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# Resilience of seagrass *Posidonia sinuosa* is negatively affected by high levels of burial of dredged material

**Theme:** Benthic Habitats and Communities

WAMSI Westport Marine Science Program

# WAMSI WESTPORT MARINE SCIENCE PROGRAM

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## ABOUT THE MARINE SCIENCE PROGRAM

The WAMSI Westport Marine Science Program (WWMSP) is a $13.5 million body of marine research funded by the WA Government. The aims of the WWMSP are to increase knowledge of Cockburn Sound in areas that will inform the environmental impact assessment of the proposed Westport development and help to manage this important and heavily used marine area into the future. Westport is the State Government's program to move container trade from Fremantle to Kwinana, and includes a new container port and associated freight, road and rail, and logistics. The WWMSP comprises more than 30 research projects in the biological, physical and social sciences that are focused on the Cockburn Sound area. They are being delivered by more than 100 scientists from the WAMSI partnership and other organisations.




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The $13.5 million WAMSI Westport Marine Science Program was funded by the Western Australian Government, Department of Transport. WAMSI partners provided significant in-kind funding to the program to increase the value to >$22 million.


**DATA**

Finalised datasets will be released as open data, and data and/or metadata will be discoverable through Data WA and the Shared Land Information Platform (SLIP).




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**FRONT COVER IMAGE**

**Theme:** Benthic habitats and communities

**Front cover image:** Seagrass (*Posidonia australis*) in Cockburn Sound. Photo courtesy of Rachel Austin (The University of Western Australia).

# Contents



*The WAMSI Westport Marine Science Program is a $13.5 million body of research that is designed to fill knowledge gaps relating to the Cockburn Sound region. It was developed with the objectives of improving the capacity to avoid, mitigate and offset environmental impacts of the proposed Westport container port development and increase the WA Government's ability to manage other pressures acting on Cockburn Sound into the future. Funding for the program has been provided by Westport (through the Department of Transport) and the science projects are being delivered by the Western Australian Marine Science Institution.*

# Figures



# Tables

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**Project**
*Theme 2, Project 2.2: Pressure-response relationships, building resilience and future proofing seagrass meadows*

## Executive Summary

Dredging activities can lead to burial of seagrass meadows. Seagrass resilience to burial disturbances remains unclear primarily due to insufficient burial threshold information. Obtaining burial thresholds can enhance impact prediction of dredging activities by providing information on burial depth and duration that seagrasses can withstand. Dredging activities are proposed for Cockburn Sound, a marine embayment in Western Australia which is dominated by the persistent seagrass *Posidonia sinuosa*. This report presents findings from a controlled single-stressor mesocosm experiment designed to derive burial thresholds for *P. sinuosa* in this region. Relevant scenarios for applying this information include, but are not limited to, burial disturbances associated with dredging, disposal of dredge spoil, sidecasting and migration of disposed sediment over time onto nearby seagrass meadows. The experiment was carried out under ambient light conditions which is unlikely to represent the light climate during dredging operations so represents solely the response to a single stressor. This is important to consider when assessing the relevance of these findings as previous experiments have shown that burial thresholds for seagrasses may be lower under conditions of reduced light availability (WAMSI DSN Project 5.5.4, Statton et al., 2017b). Findings from this report will help inform impact prediction and could be used in the development of 'burial-related' thresholds but additional pressures such as light reduction need to be considered in dredging management frameworks. This report should be read in conjunction with the report on a subsequent field experiment that assessed the effects of the same burial depths (0, 4 and 8 cm) under both ambient and low light in natural seagrass meadows (Said et al., 2024). We present the findings from the laboratory experiment but also draw on the findings from the field experiment to provide guidance for impact predictions based on the current EPA Environmental Impact Assessment (EIA) zonation scheme. The experiment comprised impact and recovery phases. In the impact phase, the response of *P. sinuosa* to sustained burial was assessed over 2, 4 and 8 weeks. In the recovery phase, sediment was removed from the burial treatments after 8 weeks and the recovery of *P. sinuosa* was examined following 5 and 8 weeks. To test the effects of burial level we established different depths: 0, 1, 4, 8 and 16 cm. These depths were selected to represent the depths that could occur in proximity to dredging operations (McMahon et al., 2017) and those expected to induce sublethal and lethal responses (Cabaço et al., 2008). The experiment utilised dredge-spoil sediment supplied by Cockburn Sound Cement to mimic sediments that are typically deposited by a cutter suction dredge during operations on Kwinana Shelf. Pots were harvested periodically to assess the effects of duration on plant response.

The key findings were:

- Burial depths of 1 and 4 cm had no adverse effects across all measured response indicators of *P. sinuosa* after 8 weeks. Based on the lack of detectable biological impact, we suggest a burial depth of ≤ 4cm could represent the outer boundary between the Zone of Influence (ZoI) and Zone of Moderate Impact (ZoMI). We have confidence of no impacts over an 8-week period.
- Burial depths of 8 and 16 cm led to sublethal impacts on *P. sinuosa* including significant declines in growth rates which, after the burial sediment was removed, did not recover within 8 weeks. Declines in photophysiology and increased redox in the sediments indicated light reduction due to sediment shading leaves and sulphide intrusion under high levels of burial are the likely mechanisms underlying declines in plant growth.
- Other sublethal indicators that are incorporated into environmental impact assessment and monitoring, including shoot density and biomass, were not significantly affected by these burial depths and no lethal responses were observed. Therefore, based on the findings from this study alone, a burial threshold for the boundary between the ZoMI and Zone of High Impact (ZoHI) cannot be quantified.
- Burial of 8 cm in a complimentary field experiment did elicit a lethal response with projected recovery estimated to be 3 to ≥5 years. Combined, the findings of this study and the field study, indicate that growth rates are a reliable early warning indicator of sublethal effects that may occur from burial.

# 1 Introduction

Foundation plant species must be able to grow and survive under dynamic conditions inherent in ecosystems. In coastal ecosystems, sediment movement is dynamic with erosion, sediment transport and deposition of particulate matter fluctuating in magnitude over time (Wolanski, 2007). Settlement of particulate matter can smother organisms with potentially negative consequences for immobile species that cannot evade burial although the severity of impact depends on the burial depth and duration coupled with the organisms' natural tolerance (Hyland et al., 1994). Causes of burial disturbances include natural events such as burrowing by animals, storms or hurricanes (Fonseca et al., 2008; Oprandi et al., 2020) and anthropogenic activities such as dredging (Cunning et al., 2019). Burial disturbances pose a concern for the structure and function of coastal ecosystem by potentially inducing mortality of foundation plant species like seagrasses (Manzanera et al., 1998). For many seagrass species, there is a lack of burial threshold data which hinders impact prediction and understanding of the subsequent environmental consequences.

Seagrasses are flowering plants that occupy a fraction of the ocean floor (0.1-0.2 %) but provide goods and services of substantial economic value (Kurniawan et al., 2020). Seagrasses have high light requirements and therefore grow in shallow coastal waters where a large proportion of human settlements exist, so human related activities are frequently identified as key drivers of seagrass decline. For instance, dredging has the potential to adversely affect seagrass health through both direct such as excavation and burial and indirect impacts including light reduction from turbid plumes and changes to sediment characteristics (Badalamenti et al., 2011). A comprehensive understanding of seagrass response to the pressure of light reduction associated with dredging has been achieved with studies investigating a range of factors e.g. magnitude, frequency and duration (Lavery et al., 2009; McMahon et al., 2013; Statton et al., 2017a). Comparatively, the response to burial impacts have largely focused on the effects of burial depth meaning that other aspects, such as duration and recovery are not well understood (Cabaço et al., 2008). This limits our capacity to predict impacts under new coastal developments.

Different species of seagrass exhibit different tolerances to burial which is influenced by the type of burial impact, their life-history and growth strategies. For instance, burial thresholds have been derived for many seagrass species and indicate that 100 % shoot mortality occurs at depths between 2-15 cm for 35 – 250 days (Cabaço et al., 2008). The broad range in both the depths and duration of burial which elicit a negative response reflect species-specific tolerances to burial with larger species tending to tolerate greater levels of burial than smaller species. For example, 16 cm of burial only affected *Enhalus acoroides* after 10 months whereas *Halodule uninervis* was impacted after 2 months (Duarte et al., 1997). This is because larger species have long leaves and greater photosynthetic area which makes them less susceptible to negative carbon balances induced by light reduction from burial. Species with vertical rhizomes are also generally more tolerant of burial because leaves can be 'relocated' to the sediment surface thereby increasing light access e.g. *Cymodocea nodosa* (Marba and Duarte, 1994). Changes to sediment attributes with burial can also impact seagrass. For instance, sediment anoxia can increase under burial and promote sulphides which are toxic to seagrasses and can induce shoot mortality (Carlson et al., 1994). Seagrass response can be affected by attributes of the burial sediment including the amount of organic matter with higher levels tending to cause greater impact in terms of growth and mortality (Terrados et al., 1999). Seagrass tolerance to disturbances can also vary among populations within species indicating a need for locally-derived burial thresholds (Salo et al., 2014). Further, very few studies have assessed the recovery capacity of seagrasses following the removal of burial. Information on seagrass impact and recovery from dredging activities is needed to advance environmental impact assessments for dredging proposals. To date, this is well advanced in relation to light reduction pressures as the spatial extent of light impacts is much greater than for burial disturbances and hence more significant in a regional and cumulative impact context (Chartrand et al., 2016).

