Sediment-effects on seagrass *Zostera muelleri* in New Zealand

A thesis submitted in fulfilment
of the requirements for the degree
of

Doctor of Philosophy
in
Biological Sciences
at

The University of Waikato

by

Iñigo Zabarte-Maeztu

2021

“The sea, once it casts its spell, holds one in its net of wonder forever”

Jacques – Yves Cousteau

Abstract

Seagrasses are ecosystem engineers that provide important ecological functions and societal economic values. Examples of the services that seagrasses provide are: sediment and coastal stability; maintenance of water quality; primary productivity for coastal ecosystems; fisheries nursery habitat; food for large herbivores; food-webs for complex marine communities; fisheries habitats; and carbon sink. They help minimise the costs of foreshore protection and maintain and support both tourism and fisheries economies. Different factors are implicated in causing the decline of seagrass ecosystems, but human activities are clearly identified as one of the major causes of seagrass decline in the world.

Humans affect this ecosystem via physical damage (e.g., harbour developments, trawling, aquaculture), introduced species, global change, and pollution (e.g., sediments, nutrients, wastewaters, herbicides, heavy metals, petrochemicals). In New Zealand, sediment is the most pervasive seagrass stressor and the most prominent cause of seagrass decline. The goal of this PhD was to determine sediment effects on the seagrass *Zostera muelleri* in terms of light attenuation and substrate physico-chemical alteration. Within this research framework, provision of assistance for successful seagrass restoration was also considered.

The principal research question for this project was to evaluate how sediment affects seagrasses and the project hypothesis was that ***sedimentation affects seagrass by altering the light climate, physically smothering the plants and modifying substrate physico-chemical composition.***

An extensive global literature review was undertaken to improve understanding of the international body of knowledge on the effects of sediment upon seagrass. Field surveys, field experiments and mesocosm experiments were used to evaluate the research objective. Field experiments were undertaken in Pāuatahanui Inlet, New Zealand. This inlet provides a wide range of seagrass cover, historical seagrass sites and substrate conditions, which makes it an excellent field laboratory to test hypotheses. Mesocosm experiments were undertaken at the University of Waikato Marine Field Station in Tauranga.

A series of observations and experiments investigated the relationship between *Z. muelleri* growth, light climate and substrate properties. Initially, correlations between receiving irradiance, substrate physicochemical variables and *Z. muelleri* traits in Pāuatahanui Inlet, were explored using an observational seasonal survey. A series of experiments followed, that used field and mesocosm-based methods that allowed deeper analysis of how sediment affects light climate and substrate properties at seagrass habitats. These results provided new insights into conditions under which seagrass declines or is unable to re-establish. The observational-based field study was undertaken in three habitat types: historical seagrass habitat, existing seagrass habitat and potential seagrass habitat and involved two field campaigns in winter and summer. A variety of substrate physicochemical variables including substrate grain size, bulk density, redox profiles, porewater nutrients, dissolved metals, receiving irradiance and temperature were measured as well as *Z. muelleri* traits such as percent plant cover, rhizome length, shoot density leaf width and length. Significant differences of substrate properties were observed between deteriorated historical habitat substrate and existing seagrass habitats and potential seagrass habitats. Increased substrate muddiness and consequent unfavorable rhizosphere conditions were implicated as causes of seagrass decline or failure to recolonize historical habitat. The results suggested for the multi-stressor effects of sediment on seagrasses, with both substrate suitability and submerged light climate for seagrass being detrimentally affected.

However, despite considering a wide range of substrate properties and irradiance, the exact mechanisms of seagrass decline could not be extracted from the data collected in the observational field survey. Further manipulative mesocosm experimentation was expected to allow more conclusive inferences to be drawn on the influence of substrate physicochemical factors and irradiance on seagrass growth and persistence. A factorial mesocosm experiment was conducted to elucidate the links between these. Two irradiance treatments; low (6.3 mol m^{-2} d^{-1}) and very low (2.3 mol m^{-2} d^{-1}), were crossed with two substrate treatments; historical substrate (42 % mud) and existing substrate (20 % mud). Seagrass growth was monitored for six weeks. Belowground biomass and rhizome growth were significantly reduced by substrate muddiness but were unaffected by irradiance. However, shoot growth was significantly affected by reduced irradiance and increased substrate muddiness as well as the synergistic interaction between both these parameters. Results suggest that *Z. muelleri* inhabiting muddy substrates has an increased irradiance demand to deal with adverse rhizosphere conditions and specifically to oxygenate the rhizosphere. Therefore, interactions between substrate and light climate, which are both affected by fine sediment pollution, should be considered when determining light thresholds for seagrass survival.

In order to further investigate the effects of site and irradiance on seagrass, a field transplanting experiment was undertaken across the previously characterised habitats in the Pāuatahanui

Inlet. The aim of this experiment was to disentangle substrate effects from other effects such as light climate and smothering. As the experiment progressed, some challenges to its successful completion emerged. Firstly, it proved impossible to reliably relocate some of the transplanted sprigs, which impeded the planned comparisons. Secondly, an incursion of the filamentous green algae *Chaetomorpha ligustica* smothered approximately half of the quadrats of one of the treatments. This is the first time, negative impacts of this species upon meadows of the New Zealand seagrass *Zostera muelleri* has been reported. *Chaetomorpha ligustica* can easily be misidentified in the field and genetic tests are required to identify this species. Hence, the need for careful identification of this green macroalga blooms in future as well as further research on growth requirements and origins of strains is desirable as it may play an important role on seagrass loss. Outcomes from this transplanting experiment allowed the conclusion to be drawn that the cumulative effect of rhizosphere deterioration, lower irradiance and close location to a source of natural sediment input during events such as storms may be the cause of the inability of seagrass to re-establish at Pāuatahanui Inlet in historical seagrass habitat.

The last experiment of the project aimed to compare the ability of the seagrass to carry out photosynthesis both in air and in water as this is potentially important for determining its vulnerability to enhanced water turbidity. To compare photosynthetic rates, oxygen (O_2) flux in water, CO_2 flux in air, and pulse amplitude modulated (PAM) fluorometry in both air and water were utilized. In water, “gross” photosynthetic O_2 evolution (GPS) as oxygen exchange averaged 2.24 µmol O_2 $m^{-2}s^{-1}$, leaf respiration rates averaged 0.44 µmol O_2 $m^{-2}s^{-1}$ and saturation irradiance 115 µmol photons $m^{-2}s^{-1}$. In air, CO_2 showed light saturated gross photosynthesis of 2.26 µmol CO_2 $m^{-2}s^{-1}$, respiration rates of 0.7 µmol CO_2 $m^{-2}s^{-1}$ and saturating irradiance 286 µmol photon $m^{-2}s^{-1}$. Compensation irradiance (E_c) is 22 µmol photons $m^{-2}s^{-1}$ and 140 µmol photons $m^{-2}s^{-1}$ when submerge and emerge showing higher photorespiration when emerged. Potential production of intertidal seagrass under submerged and emerged conditions was modeled across tidal cycles using experimental gas exchange results and field measured irradiance, using two scenarios; a high tide scenario 1 when high tide coincided with midday and low tide scenario 2 when low tide did. Respiration rate differed little between scenarios, and approximately similar amounts of net photosynthesis were predicted for emerged and submerged periods. In contrast emerged net photosynthesis was 25 times greater than submerged in the low tide scenario. These results support previous studies that have reported emerged photosynthesis as a mechanism to mitigate degraded submerged light climate, and to contribute to seagrass production estimates.

Lastly, a synthesis of new knowledge gained through this thesis, together with recently published literature is presented, which develops a new paradigm for understanding the interactive and cumulative effects of sediment on seagrass. Of particular importance are the complex interactions between irradiance and substrate muddification. This research suggests that a nuanced interpretation of fine sediment effects on seagrass, growth and persistence needs to be developed that is sensitive to the specific estuary exposure to the pollutant. Future directions for research are also suggested, which aim to build upon the research presented in this thesis and further advance understanding of the physicochemical drivers of seagrass *Zostera muelleri* loss. The information gathered from the research is available to help new methods of seagrass restoration development. This research provided evidence that enriches our knowledge of seagrass, especially estuarine seagrass ecosystems in New Zealand and this will provide an opportunity to create tools for better management of water quality and quantity targets within New Zealand to help maintain and hopefully restore this important ecosystem.

Acknowledgements

Nei ra te mihi atu ki a koutou ngā kaitiaki o Pāutahanui, otirā, ki a koutou ngā mana whenua o Porirua. Kāre e mutu ana ngā mihi ki a koutou katoa, i tautoko mai i tenei mahi rangahau, i roto i te whanga o Pāutahanui. Ko te tumanako, ka whai hua tēnei mahi rangahau, kia whakaorangia ai tēnei taonga te karepō.

Me mihi hoki au ki tōku whānau, na rātou i whakawatea i te huarahi kia hikoi ai au ki roto I tēnei ao hurihuri. Ano nei, ko tōku whānau tērā, e maanaki, e tiaki hoki ana i au i runga i tēnei haerenga.

Tēnā koutou, tēnā koutou, tēnā koutou katoa.

I have loved working in Pāuatahanui Inlet, Tauranga, Raglan, Whangarei and Waiheke seagrass meadows and I hope this research will contribute to the protection of this key ecological engineer.

Numerous people have helped me to complete this thesis. I would like to express my sincerest thanks to my supervisors Professor Ian Hawes, Dr Fleur Matheson and Professor Merilyn Manley-Harris for their time, support, encouragement and guidance throughout my PhD research. Fleur, I thank you for your boundless enthusiasm and positive energy. Merilyn, I thank you for your extreme promptness in providing feedback and your sincerity. Ian, I thank you for your wisdom, challenging my thinking and encouraging me to grow as an independent researcher. You all have inspired me and I definitely feel very lucky with my supervisory team.

I would also like to acknowledge Dr Rob Davies-Colley, for your thorough revisions to my manuscripts. Dr Chris Hickey, for the chats in the early stages of the project, Dr Iain MacDonald for allowing me to work along with you at the beautiful Waiheke Island to help out with instrument deployments, Dr Daniel Clements for letting me dive further into "Alligator weed" issue and Dr Ben Woodward for your advice and guidance on navigating an early career in science. Sanjay, Aarti and Bhavit Wadhwa for being our Indian-Kiwi family since our very first day in New Zealand. Living with you for almost two years made Laura's and my settlement in the country much easier and we recall those days as a very happy time.

It was a pleasure to work with Alex Yates from the University of Bangor during his bachelor's thesis as well as with Sarah Packard who helped me carry heavy weights when transplanting

seagrass cores. I greatly appreciate your contribution to Chapter 5, as well as your company and enthusiasm in the field.

I am indebted to the many people who assisted me for many hours in the field, thank you for giving up your time and making field work so enjoyable. Thanks specially to ***Peter Notman aka Pirate Pete***, Warrick Lyon, Owen Anderson, Ron Ovenden, Bhavit Wadhwa and Dean Stotter. Without you all, this PhD would not have been possible. A special thanks to Pirate Pete and Laura Rodriguez for moving what felt like a million mud buckets around the harbours.

I would also like to acknowledge the great people from the University of Waikato's Coastal Marine Field Station for their help when setting up the mesocosms experiment. Thanks, in particular to Dr Kaeden Leonard, David Culliford and Holly Fergurson for helping me to set up the lighting and water flow system. A huge thanks to Peter Randrup who helped me out when a considerable leak occurred without Peter something worse could have happened…

I would also like to thank my fellow students Louis Skovsholt, Theo Mouton, Sam MacCormack, Yanika Reiter, Ella Van Gol, Thang Ha, Stine Sorensen and Paola Rodriguez for the coffees, chats and quality time spent together.

I would also like to say thank you to technicians at Waikato University, in particular to Annette Rodgers (grain size), Steph Mangan, Bex Gibson, Georgina Flowers (FIA team) and Annie Barker and Amanda French (ICP-MS). Thanks are also due to NIWA lab staff Margaret McMonagle and Alice Broadhead (sulphide analysis).

Additional financial support has made my thesis work easier and also allowed me to participate in additional activities to enrich my experience. I thank NIWA for funding this PhD through its Science and Capability Development Fund (Doctoral Scholarship) and with further contributions from the Managing Mud Strategic Research program led by Dr Andrew Swales. I was also fortunate to receive several additional scholarships. Thank you to Greater Wellington Regional Council (GWRC) for supporting me with Science and Research Fund Grant in 2019 & 2020. Thanks, in particular to Dr Megan Oliver, who has provided support for the duration of this project. Additionally, I am grateful for the award of the ISBW13-NParks Travel Grant. This grant covered my registration fees for the World Seagrass Conference WSC 2018 and International Seagrass Biology Workshop ISBW 13 enabling me to attend the conference in Singapore and meet many of the internationally recognized researchers in this field. NIWA also provided support for me to attend the New Zealand Coastal Society conference in Gisborne in 2018, and the New Zealand Marine Sciences Society conference in Dunedin in 2019, allowing me to follow the cutting edge research being undertaken in Aotearoa.

I would like to thank Dr Jimmy de Fouw from Radboud University for letting me know about his project on seagrass-bivalves-sulphide interactions for which I have provided samples and I am looking forward to future collaborations.

I would also like to thank my whānau who have always encouraged me to pursue my educational aspirations even though they are literally on the opposite side of the world. Thank you to my partner Laura for her companionship and unfailing support as well as for driving, sieving, coring, and for long hours spent both in the laboratory and the field.

Contribution and publications

Publications:

- Zabarte-Maeztu, I., Matheson, F. E., Manley-Harris, M., Davies-Colley, R. J., Oliver, M., & Hawes, I. (2020). Effects of fine sediment on seagrass meadows: A Case Study of *Zostera muelleri* in Pāuatahanui Inlet, New Zealand. *Journal of Marine Science and Engineering*, *8*(9), 645.
- Zabarte-Maeztu, I., Matheson, F. E., Manley-Harris, M., Davies-Colley, R. J., Oliver, M., & Hawes, I. (2021). Interaction of substrate muddiness and low irradiance on seagrass: A mesocosm study of *Zostera muelleri*. *Aquatic Botany, h*ttps://doi.org/10.1016/j.aquabot.2021.103435.

Conferences:

- Zabarte-Maeztu, I. Matheson, F. E., Manley-Harris, M., Hawes, I. (2019). *Sediment-effect thresholds for the New Zealand seagrass Zostera muelleri: a case study in Porirua Harbour, NZ.* [Conference paper]. Presented at New Zealand Marine Sciences Society conference, 2-5 July 2019, Dunedin, New Zealand.
- Zabarte-Maeztu, I. Matheson, F. E., Manley-Harris, M., Hawes, I. (2018). *Exploring sediment effects and associated thresholds for the New Zealand seagrass Zostera muelleri.* [Poster] Presented at New Zealand Coastal Society conference, 20-23 November 2018, Gisborne, New Zealand.
- Zabarte-Maeztu, I. Matheson, F. E., Manley-Harris, M., Hawes, I. (2018, June). Sediment-effect thresholds for the seagrass *Zostera muelleri* in New Zealand. [Poster] Presented at session presented at the International Seagrass Conference, 11-17 June 2018, Singapore.

Journal article drafts:

- **Chapter 2 and 8** has been submitted to Marine Environmental Research, under the title “Sediment-effects on seagrasses: a global review and quantitative synthesis.” by Zabarte-Maeztu, I., Matheson, F. E., Manley-Harris, M., Davies-Colley, R. J., & Hawes, I.

- **Chapter 6 Short notification** has been prepared to be submitted in a peer reviewed scientific journal, under the title "First observations of *Chaetomorpha ligustica* (Cladophoraceae, Cladophorales) smothering the seagrass *Zostera muelleri* in a New Zealand estuary" by Zabarte-Maeztu, I., Matheson, F. E., D'Archino, R., Manley-Harris, M., Davies-Colley, R. J., & Hawes, I.
- Matheson, F. E., Zabarte-Maeztu, I., Mackay, G., Middleton, C., Oliver, M., Griffiths, R., & Davies-Colley, R. J. Estuary muddiness and seasonally low light constrains seagrass restoration. Manuscript in preparation.
- During fieldwork I came across seagrass flowering in different estuaries around the country and I quantified densities in some of them both at South and North islands (**Short Note, In preparation**). It may not be strictly linked to the fine sediment issue, but as aiding restoration of seagrasses was one of the higher goals of the project, it would be useful to create a seedbank.

Other research collaborations

- Gall, M., & Davies‐Colley, R. (2020). A portable underway flow‐through sampler for rapid survey of contaminated river plumes in coastal waters. Limnology and Oceanography: Methods.
- Dr Jim de Fouw: Project: An ancient detoxification mutualism – A missing link in seagrass conservation
    - This project aims to investigate the nature and relevance of the interaction between seagrasses and lucinid bivalves in coastal zones around the world. Recent work suggests that a mutualism between seagrass, lucinid bivalves and their sulphide-oxidizing endosymbiotic gill-bacteria can alleviate sulphide stress in seagrass ecosystems, that this association has existed since seagrasses evolved, and that it is common in seagrass ecosystems worldwide.
    - With this aim seagrass and bivalves samples were collected in our field sites and sent to Dr Jim de Fouw (Radboud University, The Netherlands) to develop an international investigation of the topic.

Table of Contents

List of Figures

List of Tables

List of Abbreviations

AGB	Aboveground Biomass
BGB	Belowground Biomass
CA	Carbonic Anhydrase
CCM	Carbon Concentration Mechanism
CDOM	Coloured Dissolved Organic Matter
C_i	Inorganic Carbon
DIC	Dissolved Inorganic Carbon
E_k	Saturating Irradiance
ETR	Electron Transport Ratio
ETR_{max}	Maximum Electron Transport Ratio
GPS	Gross Photosynthesis
E_c	Compensation Irradiance
IRGA	Infra-red Gas Analyzer
MDL	Maximum Depth Limit
MPB	Microphytobenthos
NPS	Net Photosynthesis
OM	Organic Matter
ORP	Oxidation Reduction Potential
PAR	Photosynthetically Available Radiation
PCA	Principal Component Analysis
PAM	Pulse Amplitude Modulated
POM	Particulate Organic Matter
PSI	Photosystem one
PSII	Photosystem two
Rubisco	Ribulose-1,5-bisphosphate carboxylase-oxygenase
RZ	Root Zone

SI	Surface Irradiance
SPM	Suspended Particulate Matter
SQG	Sediment Quality Guidelines
SS	Suspended Sediment
SSC	Suspended Sediment Concentration
SSL	Sediment Seagrass Light
TSS	Total Suspended Sediment

Chapter 1

General introduction

1.1 General introduction

Seagrass meadows are one of the most important, and threatened, ecosystems on the planet (Waycott *et al.*, 2009). They have immense ecological, and socio-economic value (Orth *et al.*, 2006; Burkholder *et al.*, 2007; Waycott *et al.*, 2009; Cunha *et al.*, 2012). However, as a result of human activities these important ecosystems are in decline (Short & Coles, 2001) and, if we are to maintain them and the ecosystem services they provide (Table 1.1), it is essential that we understand the factors that have caused their demise.

Table 1.1: Seagrass ecological and socio-economic values (adapted from Short & Coles, 2001).

Ecological values	How seagrasses provide the value	Socio-economic values
Bed sediment and coastal stability	Leaf canopy dampens water movement; rhizome & root system binds sediments and stabilises foreshore topography (Orth *et al.*, 2006; Battley *et al.*, 2011)	Reduces costs of foreshore protection
Maintaining water quality	Leaf canopy and epiphytic algae "scrub" and buffer nutrients and toxins from land run-off (Hemminga & Duarte, 2000; Duarte *et al.*, 2013)	Maintains tourist economies and local fisheries (e.g. shellfish), when inorganic sediment in the water column is reduced
Carbon sink. "Blue" carbon	Seagrasses and their sediments act as a substantial store of "blue carbon" locking up carbon that would otherwise be released to the atmosphere as carbon dioxide (Duarte *et al.*, 2010)	Mitigates climate change effects
Primary productivity for coastal ecosystems	Organic carbon production by seagrasses and epiphytic algae contributes to coastal food webs (Fourqurean *et al.*, 2012)	Supports fisheries economies and marine biodiversity
Fisheries nursery habitat & food for large herbivores	Shelter, food and food web support for commercial and non-commercial fisheries (Bertelli & Unsworth, 2014)	Supports fisheries economies and marine biodiversity

In New Zealand, fine sediment is considered to be the most pervasive contaminant affecting estuaries and sheltered coastal embayments (Green & Short, 2003; Matheson & Schwarz, 2007; Morrison *et al.*, 2009) and it is thought to have contributed substantially to documented losses of seagrass meadows in a number of New Zealand estuaries (Inglis, 2003; Matheson *et al.*, 2011). This PhD research project entitled “Sediment effects on seagrass *Zostera muelleri* in New Zealand” was established to closely examine the mechanisms by which fine sediment pollution affects the condition and resilience of seagrass in New Zealand estuaries. This research contributed to the study of interactive sediment effects and the potential identification of thresholds in terms of light attenuation and deposited sediment that can be used by resource managers to protect and restore seagrass meadows by limiting catchment fine sediment loads in the future.

Seagrasses are marine flowering plants that can grow fully submerged in the sea but some species can also tolerate regular periods of emersion at low tide. Consequently, seagrasses can be found both in subtidal and intertidal coastal zones. In comparison with terrestrial angiosperms, they exhibit low taxonomic diversity with approximately 12 genera and 60 species worldwide (Kuo & Den Hartog, 2000). All species share similar architecture and physiology and perform similar ecosystem functions**.** Seagrasses occur across the globe, in subarctic, temperate and equatorial regions, reaching their most southerly limit at Stewart Island, New Zealand (Hemminga & Duarte, 2000; Turner & Schwarz, 2006).

Seagrasses predominantly occur in shallow, sheltered coastal waters, on a variety of substrata ranging from mud through to sand and bedrock (Hemminga & Duarte, 2000; Green & Short, 2003). However, the most extensive meadows are usually found on soft substrata, often forming continuous expanses over several square kilometres. Sometimes, they can form patches, and this is often observed in areas with more wind-generated wave exposure (Inglis, 2003). Seagrasses are typically found in intertidal and shallow subtidal waters at depths between 2 and 12 m, but some species can grow in water depths of 50–60 m, depending on water clarity (Turner & Schwarz, 2006). Seagrasses have an underground root network and grow vegetatively by creeping through the substrate, continuously sending out new horizontal root runners (rhizomes) from which new stems, leaves and finer roots arise (Duarte *et al.*, 1994). Seagrass plants can also reproduce sexually, by flowering and producing seed (Hemminga & Duarte, 2000).

1.1.1 New Zealand seagrass *Zostera muelleri*

Historically, New Zealand seagrass specimens have been taxonomically referred to as *Zostera novazelandica* and/or *Z. capricorni*, but these species are now considered to be synonymous with *Zostera muelleri*, (Jacobs *et al.*, 2006) which also inhabits the coastal waters of Australia and Papua New Guinea (Jones *et al.*, 2008). In New Zealand, this species grows on silty or sandy tidal flats, in channels and river mouths in estuaries, on some coastal beaches and rocky reef platforms, and in shallow waters near offshore islands (Woods & Schiel, 1997; Ramage & Schiel, 1998; Schwarz *et al.*, 2006; Turner & Schwarz, 2006; Dos Santos & Matheson, 2017).

Zostera muelleri is a small plant compared to some of the larger-leaved seagrass species, such as *Posidonia oceanica*, *P. australis* and *Thalassia testidinum*, which grow elsewhere in the world, particularly in tropical waters. *Zostera muelleri* has thin, green, leaves which range in size from approximately 5 to 30 cm length (but are usually around 10 cm in length) and 0.1 to 0.4 cm in width (Turner & Schwarz, 2006).

In New Zealand, seagrass meadows have been recorded throughout the country, from Parengarenga Harbour in Northland to Cook’s Inlet on Stewart Island. Unfortunately, no systematic survey of seagrass distribution and abundance throughout New Zealand has been carried out. New Zealand seagrass is recorded as mostly intertidal rather than subtidal. This may reflect local extinction of subtidal beds in human-impacted estuaries, because dense subtidal meadows still occur in more pristine waters for example around offshore islands, (Schwarz *et al.*, 2006; Matheson *et al.*, 2010). New Zealand seagrass populations are thought to be predominantly perennial with clonal reproduction (Turner & Schwarz, 2006). Sexual reproduction has only been studied in two instances for *Zostera muelleri* in New Zealand (Ramage & Schiel, 1998; Dos Santos & Matheson, 2017).

In New Zealand, substantial losses of seagrass linked to human activities have been documented for Avon-Heathcote estuary (Inglis, 2003), Manukau Harbour (Turner, 1995), Tauranga Harbour (Park, 1999), Waitemata Harbour (Hayward *et al.*, 1999), Whangarei Harbour (Reed *et al.*, 2004), Eastern Bay of Islands (Matheson *et al.*, 2010; Booth, 2019) and Porirua Harbour (Matheson & Wadhwa, 2012). In Tauranga Harbour, approximately one-third of intertidal seagrass and 90% of sub-tidal seagrass were lost in the period from 1954 to 1996 and this has been linked to increased siltation of the estuary (Park, 1999).

In Porirua Harbour, approximately 40% of seagrass beds have been lost since 1980 (Matheson & Wadhwa, 2012). The largest loss (c. 32 ha) is from the head of the Pāuatahanui arm since 1980 where there is strong evidence for siltation effects.

Globally, the decline of seagrass meadows has often been linked to contamination by sediment (Orth *et al.*, 2006; Waycott *et al.*, 2009; Cunha *et al.*, 2012). This project hypothesises that excessive sediment inputs to estuaries affects the seagrass growing environment in three main ways: 1) by affecting light climate, 2) by coating and smothering plants; and 3) by altering physicochemical conditions in the rhizosphere and that these effects may interact to cause seagrass loss.

The key aim of this PhD was to examine sediment effects on the seagrass *Zostera muelleri*. Specifically, this study was focused upon the three modes of effect described above. The specific research questions and the chapters in which they were addressed are as follows:

1.2 Thesis Structure and objectives

1.2.1 Chapter 2: Sediment-effects on seagrasses: a global review and quantitative synthesis

The aim of this chapter was to establish the existing state of knowledge of sediment effects on seagrasses. An extensive global literature review and quantitative synthesis was performed in which 201 papers were scrutinized and classified by research location; species; as field, laboratory or mesocosm experiments, or reviews; and by one of three non-exclusive modes of action of sediment: 1) light climate; 2) smothering (burial), and 3) effects via rhizosphere physico-chemistry.

1.2.2 Chapter 3: Multiple effects of sediment on seagrass meadows: A case study of *Zostera muelleri* in Pāuatahanui Inlet, New Zealand.

It was hypothesised that sediment inputs to estuaries alter the growing environment for seagrass in three main ways and that these factors interact to cause seagrass loss. The relative importance of these effects was examined by a comprehensive one-off field survey in Pāuatahanui Inlet comparing substrate physicochemical conditions and irradiance at sites where (1) seagrass thrived historically but no longer grows (HS), (2) seagrass still persists (ES), and (3) seagrass has been transient in recent times and could potentially grow (PS).

This chapter examined relationships between seagrass health, light availability, and substrate physico-chemistry across the three groups of sites listed above. The chapter allowed the identification of the main physical and chemical stressors that limit seagrass health by comparison of data from sites where seagrass persists and where it is absent due to suspected sediment effects.

