



## Sediment burial stress response, bio-indicators and thresholds for a tropical multi-species seagrass assemblage

John Statton<sup>1,3</sup>, Kathryn McMahon<sup>2,3</sup>, Roisin McCallum<sup>2,3</sup>, Gary Kendrick<sup>1,3</sup>, Paul Lavery<sup>2,3</sup>

<sup>1</sup> The Oceans Institute, The University of Western Australia, Crawley, Western Australia, Australia

<sup>2</sup> Edith Cowan University, Joondalup, Western Australia, Australia

<sup>3</sup> Western Australian Marine Science Institution (WAMSI), Perth, Western Australia, Australia

### 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.

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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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**Corresponding author and Institution:** J Statton (UWA). Email address: [john.statton@uwa.edu.au](mailto:john.statton@uwa.edu.au)

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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: Vertical rhizome growth of *Halodule uninervis* after sediment burial (burial sediments have been removed to expose vertical rhizomes). (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: *Halophila ovalis* meadow in the Pilbara region of Western Australia. (Source: Kathryn McMahon)

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## Executive Summary

Changes to the amount of light and burial by the deposition of suspended sediments are presumed to be major mechanisms by which dredging can impact seagrasses. This report presents findings from a controlled burial experiment that aimed to determine the effects of burial by sediments on the growth of two seagrass species found in the northwest of Western Australia (NW WA). The report provides guidance and protocols for the application of the research outputs (e.g. seagrass burial stress:response relationships, sub-lethal and lethal bio-indicators and thresholds) to the prediction, assessment, monitoring and management of dredging programs in NW WA.

To test the effect of burial depth and duration on co-occurring tropical seagrass species we established pots containing mixed assemblages of two seagrasses that commonly co-occur in the northwest of Western Australia, *Halodule uninervis* and *Cymodocea serrulata*. Under climate-controlled tank conditions, replicate pots were subjected to five burial depth treatments (0, 8, 16, 40 and 70 mm), mimicking a range of heights that were predicted to occur close to dredging operations in WAMSI DSN Project 5.1.2 (McMahon et al. 2017) as well as covering a range expected to elicit a mortality response in tropical seagrasses determined from published research (Cabaco 2008, McMahon et al. 2017). We ran the experiment for 14 weeks, harvesting and measuring plant condition at 3, 6 and 14 weeks after sediment addition.

Three separate but linked components were used to develop sub-lethal and lethal bio-indicators and burial threshold values:

1. Under the imposed burial stress, we determined the cause-effect pathway from measurements of 17 response variables;
2. From (1), we identified those response variables that showed a consistent direction and magnitude of response with increasing duration and depth of burial to determine the most appropriate bio-indicators for use in monitoring programs; and
3. From these bio-indicators, we determined sub-lethal and lethal stress thresholds for intensity and duration of burial.

The key findings were:

- Burial depths up to 40 mm had little effect on either species of seagrass. At burial depths greater than 40 mm, burial resulted in significant effects on plant physiology, productivity, morphology and biomass, though the effects depended on the duration of burial as well as the species of seagrass.
- We identified one robust bio-indicator, vertical rhizome growth, that is appropriate for immediate incorporation into monitoring programs to identify sub-lethal burial impacts on a tropical seagrass assemblage. This has been selected based on its sensitivity and speed of response, applicability to both species studied, and consistency in direction of response with magnitude and duration of burial. However, the rate of response differed between both species. On this basis, extrapolation of results from one species to another, or using one species as a surrogate for many (i.e. a mixed assemblage), which has been normal practice, may lead to erroneous conclusions and should be avoided where possible.
- Where mixed species assemblages occur, a whole assemblage approach is warranted rather than targeting any one species, unless protection of ecological values associated with an individual species is a management goal. We have shown that *C. serrulata* is more sensitive to burial than *H. uninervis*, due to the differences in the rate of vertical rhizome elongation.
- Importantly, this bio-indicator could be useful for dredging monitoring and management programs that aim to differentiate between impacts related to burial stress from impacts related to light reduction, as this response is not elicited consistently under light reduction.

- We found that we could calculate the magnitude and duration of burial stress that caused a sub-lethal response in *C. serrulata* (>40 mm for >14 weeks) but not *H. uninervis* since plants did not show sub-lethal effects at the greatest burial depth imposed in this experiment (70 mm for 14 weeks). In addition, due to both species' rapid adaptive response to burial under the imposed light and sediment conditions, we were unable to define lethal levels of burial.

This work has led to the identification of a robust bio-indicator of sub-lethal stress that would be appropriate for use in monitoring programs and which, importantly, is distinct from sub-lethal indicators of light stress. This research also identified species-specific burial threshold values that are time dependent, under which management actions and alerts could be proposed to ensure appropriate steps are taken 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 framework outlined in the EPA'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 monitoring 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**

Impact prediction will require an understanding of the baseline sediment deposition and net burial rates at a site together with prediction of the additional pressure that will result from dredging. This, and previous studies, have highlighted that there is currently almost no field data on background burial pressure or those induced by dredging relevant to seagrasses. Pre-development surveys offer the opportunity to characterise the background natural sediment bed load, sediment dynamics (deposition, resuspension) and composition (organic content, pollutants, particle size distribution) as well as seagrass species responses to previous sedimentation history (i.e. identified by vertical rhizome elongation) that are necessary to determine appropriate thresholds to apply in impact prediction.

### **Impact Assessment**

On the basis of the plant responses we observed in our experiments and the likelihood that our burial treatments probably represent extreme events in the dredging far-field, **we conclude, therefore, that the sorts of maximum burial rates that can be estimated from field data may pose a relatively low pressure to seagrasses.**

Sediment burial levels of up to 40 mm for 14 weeks have little effect on either of the seagrass species in this study, while burial at depths of 70 mm had sub-lethal effects on *C. serrulata*. On this basis, conservative sub-lethal **tolerance thresholds for the two species can be estimated as:**

***Cymodocea serrulata*: More than 40 mm of burial for more than 14 weeks; and**

***Halodule uninervis*: More than 70 mm burial for more than 14 weeks.**

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* is more representative of colonising species with faster growth rates and smaller storage reserves. Our study clearly showed species-dependent differences in response and so **extrapolating the findings for these two species to other species is not advised**. However, in the absence of any other data, two approaches could be taken to estimate tolerances for other species:

- 1) apply a conservative approach and use the threshold for *C. serrulata* in all cases; or
- 2) take a less conservative approach and 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.

The complete absence of accurate field-based data on burial pressures makes it difficult to place the results into a meaningful context. To interpret the results, we have used estimates of field burial rates, derived from sediment trap data (McMahon et al. 2017). Sediment trap data have the limitation that they do not incorporate any resuspension of material and, therefore, they represent the gross sediment deposition. Nonetheless, on the basis of those estimates, the burial depths applied in this experiment are likely to reflect the upper range of burial depths seagrasses might experience in the field near dredging projects. Similarly, these conditions might reasonably be expected on the periphery of dredge spoil disposal sites (noting that deposition can exceed metres within spoil grounds). Natural net sediment deposition rates are highly variable (McMahon et al. 2017), and this is also reflected in different dredging campaigns where burial rates increased from 2.5 – 19 times. Similarly, measured over shorter timescales, sediment height loggers deployed in Cockburn Sound, Perth, W.A., detected changes in sediment height in the order of 1 – 8 mm hr<sup>-1</sup> (Chisholm 2009). In NW WA, across three different dredging projects, estimated average burial rates were 0.1 – 3.0 mm d<sup>-1</sup>, although some of these rates may have been influenced by post-cyclonic conditions. We observed impacts on *Cymodocea serrulata* at burial levels of 40 mm but only after 14 weeks. Therefore to reach this threshold of impact would require a minimum of 14 days of deposition with no resuspension, and for this to persist for 98 days, so a total of 3.5 – 4 months at the upper end of the average burial range. At maximum estimated burial rates from the NW WA data (0.47 – 25.4 mm d<sup>-1</sup>), it would require a minimum of 2 days of deposition persisting for 98 days, so a total of 3 months across the maximum burial range. It should be noted that data for periods of cyclone activity were removed from this analysis, though some of the upper values may have been influenced by post-cyclonic conditions).

Despite the likelihood that dredging-induced burial is unlikely to be a major stress to seagrasses in the far-field plume region, our study points to possible consequences of this sub-lethal pressure for the ecological function of seagrass meadows:

1. **Sub-lethal burial stress could drive a change in seagrass diversity.** Our findings suggest that *Halodule uninervis*, with its rapid vertical elongation rates, is able to escape burial and is likely to cope with dredging-induced burial pressures. *Cymodocea serrulata* is representative of slower growing species that cannot respond as quickly and are likely to be impacted if the burial pressure persists for extended periods, in this case for 3 – 4 months. 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 elicit a rapid response and hence better cope with increased levels of sediment deposition;
2. **Burial may have implications for the forage value of seagrasses,** especially *H. uninervis*, which is an important food source for dugongs. For *H. uninervis*, starch concentrations accounted for up to 23% of rhizome dry weight, but were significantly lower in the 40 mm and 70 mm burial treatments after 3 – 6 weeks. While the concentrations subsequently recovered, the results indicate that there may be a period of reduced carbohydrate reserves while the plant is investing resources into vertical growth. This suggests that the forage value of *H. uninervis* may be reduced during the period the plant is responding to sub-lethal burial pressures.

## **Post-Approval**

Due to the very consistent response of the vertical rhizome, this is a useful and specific bio-indicator, identifying that the plant is responding to burial stress. This would be particularly useful where dredging monitoring programs seek to differentiate between stress induced by burial or by light reduction. Four consistent bio-indicators relating to light reduction have been determined in the WAMSI Dredging Science Node project 5.5.1 that can be used in combination with the bio-indicator for sublethal burial stress. This would provide confidence to dredging operators about the source of pressure that is generating stress and allow them to adjust their operations accordingly. These stressor-specific bio-indicators can also provide confidence to resource managers that any management that may be required are appropriately targeted. Note however, that these findings relate the type of sediment and light intensities applied in this study and it needs to be confirmed whether they are robust across a range of sediment types and light intensities, an issue investigated in WAMSI DSN Project 5.5.4 (Statton et al. 2017)

## **Residual Knowledge Gaps**

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

### ***In situ* burial stress fields**

At this point in time, there are inadequate field data to accurately characterise burial pressure that seagrasses are exposed to, both with and without dredging pressure. While we conclude that the sorts of maximum burial rates estimated from field data may pose a relatively low pressure to seagrasses, there is very little understanding of whether the burial pressures occurring in field sites can exceed the limits of tolerance that we suggest here, or for how long. This remains a very significant gap in our knowledge of seagrass ecosystems and limits the ability to meaningfully apply the experimentally-derived threshold developed here in the framework of EAG7.

There is still uncertainty as to whether a single depositional event, such as that imposed in this and other experimental studies, is the sort of stress experienced by seagrasses around dredging and spoil disposal operations, and whether the laboratory-derived, single dose-response thresholds derived from them, are transferable to a dredging context. During dredging operations, sediments are more likely to accumulate over time, except for the case of dredge spoil dumping, or in locations very close to the dredging activity (near-field). Unfortunately, at this time most field data relating to burial pressures are from sediment traps which integrate over 5 – 118 days (McMahon et al. 2017) and do not allow resuspension (Storlazzi et al. 2011) and so provide a gross deposition over timescales of less than a week to a number of month. Sediment height loggers have detected changes in sediment height in the order of 1 – 8 mm hr<sup>-1</sup> but this has been undertaken in temperate locations in W.A. (Chisholm 2009) and sediments and hydrodynamics are likely to differ from NW WA. Alternatively, there are water column suspended sediment concentrations but using these to estimate burial rates requires a significant number of assumptions regarding sediment density, resuspension and the persistence of those water column concentrations. Consequently, we are unable to reliably estimate either the net burial depths that seagrasses are subjected to near dredging projects or the rate at which this burial occurs.

### **Interactive effects of burial and other pressures**

Our study was a mesocosm-based experiment in which burial pressure was addressed in isolation. In reality, burial due to dredging will usually be accompanied by simultaneous reductions in light availability and with sediments of varying composition (e.g. organic matter content, heavy metals, or anoxia). Other pressures could include grazing by dugong; globally, herbivorous mammals such as dugongs remove an average of 46% of plant biomass in a seagrass meadow (Wood et al. 2016), which would represent a significant additional stress on seagrasses attempting to cope with light reduction and sediment burial stresses. The interactive effects of burial and light reduction, and/or burial and sediment composition could be different to those of burial alone, and warrants further investigation.

## 1 Introduction

In many coastal ecosystems, burial of seagrasses is a recurrent event whether from storms, riverine inputs or bioturbating fauna (see review Cabaco et al. 2008). In these systems, burial has strong selective pressures towards species that can tolerate considerable sedimentation. As such the seagrasses living there have developed adaptations to persist with the prevailing environmental conditions (Duarte 1997, Ooi et al. 2011, Hovey et al. 2015). Other events that increase the amount of additional sediment to the system may expose seagrasses to further burial stress, outside their ecological range of tolerance. For example, following release of dredged sediment into the water column these suspended particles may settle and potentially bury seagrasses. However, under natural conditions sediment may be resuspended and deposited, with the rate of these processes dependent on the size and density of sediment particles, and local hydrodynamics (Middleton & Southard 1984). Yet, seagrasses are very effective at trapping sediments (Madsen et al. 2001), specifically through reducing resuspension once the particles are within the canopy (Gacia and Duarte 2001). Over time, direct settling of sediment particles, or secondary resuspension and deposition processes, can result in the accumulation of sediment in the seagrass meadow. Although direct measurements of sediment deposition and resuspension rates are lacking, some studies have provided estimates of the scale of sediment movement. For instance, during several dredging operations and at sites that were predicted to be impacted by increased sedimentation from dredging, estimated burial rates based on sediment trap data ranged from an average of 0.07–2.79 mm d<sup>-1</sup> with maximum rates ranging from 0.42–18.5 mm d<sup>-1</sup> (McMahon et al. 2017). These rates were estimated for non-cyclone, however the higher burial rates may have been influenced by the immediate post-cyclone conditions. In any case, the estimated rates serve to illustrate the levels of sediment movement that seagrasses are likely to experience. When measured over shorter timescales, sediment height loggers deployed in Cockburn Sound (Perth, WA), detected changes in sediment height in the order of 1–8 mm hr<sup>-1</sup> (Chisholm 2009). Consequently, an increase in sediment bed loads from marine dredging constitutes a hazard and potential risk to seagrasses worldwide (Orth et al. 2006). To tackle this problem, control measures can be introduced into dredging operations to lessen the impact on adjacent seagrass meadows (Erftemeijer and Robin Lewis III 2006). However, for most seagrass species, the thresholds of sediment burial and their potential for recovery are poorly understood, limiting the ability of coastal resource managers to recognize impacts and apply suitable mitigation strategies.

