**<0.001**	0.008	**0.011**

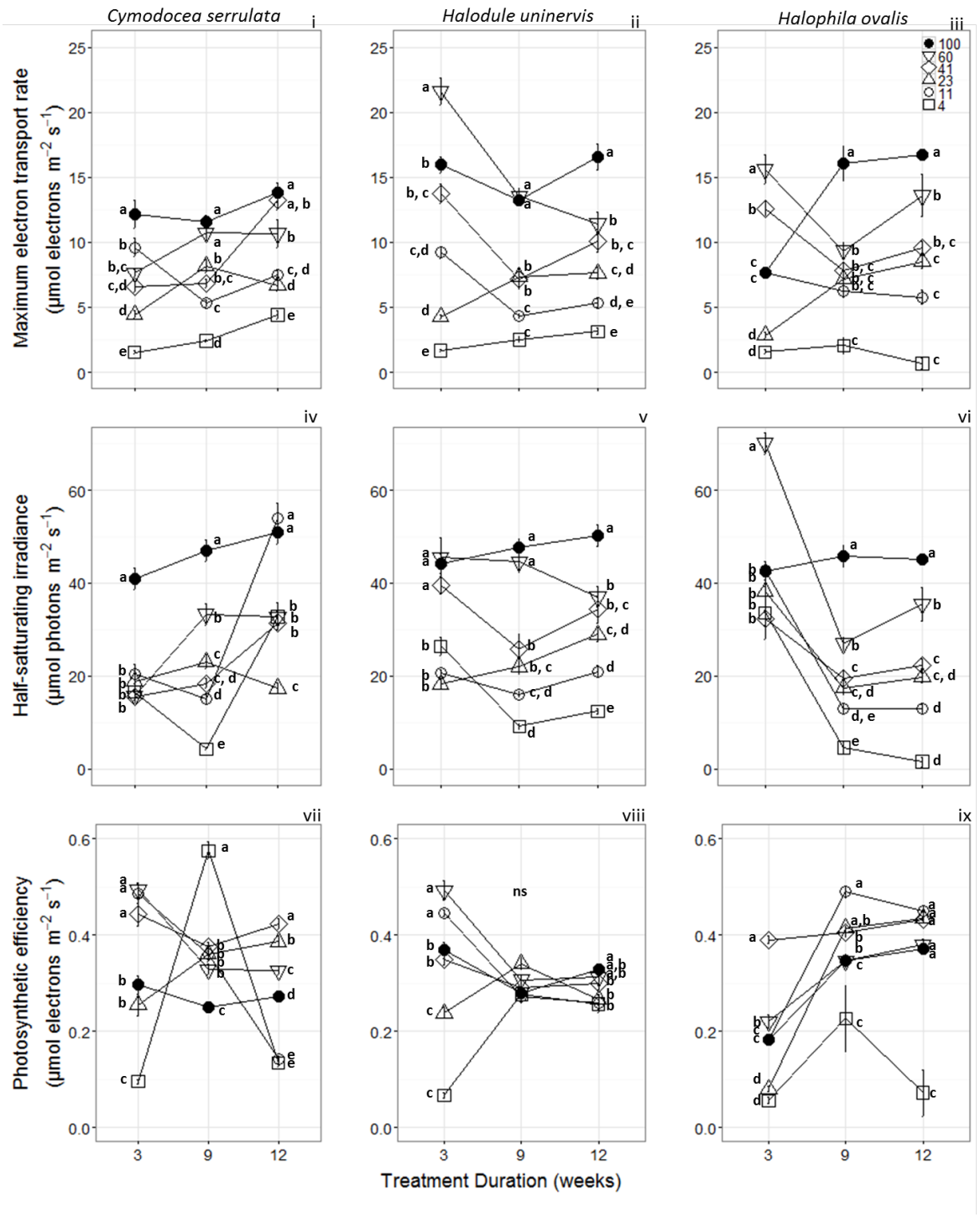


Figure 2: Effect of light reduction on seagrass photophysiological characteristics. Maximum electron transport rate (i – iii), half-saturating irradiance (iv – vi), and photosynthetic efficiency (vii – ix) for *Cymodocea serrulata* (left), *Halodule uninervis* (centre) and *Halophila ovalis* (right) at 3, 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} (2.5 mol quanta m^{-2} day^{-1}) and 4% I_{PAR} (0.9 mol quanta m^{-2} day^{-1}). Values are means (n = 12) ± S.E. Letters indicate significant differences between treatments for each species and at each time.

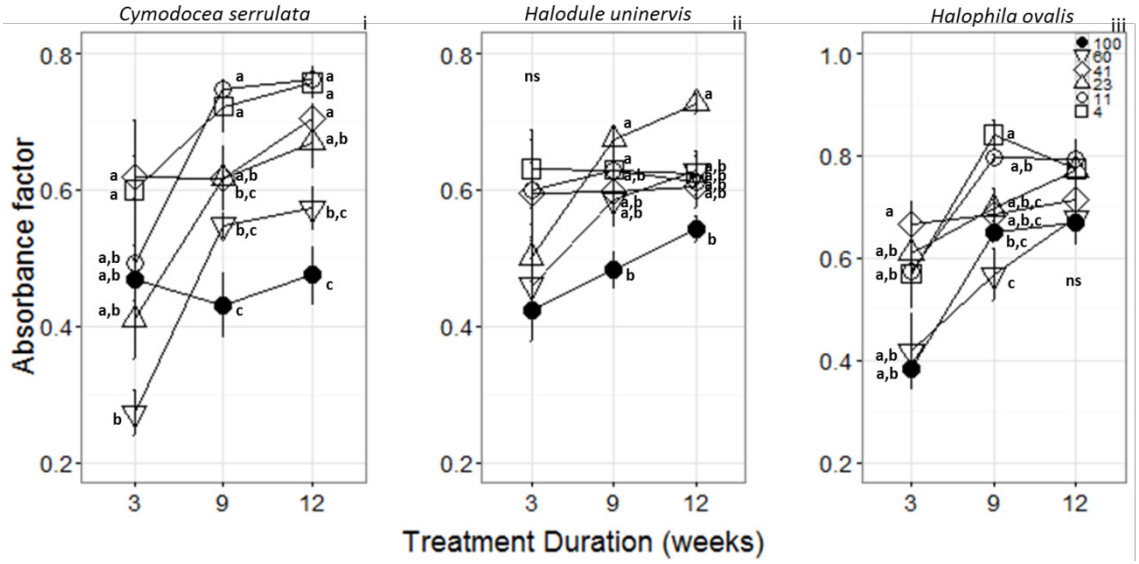


Figure 3: Leaf light absorption (Absorbance Factor) for *Cymodocea serrulata* (i), *Halodule uninervis* (ii) and *Halophila ovalis* (iii) at 3, 9 and 12 weeks after shading: 100% I_{PAR} (21.60 mol quanta m^{-2} day^{-1}), 60% I_{PAR} (13.1 mol quanta m^{-2} day^{-1}), 41% I_{PAR} (8.90 mol quanta m^{-2} day^{-1}), 23% I_{PAR} (5.02 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 = 5) ± S.E. Letters indicate significant differences between treatments for each species and at each time.

3.1.2 Leaf nutrients and isotopes

Leaf nutrient concentration and isotopic signature were affected by light reduction, in particular leaf N concentration and $\delta^{15}N$ showed a general increase with light reduction, whereas $\delta^{13}C$ decreased. However, there were species-specific differences in the magnitude of response to these variables which tended to intensify with time for all species. Both *C. serrulata* and *H. ovalis* leaf carbon (C) were not significantly affected by light reduction ($p>0.05$, Table 4), but this was not the case for *H. uninervis* (Treatment, MS=20.7, $p<0.001$, Table 4, Figure 4i, ii, iii). Leaf C was significantly higher in 4–41% treatments relative to controls. There was also a trend towards an increase in leaf C in *H. uninervis* over time (Time, MS = 9.24, $p=0.008$, Table 4). Leaf N concentration increased in response to light reduction for *C. serrulata* but the nature of the response was dependent on time (Treatment × Time, MS=0.204, p=<0.001, Table 4). At 3 weeks the 4% I_{PAR} treatment was significantly higher than the control with all other light treatments intermediate between these two groups. At 6, 9 and 12 weeks the 4 and 11% light treatments were greater than controls and all other light treatments, with the exception of 23% treatment at 12 weeks (Figure 4iv). For *H. uninervis*, leaf N concentration increased in response to light reduction and the nature of the response was dependent on time (Treatment × Time, MS=0.066, $p=0.008$, Table 4). At 3 weeks the 4% I_{PAR} treatment was significantly higher than the controls with 11–41% light treatments intermediate between these two groups (Figure 4v). At 6 weeks, leaf N was significantly greater in 4–23% treatments, and by 9 weeks the 60 and 41% light treatment were also elevated relative to controls. At 12 weeks only 4–23% treatments were significantly higher than the control with 41 and 60% treatments intermediate between these two groups. For *H. ovalis* leaf N concentration was similarly affected by light treatment over the 12 week experiment (Treatment, MS=0.895, $p<0.001$, Table 4) with plants grown in 4 – 23% light treatments showing elevated leaf N concentrations compared to controls (Figure 4vi).

Light reduction reduced the leaf CN ratio in *C. serrulata* (Treatment, MS 325, $p< 0.001$, Table 4). Leaf CN ratio decreased in 4–23% I_{PAR} for the 12 week experiment (Figure 4vii). For *H. uninervis*, leaf CN ratio also decreased in response to light reduction but the nature of the response changed over time (Treatment x Time, MS=10.8

p=0.014, Table 4). At 3 weeks there was no difference in CN ratio between control and light treatments, but at 6 weeks plants grown in 4–23% light treatments showed a decrease in leaf CN ratio. At 9 and 12 weeks 4, 11, 23 and 60% treatments were significantly lower than controls (Figure 4viii). For *H. ovalis* leaf CN ratio was also affected by light reduction (Treatment, MS=663, p< 0.001, Table 4) over the 12 week experiment. Plants grown in 4–41% light treatments had a significantly lower leaf CN ratio relative to controls (Figure 4ix).

Leaf $\delta^{13}C$ values decreased in response to light reduction but the differences, for all species, changed over time (Treatment × Time, Table 4). For *C. serrulata*, there was no change in $\delta^{13}C$ at 3 weeks, by 6 weeks plants grown in 11–41% I_{PAR} had lower (>30%) $\delta^{13}C$, at 9 weeks all light reduction treatments showed a decline in $\delta^{13}C$ (the 11 and 23% light treatments were ~40% lower, whereas the 4% I_{PAR} treatment decreased by only 17%), and at 12 weeks the 11-41% I_{PAR} treatments were significantly reduced relative to controls (up to 40% decrease but the 4% I_{PAR} was not (Figure 4x). *H. uninervis* showed a very similar pattern and magnitude of response: no change at 3 weeks but by 6 weeks the 11–41% I_{PAR} treatments showed the greatest decrease (>40%) (Figure 4xi). *H. ovalis* showed no change in $\delta^{13}C$ at 3 weeks but at 6 weeks all light shading treatments showed a similar decrease in $\delta^{13}C$, at 9 weeks plants grown in 4–41% I_{PAR} showed >25% decrease and at 12 weeks there was no difference in $\delta^{13}C$ for control and 41–60% I_{PAR} light treatment (Figure 4xii); at 12 weeks, data were unavailable for low light treatments due to insufficient sample for analysis.