1.2.3 Chapter 4: Substrate-Irradiance interactive effects on seagrass: a mesocosm study of *Zostera muelleri*.

The key aim of this chapter was to study the interactions between substrate properties and receiving irradiance on seagrass growth and survival through a factorial mesocosm experiment. Seagrass was exposed to two irradiances and two substrates (the latter from Pāuatahanui Inlet, one from an HS site and one from an ES site) with growth responses monitored for a six-week period.

1.2.4 Chapter 5: Examining the relative influence of substrate physicochemical condition versus smothering and light climate effects on seagrass growth: A transplanting experiment.

In this chapter a field transplanting experiment was performed to test if persistent alterations to substrate physico-chemistry can be the primary factor driving seagrass loss and failure to re-establish at former sites in Pāuatahanui Inlet. Seagrass growth was monitored following controlled exchanges of sediment among historical and current seagrass sites. The growth responses on the different sediment types at each site were informative. Furthermore, a test of sprigs versus intact cores as most suitable transplanting units for *Zostera muelleri* was performed. However, difficulties arose during the fieldwork including an unexpected algal bloom of *Chaetomorpha ligustica* (Chapter 6. Short notification).

1.2.5 Chapter 6: First observations of *Chaetomorpha ligustica* (Cladophoraceae, Cladophorales) smothering the seagrass *Zostera muelleri* in a New Zealand estuary

As mentioned above, during chapter 5 experimental fieldwork a *C. ligustica* bloom was detected, and the aim of this chapter is to report this as well as to give details on the identification of the filamentous alga and its potential negative effects on seagrass.

1.2.6 Chapter 7: Evaluating the effect of tidal exposure on *Zostera muelleri* photosynthesis combining gas exchange measurements and pulse amplitude-modulated (PAM) fluorometry

The key aim of this chapter was to study the effect of exposure to air or water of intertidal seagrass on its photosynthetic performance. With this aim, field and laboratory experiments were performed using oxygen flux in water, CO_2 flux in air, and pulse amplitude modulated (PAM) fluorometry in both. In addition, potential production of intertidal seagrass under submerged and emerged conditions was modeled across tidal cycles using experimental gas exchange results and field measured irradiance.

1.2.7 Chapter 8: Sediment-effects on New Zealand seagrass *Zostera muelleri* – a synthesis

The key aim of this chapter was to synthetize the work and summarize the further recommendations and applications.

Chapter 2

Sediment effects on seagrasses: a global review and quantitative synthesis

2.1 Abstract

This review collates research into sediment as a stressor of seagrass causing decline or loss and emphasizes the multiple modes of action of this contaminant. The review is based on an on-line database search that retrieved 201 articles on sediment impacts on seagrasses. Articles were classified by research location; species; as field, laboratory or mesocosm experiments, or reviews; and by one of three non-exclusive modes of action: 1) light reduction; 2) rhizosphere chemistry and 3) smothering (burial).

Most research has been undertaken in the USA and Australia followed by Spain, the Philippines and New Zealand. Thresholds determined for seagrass survival under sediment stress were primarily field studies (141 citations), followed by laboratory and mesocosm studies (24 citations) and there have been 36 reviews. The most frequently described adverse mechanism is light reduction (57 citations), followed by substrate rhizosphere chemistry (31) then smothering (surface-settled) effects (6). This chapter highlights how mud with high organic content (implying high oxygen demand) is likely to be particularly problematic, and that smaller seagrass species are particularly vulnerable to smothering. Research gaps are identified and research effort recommend as follows: multi-approach studies, and studies of chronic smothering, physico-chemical alteration and its interaction with light. Identifying the thresholds of seagrass health indicators under acute and chronic sediment loading would benefit coastal resource management, contributing to improved decision-making and enabling implementation of protective actions.

2.2 Introduction

Seagrass meadows are widely distributed in tropical and temperate coastal waters, globally (Waycott *et al.*, 2009), where they have immense ecological, and socio-economic value, supporting a wide range of ecosystem services (Short & Coles, 2001). These aquatic angiosperms are critical intertidal and subtidal habitat for many marine organisms, providing shelter, food and structural habitat, both above and below the substrate surface (Orth *et al.*, 2006). Seagrasses are ‘ecosystem engineers’ in the sense that they are able to modify their environment, particularly by damping wave action and sediment entrainment, retaining settled fines and thereby clarifying water, and by oxygenating substrates in their rhizosphere and modifying substrate chemistry (Terrados *et al.*, 1999; Enríquez *et al.*, 2001; Borum *et al.*, 2005b; Brodersen *et al.*, 2015).

Seagrasses are, however, in decline across their entire range. Waycott et al (2009) estimated that, globally, 29% of the known areal extent of seagrass has disappeared since seagrass areas were initially recorded in 1879 (Waycott *et al.*, 2009). Furthermore, rates of decline have accelerated from a median of 0.9% year^{-1} before 1940 to 7% year^{-1} since 1990 placing seagrass meadows among the most threatened ecosystems on earth (Waycott *et al.*, 2009). Whether declines are occurring mostly in subtidal versus intertidal locations, and or on open coasts versus estuaries are significant knowledge gaps. The fact that seagrass grows both sub tidally and/or in shallow but sometimes turbid waters challenges mapping at large scales using remote-sensing techniques (Waycott *et al.*, 2009).

Seagrass decline can be the result of natural or anthropogenic influences. Natural factors including extreme climatic events, such as storms, and biotic influences, for example plant diseases and avian grazing, may contribute to the permanent or temporary loss of seagrass beds (Walker *et al.*, 2007; Smale *et al.*, 2019). However, anthropogenic activities, invasive species and particularly pollution of coastal waters by mud, nutrients or toxins, is generally regarded as the principal contributor to long-lasting seagrass decline globally (Costanza *et al.*, 1997; Burkholder *et al.*, 2007; Infantes *et al.*, 2011; Cunha *et al.*, 2012). Increased loading sediments to estuaries and coastal waters, due to soil disturbance on land and dredging activities in harbours are often implicated in seagrass declines (Thrush *et al.*, 2004). Such chronic increases result in persistent changes to water clarity and sedimentary environment, whereas natural disturbances are typically episodic and allow recovery and adaptation (Cabaço *et al.*, 2008b). Seagrasses are especially vulnerable to human disturbances because they usually occupy sheltered, shallow coastal waters, particularly estuaries and sheltered embayments, locations which are especially desired for human recreation, port facilities and other activities. World-wide, approximately a billion or more people live within 50 km of the coast (Cunha *et al.*, 2012) and so potentially benefit from ecosystem-services (Table 1.1), but may contribute to loss of, seagrass habitats particularly by mobilising sediment.

In this review, we propose that sediments and associated organic matter can affect seagrasses in three main ways in three areas (Table 2.2). Firstly, as primary producers, seagrasses must have sufficient light to grow (Duarte, 1991), and this makes them vulnerable to sediments that reduce light penetration when suspended in the water column (Walker & McComb, 1992; Dennison *et al.*, 1993; Adams *et al.*, 2016; Collier *et al.*, 2016). Secondly, sediment, usually with associated organic matter, that sinks and settles on or near seagrass meadows can smother leaf surfaces, inhibiting photosynthesis by shading, imposing an oxygen demand, and restricting metabolite exchange. In extreme cases, complete burial of whole plants may initiate all of these damaging mechanisms (Cabaço *et al.*, 2008b; Munkes *et al.*, 2015; Campbell, 2016; Stevens & Robertson, 2016; Brodersen *et al.*, 2017; Benham *et al.*, 2019). Thirdly, settled sediments, particularly fine sediments (<63 µm) and associated organic matter intruded into substrate pore space detrimentally alters the physico-chemical conditions of the seagrass rhizosphere by reducing porosity, and thus permeability, and exerting an oxygen demand. Low porosity can reduce oxygen availability and therefore increase the prevalence of toxins associated with anoxia, such as hydrogen sulphide (H_2S), (Terrados *et al.*, 1999; Robertson *et al.*, 2015). Furthermore, it can expose seagrass to high concentrations of mud associated nutrients (Duarte, 1995; Burkholder *et al.*, 2007; Van Katwijk *et al.*, 2011) and contaminants (Hoven *et al.*, 1999) (Macinnis-Ng & Ralph, 2004), for example heavy metals and herbicides. Sediment pollution of coastal waters potentially results in all of these types of stress operating simultaneously, and these effects will likely interact to accelerate seagrass loss. Here, we attempt to use existing literature to determine which mechanisms stressing seagrasses in response to sedimentation are most significant, to assist with identification of remediation strategies and seagrass management.

Table 2.1: Modes of action and areas in which sediment affects seagrasses. Italic script identifies major stress factors such as *light starvation, anoxia* and *toxicants.*

Sediment	Area	Processes	Damage mechanism
Suspended	Water column	Light attenuation (interaction with other light attenuating components, CDOM*, phytoplankton)	*Light starvation*
Settled	Substrate and leaf surface	Light attenuation and diffusivity	Burial and (further) *light starvation*, smothering - *anoxia*
Intruded	Substrate pore space	Reduced pore space, oxygen demand, exposure to intruded herbicides, heavy metals and other toxicants	Substrate *anoxia* and exposure to reduced *phytotoxic compounds* to belowground structures (roots and rhizomes)

* Chromophoric dissolved organic matter (CDOM)

2.3 Approach

Three searches of the published literature were conducted using Web of Science and Google Scholar databases in order to uncover research on the effects of sediment on seagrasses as categorized above. The first search targeted suspended sediment effects on seagrass light climate, the second smothering and burial by settled sediment and the third targeted effects of sediments on porewater chemistry. The three searches included field surveys and field and mesocosm experiments, as well as review papers. The searches included literature published between 1987 and 2018, and used the following keywords: sediments, suspended solids, mud, clay, silt, anoxia, PAR, seagrass, light, burial, erosion, threshold, rhizosphere, eutrophication, nutrients, chemistry, phytotoxic, infilling, pore water, suspensoids, suspended sediment, sulphide, ammonia, pollution, *Zostera, Posidonia, Syringodium, Cymodocea. Halophila, Thalassia, Halodule, Enhalus, Phyllospadix, Amphibiolis, Thalassodendron.* Through this process, we identified 201 relevant articles, all of which were examined and then grouped according to the key sediment-related issues that were identified.

2.4 Classification of research effort

The main reported effects of sediment on seagrasses by category are (in order of frequency): light climate, substrate porewater chemistry and smothering (burial) effects. Field studies were the most frequent approach to study sediment effects (Table 2.2). There is a trend of increasing research effort over the last thirty years, and the most prolific year was 2016, a stochastic cluster, probably of little importance given that the adjacent years (2015, 2017) showed relatively low output (Figure 2.1). Australia, USA and Spain, in that order, were the countries contributing most strongly to research on sediment-effects on seagrass (Figure 2.2A). If research effort in number of papers is normalized to country populations, Australia and New Zealand contributed most (Figure 2.2B) and if normalized to length of coastline Netherlands, Portugal and Spain were most ‘productive’. *Zostera* was the most studied genus in the reviewed papers (Table 2.2). Field experiments and surveys were around five times more common than mesocosm experiments. The sediment effects that were researched included: seagrass decline, eco-services, eutrophication, management, monitoring/modelling, resilience and restoration. Sediment effects were also the focus of a number of reviews (Table 2.2). Sediment effects on seagrass growth, shoot density, biomass, physiology, and other morphological and physiological traits are summarized in the following sections.

Table 2.2: Research effort, represented as numbers of papers on the sediment effects for seagrass per category and research approach. Also, research "focus" importance and species, in numbers of papers on the sediment effects for seagrass (n=201) is recapitulated.

Category	**Number of papers**
Sediment light climate	57
Substrate chemistry	36
Sediment smothering (burial)	6
Multiple categories	102
Research approach	**Number of papers**
Field study	141
Review	36
Mesocosm & laboratory experiments	24
Focus of the study	**Number of papers**
Eco-Services	40
Management	35
Decline	30
Eutrophication	28
Resilience	28
Restoration	17
Review	13
Monitoring/Modelling	10
Genus	**Number of papers**
Multiple species	98
Zostera	47
Posidonia	18
Thalassia	12
Cymodocea	9
Halodule	9
Syringodium	6
Enhalus	2

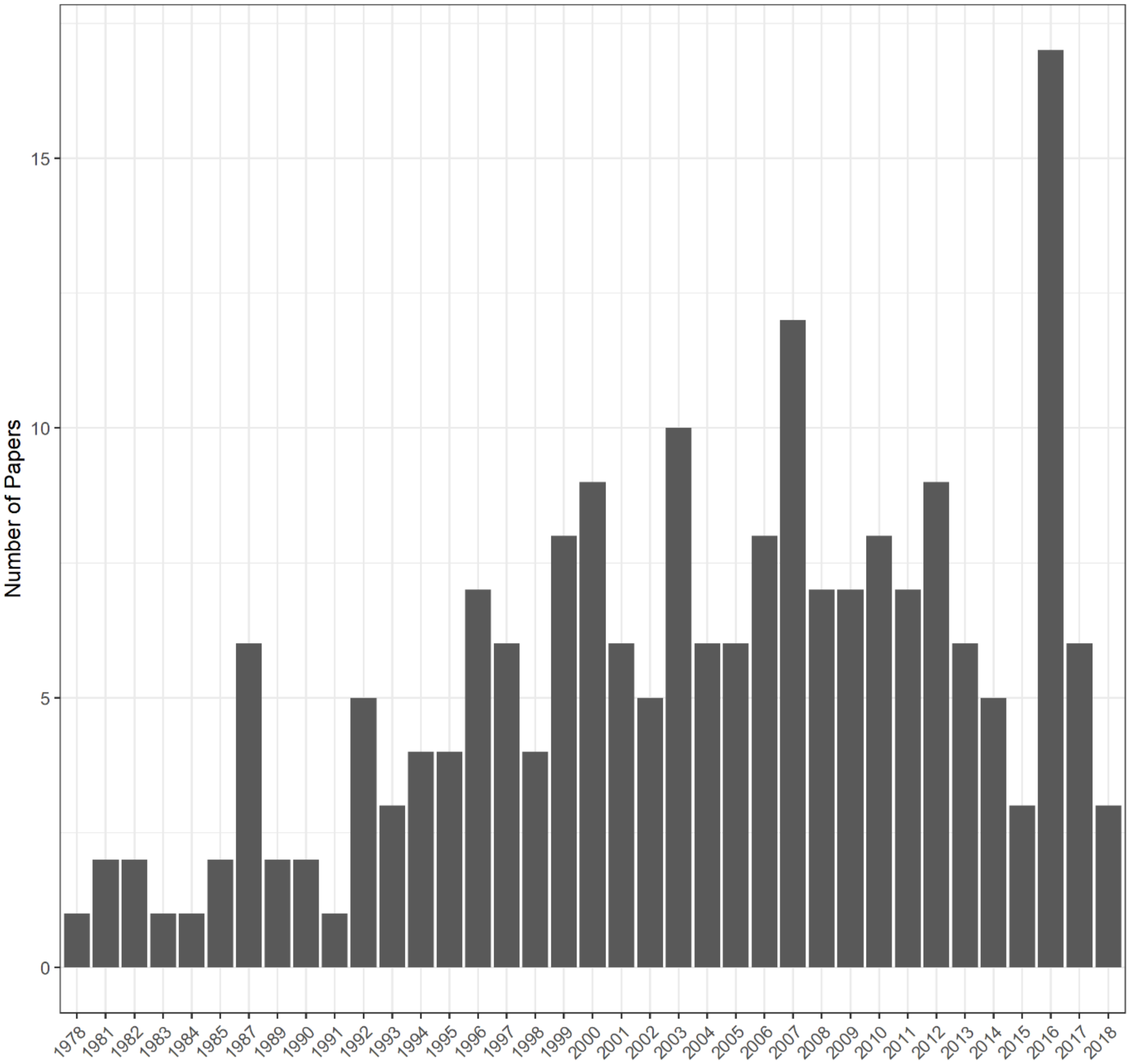


Figure 2.1: Research effort represented as numbers of papers on sediment effects on seagrass per year since 1987 (n=201).

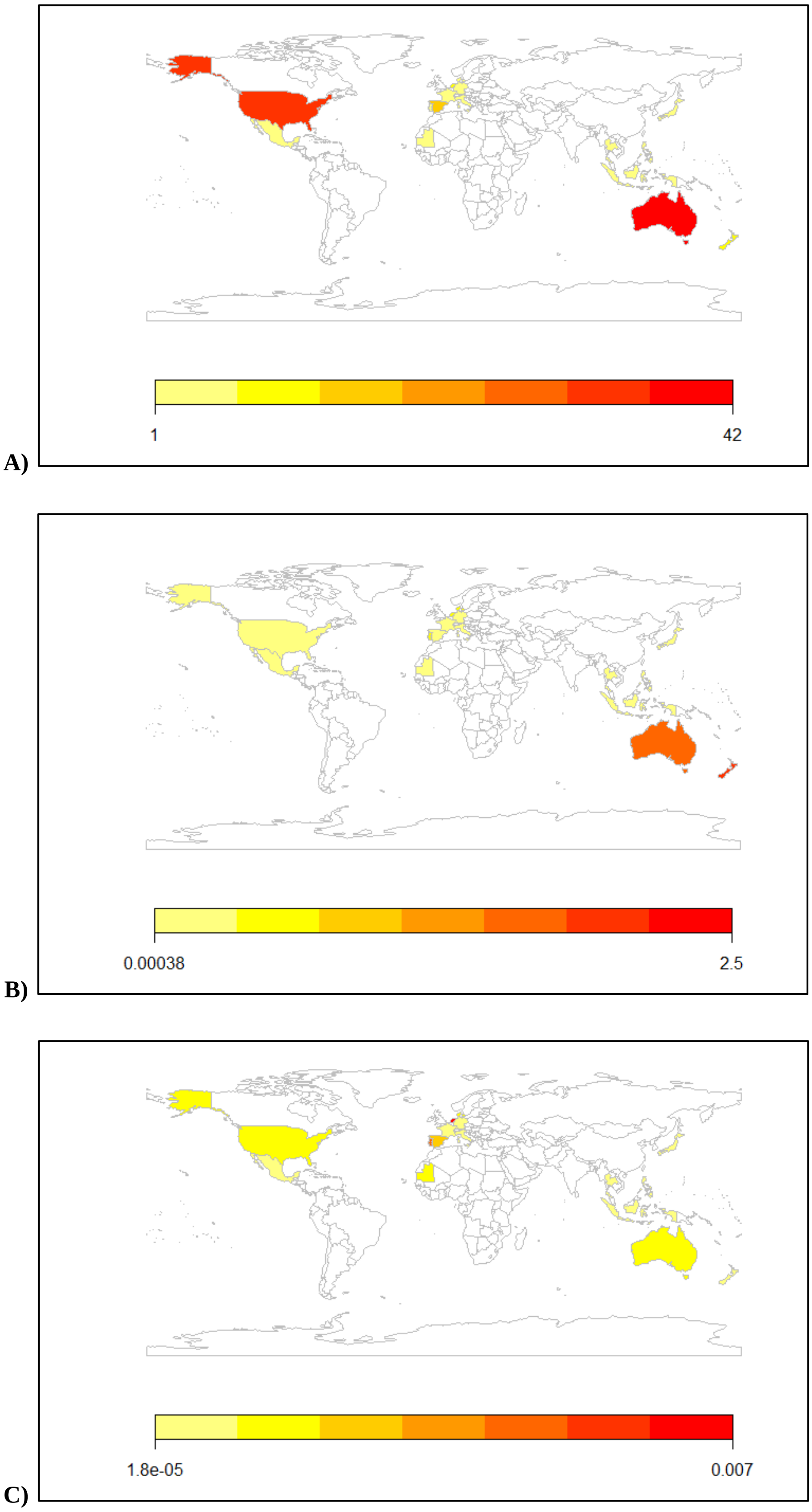


Figure 2.2: Sediment-effects on seagrass ecosystems: publication effort worldwide. A) Effort, in numbers of papers on the sediment effects on seagrass per country since 1991. Legend shows a colour gradient from a minimum of 1 to a maximum of 42 papers. Multi-country studies are not plotted, (n=137). B) Effort in number of papers normalized to country population. C) Effort normalized to countries length of coastline.

2.5 Effects of suspended sediment on light climate

2.5.1 How seagrasses respond to reduced light availability

A major cause of seagrass losses globally is the quantitative reduction of available sunlight for seagrass photosynthesis, which is the primary driver of seagrass growth (Duarte, 1991; Dennison *et al.*, 1993; Duarte *et al.*, 2004a; Ralph & Gademann, 2005; Adams *et al.*, 2016; Chartrand *et al.*, 2016; Collier *et al.*, 2016). Increased suspended sediment loads to coastal waters contribute to this by reducing diffuse sunlight penetration through the water to the sea bed (Erftemeijer & Lewis III, 2006). There are four natural constituents that attenuate light besides water itself and these interact to determine its optical quality: mineral suspended sediment (SS), coloured dissolved organic matter (CDOM), non-algal particulate organic matter (POM); and phytoplankton (Davies-Colley *et al.*, 2003). In seagrass habitat, CDOM and total suspended solids (TSS) are the constituents that mainly affect light climate (Fernandes *et al.*, 2017), although phytoplankton contribute further light attenuation in eutrophic estuaries. Kirk (1985) showed, by stochastic modelling of photon trajectories, that suspended matter reduces diffuse light penetration of water mainly by light scattering – which results in photons taking a tortuous path down through the water column so increasing their probability of extinction by absorption. Figure 2.3 illustrates how light penetration to benthic plants is reduced by high suspended particulate matter (SPM).

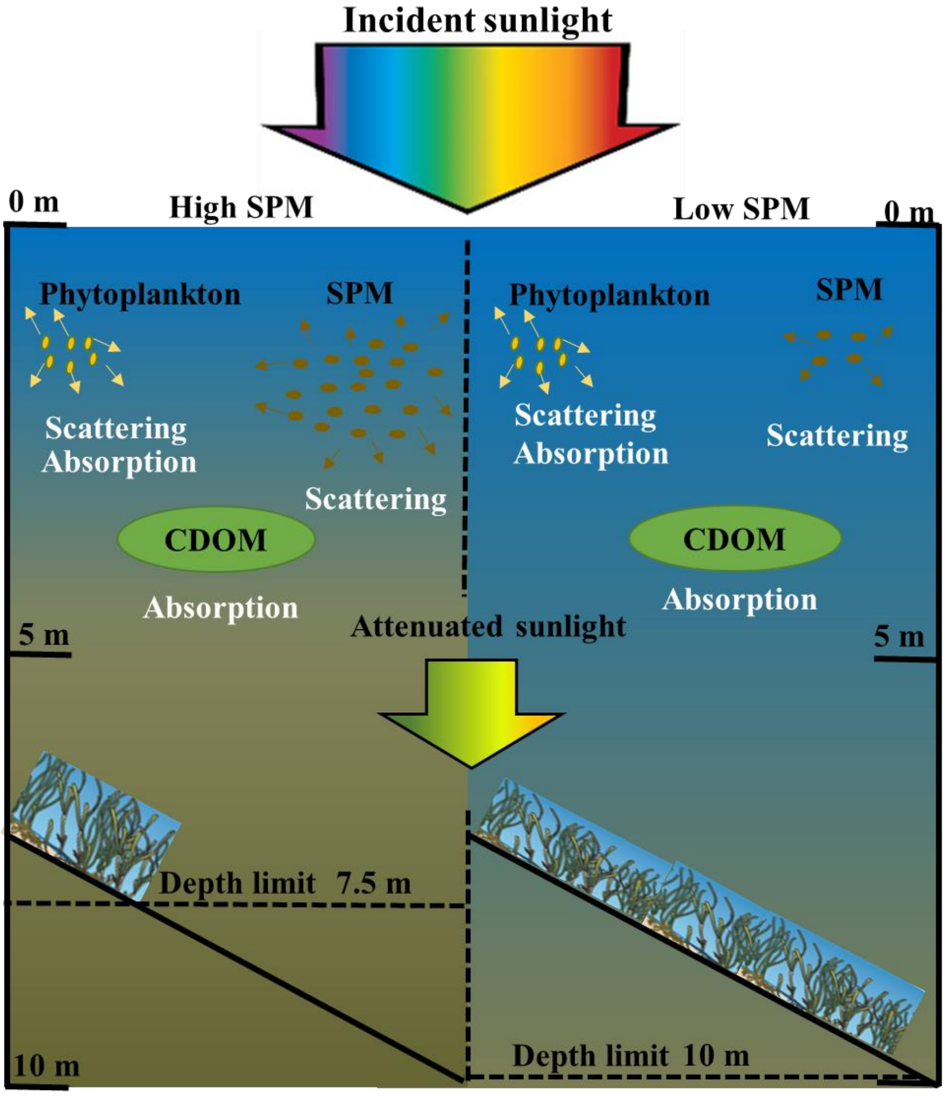


Figure 2.3: Effect on the benthic light climate of suspended particulate matter (SPM) at high versus low concentrations, in the presence of coloured dissolved organic matter (CDOM), and chlorophyll a (phytoplankton). The figure explains how light penetration to benthic plants is reduced by high fine suspended particulate matter (SPM). Consequently, a shallower depth limit is shown in presence of higher SPM.

Seagrasses initially respond to a reduction in available light by subtle changes in gene regulation (Procaccini *et al.*, 2010; Procaccini *et al.*, 2012). This results in changes to physiological parameters such as effective quantum yield (Y) and maximum photochemical efficiency (Fv/Fm) and pigments (Abal *et al.*, 1994; Kirk, 1994; Collier *et al.*, 2011). As light-limitation stress increases, this is followed by decreases in growth rates, changes to morphology and finally meadow scale reductions in abundance. Compensation irradiance (I_C), is the irradiance at which rates of organic carbon production from photosynthesis equal carbon use rates from respiration, (Bulthuis, 1987). Once use of stored carbon exceeds production, the plants start to decline and survival for a time will depend upon carbon reserves (Touchette & Burkholder, 2000). Thus, identifying the time period during which plants can persist below their compensation irradiance by consuming carbon reserves is necessary to determine their species and site-specific light requirements.

2.5.2 Critical light thresholds

A critical light threshold is defined as the minimum (steady) photosynthetically available radiation (PAR) required for a photosynthetic organism not to show decline or damage signals. A number of studies, dating back to the 1990's, provide information on the PAR required by seagrasses for persistence and growth, and clearly show considerable interspecific differences. Duarte (1991), in a global meta-analysis of seagrass depth distributions concluded that, on average, seagrasses could grow only to depths where 11% of surface-incident PAR penetrated. This shows that seagrasses are "high light" plants considering that some plants can persist on 1% of incident sunlight, the euphotic depth, (Kirk, 1994). Further review effort on the topic found similar average compensation irradiances (ca. 16% surface PAR), although the range among species varied from 3 to 30%, moreover often with congeneric species, and even the same species from different populations showing different values (Erftemeijer & Lewis III 2006).

Next, we attempted to summarize all the thresholds determined for *Z. muelleri* in particular, using the available literature in our database. We used *Zostera muelleri* (syn. *Z. capricorni* and/or *Z. novazelandica*) as an example species as there is substantial information available. Chartrand *et al.* (2016) conducted experiments in Gladstone Harbour, Australia, studying the effects of dredging activities on the light climate for seagrass. They determined that *Z. muelleri* coverage declined when light was less than 5 mol photons m^{-2} d^{-1} for periods of time longer than 4 weeks. Based on these results, an 'applied management threshold' of 6 mol photons $m^{-2}d^{-1}$ was established to protect seagrass from sediment mobilised by dredging operations in the harbour.

