



## Response of a mixed tropical seagrass assemblage to burial by inorganic and organic sediments under low light

John Statton<sup>1,3</sup>, Kathryn McMahon<sup>2,3</sup>, Roisin McCallum<sup>2,3</sup>, Manuel Vivanco Bercovich<sup>1,3</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

#### Theme 5 Report

Project 5.5.4

November 2017





## 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.

### Ownership of Intellectual property rights

Unless otherwise noted, any intellectual property rights in this publication are owned by the Western Australian Marine Science Institution, Edith Cowan University and the University of Western Australia.

### Copyright

© Western Australian Marine Science Institution

All rights reserved.

Unless otherwise noted, all material in this publication is provided under a Creative Commons Attribution 3.0 Australia Licence. (<http://creativecommons.org/licenses/by/3.0/au/deed.en>)



### 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



## Legal Notice

The Western Australian Marine Science Institution advises that the information contained in this publication comprises general statements based on scientific research. The reader is advised and needs to be aware that such information may be incomplete or unable to be used in any specific situation. This information should therefore not solely be relied on when making commercial or other decision. WAMSI and its partner organisations take no responsibility for the outcome of decisions based on information contained in this, or related, publications.

**Year of publication:** 2017

**Metadata:** <http://catalogue.aodn.org.au/geonetwork/srv/eng/metadata.show?uuid=50f82e70-3324-478a-ac92-b00a95b6cecf>

**Citation:** Statton J, McMahon KM, McCallum R, Vivanco Bercovich M Kendrick GA, Lavery PS (2017) Response of a mixed tropical seagrass assemblage to burial by inorganic and organic sediments under low light. Report of Theme 5 - Project 5.5.4 prepared for the Dredging Science Node, Western Australian Marine Science Institution, Perth, Western Australia, 28 pp.

**Author Contributions:** JS, KM, PL and GK conceived the experimental design. JS and KM supervised implementation of the experiments. JS and RM processed the experimental samples. JS and KM prepared initial drafts of the report with editorial input from PL and GK. All authors read and approved the final report.

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

**Competing Interests:** The commercial investors and data providers had no role in the data analysis, data interpretation, the decision to publish or in the preparation of the manuscript. The authors have declared that no competing interests exists.

**Acknowledgements:** We acknowledge the assistance of Paul Armstrong in establishing the research infrastructure for this project and Natasha Dunham for assistance with sample processing. Thanks to the WAMSI Dredging Science Node leadership team, Ray Masini, Kevin Crane and Ross Jones for their helpful advice. Andrea Zavala Perez and Samuel Gustin-Craig assisted in maintaining the experimental mesocosms, the UWA Marine Research Volunteers also assisted in maintaining the experimental mesocosm as well as sample processing.

**Collection permits/ethics approval:** Flora collection permit; DEC SOPP SW014729

## 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: Individual pots with mixed seagrass species in an experimental tank at the UWA Seagrass Facility. (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: A siphon hose removes fine sediments that were used to bury the seagrasses *Cymodocea serrulata* (wide leaf) and *Halodule uninervis* (narrow leaf) after sediment burial. (Source: John Statton)

# Contents

EXECUTIVE SUMMARY .....	I
CONSIDERATIONS FOR PREDICTING AND MANAGING THE IMPACTS OF DREDGING .....	II
PRE-DEVELOPMENT SURVEYS .....	II
IMPACT ASSESSMENT .....	II
POST-APPROVAL .....	III
RESIDUAL KNOWLEDGE GAPS .....	III
<b>1 INTRODUCTION .....</b>	<b>1</b>
<b>2 METHODS .....</b>	<b>2</b>
2.1 PLANT COLLECTION .....	2
2.2 EXPERIMENTAL TANK SYSTEM, DESIGN AND SETUP .....	2
2.3 INDICATORS MEASURED.....	4
2.4 PHYSIOLOGICAL INDICATORS.....	4
2.4.1 <i>Photo-physiology</i> .....	4
2.4.2 <i>Rhizome carbohydrates</i> .....	5
2.4.3 <i>Leaf nutrient and isotopic analysis</i> .....	5
2.5 PLANT AND MEADOW-SCALE INDICATORS.....	6
2.5.1 <i>Growth, biomass and morphology</i> .....	6
2.6 STATISTICAL ANALYSIS.....	6
<b>3 RESULTS.....</b>	<b>6</b>
3.1 PHYSIOLOGICAL RESPONSES.....	6
3.1.1 <i>Photophysiology</i> .....	6
3.1.2 <i>Leaf nutrients and isotopes</i> .....	8
3.1.3 <i>Carbohydrates</i> .....	10
3.2 PLANT SCALE RESPONSE.....	11
3.2.1 <i>Growth and morphology</i> .....	11
<b>4 DISCUSSION .....</b>	<b>15</b>
<b>5 REFERENCES.....</b>	<b>17</b>



## Executive Summary

Seagrasses can be negatively affected by dredging-associated deterioration in water clarity due to suspended sediments and burial by the subsequent deposition of those sediments. The majority of studies investigating these impacts of dredging have focussed on each factor in isolation. Furthermore, past studies examining the effects of burial have not examined whether the nature of the sediment can affect the plant response, despite knowledge that organic-rich sediments can produce conditions unfavourable to seagrasses. This report presents findings from a controlled sediment burial experiment that aimed to determine the effects of sediment burial using inorganic and organic-rich sediments under dredge-simulated conditions of severe light reduction on the growth and health 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. influence of burial sediments on seagrass response relationships, sub-lethal and lethal bio-indicators and thresholds) to the prediction, assessment, monitoring and management of dredging programs in northwest Western Australia.

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 mesocosm conditions, replicate pots were subjected to four sediment burial depth treatments (0, 5, 16 and 40 mm), mimicking a range of heights that were 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 (Cabaco 2008, McMahon et al. 2016; Statton et al. 2016b). Burial sediments either had no organic matter or had organic matter (4% sediment dry weight) added in the form of dried, ground (< 2 mm) seagrass leaves and mixed homogeneously within sediments prior to application in pots. We ran the experiment at  $\sim 2.4$  mol photons  $\text{m}^{-2} \text{d}^{-1}$  for 6 weeks, harvesting and measuring plant condition at the end of the experiment.

There were three separate but linked components of the study:

1. Under the imposed light and burial depths with and without organic matter, we determined the cause-effect pathway from measurements of 13 response variables;
2. While it was not a main aim to assess bio-indicators in this study, because similar treatments were tested in Experiment 5.5.2 (Statton et al. 2016b) and under severe light reduction similar to Experiment 5.5.1 and 5.5.3 (Statton et al. 2016a; Statton et al. 2016c) we identify whether the bio-indicator 'vertical rhizome length' is consistent with 5.5.2 even with different sediments and under reduced light conditions; and
3. Using the variables identified in (2), we determined sub-lethal and lethal thresholds of tolerance to different burial sediments under severe light reduction and compared these to thresholds developed in Experiment 5.5.2 (Statton et al. 2016b).

The key findings were:

- Sediment burial depths of 40 mm had significant impact on the shoot density (50% decrease) of both species of seagrass but only when organic matter was present in burial sediments. Without organic matter, burial had little effect on plant physiology, shoot density, morphology and biomass.
- Vertical rhizome growth was not an appropriate bio-indicator for monitoring burial stress in this experiment. This study reveals that under severe light limitation plants do not have the capacity for vertical rhizome growth, and consequently it is not a useful bio-indicator for identifying that the plant is responding to sub-lethal levels of burial under these conditions.
- We found that the rhizome carbohydrate reserves in seagrass plants exposed to 6 weeks of low light conditions  $\sim 2.4$  mols photons  $\text{m}^{-2} \text{d}^{-1}$  were similar in Experiment 5.5.1, 5.5.3 and this study, for both species. The carbon deficit due to reduced light was compensated by utilisation of rhizome carbohydrate reserves. Therefore, as vertical elongation is energetically costly to the plant (Statton

et al. 2016b), reduced light availability appeared to have an over-riding effect on both species ability to escape burial through rhizome elongation.

- Importantly, the lack of differentiation in bio-indicators when these stressors (burial and low light) overlap will limit resource managers' ability to determine which stressor is causing the greater impact.

This work reveals potential synergistic effects of low light and burial stress. It also identified a burial threshold value for both species (40 mm) that was lower than that found in WAMSI DSN Project 5.5.2 (Statton et al. 2016b) and is related to the composition of burial sediments (organic matter content) and the lower light availability used here, indicating that sediment characteristics of dredged sediments are an important factor to consider.

## 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 framework (EPA, 2106). 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 these three phases.

### Pre-development Surveys

Impact prediction will require an understanding of the baseline characteristics of sediments likely to be re-suspended and subsequently deposit on seagrass habitat. Relevant sediment characteristics include the organic matter content, as well as the background and dredging induced deposition and net burial rate at a site. This, and previous studies, have highlighted that there is currently almost no field data on background burial pressure or those induced by dredging. Pre-development surveys offer the opportunity to characterise the background natural sediment bed load and sediment dynamics (deposition, re-suspension) and characteristics (e.g. organic matter content) as well as seagrass species responses to previous sedimentation and light history (i.e. identified by vertical rhizome elongation and rhizome carbohydrate concentrations) that are necessary to determine appropriate thresholds to apply in impact prediction.