Leaf $\delta^{15}N$ was affected by light reduction for *C. serrulata* (Treatment, p<0.001, MS=20.56, Table 4). Leaves had higher $\delta^{15}N$ at 23 and 11% I_{PAR} compared to controls, and all other treatments including severe low light (4% I_{PAR}), over the 12 week experiment (Figure 4xiii). For *H. uninervis*, a significant effect of light reduction on $\delta^{15}N$ was dependent on time (Treatment × Time, MS=1.54, p=0.039, Table 4); there was no effect at 3 weeks, at 6 weeks 23% I_{PAR} was significantly greater than controls, at 9 weeks the 23 and 11% I_{PAR} were higher than controls and the 4% I_{PAR} was lower than 23% I_{PAR} (Figure xiv). *H. ovalis* was also affected by light treatment (Treatment, MS=5.82, p<0.001, Table 4), but only 11% I_{PAR} showed a significant increase leaf $\delta^{15}N$ compared to controls (Figure 4v)

Table 4: Results of two-way ANOVA testing for the effects of treatment (integrated daily irradiance mol photons m^{-2}), time and treatment nested within tank on leaf C and N concentrations, leaf CN ratios, $\delta^{13}C$ and $\delta^{15}N$. Note leaf samples were from pooled tank samples therefore no nesting term is applied. Bold text denotes significant differences

	Carbon (C)		Nitrogen (N)		C:N ratio		$\delta^{13}C$		$\delta^{15}N$	
	MS	p	MS	p	MS	p	MS	p	MS	p
Cymodocea serrulata										
Treatment	18.4	0.090	3.73	**<0.001**	325	**<0.001**	115	**<0.001**	22.6	**<0.001**
Time	8.67	0.240	0.087	0.239	1.3	0.926	120	**<0.001**	18.2	**<0.001**
Tank (Treatment)	9.89	0.122	0.344	**<0.001**	27.8	**0.005**	8.44	0.166	3.31	0.06
Treatment × Time	7.80	0.166	0.204	**<0.001**	16.9	0.230	10.7	**<0.001**	1.83	0.107
Halodule uninervis										
Treatment	20.7	**<0.001**	1.08	**<0.001**	164	**<0.001**	148	**<0.001**	11.7	**<0.001**
Time	9.24	**0.008**	1.69	**<0.001**	278	**<0.001**	93	**<0.001**	20.1	**<0.001**
Tank (Treatment)	7.36	**0.003**	0.106	0.162	16.1	0.217	2.20	0.129	2.20	0.129
Treatment × Time	1.98	0.585	0.066	**0.008**	10.8	**0.014**	11.3	**<0.001**	1.54	**0.039**
Halophila ovalis										
Treatment	7.95	0.062	0.895	**<0.001**	663	**<0.001**	19.3	**<0.001**	5.82	**<0.001**
Time	3.64	0.397	0.331	**<0.001**	109	**0.040**	43.5	**<0.001**	5.69	**0.002**
Tank (Treatment)	5.44	0.213	0.091	0.100	119.8	**0.003**	3.63	**0.006**	3.63	**0.006**
Treatment × Time	6.28	0.072	0.045	0.578	26.2	0.762	3.92	**0.006**	1.08	0.150

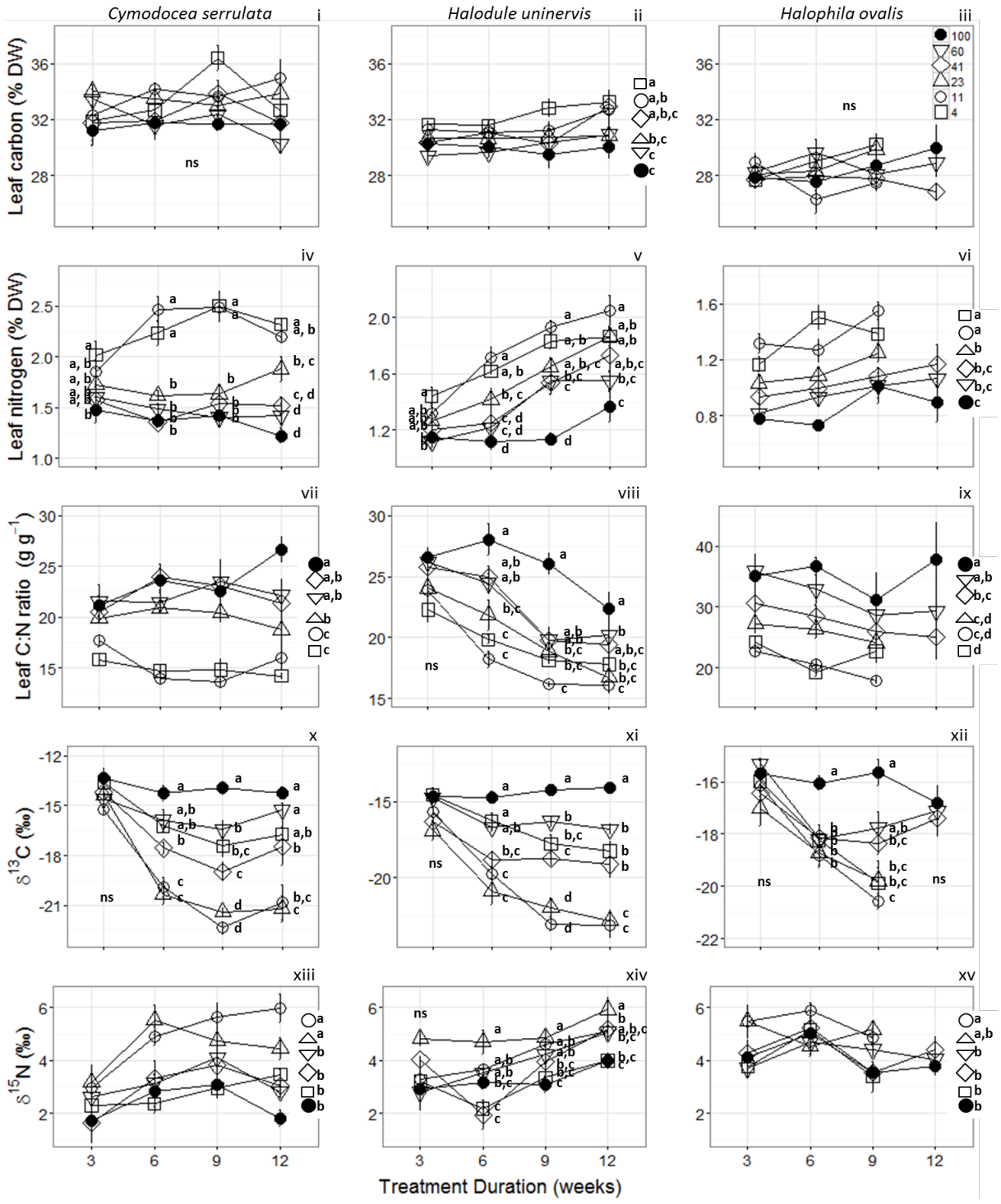


Figure 4: Effect of light reduction on seagrass leaf nutrient content. Leaf Carbon,% dry weight (i – iii), leaf Nitrogen,% dry weight (iv – vi), leaf CN ratio, g g^{-1} (vii – ix), leaf δ ^{13}C (x – xii), and δ ^{15}N (xiii – xv) for *Cymodocea serrulata* (left), *Halodule uninervis* (centre) and *Halophila ovalis* (right) at 3, 6, 9 and 12 weeks after shading; 100% I_{PAR} (21.6 mol quanta photons m^{-2} day^{-1}), 60% I_{PAR} (13.1 photons m^{-2} day^{-1}mol quanta), 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 photons m^{-2} day^{-1}mol quanta). Values are means (n = 12) ± SE. Letters within figure indicate significant differences between treatments for each species and at each time. Symbols on right of each graph indicate a Treatment effect for that species and represent each of the shading intensities. Letters outside figure indicate significant differences between treatments for a given species.

3.1.3 Carbohydrates

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 <1% starch, whereas *H. uninervis* had <6% soluble sugars and ~18% starch. In *C. serrulata* and *H. uninervis,* rhizome concentrations tended to decrease with a reduced light availability. For C. *serrulata*, soluble sugar concentration in the rhizome was affected by the main effects Treatment and Time (Treatment, MS = 458, p = < 0.001; Time, MS = 160, p = 0.08, Table 5). The higher light intensities (100, 60 and 41% I_{PAR}) had higher concentrations than the lowest light intensities (11 and 4% I_{PAR}) at all times, and in week 12, soluble carbohydrate concentrations in higher light intensities were at least 30% greater than the lowest light intensities (Figure 5i). Starch concentrations, which were an order of magnitude lower than soluble carbohydrates, were affected by treatment only (Treatment, MS = 0.036, p = 0.003, Table 5), with the lowest light intensity (4% I_{PAR}) showing ~50% less starch than the highest light intensity (Figure 5iv). For *H. uninervis* soluble carbohydrates in the rhizome were affected by the main effects treatment and time (Treatment, MS = 11.4, p = <0.001; Time, MS = 84.2, p = <0.001, Table 5). The higher light intensities (100 and 60% I_{PAR}) had at least 30% more soluble carbohydrates than 11% I_{PAR}, but were not significantly greater than 4% I_{PAR}. Soluble carbohydrate concentrations on average were highest at 9 weeks, followed by 6 weeks, whereas 3 and 12 weeks had the lowest soluble carbohydrate concentrations (Figure 5ii). In the highest light treatments (100 and 60% I_{PAR}), starch concentrations were 2–3 times higher than soluble carbohydrate concentrations (Figure 11b and 12b). Starch was affected by treatment but this was dependent on time (Treatment × Time, MS = 22.9, p = 0.004, Table 5). At 3 weeks the controls were no different from shaded treatments, but by 6 weeks the high-moderate light treatments (100, 60, 41% I_{PAR}) had more starch than plants grown at less than 23% I_{PAR}. At 9 and 12 weeks, *H. uninervis* grown in less than 41% I_{PAR} had significantly less starch than controls (Figure 5v). For *H. ovali*s, rhizome carbohydrate concentrations were not determined for low light treatments in the first 6 weeks and for all treatments at 9 and 12 weeks due to insufficient rhizome biomass for analysis. Where sufficient quantities of rhizome enabled carbohydrate analysis, there was no significant difference in soluble carbohydrate or starch concentrations between treatments or time (Table 5, Figure 5iii, vi).

Table 5: Results of two-way ANOVA testing for the effects of treatment (integrated daily irradiance mol quanta m^{-2} d^{-1}) and time on rhizome soluble carbohydrates and starch. Note soluble carbohydrates and starch were from pooled tank samples therefore no nesting term is applied. Bold text denotes significant differences.

		Soluble Carbohydrates			Starch	
		df	MS	p	MS	p
Cymodocea serrulata	Treatment	5	458	**<0.001**	0.036	**0.003**
	Time	3	160	0.080	0.035	**0.014**
	Tank (Treatment)	6	94.7	**0.001**	0.014	0.237
	Treatment ×Time	15	16.6	0.999	0.009	0.275
Halodule uninervis	Treatment	5	11.4	**<0.001**	215	**<0.001**
	Time	3	84.2	**<0.001**	457	**<0.001**
	Tank (Treatment)	6	0.673	0.973	17.5	0.540
	Treatment × Time	15	1.89	0.093	22.9	**0.004**
Halophila ovalis	Treatment	2	79.5	0.063	0.014	0.088
	Time	1	31.3	0.283	0.013	0.126
	Tank (Treatment)	3	11.6	0.748	0.007	0.237
	Treatment × Time	2	26.2	0.296	0.001	0.912