In another study, Collier *et al.* (2016) conducted experiments to determine the light thresholds required to maintain four species of seagrass, including *Z. muelleri*, at 50% and 80% protection levels for shoot density and growth rates over a 14-week period in 'cool' (23°C) or 'warm' waters (28°C) for tropical seagrasses. This study demonstrated that warmer water temperatures increased the light requirements for all four species and for both of the traits studied, with *Zostera muelleri* showing thresholds for maintaining 50% shoot density of 3-5 mol photons m^{-2} d^{-1} and 7-10 mol photons m^{-2} d^{-1} for maintaining 80% cover (Figure 2.4). The figure also suggests that at even lower temperatures, such as occur in temperate waters which fall below 10 °C in winter, the light requirements may be lower still, as reported by Bulmer *et al.*, (2016) in Kaipara, NZ.

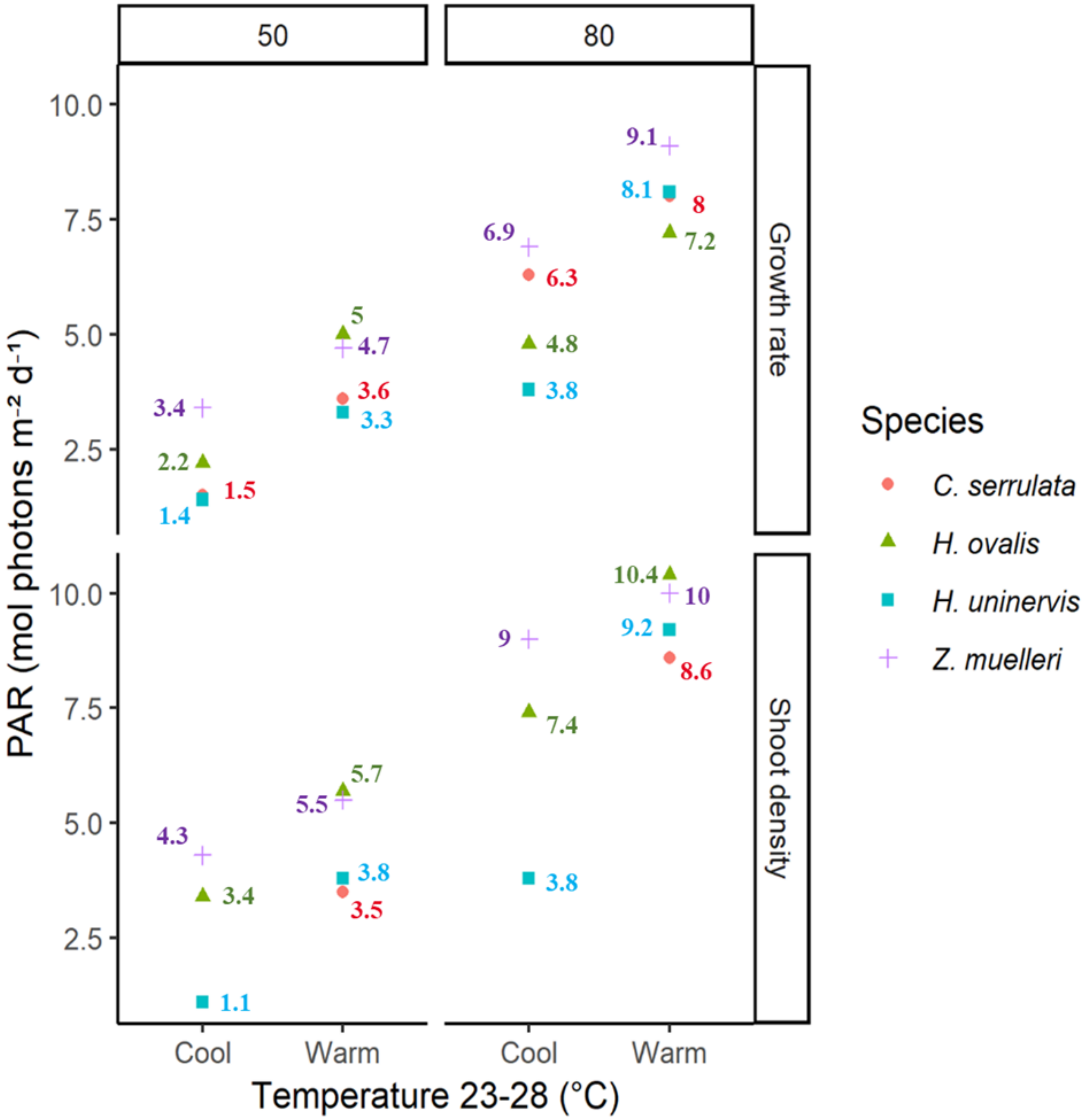


Figure 2.4: Range of critical light threshold values for 50% and 80% protection of two seagrass traits (shoot density and growth rate) under two temperatures for four species.

In Moreton Bay, Australia, the minimum light requirements (quantity and spectral quality) of the dominant seagrass *Z. muelleri* were investigated (Longstaff, 2003). Acute light reduction processes were investigated by conducting light deprivation experiments with shade screens at four monitoring sites in which temperature was 24 °C. The maximum depth limit occurred at 30% of surface light, corresponding to an annual mean of 10 mol photons $m^{-2}d^{-1}$, at three sites, and 15% of surface light, corresponding to an annual mean of 5 mol photons $m^{-2}d^{-1}$, at a fourth site.

In New Zealand, the light climate at the maximum depth limit for *Z. muelleri* was measured in Kaipara Harbour (Bulmer *et al.*, 2016). Light at the depth limit of the seagrass averaged approximately 2.1 mol photons $m^{-2}d^{-1}$ in winter (average temperature = 13.12 °C) and 4.91 mol photons $m^{-2}d^{-1}$ in summer (average temperature = 20.81 °C). This provides an indication of *Z. muelleri* minimum light requirements under cooler temperature conditions based upon the reasonable assumption that light availability is the primary factor preventing colonisation at deeper depths.

Several studies seem to be converging on an average compensation irradiance for *Z. muelleri* of around 5 mol photons $m^{-2}d^{-1}$(Flanigan & Critchley, 1996; Longstaff *et al.*, 1999; Collier *et al.*, 2011; Collier *et al.*, 2012; Bulmer *et al.*, 2016; Chartrand *et al.*, 2016; Collier *et al.*, 2016). However, a temperature gradient is indicated with lower E_c at lower water temperatures. Temperate populations show lower E_c, particularly during winter temperature troughs, compared to sub-tropical or tropical populations. Available thresholds for *Z. muelleri* and the synonymous species *Z. capricorni* and *Z. novazelandica*, are summarized in Table 2.4, with an indication of sediment composition if reported.

Table 2.3: Irradiance thresholds, compensation irradiance and required percent surface irradiance for *Zostera muelleri* or synonyms. Adapted from Matheson *et al.*, 2020 (submitted). Maximum depth limit (MDL), Percent surface irradiance (%SI) and Total suspended solids (TSS).

Study location	**Biogeography**	**Means of determination**	**PAR threshold (mol m^{-2} d^{-1}) / SI (%) / MDL (m)**	**Definition**	**Duration**	**Substrate composition**	**Reference**
NE Queensland Australia. Cape Bedford	Tropical	Measurements of light availability at mean depth limit	MDL 1.5 m	Maximum Depth Limit	November 1984	Not - available	(Coles *et al.*, 1987)
Moreton Bay, QLD, Australia. Dunwich	Tropical	Laboratory experiments to determine compensation Irradiance	1.95 mol m^{-2} d^{-1} (12 hr photoperiod) or 3.9 µmol m^{-2} d^{-1} 24h photoperiod	Irradiance when Pnet=0, i.e. compensation irradiance (E_c)	One week	Not - available	(Flanigan & Critchley, 1996)
Moreton Bay, QLD, Australia	Tropical	Measurements of light availability at mean depth limit	30%SI	%SI at MDL	Seasonal (September-August) (November-April)	Not - available	(Abal & Dennison, 1996)
Moreton Bay, QLD, Australia	Tropical	Measurements of light availability at mean depth limit	4.6	Note site specificity. Lowest value shown here	September 1997	Not-available	(Longstaff, 2003)
Moreton Bay, QLD, Australia	Tropical	Measurements of light availability at mean depth limit	5-10	Minimum long term requirement	11 months	<20% mud	Longstaff, 2003
Kaipara Harbour, North Island, NZ	Temperate	Measurements of light availability at mean depth limit	4.9 (± 0.5) (summer) 2.1 (± 0.2) (winter)	Minimum light requirements in summer and winter	60 days	Not reported	Bulmer *et al.*, 2014
Gladstone Harbour, QLD, Australia	Tropical	*In situ* shading experiments and light history monitoring over a 4-year period	6.0	To prevent measurable loss of seagrass from dredge related light attenuation	14 days during the growing season	Semi-firm substrate composition	Chartrand *et al.*, 2016

Study location	**Biogeography**	**Means of determination**	**PAR threshold (mol m^{-2} d^{-1}) / SI (%) / MDL (m)**	**Definition**	**Duration**	**Substrate composition**	**Reference**
Gladstone Harbour, QLD, Australia	Tropical	Lab. exposition quantifying response to six daily light levels in cool (23°C) and warm (28°C) temperatures	7.5 (±1.9) (c), 10.4 (±2.4) (w) 4.8 (±1.2) (c), 7.2 (±1.6) (w) 3.4 (±0.9) (c), 5.7 (±1.2) (w) 2.2 (±0.6) (cl), 5.0 (±0.7) (w)	Protect: 80% shoot density 80% growth rate 50% shoot density 50% growth rate	14 weeks	Not reported, but minimized substrate anoxia by using orchid pots and filter sock	Collier *et al.*, 2016
Whangarei and Porirua Harbours, North Island, NZ	Temperate	Comparison of light records at donor and successful transplant sites with an unsuccessful transplant site	19.2-26.9 (successful) 3.9 (unsuccessful)	To maintain a persistent seagrass cover >20%	Annually <10 days below I_c in winter	Predominantly sand. Mud WD & T: <3%^{1}, PD: 2.9^2% PT: 3.4^2-5^3%	Matheson *et al.*, 2020 submitted
Tauranga Harbour, North Island, NZ	Temperate	Laboratory experiment quantifying biomass response to five light levels at 20°C	2.9	Maintains biomass accrual Maintains biomass accrual	Up to 2 weeks 6 weeks	Predominantly sand. Mud 2.7%	Matheson *et al.*, 2020 submitted

[1] Reed et al. 2005 (0-2 cm depth)

[2] Matheson & Wadhwa 2012 (0-10 cm depth)

[3] Stevens 2017 (0-2 cm depth)

[4] Dos Santos et al. 2012 (0-10 cm depth)

2.5.3 Seagrass-Sediment-Light modelling

Seagrass-Sediment-Light (SSL) equilibrium is a concept, which expresses the balance of different processes, which occur between seagrasses and sediment (Figure 2.5). Suspended sediments attenuate light (Kirk, 1985) reducing available light for seagrass. Seagrass presence induces local reductions of near bed currents reducing turbulence and favouring deposition while dampening re-entrainment of settled mud. Models of SSL equilibrium suggest that the SSL feedback can induce bistability, that is a dynamic system that has two stable equilibrium states (Carr *et al.*, 2010). One ecosystem state occurs in which seagrass presence reduces suspended sediment concentrations and increases benthic light availability, whereas the opposite state occurs in the absence of seagrass or with low density seagrass where increments of suspended particulate matter decrease benthic light availability (De Boer, 2007; van der Heide *et al.*, 2007; van der Heide *et al.*, 2011; Adams *et al.*, 2016). Bistability can be induced only if suspended sediment is sufficiently-strongly light attenuating to slant the equilibrium towards state 2 (Figure 2.5) (Adams *et al.*, 2016).

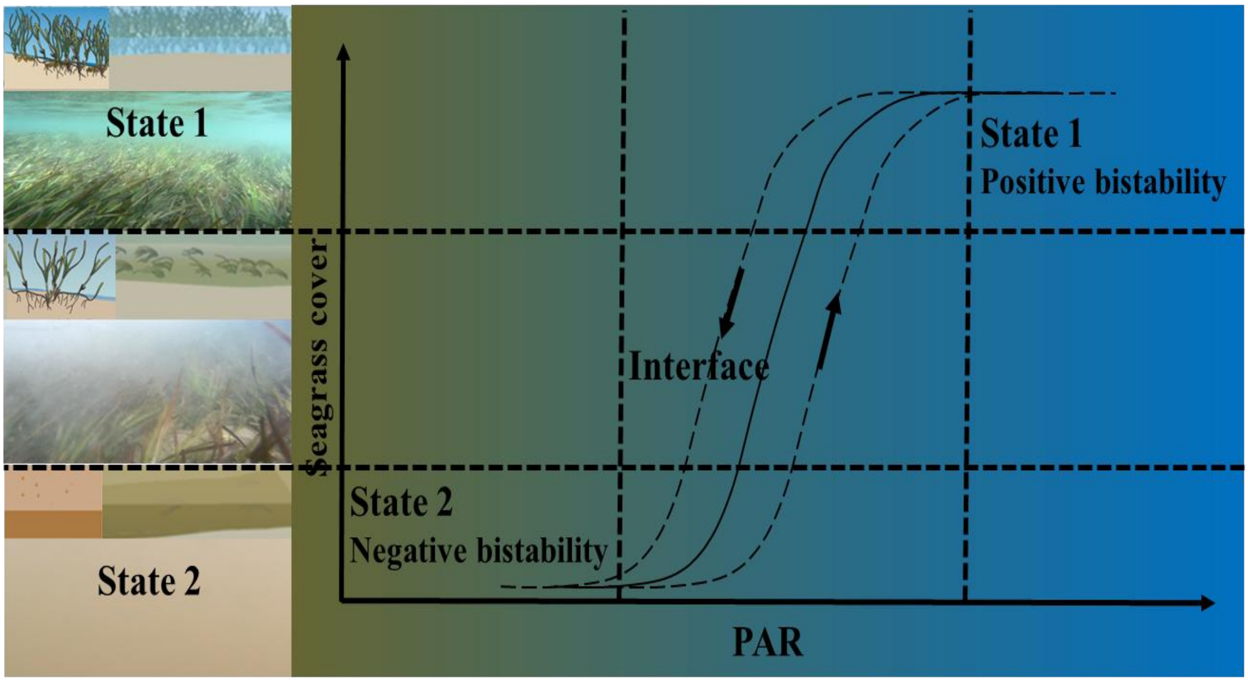


Figure 2.5: Seagrass-Sediment-Light (SSL) equilibrium. Hypothesised hysteretic relationship of seagrass cover, suspended sediment and average lighting – where seagrass influence their own light environment by protecting settled sediment from erosion. A well-vegetated state is characterised by clear overlying water and high average PAR (state 1 = positive bistability). If the average lighting should fall, eventually the seagrass cover declines to a second stable, unvegetated state (state 2 = negative bistability) characterised by low PAR due to lack of seagrass cover protecting settled sediment from re-entrainment and high light attenuation in overlying water. The average PAR would have to be greatly increased above the original threshold in order to restore the seagrass cover and return the system to the first stable state.

Adams et al. (2016) demonstrated by modelling that if the water residence time is similar to or greater than the sediment deposition time, the scale of re-establishment needs to be large enough for the feedback between seagrass, sediment and light penetration to locally improve the light climate. This calculation can be used to identify areas where this feedback is likely to generate positive bistability, and to estimate the minimum suitable meadow size in such locations. This state of bistability has been termed SSL positive feedback (De Boer, 2007). Conversely, the de-vegetated stable state can be a major barrier for restoration as shown in the Dutch Wadden Sea (van der Heide *et al.*, 2007). Future research should focus on identification of the quantitative relationships that control the SSL feedback. This is required for a better understanding of this coupled physical-ecological process, to support managers in the implementation of best practices for protection of seagrass ecosystems (Adams *et al.*, 2016).

2.6 Smothering and burial effects

Acute and chronic sediment deposition may cause seagrass decline being sediment depth and spatial extent of burial key attributes to smothering events. In acute events, whole or partial burial, typically by coarser sediment, usually extending into the sand range, can completely smother plants, depriving them of light and oxygen and causing mortality depending on the size of the species affected (Cabaço *et al.*, 2008b). Chronic effects of, generally finer, sediment deposition have been less studied with very little research reported in the literature (Brodersen *et al.*, 2017). Intense sediment burial has been implicated in widespread seagrass loss (Cabaço *et al.*, 2008b). Extreme natural events, such as cyclonic storms can mobilise large sediment loads leading to acute 'dumps' of sediment in coastal waters, and large-scale alteration of seagrass habitat (Kirkman & Kuo, 1990; Fourqurean & Rutten, 2004; Suykerbuyk *et al.*, 2016). Such heavy sediment deposition results in comprehensive and perhaps irreversible, disappearance of seagrass meadows. The construction of permanent structures, for example ports, (Ruiz & Romero, 2003), typically with local modification of hydrodynamics and sedimentary dynamics, for example by land reclamation, (Meinesz *et al.*, 1991) may exclude seagrasses through creation of unfavourable environments and burial (Meinesz *et al.*, 1991; Duarte *et al.*, 2004a). Silts and clays can lead to acute and chronic smothering of seagrass beds by impeding photosynthesis through shading and restriction of metabolite exchange, and in extreme cases, by burying whole plants (Marba & Duarte, 1994; Duarte *et al.*, 1997; Manzanera *et al.*, 1998; Mills & Fonseca, 2003) (Figure 2.6).

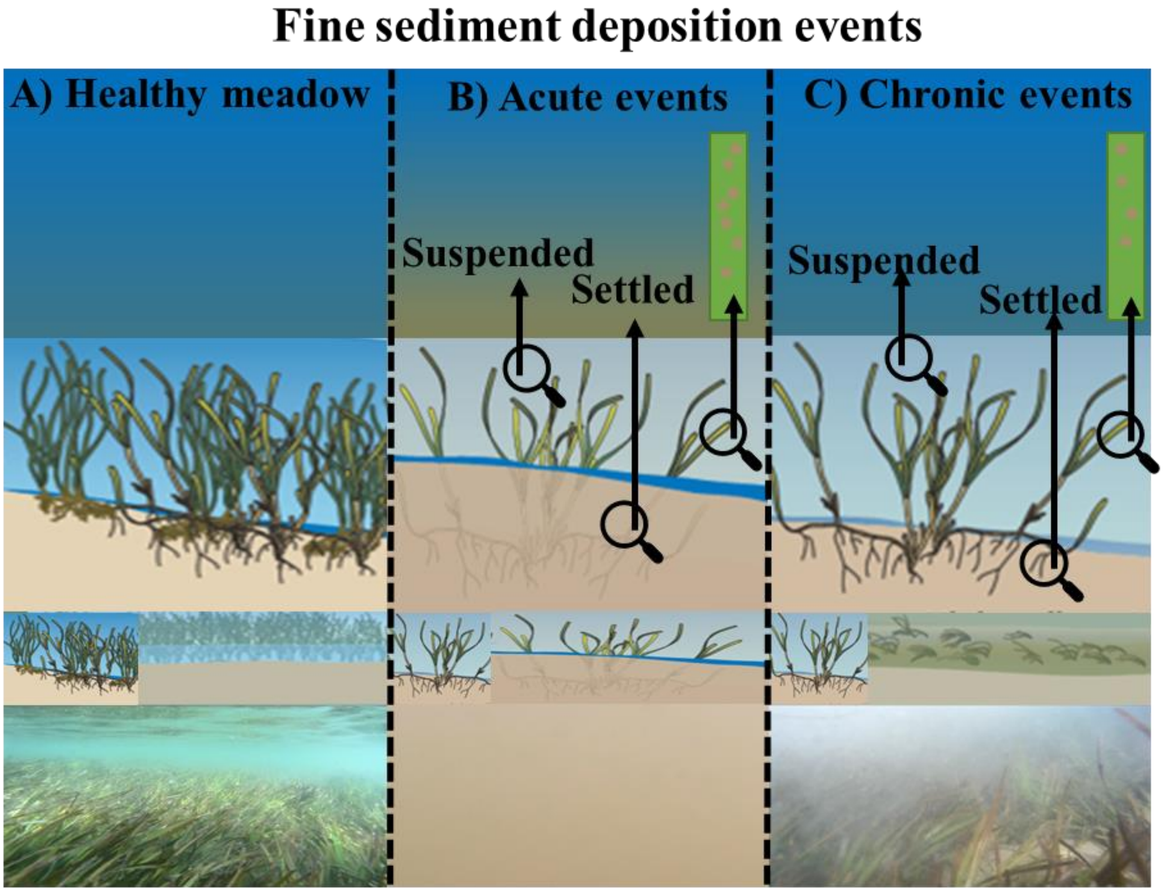


Figure 2.6: Effects of sediment deposition on seagrass according to the severity/scale of the event are shown. A) shows a healthy meadow (with little mud deposition); B) shows an acute (burial) event where seagrass is physically smothered through burial by settled sediment both (in the canopy and seafloor) impeding metabolite and gas exchange. C) shows a chronic event with, at most, partial burial but with a degree of smothering through settled sediment. Magnifying glasses zoom the location in which sediment is affecting seagrass.

Sediment burial effects (acute effects) on seagrasses were reviewed by Cabaço et al. (2008b). Several more recent studies are considered here to update that review (Table 2.5). Burial effects have been studied in approximately one-third of seagrass species. These studies indicate a wide range of tolerance to burial levels amongst different seagrasses (Table 2.5). Australian *Zostera muelleri* showed a low tolerance to burial relative to canopy height (Table 2.5). Other species of *Zostera* seem to have a higher tolerance to burial, particularly the larger species, *Z. marina* (Philippart, 1994; Cabello-Pasini *et al.*, 2002; Dumbauld & Wyllie-Echeverria, 2003; Cabaço *et al.*, 2008b). The articles reviewed here do not include reports of studies of 'chronic' pulses of less than 2 cm of sediment. To date, mainly acute sediment effects have been studied, potentially due to the technical difficulties of simulating and quantifying chronic sediment pulses. However, deposition of as little as 0.75 cm of sediment was sufficient to cause decline in shoot density in relatively small *Z. muelleri* (Benham *et al.*, 2019).

Table 2.4: Details of the experimental design to test the effects of burial on seagrass survival. Experimental burial levels causing 100% and 50% mortality are listed (Adapted from Cabaço *et al.*, (2008b).

Species	**Burial level (cm). 100% Mort.**	**Burial level (cm). 50% Mort.**	**Burial level leaf length ratio 100% Mort.**	**Burial level leaf length ratio 50% Mort.**	**Tested damage mechanism and treatments**	**Main measured seagrass response**	**Reference**
C. nodosa	13	4	0.43	0.13	- Erosion and burial treatments vs seagrass traits	- Increased shoot mortality - Increased length of the youngest vertical internode (up to 4 cm of burial) - Increased leaf turnover rate - Increased vertical growth rate - Increased leaf sheath length	Cabaço *et al.*, 2008b
C.nodosa	24	8	-	-	- Burial	- If clonal integration was preserved, the shoot density, the number of leaves, the above-ground biomass and the leaf length of did not significantly change among burial.	(Tuya *et al.*, 2013)
C. rotundata	8	2	0.53	0.13	- Burial treatments (0. 2. 4, 8, and 16 cm deposited)	- Shoot density decline - Increased vertical internode length (up to 4-8 cm of burial)	(Cabaço *et al.*, 2008b) Duarte, 1997
C. serrulata	-	2	-	0.13	- Burial treatments (0. 2. 4, 8, and 16 cm deposited)	- Initial shoot density decline in high burial levels	Cabaço *et al.*, 2008b Duarte 1997
E. acroides	-	4	-	0.13	- Burial treatments (0. 2. 4, 8, and 16 cm deposited)	- Shoot density decline only by the end of the experiment (300 days)	Cabaço *et al.*, 2008b Duarte 1997
H. uninervis	-	4	-	0.4	- Burial treatments (0. 2. 4, 8, and 16 cm deposited)	- Initial shoot density decline in high burial levels (8 and 16 cm) followed by shoot density recovery - Increased vertical internode length (up to 2 cm of burial) - Changes in age distribution	Cabaço *et al.*, 2008b Duarte 1997
H. ovalis	2	2	0.33	0.33	- Burial treatments (0. 2. 4, 8, and 16 cm deposited)	- Early increase of shoot density at intermediate burial levels (4 and 8 cm of burial)	Cabaço *et al.*, 2008b Duarte 1997

Species	**Burial level (cm). 100% Mort.**	**Burial level (cm). 50% Mort.**	**Burial level leaf length ratio 100% Mort.**	**Burial level leaf length ratio 50% Mort.**	**Tested damage mechanism and treatments**	**Main measured seagrass response**	**Reference**
P. australis	-	19.5	-	0.39	- Dredging 1999	- Increased shoot mortality - Increased sheath length in 20 cm burial level - Decreased shoot biomass and leaf growth - Decreased leaf surface area	Cabaço *et al*., 2008b
P. oceanica	14	14	0.29	0.28	- Burial intensity, frequency, timing and duration. - Reduction of water transparency - Addition of organic matter to sediments - Lethal sulphide level sand increased ammonium in sediment	- Increased shoot mortality - Decreased leaf growth and leaf length under moderate burial (6 cm) - Decreased shoot biomass and leaf no. per shoot in high burial levels (9 cm) - Decreased rhizome starch content in 3 cm burial level - Decreased leaf surface area	(Manzanera *et al.*, 1998)
P. oceanica	15	10.2	0.29	0.28	- Coastal construction and engineering	-Resuspension of silty sediments and increase of water turbidity -Irreversible replacement of the natural environment	Ruiz (P. com)
P. sinuosa	-	15.4	-	0.13	x	- Increased shoot mortality - Decreased leaf growth - Decreased sheath length and internode length	(Smith & Walker, 2002)
S. filiforme	10	4.5	0.33	0.15	- Dune migration	- Decreased shoot density - Decreased horizontal rhizome length	Cabaço *et al*., 2008b Patriquin, 1975
S. isoetifolium	-	8	-	0.27	- Burial treatments (0. 2. 4, 8, and 16 cm deposited)	- Initial shoot density decline in high burial levels (8 and 16 cm) followed by shoot density recovery - Increased vertical internode length (up to 4 and 8 cm of burial) - Changes in age distribution (increase in recruitment of young shoots (<1 yr))	Cabaço *et al*., 2008b Duarte, 1997

Species	Burial level (cm). 100% Mort.	Burial level (cm). 50% Mort.	Burial level leaf length ratio 100% Mort.	Burial level leaf length ratio 50% Mort.	Tested damage mechanism and treatments	Main measured seagrass response	Reference
T. hemprichii	-	4	-	0.16	- Burial treatments (0. 2. 4, 8, and 16 cm deposited)	- Shoot density decline - Increased vertical internode length (up to 8 cm of burial) - Changes in age distribution (selective loss of young shoots (< 1 yr) and reduced recruitment)	Cabaço *et al.*, 2008b Duarte 1997
T. testu dinium	-	5	-	0.14	- Modelling: dredging event and sulphide toxicity	- Decrease or no response of shoot density	Cabaço *et al.*, 2008b Eldridge & Morse, 2008 Koch, 1999
Z. marina	12	4	0.18	0.07	- Burial treatments	- Increased mortality - Decreased productivity (Biomass/surface area) - Decreased leaf length and leaf surface area	Cabaço *et al.*, 2008b
Z. marina	10-20	5	0.18	0.07	- Burial treatments	- Higher shoot mortality, and delayed growth and flowering, lower carbohydrate storage	(Munkes *et al.*, 2015)
Z. noltii	8	2	0.12	0.03	- Burial treatments	- Decreased shoot density - Decreased leaf and rhizome C content in high burial levels (4 cm, 8 cm and 16 cm) - Decreased leaf N content and simultaneous increase in rhizomes - Increased leaf sugar content in intermediate burial level (4 cm)	(Cabaço & Santos, 2007)
Z. noltii	-	2	-	-	-Burial and eutrophication	- Decrease in above ground biomass	(Vieira *et al.*, 2020)
Z. muelleri	0.75	-	0.06	-	- Shading and burial	- Decrease in shoot density - Decrease in rhizome growth	(Benham *et al.*, 2019)
Z. muelleri	1	-	-	-	- Burial and Eutrophication	- Decrease in leaf biomass, root biomass and shoot density	(Siciliano *et al.*, 2019)

The above studies (Table 2.4) suggest that effects of burial on seagrasses are related to plant size. Smaller plants (indexed by leaf length) seem to be more sensitive to burial than larger plants (Figure 2.7). This is supported by a study of a mixed seagrass meadow where Duarte et al (1997) described a pattern of species loss after burial-induced disturbance, in which mortality increased with decreasing seagrass size. *Zostera muelleri* species appear particularly sensitive to burial this genus tends to plot well below the trend line in figure 2.7 and far below in the case of *Z. muelleri*.