Many seagrass species are adapted to highly dynamic sedimentary environments, responding in ways that promote plant vigour during moderate burial events. For example, burial stimulated vertical growth, leaf elongation, and flowering in *Cymodocea nodosa* (Marba and Duarte, 1994, 1995) and vertical growth of *Thalassia testudinum*, *Cymodocea serrulata* and *Halodule uninervis* (Marba et al. 1994, Ooi et al. 2011). These changes can help alleviate moderate burial stress and for most species of seagrass studied so far, the strategies for tolerating small amounts of sediment burial are well known (Cabaco et al. 2008). However, deeper burial depths can cause adverse effects through a reduction in available photosynthetic area, anoxia from overlying sediments depleted of oxygen, and toxicity such as from sediments containing high levels of sulphides (see review Cabaco et al. 2008). Consequently, photosynthesis is inhibited forcing the plants to use energy resources stored within rhizomes (Alcoverro et al. 2001). These impacts are dependent on the extent (depth) of burial and also the spatial extent of the burial, because buried individuals may be supported by unaffected parts of the plants if they are connected over space (Cabaco et al. 2008, Ooi et al. 2011). Notwithstanding, the outcome of broad spatial-scale burial is mortality of shoots, a change in species composition and degradation or loss of seagrass habitat (Ooi et al. 2011, Cruz-Palacios and Van Tussenbroek 2005). However, the threshold levels of burial (both magnitude and duration of impact) leading to these responses, as well as the nature and magnitude of the adaptations are not clear for many seagrass species.

In NW Western Australia, the potential tolerance of seagrass to burial has been predicted for 7 out of 13 species (McMahon et al. 2017), with a burial of 8 cm identified as a critical level for some tropical species (Ooi et al. 2011). Tolerance to burial is considered to be size-dependent, with large genera such as *Enhalus* able to withstand greater sediment burial depths (Duarte et al. 1997, Cabaco et al. 2008), although species with the

ability to elongate vertical stems, such as *Cymodocea serrulata*, *Thalassia hemprichii*, *Syringodium isoetifolium* and *Halodule uninervis*, can overcome the impacts of sedimentation (Vermaat et al. 1995, Duarte et al. 1997, Ooi et al. 2011). However, this ability to elongate is dependent in some species on clonal integration, the sharing of resources within a ramet or connected piece of rhizome, and species such as *C. serrulata* and *S. isoetifolium* are more likely to elongate vertical stems if clonal integration is not present (Ooi et al. 2011). Therefore, in a dredging context, if a broad area is impacted and clonal integration is impaired, species would still have the ability to elongate their vertical stems. In addition, responses to sedimentation vary seasonally, so some species may be more resilient to sediment burial at different times of year (Vermaat et al. 1995). Currently, our understanding of the timescale for impact after burial of tropical seagrasses is based on just a few studies experimentally testing sediment burial and seagrass response over timescales that are either: temporally restricted and with a single sampling period (~ 1 month, Marba and Duarte 1994, Ooi et al. 2011) and, therefore, are unable to comment on longer term impacts; or very coarse timescales (2 – 10 months, Duarte et al. 1997), missing the dynamics of early plant responses. Subsequently, relevant timescales for the responses vary between weeks and up to months. To improve impact prediction and management of seagrasses during dredging operations, adequate data on seagrass tolerance and timescales of response after sedimentation is urgently required.

In this study, we examined the critical short-term (weeks) and long-term (months) response to a range of sediment burial depths, from levels expected to have a negligible impact to depths at which seagrasses would be adversely affected. The burial depth levels were pre-determined based on likely sediment burial depths plants would experience under dredging situations and the levels likely to induce a plant response in these species (Ooi et al. 2011). A review of field data from the NW of Australia (see McMahon et al. 2017) found no in situ sediment burial data so field sediment trap data were used to estimate burial rates. Average rates ranged between 0.13 – 2.73 mm d<sup>-1</sup> and maximum rates ranged between 0.47–25.4 mm d<sup>-1</sup> (assuming a bulk density of 0.7 g DW cm<sup>-3</sup>) near dredging operations, although some of these burial rates may have been influenced by post-cyclone conditions. Over several days to weeks, these levels could smother seagrasses in mm's to cm's of sediment. Published research indicated that tropical seagrasses generally show significant impacts at burial depths of 4 cm or more. Based on these estimates, a range of sediment burial depths were chosen: 0 mm, then 8, 16, 40 and 70 mm. These encompass depths approaching the minimum burial rates plants are likely to experience through to those likely to induce a mortality response, in order to determine lethal and sub-lethal thresholds. Here, we test the response of two co-occurring species of seagrasses to burial. *Halodule uninervis* is regarded as small, opportunistic/colonising species with fast growth rates and *Cymodocea serrulata*, a comparatively larger, colonising species (Kilminster et al. 2015), and both species are regarded as being capable of withstanding low-moderate levels of burial (Ooi et al. 2011). We hypothesized that low levels (<40 mm) of sediment burial would have minimal impact on photosynthesis, seagrass biomass, shoot density and survival. We also hypothesized that burial depths of 40 mm or greater would have a negative impact on physiology, biomass, shoot density and survival, and that the timescales of response, would be species-specific.

## 2 Methods

### 2.1 Plant collection

On the 27th of October 2014, the seagrasses *Cymodocea serrulata* (R. Brown) Ascherson and Magnus, *Halodule uninervis* (Forsskål) Ascherson (1882) were collected from Useless Loop (26° 6'59.12"S, 113°24'39.81"E) Shark Bay, Western Australia six and a half weeks prior to the beginning of the experiments. Both 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 the University of Western Australia's seagrass growth facility, Perth, Western Australia (1000 km or 12 h travel time). At the seagrass growth facility, ramets were prepared for planting. Preparation involved, firstly identifying ramets with one or more intact apical shoots and with at least three and up to 6 mature shoots. When a ramet had more than 6 mature shoots, additional shoots were removed using a sharp blade. If the apical shoot was damaged or missing, the ramet was discarded. On 30

October 2014, ramets were planted into square pots (28 cm sides × 30 cm deep). For each species 3 ramets were planted into each pot.

## 2.2 Experimental tank system, design and setup

Experiments were conducted in large 12 × 1800 L rectangular, fibreglass reinforced tanks. Each tank was a closed, recirculating system, with seawater recirculating from a 600 L reservoir beneath each tank. Natural seawater from a nearby unpolluted area was used to fill each tank system, with one quarter exchanges every two weeks throughout the experimental period. Seawater was circulated using an 8000 L h<sup>-1</sup> submersible pump, allowing complete replacement of water in the system 80 × per day. Within each tank, incoming seawater was spread through a diffuser (T-bar) in order to create a homogenous movement of water. The seagrass research facility received natural sunlight and is temperature controlled, set at 27°C. Seawater quality was controlled through continuous chemical and mechanical filtration. Salinity levels were monitored daily and adjusted via addition of deionised water.

To test the effect of burial depth and duration on co-occurring tropical seagrass species we installed 28 square plastic pots (28 L × 28 W × 30 D cm) in each tank. Pots contained washed river sand with 0.5% DW organic matter added in the form of dried, ground *Posidonia* seagrass leaves (see Statton et al. 2013 for further details). Plants were acclimated for approximately six weeks (42 d), at a temperature of 27°C, salinity of 36–37 ppt, and ambient light. After the acclimation period, 5 sediment burial depth treatments were applied on 12<sup>th</sup> December 2014 by adding fine (median grain size: 90 µm, grain size range: 63–120 µm), clean inorganic silica sand over the plants (Rocla Quarries Pty. Ltd., Perth W.A.). This type of sediment and range in sediment grain-size was chosen for three reasons; (i) to reflect as close as possible the fineness of sediments typically deposited within seagrass meadows found in WAMSI DSN Project 5.3 (Vanderklift et al. 2017), (ii) have sufficient density to settle out of the water column within a 24 h period and not be retained in the water column or easily resuspended during normal water flow conditions within the tank systems, and (iii) free from organic and heavy metal pollutants (Rocla Quarries Pty. Ltd., Perth W.A.) that could potentially influence the plant response to burial and bias outcomes. Fine sand was retained within the pots by plastic barriers inserted along the edge of the pot. Each barrier height was adjusted to be 10 mm above the new sediment height to prevent spillage and discourage plants from growing over the pot edges. Control pots (no addition of fine sediment) also had plastic barriers inserted to control for barrier artefacts. These burial depths were selected to mimic a range of heights that are likely to occur in the field near to dredging operations through accumulation over 3–25 d based on average burial rates (McMahon et al. 2017) as well as levels that are likely to elicit a mortality response in tropical seagrasses determined from published research (Cabaco et al. 2008, McMahon et al. 2017). Based on these estimates, a range of sediment burial depths were chosen: 0 mm (control), 8, 16, 40 and 70 mm (Table 1). These burial depths attained different relative heights for each species, as *C. serrulata* is taller than *H. uninervis* (Table 2). At 40 mm, 53% of *C. serrulata* and 68% of *H. uninervis* canopy height was covered, and at 70 mm, *H. uninervis* was completely covered. Plant harvesting was conducted at three time periods; 3 weeks (5<sup>th</sup> Jan 2015), 6 weeks (22<sup>nd</sup> Jan 2015), and 14 weeks (12<sup>th</sup> Mar) after sediment addition. At each harvest period four pots (containing both species) were removed from each tank. Each species was then placed in a separate labelled zip-lock 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.

## 2.3 Indicators measured

Indicators of seagrass status were tested throughout this experiment and these indicators ranged from sub-lethal physiological through to population level indicators (Table 3).

Table 1: Model of experimental design. Italicized number indicates number of replicate pots per treatment (burial depth, mm), tank and time. Each replicate pot contains both seagrass species (2 levels).

	Burial treatment (5 levels)	0 mm		8 mm		16 mm		40 mm		70 mm	
		1	2	1	2	1	2	1	2	1	2
<b>Monitoring Time (weeks, 3 levels)</b>	3	4	4	4	4	4	4	4	4	4	4
	6	4	4	4	4	4	4	4	4	4	4
	14	4	4	4	4	4	4	4	4	4	4

Table 2: Mean plant height of species in the study, measured on four randomly selected shoots of each species

Species	Mean plant height (mm) (n = 4)	Plant burial (% of shoot/canopy height)			
		8 mm	16 mm	40 mm	70 mm
<i>Cymodocea serrulata</i>	75.4	11	21	53	93
<i>Halodule uninervis</i>	58.8	14	27	68	119

Table 3: Summary of variables tested for each species, burial depth and duration and the replication at the tank level.

Level	Indicator Grouping	Indicator	Replicate
Physiological (sub-lethal)	Photophysiology (PAM fluorometry)	Electron Transport Rate (ETR <sub>MAX</sub> )	6
		Photochemical efficiency ( $\alpha$ )	6
		Minimum saturating irradiance ( $I_k$ )	6
	Carbohydrate reserves	Rhizome carbohydrates	3
		Rhizome starch	3
		Nutrients and isotopes	3
Plant-scale (state change)	Growth & biomass	Shoot density	4
		Shoot production rate	4
		Total biomass	4
		Aboveground biomass	4
		Belowground biomass	4
		Horizontal: Vertical rhizome biomass	4
	Morphology	Leaves per shoot	4
		Vertical rhizome length	4
		Leaf length	4
Meadow-scale (pot level)	Abundance	Leaf area	4
		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). Rapid light curves (RLCs) were used to determine the maximum rate at which electrons are transported through PSII and used for photochemistry (ETR<sub>MAX</sub>), the efficiency of electron transport ( $\alpha$ ), and the sub-saturating irradiance ( $E_k$ ). RLCs measure the effective quantum yield as a function of irradiance using the pre-installed software routine, where photosynthetic yield was measured through 9 pre-determined steps of increasing light intensity (0, 11, 26, 77, 115, 206, 317, 443 and 555  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  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 6 randomly assigned pots for each replicate treatment (2 tanks per treatment) on each sampling occasion (3, 6 and 14 weeks) prior to plant harvesting.

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 5 times) using the quantum sensor on the PAM and compared this to ambient light (light intensity for each treatment). AF was calculated as:

$$AF = \frac{(Incident_{PAR} - Transmitted_{PAR})}{Incident_{PAR}}$$

Equation 1

To determine if there were differences in AF in response to sediment burial, we measured leaf absorbance for each species in each burial treatment at the beginning of the experiment and at each time step, instead of the using the Diving-PAM's default value of 0.84. We found a difference between species but no differences between treatments so the same AF value for each species was used during the experiment.