Western Australia (WA) boasts highly diverse seagrass habitats (Carruthers et al., 2007) where the persistent species *Posidonia sinuosa* has a dominant distribution along the coast spanning approximately 5° of latitude (28°S to 33°S) from Geraldton to Busselton (Cambridge and Kuo, 1979). In the 1950s, the marine embayment of Cockburn Sound became a focal point for industrialisation in WA which led to significant losses of *P. sinuosa*. Degradation of the system led to the implementation of environmental regulation in the 1980s and by 1999, *P. sinuosa* declines amounted to approximately 79-84 % (23 $km^2$) (Kendrick et al., 2002). Better regulation and management have likely prevented any large-scale seagrass losses since 2000 (Rule 2015), however, recent mapping efforts indicate that recovery has not yet occurred at the ecosystem level (Hovey et al., 2018) and trends from long-term seagrass monitoring (2003-2023) indicate ongoing declines in condition at some sites (Webster et al., 2024). Light reduction from eutrophication was believed to be a primary driver of seagrass loss and motivated studies to improve monitoring and management of these types of disturbances in relation to seagrasses (Collier et al., 2009). Studies have derived burial thresholds for tropical seagrass species in WA e.g. *Halodule uninervis* (Statton et al, 2017) but have not yet been derived for many temperate species, including *P. sinuosa*, from within Cockburn Sound. Burial depths of 15 and 30 cm have been reported to induce respective mortalities of 50 % and 100 % of *P. sinuosa* in Western Australia but this information was from a personal communication so critical details, such as the specific origin of the plants, were not provided so it is unclear how transferable this is for the impact assessment of meadows in Cockburn Sound (Cabaço et al., 2008). Additionally, the burial depths tested (10, 15, 20, 30 cm) represent extreme levels based on a dredging context where < 10 cm burial depths are common based estimates from sediment traps deployed near dredging operations (McMahon et al., 2017). The recovery capacity of *P. sinuosa* following burial stress has not been assessed.

Dredging within Cockburn Sound is proposed as part of the Westport project, a state government program to build a new port facility to support current and future trade (https://westport.wa.gov.au/). The purpose of this study is to provide better tools to inform the impact assessment for this project and specifically, the potential impacts of dredging-related burial disturbances on *P. sinuosa*. Note that *P. australis* co-occurs with *P. sinuosa* in some areas of Cockburn Sound however, P. sinuosa is typically more abundant in the dredging footprint (i.e. where risk of burial is highest) so it was selected as the focal species. The Western Australian Environmental Protection Authority's Technical Guidance: Environmental Impact Assessment of Marine Dredging Proposals (EPA, 2021) provides a framework for Environmental Impact Assessment (EIA) of dredging. Impact prediction requires designation of spatially explicit zones to describe the predicted extent, severity and duration of impacts. The aim of this study was to derive burial threshold values that can be used to inform environmental impact assessments and dredging management plans for dredging and dredge spoil disposal areas for *P. sinuosa* meadows in the context of EPA (2021) by investigating the responses to a range of burial levels and the capacity to recover following removal of burial.

## 2 Materials and Methods

### *2.1 Plant collection*

Whole plant ramets of *P. sinuosa* were collected on 11th of May 2022 from the meadow edge at approximately 5 m depth at Woodman Point in, Western Australia (Figure 1). Ramets comprised a minimum of 3 shoots, rhizomes and roots. Plants were transported in aerated insulated containers with seawater collected *insitu* back to Edith Cowan University, Western Australia (WA). At the time of collection, salinity and temperature were 34 ppt and 19.5°C, respectively.


Figure 1. Location of Woodman Point site (orange dot) where *Posidonia sinuosa* plants were collected for the experiment which is south-west of the Cockburn Cement wash plant.

### *2.2 Experimental approach*

The experiment comprised two experimental phases, an impact and a recovery phase, to test the effects of burial depth and duration on the resilience of *P. sinuosa.* Dredge-spoil was supplied by Cockburn Sound Cement to replicate the conditions expected following operations of a cutter suction dredge on Kwinana Shelf (Appendix, Table 7-1). Pots containing adult ramets were subjected to different burial depths of 0 (control), 1, 4, 8 and 16 cm (Figure 2). Burial depths were selected based on sediment deposition rates estimated from sediment traps deployed in proximity of dredging operations (McMahon et al., 2017), as well as depths that have been reported to impact *Posidonia* spp. (Cabaço et al., 2008; Manzanera et al., 2011). Direct measures of sediment deposition and resuspension are difficult and therefore rare. Thus, burial depths derived from sediment traps can be considered as only approximates of burial depths and may not apply to all burial scenarios that unfold during dredging operations. Eight pots were set up within each tank to enable periodic harvests and assess the effect of varying durations of burial on the response of *P. sinuosa*; harvests occurred after 2, 4 and 8 weeks (Figure 3). In the recovery phase, at the 8 week point in the impact phase, burial sediment was removed from randomly selected pots to assess recovery potential of *P. sinuosa*. Recovery was assessed by comparing the condition of the plants after 8 weeks of burial impact (i.e. the start of the recovery phase) with the condition of plants at week 13 (5 weeks after removal of burial/ harvest 1) and week 16 (8 weeks after removal of burial/ harvest 2) following removal of the burial sediment (Figure 3).

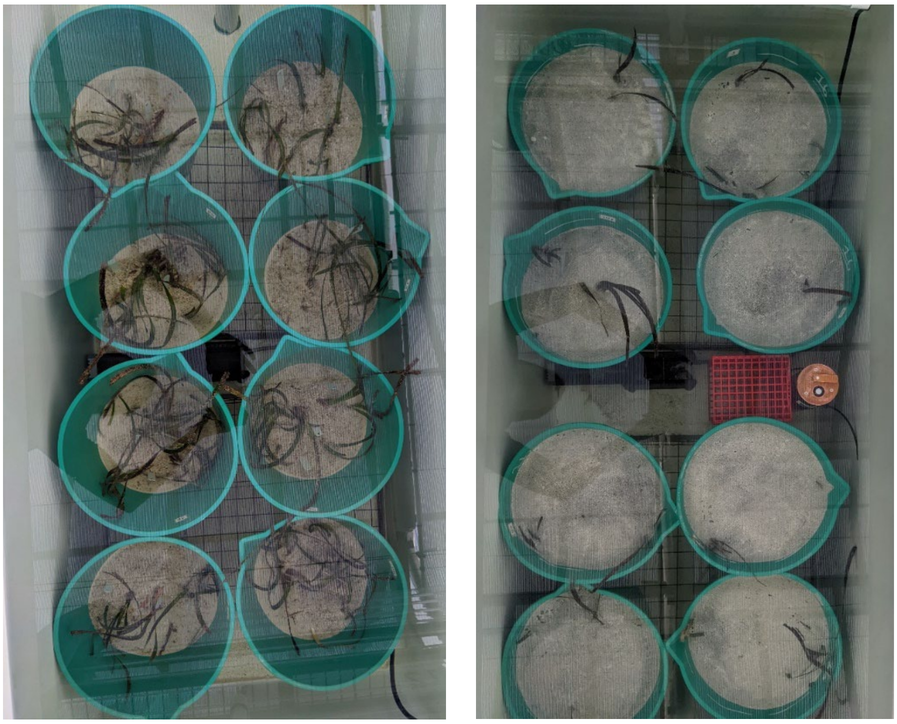

Figure 2. Photos of *Posidonia sinuosa* planted in pots to test the effects of different burial depths: 0 cm (left image) and 16 cm (right image). Note the other burial depths tested (1, 4, 8 cm) are not shown.