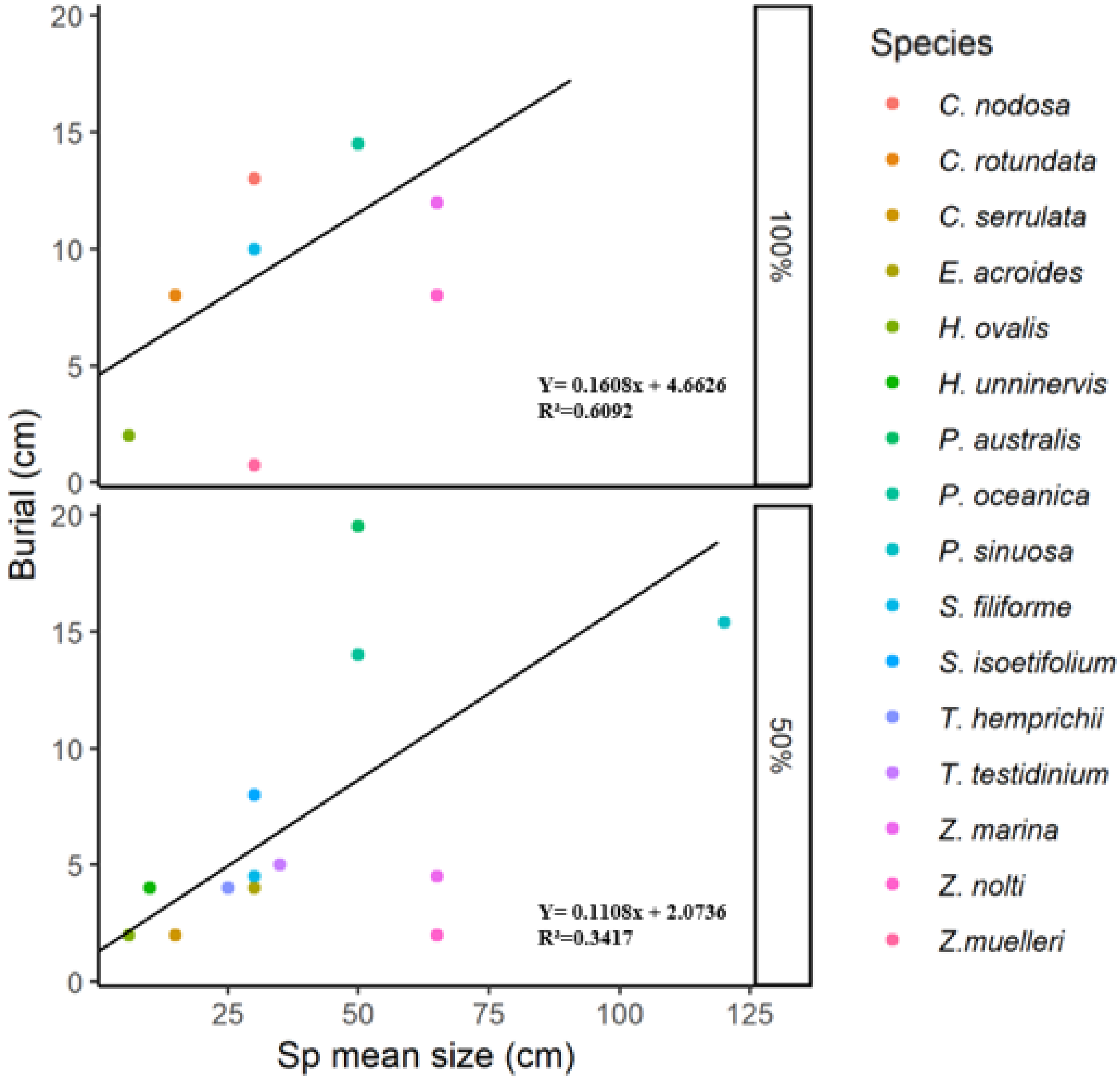


Figure 2.7: Relationship between seagrass leaf length and burial threshold to cause 100% (above) and 50% (below) mortality (Updated from (Cabaço *et al.*, 2008b) with newer literature). Lines and equations are least-squares linear regression, and neither is significant at ($p<0.05$).

The main seagrass responses to burial include a decrease in shoot density and an increase in internode length, leaf turnover rate, vertical growth rate, and leaf sheath length (Cabaço *et al.*, 2008a). Decreases in carbohydrate reserves and increases in shoot mortality indicate response to light limitation under sediment burial extending for long periods (Alcoverro *et al.*, 1999). However, light attenuation is not the only potential mechanism; sediment may also smother plants due to oxygen demand of associated organic matter and by inhibiting oxygen diffusion (Brodersen *et al.*, 2017; Benham *et al.*, 2019) and these two main mechanisms could be operating simultaneously synergistically.

The best predictors for assessing the capacity of seagrasses to tolerate burial are considered to be leaf size and rhizome diameter (Cabaço *et al.*, 2008a). However, in addition to the size of seagrass modules, (such as leaves and rhizomes but also roots, flowers and fruits), resource allocation within modules, and the life-strategy of each species (including the capacity for clonal integration or translocation of resource amongst structures) are considered to result in a differential capacity of seagrass species to survive burial (Tuya *et al.*, 2013). The capacity of seagrasses to mobilize carbon reserves during short-term burial events, is also thought to ameliorate decline rates (Duarte & Chiscano, 1999; Cabaço *et al.*, 2008b; Munkes *et al.*, 2015; Sørensen *et al.*, 2018).

The characteristics, notably grain size distribution, of sediment responsible for seagrass burial may also influence the seagrass response. For example, the inclusion of labile organic matter due to deposition of dredging materials or the erosion of the bottom exposing sediment layers depleted in oxygen (which are subsequently deposited on seagrass beds) may impose a high oxygen demand promoting anoxia as a result of bacterial respiration (Hemminga & Duarte, 2000). The grain size range of deposited sediment may also have implications for seagrass survival because oxygen is expected to penetrate faster through large pore spaces within ‘clean’ sands than where pore spaces are infilled by clay and silts (Borum *et al.*, 2005a; Benham *et al.*, 2019).

2.6.1 Nepheloid layers

An interesting phenomenon that merits further evaluation for its potential effect on light availability for seagrasses is the development of nepheloid layers that can dramatically affect light attenuation close to the sea bed (Pedersen *et al.*, 2012). These layers consist of temporarily settled partially flocculated sediment, that is easily resuspended (and may undergo many cycles of resuspension/settling with cycles of water turbulence). Pedersen et al. (2012) reported that nepheloid layers near seagrass meadows in Belize had an attenuation coefficient which exceeds the attenuation coefficient of the water column by 1.6 to >30 (average 4) times (Pedersen *et al.*, 2012). Consequently, the light received by seagrasses could be overestimated by a factor of 4 or more by extrapolating from near-surface measurements without taking into account the near-bed light attenuation (Figure 2.8). These nepheloid layers might account for some of the variance in compensation irradiance reported for seagrasses which are located in substrates with high fine partially flocculated sediments. Moreover, this type of sediment may also contribute an oxygen demand and inhibit oxygen exchange – so contributing to substrate anoxia.

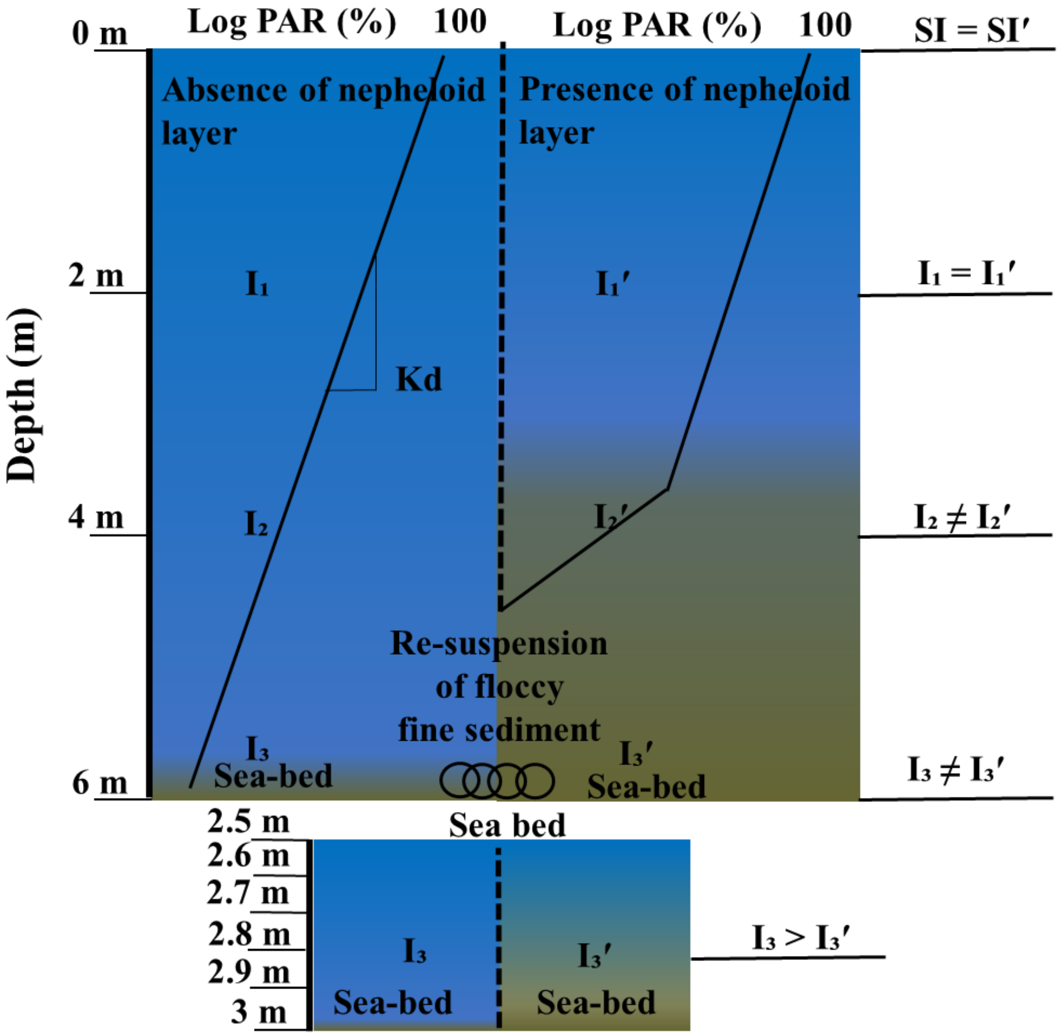


Figure 2.8: Conceptual diagram showing the effect of nepheloid layers on light reaching the seabed. Irradiance (I) at surface (SI), 1 m, 2 m, 3 m depth (I_1, I_2 and I_3) is shown. Underwater light attenuation vs depth profile under absence (left) and presence (right) of nepheloid layer is compared. Overestimation of irradiance, if the nepheloid layer is not considered is conceptually explained in the bottom of the figure. Ring shapes represent re-suspension. Right hand side figure shows PAR profiles under the absence and presence of the nepheloid layer.

2.7 Effects of intruded fine sediment on substrate pore water chemistry

Fine sediment loading of coastal environments, particularly estuaries, can potentially lead to unfavourable substrate pore water chemistry. High loads of nutrients, heavy metals, and herbicides are a concern in any anthropogenically-impacted estuary (Burkholder *et al.*, 2007). In addition, coastal substrates often naturally become anaerobic a few millimetres or centimetres below the substrate bed surface as a result of slow oxygen diffusion rates and a high microbial oxygen demand associated with mineralization of organic matter (Terrados *et al.*, 1999). Deposits of silts and clays onto coastal sediments are likely to exacerbate substrate anoxia by reducing pore space. Furthermore, increasing oxygen demand of organic matter associated with fine sediment, potentially leads to high concentrations of phytotoxic reduced compounds like hydrogen sulphide, and increases porewater concentration of heavy metals (Borum *et al.*, 2005a; Koch *et al.*, 2007a; Brodersen *et al.*, 2015; Brodersen *et al.*, 2017).

2.7.1 Fine sediment effects on substrate oxygen status and toxicity

Redox potential (Eh), also referred to as oxidation reduction potential (ORP) by some authors is the measurement of the tendency of a chemical species to acquire electrons and thereby be reduced. It is measured in millivolts (mV) relative to a reference platinum electrode. Each organic chemical species has its own intrinsic reduction potential; the more positive the potential the greater the species affinity for electrons and tendency to be reduced. ORP is a common measurement for water and soil quality. It has been used most often in soil studies to understand chemical reactions and underlying biological drivers. Redox potential profiles are used to indicate the degree of anoxia and processes that may be occurring in different horizons of the soil. Measurements have also been performed in seagrass meadow substrates.

Increased substrate anoxia has the potential to detrimentally affect seagrass performance. The effect of sediment anoxia on seagrass growth and survival was tested through a field experiment in which sucrose was added to increase the oxygen demand within the substrate porewater (Terrados *et al.*, 1999). This study was performed on different species and in different countries, including a multi-species seagrass meadow in the Philippines (Silaqui Island), a *Cymodocea nodosa* meadow in the Mediterranean Sea, Spain (Blanes), and a *Z. marina* meadow in Denmark (Roskiljde Fjord). After the addition of sucrose in all the studied meadows, redox potential decreased and hydrogen sulphide concentration in porewater increased. Increased sediment anoxia was shown to detrimentally affect all seagrass species but there were differences in response between different species and environments (Terrados *et al.*, 1999). In tropical multi-species meadows sucrose addition was reported to have a detrimental effect on *T. hemprichii* (-167.9 mV) while *H. uninervis* did not show a clear response. The redox potential of Mediterranean sediments declined significantly after sucrose addition but *C. nodosa* (-87.9 mV) survival and growth was not affected and temperate *Z. marina* showed no decline in leaf growth rates until two months of exposure to the treatment.

Other studies have demonstrated how healthy seagrass meadows can regulate sediment redox potential and conversely how susceptible they can be to anoxia when plants become stressed by other factors. The effect of photosynthetic activity by *T. testudinum* and *S. filiforme* on substrate redox potential was examined through a manipulative shading experiment in Puerto Morelos, Mexico. 73% reduction of ambient irradiance provided by density nets for five days reduced redox potential by about 45 mV by the fifth day. This result indicates that seagrasses tend to divert some of their oxygen production, which is mainly used for keeping the root meristem alive, into oxidising soils and sustaining an aerobic microbiome around their roots, which, in turn, modifies sediment redox potential. When shading (light stress) occurs seagrasses can no longer maintain an aerobic rhizosphere and consequently, substrate chemistry deteriorates (Enríquez *et al.*, 2001). Another study of multi-species(*E. acroides, Z. japonica, T. hemprichii* and *H. ovalis*) meadows in the Philippines and Vietnam has confirmed the positive effect of seagrass roots and rhizomes on substrate redox potential and thus, rhizosphere microbial processes which are key to maintenance of a healthy substrate (Marbà *et al.*, 2010).

The best-described and studied phytotoxin in seagrass literature is hydrogen sulphide (H_2S), a gas that dissolved in sediment porewater. Sulphide (sulphur in the -2 oxidation state (-II) is produced by reduction of abundant sulphate in seawater and partitions between H_2S (the toxic form), HS^- and S^{2-} forms depending on pH. Several studies both in the field and in mesocosms have demonstrated the toxic effect of this gas on seagrass and their defence responses. (Carlson Jr *et al.*, 1994; Terrados *et al.*, 1999; Holmer *et al.*, 2001; Eldridge & Morse, 2008; Brodersen *et al.*, 2015). Seagrasses are adapted to this environmental hydrogen sulphide through low resistance gas channels (aerenchyma) and carbon concentration mechanisms (CCM) involving carbonic anhydrase (CA) enzyme (Brodersen *et al.*, 2018).

A comparative study of siltation in the rootzone (1-4 cm) of *Z. noltii* inhabited versus unvegetated substrates showed that sulphate reduction to sulphide was twice as high in the vegetated sediments. The stimulation of sulphate reduction in the rootzone of *Z. noltii* was probably due to the degradation of the organic matter originating from the plants (Isaksen & Finster, 1996). This study was the first to highlight the effect that seagrasses have on rhizosphere chemistry. Muddification of bed substrates has been shown to be generally detrimental to seagrasses. Research carried out on *Cymodocea rotundata*, in South East Asia, showed that under high light availability, major changes in sediment conditions associated with, generally deleterious, muddification did not negatively affect the plant and, indeed, *increased* growth, which was attributed to increased nutrient availability. However, in instances where meadows were not dense or well-developed, even with high light availability, porewater concentrations of 1mM sulphide in response to muddification reduced the size of shoots, rhizomes and roots and elongation of horizontal rhizomes (Halun *et al.*, 2002).

Mesocosm experiments have been conducted with *Z. marina* to determine the effect of plant oxygen status upon sulphide intrusion into seagrass tissues such as roots and to determine how fast internal sulphide pools diminish after internal oxygen supplies are restored (Pedersen *et al.*, 2004). Lack of hydrogen sulphide intrusion at high internal pO_2 suggests that oxygen release from the roots ensures complete re-oxidation of sulphide in the rhizosphere. Under oxygen stress, however, the experiments clearly demonstrated intrusion of sulphide into *Z. marina* rhizomes and meristematic tissues. Thus, hydrogen sulphide intrusion only occurs when seagrass internal oxygen concentrations are low. The rate of intrusion depended on the internal pO_2 supply to roots and rhizomes. Hydrogen sulphide depletion occurred when oxygen partial pressures were re-established through leaf photosynthesis. Maximum internal sulphide concentrations reached 325 µM greatly exceeding the 1–10 µM known to inhibit mitochondrial activity in eukaryotic cells (Pedersen *et al.*, 2004). Hydrogen sulphide intrusion and low levels of oxygen may coexist in seagrass tissues because of fast internal transport of sulphide and relatively slow rates of sulphide re-oxidation. Sulphide re-oxidation within tissues is not biologically (bacterially or enzymatically) facilitated, rather it occurs via a chemical oxidation process (Pedersen *et al.*, 2004). An internal oxygen deficit caused by low water column concentrations or poor plant performance (in terms of oxygen pumping) governed by other factors, has been shown to facilitate sulphide intrusion and is implicated in sudden die-off events of *T. testudinium* (Borum *et al.*, 2005a).

Iron II (Fe^{2+}) reacts with sulphide to form very stable precipitates. Iron rich sediments have low hydrogen sulphide and low toxicity potential for seagrass. A manipulative experiment with $FeCl_2$ and OM addition was used to study relationships with sulphide, and P availability in an iron-deficient seagrass bed (Ruiz-Halpern *et al.*, 2008). $FeCl_2$ addition decreased sulphide whereas the addition of OM promoted anaerobic conditions and more sulphide. This highlights the control that iron-rich sediments can exert on sulphide effects on seagrass bed (Ruiz-Halpern *et al.*, 2008).

Research on *Z. muelleri* has shown its capacity to modify the chemical conditions of its immediate rhizosphere, through high radial oxygen (O_2) release from the base of the leaf sheath surrounding the meristematic regions of the rhizome together with alterations in pH levels (Figure 2.9). Both oxygen release and increased pH act as chemical defence mechanism against reduced phytotoxins such as hydrogen sulphide (Brodersen *et al.*, 2015). Oxygen oxidises free sulphide, while at higher pH levels less total sulphide is in the toxic H_2S form. *Z. muelleri* has been shown to have oxygen release rates of up to 500 nmol O_2 cm^{-2} h^{-1}, which can maintain an approximately 300-µm-wide plant-mediated oxic-microzone. Recent experimental results suggest that fine sediment (mud) smothering inhibits internal aeration, which promotes phytotoxic hydrogen sulphide intrusion and increases light requirements. Deposited silt and clay particles impede gas and nutrient exchange with the water column and also reduce the passive oxygen influx across the silt/clay layer leading to anoxia (Brodersen *et al.*, 2017).

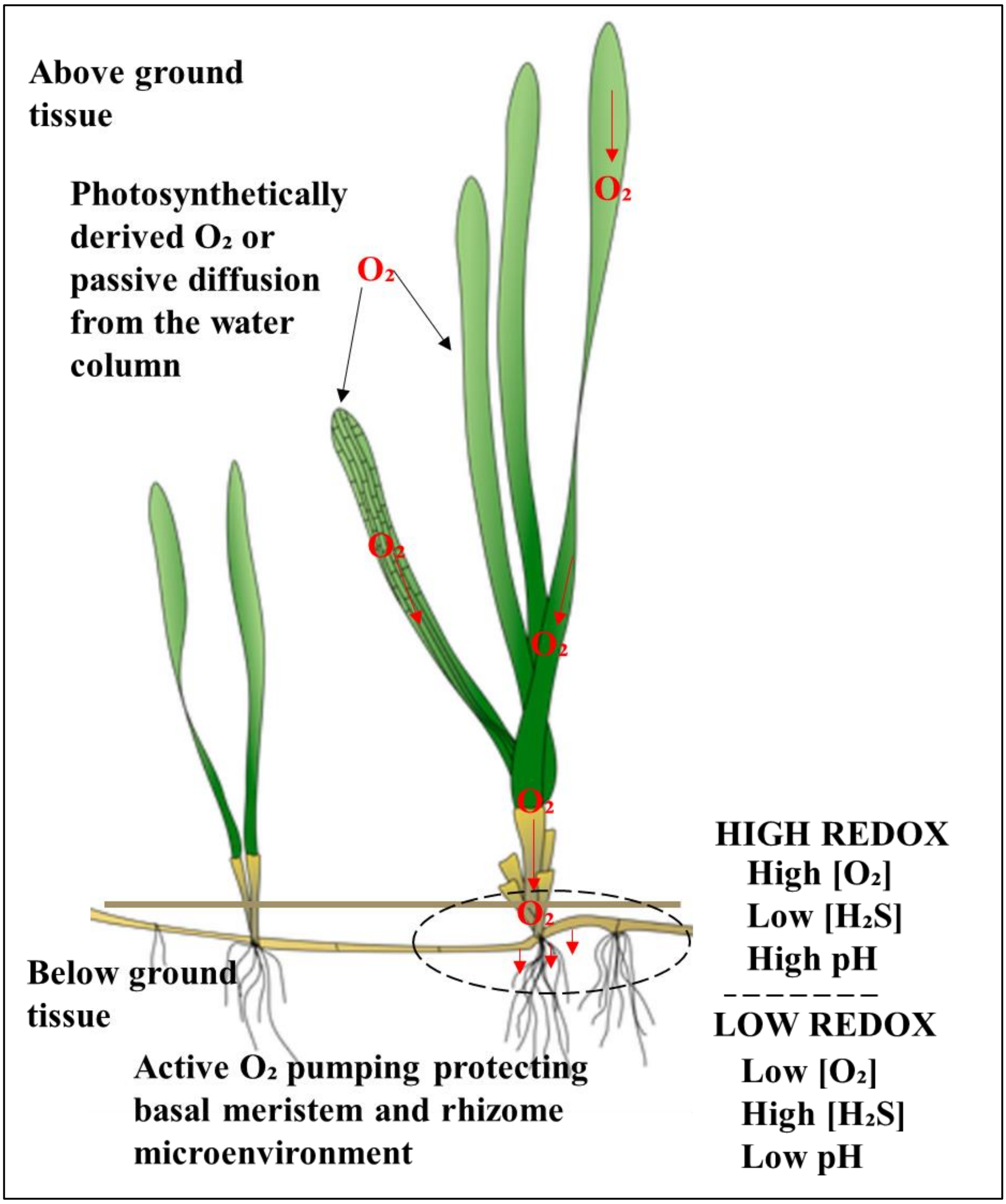


Figure 2.9: Conceptual diagram of *Zostera muelleri* showing the photosynthetically derived oxygen flux to belowground structures where oxygen is actively pumped by photosynthesising plants to form protective microenvironments in the sediments. The rhizosphere in the vicinity of roots and rhizomes shows high oxygen and pH and low hydrogen sulphide. Decreasing pH, and oxygen and an increase of H_2S moving away from rhizome and roots influence (deeper in the soil) is indicated.

Finally, altered substrate geochemistry may create conditions favouring species that compete with seagrass, leading to the decline of seagrass. In the Mediterranean Sea a detrimental influence of the macroalgae *Caulerpa taxifolia* and *C. cylindracea* species on endemic seagrass *P. oceanica* beds has been reported. The mechanism appears to be the increasing sediment organic matter pools (implying oxygen demand) and hence increased hydrogen sulphide concentrations when this species colonises the seagrass meadows. Shading of seagrass by the macroalgae might also contribute to decline of the former. Once established *Caulerpa spp* may be difficult to eradicate because the invader appears to out-compete the seagrasses in an already altered sedimentary environment (Holmer *et al.*, 2009).

2.7.2 Sediment and associated contaminants: nutrients, herbicides & heavy metals

The nutrient content of settled sediments can affect the substrate and rhizosphere porewater chemistry with potential consequences for seagrass health. Fine sediment effects on substrate porewater chemistry as well as on water column nutrients are both important for seagrass nutrition, particularly at low to moderate concentrations while at high concentrations there may be direct or indirect detrimental effects. For example, van Katwijk *et al*. (1997) have shown that high sediment porewater ammonium (NH_4^+) concentrations can be directly toxic to seagrasses. Similarly, nutrient flux from mineralisation of organic matter might fuel phytoplankton growth in the water column overlying seagrass beds creating competition for light and also a further oxygen demand when algae settle (Duarte, 1995; Burkholder *et al.*, 2007; Van Katwijk *et al.*, 2011).

The literature provides some evidence that different types of sediment settled within seagrass beds can contribute to different availabilities of dissolved nutrients. For example, Erftemeijer & Middelburg (1993) related grain size of deposited sediment to porewater nutrient concentrations. They compared porewater nutrient concentrations of two types of sediments (muddy versus coarse-grained) in a carbonate sedimentary environment in seagrass beds of South Sulawesi, Indonesia. Porewater ammonium ion (Chapter 2, Appendix) concentrations increased with sampling depth (0-30 cm) in both coarse and muddy sediments and the median values were 60.1 µM and 106.8 µM respectively. Porewater phosphate concentrations were significantly higher in the shallow sediment (0-6 cm) in comparison with the deeper fraction for both sediment types but decreased in the 6 to 30 cm depth range with median concentration of 6.1 µM (coarser) and 7.8 µM (muddier). Both Total P and N (nitrate, nitrite and ammonium) were higher in the muddier sediments due to an increased input of fines with associated nutrients from the catchment.