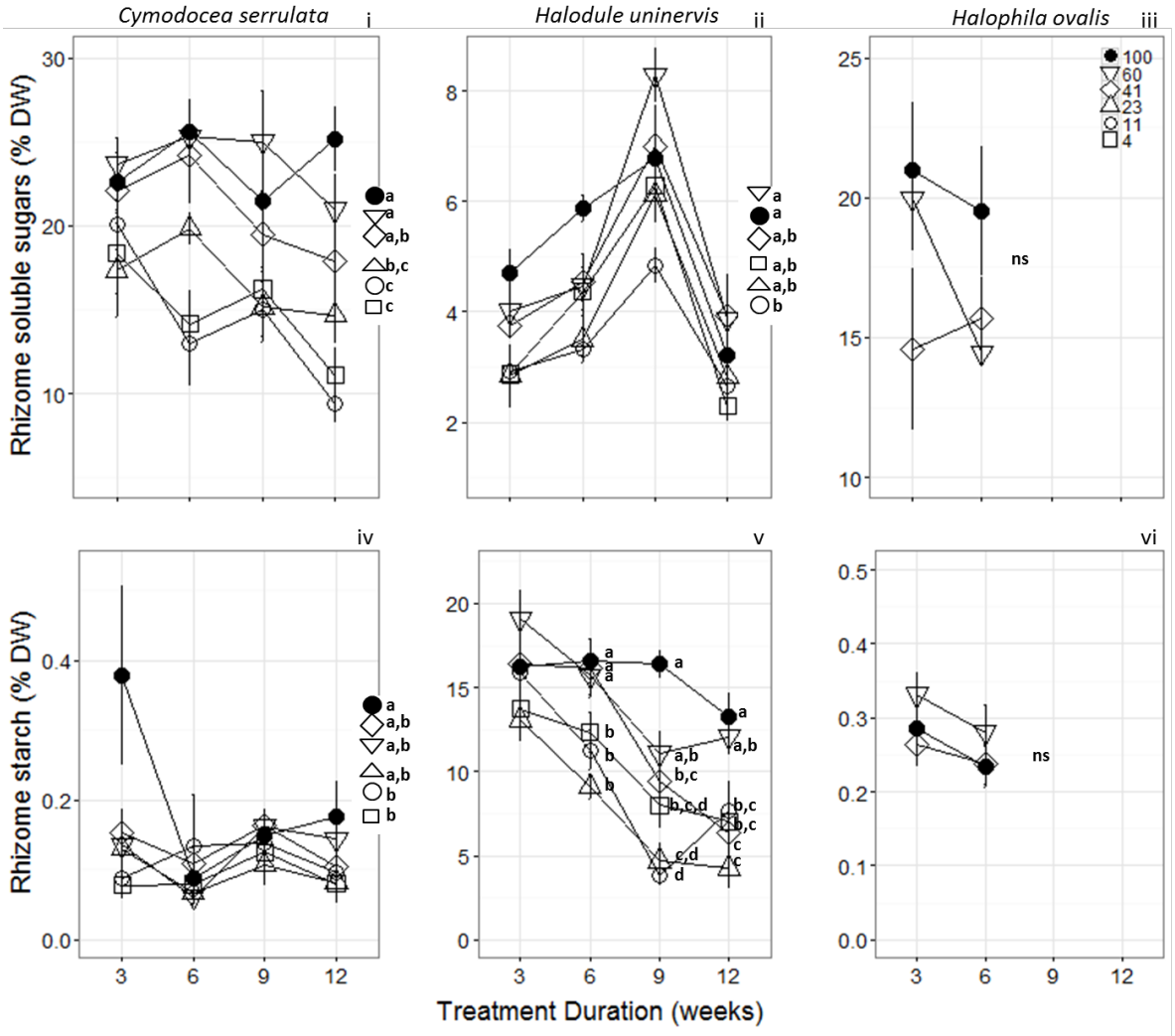


Figure 5: Effect of light reduction on rhizome carbohydrate concentrations. Rhizome soluble carbohydrate concentration,% dry weight (i – iii) and rhizome starch% dry weight (iv – vi) for *Cymodocea serrulata* (left), *Halodule uninervis* (centre) and *Halophila ovalis* (right) at 3, 6, 9 and 12 weeks 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 = 12) ± S.E. Letters within figure indicate significant differences between treatments for each species and at each time. Symbols on right of each graph indicate a Treatment effect for that species and represent each of the shading intensities. Letters outside figure indicate significant differences between treatments for a given species.

3.2 Plant scale response

3.2.1 Growth and Morphology

Plant growth rates, shoot density, leaf size and number of leaves per shoot tended to decline with a reduction in light availability but responses of each variable were not always significant for each species and responses were influenced by time. Light reduction treatments affected shoot density, but only for *H. uninervis* and after 12 weeks (Species × Treatment × Time, MS = 26, p 0.019, Table 6, Figure 6). At 12 weeks, *H. uninervis* shoot density decreased for 4–23% I_{PAR} light reduction treatments (Figure 6ii). The rate of new shoot production per ramet was affected by treatment, but this was dependent on species (Treatment × Species, MS = 0.003, p = <0.001, Table 6), so we analysed light reduction effects on each species separately. For *C. serrulata*, shoot production rate was significantly lower than controls in 4% I_{PAR} treatment whereas 11–41% I_{PAR} treatments were intermediate

between these two groups (Figure 6iv). For *H. uninervis* shoot production rate declined at light intensities lower than 41% I_{PAR} over the 12 week experiment (Figure 6v). *H. ovalis* was affected by light reduction treatments between 4–23% I_{PAR} (Figure 6vi). The rate of new shoot production was also affected by time but this differed between species (Time × Species, MS = 0.002, p = 0.023, Table 6). *C. serrulata* showed a reduction in new shoot production after 9 weeks, whereas for *H. uninervis* and *H. ovalis* impacts of light reduction were apparent after 3 weeks.

Mean leaf area per shoot was slow to change following light reductions for all species with no significant change in mean leaf area per shoot for 6 weeks (Treatment × Time, MS = 3.25, p = < 0.001, Table 6). At 9 and 12 weeks, *C. serrulata* and *H. uninervis* showed a significant decline in leaf area but only in severe low light (4% I_{PAR}, Figure 6vii, viii). *H. ovalis* also showed a significantly decrease in leaf area for severe low light but only after 12 weeks (Figure 6ix).

Mean number of leaves per shoot was also slow to change following light reductions but not for all species (Treatment × Time, MS = 0.730, p = < 0.001, Table 6). *C. serrulata* showed no change in mean leaf number per shoot over the 12 week experiment, whereas at 12 weeks, both *H. uninervis* and *H. ovalis* had one leaf less per shoot when grown at 4% I_{PAR} (Figure 6, x, xi, xii).

Table 6: Results of a four-way nested ANOVA testing for the effects of treatment (daily integrated irradiance mol quanta m^{-2} d^{-1}), species, time and treatment nested within tank on new shoot production rate, and mean number of leaves per shoot. Bold text denotes significant differences

		Shoot Density		Shoot Production		Mean Leaf Area $shoot^{-1}$		Leaves $shoot^{-1}$	
	df	MS	p	MS	p	MS	p	MS	p
Species	2	13700	**<0.001**	0.044	**<0.001**	234	**<0.001**	4.348	**<0.001**
Treatment	5	145	**<0.001**	0.023	**<0.001**	6.90	**<0.001**	1.702	**<0.001**
Time	3	318	**<0.001**	0.005	**<0.001**	3.86	**0.030**	0.442	0.099
Tank (Treatment)	6	13.1	0.980	0.002	0.108	1.23	0.769	0.145	0.745
Species × Treatment	10	100	**<0.001**	0.003	**<0.001**	2.46	**0.041**	0.191	0.526
Species × Time	6	96	**<0.001**	0.002	**0.023**	2.25	0.108	0.672	**0.004**
Treatment × Time	15	31	**0.014**	0.001	0.334	3.25	**0.001**	0.730	**<0.001**
Species × Treatment × Time	30	26	**0.019**	0.001	0.269	1.67	0.134	0.293	0.082

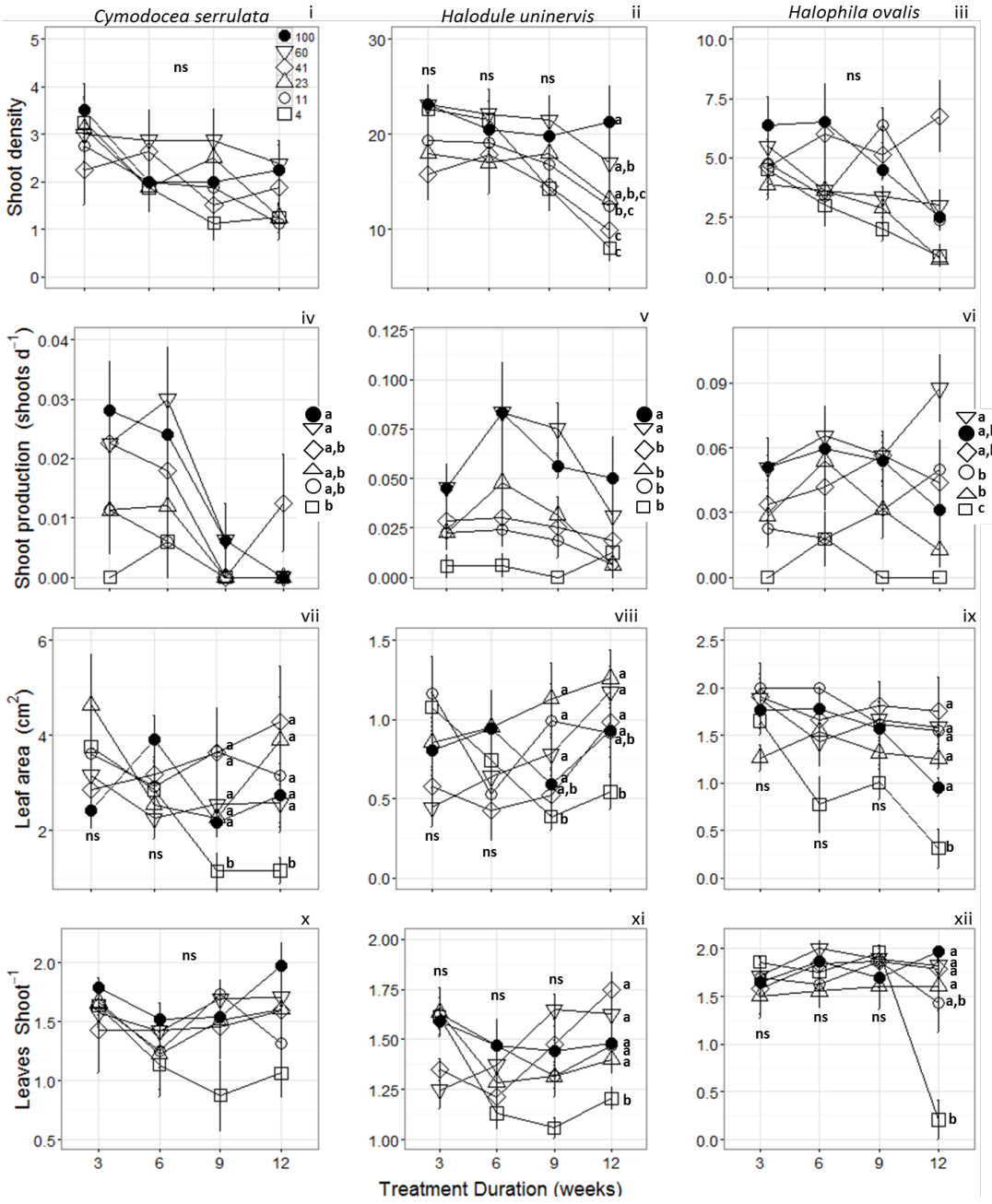


Figure 6: Shoot density (i – iii), Shoot production rate, shoots d^{-1} (iv – vi), mean leaf area, cm^2 (vii – ix), and leaves $shoot^{-1}$ (x – xii) for *Cymodocea serrulata* (left), *Halodule uninervis* (centre) and *Halophila ovalis* (right) at 3, 6, 9 and 12 weeks after shading; 100% I_{PAR} (21.6 mol quanta m^{-2} day^{-1}), 60% I_{PAR} (13.2 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) ± SE. Letters within figure indicate significant differences between treatments for each species and at each time. Symbols on right of each graph indicate a Treatment effect for that species and represent each of the shading intensities. Letters outside figure indicate significant differences between treatments for a given species.

Plant biomass, in general, decreased with a decrease in light availability, though there were species differences in the way above and below-ground biomass changed. *Cymodocea serrulata* showed no change in below-ground biomass but a reduced above-ground biomass, however, below-ground biomass was an order of magnitude greater than above-ground biomass, subsequently there was no significant effect of light reduction on total plant biomass, but this was not the case for *H. uninervis* and *H. ovalis* (Species × Treatment, MS = 0.125, p = <0.001, Table 7). The largest treatment effect was for *H. uninervis* with total plant biomass on average more than 25%

lower in 4–41% I_{PAR} light treatments compared to the controls (Figure 7ii). *H. ovalis* showed a decline in total biomass at 4 and 23% I_{PAR} but not the 11% I_{PAR} (Figure 7iii).

Above-ground biomass was affected by treatment, but the effect depended on species and times (Species x Treatment × Time, MS = 0.001, p = 0.020, Table 7). *C. serrulata* leaf biomass decreased by 80% at 9 and 12 weeks and this difference was significant only in the severe (4% I_{PAR}) low light treatment (Figure 7iv). *H. uninervis* leaf biomass decreased by 50% at 6 and 9 weeks, then by 75% at 12 weeks, but only in the severe low light treatment (Figure 7v). *H. ovalis* leaf biomass did not significantly decline relative to the controls, however, leaf biomass for control treatments did show a decline at 9 and 12 weeks (Figure 7vi).