The RLCs were analyzed by non-linear regression to obtain estimates of the maximum 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 and 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:

$$ETR = ETR_{MAX} \left(1 - e^{-(\alpha E_d / ETR_{MAX})}\right) e^{-(\beta E_d / ETR_{MAX})}$$

Equation 2

Where:

$ETR_{MAX}$  is the maximum ETR rate ( $\mu\text{mol electrons m}^{-2} \text{s}^{-1}$ );

$E_d$  is the PAR light intensity (400-700 nm) ( $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ );

$\alpha$  is the initial slope of the curve (representing photosynthetic efficiency) ( $\mu\text{mol electrons m}^{-2} \text{s}^{-1} / \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ); and

$\beta$  is the final slope of the curve (representing photoinhibition through damage to the PSII) ( $\mu\text{mol electrons m}^{-2} \text{s}^{-1} / \mu\text{mol photons m}^{-2} \text{s}^{-1}$ )

$$E_k = (ETR_{max} | \alpha)$$

Where:

$ETR_{MAX}$  is the maximum ETR rate ( $\mu\text{mol electrons m}^{-2} \text{s}^{-1}$ ); and

$\alpha$  is the initial slope of the curve (representing photosynthetic efficiency) ( $\mu\text{mol electrons m}^{-2} \text{s}^{-1} / \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ).

#### 2.4.2 Rhizome carbohydrates

Storage carbohydrates in seagrass rhizomes for each species and burial treatment were assessed at 3, 6 and 14 weeks after burial treatments were installed. Rhizome material (horizontal and vertical combined) was oven-dried and ground (ball-mill grinder). Soluble sugars and starch were then extracted using 80% (v/v) ethanol (Quarmby & Allen 1989). Soluble sugars (%DW) and starch (%DW) were analysed by colorimetric determination (420 nm) with an amylase pre-digest to convert the starch to glucose (Yemm & Willis 1954).

### 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, and 14 weeks after burial treatments were installed. Seagrass leaves were dried and ground to a fine powder using a steel ball-mill grinder. Carbon (C) and Nitrogen (N) concentrations, and  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  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,  $\delta^{15}\text{N}$  5.71‰, 41.51% C,  $\delta^{13}\text{C}$  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  $\delta^{13}\text{C}$ , and 0.3‰ for  $\delta^{15}\text{N}$ . Elemental contents of seagrass leaf samples were calculated as a percentage of dry weight.

## 2.5 Plant- and meadow-scale indicators

### 2.5.1 Growth, biomass and morphology

Growth (shoot production), shoot density, biomass, and morphology were measured on harvested plants. To assess shoot production rate (shoots ramet<sup>-1</sup> day<sup>-1</sup>), at least one ramet per species was tagged (behind the apical shoot) at the beginning of the experiment before sediment burial treatments were applied and then at each harvest time new shoots produced after the apical tag were counted. In the laboratory, shoots were counted to derive shoot density, and then separated from rhizomes (vertical and horizontal) and roots. On 3 shoots of each species in each replicate pot the following were measured on the youngest mature leaf: leaf length (from the sheath to leaf tip), and leaf width at centre of leaf. Leaf area was calculated by multiplying leaf length by leaf width. Similarly, the length of vertical rhizome from 3 shoots of each species was measured. Following this, all plant components were then dried at 60°C for 72 h in an oven. All shoots (leaves and vertical rhizomes) were weighed to obtain the dry weight of above ground biomass, while roots and horizontal rhizomes were weighed to obtain the dry weight of below ground biomass.

## 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 burial depth (fixed factor; 0, 8, 16, 40, 70 mm), Time (fixed factor; 3, 6, and 14 weeks), Species (fixed factor; *Cymodocea serrulata* and *Halodule uninervis*), and Tanks (1 and 2 (blocks)) nested within Treatment on physiology, plant growth, biomass and morphology variables. Total pot biomass summed the biomass of all species within a pot and this was 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'). If there was an interaction with species or a main effect, the *post hoc* tests were run on each species independently. 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-indicator assessment

To identify the most appropriate bio-indicators of response to burial, the variables that showed a significant effect of burial 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 depth of burial. The consistency of the response was identified by two variables. Firstly, for each burial treatment level (i.e. 8, 16, 40 and 70 mm) was there a consistent direction and magnitude of response, and secondly for each duration (i.e. 3, 6 and 14 weeks), was there a consistent direction and magnitude of response with increasing levels of burial?

## 2.8 Threshold development

To develop thresholds for sub-lethal (vertical rhizome length) and lethal (total biomass) effects of burial, a few approaches were trialled. As our statistics showed, the effect of burial was dependent on the interaction of the duration and depth of burial, so we decided not to pursue a simple single value approach such as a minimum burial threshold. Instead, we investigated approaches to develop thresholds which incorporated the duration and magnitude of burial. For each treatment (magnitude of burial depth by duration) we created a burial stress value by multiplying the depth of burial by the duration of burial in weeks. Therefore we had a different value for each burial depth ( $n = 5$ ) by duration ( $n = 3$ ) treatments. We then plotted this burial stress against the average biomass per treatment but there was no clear relationship from which to derive a threshold. Therefore we plotted the percent change in average total biomass by treatment relative to the average total biomass of the controls at that time period. This generated a useful curve from which we could derive a threshold. We repeated this for the sub-lethal indicator, vertical rhizome length.

## 3 Results

### 3.1 Physiological responses

#### 3.1.1 Photophysiology

Maximum electron transport rate,  $ETR_{MAX}$ , was affected by Treatment, but it depended on both Species and Time (Treatment  $\times$  Species  $\times$  Time,  $MS = 3864$ ,  $p = <0.001$ , Table 4), so we further analysed the Treatment and Time effects separately for each species. For *C. serrulata*, the controls remained relatively stable over the 14 week experiment and sediment burial caused no significant change in  $ETR_{MAX}$  over all time periods (Figure 1 i). In contrast, *H. uninervis* showed an increase in  $ETR_{MAX}$  after 3 weeks in all burial treatments, then only in the deepest burial treatment, 70mm, at 6 weeks. By 14 weeks there was no significant difference between burial treatments and controls (Figure 1 ii).

The half-saturating irradiance,  $E_k$ , was affected by burial depth similarly for both species (Treatment  $MS = 26304$ ,  $p = <0.001$ , Table 4). Plants buried under 8, 40 and 70 mm of sediment showed an elevated  $E_k$  relative to controls with 16 mm intermediate between these two groups (Figure 3 iii, iv,vi).  $E_k$  also showed a general decline over time for all treatments (Time  $MS = 100373$ ,  $p = <0.001$ , Table 4) which could be related to a change in light availability according to season (Summer–Autumn).

Photosynthetic efficiency,  $\alpha$  ( $\infty$ ), was affected by treatment, but this was dependent on species (Treatment  $\times$  Species,  $MS = 0.016$ ,  $p = <0.003$ , Table 4). For *H. uninervis*, there was no change in  $\alpha$  for the duration of the 14 week experiment but *C. serrulata*, in general, showed a reduced  $\alpha$  at 70 mm with intermediate values for other burial treatments (Figure 1v). *C. serrulata* also showed a general increase in  $\alpha$  over time (Species  $\times$  Time,  $MS = 0.367$ ,  $p = <0.001$ , Table 4) whereas *H. uninervis* remained relatively constant (Figure 1vi).

Table 4: Results of four-way nested ANOVA testing for the effects of treatment (sediment burial depth, mm), species, time and treatment nested within tank on photophysiology. Bold numbers denote significant differences.

	df	Electron transport rate (ETR <sub>MAX</sub> )		Photosynthetic efficiency (α)		Half-saturating irradiance (E <sub>k</sub> )	
		MS	p	MS	p	MS	p
Species	1	30127	<0.001	1.09	<0.001	841	0.299
Treatment	4	2728	<0.001	0.015	<b>0.004</b>	26304	<0.001
Time	2	6028	<0.001	0.141	<0.001	100373	<0.001
Tank [Treatment]	5	707	<b>0.024</b>	0.030	0.09	2013	0.09
Species × Treatment	4	1787	<b>0.003</b>	0.016	<b>0.003</b>	3224	0.388
Species × Time	2	18943	<0.001	0.367	<0.001	1875	0.301
Treatment × Time	8	278	0.961	0.003	0.591	2763	0.894
Species × Treatment × Time	8	3864	<0.001	0.006	0.100	9237	0.161

Table 5: Results of four-way nested ANOVA testing for the effects of treatment (sediment burial depth, mm), species, time and treatment nested within tank on leaf nutrient and isotopic ratio. Bold text denotes significant differences.

	df	Leaf carbon		Leaf nitrogen		Leaf C:N ratio		Leaf δ <sup>13</sup> C		Leaf δ <sup>15</sup> N	
		MS	p	MS	p	MS	p	MS	p	MS	p
Species	1	<b>0.012</b>	0.954	1.943	<0.001	4402	<0.001	106.8	<0.001	37.9	<0.001
Treatment	4	2.397	0.639	0.146	<b>0.048</b>	293	<b>0.007</b>	7.38	<0.001	16.4	<0.001
Time	2	15.448	<b>0.019</b>	1.949	<0.001	2794	<0.001	27.6	<0.001	86.7	<0.001
Tank [Treatment]	5	2.559	0.698	0.170	0.124	248.6	0.132	2.80	0.264	13.99	<b>0.005</b>
Species × Treatment	4	2.632	0.596	0.067	0.346	123	0.198	1.33	0.219	3.33	0.390
Species × Time	2	11.66	0.062	0.048	0.448	97	0.303	28.4	<0.001	2.81	0.419
Treatment × Time	8	4.392	0.326	0.242	<0.001	349	<0.001	1.21	0.235	3.26	0.426
Species x Treat. × Time	8	6.555	0.259	0.039	0.729	30	0.936	0.96	0.397	2.71	0.565

Table 6: Results of four-way nested ANOVA testing for the effects of treatment (sediment burial depth, mm), species, time and treatment nested within tank on rhizome soluble carbohydrates and starch. Bold text denotes significant differences.

	df	Rhizome Soluble Carbohydrate		Rhizome Starch	
		MS	p	MS	p
Species	1	7131	<0.001	17777	<0.001
Treatment	4	197	<b>0.009</b>	33	<0.001
Time	2	1804	<0.001	531	<0.001
Tank [Treatment]	5	33.96	0.853	18.7357	0.980
Species × Treatment	4	752	<0.001	504	<0.001
Species × Time	2	475	<0.001	22	<0.001
Treatment × Time	8	1140	<0.001	20	<b>0.001</b>
Species x Treatment × Time	8	1147	<0.001	6	<b>0.003</b>

Table 7: Results of four-way nested ANOVA testing for the effects of treatment (sediment burial depth, mm), species, time and treatment nested within tank on photophysiology. Bold text denotes significant differences

	df	Shoot Density		Shoot Production Rate		Leaves shoot <sup>-1</sup>	
		MS	p	MS	p	MS	p
Species	1	24180	<b>&lt;0.001</b>	3.42	<b>&lt;0.001</b>	3.646	<b>&lt;0.001</b>
Treatment	4	232	<b>&lt;0.001</b>	0.041	<b>0.008</b>	0.272	0.170
Time	2	49	0.263	0.166	<b>&lt;0.001</b>	1.230	<b>&lt;0.001</b>
Tank [Treatment]	5	232	0.720	0.027	0.441	0.325	0.204
Species × Treatment	4	227	<b>&lt;0.001</b>	0.044	<b>0.005</b>	0.471	<b>0.027</b>
Species × Time	2	311	<b>&lt;0.001</b>	0.151	<b>&lt;0.001</b>	0.560	<b>0.038</b>
Treatment × Time	8	54	0.162	0.003	0.980	0.951	<b>&lt;0.001</b>
Species x Treatment× Time	8	55	0.152	0.011	0.966	0.108	0.742

Table 8: Results of four-way nested ANOVA testing for the effects of treatment (sediment burial depth, mm), species, time and treatment nested within tank on photophysiology. Bold text denotes significant differences.

	df	Leaf Length		Leaf Area		Vertical Rhizome Length	
		MS	p	MS	p	MS	p
Species	1	4506	<b>0.004</b>	7.21e <sup>8</sup>	<b>&lt;0.001</b>	800	<b>&lt;0.001</b>
Treatment	4	2504	<b>0.001</b>	70342	<b>&lt;0.001</b>	4261	<b>&lt;0.001</b>
Time	2	42	0.922	299	0.968	1998	<b>&lt;0.001</b>
Tank [Treatment]	5	1048	0.086	15747	0.862	195	0.061
Species × Treatment	4	335	0.633	24870	<b>0.032</b>	311	<b>&lt;0.001</b>
Species × Time	2	11	0.978	12272	0.267	886	0.093
Treatment × Time	8	577	0.359	21902	<b>0.018</b>	979	<b>&lt;0.001</b>
Species x Treatment × Time	8	1063	<b>0.043</b>	17787	0.057	45	0.266

Table 9: Results of four-way nested ANOVA testing for the effects of treatment (sediment burial depth, mm), species, time and treatment nested within tank on photophysiology. Bold text denotes significant differences.

	df	Total Biomass		Above-ground Biomass		Below-ground Biomass		Pot Biomass	
		MS	p	MS	p	MS	p	MS	p
Species	1	9.178	<b>&lt;0.001</b>	0.288	<b>&lt;0.001</b>	6.215	<b>&lt;0.001</b>	NA	NA
Treatment	4	0.164	0.0916	0.005	0.472	0.117	0.067	0.328	<b>&lt;0.001</b>
Time	2	6.911	<b>&lt;0.001</b>	0.004	0.481	7.047	<b>&lt;0.001</b>	13.822	0.981
Tank [Treatment]	5	0.209	0.388	0.015	0.099	0.149	0.430	0.419	0.395
Species × Treatment	4	0.307	<b>0.005</b>	0.013	<b>0.049</b>	0.196	<b>0.006</b>	NA	NA
Species × Time	2	1.297	<b>&lt;0.001</b>	0.129	<b>&lt;0.001</b>	0.614	<b>&lt;0.001</b>	NA	NA
Treatment × Time	8	0.170	<b>0.037</b>	0.012	<b>0.031</b>	0.121	<b>0.023</b>	0.340	<b>0.029</b>
Species × Treatment × Time	8	0.214	<b>0.009</b>	0.006	0.327	0.167	<b>0.002</b>	NA	NA