### Impact Assessment

In predicting the impact of dredging on seagrasses, the effects of sediment organic matter content should be taken into account. Despite earlier findings (Statton et al. 2016b) that seagrasses were tolerant of burial up to 70 mm for several weeks, this study has shown that this tolerance is reduced as the organic matter content of sediment increases and by the interactive effects of low light conditions. Sediment burial levels of up to 40 mm for 6 weeks with organic-rich sediments (4% sediment dry weight) resulted in 50% shoot mortality on both seagrass species in this study. On this basis, conservative sub-lethal **tolerance thresholds can be estimated as:**

**for *Cymodocea serrulata* and *Halodule uninervis*: 40 mm of burial for 6 weeks with organic rich (>4% organic matter) sediments**

The species used in these experiments 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.



The complete absence of accurate field-based data on burial pressures and organic matter content of burial sediments 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. 2016), which have significant limitations as discussed in that report. Based on those data, the maximum estimated sediment deposition rates at seagrass sites predicted to be impacted by dredging project in NW WA were 0.42–18.5 mm d<sup>-1</sup> (McMahon et al. 2015). Therefore, achieving a burial depth of 40 mm would require between 2 and 95 days, with no resuspension and for those conditions to then persist for over 40 days. On the basis of these estimates, the burial depths applied in this burial experiment are likely to reflect the upper range of burial depths seagrasses will experience near dredging projects and are more likely to occur in the near-field or dredge spoil disposal areas. Nonetheless, reliable field data on net sediment deposition rates is required to verify this.

## **Post-Approval**

Statton et al. (2016b) found vertical rhizome elongation was an excellent indicator of burial stress when plants were grown under ambient light. However, the lack of response of this variable when plants were buried under low light conditions indicates that there is no consistently reliable bio-indicator of sub-lethal burial stress and that sediment organic content and light conditions need to be taken into account. This outcome is important to dredging monitoring programs that aim to identify whether impacts are related to burial stress or light reduction. The lack of differentiation in bio-indicators between stressors limits resource managers' ability to determine which stressor is causing the greater impact.

From our results, it is clear that burial by organic rich sediments adds an increased stress under low light conditions and therefore, the impacts on seagrasses are likely to be more severe. Prediction of impacts based on light thresholds due to the ease of collection of information on light intensity thresholds alone may underestimate the impacts of dredging if burial stress, particularly by organic-rich sediments, is also present.

## **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**

There are inadequate field data to accurately characterise burial pressure that seagrasses are exposed to with and without dredging pressure. While the sorts of maximum burial rates estimated from field data appear to pose a relatively low pressure to seagrasses, there is 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 EPA (2016).

Uncertainty remains as to whether a single depositional event, such as that imposed in this and other experimental studies, reflects the stress experienced by seagrasses near dredge plumes, and whether the 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. 2016) and do not allow re-suspension (Storlazzi et al. 2011) and so provide a gross deposition over week to month timescales. Alternatively, there are water column suspended sediment concentrations but using these to estimate burial rates requires a significant number of assumptions regarding sediment density, re-suspension 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. Similarly, the concentration of organic matter in sediments that were to be dredged was shown to vary between 3 – 5 % sediment dry weight (McMahon et al. 2015). However, the type or source of organic matter (high quality and fast decomposing vs.

low quality and slow decomposing) is less well known and may influence the plant response (e.g. Statton et al. 2012 and references therein).

### **Interactive effects of burial and light reduction pressures**

Our study imposed burial pressure with two sediment types and under one level of severe light stress. The findings clearly showed that at this high level of light reduction and at 4% organic matter, the organic content of strongly influences the effect of burial. However, it remains unclear if these affects will occur at lower levels of organic content or at lower levels of light reduction. It is clear however that thresholds derived from single-pressure experiments are highly likely to underestimate impacts and therefore more extensive research into the interactive effects of dredging-induced pressures is required.

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

Our study was a mesocosm-based experiment in which burial pressure was addressed with two sediment types and under one level of severe light stress. 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 trialled here, and warrants further investigation.

.

# 1 Introduction

Coastal, port and channel dredging activities have resulted in direct seagrass loss in many locations worldwide, with conservative estimates amounting to losses greater than 20 000 ha. (Erftemeijer & Lewis, 2006). Apart from direct removal of seagrasses the associated reductions in water clarity through sediment re-suspension, as well as subsequent burial of plants when suspended sediment particles settle out of the water column, can also affect seagrass survival and distribution (Erftemeijer & Lewis, 2006). While seagrass meadows show some capacity to tolerate light reduction (Ralph et al 2008) and burial (Cabaço et al., 2008, Duarte et al. 1997, (Tuya et al 2013, Ooi et al 2011; WAMSI DSN Project 5.5.1) for short periods via physiological and morphological adjustments, the combined effects of these stressors has not been investigated. In addition, the type of sediments introduced into seagrass meadows from dredging operations can also vary with respect to the particle size and amount of organic matter, which may elicit different responses.

One of the most ecologically damaging consequences of dredging on primary producers is the reduction in light availability for photosynthesis (Erftemeijer and Lewis 2006). Not surprisingly, there are many published studies that address the different scales of response to light reduction from cellular to population level, and these responses have been summarized in reviews (Ralph et al. 2008; McMahon et al. 2013). Burial is also an important pressure produced by dredging, yet the impacts on seagrasses have been less well studied. Current knowledge indicates that resilience of seagrasses to burial can vary in accordance with species-specific features (fast growth rates, WAMSI DSN Project 5.5.2, magnitude of burial depth (Marba and Duarte 1994; Ooi et al. 2011; WAMSI DSN Project 5.5.2) and event duration (Cabaço et al. 2008, WAMSI DSN Project 5.5.2). In general, a burial depth of 8 cm has been identified as a critical level for some tropical species, showing signs of impact within one month after burial (Ooi et al. 2011). However, studies that have addressed the impacts of burial have typically done so independent of other dredging related stresses, such as low light. For example, under ambient light conditions seagrasses show some resilience to moderate levels of burial stress (<8 cm), with some species responding in ways that promote plant vigour. In *Cymodocea nodosa* for example, burial stimulated vertical growth, leaf elongation, and flowering (Marba and Duarte 1994, 1995) while in *Cymodocea serrulata* and *Halodule uninervis* vertical growth was stimulated (Ooi et al. 2011; WAMSI DSN Project 5.5.2). However, these studies do not represent the plant response under a dredging scenario, where light availability is also limited, and therefore may overestimate the plant response and threshold of tolerance to burial.

Little consideration has been given to the effect of different sediments on burial responses, despite evidence of negative interactions between plants and sediments. For example, where fine sediments have been deposited on top of seagrass meadows, porewater flow tends to be restricted, leading to changes in the redox state of the sediments and meadow decline (Terrados et al. 1998). In addition, increased levels of organic matter in sediments can result in oxygen depletion and production of hydrogen sulphide, a known phytotoxin (Holmer and Hasler-Sheetal 2014). Sulphide intrusion can decrease plant photosynthetic activity, leaf elongation rate, carbohydrate reserves and above-ground biomass (Holmer and Bondgaard 2001). Although, plants have an ability to counter the formation of reduced toxic compounds close to the root zone by delivering photosynthetically derived oxygen to the sediments via roots (Borum et al. 2005), sediments that are organic-rich can increase the respiratory load of the sediments beyond a plant's oxidative capability (Holmer et al. 2005). Similarly, a reduction in a plant's capacity to photosynthesize (e.g. low light and/or burial) can also decrease oxygen production and transport to the root zone (Ralph et al. 2007). Given that seagrasses may experience multiple stresses under a dredging operation, our current understanding, from a single stress approach, may potentially underestimate critical burial depths.

In this study, we examined the critical short-term (weeks) response of two co-occurring species of seagrasses to burial stress with inorganic and organic-rich sediments under reduced light conditions that simulate a dredging scenario. *Halodule uninervis* is regarded as small, opportunistic/colonising species with fast growth rates and *C. serrulata* is a comparatively larger, colonising species (Kilminster et al. 2015). Both species are regarded as being capable of withstanding low-moderate levels of burial under ambient light conditions (Ooi et al. 2011; WAMSI

DSN Project 5.5.2 (Statton et al. 2017b)). Here we subjected them to reduced light conditions ( $\sim 2.4$  mol photons  $\text{m}^{-2} \text{d}^{-1}$ ) simulating light availability under a typical dredge plume in the far field (McMahon et al. 2016), which has been shown to cause a negative plant response for both species (WAMSI DSN Project 5.5.1 (Statton et al. 2017a), 5.5.3 (Statton et al. 2017c). The burial depth levels were pre-determined based on a sediment burial depths plants could potentially experience under dredging situations and the levels likely to induce a plant response in these species (WAMSI DSN Project 5.5.2). WAMSI DSN Project 5.5.2 informed us that under ambient light conditions, low sediment burial levels ( $<40$  mm) appear to have little effect (or positive effects) for both *C. serrulata* and *H. uninervis*. At 40 mm of sediment burial and above, *C. serrulata* was negatively affected, showing reduced shoot density and shoot production rate whilst *H. uninervis* showed a positive growth response up to 70 mm (WAMSI DSN Project 5.5.2). In that experiment, burial pressure was imposed using fine inorganic sediments. However the interactive effect of sediment burial with organic-rich sediment could be different to the earlier findings, due to differences in the sediment biogeochemistry when organic matter is present. Available data for the NW of Western Australia show that natural sediment organic matter concentrations range from 3–5% of sediment dry weight, with a mean of  $\sim 4\%$  of sediment dry weight (WAMSI DSN Project 5.1.1).