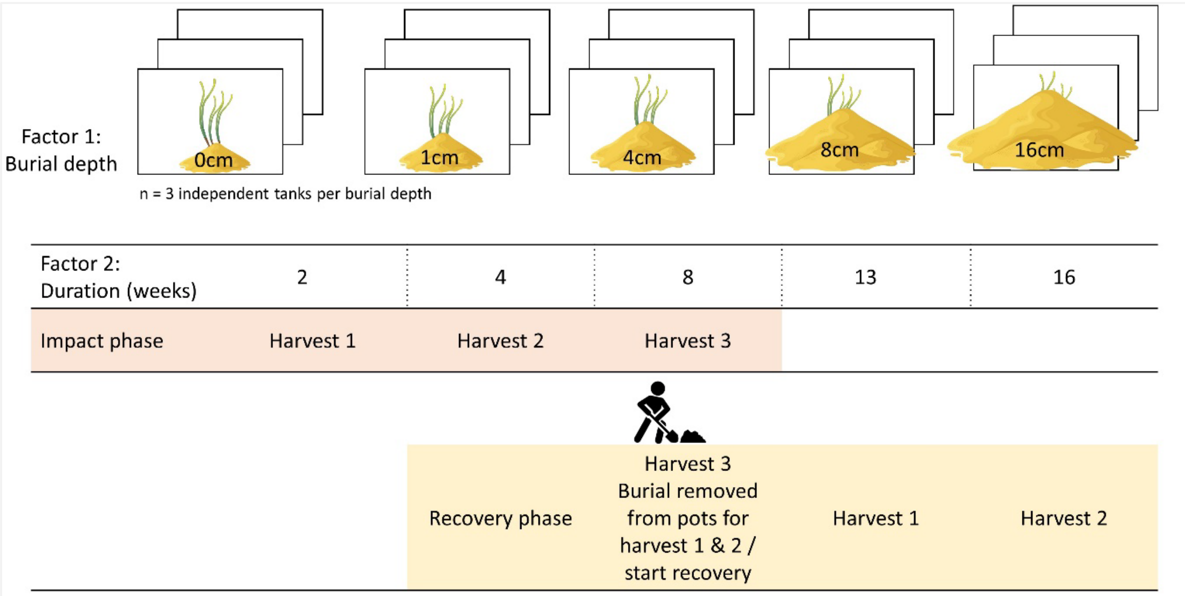


Figure 3. Experimental set up to test the effects of burial depth and duration on *Posidonia sinuosa*. There were three independent replicate tanks per burial depth (0, 1, 4, 8, 16 cm) and timing of periodic harvests (2, 4, 8 weeks) during an impact phase and the start of the recovery phase (week 8) where burial was removed from some pots and recovery was assessed (after 5 weeks, 8 weeks).

There were 3 replicate tanks for each of the 5 burial levels (n=15) which contained 8 individual pots. Each tank set up was independent and comprised a main tank (volume: 850 L) and sump tank (volume: 100L). Seawater was circulated through both tanks by aquarium pumps (1 per tank). Each tank contained 8 pots and within each pot, the number of ramets varied (2-3) such that the total number of shoots per pot was ≥ 10 (min: 11, max: 26). Before planting, epiphytes were gently removed from the leaves to reduce light attenuation (Burt et al., 1995) and the roots were trimmed to stimulate new root growth (Hovey et al., 2011). Unsorted sediment compromising multiple grain sizes provides suitable sediment conditions for growth and survival of seagrass in aquaria (Statton et al., 2013). Thus, pots were filled with Gingin quartz sand (https://www.soilsaintsoils.com.au/Products/Soils-Sands/Sands/Gin-Gin-Quartz) to a depth of 5 cm. Treated dredge sand was used for the burial treatment to mimic the type of sediment most likely to be deposited during operations of a cutter suction dredge on Kwinana Shelf (Kemp, *pers comm*, 2023, Appendix, Table 7-1). The experiment was conducted in an outdoor mesocosm facility with access to natural sunlight and exposure to the natural day/night photoperiod. Plants were acclimated to aquarium conditions for 1 week. During acclimation and the experimental period (8 weeks), salinity was maintained at 34 (± 0.2) ppt and water temperature was set to 19.5°C, mimicking field collection conditions which occurred in autumn (May). Pilot trials indicated the need to acclimate plants to the light conditions in the facility. Therefore, the amount of light the plants were receiving was increased slowly, starting with 24 hours under shade reduction cloths of decreasing intensities: 90%, 70%, 50%, 30%. For the remainder of the experiment, plants were exposed to an average irradiance of 9.65 (± 0.38) moles $m^{-2}$ $day^{-1}$ although this was variable; ranging from 2.73 to 20.36 moles $m^{-2}$ $day^{-1}$.

### *2.3 Seagrass response variables*

To assess the effects of burial on seagrass, multiple response indicators were measured over a range of plant scales and time-points during the impact and recovery phases (Table 2-1). Photosynthetic parameters were measured using a Waltz™ pulse amplitude modulation (PAM) fluorometer and included maximum quantum yield ($F_v/F_m$) and electron transport rate (ETR). All measures were done on the area of the leaf that was green and for which an adequate fluorescence signal (≥ 100) could be obtained. $F_v/F_m$ was measured pre-dawn on dark-acclimated leaves to represent the maximum potential of photosystem II (PSII) as all reaction centres are open (Murphy et al., 2003). The formula for calculating the ETR was: ETR = Y x Ei x AF x 0.5, where Ei is the incident irradiance (µmol photons $m^{-2}$ $s^{-1}$), AF is the absorption factor, 0.5 is the fraction of photons absorbed by PSII in plant and data was expressed as (µmol electrons $m^{-2}$ $s^{-1}$) (Beer et al., 2014). Yield measurements for calculating the ETR was taken after approximately 5 hours of illumination. To calculate AF, ambient light was measured at the seagrass canopy and with a leaf covering the light sensor and then the difference between the two was divided by ambient light. As light varied over the course of the treatment, there was a need to standardise the ETR values so these were expressed relative to the controls by dividing the values for the plants under burial treatment by the values for the controls to derive a relative ETR (rETR).

Shoot growth was estimated using the hole punch method (Short and Duarte, 2001). On day 0, 1-2 sheaths were hole punched within each pot and the number of leaves and number of shoots were counted then summed to derive a total number per pot (total at start). At the time of each harvest, leaves and shoots were recounted and then the relative change for both variables were calculated separately (the difference in the totals at the time of harvest and start expressed as a percentage relative to the total at the start). The length of new tissue (leaf and sheath) produced was measured (mm) and then expressed as a shoot extension rate per day (mm $day^{-1}$). Plants from each pot were sorted into separate categories: living leaf, dead leaf, living sheath, dead sheath, rhizome, root and if applicable, new tissue (leaf and sheath) produced. This material was dried in a 60°C oven for 48 hours and weighed (g) to obtain a dry weight estimate of biomass. Samples of a portion of the dried rhizome material (~5 g) were sent to the University of Queensland and analysed for soluble sugar and starch concentrations. Total carbohydrates were calculated as the sum of soluble sugar and starch and expressed as a % (Table 2-1).

Table 2-1. Summary of seagrass indicators measured across different scales of response

| Scale of seagrass response | Indicator | Measure |
|---|---|---|
| Small (cellular-leaf-plant, sub-lethal) | Maximum quantum yield | 1 ramet per pot |
| | Electron Transport Rate (relative to controls) | 1 ramet per pot |
| | Total Carbohydrates (%) | 1 ramet per pot |
| Intermediate (plant-patch-meadow, sub-lethal) | Relative change in leaf density (%) | $\frac{\text{Number leaves (at time of harvest)} - \text{Number leaves (initial)}}{\text{Number leaves (initial)}} \times 100$ |
| | Shoot extension rate | 1 ramet per pot |
| Intermediate to landscape (meadow-ecosystem-ecoregion) | Relative change in shoot density (%) | $\frac{\text{Number shoots (at time of harvest)} - \text{Number shoots (initial)}}{\text{Number shoots (initial)}} \times 100$ |
| | Biomass (by individual category and above, below, total) | Above: total weights of living leaf, dead leaf, living sheath, dead sheath; Below: rhizome, root; Total: above + below |

Redox potential and oxygen were measured to indicate variation in sediment conditions that can be modified during burial (Eldridge et al., 2004; Enríquez et al., 2001). Redox potential measurements were conducted using a universal meter (WTW MultiLine P4) with a WTW Sentix ORP probe attached to it. Oxygen measurements were taken using a Thermoscientific Orion RDO Dissolved oxygen probe. Both oxygen and redox were measured in the sediment just below the surface and at the rhizome-root interface.

### *2.4 Statistical analyses*

Two datasets were created for each experimental phase (impact, recovery) and analysed separately. The impact dataset comprised data collected from three harvests under each burial depth (Levels = 0, 1, 4, 8, 16 cm) over varying durations of impact (Levels = 2, 4 and 8 weeks). The end point of the impact phase (8 weeks) was also the starting point of the recovery phase and so this dataset comprised data from the starting point (8 weeks of impact), a harvest that occurred after the burial was removed and over varying durations (Levels = 5, 8 weeks (or weeks 13, 16)). Separate PERMANOVA analyses were conducted on each dataset to test the effects of burial depth, duration and the interaction on the response of *P. sinuosa* based on a similarity matrix created from Euclidean distances between samples. The replicate tanks were the experimental units (n=3) for each burial depth. The value for each response variable is the sum or the average for this variable obtained from the pot harvested from each tank. Separate univariate PERMANOVA analyses were carried out on each response variable. Primer v7 statistical package and PERMANOVA+ were used for all analyses.