The concepts of ‘muddification’ and ‘sandification’ have been used to describe relationships between substrate characteristics and seagrass performance. Sandification refers to relative increase in sand owing to decrease in fine sediments and organic content supply, whilst muddification refers to the opposite trend of an *increase* in fine sediments and organic content, thus reducing the sand fraction (Van Katwijk *et al.*, 2010). Muddification may increase the nutrient load as smaller particles have a larger surface area to volume ratio so potentially can sorb more nutrient onto their surfaces. Clay particles, particularly layer clays, have a very large surface area per unit mass, and can adsorb many chemical species. Muddification may promote seagrass development due to nitrogen release (as ammoniacal-N) from mineralisation of associated organic matter. Increased growth of seagrass would be expected where nitrogen is limiting for growth, but reduced growth where muddification results in toxicity or enhances the growth of competing (shading) algae and phytoplankton. On the other hand, sandification may promote recovery of seagrass beds where substrates have become too muddy for seagrass habitat (Van Katwijk *et al.*, 2010).

Besides nutrients, the effect of sediment-bound pesticides and herbicides on seagrass meadows needs to be better studied and understood. Very few reports exist of sediment-bound herbicide effects on seagrasses (Dos Santos, 2011). In New Zealand, the cumulative presence of the herbicides cyanazine, trifluralin and chlorotoluron with a maximum concentration of 132 µg/kg was shown to have a detrimental effect on seagrass. However, no relationship was found with other condition metrics and toxicity thresholds are still lacking (Dos Santos, 2011). Atrazine, diuron, lindane, dieldrin, DDT and DDE have been detected in sediments from the Great Barrier Reef World Heritage Area in Queensland, Australia, with diuron the herbicide of most concern considering its known toxicity to seagrasses (Haynes *et al.*, 2000a; Dos Santos, 2011). The effect of diuron herbicide [DCMU; 3-(3,4-dichlorophenyl)-1,1-dimethylurea] on *Cymodocea serrulata, Halophila ovalis* and *Z. muelleri* in water was tested in aquaria over a 10-day period. Exposure to 10 and 100 µg/L diuron provoked decline in effective quantum yield after the first 24 h of exposure (0.1-1 µg/L) for *H. ovalis and Z. muelleri.* Five days exposure to 10-100 µg/L diuron were required to cause a detectable response in *C. serrulata*. The results of this study indicate that exposure to diuron is a specie-dependent, potential risk to seagrass. (Haynes *et al.,* 2000b).

Phytotoxic effect thresholds for sediment-bound chemicals, are poorly known for seagrasses. Toxicity values for single chemicals such as tributyltin (TBT) need to be further investigated (Jensen *et al.*, 2004). Our dataset shows just one report of a study in which a whole sediment toxicity test was conducted with seagrasses (Hoven *et al.*, 1999). More studies with a spectrum of toxicants are needed to assess potential synergistic effects of multiple chemicals as well as studies with isolated toxicants.

Heavy metals enter seagrass meadows mainly adsorbed to fine sediment surfaces or incorporated in organic matter via weathering, erosion, urban runoff, effluents and dredging (Haynes *et al.*, 2000a). Dredging in particular, releases heavy metals from anoxic deeper layers of the bed sediments, re-mobilizing them and making them bioavailable (Batley, 1987). Bioavailability determination for metals is challenging due to their transient and highly variable residence time in waters, sediments, seagrasses and epiphytes (Haynes *et al.*, 2000a). Heavy metals uptake from interstitial water into seagrass roots and rhizomes, toxicology and acclimation are poorly understood (Larkum *et al.*, 2006).

Heavy metals research has been focused on bioaccumulation and few studies have described the physiological impact on seagrasses (Macinnis-Ng & Ralph, 2004). Three populations of *Zostera muelleri* exposed to copper in Sydney region showed worse photosynthetic efficiency (Macinnis-Ng & Ralph, 2004). Reviewed literature underlines the importance of further experimentation on the possible synergistic effect of metals contamination with other stressors such as turbidity, eutrophication and herbicides.

2.8 Overall status of knowledge, research gaps and implications for management and restoration.

2.8.1 Effects of suspended sediment on light climate

For effective management and protection of seagrass habitats, the derivation and publication of light threshold values and models for particular species, including *Zostera muelleri*, has been an important advance in recent years. However, a limitation and significant knowledge gap is understanding how the multiple stressors associated with sediment may affect light thresholds (Table 2.6). Suggested further research to fill current gaps and support management applications include:

- Combination of light threshold with effective sub-lethal biological indicators of light stress such as P-I curves $_{r}$ETR (electron transport rates), F_v/F_m and other physiological traits. Day response recommended rather than weeks (McMahon *et al.*, 2013).
- Development of molecular indicators of sub-lethal seagrass light stress as a promising and under-studied approach (Macreadie *et al.*, 2014a).

- Temperature is known to influence temperate seagrass meadow dynamics and metabolism owing to a strong seasonal influence on rates of chemical reactions including photosynthesis. Seasonal fluctuations need further research in terms of light requirements which are also highly seasonal in the temperate zone (Hoeffle *et al.*, 2011; Rasheed & Unsworth, 2011; Smale *et al.*, 2019). Temperature effect on light requirements need to be studied in more species and in greater detail to understand how temperature modifies seagrass light thresholds (Chartrand *et al.*, 2016), usually such that compensation irradiance (E_c) increases with temperature (Lee *et al.*, 2007; Collier *et al.*, 2012).

The cumulative nature of long term impacts such as poor water quality and muddiness needs to be taken into account to manage chronically stressed seagrass meadows (Chartrand *et al.*, 2016) as well as day to day variation which may reduce irradiance below plant E_c for a few days causing decline (Matheson *et al.*, 2020 submitted). Time distribution of light climate (seasonality and site-specificity), as well as interactive or simultaneous perturbations, may need to be considered when it comes to threshold determination and management (Kemp *et al.*, 2004).

2.8.2 Smothering and burial effects

Species for which a sediment burial threshold has been experimentally determined comprise only a third of seagrass flora. Moreover, only acute sediment pulses have been investigated for this third of the seagrass flora and only two studies of chronic pulses exist (Brodersen *et al.*, 2017; Benham *et al.*, 2019). Despite the fact that, there are allometric relationships reported in literature (Cabaço *et al.*, 2008b), further experimentation is desirable in order to derive smothering and burial thresholds for other seagrass species and to support decision-making in seagrass management. Much more work on chronic fine sedimentation is required in order to understand mechanisms of stress.

Information is needed on effects of seagrass burial by settled mud layers. Partial shading seems likely to be involved (Pedersen et al., 2012), but, additionally, recently-settled mud may impose an oxygen demand and consequent stress on plants. Not only burial but nepheloid layers compromising irradiance may interact with settled fine particles both on the leaves and on the seafloor surface compromising oxygen exchange (Table 2.6).

2.8.3 Intruded/Deposited fine sediment effects on bed substrate pore water chemistry

Research on seagrass meadow biogeochemistry needs to investigate phytotoxins associated with sediments using traditional approaches such as monitoring as well as bioassays to better understand the stress imposed by sediment-associated chemicals. However, identifying and quantifying the contributions of the various stressors related to seagrass declines is challenging. There are particular information gaps around *interactions* of various chemical stressors, such as sulphide and nitrate ions, with the light environment and plant photosynthesis. In the case of common nearshore toxic contaminants, there is a need for baseline fate and effect information (Daughton, 2005). Magnitude, temporal variability, biological significance of the chemicals (bioavailability), tissue incorporation and geographical scale are all needed to interpret soft sediment seagrass ecosystems response (Hemminga & Duarte, 2000; Birch *et al.*, 2017).

Table 2.5: Summary of sediment effects on seagrasses: research status, suggested gaps and potential approaches.

Sediment effect	Mechanisms	What do we know?	Knowledge gaps	Potential approaches
Light climate	- Underwater **light reduction** through **suspended** sediment.	- Light *thresholds* for several species are available. - Threshold are determined as MDL reaching irradiance, %SI reaching the seafloor or manipulating received light doses with shade cloths experiments both in field and mesocosms designs… -SSL modelling.	- Better understanding of multiple stressors **interactions** and cumulative effects (temperature, eutrophication, fines). - Cumulative nature of long term impacts understanding of temporal fluctuation. - *Short term disturbances* such as plume monitoring and its effects on seagrass meadows	- Sub-lethal bio indicators. Physiological traits Molecular indicators - Plumes (short term disturbances) monitoring new methodologies (PUFTS) (Gall & Davies‐Colley, 2020) - unified procedures to best support managers and inform decision making.
Smothering & burial	**- Oxygen deprivation** through settled sediment both in the leaves and in the seafloor (belowground tissues and sheaths.	- 1/3 of seagrass species' burial *thresholds* determined. - Allometric relationships reported. - Species size critically important against burial. - Nepheloid layers occur and plant reaching irradiance overestimation potentially has occurred.	- Mainly acute burial (eg., > 2-3 mm) deposited fines layers effects have been quantified and manipulatively simulated in mesocosms and field experiments. 2/3 of seagrass flora left. - No quantitative data available for many species and also the potential *interactions* with other factors such as again (temperature and eutrophication) - Chronic effects of fine sediment re-suspension and loadings (e.g, <2mm) effects is poorly studied and simulated due to its difficulties to work at this small scale. -Burial and smothering interactions with other factors such as light, eutrophication and substrate physico-chemistry are lacking or poorly studied.	- Mesocosms set ups were small scale manipulation is do-able and fines can be monitored. -Flume experiments on erodibility of substrate to further research nepheloid layers using portable erodibility measuring systems (EROMES). - Burial for 2/3 of flora + interactions with substrate quality and nutrient regimes, phytotoxins, etc.
Rhizosphere alteration	- **Toxicity** and **anoxia** though intruded fine sediment.	- Anoxia and hydrogen sulphide phytotoxicity fairly well documented.	- Information for sediment bound heavy metals such as (copper, lead, zinc…) and herbicides (tributylin, Diuron…) is lacking or poorly studied for the majority of the seagrass flora. -*Interactions* with other stressors will improve understanding of damage mechanisms.	-Bio-assays with commonly terrestrial herbicides which potentially reach estuaries and embayments. - Bio-assays with heavy metals as well as other redox-associated toxins and *interactive* effects with light dose,

2.9 Conclusions

The three ways in which sediment can affect seagrass growing conditions, are summarized in Figure 2.10 which outlines the structure of this review. Specifically, fine sediments associated with appreciable organic matter are likely to be particularly problematic, and smaller seagrass species are possibly more vulnerable to fine sedimentation. These three categories of effect may operate simultaneously and interact.

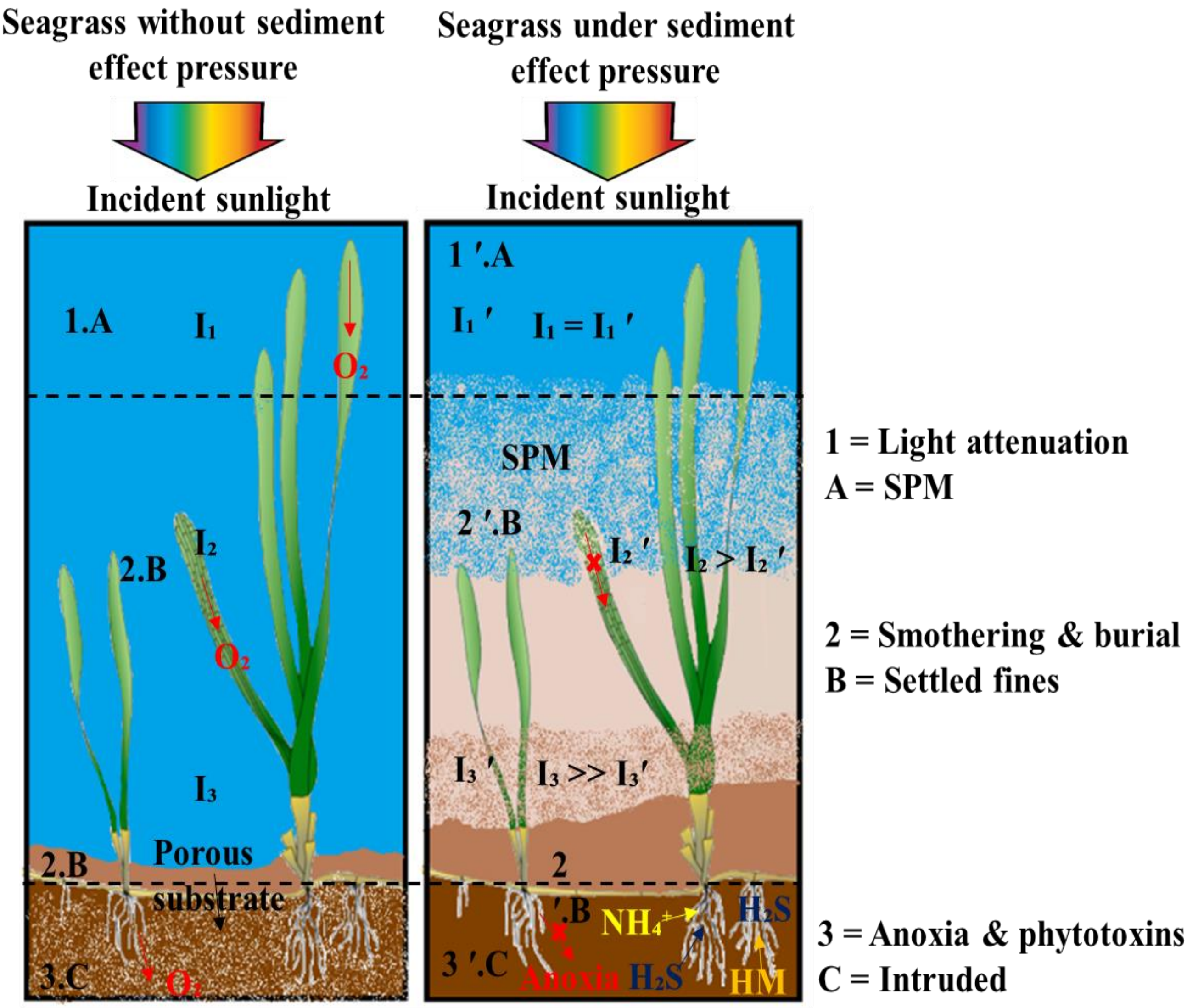


Figure 2.10: Conceptual diagram showing the three main effects of sediment on seagrasses. Numbers indicate the damage mechanisms and letters indicate the locality where sediment acts detrimentally against seagrass **1)** Effect on **light climate** by suspended sediment**, 2) Burial and smothering** by deposited settled sediment and **3) alterations to substrate chemistry** by intruded fine sediment**. A) Water column, B)** Settled sediment on leaf surfaces or seafloor**, C)** Sediment intruded into the substrate pores**.** These three effects of mud may **interact** such that seagrass meadows simultaneously stressed in three main localities by several stressors associated with sediment.

*Irradiance (I), Suspended Particulate matter (SPM), Heavy Metals (HM).

Research gaps that we recommend for study as an outcome of this review are as follows: **multi-approach studies** including mesocosms, field work and reviews addressing the three key location of mud effects (water column, settled and intruded). Further effort is desirable where gaps have been identified in the literature (Table 2.6). Studying the three hypothesized interactive effects is key to improving knowledge and furthering our understanding of seagrass declines. We suggest that more effort towards **elucidating mechanisms** of mud effect on seagrasses in different locations and at different ‘intensities’ is needed.

To sum up, we suggest that sediment effects may provoke interactive and/or cascade effects related to light reduction, oxygen demand, changes in bed sediment chemistry and smothering causing multiple stresses eventually leading to decline (Figure 2.10). Understanding the multi-faceted effects of sediments and their interactions is important for seagrass management. Managers face the challenge of dealing with sedimentation problems to protect and conserve seagrasses, and the suggested research topics should inform decision-making.

Chapter 3

Multiple effects of sediment on seagrass meadows: A case study of *Zostera muelleri* in Pāuatahanui Inlet, New Zealand.

Note: this chapter has been published as:

Zabarte-Maeztu, I., Matheson, F. E., Manley-Harris, M., Davies-Colley, R. J., Oliver, M., & Hawes, I. (2020). Effects of fine sediment on seagrass meadows: A Case Study of *Zostera muelleri* in Pāuatahanui Inlet, New Zealand. *Journal of Marine Science and Engineering*, *8*(9), 645.

3.1 Abstract

Estuaries are amongst the most productive environments in the world, with seagrass meadows providing numerous ecological services. Seagrass meadows are, however vulnerable to fine sediment (mud) pollution, with impacts usually, attributed to reduction in submerged light. Here we studied two non-exclusive hypotheses, that mud particles (<63 µm) impacts seagrasses through both (1) the light climate and (2) changes in substrate physico-chemistry. We tested these hypothesis in Pāuatahanui Inlet, New Zealand, by comparing seagrass presence, abundance and health, together with light climate and substrate physico-chemistry at contrasting habitats where: 1) seagrass used to thrive but no longer grows (historical seagrass, HS), 2) seagrass still persists (existing seagrass, ES) and 3) seagrass has been present recently, but not currently (potential seagrass, PS). HS substrate had significantly higher mud (35% average), bulk density (1.5g cm^{-3}), porewater ammonium concentration (65 µM), and more reduced redox profile (negative redox at only 2 cm soil depth) as well as a lower light availability when submerged compared to other habitats, while daily light exposure seems sufficient in the three habitats. This suggests that failure of seagrass to recolonize HS habitat may reflect substrate muddiness and consequent unfavorable rhizosphere conditions. Our results suggest the possibility of multi-stressor effects of fine sediment on seagrasses, with both substrate suitability and submerged light climate for seagrass being detrimentally affected.

3.2 Introduction

Seagrass meadows are one of the most important, and threatened, ecosystems on the planet (Waycott *et al.*, 2009). They have immense ecological and socio-economic value (Orth *et al.*, 2006; Burkholder *et al.*, 2007; Waycott *et al.*, 2009; Cunha *et al.*, 2012), yet, as a result of human activities these ecosystems are increasingly threatened (Short & Coles, 2001).

A major contributor to global anthropogenic stress on seagrass is the reduction of the available light, the primary driver of seagrass growth (Duarte, 1991; Dennison *et al.*, 1993; Duarte *et al.*, 2004a; Ralph & Gademann, 2005; Adams *et al.*, 2016; Chartrand *et al.*, 2016; Collier *et al.*, 2016). The usual cause of underwater light reduction is increased suspended sediment loads in coastal waters. Natural events, such as severe rain and windstorms can provoke sediment movement to and within coastal systems leading to acute, large-scale alteration of seagrass habitat (Kirkman & Kuo, 1990; Fourqurean & Rutten, 2004; Suykerbuyk *et al.*, 2016). However, acute and chronic human-induced impacts, resulting from land clearance and other activities that increase fine sediment concentrations, can result in complete and perhaps irreversible, extinction of seagrass meadows (Short & Wyllie-Echeverria, 1996; Erftemeijer & Lewis III, 2006; Cabaço *et al.*, 2008b; Benham *et al.*, 2019).

While impacts of suspended sediments on seagrasses via reduced underwater light exposure are widely reported, sediments may also affect seagrasses after they settle. Fine sediment initially settled as nepheloid layers, continues to shade seagrasses (Pedersen *et al.*, 2012), and may also restrict solute flux, and result in physiological stress for example by causing hypoxia (Brodersen *et al.*, 2017), or drive indirect impacts via changes to substrate biogeochemistry. Seagrass substrate typically becomes anaerobic a few millimetres or centimetres below the bed surface as a result of slow oxygen diffusion rates and a high microbial oxygen demand associated with mineralization of organic matter within the rhizosphere (Terrados *et al.*, 1999). Deposits of silts and clays may exacerbate substrate anoxia by filling pore spaces, thus reducing diffusivity, and by the associated additions of oxygen demanding organic matter. Anoxia may lead to high porewater concentrations of phytotoxic compounds such as sulphides and metals (Borum *et al.*, 2005a; Koch *et al.*, 2007a; Brodersen *et al.*, 2015).

In New Zealand, fine sediments are considered to be *the* most pervasive contaminant affecting estuaries and sheltered coastal embayments (Green & Short, 2003; Thrush *et al.*, 2004; Matheson & Schwarz, 2007; Morrison *et al.*, 2009). Sediment is thought to have contributed substantially to documented losses of seagrass meadows in a number of New Zealand estuaries (Inglis, 2003; Matheson *et al.*, 2011). In Pāuatahanui Inlet, ca. 39 hectares of seagrass, that were present in the inner estuary in ca. 1980 (Healy, 1980) and earlier, have been lost subsequently. This loss has been tentatively, but plausibly, attributed to catchment development and increased sedimentation and eutrophication of the estuary (Matheson & Wadhwa, 2012). Nevertheless, in the better-flushed, outer part of this estuary seagrass beds still persist and are a prominent feature of the intertidal zone. For this study, we used the gradient of seagrass-sediment conditions evident within the estuary to examine the potential causes of seagrass decline and failure to recover by comparing light climate and substrate physical and chemical characteristics at habitats with and without seagrass. We hypothesized that high fine sediment affects seagrass through multiple-stresses caused by deposited sediment in addition to light attenuation by suspended sediment alone. To test our hypothesis, we quantified and compared habitat (light, substrate grain size and biogeochemistry) at habitats with and without seagrass in Pāuatahanui Inlet. Specifically we targeted 1) sites in the inner estuary where seagrass used to thrive but no longer grows (historical seagrass, HS), 2) sites where seagrass still persists in the outer estuary (existing seagrass, ES) and 3) sites in the outer estuary, adjacent to existing seagrass beds, where seagrass has been present in recent years but where seagrass is not currently growing (potential seagrass, PS). Planned comparisons among these sites potentially would allow disentanglement of the effect of anthropogenic sediments on biogeochemistry in the absence of seagrass, the impacts of seagrass on biogeochemistry in the absence of anthropogenic sedimentation and the effects of habitat on light regime.

3.3 Materials and Methods

3.3.1 Study location

Pāuatahanui Inlet is a natural inlet and wildlife reserve on New Zealand's North Island southwestern coast (Figure 3.1). It is the eastern arm of Porirua Harbour which has a total catchment area of 199 km², comprising a land area of 185 km² and a harbour area of 14 km². The maximum elevation of the catchment is 530 m at the head of the Horokiri sub-catchment and mean altitude is 150 m. Average annual rainfall is 1200 mm and the mean air temperature is 12.9 °C, with prevailing winds from the North and North-West (Blaschke *et al.*, 2010).

The Pāuatahanui catchment is 109 km² (Milne & Warr, 2007), and has six sub-catchments. The Pāuatahanui, Horokiri and Kakaho streams are the major sources of sediment (Figure 3.1).

Within these, predominantly pastoral sub-catchments, soil erosion and runoff has resulted in high downstream sedimentation rates and land use has driven moderate eutrophication (Stevens & Robertson, 2016). These inputs have almost certainly contributed to estuary infilling and have likely contributed to the loss of seagrass meadows from inner parts of the inlet (HS habitat), which occured some time after 1980 (Matheson & Wadhwa, 2012). From 1974 to 2009 the mean sedimentation rate for Pāuatahanui Inlet was 9.1 mm yr^{-1} (Gibb & Cox, 2009) which is high compared to rates of <1 mm yr^{-1} expected in natural, undeveloped catchments in this region and elsewhere (Swales *et al.*, 2005; Townsend & Lohrer, 2015). In the better-flushed, outer part of this estuary, seagrass beds still persist and are a prominent feature of the intertidal zone (ES). Seagrasses are very dynamic plants (Waycott *et al.*, 2005; Duarte *et al.*, 2007) and have been present in the southern PS areas in the recent past, but were not growing there during this study (Chapter 3, Appendix). From 2012, Greater Wellington Regional Council (GWRC) implemented a catchment management plan to reduce sediment loads with a target average sedimentation rate of 1 mm yr^{-1}. The latest data suggests that this rate was close to being achieved in 2016 (Stevens & Robertson, 2016). However, while this strategy may limit further deterioration in estuary conditions, it does not address the legacy effects of sediment previously washed into the inlet.

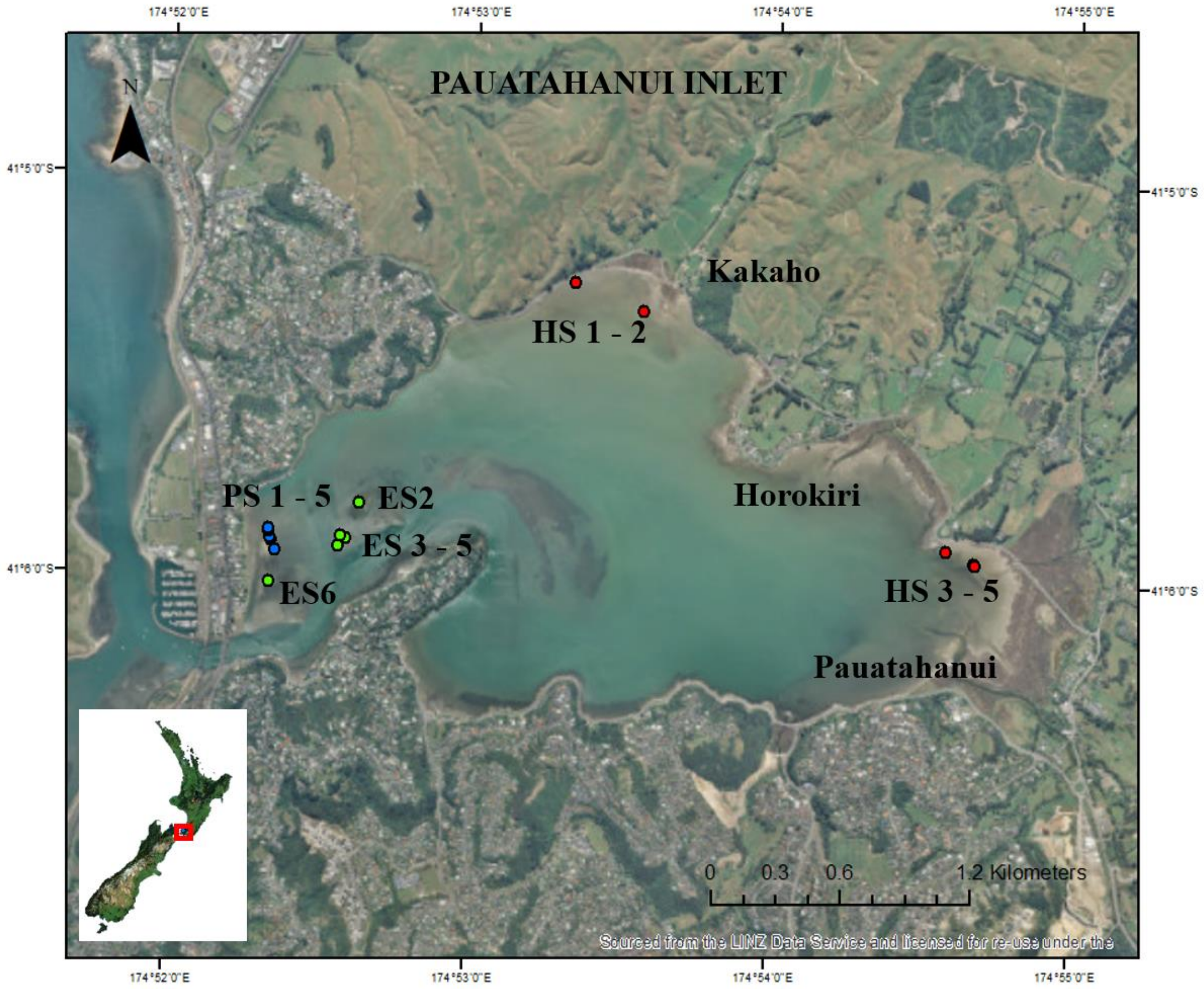


Figure 3.1: Map of Pāuatahanui Inlet (North Island, New Zealand). Locations of the 15 sampling sites are shown. Sites in the inner Pāuatahanui Inlet are where seagrass occurred historically but no longer grows (red, HS 1-5), sites in the outer estuary are where seagrass continues to persist (green, ES 2-6) and sites in the outer estuary where seagrass has been transient but the environment is sufficiently similar to ES to be considered potential seagrass habitat (blue, PS 1-5). Kakaho, Horokiri and Pāuatahanui streams are major sources of water and sediment to the Inlet.