## 2 Methods

### 2.1 Plant Collection

On the 21 May 2015,  $\sim 1000$  ramets each of the seagrasses *Cymodocea serrulata* (R. Brown) Ascherson and Magnus, *Halodule uninervis* (Forsskål) Ascherson (1882) were collected by gently excavating by hand, from Useless Loop, Shark Bay ( $26^{\circ} 07' 08''\text{S}$ ,  $113^{\circ} 24' 57''\text{E}$ ) Western Australia 12 weeks prior to the beginning of the experiments. Ramets were then placed in aerated and insulated containers filled with seawater for transport to University of Western Australia's seagrass growth facility, Perth, Western Australia (1000 km or 12 h travel time). At the seagrass growth facility ramets, consisting of one or more intact apical shoots and with at least three and up to six mature shoots, were prepared for planting. When a ramet had more than six mature shoots, additional shoots were removed using a sharp blade. If the apical shoot was damaged or missing, the ramet was discarded. On 23 May 2015, three ramets of each species were planted into a single square pot (280 mm sides  $\times$  300 mm deep), and a total of 56 pots were planted.

### 2.2 Experimental tank system, design and setup

The mesocosm system consisted of  $2 \times 1800$  L rectangular, fibreglass reinforced plastic tanks, each receiving ambient light. Each 1800 L tank was a closed, recirculating system, with seawater recirculating from a 600 L reservoir beneath each tank. Natural seawater from a nearby unpolluted area was used to fill each tank system, with one quarter exchanges every 2 weeks throughout the experimental period. Seawater was circulated using an  $8000 \text{ L h}^{-1}$  submersible pump, allowing complete replacement of water in the system  $80\times$  per day. Within each tank, incoming seawater was spread through a diffuser (T-bar) in order to create a homogenous movement of water. The seagrass research facility is temperature controlled with temperature set at  $27^{\circ}\text{C}$ . 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 organic matter content under reduced light conditions on co-occurring tropical seagrass species, we installed 28 square plastic pots (28 L  $\times$  28 W  $\times$  30 D cm) in each tank. Pots contained washed river sand and 0.5% DW organic matter added in the form of dried, ground *Posidonia* seagrass leaves (Statton et al. 2013). Plants were acclimated for approximately six weeks, at a temperature of  $27^{\circ}\text{C}$ , salinity of 36–37 ppt, and ambient light. After the acclimation period, the water re-circulating system was turned off and clean, inorganic silica sand, purchased from Rocla Quarry Products (median particle size of  $90 \mu\text{m}$ , grain size range: 63–120  $\mu\text{m}$ ) was applied.

This range in sediment grain-size (63–120  $\mu\text{m}$ ), which represents very fine to very coarse sand (Wentworth 1922), was chosen because the median size is broadly similar particle size distribution measured during dredging

(see WAMSI DSN Project 4.1 and Jones et al. (2016)). Silt-sized particles ( $<62.54 \mu\text{m}$ ) and clay sized particles ( $<4 \mu\text{m}$ ) were not used, as these sediment sizes would not fall out of suspension fast enough (according to particle-size specific settling velocities) during the period where the recirculation systems within the tanks were turned off to allow application of the sediment. Also, the normal water flow conditions within the tank systems could continually re-suspend finer silts and clays, eroding (removing) the sediment layer and generating turbidity within the tanks (see Discussion). The burial depths used (up to 40 mm) spanned a range which would most likely elicit a response in tropical seagrasses, as determined from published research (Cabaco et al. 2008, WAMSI DSN Project 5.5.2). This allows determination of lowest effect concentrations which, as Harris et al (2014) note, is far more useful in the regulatory sphere than a conclusion that no effect occurs at environmentally relevant concentrations, because an LOEC enables the regulators to impose more accurate and meaningful safety limits. During the sediment application process the fine sediments were retained within the pots by plastic barriers inserted along the edge. Each barrier height was adjusted to be 10 mm above the intended post-burial sediment height to prevent spillage and to discourage plants from growing over the pot edges. Control pots (no addition of fine sediment or fine sediment with organic matter) also had plastic barriers inserted to control for barrier artefacts. Burial depths mimicked a range of heights likely to occur in the field near to dredging operations through accumulation over 3–25 d based on average burial rates (WAMSI DSN Project 5.1.1) as well as being levels likely to induce response in the test species (WAMSI DSN Project 5.5.2). Based on these estimates, the sediment burial depths were 0 mm (control), 5, 16, and 40 mm (Table 1). Fine sediments applied to the seagrasses either had no organic matter (denoted -OM) or had organic matter (4% sediment dry weight) added in the form of dried seagrass leaves and mixed homogeneously within sediments prior to application in pots (denoted +OM). There were six burial x organic matter treatments and a control (Table 1). Three replicate pots were assigned a burial treatment and replicated in 2 tanks. The burial depths attained different relative heights for each species, as *C. serrulata* is taller than *H. uninervis* (Table 2). In the 40 mm burial treatment, 53% of the *C. serrulata* canopy height, and 68% of *H. uninervis* canopy height was covered.

To maintain reduced light levels of ambient light, two layers of high density shade cloth (70%) were applied to each tank. A light logger (HOBO PAR light loggers) was placed within a tank and recorded a mean of  $2.45 \pm 0.18 \text{ mol photons m}^{-2} \text{ d}^{-1}$  over the six week duration of the experiment (Table 3). After 6 weeks all the seagrass material in each pot was harvested. 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  $-20^\circ\text{C}$  freezer for later processing. All variables were measured at the end of the experiment only.

Table 1. Model of experimental design. Italicized number indicates number of replicate pots per treatment (burial depth, mm), organic matter (no organic matter [-OM], with organic matter at 4% sediment dry weight [+OM]) and tank. Each replicate pot contains both seagrass species (2 levels).

Burial treatment (5 levels)		0 mm		5 mm		16 mm		40 mm	
Tank (2 levels)		1	2	1	2	1	2	1	2
Organic matter (2 levels)	- OM	3	3	3	3	3	3	3	3
	+ OM			3	3	3	3	3	3

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 = 3)	Plant burial (% of shoot/canopy height)		
		5 mm	16 mm	40 mm
<i>C. serrulata</i>	75.4	7	21	53
<i>H. uninervis</i>	58.8	9	27	68

Table 3. Summary of the quantity of light received during the 6 week light deprivation period. Light received was averaged over 14 days (mol photons m<sup>-2</sup> d<sup>-1</sup> (± 1SE))

Time (weeks)	Mean total daily light (mol photons m <sup>-2</sup> d <sup>-1</sup> ((± 1SE))
2	2.11 (0.32)
4	2.49 (0.44)
6	2.74 (0.36)
Average over 6 weeks	2.45 (0.18)

### 2.3 Indicators measured

Indicators of seagrass status were measured throughout this experiment and these indicators ranged from sub-lethal physiological through to population level indicators (Table 4).

Table 4. Summary of indicators 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 (α)	6
		Minimum saturating irradiance (I <sub>k</sub> )	6
	Carbohydrate reserves	Rhizome carbohydrates	3
		Rhizome starch	3
		Nutrients and isotopes	3
		Shoot density	3
Plant-scale (state change)	Growth and biomass	Total biomass	3
		Aboveground biomass	3
		Belowground biomass	3
		Vertical rhizome length	3
	Morphology	Leaf area	3
Meadow-scale (pot level)	Abundance	Total (pot) biomass	3

### 2.4 Physiological indicators

#### 2.4.1 Photo-physiology

Photosynthetic characteristics were measured using a pulse-amplitude modulated (PAM) fluorometer (DIVING-PAM, Walz GmbH, Effeltrich, Germany). We used rapid light curves (RLCs) to determine the rate at which electrons are transported through photosystem II (PSII) at 9 pre-determined steps of increasing light intensity (0, 11, 26, 77, 115, 206, 317, 443 and 555 μmol photons m<sup>-2</sup> s<sup>-1</sup> for 10 s). Measurements were made by placing the fiber optic probe 3 mm away from the leaf using a spacer. RLCs were performed on 1 fully expanded leaf from a mature shoot for each species contained within a pot and replicated on 6 randomly assigned shoots from each replicate treatment.

Electron transport rates in  $\mu\text{mol electrons m}^{-2} \text{ s}^{-1}$  were calculated at each light level according to:

$$\text{ETR} = \phi_{PSII} \times \text{PPFD} \times 0.5 \times \text{AF} \quad \text{Equation 1}$$

Where:

$\phi_{PSII}$  is the effective quantum yield of Photosystem II (PSII, Genty et al. (1989);

PPFD is the photosynthetic photon flux density at each point of the RLC curve;

0.5 is a constant assuming that the photons absorbed are equally partitioned between PSII and PSI (Genty et al., 1989); and

AF is the absorbance factor or the fraction of PPFD absorbed by the leaf.

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:

$$\text{AF} = \frac{(\text{Incident}_{\text{PAR}} - \text{Transmitted}_{\text{PAR}})}{\text{Incident}_{\text{PAR}}} \quad \text{Equation 2}$$

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. We found a difference between species but no differences between treatments so the same AF value for each species was used for the entire experiment.

To quantitatively compare RLCs, they need to be described by several characteristics such as  $\alpha$  (the initial slope of the curve representing photosynthetic efficiency), the sub-saturating irradiance ( $E_k$ , which is the irradiance level at which photosynthesis starts to become saturated) and  $\text{ETR}_{\text{MAX}}$  (the maximum ETR) (Ralph & Gademann 2005)

Curve fitting was performed in R using the 'nls' routine (R Development Core Team, 2008) by fitting the data to a double exponential decay function (Platt et al. 1980) as described in Ralph and Gademann (2005):

$$P = P_S(1 - e^{-(\alpha E_d / \text{ETR}_{\text{MAX}})})e^{-(\beta E_d / \text{ETR}_{\text{MAX}})} \quad \text{Equation 3}$$

Where:

$\text{ETR}_{\text{MAX}}$  is the maximum ETR rate;

$E_d$  is the PAR light intensity (400–700 nm)

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

$\beta$  is the final slope of the curve (representing photoinhibition through damage to the PSII)

#### 2.4.2 Rhizome carbohydrates

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 and 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 ND Willis 1954).