# 3 Results

## *3.1* IMPACT PHASE

### *3.1.1 Physiological responses*

During the impact phase, maximum quantum yield and electron transport rates of *P. sinuosa* were significantly affected by burial depth and the effects were independent of duration (Table 2). Maximum quantum yield ($F_v/F_m$) was significantly lower in the deepest burial treatment of 16 cm (0.30 ± 0.08) compared to the controls (0.72 ± 0.01), 1 cm (0.55 ± 0.13) and 4 cm (0.66 ± 0.03) (Figure 4A). Relative electron transport rate (rETR) was affected by burial (Burial, p=0.003, Table 2), with lower rETR for plants buried under 16 cm (0.55 ± 0.13) compared to burial levels of 1 cm (0.91 ± 0.05) and 4 cm (0.97 ± 0.05) (Figure 4B). Duration had significant independent effects on maximum quantum yield but not relative electron transport (Table 3-1). Relative to maximum quantum yields at the start of the experiment (0.72 ± 0.01), there was a significant decline in $F_v/F_m$ after 2 (0.59 ± 0.06), 4 (0.48 ± 0.07) and 8 weeks (0.55 ± 0.07, Figure 6D). Total carbohydrates was not affected by any factors (Table 3-1).

### *3.1.2 Plant and meadow scale responses*

Shoot extension rates were affected by burial depth (Burial, p=0.0001, Table 3-1) and significantly reduced by burial of 8 cm (0.5 ± 0.14) and 16 cm (0.28 ± 0.06) compared to the controls (1.02 ± 0.1),1 cm (1.15 ± 0.18) and 4 cm (0.95 ± 0.16) burial depths (Figure 4C). Duration also affected shoot extension rates which reduced over time; 0.92 ± 0.14 after 2 weeks and 0.57 ± 0.13 after 8 weeks (Figure 6E). Meadow-scale indicators such as change in leaves, shoots, above-ground, below-ground and total-biomass, were not affected by any factors (Table 3-1).

### *3.1.3 Changes in oxygen and redox at the rhizome-root interface*

Oxygen was affected by the interaction between burial and duration but not across all burial levels and not all durations (B x D, $p<0.05$, Table 3-1). Across all durations of impact, oxygen was significantly higher in the controls (5.07 ± 0.7; 6.47 ± 0.3; 5.03 ± 0.8) but only compared to values in the deepest burial depth of 16 cm (2.13 ± 0.5; 1.57 ± 0.2; 0.4 ± 0.1) (Figure 5A). Within the 4, 8 and 16 cm treatments, oxygen declined but the timing of significant differences varied. For instance, oxygen levels were significantly higher in week 2 (4 cm: 4.07 ± 0.5; 8 cm: 4.23 ± 0.2; 16 cm: 2.1 ± 0.5) compared to week 4 for 4 cm and 8 cm (4 cm: 2.5 ± 0.2; 8 cm: 1.07 ± 0.8) and compared to week 8 for 16 cm (0.4 ± 0.1) (Figure 5A). Redox was altered by burial only (Burial, p=0.0002, Table 3-1) and was significantly more negative for plants buried under 16 cm (-272 ± 29.9) compared to the controls (99.9 ± 21.6), 1 (84 ± 26.8) and 4 cm (-165.4 ± 37.7) but not the 8 cm (-255.9 ± 35.4) (Figure 5B).

## 3.2 RECOVERY PHASE

### *3.2.1 Physiological responses*

During recovery, there was no effect of burial treatment on any of the photophysiological indicators suggesting recovery had occurred in relation to burial (Table 3-1). $F_v/F_m$ was affected by duration and declined over the recovery period from 0.55±0.07 at the start of recovery to 0.45±0.1 after 4 weeks and 0.34±0.09 after 8 weeks (Figure 6D, $p<0.05$, Table 3-1). Relative electron transport rates and total carbohydrates were not affected by any factors (Table 3-1).

### *3.2.2 Plant and meadow scale responses*

As leaf extension rates were affected by burial only there is no indication of recovery ($p<0.05$, Table 3-1). Extension rates for the 1 and 4 cm burial depths remained within control levels whereas plants that were previously buried under 8 and 16 cm the rates (0.06 ± 0.02; 0.2 ± 0.07) continued to be significantly lower than both the controls and the other burial treatments (0 cm: 0.99 ± 0.18; 1 cm: 0.52 ± 018; 4 cm: 0.7 ± 0.19) (Figure 4C). Duration affected the change in shoots ($p<0.05$, Table 3-1), pairwise comparisons showed that compared to the start of recovery (8 weeks) (-11 % ± 3), fewer shoots were lost after 5 weeks (-7 ± 2) without burial whereas more were lost after 8 weeks (-23 % ± 4, Figure 6F).

### *3.2.3 Changes in oxygen and redox at the rhizome-root interface*

Oxygen and redox were affected by burial depth but it depended on duration (B x D, p=<0.05, Table 3-1). For oxygen, this interaction was driven by differences at the start of the recovery phase and after 8 weeks without burial (week 16), oxygen was significantly lower in 16 cm treatment (3.47 ± 0.2) compared to the controls (5.27 ± 0.2) (Figure 5A). The interaction for redox was also driven by differences between treatments at the start of the recovery phase but by weeks 13 and week 16 the differences were no longer significant (Figure 5B).

Table 3-1. Mains results of PERMANOVA testing for the effects of burial depth (0, 1, 4, 8, 16 cm) and duration (weeks) on response variables of adult *Posidonia sinuosa* during impact and recovery phases and on oxygen (mg/L) and redox (mV) at the sediment surface and rhizome root interface. p-value (α)=0.05 unless otherwise stated. Significant comparisons are indicated in bold text, n.s. = not significant.

| | **Maximum quantum yield** | | | | | | **Relative electron transport rate** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Impact (0.01)** | | | **Recovery (0.01)** | | | **Impact (0.01)** | | | **Recovery (0.01)** | | |
| Source | df | SS | α | df | SS | α | df | SS | α | df | SS | α |
| Burial | 4 | 1.33 | **<0.01** | 4 | 0.99 | n.s. | 3 | 1.21 | **<0.01** | 3 | 1.64 | n.s. |
| Duration | 3 | 0.46 | **<0.01** | 2 | 1.12 | **<0.01** | 3 | 0.69 | n.s. | 2 | 0.43 | n.s. |
| B x D | 12 | 0.46 | n.s. | 8 | 0.53 | n.s. | 9 | 0.93 | n.s. | 6 | 0.81 | n.s. |
| Residual | 40 | 0.94 | | 30 | 2.25 | | 32 | 2.22 | | 24 | 7.18 | |
| Total | 59 | 3.18 | | 44 | 4.89 | | 47 | 5.05 | | 35 | 10.06 | |
| | **Total carbohydrates** | | | | | | **Extension rates** | | | | | |
| | **Impact** | | | **Recovery** | | | **Impact** | | | **Recovery** | | |
| Source | df | SS | α | df | SS | α | df | SS | α | df | SS | α |
| Burial | 4 | 133.47 | n.s. | 4 | 226.24 | n.s. | 4 | 5.01 | **<0.05** | 4 | 3.46 | **<0.05** |
| Duration | 2 | 17.57 | n.s. | 2 | 95.73 | n.s. | 2 | 1.07 | **<0.05** | 2 | 0.43 | n.s. |
| B x D | 8 | 92.28 | n.s. | 8 | 219.75 | n.s. | 8 | 1.46 | n.s. | 8 | 0.50 | n.s. |
| Residual | 30 | 831.75 | | 30 | 696.93 | | 30 | 4.10 | | 30 | 4.13 | |
| Total | 44 | 1075.10 | | 44 | 1238.50 | | 44 | 11.64 | | 44 | 8.51 | |
| | **Change in shoots (%)** | | | | | | **Total biomass (excl dead sheath)** | | | | | |
| | **Impact** | | | **Recovery** | | | **Impact** | | | **Recovery** | | |
| Source | df | SS | α | df | SS | α | df | SS | α | df | SS | α |
| Burial | 4 | 218.61 | n.s. | 4 | 27.68 | n.s. | 4 | 3.08 | n.s. | 4 | 9.33 | n.s. |
| Duration | 2 | 160.13 | n.s. | 2 | 32.28 | **<0.05** | 2 | 4.83 | n.s. | 1 | 1.35 | n.s. |
| B x D | 8 | 2619.5 | n.s. | 8 | 47.31 | n.s. | 8 | 2.02 | n.s. | 4 | 2.20 | n.s. |
| Residual | 30 | 5946.9 | | 28 | 102.78 | | 30 | 46.17 | | 20 | 31 | |
| Total | 44 | 8945.1 | | 42 | 210.73 | | 44 | 56.10 | | 29 | 43.89 | |

| | Oxygen at the rhizome-root interface | | | | | | Redox at the rhizome-root interface | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Impact | | | Recovery | | | Impact | | | Recovery | | |
| Source | df | SS | α | df | SS | α | df | SS | α | df | SS | α |
| Burial | 4 | 95.84 | **<0.05** | 4 | 27.41 | **<0.05** | 4 | 24564 | **<0.05** | 4 | 20548 | n.s. |
| Duration | 2 | 2.79 | n.s. | 2 | 19.26 | **<0.05** | 2 | 5862.50 | n.s. | 2 | 59918 | **<0.05** |
| B x D | 8 | 29.67 | **<0.05** | 8 | 29.68 | **<0.05** | 8 | 31687 | n.s. | 8 | 80404 | **<0.05** |
| Residual | 30 | 31.88 | | 30 | 27.43 | | 30 | 205950 | | 30 | 86804 | |
| Total | 44 | 160.18 | | 44 | 103.78 | | 44 | 489150 | | 44 | 247670 | |