Below-ground biomass was affected by treatment, but not for all species (Species × Treatment, MS = 0.094, p = <0.001, Table 7). While *C. serrulata* below-ground biomass was unaffected by treatment (Figure 7vii), *H. uninervis* below-ground biomass significantly declined by more than 35% for 4–41% I_{PAR} light treatments (Figure 7viii). *H. ovalis* below-ground biomass showed a significant difference from the control in the 4 and 23% I_{PAR} treatments, with more than 50% less below-ground biomass in these treatments (Figure 7ix).

Table 7: Results of a four-way nested ANOVA testing for the effects of treatment (daily integrated irradiance mol quanta m^{-2} d^{-1}), species, time and treatment nested within tank on total plant, above-ground and below-ground biomass; and results of three-way nested ANOVA testing for the effects of treatment (integrated daily irradiance mol quanta m^{-2} d^{-1}) and time on total pot biomass. Bold text denotes significant difference

		Total Biomass		Above-ground Biomass		Below-ground Biomass		Total Biomass (Pot)	
	df	MS	p	MS	p	MS	p	MS	p
Species	2	6.82	**<0.001**	0.011	**<0.001**	6.93	**<0.001**	NA	NA
Treatment	5	0.191	**<0.001**	0.007	**<0.001**	0.157	**<0.001**	0.650	**<0.001**
Time	3	0.070	**0.021**	0.020	**<0.001**	0.026	0.230	0.310	**0.001**
Tank (Treatment)	6	0.019	0.875	0.001	0.290	0.154	0.942	0.065	0.431
Species × Treatment	10	0.125	**<0.001**	0.004	**<0.001**	0.094	**<0.001**	NA	NA
Species × Time	6	0.018	0.536	0.002	**0.002**	0.013	0.647	NA	NA
Treatment × Time	15	0.033	0.087	0.002	**<0.001**	0.028	0.084	0.108	**0.026**
Species × Treatment × Time	30	0.019	0.633	0.001	**0.020**	0.016	0.593	NA	NA

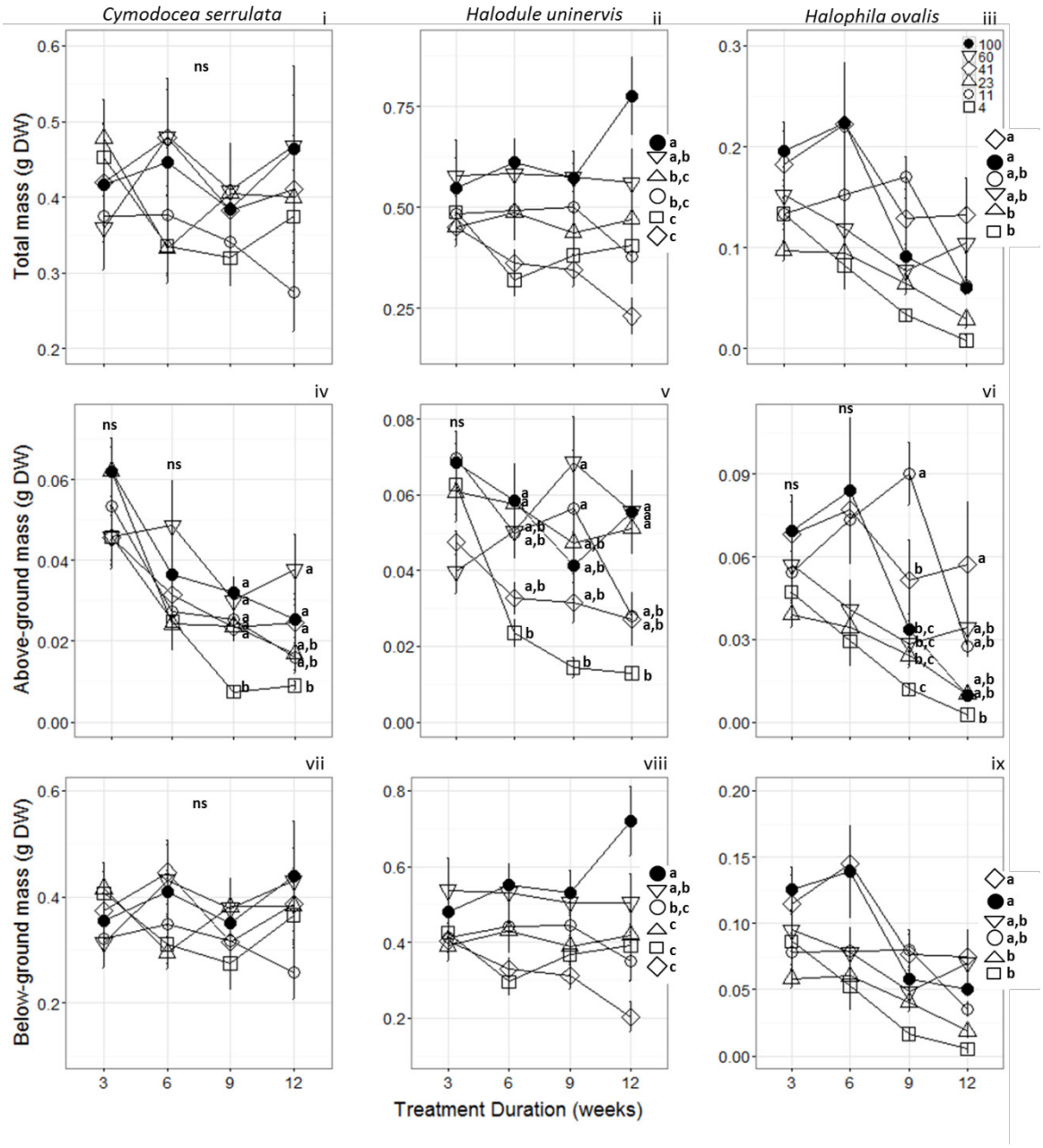


Figure 7: Total biomass, g dry weight (i – iii), above-ground biomass, g dry weight (iv – vi), below-ground biomass, g dry weight (vii – ix) for *Cymodocea serrulata* (left), *Halodule uninervis* (centre) and *Halophila ovalis* (right) at 3, 6, 9 and 12 weeks after shading; 100% I_{PAR} (21.6 mol quanta 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. Symbols on right of each graph indicate a Treatment effect for that species and represent each of the shading intensities. Letters outside figure indicate significant differences between treatments for a given species.

3.3 Meadow-scale (pot level)

3.3.1 Plant Abundance

The total biomass (all species combined) declined with light reduction but this was dependent on the treatment and time (Treatment × Time, MS = 0.108, p = 0.026, Table 5). At three weeks, no significant reduction in biomass was detected, but by six weeks both the 23% and 4% were significantly lower than the controls, with the

remaining treatments intermediate between these two groups. By 12 weeks all treatments, apart from the 60% were significantly lower than the controls (Figure 8).

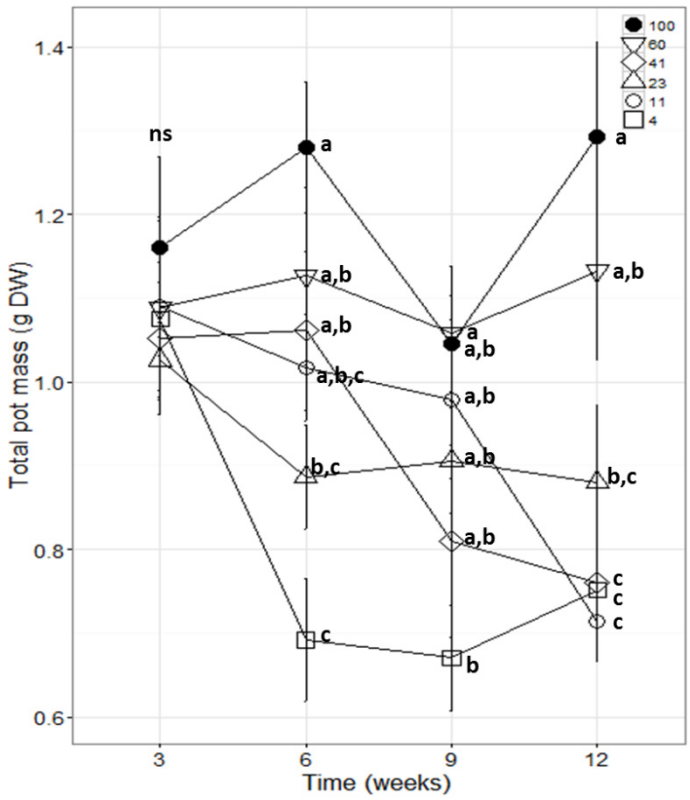


Figure 8: Total biomass, g dry weight, for all species combined within each pot at 3, 6, 9 and 12 weeks after shading; 100% I_{PAR} (21.6 mol quanta m^{-2} day^{-1}), 60% I_{PAR} (13.1 mol quanta m^{-2} day^{-1}), 41% I_{PAR} (8.9 mol quanta m^{-2} day^{-1}), 23% I_{PAR} (5.0 mol quanta m^{-2} day^{-1}), 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.

3.4 Bio-indicators

3.4.1 *Cymodocea serrulata*

A variable is useful as a bio-indicator when it shows a consistent direction of response with increased duration and magnitude of light reduction. The variables that responded most consistently were rhizome soluble carbohydrates, shoot production, mean leaf area and total above-ground biomass (Figure 9h, j, k, l and Table 8). They all reduced with increased magnitude and duration of light reduction. The best early warning indicator, as it responded within three weeks, was rhizome soluble carbohydrates and shoot production, and these both responded at the highest level of light received (13 mol quanta m^{-2} d^{-1}). Whereas mean leaf area and above-ground biomass responded at 9 weeks, so would be considered later warning indicators, and this was only observed with very high levels of light reduction where plants received less than 2.3 mol quanta m^{-2} d^{-1}. Some other variables did show a consistent response, but only from particular levels of light reduction. ETR_{MAX} showed a consistent decline but only in treatments that received 5 mol quanta m^{-2} d^{-1} or less (Figure 21d, Table 8). Likewise the nitrogen content showed a consistent increase, but only in treatments that received 2.3 mol quanta m^{-2} d^{-1} or less (Figure 9f, Table 8).

3.4.2 *Halodule uninervis*

The variables that responded most consistently for *Halodule uninervis* were the photosynthetic parameters E_k and ETR_{MAX} as well as shoot production and leaves per shoot (Figure 10c, d, k, l). Early warning indicators for

Halodule were E_k and shoot production as they responded consistently from three weeks. Declines in shoot production were observed across all time periods when plants received 8.9 mol quanta m^{-2} d^{-1} or less, but for E_k the light reduction level at which a decline was observed varied depending on the duration of stress. It declined when plants received 5 mol quanta m^{-2} d^{-1} or less at 3 weeks, then 8.9 mol quanta m^{-2} d^{-1} or less at 9 weeks, and 13.1 mol quanta m^{-2} d^{-1} or less at 12 weeks. ETR_{MAX} and leaves per shoot are considered later warning indicators as they declined significantly after 9 and 12 weeks, respectively. Mean leaf area also declined, but only consistently at 12 weeks (Table 8). The starch content in rhizomes was consistently less than the controls (Figure 10i) from 6 weeks of light reduction, but there was not a consistent pattern in the magnitude of the response with increasing light reduction (Table 8).

3.4.3 *Halophila ovalis*

There were not as many significant responses to the light reduction treatment in *Halophila ovalis*, and of those, only two showed a consistent response to light reduction, the leaf carbon isotope ratio and nitrogen content. Leaf nitrogen increased at 3 weeks, and significantly when plants received 2.3 mol quanta m^{-2} d^{-1} or less, whereas the leaf carbon isotope ratio declined at 6 weeks in all light reduction treatments (Figure 11e, f, Table 8).