3.3.2 Field sampling

Five sites in each of historical, existing and potential seagrass habitats (HS, ES, PS respectively) were sampled in winter (23-31/8/2018) and again in summer (8-15/2/2019). At each site substrate condition, light climate and seagrass traits were measured. A 10 m transect was laid out parallel to the shore in the mid intertidal zone, along which five equidistant 0.5 x 0.5 m² sampling plots were located. At each plot, a photograph was taken for subsequent determination of seagrass % cover and two 12 cm diameter cores, each 10 cm deep, were randomly collected for laboratory analysis of substrate properties and seagrass traits. In the first, third and fifth plot of each transect, porewater was extracted at two soil depth ranges (0-5 and 5-10 cm) using a hollow, 10 cm stainless steel cannula drilled with 1 mm diameter pores at intervals of 3 mm. Extracted porewater was passed promptly through a 0.45 µm pore filter on site and then stored in a vial out of sunlight at 0°C (chilled by slush ice), before freezing on return to shore base within 3 h. One 50 ml vial and one 15 ml vial of porewater was extracted per plot; the first for nutrient analysis and the second for hydrogen sulphide analysis. Two drops of zinc acetate (2M) were added to the 15 ml vials prior to sampling to capture free sulphides as ZnS precipitate (APHA 4500-S2-D). Adjacent to each transect at least two redox potential (Eh) profiles were measured with readings taken at 0, 2, 4, 6 and 8 cm depths. We used a platinum redox electrode to measure potential versus an Ag/AgCl reference electrode filled with 3M KCl solution. Field observations showed that most seagrass roots and rhizomes were confined to the upper 4 cm of the substrate, and we define this as the root zone (RZ). Average RZ Eh was calculated as the mean of the 0, 2 and 4 cm values.

Sensors were deployed for long term (months) monitoring of light, temperature and water level. An ECOPAR™ (http://www.seabird.com/ecopar) sensor was deployed at one site in each of HS, PS and ES habitats to directly measure photosynthetically available radiation (PAR) (mol m^{-2} d^{-1}). To address variability within habitats, HOBO loggers (http://www.onsetcomp.com/), measuring luminous flux (lux) and temperature, were deployed adjacent to the first plot at each site. Luminous flux was converted to PAR following (Thimijan & Heins, 1983) and validated by running each Hobo logger alongside a calibrated ECO-PAR sensor. A U20L water level data logger (Onset HOBO) was deployed at one site in each habitat to record water level.

Loggers were deployed from 23/8/18 to 3/10/18 (winter) and from 8/2/19 to 21/3/19 (summer), in both cases covering a complete lunar (semi-diurnal tide) cycle. Water level data were used to calculate the periods of immersion and emersion and the received light was calculated separately for these two periods. Unfortunately, HOBO loggers were lost from sites: HS2, PS4 and PS5 in winter and; PS3 and HS1 in summer.

3.3.3 Laboratory analysis

Substrate organic matter content (% OM) was estimated as weight loss-on-ignition through combustion (450 °C for 4 h) (Mook & Hoskin, 1982) and bulk density (g m^{-3}) was determined as weight of dry solids in a known pre-determined volume. Grain size was measured using a laser diffraction particle size analyser (Malvern Mastersizer 2000) over the particle size range 0.05–2000 µm (Singer *et al.*, 1988). We used grain size categories as defined by (Folk, 1968), and defined Mud (silt + clay) as <63 µm particles. Porewater samples were analysed for dissolved inorganic nutrients: nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+) and phosphate (PO_4^{3-}) ion concentrations using standard colorimetric techniques on a Lachat Quick Chem 8000 series flow injection analyser (FIA) + (Zellweger Analytics Inc. Milwaukee, Wisconsin, 53218, USA).

Seagrass cover was estimated to the nearest 5% by visual inspection of photographs following the approach recommended by (Short & Coles, 2001). Shoot density was determined by counting the number of seagrass shoots in each core. Plant material was extracted from each core from ES, rinsed with water and separated into aboveground biomass (AGB – shoots and leaves) and belowground biomass (BGB - roots and rhizomes), respectively. Plant samples were dried at 80 °C to constant weight to determine biomass per unit area (g m^{-2}).

3.3.4 Data analysis

Statistical analysis of data was performed using the R statistical package (R Team 2016). Normality of data was tested, and parameters log-transformed to reduce skewness as appropriate. Homogeneity of variance was examined using Cochran tests and when required data was log-transformed.

If not stated differently, mean values are presented with standard errors of the mean (mean ± SE). One way-ANOVA and Two way-ANOVA (with post-hoc Tukey HSD tests) were used to detect significant differences in seagrass traits, light availability and substrate properties between habitats and times. Pearson’s product-moment correlation coefficients were calculated to indicate associations between habitat and seagrass variables, and, where nonlinear relationships were apparent, we applied logistic or logarithmic (power-law) models, and report goodness of fit (R^2). Multidimensional scaling analyses using principle component analysis (PCA), were used to illustrate differences in substrate and light as a function of Habitat and treatment.

We used an existing hydrodynamic model for Porirua Harbour (J. Oldman, pers. comm, based on Oldman *et al.*, 2014) to characterise hydrodynamic condition. The model predicts current velocity, wave period, wave height, salinity and suspended sediment concentration

(SSC) and deposition across both arms of the harbour, at 30 min intervals. The model uses wind, precipitation and insolation as meteorological drivers, and we summarised predictions for 2010, which is considered a typical year, using existing bathymetry. Predictions were averaged to provide annual mean (± SE) values for each modelled characteristic.

3.4 Results

3.4.1 Substrate conditions

Substrate grain size composition showed significant differences between HS and both ES and PS habitats, which did not differ significantly from each other, but showed little seasonality (Table 3.1). HS sites had high average substrate mud contents (33.8 - 38.8 %), compared to ES and PS (average values ranging from 11.1 - 15.3 % and 10.3 - 13.6 % respectively) (Table 3.1). Fine sand was the dominant size fraction at ES and PS, whereas HS has significantly lower fine sand content and more coarse sand, particularly in winter (Table 3.1).

A Principal Components Analysis (PCA) of grain size category distribution across sites separates HS from ES and PS primarily along Axis-1 which correlates with mud vs fine sand. Axis-2 correlates with coarse sand, which was, on average, higher and more variable at HS than other habitats. Amongst HS sites, HS2 was notable higher for coarse sand, reflecting proximity to an inflowing stream. Overall, the PCA showed greater heterogeneity of grain size among HS than either PS or ES, (which were not significantly different). HS had a strong tendency for high proportion of very fine particles, and relatively low fractions of intermediate-sized particles (sand and fine sand) (Figure 3.2).

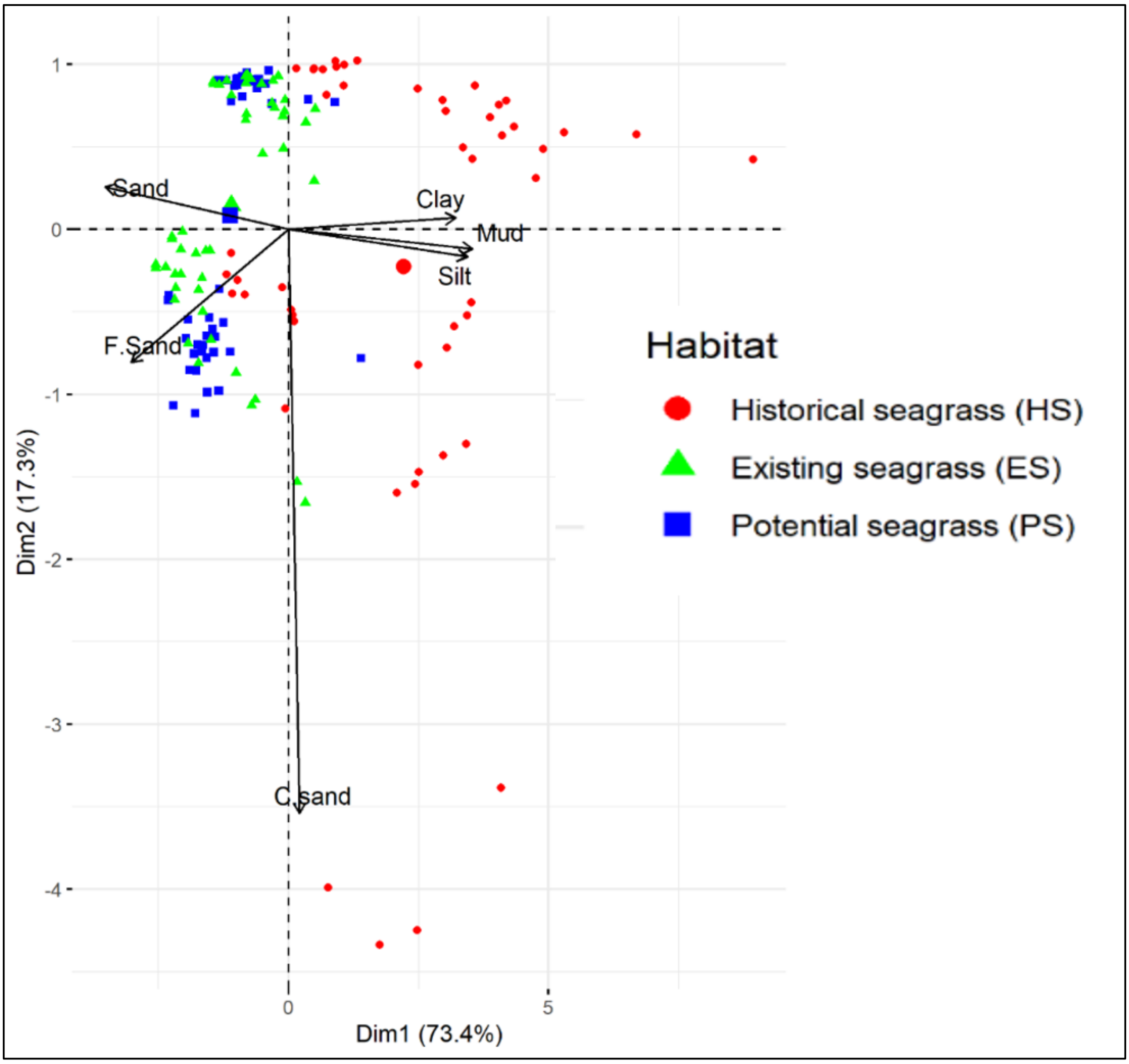


Figure 3.2: Principal component analysis (PCA) of the grain size categories for HS, ES and PS habitats. Dim1 and Dim2 together explain 90.7 % of the variation on measured grain sizes. Dim1 explains 73.4% of the variability and Dim2 the 17.3%. Enlarged points show averages for HS, ES and PS.

Table 3.1: Substrate properties, porewater nutrients and porewater sulphide concentrations at historical seagrass (HS), existing seagrass, (ES) and potential seagrass (PS). Values are means (±SE). Two sampling depths were applied for porewater chemistry. Significant differences ($p<0.05$, Tukey HSD test) among habitats and times for a particular substrate property are indicated by different alphabetic superscripts. ANOVA tables can be found in (Chapter 3, Appendix).

Substrate properties

Habitat	**Times**	**% Mud**	**% Silt**	**% Clay**	**% Sand**	**% Fine sand**	**% Coarse sand**	**Bulk density (g cm^{-3})**	**% Organic matter**	**Eh (mV) mean (0-10 cm) RZ (Rootzone 0-4 cm)**
HS	Winter	33.7 ± 5.8^{b}	27.5 ± 2.5^{c}	6.3 ± 0.8^{e}	65.9 ± 3.2^{a}	48.1 ± 4.1^{a}	17.8 ± 2.7^{d}	1.32 ± 0.05^{b}	1.79 ± 0.13^{d}	-79.8 ± 13.1^{a} Eh (RZ) $-35.7^{a'}$
	Summer	38.8 ± 6.0^{b}	30.8 ± 1.9^{c}	8.0 ± 0.9^{e}	61.2 ± 2.6^{a}	49.9 ± 3.2^{a}	11.3 ± 0.9^{b}	1.63 ± 0.07^{c}	1.60 ± 0.09^{c}	-71.6 ± 17.3^{a} Eh (RZ) $8.9^{b'}$
ES	Winter	11.1 ± 2.1^{a}	9.1 ± 0.7^{a}	2.0 ± 0.2^{a}	88.7 ± 0.9^{c}	78.5 ± 1.8^{b}	10.1 ± 0.9^{b}	1.01 ± 0.03^{a}	1.53 ± 0.14^{c}	-33.8 ± 11.4^{b} Eh (RZ) $22.6^{c'}$
	Summer	15.3 ± 1.7^{a}	12.1 ± 0.7^{b}	3.3 ± 0.1^{c}	84.7 ± 0.8^{b}	73.7 ± 1.4^{b}	10.5 ± 0.4^{b}	1.28 ± 0.05^{b}	1.71 ± 0.09^{d}	-22.3 ± 21.5^{b} Eh (RZ) $13.6^{c'}$
PS	Winter	10.3 ± 0.8^{a}	7.7 ± 0.7^{a}	2.6 ± 0.2^{b}	87.4 ± 1^{c}	74.8 ± 1.2^{b}	12.6 ± 0.4^{c}	1.09 ± 0.05^{a}	1.39 ± 0.04^{b}	-14.4 ± 14.8^{c} Eh (RZ) $40.6^{d'}$
	Summer	13.6 ± 1.0^{a}	8.9 ± 0.8^{a}	4.7 ± 0.4^{d}	86.4 ± 0.7^{c}	76.9 ± 0.8^{b}	9.5 ± 0.3^{a}	1.16 ± 0.05^{a}	1.14 ± 0.02^{a}	-41.6 ± 22.2^{b} Eh (RZ) $5.6^{b'}$

Substrate porewater

Habitat	**Times**	**$[PO_4]^{3-}$ µM (0-5 cm)**	**$[PO_4]^{3-}$ µM (5-10 cm)**	**$[NH_4]^{+}$ µM (0-5 cm)**	**$[NH_4]^{+}$ µM (5-10 cm)**	**$[H_2S]$ µM (0-5 cm)**	**$[H_2S]$ µM (5-10 cm)**
HS	Winter	0.04 ± 0.004^{a}	0.27 ± 0.03^{d}	12.47 ± 3.49^{b}	61.43 ± 5.93^{c}	1.11 ± 0.29^{b}	2.69 ± 0.53^{d}
	Summer	0.40 ± 0.09^{e}	0.24 ± 0.07^{d}	51.72 ± 21.23^{c}	71.52 ± 15.14^{c}	2.89 ± 0.18^{d}	3.22 ± 0.12^{e}
ES	Winter	0.05 ± 0.01^{b}	0.29 ± 0.06^{d}	5.71 ± 1.89^{a}	22.12 ± 4.93^{b}	0.83 ± 0.22^{a}	3.23 ± 0.71^{e}
	Summer	0.29 ± 0.04^{d}	0.25 ± 0.04^{d}	12.70 ± 3.05^{b}	14.97 ± 5.21^{b}	2.10 ± 0.19^{c}	2.61 ± 0.23^{d}
PS	Winter	0.07 ± 0.01^{b}	0.12 ± 0.01^{c}	8.76 ± 1.33^{a}	21.84 ± 1.44^{b}	1.55 ± 0.41^{b}	1.62 ± 0.47^{b}
	Summer	0.17 ± 0.04^{c}	0.16 ± 0.01^{c}	14.41 ± 2.77^{b}	14.53 ± 3.16^{b}	1.58 ± 0.21^{b}	1.46 ± 0.14^{b}

3.4.2 Substrate bulk density, organic matter and redox potential

Substrate bulk density was higher at HS than PS and ES (which were similar) and substrates at all sites were denser in summer than in winter (Table 3.1). The latter could indicate intrusion of fine particles into substrate at sites driven by generally higher concentrations associated with strong summer storms and increased runoff from the land. Substrate organic content was significantly higher at HS and ES than PS. HS had significantly higher organic content compared to ES during winter and significantly lower during summer (Table 3.1). These patterns suggest organic content at ES is related to seagrass productivity during summer, whereas organic content at HS is linked to catchment runoff and fine sediment (and organic) inputs during winter.

Redox (Eh) profiles all had similar gradients (Figure 3.3), but were more negative at HS than at PS and ES during winter, profiles were very similar for all habitats in summer (Figure 3.3). Eh values became negative between 0-2 cm depth for HS in winter, while in summer, and in both times for PS and ES, negative values commenced at greater depth, between 2-4 cm (Figure 3.3).

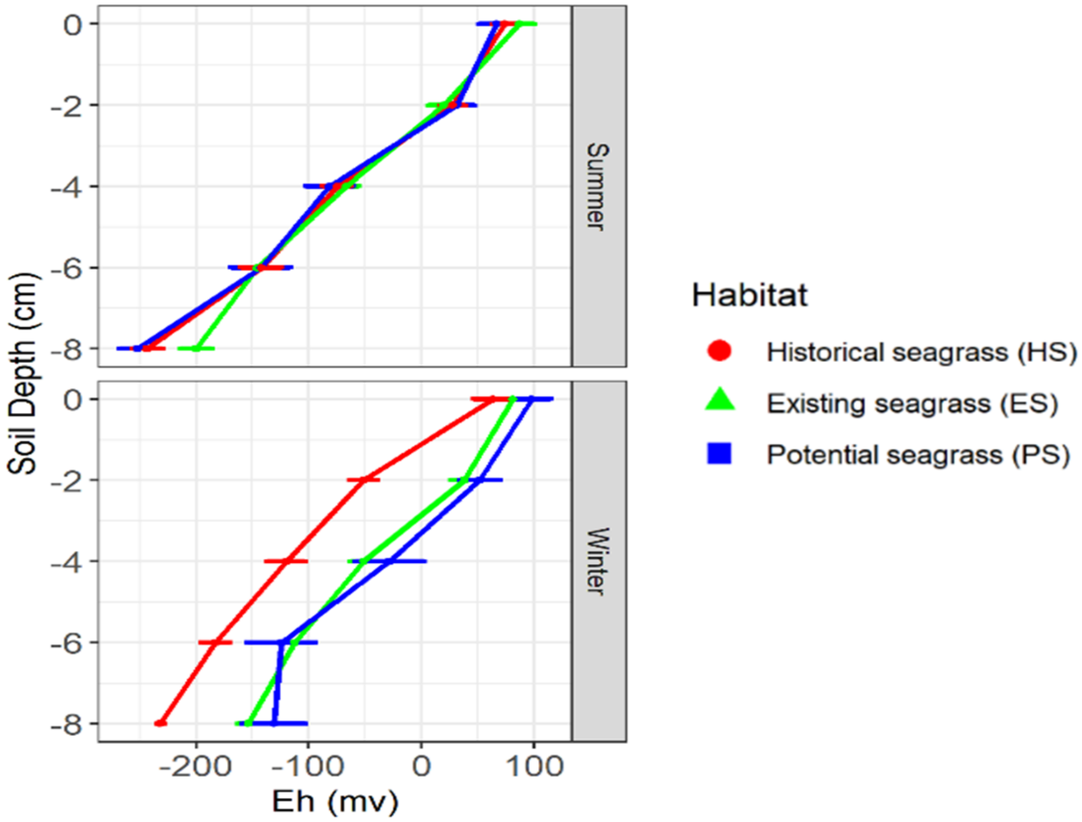


Figure 3.3: Redox potential profiles (means ±SE) for historical, potential and seagrass habitats in summer and in winter.

3.4.3 Porewater chemistry

Porewater analyses for [NO_3^-] and [NO_2^-] were below detection limits (<0.01 µM) at all sites, in both times and at both depth ranges (Table 3.1). The very low levels of oxidised forms of nitrogen is consistent with strongly reducing conditions at all sites. Deeper (5-10 cm) [PO_4^{3-}] concentrations varied little from winter to summer, and were similar to near-surface (0-5 cm) values in summer. In winter [PO_4^{3-}] was lower near the surface in all habitats. Overall, [PO_4^{3-}] was lower in PS than either HS or ES. A similar times depth pattern emerged for both [$NH_4^{+]}$ and sulphide, with concentrations at depth similar in both winter and summer, but with near surface (0-5 cm) values significantly lower in winter. [NH_4^+] was similar at ES and PS but was considerably higher for all date/depth combinations in the HS habitat. Hydrogen sulphide, tended to have lower concentrations at PS than either HS or ES habitats, which were similar.

3.4.4 Relationship of habitat to substrate characteristics and light

A PCA ordination of all parameters related to sediment impacts effectively segregates the three habitats along PCA axis 1, which is linked to % mud, total suspended solids (TSS), PAR and Eh (Figure 3.4). HS sites had high muddiness and suspended sediment concentrations (and low Eh and PAR) whereas sites in PS and ES habitat had lower muddiness and higher PAR and redox potential. PCA Axis 2 separates mostly within habitat, and is driven by variation in pore water chemistry. The degree of scatter along this axis indicates more variability in pore water chemistry within ES and HS than PS.

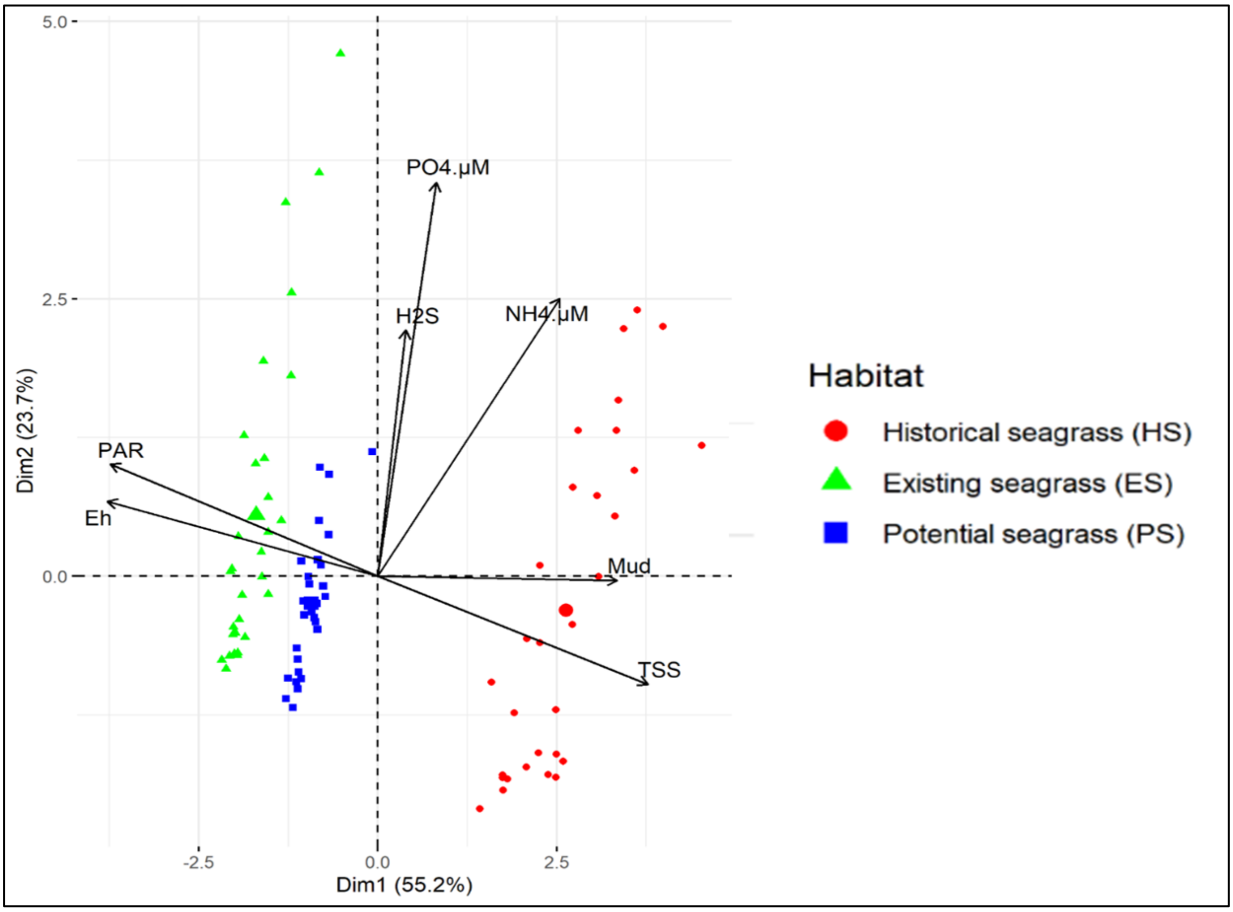


Figure 3.4: Principal component analysis (PCA) of porewater nutrients, sulphide, PAR, Redox and % Mud from HS, ES and PS. Dim1 and Dim2 of the PCA explain 55.2% and 23.7% of the variation respectively. Enlarged points show averages for HS, ES and PS.

3.4.5 Light availability

Our light data shows that all habitats receive > 90% of their daily light dose while emerged (Table 3.2) reflecting high light attenuation in the (often muddy) water during submersion under higher than mid-tide water levels. During winter, total light received both immersed and emerged increased from ES, through PS to HS, whereas in summer this order was reversed (Table 3.2). The last is surprising, and our summer PAR values might be biased (low) because of shading of sensors due to fouling by drifting macroalgae (*Ulva* sp) that were frequently observed during field work in this time. Data as a time series plot and the number of days below a daily average compensation irradiance level (E_c) are shown in (Chapter 3, Appendix).

HS also experienced a relatively high number of days (8), for the whole deployment, when light availability was on average below a compensation irradiance (E_c) 1.9 mol m^{-2} d^{-1} assuming 12-hr photoperiod (Flanigan & Critchley, 1996).

Table 3.2: Light availability at HS, PS and ES during winter and summer deployments. Values are daily mean PAR (±SE). PAR dose when submerged and emerged are shown as well as the number of days each habitat was below a compensation irradiance for *Zostera muelleri* (E_c, 1.9 mol m^{-2} d^{-1}) during deployments. The value given is the total number of days and the figure in parentheses is the longest consecutive period. Significantly different mean values (ANOVA, Tukey HSD, $p<0.05$) among habitats and times are indicated by different alphabetic superscripts and (*) respectively. ANOVA tables can be found in (Chapter 3, Appendix).