#### 2.4.3 Leaf nutrient and isotopic analysis

Seagrass leaves were dried and ground to a fine powder using a steel ball-mill. 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

Shoot density, biomass, and morphology were measured on harvested plants. 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 leaf length (from the sheath to leaf tip) and leaf width at centre of leaf was measured on the youngest mature leaf. Leaf area was calculated by multiplying leaf length by leaf width. Similarly, the length of vertical rhizome from 3 shoots of *C. serrulata* and *H. uninervis* were measured to obtain vertical rhizome length. 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, 5, 16, 40 mm), organic matter (OM) content (fixed factor; -OM and +OM), Species (fixed factor; *C. serrulata* and *H. uninervis*), and Tanks (1 and 2 (blocks)) nested within Burial  $\times$  OM, on physiology, morphology, shoot density and biomass variables. Total pot biomass summed the biomass of all species within a pot, this was analysed using a three-way nested ANOVA (burial, organic matter content, Tank (burial  $\times$  OM)). Following a significant main effect or interaction, a Tukey's post hoc test was used to test for significant differences in treatment means (. 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 (R core team), and transformed where appropriate.

## 3 Results

### 3.1 Physiological responses

#### 3.1.1 Photophysiology

Maximum electron transport rate, ETRMAX, was affected by burial depth and organic matter content, but only for *H. uninervis* (Burial  $\times$  Species, MS = 87.8,  $p = 0.017$ ; OM  $\times$  Species, MS = 95.8,  $p = 0.049$ , Table 5). ETRMAX did not significantly differ from controls in any treatments but was significantly lower in 16 mm burial depth without organic matter than 40 mm with organic matter (Figure 1 ii). Photosynthetic efficiency ( $\infty$ ) and  $E_k$ , were not affected by burial depth or organic matter content (Figure 1 iii–vi, Table 5). For *C. serrulata*, photosynthetic efficiency was generally less than *H. uninervis*, but there was no difference in  $E_k$  (Table 5).



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 photophysiology. Bold numbers denote significant differences.

	df	Electron transport rate (ETR)		Photosynthetic efficiency ( $\alpha$ )		Half saturating irradiance ( $E_k$ )	
		MS	p	MS	p	MS	p
Species	1	745.4	<b>&lt;0.001</b>	0.025	<b>&lt;0.001</b>	400.1	0.196
Burial	3	45.8	0.1395	0.001	0.604	216	0.437
Organic Matter (OM)	1	11.8	0.489	0.001	0.383	694	0.090
Tank [Burial $\times$ OM]	7	30.56	0.532	0.005	<b>0.005</b>	845.1	<b>&lt;0.001</b>
Species $\times$ Burial	3	87.8	<b>0.017</b>	0.003	0.121	180.1	0.518
Species $\times$ OM	1	95.8	<b>0.049</b>	0.001	0.161	137.5	0.180
Burial $\times$ OM	2	1.1	0.956	0.001	0.657	238.1	0.368
Species $\times$ Burial $\times$ OM	2	69.7	0.063	0.001	0.899	284.2	0.304

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 leaf nutrient and isotopic ratio. Bold text denotes significant differences.

	df	Leaf carbon		Leaf nitrogen		Leaf C:N ratio		$\delta^{13}\text{C}$		$\delta^{15}\text{N}$	
		MS	p	MS	p	MS	p	MS	P	MS	p
Species	1	148.8	<b>&lt;0.001</b>	0.410	<b>0.004</b>	35.1	0.176	81.7	<b>&lt;0.001</b>	16.43	<b>0.009</b>
Burial	3	9.92	<b>0.007</b>	0.224	<b>0.003</b>	125.9	<b>&lt;0.001</b>	1.62	0.220	4.83	0.104
Organic Matter (OM)	1	0.41	0.6761	0.553	<b>&lt;0.001</b>	310.8	<b>&lt;0.001</b>	0.46	0.410	0.38	0.682
Tank [Burial $\times$ OM]	7	6.818	0.136	0.060	0.256	11.88	0.148	2.06	0.309	2.70	0.381
Species $\times$ Burial	3	8.77	<b>0.013</b>	0.019	0.741	8.75	0.708	0.83	0.300	0.18	0.971
Species $\times$ OM	1	4.36	0.169	0.001	0.921	3.37	0.673	1.32	0.166	4.18	0.179
Burial $\times$ OM	2	1.61	0.493	0.080	0.176	37.8	0.142	1.20	0.175	0.11	0.954
Species $\times$ Burial $\times$ OM	2	0.05	0.976	0.014	0.729	20.5	0.343	0.81	0.306	6.12	0.075

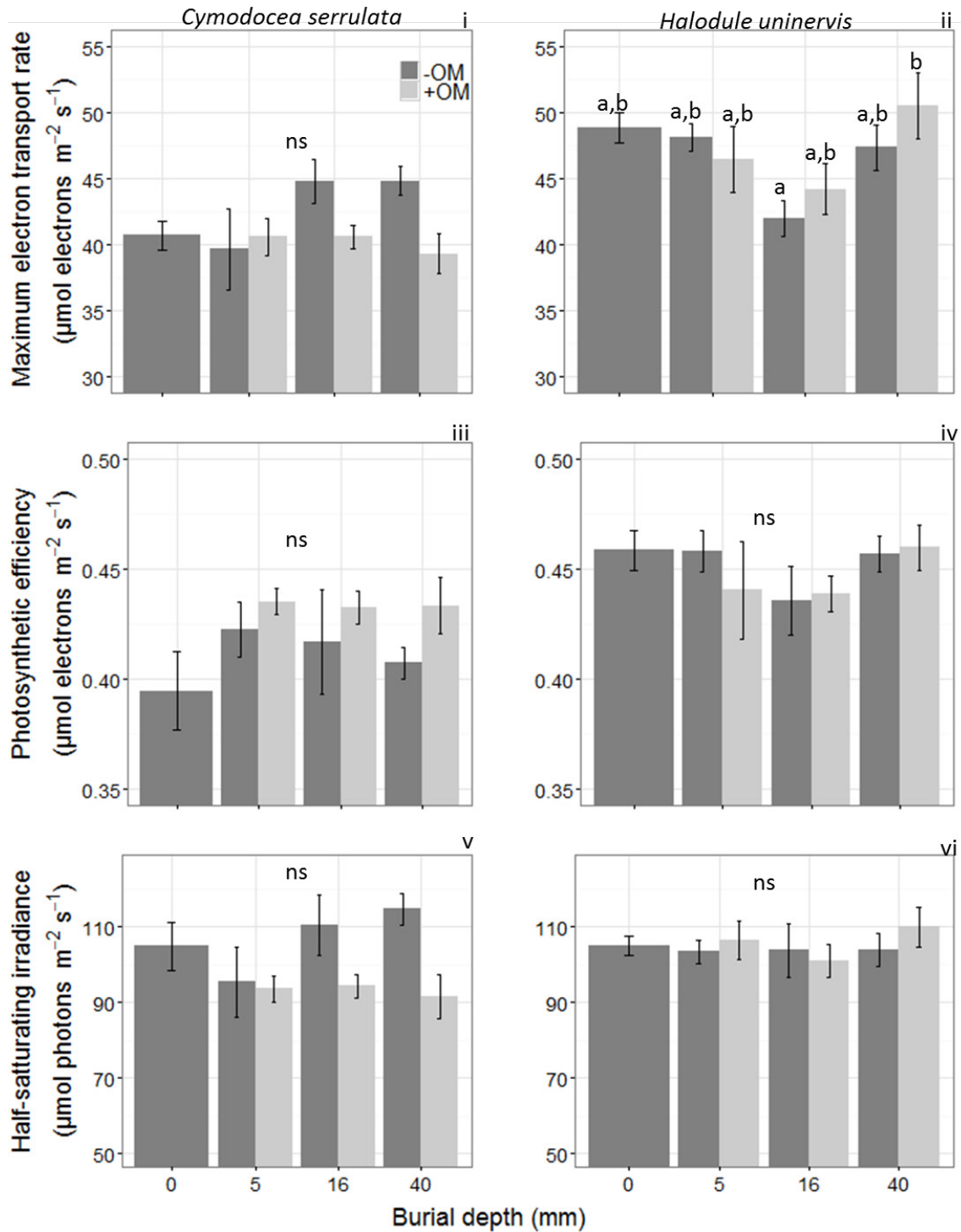


Figure 1. Maximum electron transport rate,  $\text{ETR}_{\text{MAX}}$  (i, ii), photosynthetic efficiency ( $\alpha$ ) (iii, iv), and half-saturating irradiance,  $E_k$  and (v, vi) of *C. serrulata* and *Halodule uninervis* after 6 weeks of burial under 5, 16, and 40 mm of sediment either with very low organic matter content (-OM) or sediment with added organic matter (+OM). Values are means pooled across tanks ( $n = 12$ )  $\pm$  SE. Letters denote significant differences. ns indicates no significant difference at 0.05 level.