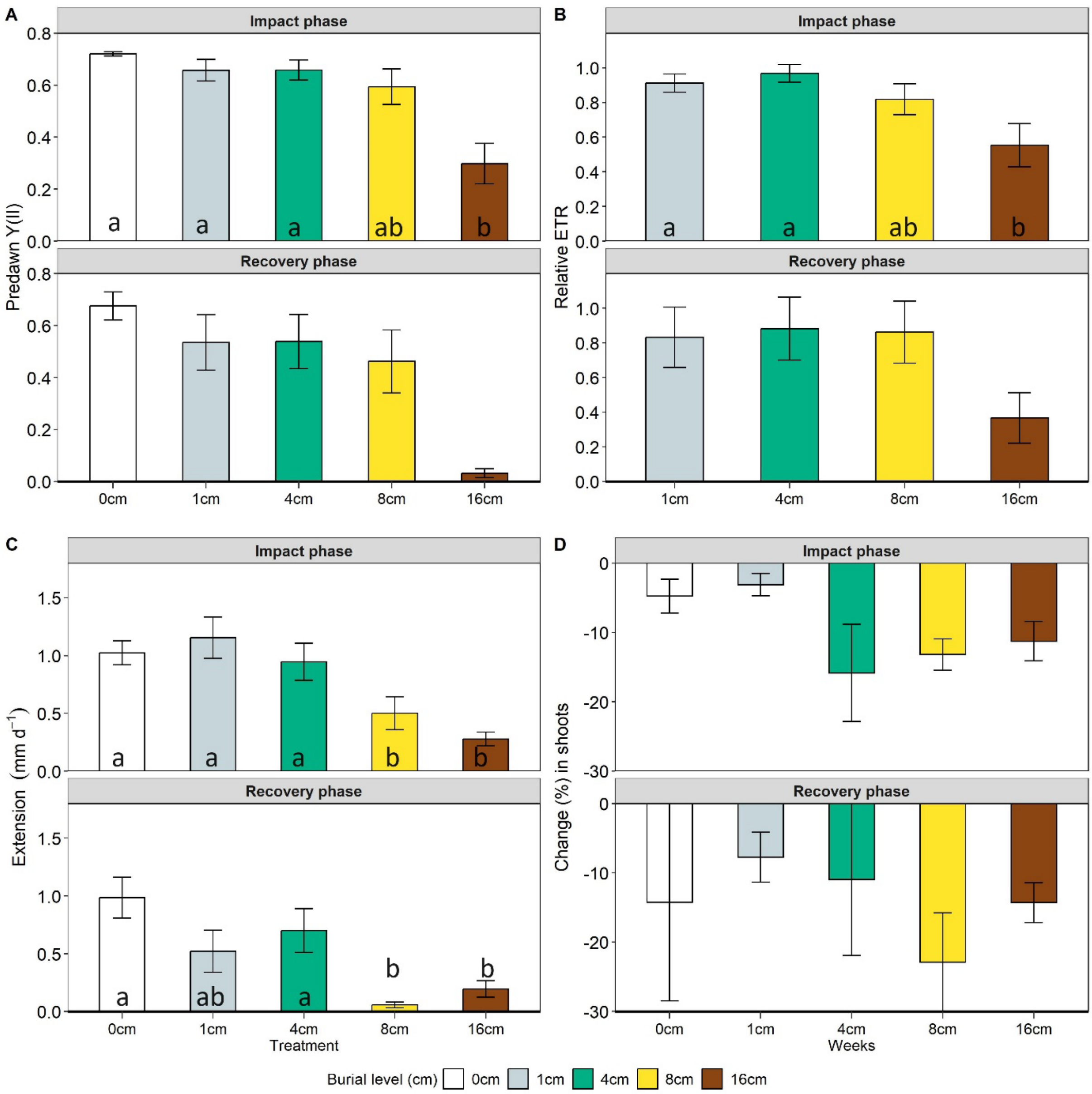


Figure 4. Effects of burial depths (0, 1, 4, 8 and 16 cm) on seagrass *Posidonia sinuosa* maximum quantum yield (A), relative electron transport rate (B), extension rates (C) and change in shoots (D). Letters on graphs indicate significant PERMANOVA pairwise comparisons for differences in response between the burial depths.

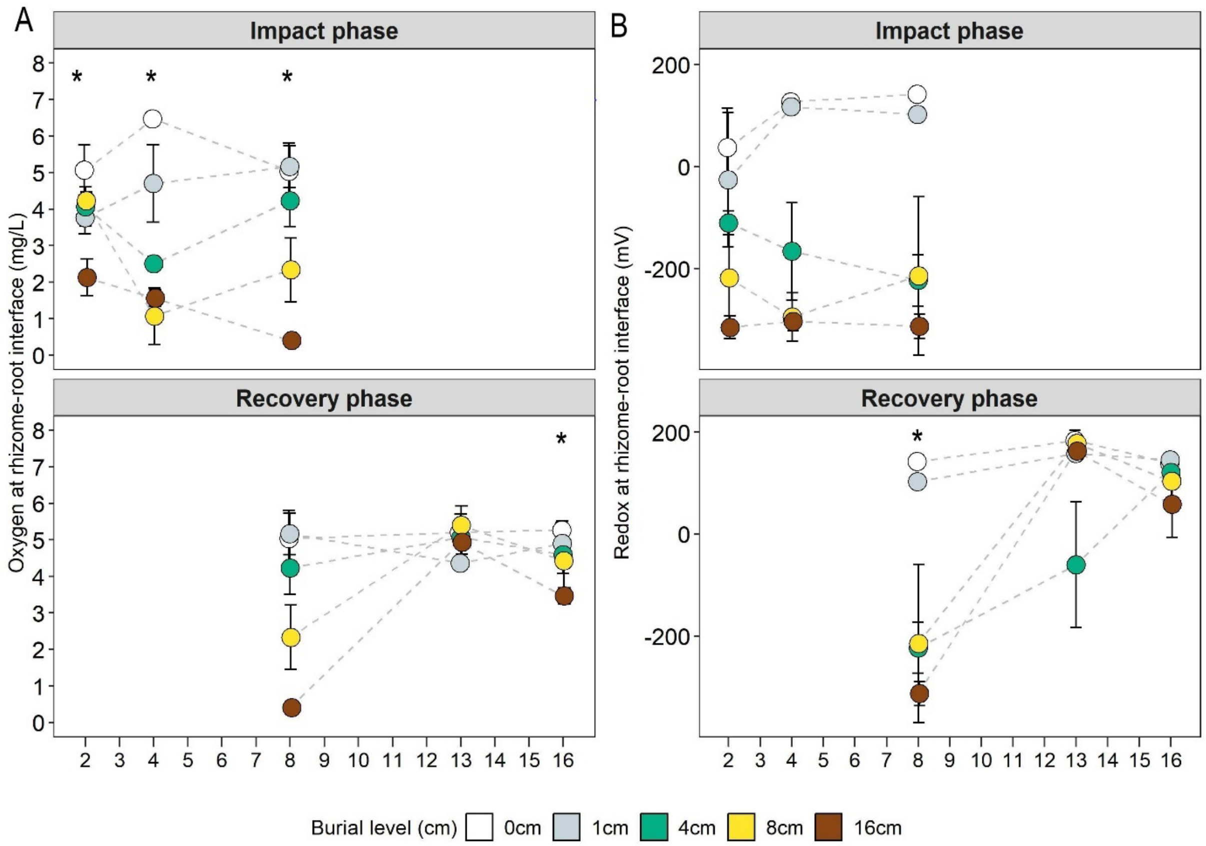


Figure 5. Average (± SE) oxygen (A) and redox (B) measured at the rhizome-root interface under various burial depths (0, 1, 4, 8 and 16 cm) during an Impact phase (2, 4 and 8 weeks) and during a recovery phase after burial was removed (5 weeks without burial or week 13 and 8 weeks without burial or week 16). Stars indicate significant pairwise comparisons between the different burial depth groups.