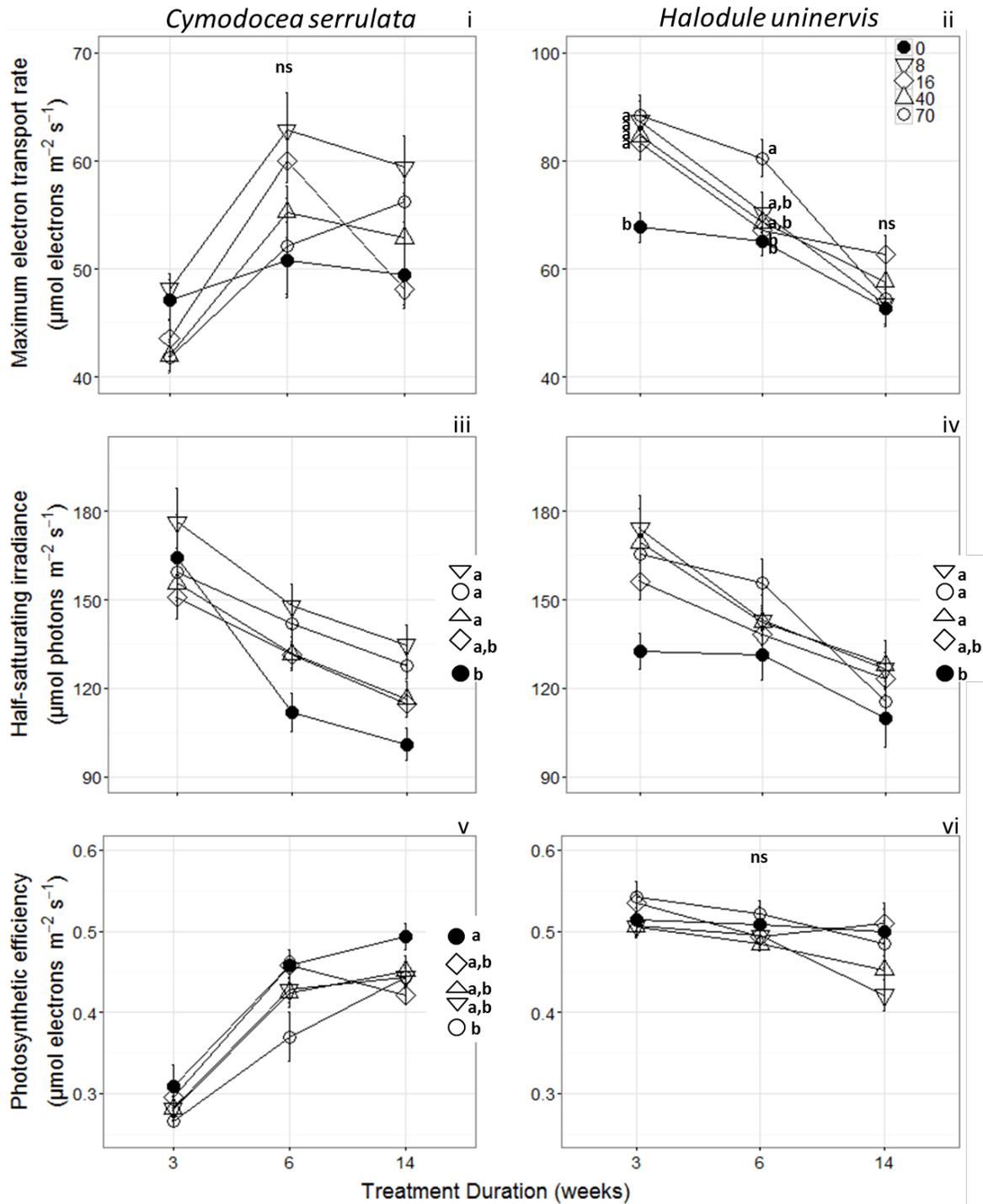


Figure 1. Maximum electron transport rate,  $ETR_{MAX}$  (i, ii), half-saturating irradiance,  $E_k$  (iii, iv) and photosynthetic efficiency,  $\alpha$  (v, vi) of *Cymodocea serrulata* and *Halodule uninervis* at 3, 6 and 14 weeks of burial at 0 (control), 8, 16, 40, and 70 mm. Values are means pooled across tanks ( $n = 12$ )  $\pm$  SE. Letters denote significant differences. Letters outside figure indicate significant differences between treatments pooled ( $n = 36$ ) over all time periods for a given species.

### 3.1.2 Leaf nutrients and isotopes

Leaf nutrient concentration and isotopic signature were affected by light reduction but the responses varied with burial depth. While leaf carbon concentration did not change with burial, leaf N concentration generally decreased for both species after 14 weeks in a burial depths up to 40 mm; at deeper burial depth (70 mm) leaf N increased but was only significantly different to treatments less than 40 mm and not significantly different

from the controls (Table 5, Figure 2 iii, iv). Accordingly, these changes in leaf N concentration effected leaf C:N ratio (Table 5, Figure 2 v, vi). For both species, leaf carbon isotopic ratio was affected by burial depth (Treatment effect, Table 5). Burial depths of 8 mm showed a significant increase in  $\delta^{13}\text{C}$  relative to controls, whereas all other treatments were intermediate between the two groups (Figure 2 vii, viii). There was also a time effect which differed between species (Species  $\times$  Time, Table 5), with *H. uninervis* showing an increase in  $\delta^{13}\text{C}$  by the end of the experimental period, whereas *C. serrulata* remained unchanged. Similarly, for both species, leaf nitrogen isotope ratio was affected by burial depth (Treatment effect, Table 5), with 8 and 16 mm burial depths resulting in a significant increase in  $\delta^{15}\text{N}$  compared to controls, whereas 40 and 70 mm treatments were intermediate between the two groups (Figure 2 ix, x).

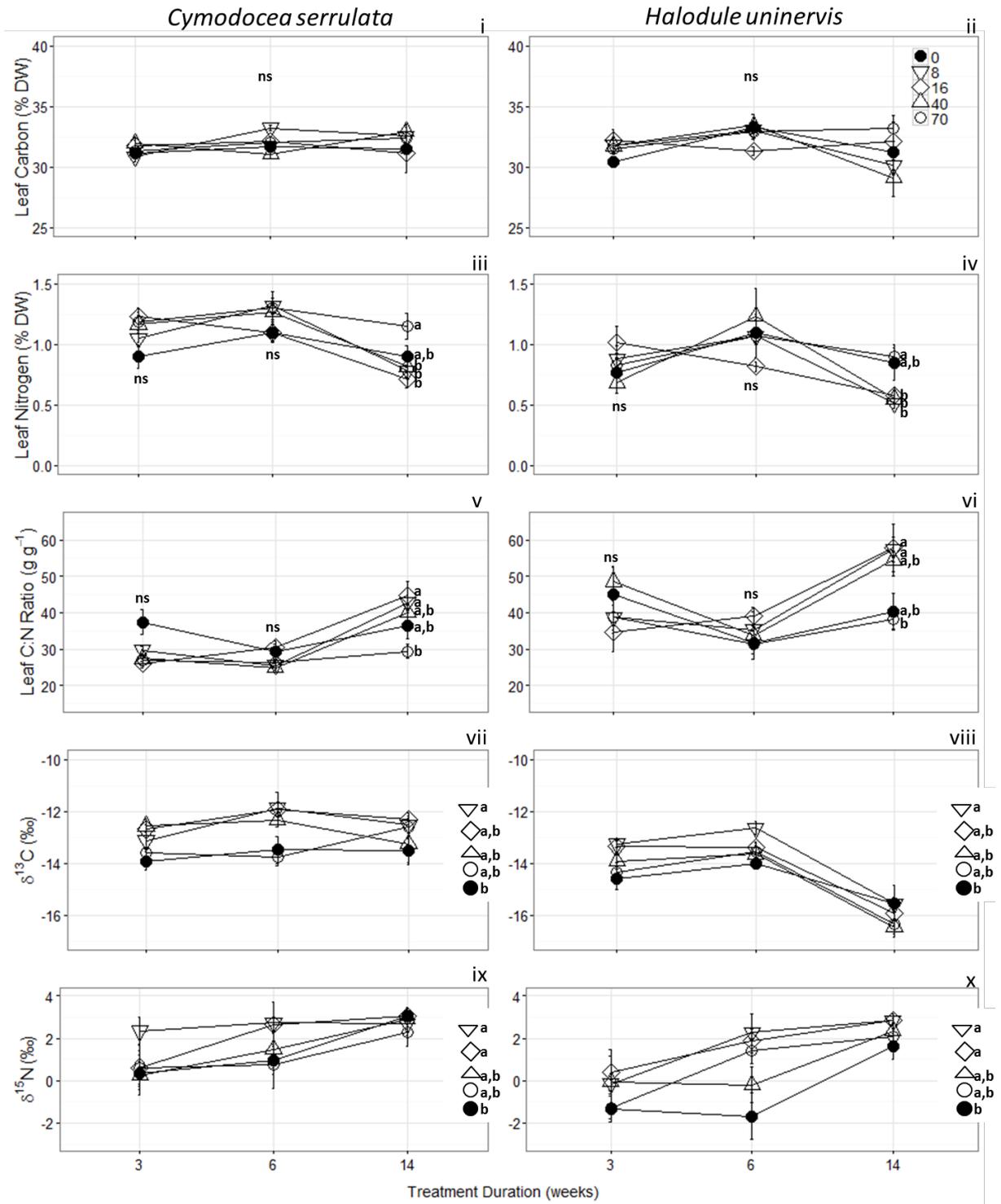


Figure 2. Leaf carbon (C) concentration, % DW (i, ii), nitrogen (N) concentration, % DW (iii, iv), C:N ratio, g g<sup>-1</sup> (v, vi), carbon isotope ratio (vii, viii), and nitrogen isotope ratio (ix, x) of *Cymodocea serrulata* (left) and *Halodule uninervis* (right) at 3, 6 and 14 weeks after burial at 0 (control), 8, 16, 40, and 70 mm. Values are means pooled across tanks (n = 12) ± SE. Letters denote significant differences. Letters outside figure indicate significant differences between treatments pooled (n = 36) over all time periods for a given species.

### 3.1.3 Carbohydrates

Soluble carbohydrates within horizontal and vertical rhizomes were affected by Treatment, but it depended on both Species and Time (Treatment × Time × Species, MS = 225,  $p = <0.001$ , Table 6). For rhizome soluble carbohydrates in *C. serrulata*, there were no effects at 3 weeks, but at 6 weeks there was up to ~50% decline relative to controls in the 40–70 mm burial treatments. However, by 14 weeks, the pattern changed, with an increase in rhizome soluble carbohydrates relative to controls in the 16 and 40 mm burial treatments but no significant differences in the 8 and 70 mm burial treatments (Figure 3 i). For *H. uninervis* no significant differences were detected among treatments until 14 weeks, at this time only the low burial treatments (8 and 16 mm) had reduced soluble carbohydrates relative to controls (Figure 3 ii).

Rhizome starch concentrations were affected by Treatment, but it depended on both Species and Time (Treatment × Time × Species, MS = 225,  $p = <0.001$ , Table 6). In general, rhizome starch concentrations were lower for *C. serrulata* (<1% DW) compared to *H. uninervis* (up to 28% DW). The rhizome starch of *C. serrulata* was lower in all burial treatments relative to controls, but only at 6 weeks of burial. All other time periods had no significant differences among treatments (Figure 3 iii). For *H. uninervis*, starch concentrations were significantly lower in the 70 mm burial treatment at 3 weeks, and lower in both the 40 and 70 mm burial treatments at 6 weeks, but by 14 weeks there was no difference between controls and burial treatments (Figure 3 iv).

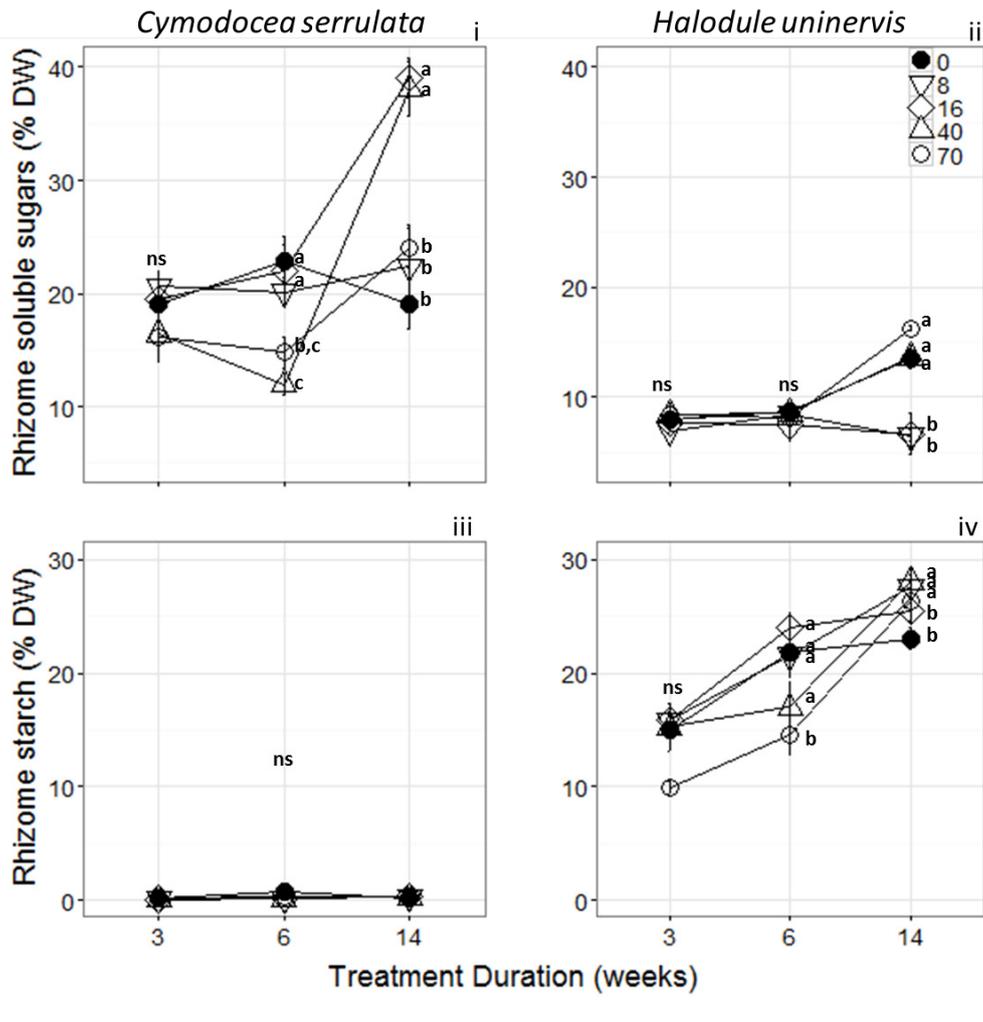


Figure 3. Rhizome soluble sugars, % DW (i, ii) and rhizome starch, % DW (iii, iv) of *Cymodocea serrulata* (left) and *Halodule uninervis* (right) at 3, 6 and 14 weeks after burial at 0 (control), 8, 16, 40, and 70 mm. Values are means pooled across tanks ( $n = 12$ ) ± SE. Letters denote significant differences.