### 3.1.2 Leaf nutrients and isotopes

Leaf nutrient concentration and isotopic signature were affected by burial depth and organic matter content but the responses varied with indicator measured. Leaf carbon concentration decreased when *C. serrulata* was buried regardless of organic matter content (Burial  $\times$  Species,  $\text{MS} = 8.77$ ,  $p = 0.013$ , Table 6) but in *H. uninervis*

was not affected by burial depth (Figure 2 i, ii). Leaf N concentration generally increased for both species with a burial depth of 40 mm (Burial effect, MS = 0.224,  $p = 0.003$ , Table 6), and when organic matter was present (OM effect, MS = 0.553,  $p < 0.001$ , Figure 2 iii, iv, Table 6). Accordingly, these changes in leaf N concentration affected leaf C:N ratio (Table 6, Figure 2 v, vi). For both species, leaf carbon and nitrogen isotopic ratios were not affected by burial depth or organic matter content, but there were differences between species, with *H. uninervis* showing lower  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotopic values compared to *C. serrulata* (Species effect, Figure 2 vii–x, Table 6).

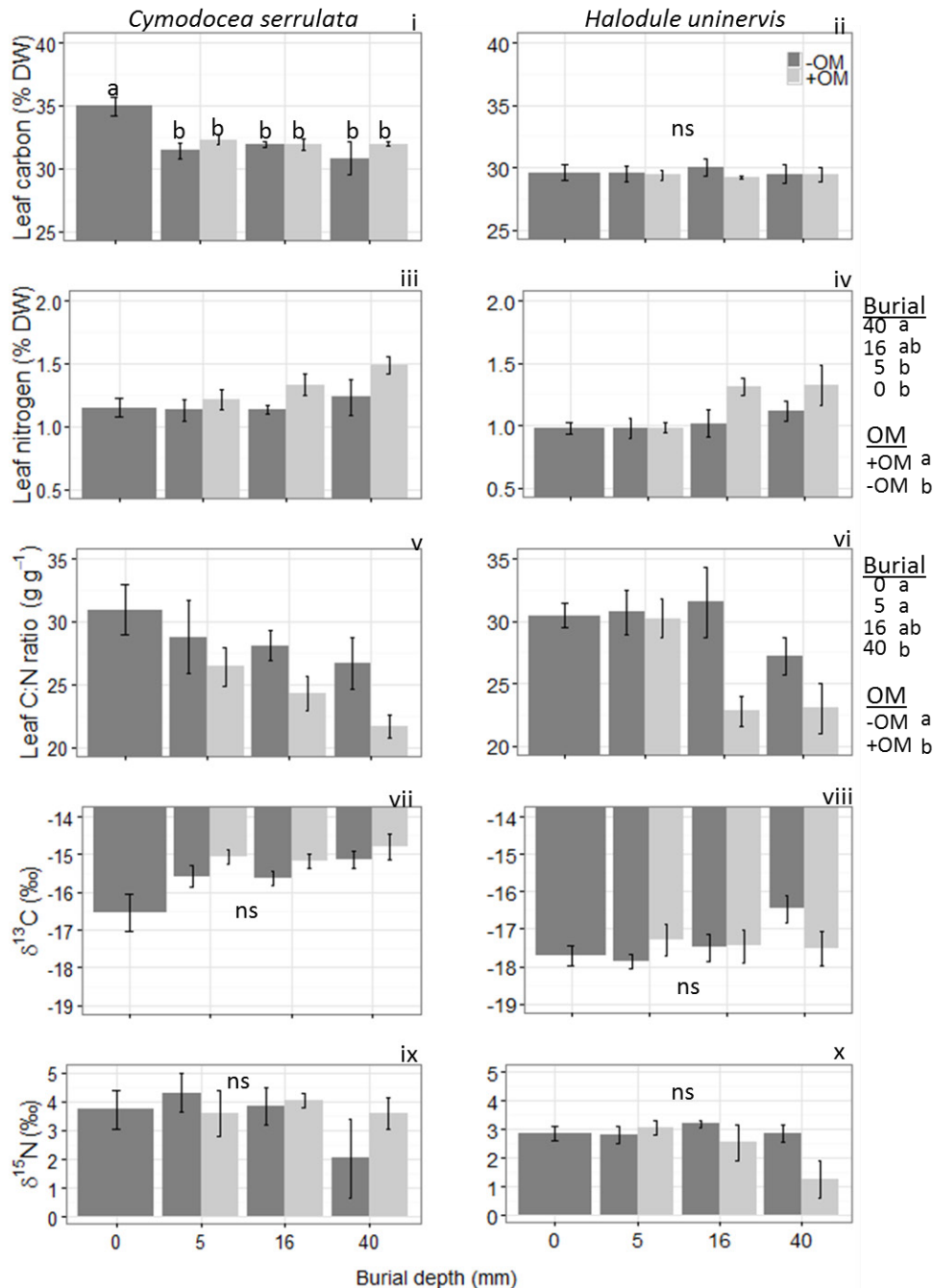


Figure 2. Leaf carbon (C) concentration, % DW (i, ii), nitrogen (N) concentration, % DW (iii, iv), C:N ratio, g g<sup>-1</sup> (v, vi), carbon isotope ratio (vii, viii), and nitrogen isotope ratio (ix, x) of *C. serrulata* (left) and *H. uninervis* (right) after 6 weeks of burial under 5, 16, and 40 mm of sediment either with very low organic matter content (-OM) or sediment with added organic matter (+OM). Values are means pooled across tanks ( $n = 6$ )  $\pm$  SE. Letters denote significant differences. ns indicates no significant difference at 0.05 level. Letters outside figure indicate significant differences between burial main effect pooled ( $n = 24$ ) and organic matter main effect ( $n = 28$ ) over all time periods for both species.

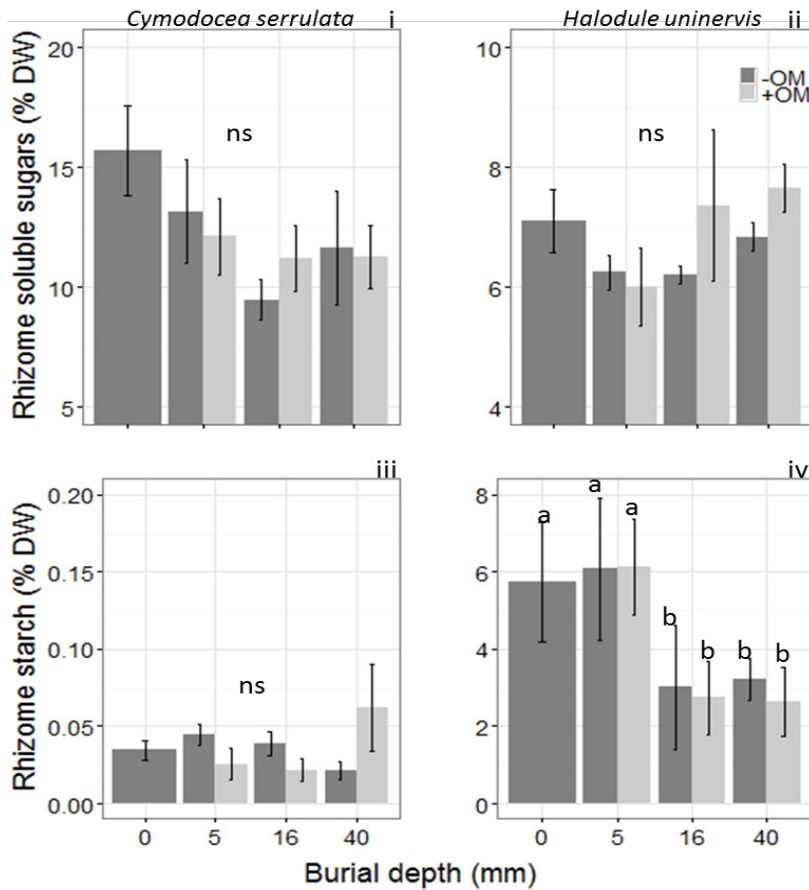


Figure 3. Rhizome soluble sugars, % DW (i, ii) and rhizome starch, % DW (iii, iv) of *C. serrulata* (left) and *H. uninervis* (right) after 6 weeks of burial under 5, 16, and 40 mm of sediment either with very low organic matter content (-OM) or sediment with added organic matter (+OM). Values are means pooled across tanks ( $n = 6$ )  $\pm$  SE. Letters denote significant differences. ns indicates no significant difference at 0.05 level.

### 3.1.3 Carbohydrates

The proportions of different rhizome carbohydrates (soluble sugars and starch) differed in *C. serrulata* and *H. uninervis*; *C. serrulata* had >15% soluble sugars compared to <0.04% starch whereas *H. uninervis* had relatively similar proportions; ~7% soluble sugars and ~6% starch. Soluble carbohydrates within rhizomes were not affected by burial depth or organic matter content, regardless of species (Figure 3 i, ii, Table 7). Rhizome starch concentrations were affected by burial depth, but only for *H. uninervis* (Burial  $\times$  Species, MS = 16.4,  $p = 0.029$ , Table 7). For *H. uninervis*, starch concentrations were significantly lower in the 16 mm and 40 mm burial depth regardless of organic matter content (Figure 3 iv).