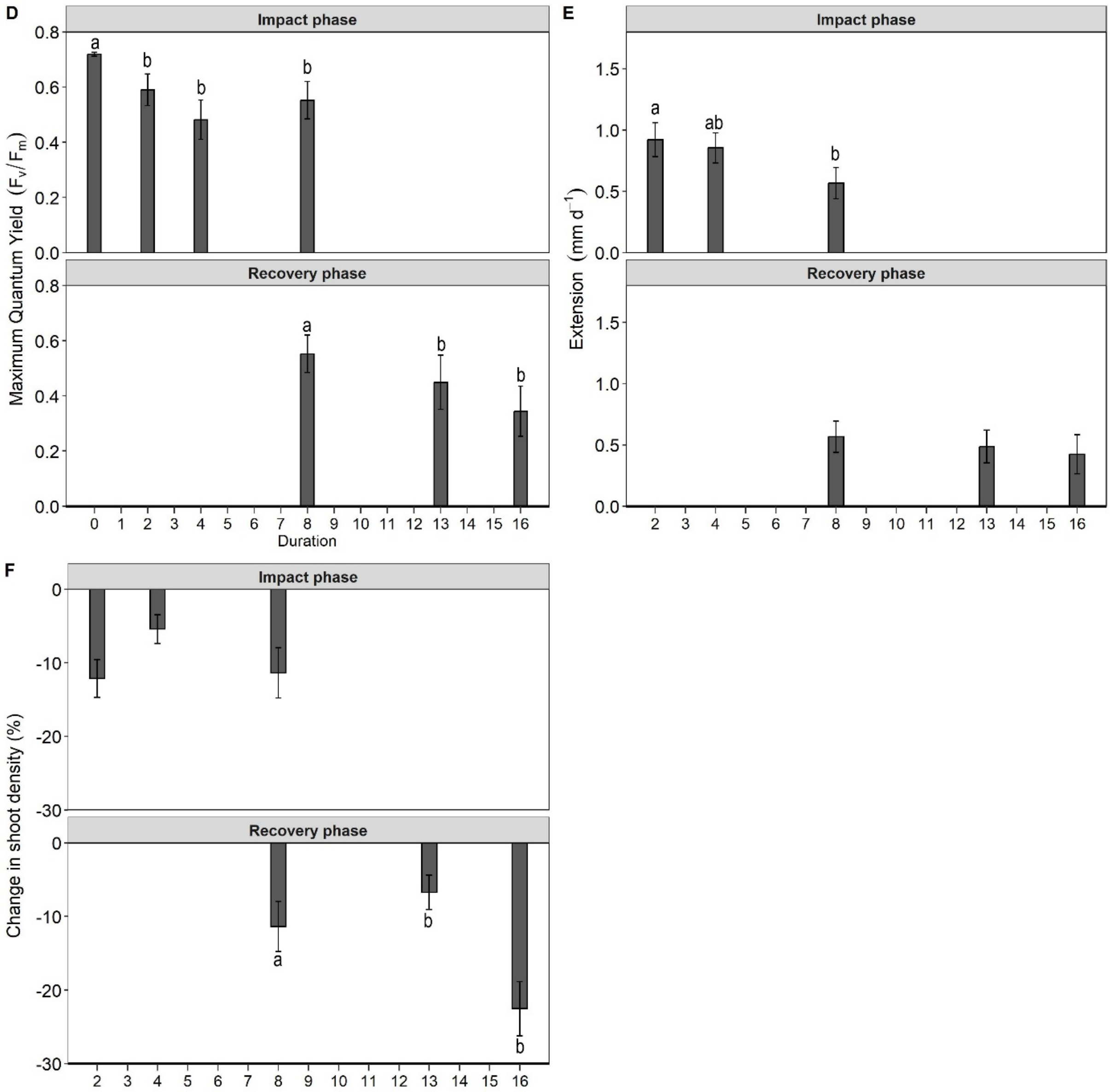


Figure 6. Effects of duration on the response of seagrass *Posidonia sinuosa* during an impact phase and a recovery phase. Indicators shown include: maximum quantum yield (D), extension rates (E) and change in shoots (F). Letters on graphs indicate significant PERMANOVA pairwise comparisons for duration (weeks).

# 4 Discussion

Depth of sediment significantly influenced *Posidonia sinuosa's* response to burial. No impact was observed at burial depths of up to and including 4 cm after 8 weeks. Burial depths of 8 cm and 16 cm led to significant declines in growth rates which did not recover after burial was removed, but these reductions did not manifest in significant shoot density or biomass declines. These results imply that *P. sinuosa* can tolerate these more extreme depths of burial (8, 16 cm) over 8 weeks without lethal effects but its capacity to grow is compromised. Complementing these findings, significant declines in leaf density were observed after one month under burial of 8 cm in another WAMSI Westport experiment that was conducted in the field in Cockburn Sound indicating burial of 8 cm can cause lethal impacts (Said et al., 2024). Therefore, we suggest that growth rates are a reliable early warning indicator of sublethal effects that may occur from burial. The incorporation of early warning indicators into monitoring programs may be valuable for predicting ecosystem collapse although the proposed value should be assessed against management capacity (e.g. alignment between monitoring frequency and indicator response time) and importantly, with the knowledge that collapses can also occur without warning (Lindemayer et al., 2016). These findings are applied to WA's Dredging Guidance Framework (EPA, 2021).

## *4.1 Light reduction and sulphide intrusion as mechanisms underlying declining plant condition under high levels of burial*

It is important to identify the mechanisms underlying plant response to pre-empt surpassing ecological thresholds (Chaves and Oliveira, 2004). Oxygen and redox were significantly altered by burial depths of 8 and 16 cm and these treatments induced sublethal effects by reducing plant growth. These changes to sediment attributes are known to affect seagrass health and recovery. For example, plants can become more susceptible to sulphide intrusion when light reduction associated with burial reduces photosynthesis, and therefore the production of oxygen which is essential to re-oxidise sulphide (Eldridge et al., 2004). Light reduction was greatest in the 16 cm treatment as sediment covered approx. 70% of the leaf surface area and the plants' response (i.e. declines in photophysiology and growth) allude to a vulnerability to sulphide intrusion. Interestingly, photophysiology of plants under 8 cm were not affected, which implied that there was sufficient light to support photosynthesis, however, the manifestation of significant reductions in growth suggested that photosynthesis was insufficient to support overall function. In the recovery phase, oxygen at the rhizome-root interface remained depleted even after burial was removed and shoot extension rates did not recover from the 8 and 16cm treatments within 8 weeks of the burial sediment being removed. The re-oxidisation of sulphide is generally slow in seagrasses even when oxygen is available in adequate supply (Pedersen et al., 2004). Hence, it is possible that the lack of recovery in relation to growth rates was related to ongoing damage caused by retained sulphides. Conjointly, these results align with other studies indicating that light reduction and sulphide intrusion are important mechanisms underlying seagrass decline in relation to high levels of burial (Carlson et al., 1994).

Overall, there were no adverse effects of burial depths 1 and 4 cm on *P. sinuosa*. These results contrast with the responses of *P. oceanica* under 4 cm of burial which depending on the study lost approximately 65% (Manzanera et al., 2011) to 100 % of shoots (Gera et al., 2014) after 105 days compared to 56 days in this study. Manzanera et al. (2011) suggested that low sedimentation rates of the habitat where the study was conducted resulted in plants having lower resilience to burial. During plant collections for this study, it was noted the plants were growing in fine sediment and in some cases, the first 2-3 shoots of plants at the edge of the meadow were 'hanging' loosely in the water column as if sediment had recently moved out of the area and the water was also turbid. Plants under these conditions have been shown to be able to modify their physiology and morphology to maximise photosynthetic capacity and growth e.g. increased total chlorophyll content (Maxwell et al., 2014). It is possible that the combination of dynamic sediment conditions and turbid water has resulted in the plants in this study having greater tolerance to higher depths of burial. Certainly, the slight impact to photophysiology under 8 cm of burial implies that the plants may regularly be exposed to low light conditions and may be more tolerant of light reduction associated with burial stress. Therefore, these results imply that burial stress may have differential impacts on seagrass meadows throughout Cockburn Sound and Owen Anchorage, and more broadly, depending on site-specific factors and that the outcomes can be highly localised depending on where dredging occurs.