Cymodocea serrulata

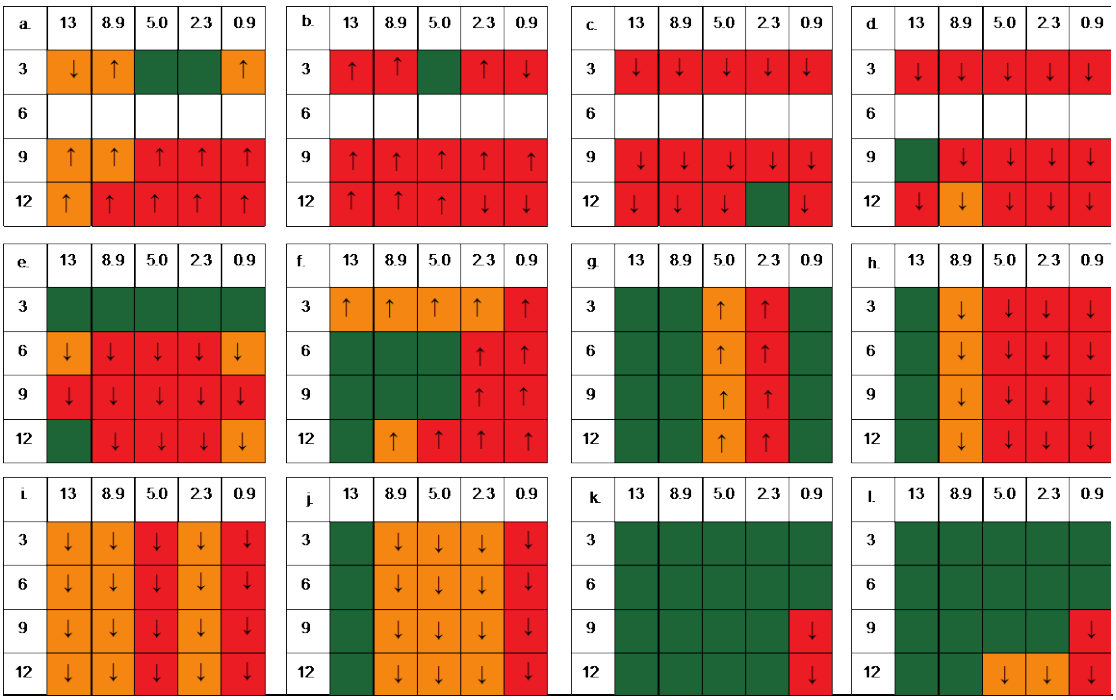


Figure 9: Summary of significance of responses and direction of response for all variables that showed a significant effect or interacting effect of treatment for the species Cymodocea serrulata. Each large box represents a different variable a. Absorbance factor, b. alpha. c. Half-saturating irradiance, d. Maximum electron transport rate, e. Carbon isotope ratio, f. Nitrogen content (% DW), g. Nitrogen isotope ratio, h. Rhizome soluble carbohydrate content (% DW), i. Rhizome starch content, j. Shoot production, k. Mean leaf area per shoot (cm2), l. Above-ground biomass. Within each large box the magnitude of light reduction increases on the top axis from an total daily average light of 13 down to 0.9 mols PAR m-2 d-1. The duration of light reduction increases on the left axis, from 3 down to 12 weeks. A green coloured box indicates no significant difference to the controls, an orange coloured box indicates it is at an intermediate level and a red coloured box indicates that it is significantly different to the control. 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.

Halodule uninervis.

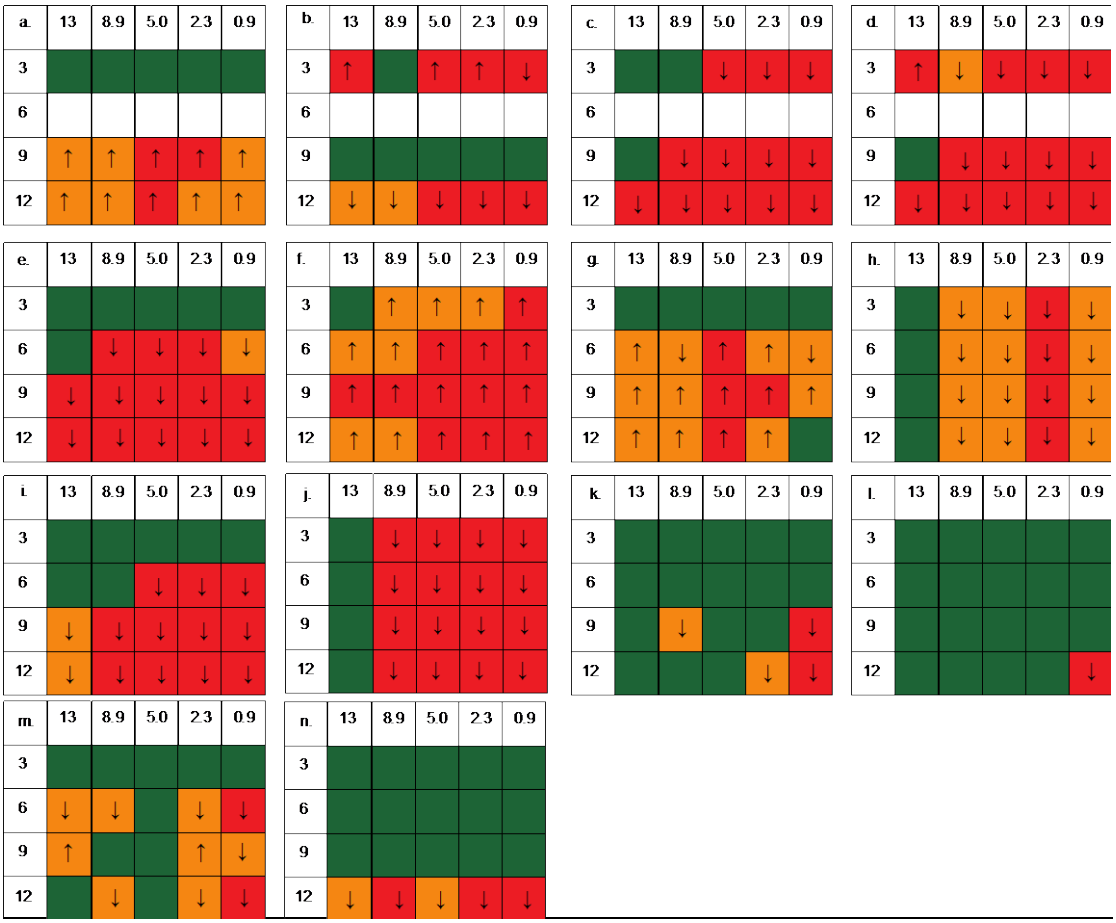


Figure 10: Summary of significance of responses and direction of response for all variables that showed a significant effect or interacting effect of treatment for the species *Halodule uninervis*. Each large box represents a different variable a. Absorbance factor, b. alpha. c. Half-saturating irradiance, d. Maximum electron transport rate, e. Carbon isotope ratio, f. Nitrogen content (% DW), g. Nitrogen isotope ratio h. Rhizome soluble carbohydrate content (% DW), i. Rhizome starch content, j. Shoot production, k. Mean leaf area per shoot (cm^2), l. Leaves per shoot, m. Above-ground biomass and n. Shoot density. Within each large box the magnitude of light reduction increases on the top axis from a total daily average light of 13 down to 0.9 mols PAR m^{-2} d^{-1}. The duration of light reduction increases on the left axis, from 3 down to 12 weeks. A green coloured box indicates no significant difference to the controls, an orange coloured box indicates it is at an intermediate level and a red coloured box indicates that it is significantly different to the control. 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.

Halophila ovalis

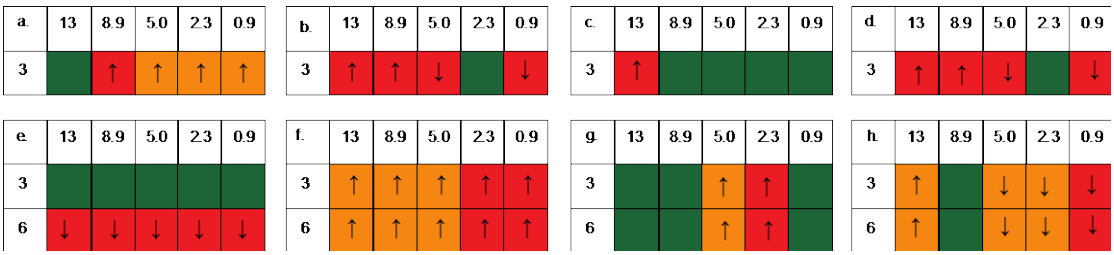


Figure 11: Summary of significance of responses and direction of response for all variables that showed a significant effect or interacting effect of treatment for the species *Halophila*. Each large box represents a different variable a. Absorbance factor, b. alpha. c. Half-saturating irradiance, d. Maximum electron transport rate, e. Carbon isotope ratio, f. Nitrogen content (% DW), g. Nitrogen isotope ratio, h. Shoot production. Within each large box the magnitude of light reduction increases on the top axis from a total daily average light of 13 down to 0.9 mols PAR m^{-2} d^{-1}. The duration of light reduction increases on the left axis, from 3 down to 6 weeks. A green coloured box indicates no significant difference to the controls, an orange coloured box indicates it is at an intermediate level and a red coloured box indicates that it is significantly different to the control. 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.

Tables 8. Consistency of potential bio-indicators to light reduction stress for *Cymodocea serrulata, Halodule uninervis* and *Halophila ovalis*. Bold variables responded most consistently and in the same direction to increasing durations (weeks) and magnitudes of light reduction (mol quanta m^{-2} d^{-1})

Variable	***Cymodocea serrulata***		***Halodule uninervis***		***Halophila ovalis*.**	
	duration	**magnitude**	**duration**	**magnitude**	**duration**	**magnitude**
	mol quanta m^{-2}	week	mol quanta m^{-2}	week	mol quanta m^{-2}	
Absorbance Factor (AF)	**from 8.9**	**from 9**	N	N		
Alpha ($\propto$)	N	N	N	only at 12	na	na
Half saturating irradiance (E_k)	N	N	**from 13.1**	**from 3**	Na	na
Maximum ETR (ETR_{MAX})	**from 5**	**from 9**	**from 8.9**	**from 9**	na	na
Carbon isotope ratio (C:N)	N	N	N	N	**from 13.1**	**from 6**
Nitrogen content (% DW)	**from 2.3**	**from 3**	from 2.3	N	**from 13.1**	**from 3**
Nitrogen isotope ratio ($\delta^{15}N$)	N	N	N	N	from 5	N
Rhizome soluble carbohydrates	**from 13.1**	**from 3**	from 8.9	N		
Rhizome starch	from 13.1	N	from 13	N		
Shoot production	**from 13.1**	**from 3**	**from 8.9**	**from 3**	from 5	N
Mean leaf area per shoot	**from 0.9**	**from 9**	**from 2.3**	**only at 12**		
Leaves per shoot			**from 0.9**	**only at 12**		
Above-ground biomass	**from 2.3**	**from 9**	N	N		
Shoot density			from 13	N		

3.4.4 *Comparison among species*

There were a few bio-indicators which were identified in more than one species. At the molecular level these were the photosynthetic parameters ETR_{MAX} and the absorbance factor (AF), at the tissue level this was shoot nitrogen content, at the ramet level shoot production and leaf area and only above-ground biomass at the community or meadow level (Figure 12).