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 rhizome soluble carbohydrates and starch. Bold text denotes significant differences.

	df	Rhizome soluble carbohydrate		Rhizome starch	
		MS	p	MS	p
Species	1	593	<b>&lt;0.001</b>	369	<b>&lt;0.001</b>
Burial	3	21.3	0.110	16.4	<b>0.029</b>
Organic Matter (OM)	1	2.2	0.642	0.3	0.814
Tank [Burial × OM]	7	22.8	0.258	2.37	0.980
Species × Burial	3	22.8	0.092	16.4	<b>0.029</b>
Species × OM	1	1.0	0.750	0.3	0.811
Burial × OM	2	6.5	0.530	0.1	0.978
Species × Burial × OM	2	1.3	0.883	0.2	0.967

### 3.2 Plant Scale Response

#### 3.2.1 Growth and morphology

Leaf area was 4× times greater for *C. serrulata* than for *H. uninervis* but for both species was unaffected by burial depth and organic matter content (Figure 4 i, ii, Table 8). Vertical rhizome length was also unaffected by burial depth and organic matter content (Figure 4 iii, iv, Table 8). The vertical rhizome growth of plants was generally less than 10 mm and more related to growth after planting (i.e. ramets were planted 2-3 cm below the sediment surface) rather than to vertical growth during the experimental treatments period. Sediment burial depth and organic matter negatively affected shoot density for both species (Burial × OM, MS = 23.9, p = 0.029, Table 8). Shoot density in the 40 mm burial treatment with organic matter was ~50% that of controls and all other treatments (Figure 5 I, ii).

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 area		Vertical rhizome length		Shoot density	
		MS	P	MS	p	MS	p
Species	1	577.2	<b>&lt;0.001</b>	154.4	<b>0.019</b>	646	<b>&lt;0.001</b>
Burial	3	3.8	0.123	9.54	0.788	29.9	<b>0.005</b>
Organic Matter (OM)	1	4.2	0.143	83.7	0.083	11.4	0.187
Tank [Burial × OM]	7	1.12	0.997	81.28	<b>0.003</b>	8.12	0.807
Species × Burial	3	2.2	0.342	11.6	0.734	5.2	0.494
Species × OM	1	0.2	0.753	7.44	0.602	4.3	0.418
Burial × OM	2	0.4	0.828	30.3	0.333	23.9	<b>0.029</b>
Species × Burial × OM	2	0.4	0.801	42.5	0.216	12.2	0.159

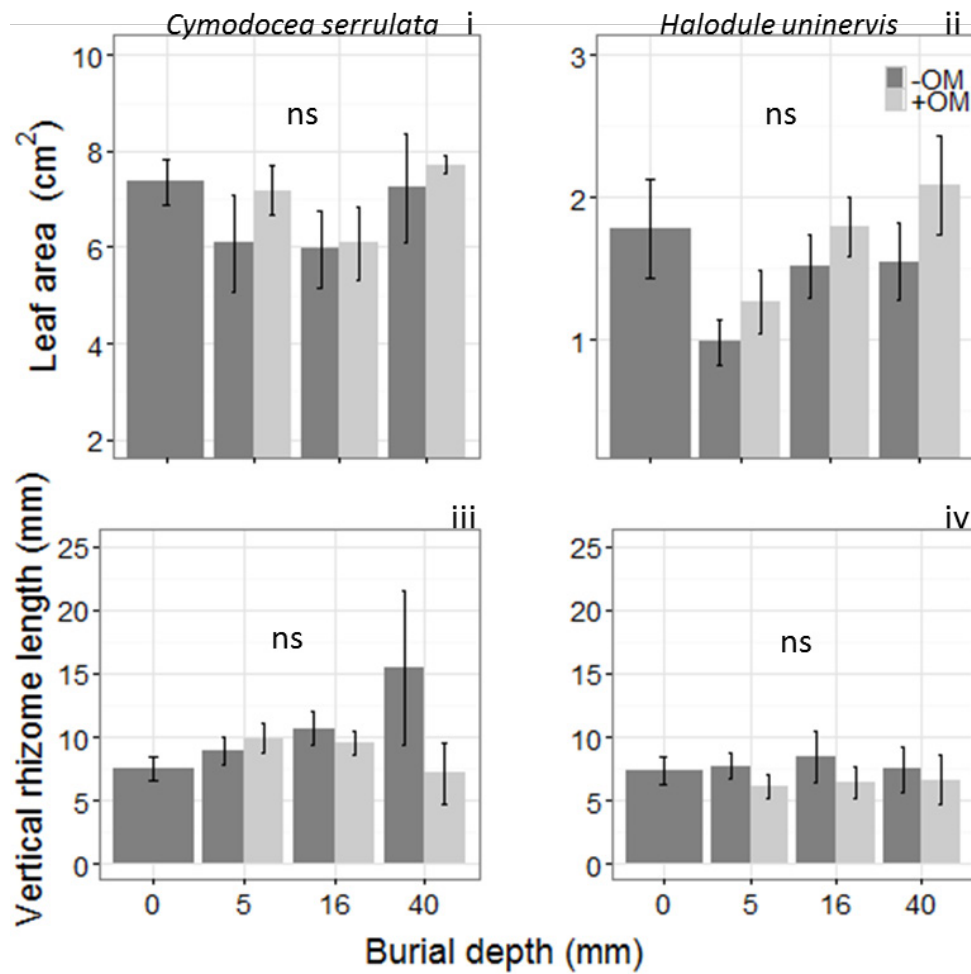


Figure 4. Leaf area, cm<sup>2</sup> (i, ii), vertical rhizome length (mm) (iii, iv) of *C. serrulata* (left) and *H. uninervis* (right) after 6 weeks of burial under 5, 16, and 40 mm of sediment either with very low organic matter content (-OM) or sediment with added organic matter (+OM). Values are means pooled across tanks (n = 8) ± SE. Letters, if shown denote significant differences. ns indicates no significant difference at 0.05 level.

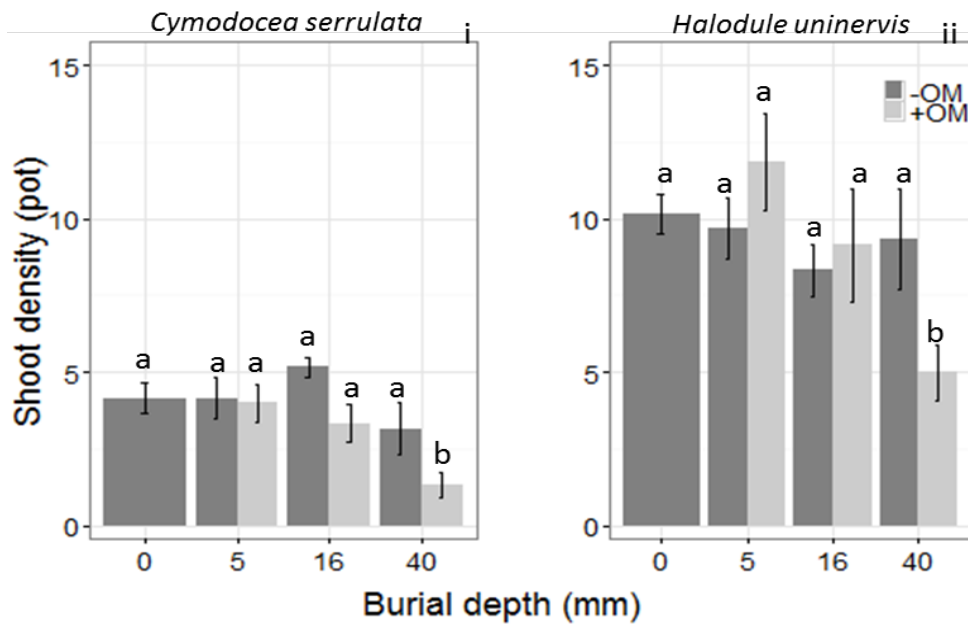


Figure 5. Shoot density (i, ii) of *C. serrulata* (left) and *Halodule uninervis* (right) after 6 weeks of burial under 5, 16, and 40 mm of sediment either with very low organic matter content (-OM) or sediment with added organic matter (+OM). Values are means pooled across tanks (n = 8)  $\pm$  SE. Letters, if shown denote significant differences. ns indicates no significant difference at 0.05 level.

Plant biomass (total, above- and below-ground) for each species was not significantly affected by burial depth or organic matter content (Figure 6, Table 9). Total pot biomass (both species combined) showed a significant effect of burial depth (Figure 7, Table 9) though no treatments were significantly different to the controls; only the 5 and 40 mm burial depths differed from each other.

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.