### *4.2 Designating zones for dredging impact prediction*

Environmental impact assessment and monitoring of marine dredging proposals in Western Australia is guided by the Environmental Protection Authority's (EPA) Technical Guidance (EPA, 2021). A spatial zonation scheme is used to help proponents define areas where the spatial extent, severity and duration of impacts from operations are expected in relation to their effects on sensitive components of the environment. The scheme comprises three zones: Zone of Influence (ZoI), Zone of Moderate Impact (ZoMI) and Zone of High Impact (ZoHI) (Figure 7). The ZoI is defined as the area where no detectible impacts occur to the biota including no sublethal impacts, the ZoMI is defined as the area where there can be sublethal and/or lethal impacts that are recoverable within 5 years and the ZoHI is the area where lethal impact is predicted that will not recover in 5 years or is irreversible (EPA, 2021, (Figure 7). Data is used to model and predict the spatial extent of the pressures from a particular dredging activity and the boundary of the zones are set based on what levels are likely to trigger a biological response. When providing guidance on potential thresholds for burial impacts from dredging-related activities, we have interpreted responses variables at the sublethal and lethal scales based on the response after a set duration of stress. Although we provide suggestions based on this study for thresholds relevant to the different zones, we recognise that multiple pressure fields are generated during dredging activities and this study has developed thresholds for burial under ambient light conditions. As burial pressure fields generally have a smaller spatial footprint compared to turbidity or light-related pressure fields (BMT, 2020; Jones et al 2019), it is more likely that light or turbidity thresholds will be used to designate the location of the zones, but it is still of benefit to be able to incorporate these burial thresholds into impact prediction and dredging management.

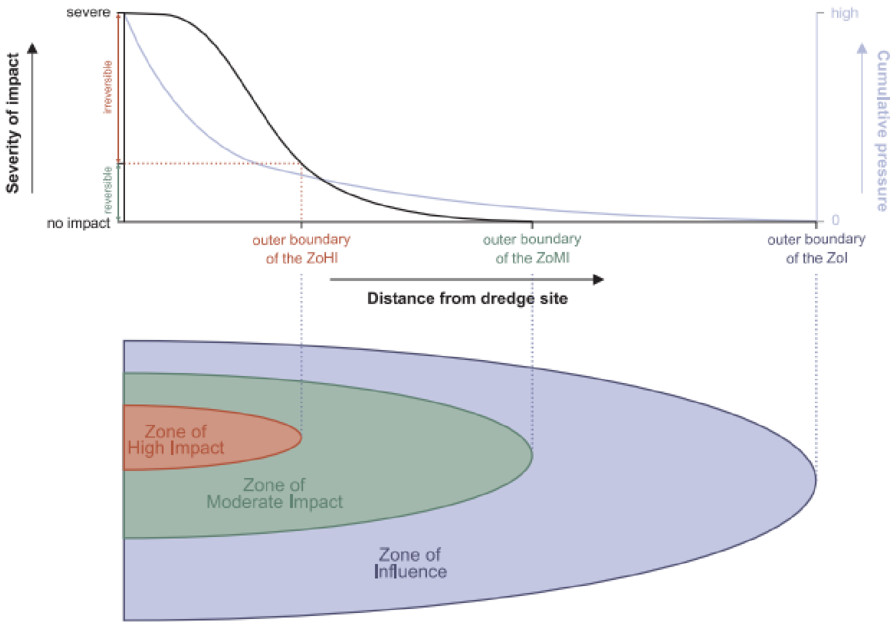


Figure 7. Spatial zonation scheme indicating the degree of change in environmental quality associated with dredging (grey line) and resultant impact to benthic communities (black line) with increasing distance from the dredging site. Outer boundaries of the Zone of High Impact (ZoHI), Zone of Moderate Impact (ZoMI) and Zone of Influence (ZoI) are shown in relation to predicted changes in environmental quality and impacts on biota. Source EPA (2021).

*Zone of Influence*

In this study, no indicators were significantly affected under burial depths of 4 cm. Therefore, for burial impacts we suggest that the ZoI could be designated at ≤ 4 cm of burial depth and we are confident of this tolerance for a duration of ≤ 8 weeks. Note that the same recommendation was made based on the lack of detectible impacts under this depth of burial in the related field experiment (Said et al., 2024).

*Zone of Moderate and High Impact*

Any sublethal or lethal impacts in the ZoMI must show recovery within 5 years (EPA, 2021). Interestingly once the burial stress was removed in this study, the maximum quantum yield of the plants that were previously buried under 8 and 16 cm increased after five weeks, indicating that plants had recovered photosynthetically. No recovery of growth rates occurred in this short timeframe and we are not in the position to make a prediction about how long this recovery would take considering the treatments were under ambient light and slightly lower than has been observed in the field (Collier et al., 2009).

Sublethal effects occurred under burial depths of 8 and 16 cm for the indicator, shoot extension rates, and photophysiology measures only were impacted under 16 cm of burial. As there were no impacts to shoot density and biomass, we cannot suggest a burial threshold for the outer boundary of the ZoHI from this study. However, in a related field experiment as part of the WAMSI Westport program, Said et al. (2024) measured significant leaf loss under 8 cm of burial providing justification that this level of pressure could be used to set the outer boundary of the ZoHI, with 4 cm as the threshold for the outer boundary of the ZoMI. This study and the field study did not test the response of *P. sinuosa* to burial levels between 4 cm to 8 cm, suggesting 4cm as the threshold is a conservative approach.

### *4.3 Implications for management considering study limitations*

Early warning indicators are useful when actively managing the environment to intervene before an ecological threshold is crossed (Carpenter et al., 2011). Burial depth of 8 cm elicited significant sublethal impacts including reductions in shoot extension rates (this study) and shoot density (field experiment, Said et al., 2024). The alignment in the observations suggests that shoot extension rates are an appropriate early warning indicator of seagrass decline. This indicator could be incorporated into seagrass monitoring programs when burial pressure is a concern and there is a need to identify any impacts from this pressure. The ability to intervene before lethal impacts is valuable especially for reducing the overall impact and reducing the recovery time. Measuring changes in sediment oxygen and redox levels in this study helped us to understand the conditions being modified by the dredged sediment and the particular stressor(s) plants were responding to such as potentially higher levels of sediment sulphide due to the reduced oxygen and redox.

The burial sediment was dredge-spoil sediment supplied by Cockburn Sound Cement to mimic sediments that are typically deposited by a cutter suction dredge during operations on Kwinana Shelf (Appendix, Table 7-1). Therefore, the tolerance and thresholds should be considered in the context of these conditions. Previous WAMSI experiments have shown that organic matter of 4 % of DW can worsen the impacts on seagrass from burial compared to lower organic matter sediments (Statton et al., 2017). Therefore, to help the impact(s) from burial associated with dredging, it is important to characterise not only the dredged sediment but also the sediment conditions that the seagrass is growing in. In relation to sediment disposal, considering areas with high water movement to facilitate the natural 'removal' of sediments might also be useful to reduce the likelihood of burial (Savioli et al., 2020).

In Owen Anchorage, *P. sinuosa* co-occurs with *P. australis.* As these two species both have similar life history strategies including being slow growing, we might expect the response of *P. australis* to be similar to *P. sinuosa*. A study in 2019 found that transplanted sprigs of *P. australis* from Cockburn Sound suffered increased mortality under burial depths of 8 and 16 cm (Chisholm, 2019). This suggests our findings may have some applicability for predicting likely impacts to this species from burial. Note that colonising species such as *Halophila ovalis* can also be found in Cockburn Sound but these smaller species are less resistant to burial so these thresholds should not be applied in relation to assessing their impact.

Duration significantly affected some of the indicators for control plants suggesting that the aquarium conditions were stressful for the plants. This can occur in experimental studies and especially for large, slow-growing species (Jiménez-Ramos et al., 2022; Marín-Guirao et al., 2011). Residual factors also accounted for some of the variation in the responses. After investigating the differences in light quality between the field which may have caused the decline over time with some parameters. However, the plant responses we observed were similar to those published in other studies (Cabaço et al., 2008), providing confidence in our results. A total of eight flowers were produced during the experiment across the 0, 1 and 8 cm treatments and this timing coincided with *insitu* observations suggesting their phenology was not disrupted by the aquaria conditions. Flowers developed on 7 plants equivalent to 2.3 % plants flowering out of the total number of plants (n=294) and is consistent with previously reports of patchy flowering for this species e.g. 0-0.31% of flowering shoots $yr^{-1}$ (Marbá and Walker, 1999). Therefore, whilst we acknowledge that the aquarium conditions accounted for some of the stress induced in the plants, we have confidence in the conclusions drawn in relation to the effects of sediment burial on *P. sinuosa*.

Figure 8. Flowers of *Posidonia sinuosa* that emerged under a controlled experiment designed to test the effects of burial levels (0, 1, 4, 8, 16 cm). Flowers (n=8) were observed across the 0, 1 and 8 cm treatments.