## 3.2 Plant scale response

### 3.2.1 Growth and Morphology

Sediment burial negatively affected shoot density, but only for *C. serrulata* and after 14 weeks (Species × Treatment, MS = 227,  $p < 0.001$ ; Species × Time MS = 311,  $p < 0.001$ , Table 7). Shoot density in 40 mm burial treatments was ~ 50% that of controls (Figure 4 i). For *H. uninervis*, shoot density increased at 6 weeks in the 40 mm burial (Figure 4 ii). At 14 weeks there was a similar pattern of response but it was not significant.

The rate of new shoot production per ramet was affected by treatment, but this was dependent on species (Treatment × Species, MS = 0.003,  $p < 0.001$ ; Species × Time MS = 311,  $p < 0.001$ , Table 7). For *C. serrulata*, there was no significant difference between controls and burial treatments during the experiment (Figure 4 iii) but for *H. uninervis* there was a trend towards increasing shoot production with 16 mm of burial, though this was only significantly different from 70 mm burial treatment and not the controls and 40 mm (Fig 4 iv).

The number of leaves per shoot was significantly affected by burial, but the response was dependent on the time (Treatment × Time, Table 7) and species (Treatment × Species, Table 7). For *C. serrulata* at 3 weeks, the 70 mm burial treatment was significantly less than the controls, though at 6 weeks there was no significant differences relative to the controls and by 14 weeks, the 8–40 mm burial treatments were less than the controls (Figure 4v). *H. uninervis* responded in a different direction, and only at 3 weeks. At this time there was a trend of increasing leaves per shoot, but only the 16 mm burial treatment was significantly greater than the controls (Figure 4 vi).

Leaf length increased with burial depth, but it depended on both Species and Time (Treatment × Time × Species, MS = 1063,  $p = 0.043$ , Table 8). Leaf length was unaffected by burial at 3 weeks, but by 6 weeks in the 70 mm burial treatment it increased by 30%, and by 14 weeks it increased by 40% relative to controls. Leaf length was intermediate between the two groups in the 40 mm burial treatment (Figure 5a (i)). *H. uninervis* showed a more rapid response, increasing leaf length by 30% within the first 3 weeks in the 40 and 70 mm burial treatments and the 8 and 16 mm burial treatments were intermediate between these two groups (Fig 5a (ii)). However, after 6 weeks there was no significant difference. Leaf area was similarly affected as leaf length, consequently a similar pattern of increase occurred with burial treatment (Table 8, Figure 5a (iii) and (iv)).

Vertical rhizome length increased with burial depth, but it depended on both Species (Treatment × Species, MS = 311,  $p < 0.001$ , Table 8), and Time (Treatment × Time, MS = 979,  $p < 0.001$ , Table 8). For *C. serrulata* and *H. uninervis* the vertical rhizome length of controls were less than 10 mm and remained relatively constant during the experiment. For *C. serrulata*, there was no change in vertical rhizome length at 3 weeks, but by 6 weeks the vertical rhizomes of plants buried under 70 mm of sediment had grown vertically by more than 25 mm, and more than 20 mm for 40 mm burial depth (Figure 5a (v)). These lengths increased again at by 14 weeks. The 16 mm treatment also showed an increase but it was intermediate between the control and 40 mm treatment. In contrast, *H. uninervis* showed a significant but small increase (10 mm) in vertical rhizome length at 3 weeks for the 70 mm treatment only. At 6 weeks this had increased to a vertical rhizome length of 30 mm and 20 mm for the deeper burial treatments (70 and 40 mm, respectively) and at 14 weeks, the vertical rhizome length had increased further to almost 55 mm in the 70 mm burial treatment and 35 mm in the 40 mm burial treatment (Figure 5a (vi)). Photographic examples of seagrass response at the end of the experiment can be seen in Figure 5b.

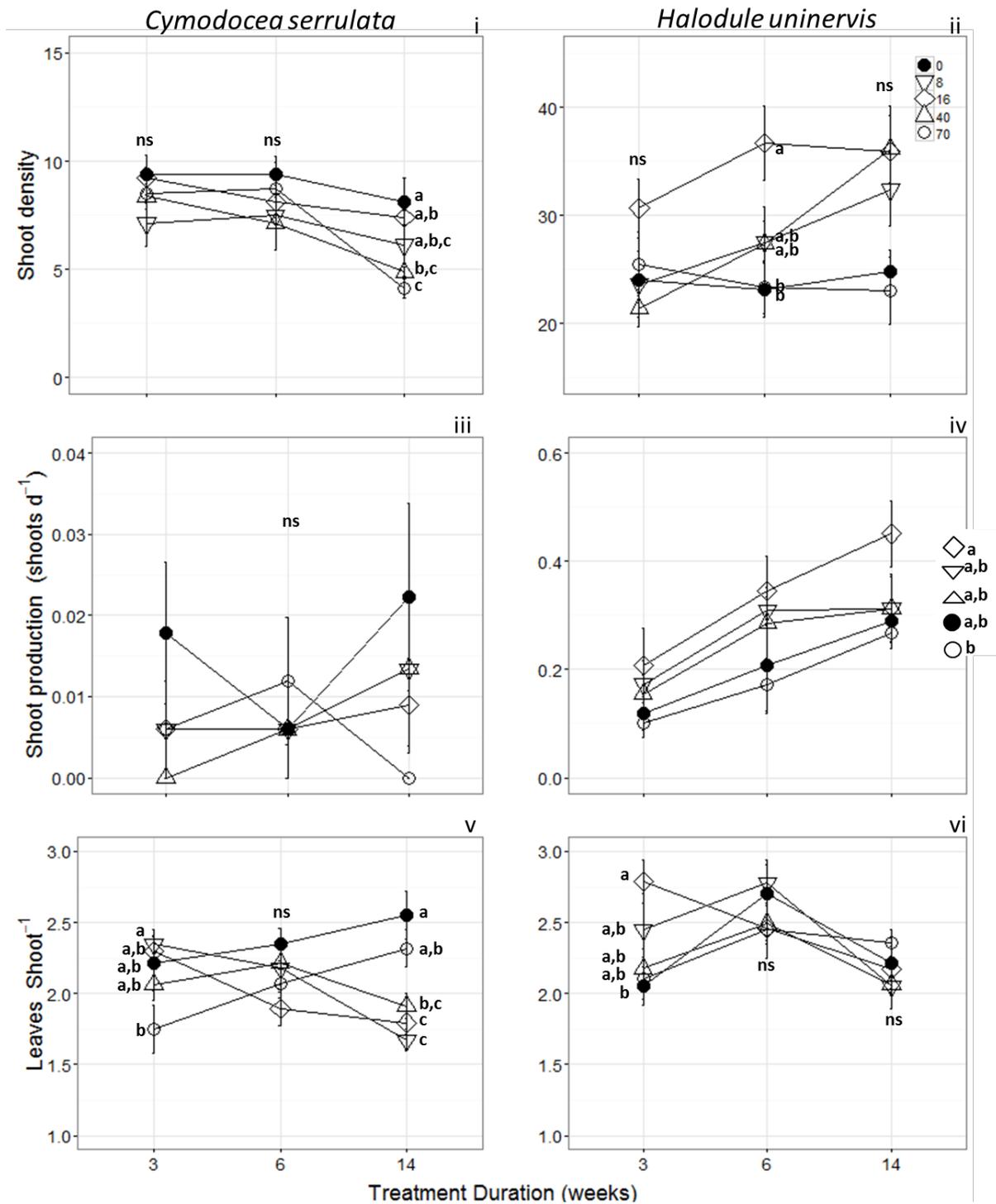


Figure 4: Shoot density (i, ii), shoot production, shoots d<sup>-1</sup> (iii, iv), and leaves shoot<sup>-1</sup> (v, vi) of *Cymodocea serrulata* (left) and *Halodule uninervis* (right) at 3, 6 and 14 weeks after burial at 0 (control), 8, 16, 40, and 70 mm. Values are means pooled across tanks (n = 12) ± SE. Letters denote significant differences. Letters outside figure indicate significant differences between treatments pooled (n = 36) over all time periods for a given species.

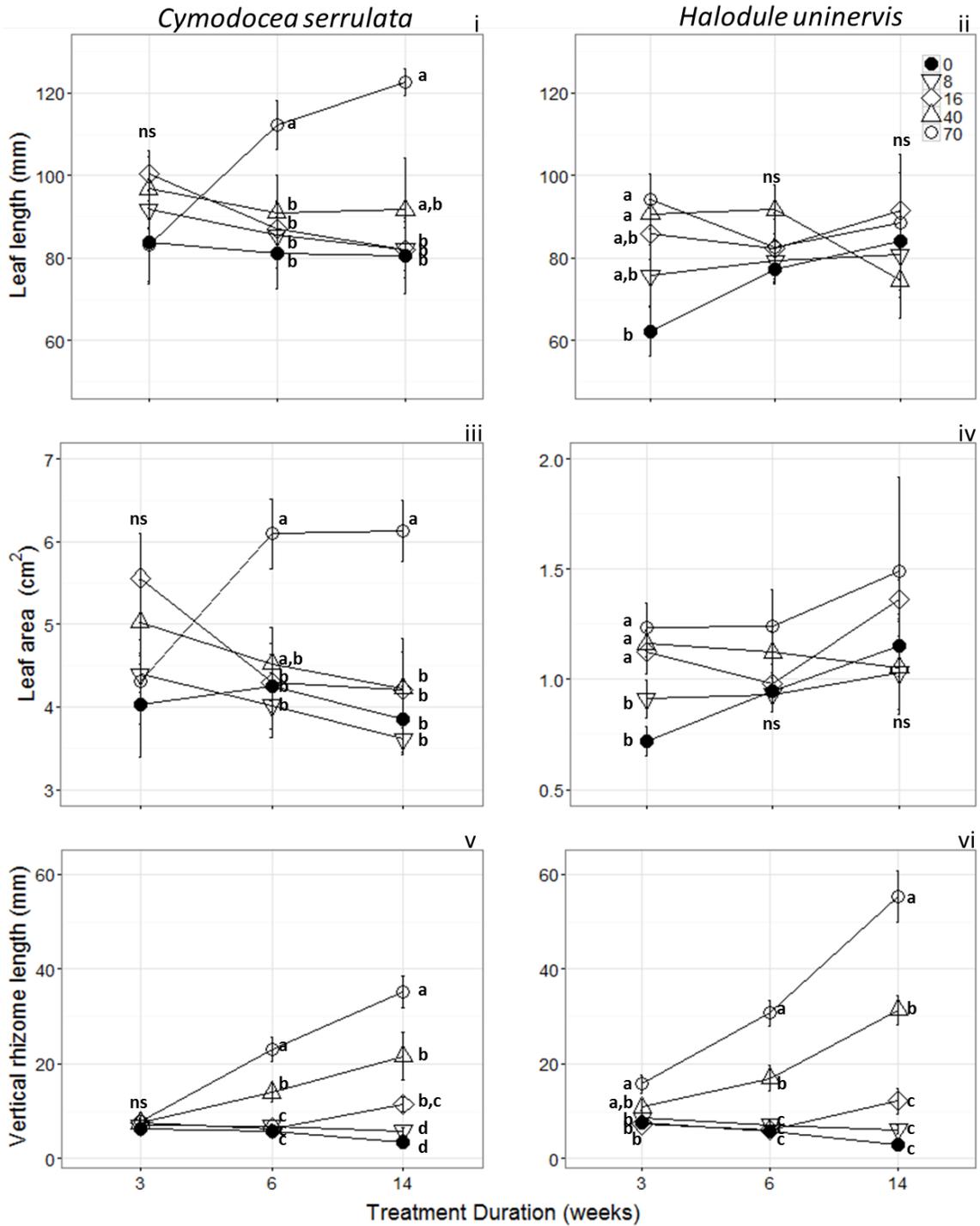


Figure 5a: Leaf length, mm (i, ii), leaf area, cm<sup>2</sup> (iii, iv), and vertical rhizome length, mm (v, vi) of *C. serrulata* and *H. uninervis* at 3, 6 and 14 weeks after burial at 0 (control), 8, 16, 40, and 70mm. Values are means pooled across tanks (n=12) ± SE. Letters denote significant differences.