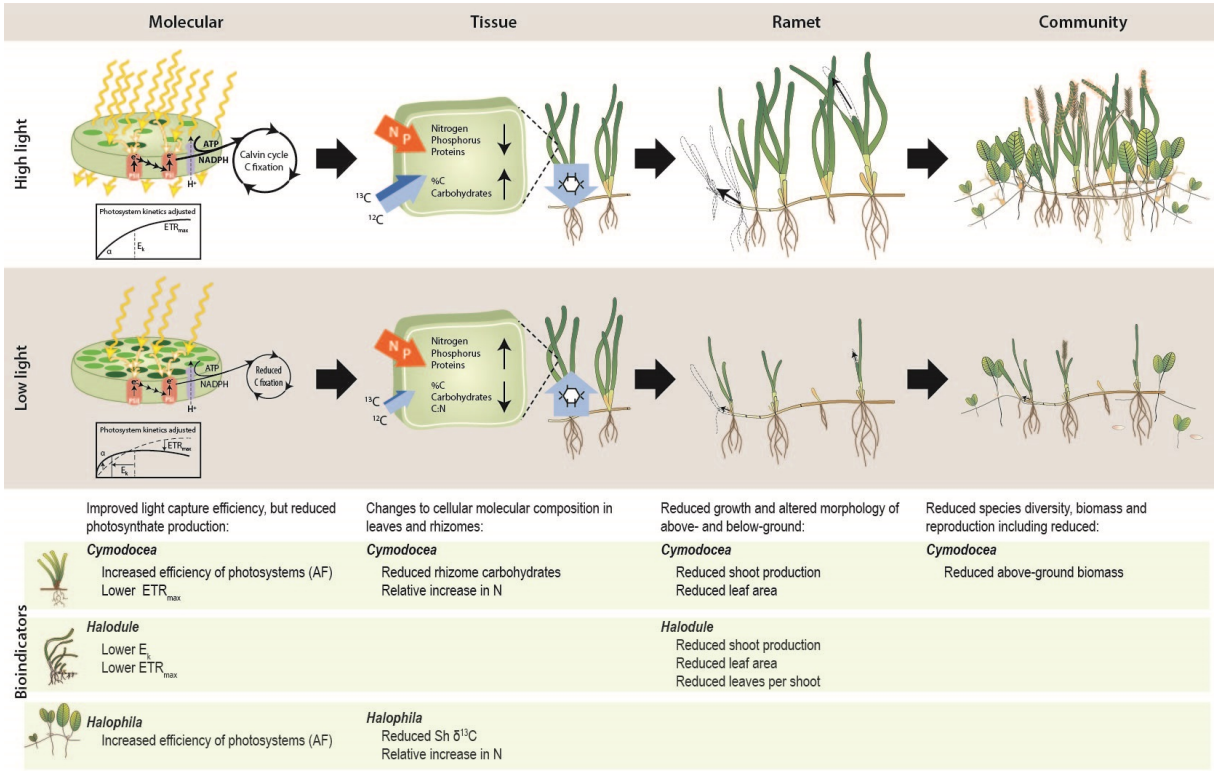


Figure 12: Plant and community response pathway to increased magnitude and duration of light reduction, based on the findings from the light reduction experiment.

3.5 Thresholds

Based on outcomes from our bio-indicator assessment, four variables were deemed appropriate for use in the development of thresholds of low light stress: two sub-lethal indicators, ETR_{MAX} and total rhizome carbohydrates (soluble sugars plus starch), which were assessed independently for each species; and two lethal indicators, total biomass of all species pooled, and the above-ground biomass of each species considered independently. Our comparison of control data at each time-step (duration; n = 8) against the total control data set (n = 32), indicated that the median values of each duration never fell below the 20th percentile (P_{20}) of the total control data set (Table 9), but that in some instances they did fall below the P_{50}. This provided confidence that P_{20} was appropriate for use as the threshold value across our indicators.

Table 9: 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 P_{50} but not P_{20}. On this basis, P_{20} was used as the nominal 'threshold value'.

	All species				***Cymodocea***				***Halodule***				***Halophila***			
Variable	**3**	**6**	**9**	**12**	**3**	**6**	**9**	**12**	**3**	**6**	**9**	**12**	**3**	**6**	**9**	**12**
ETR_{MAX}					20		20	50	50		20	50	20		50	50
Rhizome total carbohydrates					20	50	20	50	50	50	50	20	20	50		
Above-ground biomass					50	50	20	20	50	20	20	20	20	50		
Total biomass	50	50	50	20												

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 P_{20} of the controls. When plants receive 13 mol quanta m^{-2} d^{-1}, irrespective of the duration, the total biomass is maintained around the P_{50} of the controls, but as the magnitude of light reduction increases, the P_{50} of the treatments dropped below the P_{20} trigger level (Figure 13). After six weeks this occurs below 5 mol quanta m^{-2} d^{-1} and from nine weeks onwards, this occurs below 8.9 mol quanta m^{-2} d^{-1}.

3.5.2 *Cymodocea serrulata*

For *Cymodocea serrulata* the lethal threshold for a light reduction impact based on above-ground biomass was not triggered with three weeks of light reduction at any magnitude, but with 6 and 9 weeks of light reduction it was triggered when plants received intermediate levels of light reduction <5 mol PAR m^{-2} d^{-1} and 8.9 mol quanta m^{-2} d^{-1}, respectively, but not at the very low levels of 2.3 mol quanta m^{-2} d^{-1} and less (Figure 14). However, with 12 weeks of light reduction this threshold was triggered with light reduction treatments less than 8.9 mol quanta m^{-2} d^{-1}.

For the sub-lethal indicator, rhizome total carbohydrates, treatments fell below the trigger value at durations of 3 weeks of light reduction when the light plants were receiving <5 mol quanta m^{-2} d^{-1}, and at 9 and 12 weeks of light reduction when plants received less than 8.9 mol quanta m^{-2} d^{-1} (Figure 15). For ETR_{MAX}, the threshold was triggered at 3 weeks under all light reduction treatments, but at 9 weeks for light treatments that received less than 13 mol quanta m^{-2} d^{-1} and at 12 weeks for light treatments that received less than 8.9 mol quanta m^{-2} d^{-1} (Figure 16). This highlights the ability of *Cymodocea* to acclimate to low amounts of light reduction (i.e. when they receive 8.9 mol quanta m^{-2} d^{-1} or more), and that this threshold is only suitable as an early warning indicator in the first three weeks.

3.5.3 *Halodule uninervis*

The lethal threshold for *Halodule uninervis* for a light reduction impact only occurred with 12 weeks of light reduction of 2.3 mol quanta m^{-2} d^{-1} or less (Figure 14). The sub-lethal indicator of total rhizome carbohydrates was triggered earlier at 3 weeks, but only in the treatments that received 5 and 0.9 mol quanta m^{-2} d^{-1} of light (Figure 15). At 6 weeks, treatments below 8.9 mol quanta m^{-2} d^{-1} were triggered, at 9 weeks all treatments that received 8.9 mol quanta m^{-2} d^{-1} or less were triggered, and by 12 weeks, all light reduction treatments were triggered. The other sub-lethal indicator, ETR_{MAX} was triggered in treatments that received less than 8.9 mol quanta m^{-2} d^{-1} at three and six weeks, and for all treatments at 12 weeks (Figure 16).

3.5.4 *Halophila ovalis*

The lethal threshold of above-ground biomass for *Halophila* was triggered at 3 weeks, and only for the lowest light treatment. The median of all the treatments never fell below the P_{20} of the controls (Figure 14), except for 13 mol quanta m^{-2} d^{-1} at 6 weeks, but it is not clear if this is related to light, since lower light treatments were unaffected. For *Halophila,* there was not enough material to accurately assess a sub-lethal threshold for total rhizome carbohydrates, but there was for ETR_{MAX}, which was triggered only at the 5 and 0.9 mol quanta m^{-2} d^{-1} at 3 weeks, and for light reduction treatments less than 5 mol quanta m^{-2} d^{-1} at 9 and 12 weeks duration (Figure 16).

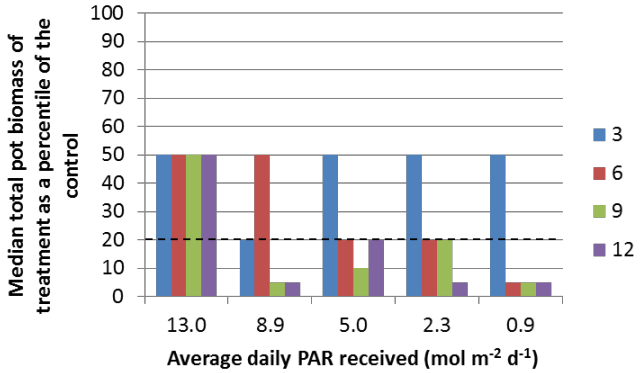
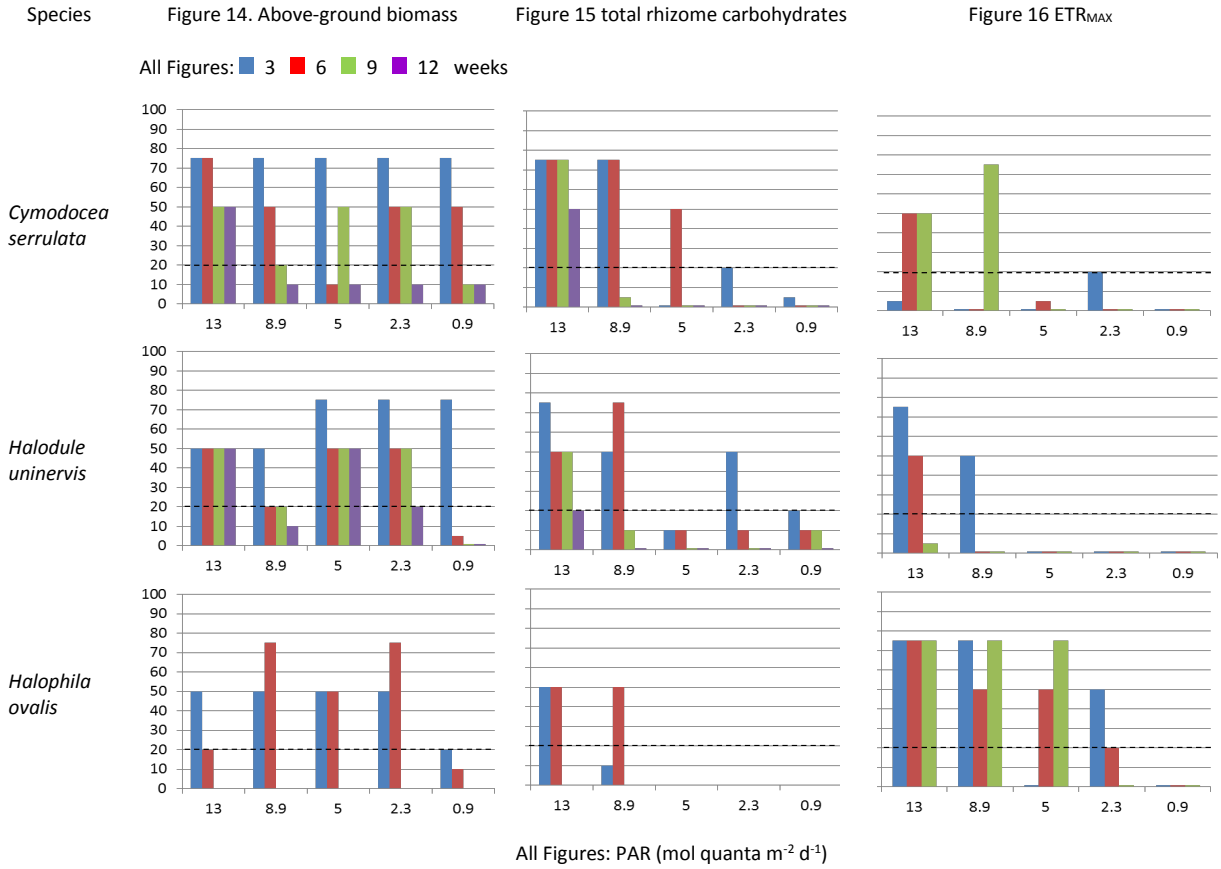


Figure 13. Light reduction threshold values for total seagrass biomass. The figure shows the total pot biomass (all species pooled) at different intensities and durations of light reduction relative to the percentiles of the controls. The dashed line denotes the 20th percentile impact trigger value. For each magnitude of light reduction, the earliest duration at which the 20th percentile trigger value is breached represents a threshold value.



Light reduction threshold values for above ground seagrass biomass (Figure 14) ,total rhizome carbohydrate concentration (Figure 15), and maximum ETR (ETR_{MAX}) at different intensities and durations of light reduction for *Cymodocea serrulata*, *Halodule uninervis*, and *Halophila ovalis* relative to the percentiles of the controls. The dashed line denotes the 20th percentile impact trigger value. For each magnitude of light reduction, the earliest duration at which the 20th percentile trigger value is breached represents a threshold value

4 Discussion

4.1 Key Findings

The three tropical seagrass species found in north west Western Australia demonstrated broad strategies for tolerance to short term light reductions, which is consistent with findings for other species and locations, including both tropical and temperate seagrasses (Lavery et al. 2009; Collier et al. 2012). We hypothesized that there would be threshold intensities and durations of light reduction indicating sub-lethal and lethal effects, and that the magnitude and time required for the response to occur will be species dependent. As expected, the light intensity and timeline for detectable impacts differed considerably amongst the three species. Here, we examined the range of response indicators (physiological–plant scale) across a gradient in light levels over time to determine the most appropriate indicator variables (bio-indicators) and then used these bio-indicators to identify at which intensity and duration of light reduction there was an impact to seagrasses. Furthermore, by applying treatments to a mixed species assemblage, a more realistic experimental approach to exploring the response of mixed species meadows, this study highlights how differences amongst these co-occurring tropical seagrass species could potentially influence dredging mitigation strategies in mixed seagrass assemblages, which are typical of the north west of WA.