# 5 Conclusions

We conclude that low and moderate burial disturbances (1 and 4 cm) are unlikely to impact *Posidonia sinuosa*. High intensity (≥ 8 cm) and sustained burial pose a risk to the growth and population dynamics via reduced shoot extension rates (this study) and lethal responses (field study). Plant degradation under higher burial was likely due to impacts from light reduction as leaves were buried and had reduced area exposed to light and from sulphide intrusion. These results emerged under ambient light conditions which is unlikely to represent the light climate that would be expected during dredging operations. On this basis, we propose that the response to the cumulative impacts of low light and burial could be more severe than measured in this experiment. Further research to investigate how low light conditions modifies the tolerance of *P. sinuosa* to burial is warranted for developing thorough dredging-related thresholds. Future experiments aimed at burial threshold development should incorporate other stressors such as differing levels of organic matter and varying temperature levels to explore these cumulative stressors.

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# 7 Appendices

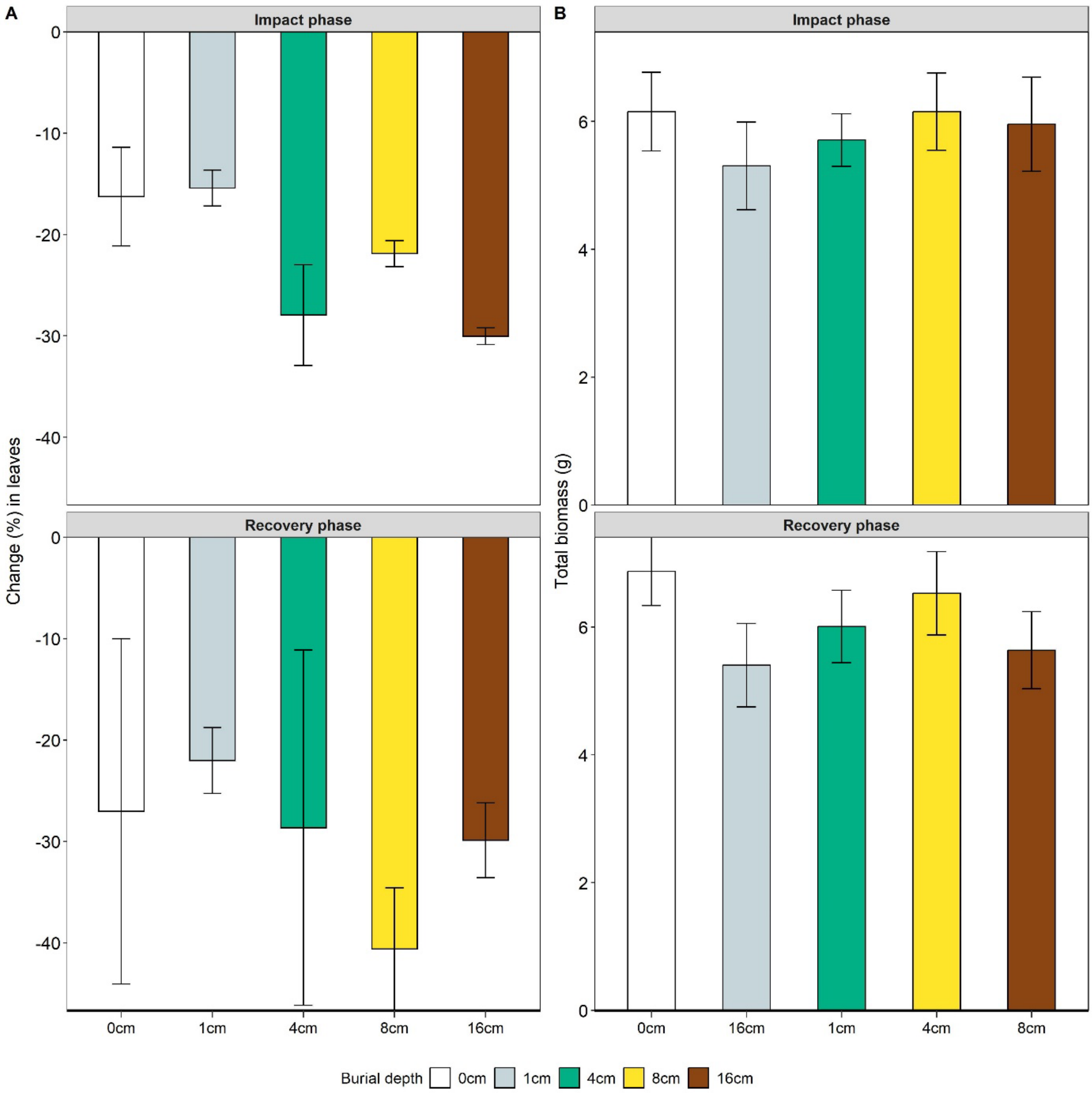


Figure A1. Effects of burial depths (0, 1, 4, 8 and 16 cm) on seagrass *Posidonia sinuosa* change in leaf density (A) and total biomass (B). Letters on graphs indicate significant PERMANOVA pairwise comparisons for differences in response between the burial depths.

Table 7-1. Sediment characteristics (percent sediment grain size, percent organic matter and percent calcium carbonates) for dredge spoil used as burial treatment.

| Sediment grain size (%) | | | Organic matter (%) | Calcium carbonates (%) |
|---|---|---|---|---|
| Silts & clays (<63µm) | Fine-medium sands (63-500 µm) | Coarse sands (500-63 µm) | | |
| 1.2 | 74.9 | 23.8 | 3.7 | 28.5 |

Table 7-2. Mains results of PERMANOVA testing for the effects of Burial (0, 1, 4, 8, 16 cm) and Duration (weeks) on response variables of adult Posidonia sinuosa during Impact and Recover phases and on oxygen (mg/L) and redox (mV) at the sediment surface. p-value (α)=0.05 unless otherwise stated. Significant comparisons are indicated in bold text.

| | **Change in leaves (%)** | | | | | | **Above-ground biomass** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Impact (0.01)** | | | **Recovery** | | | **Impact** | | | **Recovery** | | |
| Source | df | SS | α | df | SS | α | df | SS | α | df | SS | α |
| Burial | 4 | 186.96 | n.s. | | | | 4 | 1.19 | n.s. | 3 | 5.32 | n.s. |
| Duration | 2 | 1058.7 | n.s. | | | | 2 | 1.13 | n.s. | 2 | 34.03 | **<0.05** |
| B x D | 8 | 1961.4 | n.s. | | | | 8 | 0.58 | n.s. | 6 | 3.34 | n.s. |
| Residual | 30 | 3768.4 | | | | | 30 | 0.07 | | 24 | 32.96 | |
| Total | 44 | 6975.5 | | | | | 44 | 0.26 | | 35 | 75.27 | |
| | **Below-ground biomass** | | | | | | **Total biomass (excl dead sheath)** | | | | | |
| | **Impact** | | | **Recovery** | | | **Impact** | | | **Recovery** | | |
| Source | df | SS | α | df | SS | α | df | SS | α | df | SS | α |
| Burial | 4 | 3.24 | n.s. | 4 | 2.66 | n.s. | 4 | 3.08 | n.s. | 4 | 9.33 | n.s. |
| Duration | 2 | 9.98 | n.s. | 2 | 110.55 | **<0.05** | 2 | 4.83 | n.s. | 1 | 1.35 | n.s. |
| B x D | 8 | 2.55 | n.s. | 8 | 2.53 | n.s. | 8 | 2.02 | n.s. | 4 | 2.20 | n.s. |
| Residual | 30 | 97.52 | | 30 | 47.34 | | 30 | 46.17 | | 20 | 31.01 | |
| Total | 44 | 113.29 | | 44 | 163.07 | | 44 | 56.10 | | 29 | 43.89 | |
| | **Oxygen at the sediment surface** | | | | | | **Redox at the sediment surface** | | | | | |
| | **Impact** | | | **Recovery** | | | **Impact** | | | **Recovery** | | |
| Source | df | SS | α | df | SS | α | df | SS | α | df | SS | α |
| Burial | 4 | 2.93 | n.s. | 4 | 4.34 | n.s. | 4 | 5063.20 | n.s. | 4 | 412.76 | n.s. |
| Duration | 2 | 44.86 | **<0.05** | 2 | 10.09 | **<0.05** | 2 | 4240.80 | n.s. | 2 | 40730 | **<0.05** |
| B x D | 8 | 6.88 | n.s. | 8 | 5.40 | n.s. | 8 | 17492 | n.s. | 8 | 5982 | n.s. |
| Residual | 30 | 19.57 | | 30 | 21.33 | | 30 | 61987 | | 30 | 30239 | |
| Total | 44 | 74.23 | | 44 | 41.16 | | 44 | 88783 | | 44 | 77363 | |

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|---|---|
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