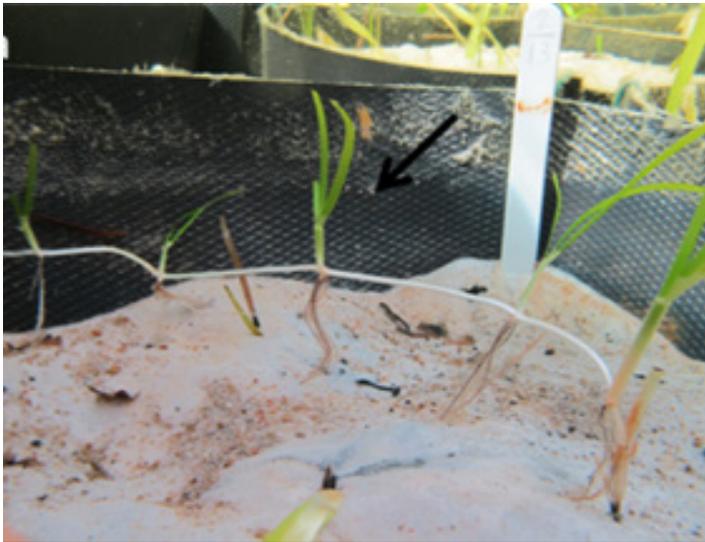


Figure 5b:

Upper image — Example of vertical rhizome growth of *H. uninervis* after sediment burial. Note burial sediments have been removed to expose vertical rhizomes.

Centre image — *H. uninervis* ramet in 70 mm sediment burial treatment after removal of sediment – black arrow identifies sediment burial height. Note: initiation of vertical growth can be seen from shoots on far right of image, white rhizome indicate new growth at the new sediment height (ie 70 mm) and roots are exposed.

Lower Image. Vertical growth (15mm, arrow) of *Cymodocea serrulata* at 70 mm burial depth. Photographs courtesy of John Statton (UWA))

Total plant biomass for each species was altered by burial treatments, but not at all times and not always in the same direction (Species  $\times$  Treatment  $\times$  Species  $\times$  Time, MS = 0.214,  $p = 0.009$ , Table 9). For *C. serrulata* at 3 and 6 weeks total plant biomass was not affected by burial treatment, but by 14 weeks, plants grown in the deepest burial treatments showed a significant decrease in biomass (~ 50% decline) relative to controls (Figure 6 i). Total biomass for *H. uninervis* was also unaffected by burial depth for the first 6 weeks, but after 14 weeks, plants grown in 40 mm burial treatments showed a significant increase in biomass relative to controls (Figure 6 ii). The decrease in total plant biomass for *C. serrulata* was driven by both above-ground biomass (Table 8, Figure 6 iii) and below-ground biomass (Table 9, Figure 6 v) whereas the increase for *H. uninervis* was a result of an increase in below ground biomass only (Table 9, Figure 6 iv, vi).

Total plant biomass (all species combined) was altered by burial treatments, but not at all times (Figure 7). By 14 weeks, plants grown in the deepest burial treatment (70 mm) showed a lower biomass (~ 20% lower) than the controls, though still increasing. All other treatments were not significantly different to the controls.

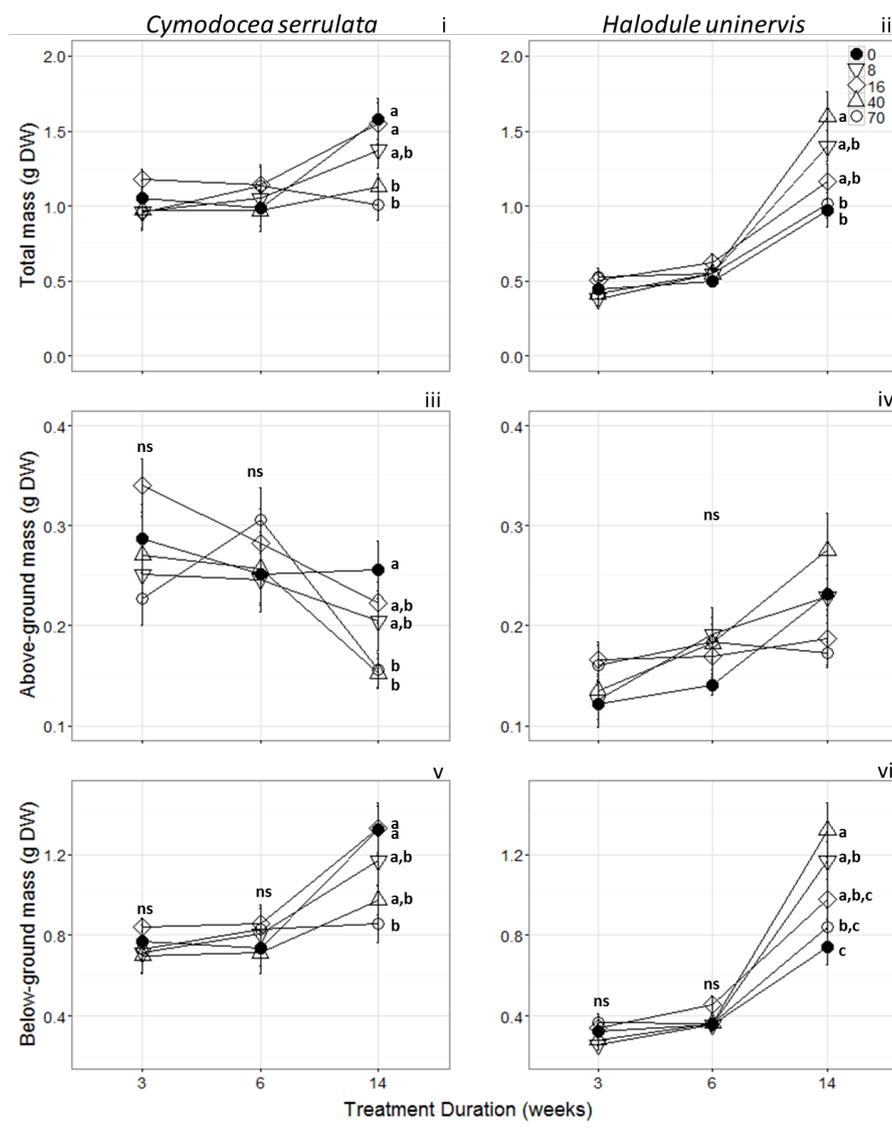


Figure 6: Total biomass, g DW (i, ii), above-ground biomass, g DW (iii, iv), and below-ground biomass, g DW (v, vi) of *Cymodocea serrulata* (left) and *Halodule uninervis* (right) at 3, 6 and 14 weeks after burial at 0 (control), 8, 16, 40, and 70 mm. Values are means pooled across tanks ( $n = 12$ )  $\pm$  SE. Letters denote significant differences.

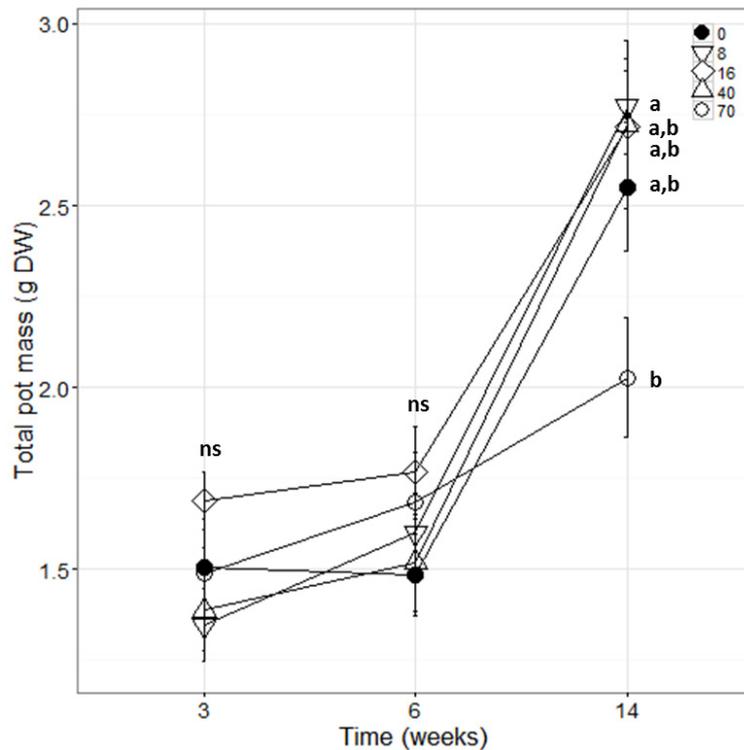


Figure 7: Total pot biomass, g pot<sup>-1</sup> both species combined (*Cymodocea serrulata* and *Halodule uninervis*) at 3, 6 and 14 weeks after burial at 0 (control), 8, 16, 40, and 70 mm. Values are means pooled across tanks (n = 12) ± SE. Letters denote significant differences.

### 3.3 Bio-indicators

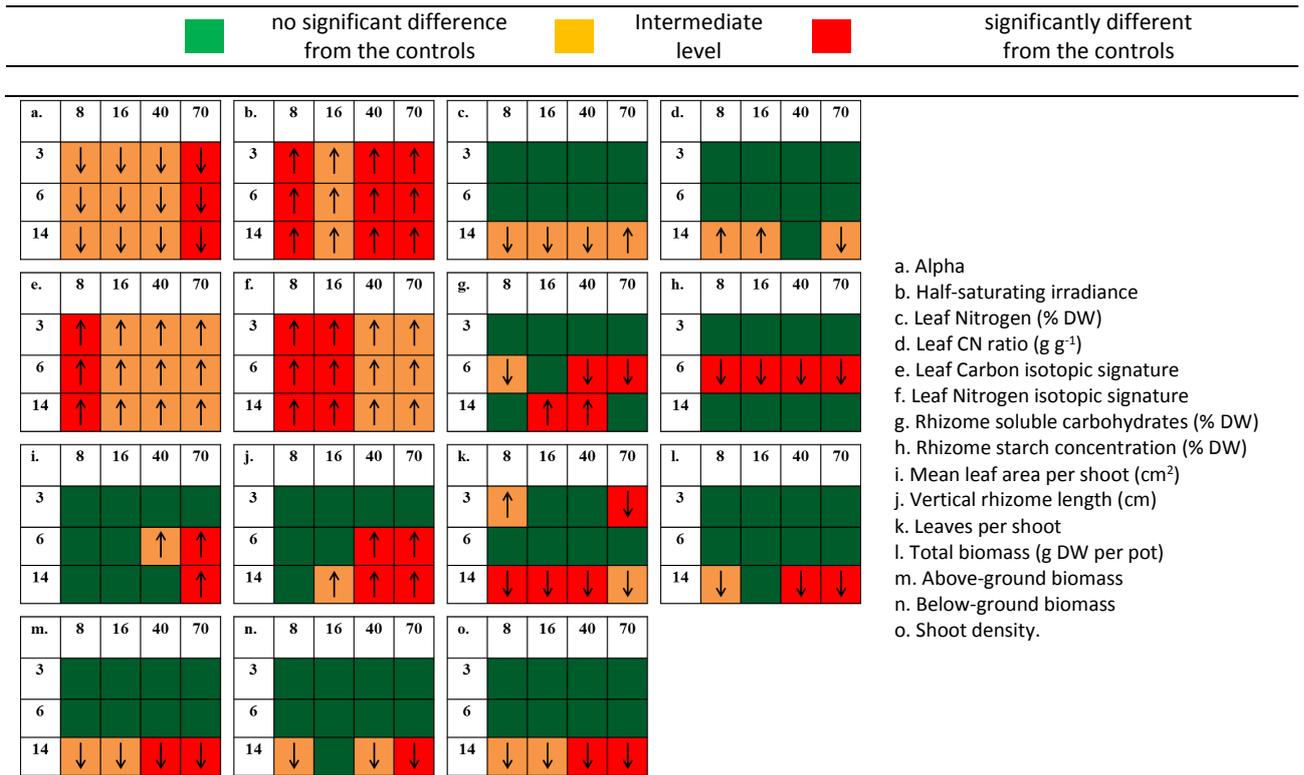
Four variables responded consistently to burial stress for *C. serrulata*. The photosynthetic parameter  $\alpha$  declined significantly with burial, the vertical rhizome length increased and the above-ground biomass and shoot density declined. These variables span a range of response times. In all burial treatments  $\alpha$  responded after 3 weeks. Vertical rhizome length responded by 6 weeks in the 40 mm and 70 mm burial treatments, and by 14 weeks in the 16 mm burial treatment. Finally, the above-ground biomass and shoot density responded by 14 weeks, across all burial treatments, and these decreased with greater burial depth (Figure 8, Table 10). All these 4 variables were consistent in the direction and magnitude of response with the duration and depth of burial.

For *H. uninervis* our ability to develop bio-indicators was limited. Only one variable responded consistently with the duration and magnitude of burial, the increase in vertical rhizome length. This was obvious from 3 weeks, and was maintained throughout the experiment. No other variables responded consistently with increasing duration or magnitude of burial (Figure 9, Table 10). Some variables responded at 3 weeks but by 14 weeks, there were no significant differences (e.g.  $ETR_{MAX}$ , Rhizome starch and Mean leaf area per shoot). Other variables such as shoot production responded in different directions depending on the magnitude of burial.

Tables 10. Potential bio-indicators of burial stress in the seagrass *Cymodocea serrulata*. Bolded and underlined variables responded most consistently and in the same direction to increasing durations and magnitudes of light reduction.