4.1.1 Response

The three species showed clear photophysiological responses to reduced light availability, and these changes are consistent with photoacclimation in other seagrass species (Ralph et al. 1998; Schwarz & Hellblom 2002; Campbell et al. 2003; Ralph & Gademann 2005). Leaf absorbance and light capture efficiency ($\propto$) increased, and the rate of photosynthesis (ETR_{MAX}) and light intensity at which photosynthesis was half-saturated (E_k), decreased. These physiological photoacclimation responses to light reduction are typically driven by an increase in chlorophyll content and a decrease in the chlorophyll a:b ratio (Dennison & Alberte 1982, 1985; Abal et al. 1994; Collier et al. 2012). Such adjustments in light capture efficiency, and maintenance of photosynthetic carbon fixation under reduced light conditions, are frequently highlighted as important mechanisms with which seagrasses respond to reductions in light availability (Dennison & Alberte 1982, 1985; Ruiz & Romero 2001; Ralph et al. 2007; McMahon et al. 2013).

The capacity to store carbohydrates is an important and distinctive feature of many seagrasses, and their responses should be considered in terms of availability of storage reserves. The formation of carbohydrate reserves requires light to be near to saturation for sufficiently long periods (Alcoverro et al. 2001), so near-peak reserves were likely at the beginning of this experiment after an acclimation period of more than 6 weeks above saturating light intensities for all species. The substantial depletion (>50%) of stored soluble sugars (in *C. serrulata*) and soluble sugars and starch (in *H. uninervis*) during the experiment under moderately to severely reduced light conditions (4–41% I_{PAR}), reflects their importance for overcoming episodes of light reduction.

There were also plant nutritional changes with decreasing light availability, including an increase in leaf N, and a corresponding decrease in leaf CN ratio. Similar responses have been observed in *ex situ* experiments for several temperate and tropical species (Grice et al. 1996; Peralta et al. 2002). Under reduced photosynthesis, plants may continue to uptake nutrients even when growth is reduced due to some limiting factor, such as low light. However, assimilation of these nutrients, for example, via nitrate reductase activity, is energetically costly (Touchette & Burkholder 2000), thereby favouring storage of N. An important consequence of N storage at reduced light, and which has not yet been reported for seagrasses, is an increase in the $\delta^{15}N$ signature (at low 23% I_{PAR} and very low 11% I_{PAR} light), indicating ^{15}N enrichment relative to higher light levels and severe low light. Enrichment under reduced light has been reported in phytoplankton, where some diatoms can store three times more nitrate at low growth rates (in low light) than at high growth rates (in high light) (Needoba & Harrison 2004). Needoba & Harrison (2004) suggested the higher internal nitrate pool in the low light cultures results in a higher uptake rate-to-assimilation rate ratio (relative to high light cultures), causing an increase in the internal pool. The benefits of storing a large internal nitrate pool, which may be applicable to these species of seagrass,

could include surviving low nitrate concentrations in a disturbed or patchy environment both in terms of light and nutrient availability. However, under severe low light, it appears all seagrass species discriminate against uptake of the heavier $\delta^{15}N$, suggesting nutrient uptake ceases completely. Another explanation for the differences in leaf N concentration is that reduced N concentrations at higher light intensities could indicate N limitation (<1.8% DW, Duarte 1990). However, continued growth of plants over the experiment at higher light levels suggests that there was faster utilisation of N than uptake, so that stored N resources in leaves were gradually depleted during growth, but may not be limiting in the environment.

Leaf $\delta^{13}C$ showed a strong and expected response – as light becomes more limiting, carbon availability becomes less limiting to photosynthesis, and so the heavier isotope is discriminated against leading to a lower or more negative $\delta^{13}C$ value (Cooper & DeNiro 1989; Ralph et al. 2007). Our values for control plants were at the mid-low range of those reported in the literature (see Hemminga & Mateo 1996; Fourqurean et al. 2015), with light shading treatment lowering $\delta^{13}C$ values to levels beyond what has been reported in the literature for these three species.

Under severely shaded conditions (4% I_{PAR}), leaf area, number of leaves per shoot, and the above-ground biomass were reduced relative to controls, suggesting an impact from this shading intensity, with plants trending towards complete loss. By maintaining photosynthetically active material in the moderate to very low light intensities plants improve light capture. However, because seagrasses fix less carbon under reduced light conditions, survival under these sub-optimal conditions requires adjustments to the carbon-budget to maintain a positive balance. Under moderate to very low light intensities it appears all species were able to draw on significant rhizome carbohydrate reserves to maintain a positive carbon balance. However, despite leaf material remaining photosynthetically active for plants grown at 4% I_{PAR}, at the reduced photosynthetic rates they could not balance the high rates of respiration, and therefore were too energetically costly to maintain (Fourqurean & Zieman 1991).

In severe low light conditions (4% I_{PAR}), the seagrass abundance and assemblage structure shifted from one of high abundance, with all species present, to an assemblage that had an overall reduced abundance (total biomass), and dominated by only two species (*H. uninervis*, *C. serrulata*). This decrease in diversity, and increase in species dominance, appeared to be driven by a collapse of *H. ovalis*. Because severe and prolonged light reduction can occur during dredging operations we would have expected to see a shift in species assemblage away from a species-rich bed to one dominated by fewer or a single tolerant species. However, in this study, for at least three months of severe low light, both *C. serrulata* and *H. uninervis* persisted, albeit at a reduced abundance.

4.1.2 Timescales of response

The light stress–response pathway occurred in the following order; increased light capture efficiency and reduced photosynthesis, reduction in carbohydrate concentrations due to remobilization from storage, reduced production and biomass, and overall reduced species biomass. In general, the time taken for each seagrass species to respond to light reduction was dependent on the shading intensity. The lowest light treatment, 4% I_{PAR} (0.9 mol quanta m^{-2} d^{-1}) tended to impact physiology and growth within three weeks (our first sampling time) for all species. However, at higher light intensities early growth responses were not evident across all species. For example, new shoot production rate was not significantly affected for *C. serrulata* by light levels greater than 4% I_{PAR}, but for *H. ovalis* decreased at 3 weeks in 23% I_{PAR} or less (< 5 mol quanta m^{-2} d^{-1}) and for *H. uninervis* at 41% I_{PAR} or less (8.9 mol quanta m^{-2} d^{-1}). Interestingly, storage carbohydrates for *C. serrulata* and *H. uninervis* in this study were much higher than in other mesocosm studies using the same species (e.g. Collier et al. 2012). For *C. serrulata*, carbohydrates provide a greater capacity to withstand the effects of shading and could be a causal reason behind the slow above-ground growth and biomass response to shading. While *H. uninervis* also had relatively high carbohydrate reserves, compared to *C. serrulata*, it is considered a weak integrator across different ramets (Ooi et al. 2011), which may explain its earlier responses to light reductions.

Although we did not measure photophysiology parameters over a timescale shorter than 3 weeks, changes can

occur within seconds to minutes following changes in light availability (Beer et al. 2006). Under prolonged light limitation, these changes can be sustained to maintain carbon capture efficiency (Schwarz et al. 2000) and allow plants to persist for weeks to months (Table 12). Greater assimilation of the lighter carbon isotope from 6 weeks onwards suggests that all species were adapting to reduced light conditions to maintain carbon capture at a lower energetic cost. In this study, responses were detected within 3 weeks for all photophysiological variables, though the plants continued to show responses beyond that, up to at least 12 weeks, indicating that they do not necessarily move rapidly to a stable alternative physiological condition.

The rapid onset of utilisation and continuous depletion of rhizome carbohydrates prolonged seagrass survival under reduced light conditions. Carbohydrate reserves can be re-allocated from storage organs within days for fast-growing species, but can be exhausted quickly (Longstaff et al. 1999). The importance of carbohydrate reserves is greater for seagrasses with a larger belowground biomass, such as *Thalassia* and *Posidonia*, due to their enhanced capacity to store carbohydrates (Czerny & Dunton 1995; Longstaff et al. 1999). Large species like *Posidonia* and *Amphibolis* can persist for several months (Collier et al. 2009; Lavery et al. 2009), whereas seagrasses such as *Halophila* and *Halodule* tend not to be able to survive for long periods because they have small rhizomes and therefore less capacity to store carbohydrates (Table 12).

New shoot production rate was affected after 3 weeks under reduced light levels and was evident for all species. In contrast, there were inconsistent changes in shoot density across species with reduced light levels. This suggests that, at the plant scale, changes in the rate of growth could potentially be an early warning indicator compared to absolute abundance of shoots at any one time. This is consistent with previous findings on four tropical seagrass species from eastern Australia, where low light stress altered leaf growth rates for each species within less than 10 days, whereas shoot density showed decline 40 days later (Collier et al. 2012).

Table 12 Time period that seagrass species can *survive* under light availabilities below their minimum light requirements (as indicated for each species in Table 1), expressed as % of surface irradiance (SI). When applicable, daily integrated photosynthetic photon flux density mol m^{-2} is also included.

Species	Light availability % (I_{PAR})	Daily integrated irradiance (mol m^{-2} d^{-1})	Period survived (months)	Reference
Halodule pinifolia	0–0.16	0–0.175	3*	Longstaff & Dennison (1999)
Halodule wrightii	0	0	<2*	Biber et al. (2009)
Halophila ovalis	0	0	1*	Longstaff et al. (1999)
Halophila ovalis	0–0.16	0–0.175	1*	Longstaff & Dennison (1999)
Zostera marina	0–N/A	0–3.02	2–3.5*	Biber et al. (2009)
Heterozostera tasmanica	2		2–4*	Bulthius (1983)
Zostera noltii	1	0.6	0.5*	Peralta et al. (2002)
Halodule uninervis	5		>1#	Grice et al. 1996)
Halophila spinulosa	5		>1#	Grice et al. (1996)
Posidonia sinuosa	<5	0.2–1.6	>7#	Collier et al. (2009)
Syringodium isoetifolium	5		>1#	Grice et al. (1996)
Zostera capricorni	5		>1#	Grice et al. (1996)
Zostera capricornii	5		>2#	Abal et al. (1994)
Heterozostera tasmanica	9		10*	Bulthius (1983)
Posidonia australis	<10		9*	Fitzpatrick & Kirkman (1995)
Thalassia testudinum	10	3.5	11*	Czerny & Dunton (1995)
Posidonia oceanica	10.4	1.75–4.9	4*	Ruiz & Romero (2001)
Posidonia sinuosa	12% ambient		24*	Gordon et al. (1994)
Amphibolis griffithii	5–19% ambient	0.9–7.4	9*	Lavery et al. (2009)
Halodule wrightii	13-15	4.5–5.6	9*	Czerny & Dunton (1995)

Experiment ended before complete mortality

* Experiment continued until complete mortality

4.1.3 Species differences

All species responded to light reductions, but the timing and magnitude of response differed considerably amongst the species. *H. uninervis* was the most responsive to light reduction followed by *H. ovalis* then *C. serrulata*. *H. uninervis* had lower shoot production and biomass after 3 weeks at moderate light intensities, 41% I_{PAR} (8.9 mol quanta m^{-2} d^{-1}), *H. ovalis* had reduced shoot production and biomass when light intensities were at 11% I_{PAR} (5 mol quanta m^{-2} d^{-1}), whereas *C. serrulata* showed reduced biomass and shoot production at 11 and 4% I_{PAR} (2.3 and 0.9 mol quanta m^{-2} d^{-1}), respectively. For these species, the differences in the magnitude and rate of response likely reflect the differences in carbohydrate storage reserves.

The growth response of *C. serrulata* to light reduction was much less pronounced than that of *H. uninervis* and *H. ovalis*, and occurred more slowly. This lag in response could be related to greater availability of carbohydrates in *C. serrulata*. *C. serrulata* has larger rhizome biomass than both *H. uninervis* and *H. ovalis*, increasing its capacity for carbohydrate storage. We recorded high concentrations of carbohydrates within *C. serrulata* compared to both *H. uninervis* and *H. ovalis*. The high concentrations and utilisation of carbohydrates prolonged the time before any morphological adjustments were needed to compensate for reduced carbon fixation under low light conditions (i.e. it provided a 'buffer'). Interestingly, the rapid growth response of *H. uninervis* to reduced light availability could also reflect the reduced concentration of soluble carbohydrates relative to starch when compared to both *H. ovalis* and *C. serrulata*. Soluble carbohydrates are mobilized more readily and are less energetically costly to hydrolize than starch (Touchette & Burkholder 2000). Eventually, *H. uninervis* begun starch utilisation at 6 weeks compared to 3 weeks for soluble carbohydrates. With a lower availability of soluble carbohydrates, *H. uninervis* potentially exhausted these storage reserves rapidly, and thus had to switch to more energetically costly mobilisation of starch. This could have prompted early structural changes (i.e. reduce leaf biomass and shoot production) to limit the carbon demand from metabolically active plant material.