		Total biomass		Above-ground biomass		Below-ground biomass		Total (pot) biomass	
	df	MS	p	MS	p	MS	p	MS	P
Species	1	3.04	<b>&lt;0.001</b>	0.045	<b>&lt;0.001</b>	1.922	<b>&lt;0.001</b>	NA	NA
Burial	3	0.078	0.149	0.001	0.438	0.052	0.159	0.333	<b>0.020</b>
Organic Matter (OM)	1	0.000	0.987	0.002	0.242	0.004	0.713	0.041	0.501
Tank [Burial $\times$ OM]	7	0.056	0.684	0.003	0.270	0.035	0.722	0.068	0.651
Species $\times$ Burial	3	0.026	0.611	0.001	0.660	0.016	0.664	NA	NA
Species $\times$ OM	1	0.003	0.792	0.002	0.259	0.011	0.543	NA	NA
Burial $\times$ OM	2	0.044	0.362	0.001	0.462	0.034	0.321	0.183	0.142
Species $\times$ Burial $\times$ OM	2	0.023	0.579	0.001	0.881	0.024	0.465	NA	NA

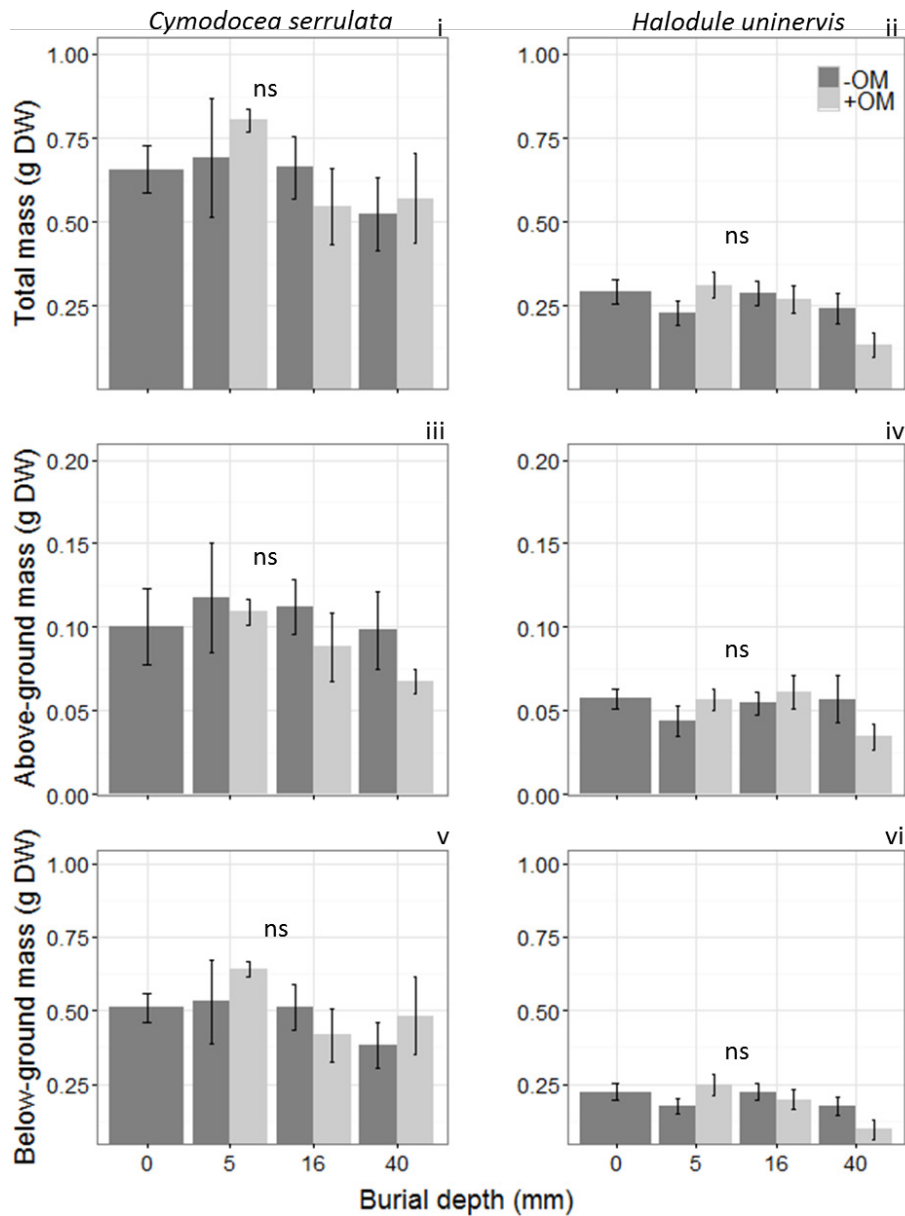


Figure 6. Total biomass, g DW (i, ii), above-ground biomass, g DW (iii, iv), and below-ground biomass, g DW (v, vi) of *C. serrulata* (left) and *Halodule uninervis* (right) after 6 weeks of burial under 5, 16, and 40 mm of sediment either with very low organic matter content (-OM) or sediment with added organic matter (+OM). Values are means pooled across tanks ( $n = 8$ )  $\pm$  SE. Letters, if shown denote significant differences. ns indicates no significant difference at 0.05 level.



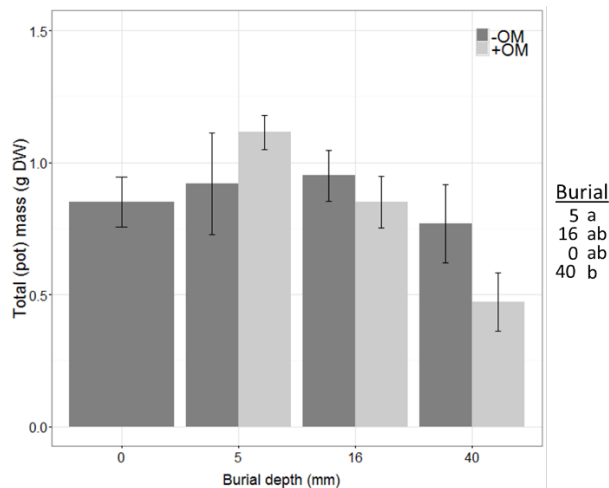


Figure 7. Total pot biomass, g pot<sup>-1</sup> both species combined (*C. serrulata* and *Halodule uninervis*) after 6 weeks of burial under 5, 16, and 40 mm of sediment either with very low organic matter content (-OM) or sediment with added organic matter (+OM). Values are means pooled across tanks (n = 8) ± SE. Letters denote significant differences. ns indicates no significant difference at 0.05 level. Letters outside figure indicate significant differences between burial main effect pooled (n = 16).

## 4 Discussion

Under reduced light conditions, burial with organic-rich sediments caused strong effects on shoot density and physiology of both seagrass species. Both species showed a ~50% decrease in shoot density after 6 weeks when plants were buried under 40 mm of sediment which had been enriched with organic material. This contrasts with the results of WAMSI DSN Project 5.5.2, where under ambient light and burial by sediments with a very low organic content, *H. uninervis* tolerated a burial depth of 70 mm for 14 weeks without adverse effects, whereas *C. serrulata* tolerated burial up to 70 mm for 6 weeks before plants were negatively impacted. Despite the clear differences in size of both species (with a burial depth of 40 mm corresponding to 53% of plant height for *C. serrulata* and 68% for *H. uninervis*), neither species was tolerant of organic-enriched sediments under the light conditions used. A larger size, or a small size and fast growth rates coupled with the ability to vertically elongate, have been identified as key features for the resilience to burial stress (Cabaco et al. 2008; WAMSI DSN Project 5.3.2). Our work has clearly shown that under severe light reduction the ability to vertically elongate appears to be hampered. Therefore, under a dredging scenario, the threshold burial depth will be influenced by both the composition of burial sediments and the degree of light availability limiting a plant's ability to grow vertically and escape burial.

The composition of burial sediments can have a significant influence on a plant's tolerance to burial stress. In a field study, a burial depth of 40–80 mm imposed severe stress for both *C. serrulata* and *H. uninervis*, (50% shoot mortality, Ooi et al. 2011) In this study, we found a similar loss in shoot density (~50% loss) at 40 mm of burial, but only with organic-enriched sediments. Sediments added to plants in the field (Ooi et al. 2011) had high concentrations of organic matter, 3% sediment dry weight, compared to 4% sediment dry weight in this study. Organic-enriched sediments potentially increase the exposure of leaves and below-ground organs 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). Although we did not explicitly measure the mechanisms leading to plant decline when plants were buried under organic-enriched sediments, the outcomes provide some insight suggesting that threshold burial depths change with different compositions of burial sediments. By increasing our understanding of how different burial sediment compositions affect plant responses, we will improve our ability to predict more appropriate dredging-related thresholds.

Neither seagrass species showed an increase in vigour (shoot production rate, shoot density, biomass) with moderate burial depths, despite a reasonable expectation that they would be based on burial under ambient light conditions (WAMSI DSN Project 5.5.2; Statton et al. 2017b). Moderate burial has been found to stimulate photosynthesis, growth, reproduction and morphological changes for various grasses in inland and coastal dunes (Zhang and Maun 1990, Yuan et al. 1993). 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*, *C. serrulata*, and *Halodule uninervis* (Marba et al. 1994, Ooi et al. 2011; WAMSI DSN Project 5.5.2). However, in this study both species were exposed to severe light reduction, simulating light levels nearby a dredging operation. Low light appeared to have an over-riding effect on both species' ability to respond positively to burial. Vertical elongation is potentially costly to the plant in terms of carbon re-allocation (WAMSI DSN Project 5.5.2), particularly if clonal integration is impaired. We found that rhizome carbohydrate reserves of control plants in this study (~ 15% DW both species) were similar to that of plants exposed to severe low light for six weeks under constant, artificial light (~ 14% DW *C. serrulata* and ~16% DW *H. uninervis* WAMSI DSN Project 5.5.1) and natural light (6% DW *C. serrulata*, 13% DW *H. uninervis*, WAMSI DSN Project 5.3.3). Therefore, vertical elongation may not be purely a function of burial, but rather is triggered by burial, and as long as there is sufficient light to support the energetic requirements of vertical growth.

In WAMSI DSN Project 5.5.2 (Statton et al. 2017b) vertical rhizome growth was identified as an excellent bio-indicator of sediment burial stress, which importantly differed from the 4 consistent bio-indicators relating to light reduction determined in WAMSI DSN project 5.5.1 (Statton et al. 2017a). However, this study shows that under severe light limitation vertical rhizome growth is not a useful bio-indicator for identifying that the plant is responding to sub-lethal levels of burial. The lack of differentiation in bio-indicators when these stressors overlap will limit resource managers' ability to determine which stressor is causing the greater impact. While resource managers may be inclined to defer to bio-indicators and thresholds of light limitation (WAMSI DSN Project 5.5.1) to detect and manage an impact from dredging on benthic primary producers such as seagrasses the additive or potentially synergistic effect of burial stress and low light could result in more severe impacts on plants beyond that which might be identified from the 4 light bio-indicators, and predicted from light thresholds alone. This is an important finding and suggests that we are unable to provide appropriate thresholds for the combined impacts of light reduction and sediment burial at this point. Future work should focus on understanding and identifying potential bio-indicators appropriate to synergistic stresses such as light limitation and burial, as well as developing thresholds that take both stresses into account.