Variable	<i>Cymodocea serrulata</i>		<i>Halodule uninervis</i>	
	duration	magnitude	duration	magnitude
Alpha	<u>Y</u>	<u>Y</u>		
ETR <sub>MAX</sub>			N	N
Half saturating irradiance	Y	N	Y	N
Leaf N	N	N	N	N
Leaf C:N	N	N	N	N
Carbon isotope ratio	Y	N	Y	N
Nitrogen isotope ratio	Y	N	Y	N
Rhizome soluble carbohydrates	N	N	Y	N
Rhizome starch	N	Y	N	Y
Mean leaf area per shoot (cm <sup>2</sup> )	N	Y	N	Y
Vertical rhizome length	<u>Y</u>	<u>Y</u>	<u>Y</u>	<u>Y</u>
Leaves per shoot	N	N	N	N
Shoot production			Y	N
Total biomass	Y	N	Y	N
Above-ground biomass	<u>Y</u>	<u>Y</u>		
Below-ground biomass	Y	N	Y	N
Shoot density	<u>Y</u>	<u>Y</u>	N	N

*Cymodocea serrulata* (Figure 8)



*Halodule uninervis* (Figure 9)

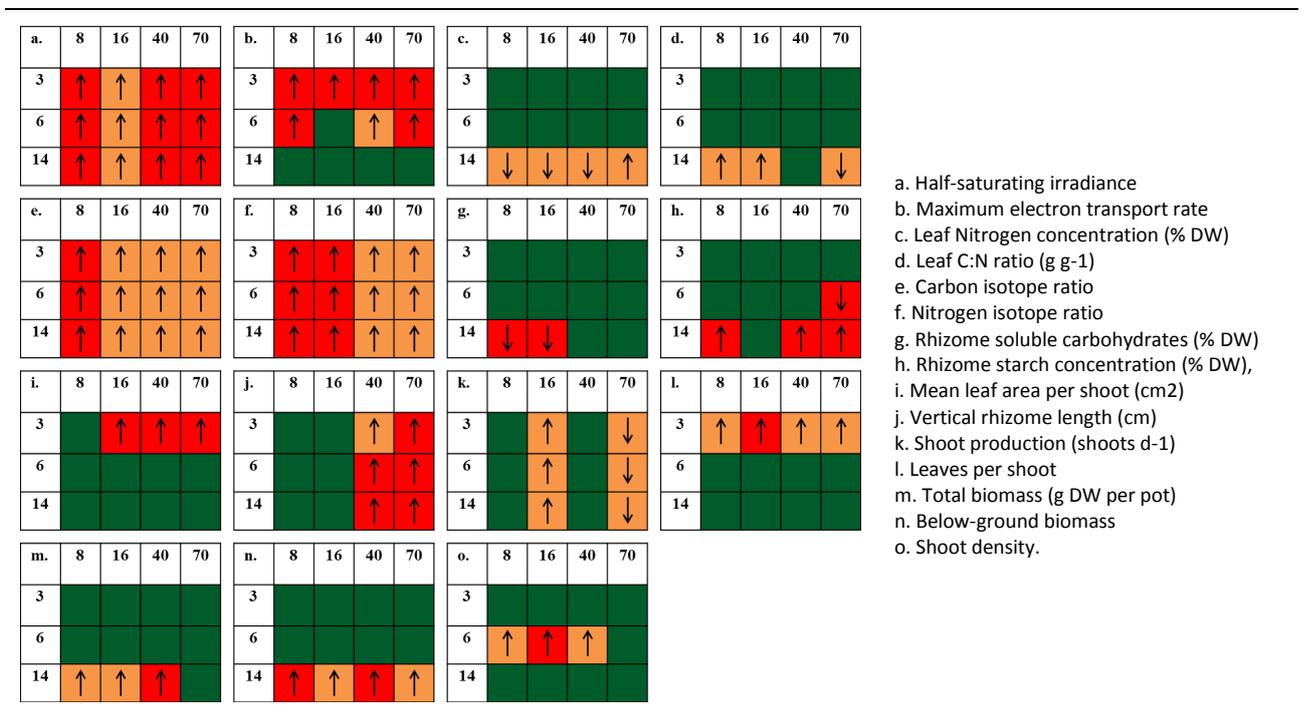


Figure 8 and 9. Summary of the significance and direction of response for all variables that showed a significant effect or interacting effect of burial treatment for the species *Cymodocea serrulata* and *Halodule uninervis*. Each large box represents a different variable. Within each large box the magnitude of burial increases on the top axis from 8mm to 70 mm burial depth. The duration of burial increases on the left axis, from 3 down to 14 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. A white box indicates no samples were collected at that time period. Within each coloured box the arrow indicates the direction of response either increasing or decreasing relative to the control.

### 3.4 Plant and community responses

We were able to develop a plant-response pathway for *C. serrulata* and *H. uninervis* for sediment burial (Figure 10). *C. serrulata* reduced the efficiency of photosynthesis by reducing  $\alpha$  and increasing the irradiance at which photosynthesis saturated ( $E_k$ ), but there were no effects on the maximum electron transport rate ( $ETR_{MAX}$ ). This occurred throughout the entire experiment. The number of leaves per shoot declined initially only in the high burial treatment at 3 weeks, this stabilised at 6 weeks, and then all treatments declined following 14 weeks of burial. Leaf area and vertical rhizome length also increased in the high burial treatments at 6 weeks and this continued through to 14 weeks. These changes may have been supported by changes in the rhizome starch, which declined through conversion to sugars across all burial treatments at 6 weeks, but despite this, soluble sugars still declined in the high burial treatments at 6 weeks, and persisted in some of these treatments to 14 weeks. By 14 weeks the biomass and shoot density had declined significantly in the high (40–70 mm) burial treatments.

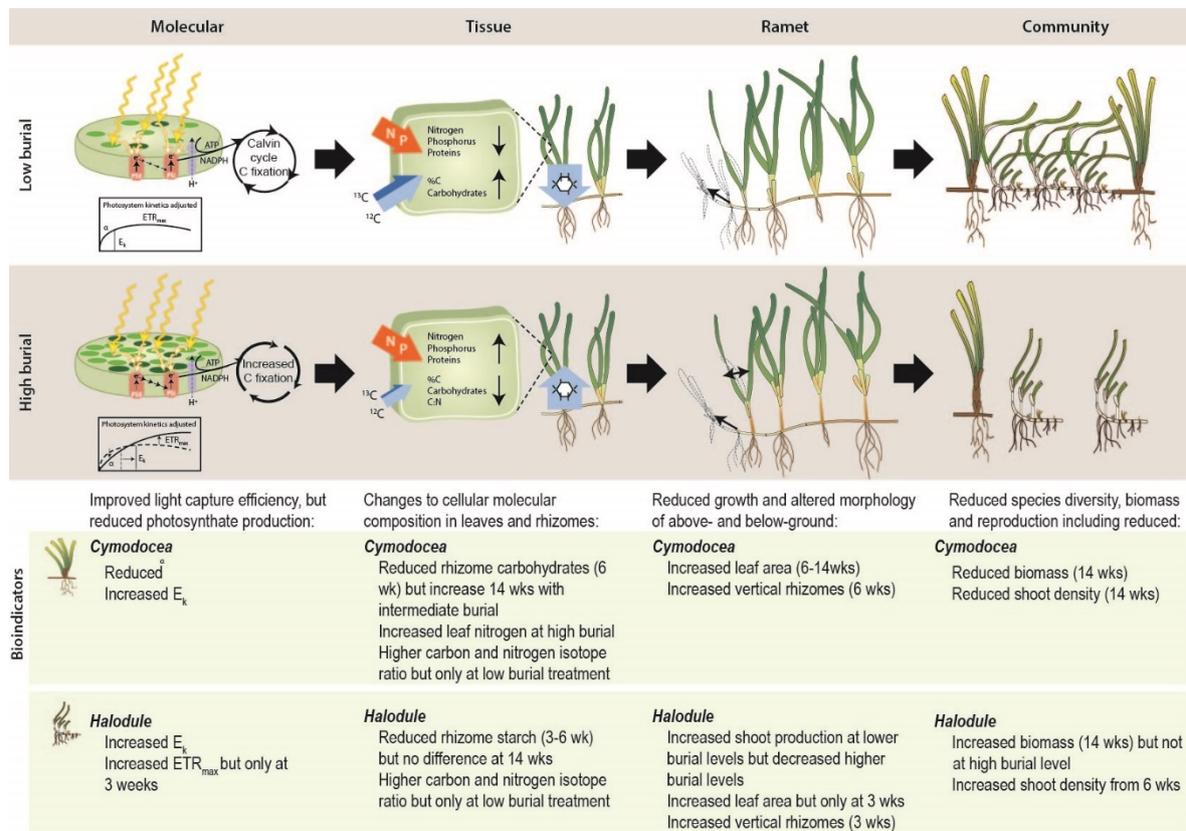


Figure 10. Plant and community response pathway to increased magnitude and duration of burial, based on the findings from the burial experiment.

### 3.5 Thresholds

Low levels of burial (8 mm) over 14 weeks (i.e. 8 mm × 14 weeks = 112 mm weeks of burial) stimulated the biomass of *H. uninervis*, where the biomass was enhanced by >40% relative to controls. A change of 14% was explained by the variation in the controls, so as a conservative measure to be confident of detecting change, we have suggested that a 20% change in the total biomass of *H. uninervis* (as indicated by the dashed lines in Figure 11) is within the natural variation you are likely to find, and points within this bar do not indicate stimulation or impacts of burial. In contrast, *C. serrulata* was negatively impacted by burial with a 30–50% reduction in total biomass but only following 14 weeks of burial with 40 mm or more sediment (i.e. 40 mm × 14 weeks = 560 mm

weeks of burial). A change of 12% was explained by the variation in the controls, and once again we suggest that a 20% change is within the natural variation that you are likely to find.

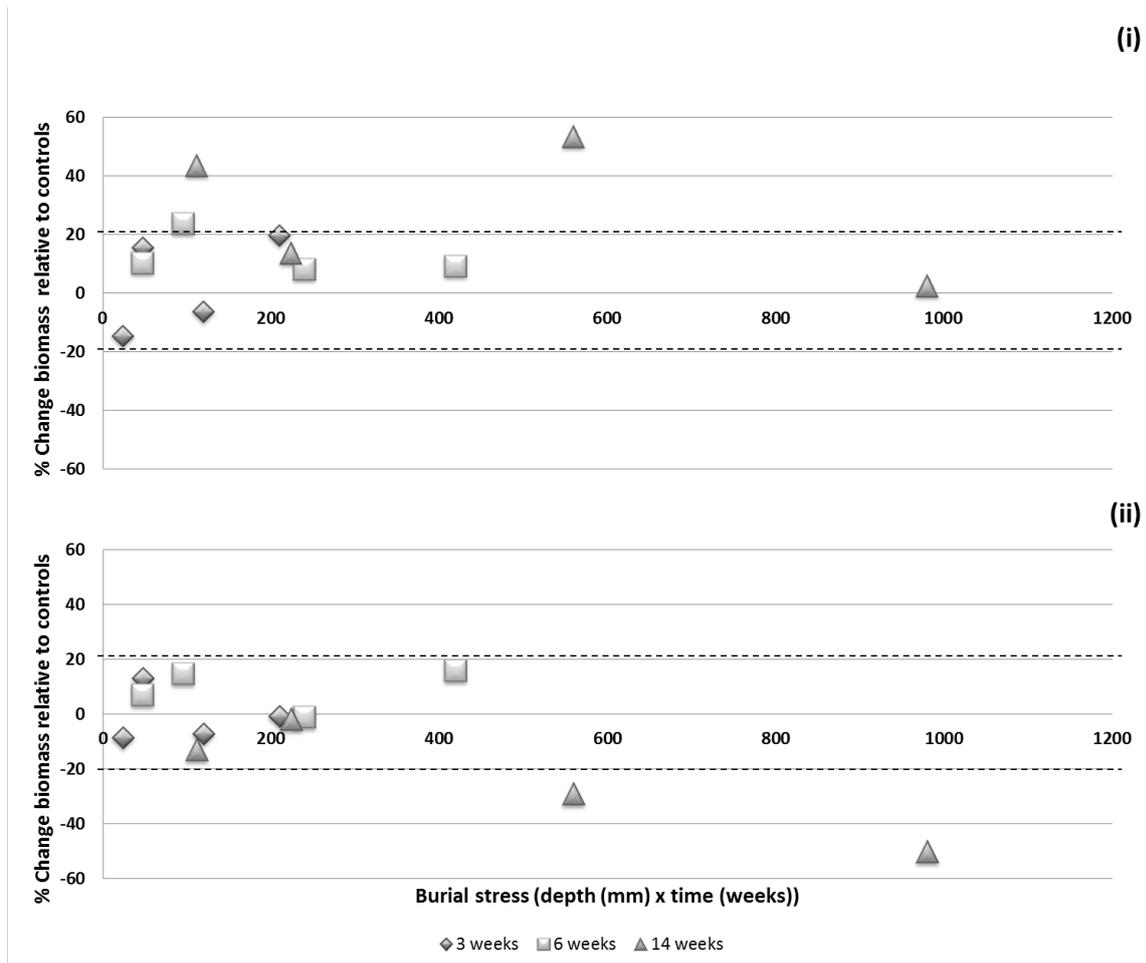


Figure 11. Burial stress thresholds based on the depth and duration of burial for *H. uninervis* (top) and *C. serrulata* (Bottom). These thresholds are based on the percent change in the treatments relative to the controls. The dashed lines indicate the expected natural variation around the controls, hence the points outside this bar indicate stimulation (positive direction) or inhibition (negative direction) due to burial stress.

The vertical rhizome length was the most consistent sublethal variable to respond to burial. There was a very clear response to increased depth and duration of burial, with *H. uninervis* responding at a greater rate to increased magnitude and duration of burial than *C. serrulata* (Figure 12). For *H. uninervis*, the background variation in vertical rhizome length was 12%, therefore we considered this the background variation range, as indicated by the dashed lines in the plot (Figure 13). We see a response to burial following 112 units or more of burial stress (3 and 6 weeks at 40 and 70 mm burial depth, or 14 weeks at all treatments). The background variation in *C. serrulata* was similar and it responded at the same level of burial stress, 112 units (70 mm, 14 weeks) but this was not consistent across all treatments until 224 units of burial stress was reached (6 and 14 weeks, 40 mm and above).