Habitat	Photosynthetically available radiation when submerged (mol m^{-2} d^{-1})		Photosynthetically available radiation when emerged (mol m^{-2} d^{-1})		Number of days during which mean PAR was below compensation irradiance (days)	
	Winter	**Summer**	**Winter**	**Summer**	**Winter**	**Summer**
HS	2.2 ± 0.2^{a}	$5.2\pm0.2^{c*}$	30.9 ± 2^{a}	60 ± 2.5^{c}	$6\ (2)^{b*}$	$3\ (1)^{b}$
ES	4.4 ± 0.3^{c}	4.0 ± 0.2^{c}	44.3 ± 2^{c}	46.1 ± 2^{a}	$3\ (1)^{a*}$	$1\ (0)^{a}$
PS	3.5 ± 0.2^{b}	$4.6 \pm 0.2^{c*}$	33.0 ± 2^{b}	53.1 ± 2.4^{b}	$5\ (0)^{a*}$	$0\ (0)^{a}$

3.4.6 Hydrodynamic model results and background information

Current velocity, wave period and salinity were predicted to be similar at ES and PS, and higher in comparison with HS. In contrast, suspended sediment concentration (SSC) and sediment deposition rates were predicted to be significantly lower at ES and PS compared to HS (Table 3.3).

Table 3.3: Predicted hydrodynamics parameters and salinity for historical seagrass (HS), existing seagrass (ES) and potential seagrass (PS) using the model of Oldman et al. (2014). Values are means (±SE). Significant differences ($p<0.05$, Tukey HSD) are indicated by different alphabetic superscripts. Suspended Sediment Concentration (SSC). ANOVA tables can be found in (Chapter 3, Appendix).

Habitat	Current velocity (m s^{-1})	Wave period (s)	Salinity (PSU)	SSC (mg L^{-1})	Deposition (mm yr^{-1})
HS	0.05 ± 0.003^{a}	0.59 ± 0.010^{a}	18.67 ± 0.76^{a}	10.72 ± 0.570^{b}	6.4 ± 2.10^{c}
ES	0.15 ± 0.010^{c}	0.66 ± 0.010^{c}	25.63 ± 0.24^{b}	2.35 ± 0.095^{a}	3.6 ± 0.90^{b}
PS	0.08 ± 0.005^{b}	0.61 ± 0.001^{b}	25.99 ± 0.01^{b}	2.17 ± 0.020^{a}	1.22 ± 0.02^{a}

3.4.7 Seagrass traits

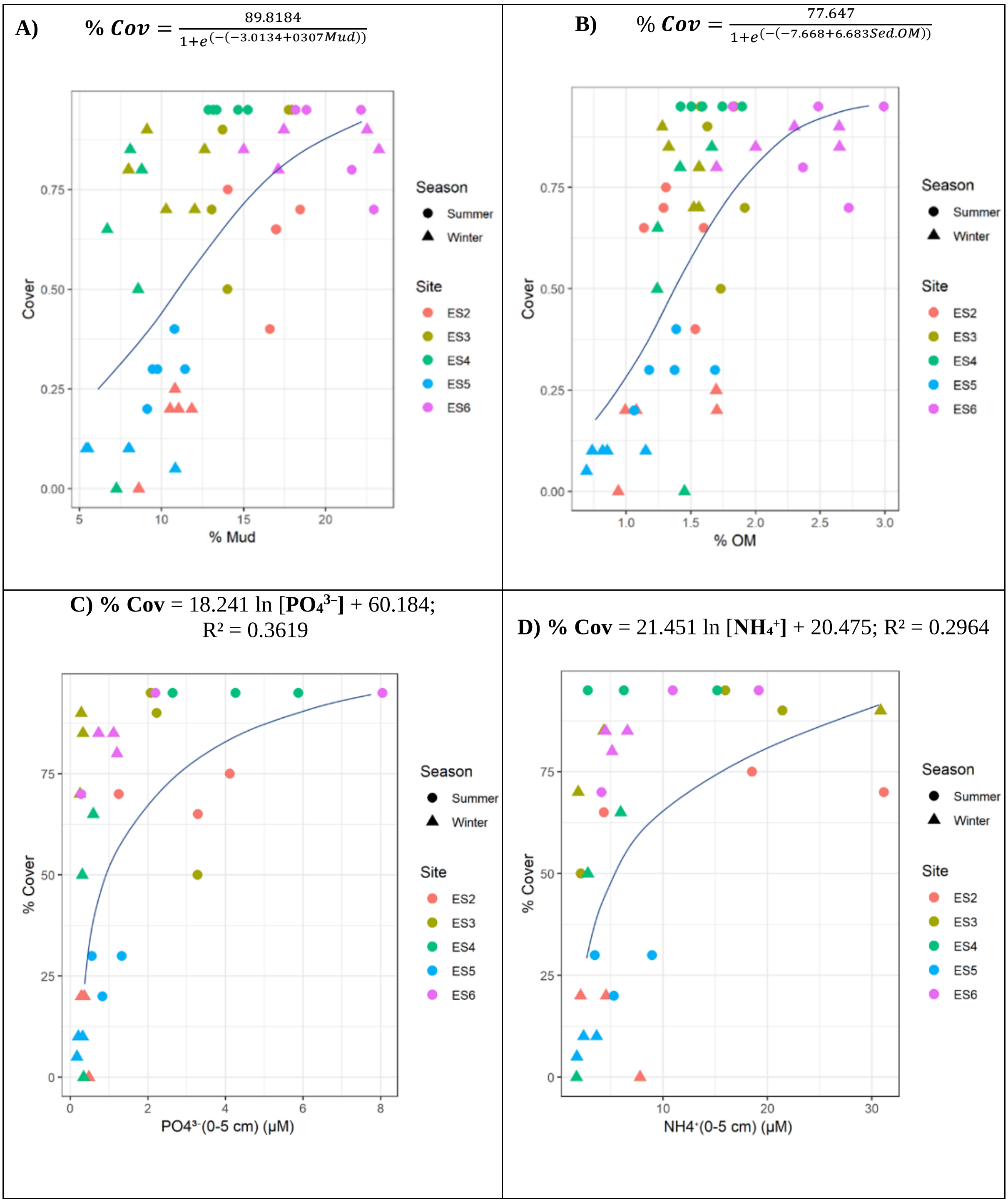
At ES sites seagrass cover ranged from 10 – 95 %, shoot density from 172 – 700 shoots m^{-2} and total biomass from 33 - 243 g m^{-2} (Table 3.4). Belowground biomass was always higher than aboveground biomass with the average summer and winter BGB:AGB ratio varying slightly (15.9 versus 17.9, differences not significant). BGB and total biomass were significantly higher in summer (average 150.7 g m^{-2}) than in winter (87.8 g m^{-2}), whereas AGB was not significantly higher in summer than in winter.

Table 3.4: Seagrass traits at existing seagrass habitat (ES). Statistical parameters are: mean (±SE), standard deviation (SD), maximum, minimum and median. Significant seasonality (ANOVA, Tukey HSD, $p<0.05$) in traits is indicated by different alphabetic superscripts. ANOVA tables can be found in (Chapter 3, Appendix).

Trait	Time	Mean ± SE	SD	Max	Min	Median
% Cover	Winter	49.4 ± 7.1^{a}	35.5	90	0	65
	Summer	71.0 ± 5.2^{b}	26.1	95	0	75
Shoot density (m^{-2})	Winter	444 ± 51.7^{a}	258	828	0	414
	Summer	670 ± 51.7^{b}	240	1401	159	700
AGB (g m^{-2})	Winter	9.4 ± 1.6^{a}	7.9	22.7	0.13	6.1
	Summer	10.2 ± 1.0^{a}	5.0	22.7	2.1	11.3
BGB (g m^{-2})	Winter	87.8 ± 15.9^{a}	79.5	302.5	1.9	46.9
	Summer	150.7 ± 24.2^{b}	121	488.8	4.6	111.5
BGB/AGB	Winter	17.9 ± 4.5^{a}	22.3	95.1	2.04	11.5
	Summer	15.9 ± 2.3^{a}	11.6	44.8	0.7	13.4
Biomass (g m^{-2})	Winter	97.2 ± 17^{a}	84.8	321.6	2.5	64.5
	Summer	161 ± 24.6^{b}	123.2	500	10.9	122.9

3.4.8 Seagrass relationships with substrate conditions

Seagrass cover increase was associated with an increase in % mud, % OM and recycled dissolved inorganic nutrients. % Mud and % OM followed a logistic relationship (Figure 3.5A & 3.5B) whereas a saturation relationship was the best fit for porewater [PO_4^{3-}] and [NH_4^+] (Figure 3.5C & 3.5D). Maximum seagrass cover occurred where substrate mud content was in the range 13-23% and organic content 1.3-3% with optimums of 13% mud and 1.3% OM. Porewater recycled nutrients followed saturation relationships with saturation concentrations of 2 and 8 µM for [PO_4^{3-}] and [NH_4^+] respectively. Shoot density also showed saturation relationships with ammonium ion (Figure 3.5D). None of the seagrass traits followed a significant relationship with PAR presumably because no ES sites were strongly light-limited. Mutual scatter plots between different biomass indices and substrate conditions and porewater chemistry are shown in the (Chapter 3, Appendix).



A) $\% \, Cov = \frac{89.8184}{1+e^{(-(-3.0134+0307Mud))}}$

B) $\% \, Cov = \frac{77.647}{1+e^{(-(-7.668+6.683Sed.OM))}}$

C) % Cov = 18.241 ln $[\mathbf{PO_4^{3-}}]$ + 60.184; $R^2 = 0.3619$

D) % Cov = 21.451 ln $[\mathbf{NH_4^+}]$ + 20.475; $R^2 = 0.2964$

Figure 3.5: Scatter plots of seagrass biomass versus substrate conditions. A) Seagrass cover versus substrate % mud. B) Seagrass cover versus substrate % organic matter. C) Seagrass cover vs porewater $[PO_4^{3-}]$. D) Seagrass cover versus porewater $[NH_4^+]$. Sites are grouped by colour and season by shape. Equations show logistic and logarithmic curve fits. A, B, C and D shown with log scales.

3.5 Discussion

3.5.1 Multiple effects of sediment on seagrass

Our study addressed relationships between seagrasses, fine sediment, light climate, and rhizosphere conditions by comparing three habitats, HS where seagrass was present many years ago and has never recovered, ES where seagrass is currently present and PS where seagrass has been present in recent years but is currently absent. We used these data to study and interpret the potential effects of fine sediment and seagrass on light climate and rhizosphere condition as well as its interactions at HS, ES and PS habitats in Pāuatahanui Inlet.

Historical substrates had significantly higher mud content (34-39%), bulk density (1.3-1.6 g cm^{-3}), porewater ammonium concentration (13-72 µM), a more reduced redox profile (negative redox at 2 cm soil depth), and higher sedimentation rates (6 mm yr^{-1}) than both PS and ES (which were broadly similar). Differences in substrate organic content were less clear, and while HS had significantly higher organic content than ES during winter, the opposite pattern occurred during summer, while organic content at PS sites was consistently lower than the other two sites, but again slightly, but significantly, lower in summer than in winter. We infer that during the warmer summer months the mineralisation of organic matter in the two seagrass-free sites temporarily exceeds accumulation, while the summer growth in seagrass cover causes an increase in substrate OM. Accumulation of phosphate and ammonium ions in the upper substrate at all sites during summer is consistent with increased mineralization of OM in this time.

That the presence of seagrass is a significant source of organic material is supported by the higher substrate OM concentrations at ES than PS, and this is well established in the literature (Romero *et al.*, 1994; Gacia & Duarte, 2001; Holmer *et al.*, 2001; Gacia *et al.*, 2003; Larkum *et al.*, 2006; Fourqurean *et al.*, 2012). That the organic content at ES exceeds that at HS at times, suggests that the amount of organic material *per se* is unlikely to preclude seagrass at HS. The simplest explanation of the more reduced nature of sediments at HS, and the accumulation of reduced chemical species may relate to the reduced diffusivity that accompanies the higher proportion of mud (and greater bulk density implying lower pore space) at HS compared to the ES substrate. Under seagrass, root oxygen release (Terrados *et al.*, 1999; Borum *et al.*, 2005b; Brodersen *et al.*, 2015; Brodersen *et al.*, 2017; Brodersen *et al.*, 2018) together with enhanced burrowing by invertebrates, mainly bivalves such as *Austrovenus* *stutchburyi* and *Macocoma liliana* that we observed in ES samples (Lohrer *et al.*, 2004; Lohrer *et al.*, 2010; Lohrer *et al.*, 2016) are processes expected to make redox potential less negative.

Unfavourable alterations to substrate chemistry resulting from fine sediment deposition is a likely cause of the failure of seagrass to colonise at HS while persisting at ES. Our results suggest that the significantly higher mud content, bulk density and more reduced conditions of the substrate at HS, is likely to be associated with a lower availability of interstitial oxygen compared to substrates at ES and PS. A poor substrate oxygen status, linked to denser substrates and higher oxygen-demanding organic matter content, can affect the capability of seagrasses to grow and is a potential cause of seagrass demise (Pérez *et al.*, 2007). Nevertheless, there appear to be strong differences between species in their ability to tolerate anoxia (Terrados *et al.*, 1999). A Mediterranean species, *C. nodosa*, did not die after sucrose additions to generate anoxia, in a manipulative experiment, and temperate *Z. marina* did not show leaf growth reduction until 2 months post treatment. However, *Thalassia hemprichii* suffered mortality in a multi-specific meadow under a similar experimental regime (Terrados *et al.*, 1999). Substrate redox values previously measured in substrates under seagrass ranged from -108 to 55 mV in three New Zealand estuaries (Matheson & Schwarz, 2007). At HS in Pāuatahanui Inlet we measured values ranging from -230 to 70 mV and -50 mV at a substrate depth of just 2cm below the surface indicating very reducing conditions. The redox values are also lower than the typical range reported for seagrass substrates of -100 and 200 mV in the first 10 cm of the substrate by (Terrados *et al.*, 1999).

Despite the more reducing conditions in HS substrates in winter, we did not find consistently increased concentrations of compounds that tend to accumulate under these conditions, that is hydrogen sulphide, ammonium and phosphate ions (Borum *et al.*, 2005a; Koch *et al.*, 2007b; Brodersen *et al.*, 2015). Only substrate ammonium ion concentrations were higher in HS than other habitats. In marine substrates, sulphate reduction is a major pathway for the mineralization of the organic matter leading to production of hydrogen sulphide (Holmer *et al.*, 2001; Duarte *et al.*, 2004a). However, our results for porewater hydrogen sulphide analysis showed values under 4 µM at HS, ES and PS, which is below the 10 and 13 µM thresholds considered to trigger decline in seagrasses (Calleja *et al.*, 2007; Krause-Jensen *et al.*, 2011). This may be explained by the fact that iron II (Fe^{2+}) reacts with sulphide to form very stable precipitates (Nielsen *et al*., 2005). Iron rich substrates have low hydrogen sulphide and low toxicity for seagrass (Ruiz-Halpern *et al.*, 2008). HS substrate samples exhibited dark colours suggesting the presence of iron sulphide. Iron solubilization ($Fe^{3+} \rightarrow Fe^{2+}$) occurs at -

47 mV redox potential which seems consistent with the values of Eh at PS and ES. Levels of Iron in Pāuatahanui Inlet were reported to be typical of other New Zealand estuaries (Stoffers *et al.*, 1983; Glasby *et al.*, 1990; Blaschke *et al.*, 2010) however, further research seems desirable perhaps combined with determination of concentration of potentially phytotoxic heavy metals such as zinc, lead and copper (Macinnis-Ng & Ralph, 2004) for which we had no data at our sites. Substrate chromium, copper, lead and zinc were determined to have maximum values of (1.68, 1.1, 3.3, 9.7 mg L^{-1}) at other sites in Pāuatahanui Inlet (Hooper, 2002). These copper, and lead concentrations exceed the reported thresholds of 1mg L^{-1} reported to have incipient effects on seagrass physiological traits (Macinnis-Ng & Ralph, 2004).

For ammonium ion, which is the most common form of N in pore waters of New Zealand estuaries (e.g., Tay *et al.*, 2013), concentrations at HS (up to 71 μM) were higher than at ES and PS and no seagrass was found to thrive at sites over 30.8 μM. However, concentration at all sites were below levels considered phytotoxic for the related species *Z.nolti* (200-4000 μM) (Brun *et al.*, 2002; Govers *et al.*, 2014). Therefore, direct hydrogen sulphide and/or ammonium ion phytotoxicity appears unlikely to have contributed to seagrass loss and failure to re-stablish at HS in Pāuatahanui Inlet. The lack of apparent influence of sulphide and ammonia is consistent with the PCA plot for different sites showing strong separation of habitat along ‘mud’ gradients, but separation along pore-water chemistry only *within* habitats.

Nevertheless, the elevated anoxia of HS substrates implies increased production and release of oxygen into the rhizosphere, which, in turn implies an increased light demand by seagrasses, in order to oxygenate the rhizosphere, were they to re- establish. Prolonged or sudden degradation of the oxic-microshield protecting the vital basal meristems may be the initial external chemical mechanism behind seagrass die-off events in highly reduced marine substrates (Brodersen *et al.*, 2017) such as HS in Pāuatahanui Inlet.

Reduced underwater light availability is often considered the leading cause of seagrass decline in estuaries suffering high sediment pollution. Increased suspended sediment loads to coastal waters have an indirect negative effect on seagrasses by reducing the available light penetrating through the water to the sea bed (Duarte, 1991; Erftemeijer & Lewis III, 2006). The Pāuatahanui Inlet study sites were located in the intertidal zone, whereas many studies that have implicated light limitation have been carried out at subtidal locations or with specimens cultivated in permanently submerged conditions (Longstaff *et al.,* 1999; Bulmer *et al*., 2016; Chartrand *et al*., 2016; Collier et al., 2016). In intertidal locations, the irradiance during the emerged periods is much higher than when immersed (by ca. 10-fold in winter and 12-fold in summer, in our study) which can compensate for poor light penetration of muddy water during immersion. Being at the same tidal height, we found no significant differences in emerged irradiance between the three habitats in either summer or winter. Any effect of sediment mediated through attenuation of downwelling irradiance therefore must be expressed in the submerged irradiance, which was lowest at HS habitats during winter (but not summer), and highest in the ES habitat, perhaps reflecting both proximity of sediment sources (to HS) and the capacity of seagrasses (at ES) to enhance sedimentation and thus generate a clearer overhead environment (De Boer, 2007). The efficiency of light use under submerged and emerged conditions becomes critical in evaluating the role of light limitation, since the effect of the elevated levels of fines on seagrass light climate at HS sites appears to be relatively small and seasonal.

Daily emerged light dose, has been proven to be key to providing resilience to inter-tidal seagrasses (Vermaat *et al.*, 1997; Schwarz, 2004; Drylie *et al.*, 2018). However, at HS sites light during emersion was high and yet seagrass has not re-stablished there suggesting that emerged production can not sustain the plants or, more likely, that other factors are dominant. Considering only the submerged irradiance, the average winter value for HS is close to the PAR determined at maximum depth limit for subtidal specimens of this species, in winter, in Kaipara Harbour (2.1 ± 0.19 mol $m^{-2}d^{-1}$) (Bulmer *et al.*, 2016). However, our sites in Pāuatahanui Inlet, including HS, experience on average at least this amount of light when submerged and a higher amount when emerged, thus leading to an expectation of net photosynthetic gains, year round (Schwarz, 2004). Furthermore, during summer, HS, ES and PS experienced 5.2, 4.0 and 4.6 mol $m^{-2}d^{-1}$ when immersed compared to 4.9 mol $m^{-2}d^{-1}$ for plants at the depth limit in Kaipara Harbour (Bulmer *et al.*, 2016). Light availability alone therefore does not appear to explain the failure of seagrass to re-establish, at HS in Pāuatahanui Inlet.

In addition to low winter light availability and alteration to substrate physico-chemistry, periodic smothering of seagrass plants by sedimentation events may have also contributed to seagrass loss, and failure to recover, at HS. Recently settled fine sediment within nepheloid layers not only shades seagrass (e.g. (Pedersen *et al.*, 2012)), but may also exert an oxygen demand and inhibit oxygen transfer so contributing to deoxygenation. Seagrass was present at HS around 1980 (Healy, 1980) but disappeared sometime afterwards (Matheson & Wadhwa, 2012). A large acute sedimentation event occurred in the inlet in 1981 which delivered a sediment load of about 40,000 tonnes (Oldman et al., 2014). This event may have caused catastrophic, and acute, loss of seagrass from the inner part of the inlet, where plants have not subsequently re-established. However, it is also apparent that HS continues to experience chronically elevated fine sediment. The rate of sediment accumulation in most NZ estuaries under natural conditions is below 1 mm yr^{-1} (Townsend & Lohrer, 2015). However, HS experienced fine sediment deposition of about 6 mm yr^{-1} compared to rates of 3.66 and 1.22 mm yr^{-1} at ES and PS respectively. Shoot density and rhizome growth in *Zostera muelleri* have been shown to significantly decline under 5 mm burial treatments (Benham *et al.*, 2019).

Intertidal flats of relatively undeveloped estuaries with healthy seagrass meadows are generally characterised by sandy substrates with minimal mud content (Matheson & Schwarz, 2007; Swales *et al*., 2007). We have shown here that multi stressor effects of mud are strong determinants of seagrass growth and persistance. Moreover, fine sediment often interacts with other factors such as nutrient enrichment leading to increased organic matter which may interact with mud loading by both increased shading by phytoplankton and epiphytes and extra oxygen demand, further complicating seagrass response (Walker & McComb, 1992; Gladstone-Gallagher *et al.*, 2018)

We also showed seagrass grew at ES sites throughout the year, but plant cover, shoot density and biomass were 1.4-fold, 1.5-fold and 1.7-fold higher in summer than in winter. For ES sites maximum seagrass cover occurred in an intermediate range of substrate mud content ranging from 13-23%. Substrate grain size has been proposed as an influence on seagrass growth (Kenworthy & Fonseca, 1977; Short, 1987; Koch, 2001; Short *et al.*, 2002; Leschen *et al.*, 2010; Krause-Jensen *et al.*, 2011; Moksnes *et al.*, 2018). The substrate mud range (13-23%) in which seagrass grows in Pāuatahanui Inlet is higher than the silt (only) threshold determined for this species in another New Zealand estuary, Tauranga Harbour, of 13% (Park and Donald 1994). This suggest that *Zostera muelleri* may have a broader tolerance for mud than we thought, initially, based on the Tauranga experience. This suitable substrate muddiness range is local and may vary for different estuaries depending on the nature of the mud and interaction with other stressors, for example grain size, %OM, oxygen demand, pore water conditions. Studies in other countries report a wide range of substrate muddiness thresholds ranging from 13 to 70 % (Table 3.5). Tolerances of seagrasses to substrate muddiness are likely to vary between species and between geographical locations due to interactions with other biogeochemical characteristics of the substrates (Krause-Jensen *et al.*, 2011).

Table 3.5: Percent substrate mud ranges for *Zostera* genus occurrence reported in the literature.

Study location	**Seagrass species**	**Means of determination**	**Substrate %mud where seagrass thrives**	**References**
Chesapeake Bay, Virginia, USA	*Z. marina*	-	15	Orth (1977)
New Bedford Harbour, USA	*Z. marina*	-	<70%	(Short *et al.*, 2002)
Boston Harbour,USA	*Z. marina*	-	<35%	(Leschen *et al.*, 2010)
42 Sites, Denmark	*Z. marina*	Wet sieving	<13%	(Krause-Jensen *et al.*, 2011)
NW coast, Sweden	*Z. marina*	Wet sieving	35%	(Moksnes *et al.*, 2018)
Königshafen, Sylt, Germany	*Z. noltii*	-	1.3 - 4.8 %	(Widdows *et al.*, 2008)
Oosterschelde, Netherlands	*Z. noltii*	Laser analyzer	130 µm	(Govers *et al.*, 2014)
Tauranga Harbour, New Zealand	*Z. muelleri*	-	<13 % silt only threshold.	(Park & Donald 1994)
Western Port, Australia	*Z. muelleri*	-	0.5-72 %	(Koch, 2001)
Porirua Harbour, New Zealand	*Z. muelleri*	Laser analyzer	8-23%	This study

* = Not defined.

3.6 Conclusion

In conclusion, we associate loss of the seagrass, *Zostera muelleri*, in Pāuatahanui Inlet to estuary pollution with fine sediment which exerts multiple stresses including altered substrate physico-chemistry, and light reduction. We recommend further experiments to isolate mechanisms of mud damage to seagrass and to improve our understanding on its interactions with other factors such as light deprivation and eutrophication. This, will assist policy makers to better manage this pollutant for seagrass protection and restoration.

Chapter 4

Substrate-Irradiance interactive effects on seagrass: a mesocosm study of *Zostera muelleri*.

Note: this chapter has been published as:

Zabarte-Maeztu, I., Matheson, F. E., Manley-Harris, M., Davies-Colley, R. J., & Hawes, I. (2021). Interaction of substrate muddiness and low irradiance on seagrass: a mesocosm study of *Zostera muelleri*. *Aquatic Botany*, 103435.

4.1 Abstract

Seagrass meadows are important estuarine habitats and in recent decades, have suffered global declines. Fine sediment pollution is a recognised major cause of decline, with impacts usually attributed to reduction of photosynthetically available radiation (PAR), or effects of burial. However, it has been speculated that intruded fine sediment affects the seagrass rhizosphere and this interacts with reduced irradiance to affect seagrass performance. In a 2 x 2 factorial mesocosm experiment, we examined the interaction between substrate "muddiness" and irradiance dose on seagrass growth and survival over a six-week period. Seagrass *Zostera muelleri* was grown on two substrates from the same estuary: (1) an inner estuary substrate with high mud content (42%) from a location where seagrass formerly grew (historical seagrass, HS); and (2) an outer estuary substrate with moderate mud content (20%) from a location at which seagrass persists (existing seagrass, ES). Two irradiance levels were used; (1) low light (6.3 mol m^{-2} d^{-1}, LL) and (2) very low light (2.3 mol m^{-2} d^{-1}, VL). These lie slightly above a published compensation irradiance (E_c) of 1.9 mol m^{-2} d^{-1} (assuming a 12-hr photoperiod). Belowground biomass and rhizome growth were significantly reduced by substrate muddiness but not detectably affected by irradiance. However, shoot growth, was reduced by *both* reduced irradiance and increased substrate muddiness with significant synergistic interaction. We conclude that *Z. muelleri* inhabiting muddified substrates requires an increased minimum irradiance to deal with an adverse rhizosphere. Interactions between substrate and light climate, both of which are affected by fine sediment pollution, should be considered when determining light thresholds for seagrass survival.

4.2 Introduction

Seagrass meadows are widely distributed in tropical and temperate coastal waters, worldwide (Waycott *et al.*, 2009), where they have immense ecological, and socio-economic value, supporting a wide range of ecosystem services (Orth *et al.*, 2006). These aquatic angiosperms are critical intertidal and subtidal habitat for many marine organisms, providing shelter, food and structural habitat, both above and below the sediment surface (Jackson *et al.*, 2001; Orth *et al.*, 2006; Bertelli & Unsworth, 2014; Morrison *et al.*, 2014). As 'ecological engineers', seagrasses are also able to modify their environment, for example by damping wave action and sediment erosion (Bos *et al.*, 2007; Battley *et al.*, 2011), oxygenating their rhizospheres and modifying sediment chemistry (Terrados *et al.*, 1999; Enríquez *et al.*, 2001; Borum *et al.*, 2005b; Marbà *et al.*, 2010; Brodersen *et al.*, 2015).