Placing the findings of this study and those of WAMSI DSN Project 5.5.2 (Statton et al. 2017b) into a field context is made difficult by the almost total absence of reliable data on sediment deposition and resuspension rates in the field. By necessity, estimates of sediment deposition rates for dredging projects in NW WA have been based on either sediment trap data or suspended sediment load data (WAMSI DSN Project 5.2; McMahon et al. 2016; WAMSI DSN Project 4.1 and Jones et al. 2016)) requiring significant assumptions, which cannot be validated due to the lack of field data on sediment deposition rates. Those estimates indicated that seagrasses were unlikely to experience the sorts of rapid and deep sediment burials they were subjected to in the mesocosm experiments reported here. On the basis of the plant responses observed here, we conclude that under reduced light conditions burial of 40 mm or more can negatively affect seagrasses, if the sediment has moderate organic content (>4%). However, these conditions are most likely to occur in the dredging near-field or spoil dump grounds. Our burial treatments probably represent extreme events in the dredging far-field and so the burial rates that we can estimate seagrass will experience in the far-field are likely to pose a relatively low pressure. Notwithstanding the above, there is a critical need for reliable field data on net sediment deposition rates around dredging projects in order to place biological response data, be it seagrass, corals or filter-feeders, into context.

## 5 References

- Borum J, Pedersen O, Greve TM, Frankovich TA, Zieman JC, Fourqurean JW, Madden CJ (2005) The potential role of plant oxygen and sulphide dynamics in die-off events of the tropical seagrass, *Thalassia testudinum*. *Journal of Ecology* 93:148-158
- Cabaço S, Santos R, Duarte CM (2008) The impact of sediment burial and erosion on seagrasses: a review. *Estuarine, Coastal and Shelf Science* 79:354-366
- Cruz-Palacios V, Van Tussenbroek BI (2005) Simulation of hurricane-like disturbances on a Caribbean seagrass bed. *Journal of Experimental Marine Biology Ecology* 324:44-60
- Duarte CM, Terrados J, Agawin NS, Fortes MD, Bach S and Kenworthy WJ (1997) Response of a mixed Philippine seagrass meadow to experimental burial. *Marine Ecology Progress Series* 147:285-294
- Erftemeijer PL, Lewis RR (2006) Environmental impacts of dredging on seagrasses: a review. *Marine Pollution Bulletin* 52:1553-1572
- de Mendiburu, F. and de Mendiburu, M.F. (2015) Package 'agricolae', Version.
- Environmental Protection Authority (2016) *Technical Guidance: Environmental Impact Assessment of Marine Dredging Proposals*. EPA, Western Australia.
- Gacia E, Duarte CM (2001) Sediment retention by a Mediterranean *Posidonia oceanica* meadow: the balance between deposition and resuspension. *Estuarine, Coastal and Shelf Science* 52:505-514
- Harris CA, Scott AP, Johnson AC, Panter GH, Sheahan D, Roberts M, Sumpter JP (2014) Principles of Sound Ecotoxicology. *Environmental Science and Technology* 48:3100-3111
- Holmer M, Bondgaard EJ (2001) Photosynthetic and growth response of eelgrass to low oxygen and high sulfide concentrations during hypoxic events. *Aquatic Botany* 70:29-38
- Holmer M, Frederiksen MS, Møllegaard H (2005) Sulfur accumulation in eelgrass (*Zostera marina*) and effect of sulfur on eelgrass growth. *Aquatic Botany* 81:367-379
- Holmer M, Hasler-Sheetal H (2014) Sulfide intrusion in seagrasses assessed by stable sulfur isotopes—A synthesis of current results. *Frontiers in Marine Science* 1:64
- Holmer M, Pedersen O, Ikejima K (2006) Sulfur cycling and sulfide intrusion in mixed Southeast Asian tropical seagrass meadows. *Botanica Marina* 49:91-102
- Hovey RK, Statton J, Fraser MW, Ruiz-Montoya L, Zavala-Perez A, Rees M, Stoddart J, Kendrick GA (2015) Strategy for assessing impacts in ephemeral tropical seagrasses. *Marine Pollution Bulletin* 101:594-599
- Kilminster K, McMahon K, Waycott M, Kendrick GA, Scanes P, McKenzie L, O'Brien KR, Lyons M, Ferguson A, Maxwell P (2015) Unravelling complexity in seagrass systems for management: Australia as a microcosm. *Science of the Total Environment* 534:97-109
- Madsen JD, Chambers PA, James WF, Koch EW, Westlake DF (2001) The interaction between water movement, sediment dynamics and submersed macrophytes. *Hydrobiologia* 444:71-84
- Marba N, Duarte CM (1994) Growth response of the seagrass *Cymodocea nodosa* to experimental burial and erosion. *Marine Ecology Progress Series* 107:307-311
- Marba N, Duarte CM (1995) Coupling of seagrass (*Cymodocea nodosa*) patch dynamics to subaqueous dune migration. *J Ecology*:381-389
- McMahon K, Lavery P, McCallum R, Hernawan U (2016) Current state of knowledge regarding the effects of dredging-related 'pressure' on seagrasses. Report of Theme 5 - Project 5.1.1 prepared for the Dredging Science Node, Western Australian Marine Science Institution, Perth, Western Australia, 58 pp.
- Middleton GV, Southard JB (1984) Mechanics of sediment movement. Society of Economic Paleontologists and Mineralogists, Rhode Island, USA
- Mills KE, Fonseca MS (2003) Mortality and productivity of eelgrass *Zostera marina* under conditions of experimental burial with two sediment types. *Marine Ecology Progress Series* 255:127-134
- Ooi JLS, Kendrick GA, Van Niel KP (2011) Effects of sediment burial on tropical ruderal seagrasses are moderated by clonal integration. *Continental Shelf Research* 31:1945-1954
- Orth RJ, Carruthers TJ, Dennison WC, Duarte CM, Fourqurean JW, Heck KL, Hughes AR, Kendrick GA, Kenworthy WJ, Olyarnik S (2006) A global crisis for seagrass ecosystems. *Bioscience* 56:987-996
- Pedersen O, Binzer T, Borum J (2004) Sulphide intrusion in eelgrass (*Zostera marina* L.). *Plant, Cell & Environment* 27:595-602
- Quarmby C, Allen SE (1989) Organic constituents. In: Allen S, Grimshaw H, Parkinson J, Quarmby C (eds) *Chemical Analysis of Ecological Materials*. Blackwell Scientific, Oxford
- Ralph PJ, Gademann R (2005) Rapid light curves: A powerful tool to assess photosynthetic activity. *Aquatic Botany* 82:222-237
- Statton J, McMahon KM, McCallum R, Armstrong P, Dunham N, Strydom S, Kendrick GA, Lavery PS (2017a). Determining light stress:response bio-indicators and thresholds for a tropical multi-species seagrass assemblage. Final Report for WAMSI 5.5.1 of the WAMSI Dredging Science Node

- Statton J, McMahon KM, McCallum R, Kendrick GA, Lavery PS. (2017b). Sediment burial stress: response bioindicators and thresholds for a tropical multi-species seagrass assemblage. Final Report for Project 5.5.2 of the WAMSI Dredging Science Node.
- Statton J, McMahon KM, Kendrick GA, Lavery PS. (2017c). Response and recovery of a mixed tropical seagrass assemblage to variation in the frequency and magnitude of light deprivation. Report for Project 5.5.3 of the WAMSI Dredging Science Node.
- Terrados J, Duarte CM, Fortes MD, Borum J, Agawin NSR, Bach S, Thampanya U, Kamp-Nielsen L, Kenworthy WJ, Geertz-Hansen O (1998) Changes in community structure and biomass of seagrass communities along gradients of siltation in SE Asia. *Estuarine, Coastal and Shelf Science* 46:757-768
- Tuya F, Espino F, Terrados J (2013) Preservation of seagrass clonal integration buffers against burial stress. *Journal of Experimental Marine Biology and Ecology* 439:42-46
- Vermaat J, Agawin N, Duarte C, Fortes M, Marba N, Uri J (1995) Meadow maintenance, growth and productivity of a mixed Philippine seagrass bed. *Marine Ecology Progress Series* 124:215-225
- Wood KA, O'Hare MT, McDonald C, Searle KR, F Daunt F, Stillman RA (2016) Herbivore regulation of plant abundance in aquatic ecosystems. *Biological Reviews*, 2016 doi: 10.1111/brv.12272
- Yemm E, Willis A (1954) The estimation of carbohydrates in plant extracts by anthrone. *Journal of Biochemistry* 57:508-514
- Yuan T, Maun M, Hopkins WG (1993) Effects of sand accretion on photosynthesis, leaf-water potential and morphology of two dune grasses. *Functional Ecology*:676-682
- Zhang J, Maun M (1990) Effects of sand burial on seed germination, seedling emergence, survival, and growth. *Canadian Journal of Botany* 68:304-310
- Walker D, Dennison W, Edgar G (1999) Status of Australian seagrass research and knowledge. *Seagrass in Australia Strategic review and development of an R&D plan CSIRO, Canberra Australia*:1-2