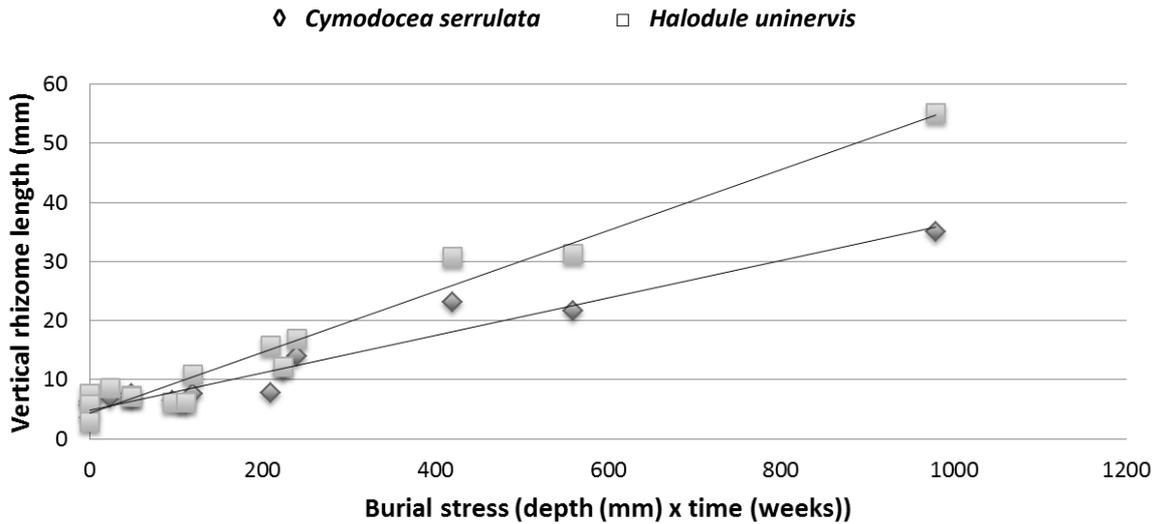


Figure 12. The relationship between burial stress (depth of burial x time) and vertical rhizome length for *H. uninervis* ( $y = 0.0514x + 4.4075$ ,  $R^2 = 0.9669$ ) and *C. serrulata* ( $y = 0.0316x + 4.8312$ ,  $R^2 = 0.9438$ ). *H. uninervis* responds at a faster rate compared to *C. serrulata*. Equations are the linear fit.

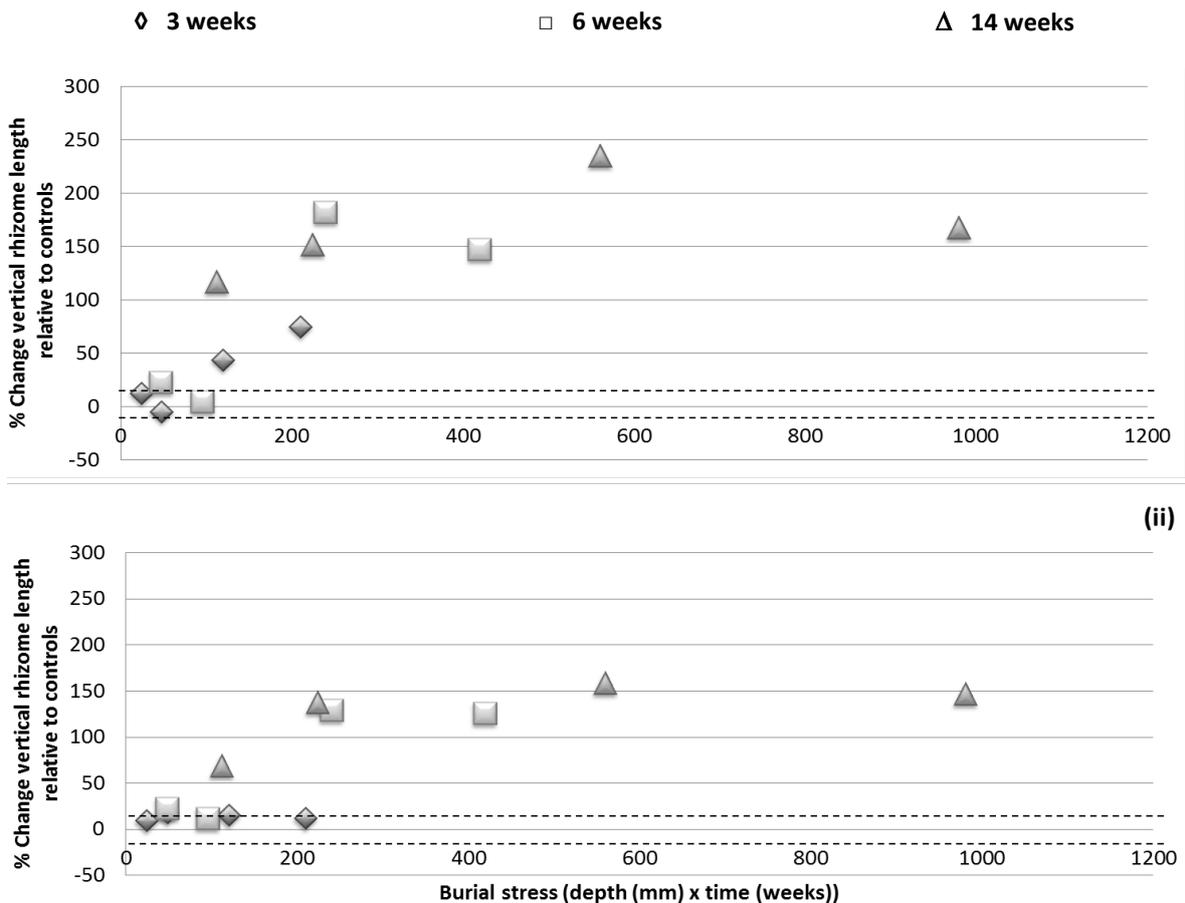


Figure 13. Sub-lethal burial stress thresholds for vertical rhizome length based on the depth and duration of burial for *H. uninervis* (top) and *C. serrulata* (Bottom). These thresholds are based on the percent change in the treatments relative to the controls. The dashed lines indicate the expected natural variation around the controls, hence the points outside this bar indicate stimulation (positive direction) or inhibition (negative direction) to burial stress.

## 4 Discussion

Sediment burial caused strong effects on physiology, growth and morphology of both seagrass species, though the magnitude and timeline of response was species specific. *Halodule uninervis* tolerated a burial depth of 70 mm for 14 weeks without adverse effects, whereas *Cymodocea serrulata* tolerated up to 70 mm of burial for 6 weeks but after 14 weeks could only tolerate 16 mm of burial before plants were negatively impacted. Despite the clear differences in size of both species, which corresponded to 21% of plant height for *C. serrulata* and 119% for *H. uninervis* (Table 1), the smaller *H. uninervis* tolerated greater burial depths than *C. serrulata* which was 30% larger. A larger size and the ability to vertically elongate have been identified as key features for the resilience to burial stress (Cabaco et al. 2008). Our work has shown that a smaller, faster growing plant with the ability to vertically elongate is resilient to burial stress and performs better than a larger plant, which also has the ability to vertically elongate but cannot respond at as fast a rate. Therefore, resilience to burial stress is related to size, as well as the ability to vertically elongate and the rate at which this occurs. Smaller species can be more resilient and acclimate to conditions, even after high levels of burial have been applied (e.g. 160% of leaf height, *Halodule uninervis*, Ooi et al. 2011), as long as there is adequate light availability to enable fast growth rates for vertical rhizome growth and leaf elongation.

*Halodule uninervis* was the seagrass species most tolerant to burial, showing increased vigour (shoot production rate, shoot density, biomass) with moderate burial depths (40 mm). Similarly, moderate burial stimulates photosynthesis, growth, reproduction and morphological changes for various grasses in inland and coastal dunes (Disraeli 1984, Zhang and Maun 1990, Yuan et al. 1993, Bach 2000). For seagrasses, moderate burial also encourages vertical growth, leaf production and flowering in *Cymodocea nodosa* (Marba and Duarte 1994, 1995) and vertical growth of *Thalassia testudinum*, *Cymodocea serrulata*, and *Halodule uninervis* (Marba et al. 1994, Ooi et al. 2011). Despite the higher sensitivity to burial showed by *Cymodocea serrulata* i.e. 50% mortality when buried up to 20 mm (in field conditions, Duarte et al. 1997) or up to 40 mm (in controlled tank conditions, this study), the adaptive response of this species facilitates an enhanced capacity for recovery (Duarte et al. 1997), resulting in a long-term ability to cope with burial. Areas with low levels of burial may see a dominance of *Halodule*, and if low levels of burial occur during dredging, then there may be a shift in species composition.

Despite high levels of burial relative to plant height, survival of both species can be attributed to their ability to respond rapidly, both physiologically and morphologically. Both species showed increased vertical rhizome growth and elongation in response to burial, but there was either no change in photosynthesis as measured by ETR<sub>MAX</sub> (e.g. *C. serrulata*), or increased photosynthesis (e.g. *H. uninervis*) with sediment burial. These results show that photosynthesis of exposed leaves (leaf apices exposed to light and not buried) does not appear to be negatively impacted when other parts of the leaf are buried. In response to a loss of photosynthetic area caused by partial burial of leaves, both species utilized rhizome carbohydrate reserves—even though the carbohydrates available to each species differed, with soluble sugars more abundant in rhizomes of *C. serrulata*, and starch more abundant in *H. uninervis*. At a burial depth of 40 mm, both species utilized up to 50% of their carbohydrate reserves relative to controls, and the mean leaf length and leaf area increased, thereby overcoming the loss of photosynthetic capacity and reducing the potential for sulphide toxicity caused by burial (Pedersen et al. 2004, Borum et al. 2005, Holmer et al. 2006). These increases in leaf length resulted in a 53% increase in plant height for *C. serrulata* and 68% for *H. uninervis*. Ooi et al. (2011) found that the threshold burial depth (80 mm) for *C. serrulata* and *H. uninervis* corresponded to 60 and 80% of plant height, respectively. Clearly, the similarity in our values of burial depth as a percentage of plant height could be close to the threshold values for both species.

Vertical rhizome growth also increased dramatically (>200% for both species), but with faster rates of change in *H. uninervis* matching vertical burial depths. Rhizome growth facilitates the vertical migration of shoots to new sediment heights (Figure 5, Terrados et al. 1999), and some species (*C. serrulata* and *S. isoetifolium*) only elongate vertically if clonal integration is not present which, in a dredging context, could represent broad spatial-scale burial (Ooi et al. 2011). However, vertical elongation is potentially costly to the plant in terms of carbon re-allocation, particularly if clonal integration is impaired. We measured an increased investment in vertical rhizome

growth potentially at the expense of horizontal growth, an example of a change in resource partitioning between these plant components. Interestingly, the concentration of carbohydrates in vertical compared to horizontal rhizomes was also enhanced at the end of the 14 weeks, suggesting the vertical rhizome is not only a structure that elevates shoots to new sediment heights, but also has another functional role as an energy storage organ. Carbohydrate-enriched vertical rhizomes may be an important adaptive feature of disturbance-driven species. Re-allocating storage reserves close to leaves may facilitate more efficient translocation of resources and aid in more rapid recovery in the event of another burial episode, improving the plants potential for survival and recovery.

Due to the very consistent response of the vertical rhizome growth, this is an excellent bio-indicator, identifying that the plant is responding to sub-lethal levels of burial. This response could be applied in dredging monitoring programs that aim to identify whether impacts are related to burial stress or light reduction. Four consistent bio-indicators relating to light reduction have been determined in the WAMSI DSN Project 5.5.1, and these differ to the bio-indicator for sublethal burial 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. These differences in bio-indicators between stresses allow resource managers to determine which stressor is causing the greater impact.

In NW Australia, the level of sedimentation or burial that species can cope with has been studied for 7 out of 13 species, including *C. serrulata* and *H. uninervis* (see WAMSI DSN Project 5.5.1). Ooi et al. (2011) reported that a burial of 20 and 40 mm, respectively, imposes severe stress (50% mortality) in *C. serrulata* and *H. uninervis*. In contrast, in this study, *C. serrulata* only suffered a ~50% loss of biomass and shoot density (relative to controls) at 40-70 mm burial depth, and *H. uninervis* was unaffected by burial of up to 70 mm. One possible explanation for these differences among studies may be due to differences in the sediments used. Sediments added to plants in the field (Ooi et al. 2011) had high concentrations of organic matter (3% sediment DW compared to 0% in sediments used in this study). Organic-enriched sediments potentially increase exposure of plants to anoxia, through microbial decomposition of the organic matter in the overlying sediment, and toxicity in sediments containing high levels of sulphides (Cabaco et al. 2008). Despite this, complete loss for these species did not occur even when 80–160 mm of sediment was applied (Duarte et al. 1997, Ooi et al. 2011). However, Ooi et al. (2011) was unable to comment on longer term impacts of deeper burial due to the temporal restriction in sampling (27 d), while Duarte et al. (1997) also missed the longer-term impacts of these deeper burial depths since added sediments were eroded to control heights within the 10 month duration of the trial. Our finding confirms long term survival (up to 14 weeks) of *H. uninervis* and *C. serrulata* at burial depths of up to 70 mm. Indeed, previous reports of plant decline at 80 mm for *C. serrulata* and *H. uninervis* may in fact be a critical threshold burial depth for these species (Ooi et al. 2011), although there still remains insufficient data on seagrass tolerance over relevant timescales of plant response after burial. Notwithstanding the above limitations, the existing thresholds provide some insights into the range of burial depths appropriate for developing dredging-related thresholds.

## 5 Conclusion

In the absence of more comprehensive field data on the burial pressures created by dredging, or on the interactive effects of burial and light reduction, we conclude that dredging-induced burial is unlikely to be a significant cause of seagrass loss in the far-field environment. It is possible that burial pressures above the previously reported threshold of 80 mm could occur in dredge spoil dumping grounds and in the near-field. We also conclude that sub-lethal burial pressures may result in a change in seagrass species composition, away from slow-growing and more persistent species towards fast-growing, colonising species.

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