4.2 Bio-indicators

A robust monitoring program will benefit from having a range of indicators that can: (1) provide early warning detection of altered conditions, (2) indicate sub-lethal stress that warns of forthcoming loss, and (3) appropriate indicators of plant-scale changes for defining and explaining the ecological impacts. In controlled laboratory conditions we tested 18 variables of seagrass status, ranging from early warning, sub-lethal (physiological), through to plant-scale (or state change) losses, which were broadly sorted into 5 categories (Figure 1, Table 2). These indicators were chosen because they are expected to respond to light reduction at different temporal scales, with sub-lethal, physiological indicators able to respond from seconds to months thereby acting as early warning and sub-lethal indicators, while detection of the plant-scale effects usually take many weeks to months (McMahon et al. 2013).

This experiment identified five response variables which clearly and consistently responded to light reduction in at least two of the three species, and should be relatively robust bio-indicators). Combined, these included indicators from four out of the five categories of variable we measured (Table 2):

- photophysiological: maximum photosynthetic rate (ETR_{MAX}) and increased efficiency of the photosystems (AF),
- physiological: rhizome carbohydrate concentration and leaf N,
- morphological: leaf area, and
- growth/biomass: shoot production and above-ground biomass (at the plant-scale).

However, the bio-indicator for the abundance category (i.e. biomass at the pot level for all species combined) did not show a consistent direction of response to the magnitude and duration of light reduction. This is likely because of the observed variation in response of biomass between species, and in particular, as a result of the collapse of *H. ovalis* mid-way through the experiment. Nonetheless, the similar direction of response for these variables follows patterns observed for other species of seagrass (McMahon et al. 2013), supporting their use as relevant and appropriate bio-indicators. We recommend that an ideal complement of bio-indicators would

include: (1) photosynthetic variables (ETR_{MAX}) which respond very early to light reduction and reflect changes at the photophysiological scale, (2) rhizome carbohydrates, leaf N, mean leaf area, and shoot production, which respond over longer time-scales and reflect sub-lethal changes at the scale of the ramet, and (3) shoot density or above-ground biomass, which reflects changes at the plant-scale.

While the above variables are potentially useful as bio-indicators, in the following, we have included recommendations and cautionary notes for some of these bio-indicators, as well other variables that show potential. They will require careful consideration before their incorporation into monitoring programs. We do not recommend the use of AF at this time, as this variable has similar attributes to the photosynthetic parameters ($\propto$, E_k), such that appropriate assessment will require incorporating other untested (this study) and less responsive variables (McMahon et al. 2013). Many other response variables, for example photosynthetic efficiency, half-saturating irradiance (E_k), carbon isotope ratio ($\delta\,^{13}C$), nitrogen isotope ratio ($\delta\,^{15}N$), rhizome sugars and starch, and shoot density, show potential for use as bio-indicators for at least one of the species tested. Photosynthetic parameters are quick to respond to light reduction (Beer et al. 2006), and can be interpreted as the plant increasing light capture efficiency by increasing the number and/or size of chlorophyll *a* pigments, thereby reducing the amount of light required to saturate photosynthesis. Such adaptive responses can change with increasing magnitude of light reduction up to a maximum, where pigment light absorption efficiency decreases non-linearly as pigment content per unit area increases. However, a change in seagrass optical properties (leaf thickness, specific leaf area) as a result of light reduction can also influence light absorption and potentially counterbalance pigment self-shading (Enriquez 2005). While this requires further validation for many species of seagrass, it does suggest that the potential usefulness of $\propto$ and E_k as early warning indicators cannot be appropriately assessed without incorporating other interacting variables.

Similarly, isotopic signatures will also require an understanding of background conditions, as well as plant or site history. For example, under light-limited conditions carbon availability becomes less limiting to photosynthesis and the heavier C isotope (^{13}C) is more strongly discriminated against, leading to lower $\delta^{13}C$ values (Cooper & DeNiro 1989; Ralph et al. 2007). However, to accurately detect this requires knowledge of the rate of leaf tissue replacement. For fast-growing tropical species, leaf turnover rates are typically high under optimal conditions but are reduced under low light conditions, prolonging detection of the response. In addition, if the plants reallocate stored carbohydrates to assist growth (as observed in this experiment), then the shoot $\delta^{13}C$ signal will reflect $\delta^{13}C$ signature of carbon that was assimilated under previous light conditions, potentially masking the response. Since changes in shoot $\delta^{13}C$ occurred after a long exposure (6 weeks) to low light it is unlikely to be a useful early warning indicator of reduced light, but would be a good indicator of long term effects of low light and recovery post-dredging.

Starch, rather than soluble carbohydrates, was the most abundant energy storage compound in rhizomes of *H. uninervis*. Starch may therefore be a useful bio-indicator for this species. However, under severe light reduction, starch utilisation may be inhibited under anaerobic conditions (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.

In some variables, there was either a different direction of response than anticipated, or there was no response where one would be expected – but these were easily explained. For example, the $\delta\,^{15}N$ signature at low (23% I_{PAR}) and very low (11% I_{PAR}) light, indicated ^{15}N enrichment relative to higher light levels and severe low light (4% I_{PAR}). Enrichment under reduced light has been reported in phytoplankton, where the higher internal nitrate pool in the low light cultures results in a higher uptake rate-to-assimilation rate ratio (relative to high light cultures), causing an increase in the internal N pool (Neeboda & Harrison 2004). The benefits of storing a large internal nitrate pool, which may be directly applicable to these species of seagrass, could include surviving low nitrate concentrations in a disturbed or patchy environment both in terms of light and nutrient availability. Under severe low light however, it appears all seagrass species discriminate against uptake of the heavier $\delta^{15}N$ suggesting nutrient uptake ceases completely, and with a signature reflecting that of controls, potentially confounds its use as a bio-indicator.

Shoot density for *C. serrulata* was non-responsive to light reduction, and there was only a small change in biomass after 12 weeks despite a reasonable theoretical expectation it should decline (Collier et al. 2012; McMahon et al. 2013). Even after 3 months of severe light limitation shoot densities remained unchanged. *C. serrulata* is considered the slowest growing species in our experiment and may not have responded consistently to light reduction as the duration of light reduction may have been insufficient to cause an effect. In addition, the inherent size of each species and available pot area meant that there were differences in starting shoot densities. For *C. serrulata*, the large internode distance between shoots resulted in relatively low starting shoot densities compared to *H. uninervis*, and such low shoot numbers may have influenced our ability to detect a change in shoot survival. In addition, physiological integration amongst shoots within a ramet, and therefore the potential for translocation of carbohydrate resources within the plant, may have been greater in *C. serrulata* compared to the other species. Rhizome carbohydrate concentrations of *C. serrulata* were high relative to *H. uninervis* and *H. ovalis* such that resources were readily available, thereby maintaining shoot densities similar to that of higher light treatments.

The long-term health of one species, *H. ovalis* in our tank system was questionable, hampering our ability to identify appropriate bio-indicators for this species beyond 6 weeks of the shading experiment. Across all tanks *H. ovalis* grew exceptionally well and was in good health for the 6 week acclimation period (before the experiment began), and then for the first 6 weeks of the experimental light reduction. The biomass in the controls also remained constant. However, after this time, controls and all treatments showed a consistent decline in biomass. Under field conditions, this species shows rapid growth and ramets are typically short-lived. For *H. ovalis*, the long term acclimation period may be unnecessary and clearly influenced our ability to appropriately assess the range of response variables for this species over time. Further experiments with *H. ovalis* should reduce the acclimation period to two weeks (the time we observed new shoot production for this species), suggesting plants were established and growing in our tank systems.

4.3 Thresholds

Using a percentiles approach we found that we could calculate the magnitude of sub-lethal and lethal light thresholds and duration (Table 13). 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 compliance (e.g. dredging). However, some precautions are recommended. For early warning indicators (e.g. ETR_{MAX}) of seagrass stress, a light-based trigger value for impacts on one particular species will likely be appropriate to encompass responses of all species. That is all species showed similar magnitude of photophysiological responses and timescales of responses, indicating that the plants were making physiological adjustments to avoid an imminent effect of light reduction. For sub-lethal indicators, we suggest species-specific trigger values would be more appropriate since *C. serrulata* (soluble sugars) and *H. uninervis* (starch) utilised different forms of rhizome non-structural carbohydrates as an energy source to cope with light reduction and, therefore, the rate at which each form is utilized differed between species (3 weeks for soluble sugars in *C. serrulata* and 6 weeks for starch in *H. uninervis*). On the other hand, trigger values for lethal impacts 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. For example, the threshold trigger value for lethal impacts (above-ground biomass) for all species combined was ≤8.9 mol quanta m^{-2} d^{-1} after 3 weeks, and ≤13 mol quanta m^{-2} d^{-1} after 6 weeks, but for *H. uninervis* alone the trigger value was ≤2.3 mol quanta m^{-2} d^{-1} after 12 weeks. 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. 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.

When deciding appropriate light thresholds to apply under circumstances where there are mixed seagrass assemblages, it may be necessary to assess the dominance, ecological and economic-value of each species, along with the capacity of each species to recover (and timeframes of recovery) if they were to be impacted. On the other hand, trigger values for lethal impacts are best viewed at the level of the seagrass assemblage rather than assigned to individual species (Posthuma et al. 2002).

Table 13: Threshold values based on magnitude and duration of light reductions for Sub-lethal (ETR_{MAX} and Rhizome total carbohydrates) and Lethal (above-ground biomass for individual species or total biomass of all species pooled) indicators of light reduction stress in three north west Western Australian seagrass species

Species	ETR_{MAX} PAR		Total Rhizome Carbohydrates PAR		Above Ground Biomass PAR		Total Biomass PPFD	
	mol m^{-2} d^{-1}	Wks	mol m^{-2} d^{-1}	Wks	mol m^{-2} d^{-1}	Wks	mol m^{-2} d^{-1}	Wks
Cymodocea serrulata								
	5.0	3	2.3	3	8.9	12	NA	
			8.9	9				
Halodule uninervis								
	5.0	3	5.0	3	0.9	6	NA	
	8.9	6	8.9	9	2.3	12	NA	
	13.1	12	13.1	12				
Halophila ovalis								
	0.9	3	NA		0.9	3	NA	
	2.3	9						
All species							5	6
							8.9	9

5 Conclusion

We examined a range of response indicators (physiological – plant scale) across a gradient in light levels over time to determine firstly, which variables could be used as robust indicators to evaluate change and secondly, what are the threshold intensities and durations of light reduction that trigger sub-lethal and lethal responses for each species within a mixed seagrass assemblage. We hypothesized that there will be a threshold intensity and duration of light reduction indicating sub-lethal and lethal effects, and that the magnitude and time required for the response to occur will depend upon species. Despite all species showing a similar capacity to photoacclimate to reduced light conditions, the intensity and timescale for changes in photophysiology, physiology, growth, biomass and morphology differed among species. Out of 18 potential bio-indicator variables, we identified four as robust bio-indicators across sub-lethal to lethal scales of response as well as several others for consideration. Subsequently, we used the four variables to develop trigger values (based on percentile differences from controls) and determine the magnitude and duration of light reduction at which these trigger values were exceeded. While we anticipate that these findings can contribute to the development of water quality guidelines for tropical seagrasses, in particular, in relation to short-term water quality compliance (e.g. dredging) in NW Western Australia, we recommend a conservative interpretation of the thresholds is warranted; that is, protection of seagrass is more likely to be achieved by assuming effects occur at higher daily light intensities and at shorter times of light reductions. These light thresholds were developed under experimental tank conditions with constant light availability and the transferability of these thresholds to *in situ* habitats where daily light fluctuates, both naturally and under dredging operations, is a critical knowledge gap that requires further investigation.

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