Seagrasses are, however, in decline across their range. It is estimated that 29% of the known areal extent of seagrass has disappeared globally since 1879 (Waycott *et al.*, 2011). Furthermore, rates of decline have accelerated from a median of 0.9 % year^{-1} before 1940 to 7% year^{-1} since 1990, placing seagrass meadows among the most threatened ecosystems on earth (Waycott *et al.*, 2009). Seagrass decline can be the result of natural or anthropogenic influences. Natural factors such as cyclonic storms, for example hurricanes and typhoons, and biotic influences, for example plant diseases, avian grazing and invasive species, may contribute to the permanent or temporary loss of seagrass beds (Walker *et al.*, 2007; Infantes *et al.*, 2011). However, human-related activities, particularly those that cause sediment pollution of coastal waters, are generally regarded as the major contributors to long-lasting seagrass decline (Costanza *et al.*, 1997; Burkholder *et al.*, 2007; Cunha *et al.*, 2012). Activities related to enhanced supply of fine sediments to estuaries and coastal waters, such as soil disturbance and land clearing in the upper catchment and dredging activities in harbours that disturb or displace coastal sediment deposits are often implicated in seagrass declines (Erftemeijer & Lewis III, 2006; Bainbridge *et al.*, 2018).

Terrigenous fine sediment is a ubiquitous pollutant in coastal waters and in New Zealand, is considered to be the most pervasive contaminant affecting estuaries and sheltered coastal embayments (Inglis, 2003; Thrush *et al.*, 2004; Lohrer *et al.*, 2006; Matheson & Schwarz, 2007; Matheson *et al.*, 2010; Drylie *et al.*, 2018; Gladstone-Gallagher *et al.*, 2018; Dudley *et al.*, 2020). It is also thought to have contributed substantially to documented losses of seagrass *Zostera muelleri* (Inglis, 2003; Matheson & Schwarz, 2007). Losses have been reported for Avon-Heathcote estuary (Inglis, 2003), Manukau Harbour (Turner, 1995), Tauranga Harbour

(Park, 2016), Waitemata Harbour (Hayward *et al.*, 1999) Whangarei Harbour (Reed *et al.*, 2004), Eastern Bay of Islands (Matheson *et al.*, 2010; Booth, 2019) and Porirua Harbour (Matheson & Wadhwa, 2012). In Tauranga Harbour, approximately one-third of intertidal seagrass and 90% of sub-tidal seagrass were lost in the period from 1954 to 1996 and this has been linked to increased sediment loading of the estuary (Park, 1999).

Fine sediment impacts seagrasses through reduction of photosynthetically available radiation (PAR), due to suspended particles increasing the rate of attenuation through the water-column (Davies‐Colley & Smith, 2001). This is caused mainly by increased light scattering, which increases the probability of photon extinction by absorption (Kirk, 1985, 1994). Reduced PAR availability affects the growth of seagrasses and other benthic primary producers (Erftemeijer & Lewis III, 2006; De Boer, 2007; Adams *et al.*, 2016; Bainbridge *et al.*, 2018).

Early work suggested that minimum PAR for seagrass species ranged from 4 to 29% of surface irradiance (Duarte, 1991; Dennison *et al.*, 1993; Erftemeijer & Lewis III, 2006). For *Z. muelleri* recent studies have measured minimum mean daily irradiance dose for seagrass persistence over time periods ranging from weeks to months (Longstaff, 2003; Collier *et al.*, 2011; Collier *et al.*, 2012; Bulmer *et al.*, 2016; Chartrand *et al.*, 2016; Collier *et al.*, 2016). In New Zealand, the light climate at the maximum depth limit for *Z. muelleri* in Kaipara Harbour (Bulmer *et al.*, 2016) was measured as an indication of minimum mean daily irradiance dose. Measurements of 2.1 mol photons $m^{-2}d^{-1}$ in winter (average temperature = 13 °C) and 4.91 mol photons $m^{-2}d^{-1}$ in summer (average temperature = 20 °C). Compensation irradiance (E_c) of *Z. muelleri,* as a another indicator of the minimum required daily irradiance dose, was found to lie slightly below these values (1.95 mol m^{-2} d^{-1}, assuming a 12 hr photo-period) in an Australian laboratory study conducted, at a temperature of 25℃ (Flanigan & Critchley, 1996). When suspended, fine sediments may reduce PAR to below these thresholds.

Fine sediment deposition may also cause seagrass decline through whole or partial burial, smothering plants and further shading them and reducing oxygen concentrations (Cabaço *et al.*, 2008b; Bainbridge *et al.*, 2018). Acute 'dumps' of fine sediment in coastal waters provoked by extreme natural events or construction, have been widely reported to cause large-scale alteration of seagrass habitat and, perhaps, irreversible disappearance (Kirkman & Kuo, 1990; Fourqurean & Rutten, 2004; Suykerbuyk *et al.*, 2016).

Studies indicate a range of tolerance to burial in different taxa from 1 to 20 cm (Cabaço & Santos, 2007; Brodersen *et al.*, 2017; Benham *et al.*, 2019). Burial tolerance seems to be related to plant size (Duarte *et al.*, 1997; Cabaço *et al.*, 2008b), with smaller plants generally more sensitive to burial than larger plants. In the case of *Zostera muelleri,* as little as 0.75-1 cm of sediment deposition has been reported to cause damage in Australia and New Zealand (Benham *et al.*, 2019; Siciliano *et al.*, 2020) respectively.

Even less is known about the effects of fine sediments intruded into the substrate, which can potentially modify rhizosphere physico-chemistry, and the interactive effects this modification may have on seagrass light requirements. "Muddification" is a term used to describe the increase in fine sediments and organic content at the expense of sand (Van Katwijk *et al.*, 2010), usually as a consequence of years of estuaries infilling with terrigenous sediments resulting from land development, agricultural activities and deforestation. Muddified substrate shows lower oxygen diffusivity due to smaller pore spaces and higher bulk densities. This effect combined with the increased oxygen demand associated with organic content of fine sediments (Bainbridge *et al.*, 2018) results, ultimately, in poorly oxygenated or anoxic substrate (Brodersen *et al.*, 2017). It can also elevate concentrations of phytotoxins such as heavy metals (Bonanno & Orlando-Bonaca, 2018), sulphide (Pedersen *et al.*, 2004; Borum *et al.*, 2005a) and herbicides (Haynes *et al.*, 2000a). Although seagrass can oxygenate their immediate rhizosphere, this is dependent on maintaining high rates of photosynthesis (Brodersen *et al.*, 2015; Brodersen *et al.*, 2017; Brodersen *et al.*, 2018),

To test the effects of substrate alteration and light attenuation both individually and combined, the interactive effects of PAR reduction and substrate "muddification" on the growth of the seagrass *Zostera muelleri* were studied in a factorial (2 x 2) mesocosm experiment. *Z. muelleri* sprigs were planted into two natural substrates, sourced from a single New Zealand estuary but differing in muddiness, and exposed to two different irradiance levels, both close to known persistence irradiance. Plant growth responses and substrate properties were monitored non-destructively for six-weeks, culminating in harvest of plant biomass at experiment completion. Growth of above and belowground tissues was compared.

4.3 Materials and Methods

4.3.1 Substrate and plant collection

Two substrate types from Pāuatahanui Inlet, North Island, New Zealand were collected (August 2019): (1) an inner estuary substrate with high mud content (42%, Table 4.1) where seagrass formerly grew (historical seagrass, HS); and (2) an outer estuary substrate with moderate mud content (20%, Table 4.1) from a location at which seagrass persists (existing seagrass, ES) (Figure 4.1A). Previous research has associated fine sediment pollution with seagrass loss in this inlet after 1980 (Matheson & Wadhwa, 2012). Top 5 cm of collected substrates were transferred to the mesocosms at the University of Waikato field laboratory in Tauranga, New Zealand for incubations.

The extracted top 5cm of substrates were sieved (2 mm Ø) to remove infauna and shell fragments and left to equilibrate for 5 days for stabilization of oxygen profiles prior to planting the seagrass at average ambient temperature 14.8 °C and at two irradiance levels close to the E_c for *Z.muelleri* low (6.3 mol photons $m^{-2}d^{-1}$, LL) and very low (2.3 mol m^{-2} d^{-1}, VL) PAR. Water temperature and light (lux) were monitored at 30 minute intervals using HOBO loggers (http://www.onsetcomp.com/) and PAR was measured on three occasions (beginning, middle and end of experiment) with a Li-Cor 192 Quantum Sensor (Li-Cor, Inc., US). Dissolved oxygen in water and sediment was measured three times per week and at three replicates per treatment, using a Needle Type oxygen microsensor, NTH-PSt7 (http://www.presense.com/). Substrate oxygen concentration was profiled every mm from 0 to 40 mm depth, which corresponded to the seagrass rootzone.

Vegetative fragments (sprigs) comprising a portion of rhizome with apical meristem and three leaf shoots were collected at low tide from Sulphur Point, Tauranga Harbour (Figure 4.1B), and immediately transported to the laboratory in seawater. Prior to transplanting, seagrass rhizome length was measured, and the numbers of shoots and internodes were recorded. Sprigs were transplanted individually into each tank and allowed to grow for 6 weeks before being harvested.

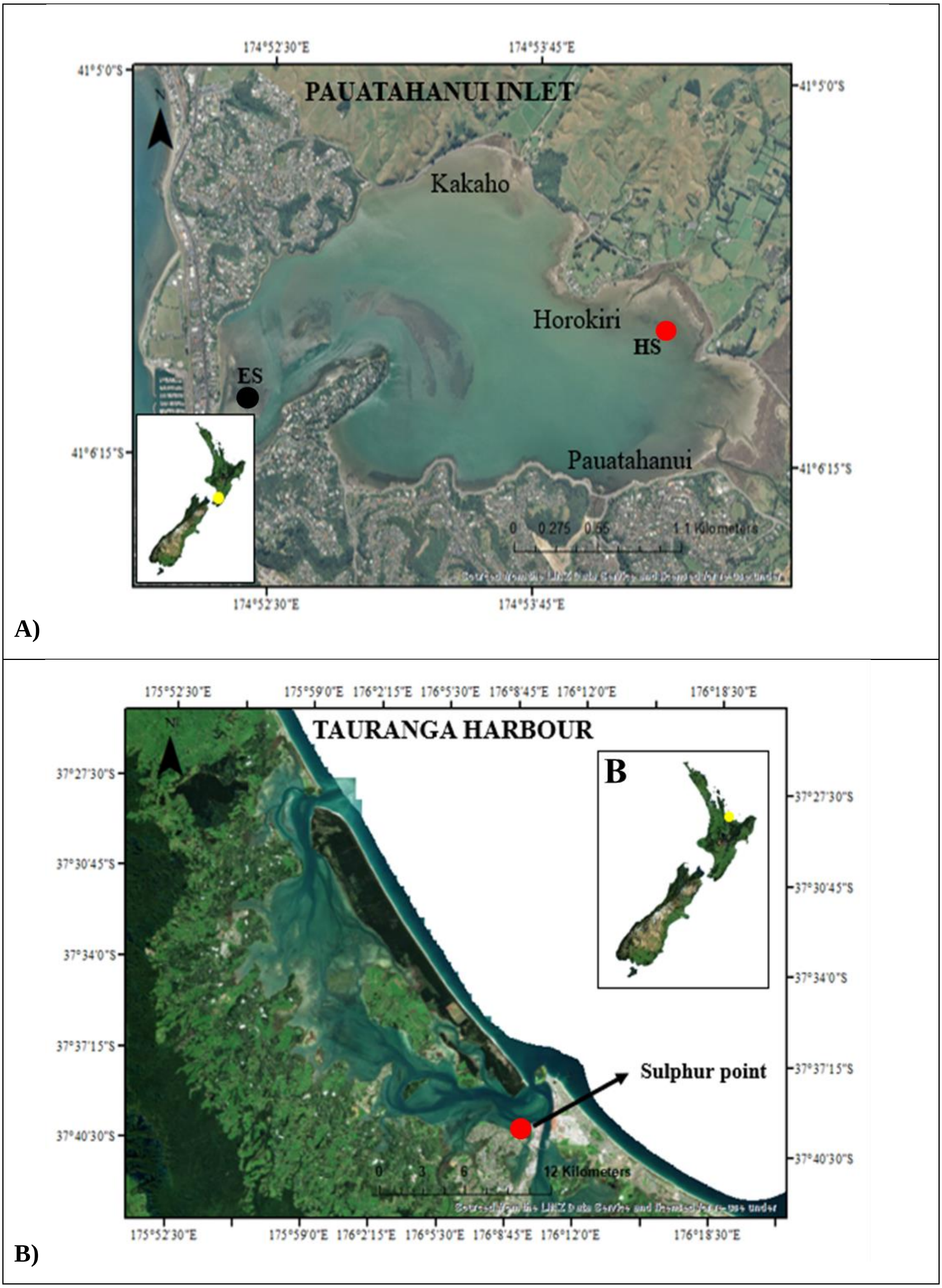


Figure 4.1: A) Map of Pāuatahanui Inlet (North Island, New Zealand) indicating the location where the two substrate types were collected: site in the inner estuary of Pāuatahanui Inlet where seagrass occurred historically but no longer grows (HS, red dot), and site in the outer estuary where seagrass continues to persist (ES, black dot). Kakaho, Horokiri and Pāuatahanui are the three major sediment sources. B) Map of Tauranga Harbour (North Island, New Zealand) indicating the locations of the meadow from which sprigs were collected (Red dot).

4.3.2 Experimental design and mesocosms setup

The mesocosm experiment was conducted in an indoor, recirculating mesocosm system using filtered seawater from Tauranga Harbour. The water content of each tank was renewed every two to three days and oxygenated and tanks were cleaned for epiphytes every two days. The mesocosm system comprised 40 tanks arranged in two sub-systems, each supporting 20 x 1.8L tanks mimicking subtidal conditions. Tank treatments were randomised within each sub-system. A 16:8 hour light:dark cycle was applied using J Series Cyanosis 1200 mm tubes (clear 4000K-4500K AC220-240V CRI90 http://www.ecopoint.co.nz) positioned above each subsystem. Irradiance was adjusted to provide a PAR of 2.3 mol m^{-2} d^{-1} (VL – very low) to one subsystem and 6.3 mol m^{-2} d^{-1} (LL – low light) to the other. It is likely that these irradiances are approaching the minimum for persistence of *Z. muelleri* and were chosen to increase the chance of detecting a light-sediment quality interaction. Under each irradiance, each of the two substrates, HS and ES were used to give a total of four treatments, each replicated 10 times (Figure 4.2). One sprig of freshly collected seagrass was transplanted into each treatment.

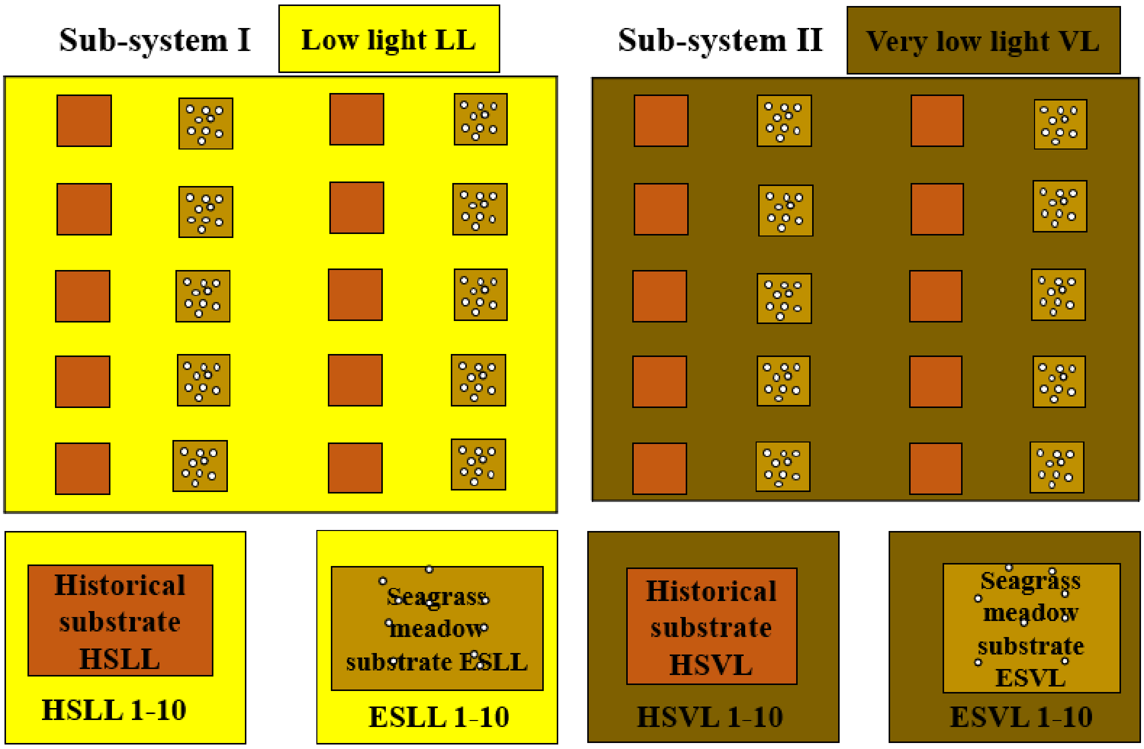


Figure 4.2: Diagram showing the distribution of the two substrate types and the two PAR levels within the mesocosms sub-systems. Tanks are set out regularly for diagrammatic clarity, but, for the experiment, their position was randomized. The four treatments are coded as: historical seagrass substrate under low light (HSLL), existing seagrass substrate under low light (ESLL), historical substrate under very low light (HSVL) and existing seagrass substrate under very low light (ESVL). One sprig per tank was transplanted (10 replicates per treatment). Low light (LL) was 6.3 mol photons $m^{-2}d^{-1}$ Very low light (VL) was 2.3 mol photons $m^{-2}d^{-1}$. White points symbolize the greater porosity of ES substrate.

4.3.3 Monitoring and laboratory analysis

4.3.3.1 Seagrass condition

Plant material was extracted from each tank, rinsed with water, and separated into aboveground biomass (AGB - shoots and leaves) and belowground biomass (BGB - roots and rhizomes), respectively. Plant samples were dried at 80 °C to constant weight to determine biomass per unit area (g m^{-2}) (Short & Coles, 2001).

Plant morphometric parameters were measured from digital images of each harvested plant using Image J software (https:/imagej.nih.gov/ij/). Images were calibrated using scale bars included in each image, and estimates of rhizome length, leaf length and leaf width were calculated to +/- 0.1mm. Increase in shoot number was calculated as the difference between the number of shoots at the end of the experiment and the number of shoot when transplanted. Rhizome length was used as the primary measure of initial plant size because of its importance in determining growth responses to disturbance in *Z. muelleri* (Macreadie *et al.*, 2014b) Senescent leaves were defined as those with > 50 % of leaf length lacking any green coloration and a “senescence ratio” was calculated for each plant as the ratio of senescent to total leaves.

4.3.3.2 Substrate condition

Substrate organic matter (% OM), was estimated as weight loss-on-ignition through combustion (450 °C for 4 h) and bulk density (g m^{-3}) was determined as weight of dry solids in a known volume substrate. Grain size was measured using a laser diffraction particle size analyser (Malvern Mastersizer 2000) and percent (%) mud particles (<63 µm) calculated. Porewater samples were extracted using a syringe to draw water through a hollow, 10 cm long and 4 mm diameter stainless steel cannula drilled with 1 mm diameter pores at intervals of 3 mm (McGlathery *et al.*, 2001). Extracted porewater was passed promptly through a (0.45 µm) pore filter for subsequent analysis of dissolved inorganic nutrients: nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+) and phosphate (PO_4^{3-}) ion concentrations using standard colorimetric techniques on a Lachat Quick Chem 8000 series flow injection analyser (FIA) + (Zellweger Analytics Inc. Milwaukee, Wisconsin, 53218, USA). Total sulphide was determined using the “methylene blue” APHA method (APHA 4500-S2-D).

Trace metal analysis was performed on transplanted sprigs and substrate samples to evaluate potential phytotoxicity, using an Agilent 8900 Inductively Coupled Plasma – Mass Spectrometer (ICP-MS; Agilent Technologies, Santa Clara, California, USA) controlled by a

MassHunter Workstation (version 4.5). Substrate concentrations were evaluated for toxicity following ANZECC (2000) which are Australasian guidelines of general estuary health, non seagrass specific.

4.3.4 Data analysis

Statistical analysis of data was performed using the R statistical package (v 3.6.2) in R Studio. If not stated otherwise, all mean values are presented with standard errors of the mean (mean ± SE). Irradiance and substrate (both fixed factors) effects were tested using a two way-ANOVA (Zar, 1984), followed by the post hoc Tukey tests for multiple comparisons (Tukey, 1977) in the case of a significant ANOVA result (with significance level set, conventionally, at 5% probability of type I error).

In all the analyses, homogeneity of variance was examined using the Cochran test and when required data was log-transformed.

4.4 Results

4.4.1 Substrate and PAR

Substrate quality parameters and PAR per treatment as well as substrate trace metal concentrations are shown in Table 4.1, Table 4.2 & Chapter 4, Appendix; these reveal that HS substrate was muddier and denser than ES substrate, which concurs with the findings in Chapter 3.

Table 4.1: Physico-chemical characteristics of the two substrates. Values are means (±SE). Significant differences ($p<0.05$, Tukey HSD test) among treatments are indicated by different alphabetic superscripts. ANOVA table can be found in (Chapter 3, Appendix).

Treatment	Sediment mud (%)	Sediment organic matter (%)	Sediment bulk density (g cm^{-3})	Sediment porewater [H_2S] (µM)	Sediment porewater [NH_4^+] (µM)	Sediment porewater [PO_4^{3-}] (µM)
Historical substrate HS	42 ± 1.7 ^{b}	2.3 ± 0.2 ^{a}	1.6 ± 0.1 ^{b}	2.6 ± 0.6 ^{b}	10.6 ± 1.5^b	3.1 ± 1.7 ^{a}
Existing substrate ES	20 ± 0.9 ^{a}	2.4 ± 0.1 ^{a}	1.0 ± 0.04 ^{a}	1.4 ± 0.4 ^{a}	5.4 ± 0.6 ^{a}	2.3 ± 1.2 ^{a}

Table 4.2: Temperature and Photosynthetically Available Radiation (PAR) values for the treatments. Values are means (±SE). These span a published compensation irradiance (E_c) of 1.95 mol photons m^{-2} d^{-1} assuming 12-h photoperiod (Flanigan & Critchley, 1996).

Treatment	Water temperature (°C)	Photosynthetically Available Radiation (PAR) (mol photons $m^{-2}d^{-1}$)
Low Light LL	14.8 ± 0.7 ^{a}	6.3 ± 0.6 ^{b}
Very Low Light VL	14.8 ± 0.7 ^{a}	2.3 ± 0.8 ^{a}

4.4.2 Seagrass response to experimental treatments

Response of certain seagrass metrics to different treatments is shown in Figure 4.3. The treatment combinations HSLL, ESLL and ESVL had similar shoot production of 3.3 to 3.9 shoots $tank^{-1}$ whereas HSVL (muddy substrate under very low light) had much lower shoot production averaging just 0.7 shoots $tank^{-1}$ (Figure 4.3). Rhizome growth was ordered ESLL> ESVL > HSLL >HSVL (with significant change at each step) (Figure 4.3) suggesting that substrate predominated over irradiance in influencing rhizome growth. HSVL was the only treatment with negative rhizome growth (i.e. loss of rhizome biomass). At harvest, belowground biomass (BGB was lower in VL treatments than in LL, suggesting an effect of irradiance. Above ground biomass (AGB) was lowest in HSVL (as expected), but there was high variance and no significant differences were detected among treatments (Figure 4.3). Total biomass, for which data is given in Chapter 4, Appendix, followed BGB and was significantly lower in HS compared to ES treatments, with no significant effect of irradiance. Senescence ratio was not significantly different among the four treatments (Chapter 4, Appendix).

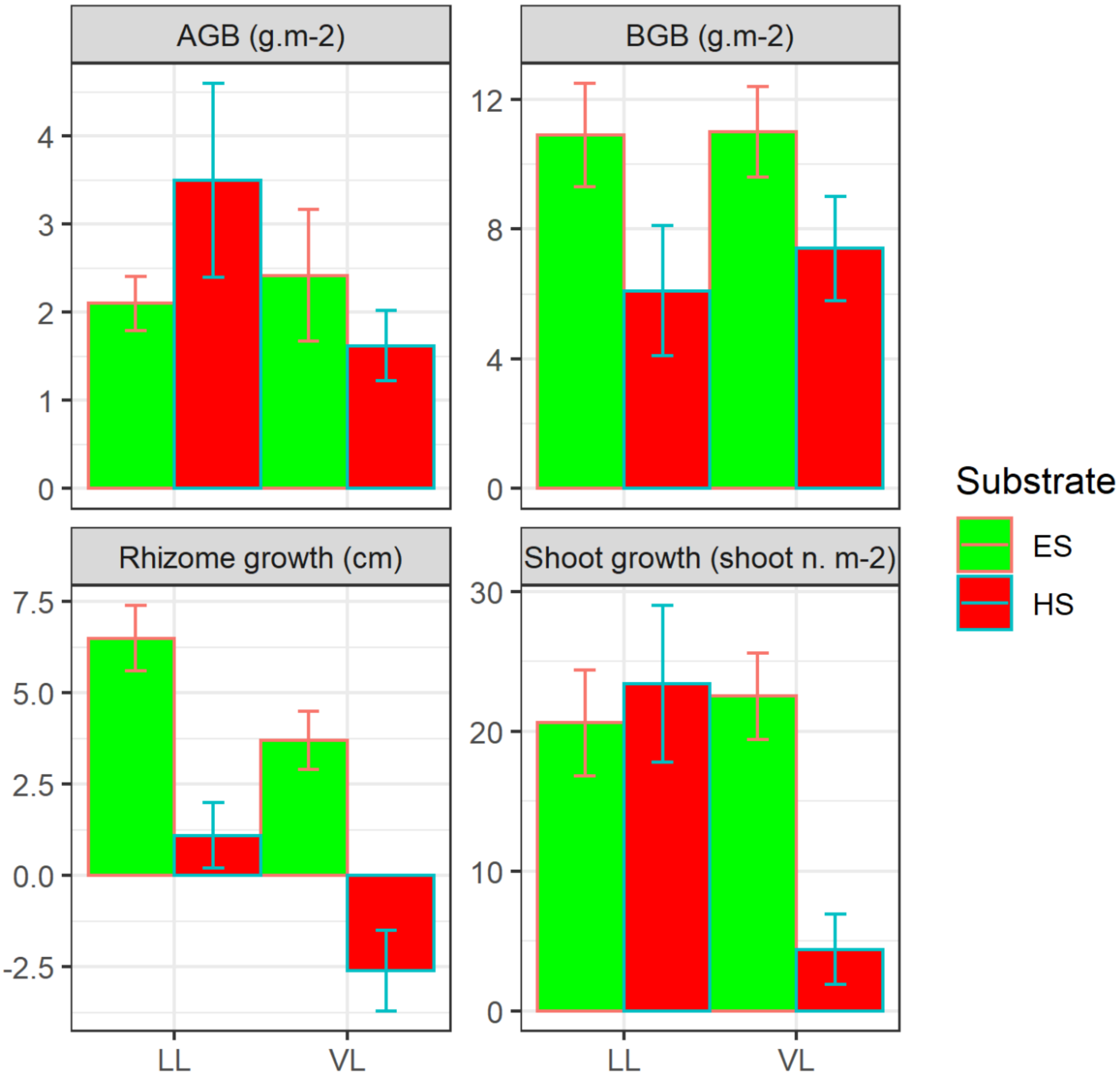
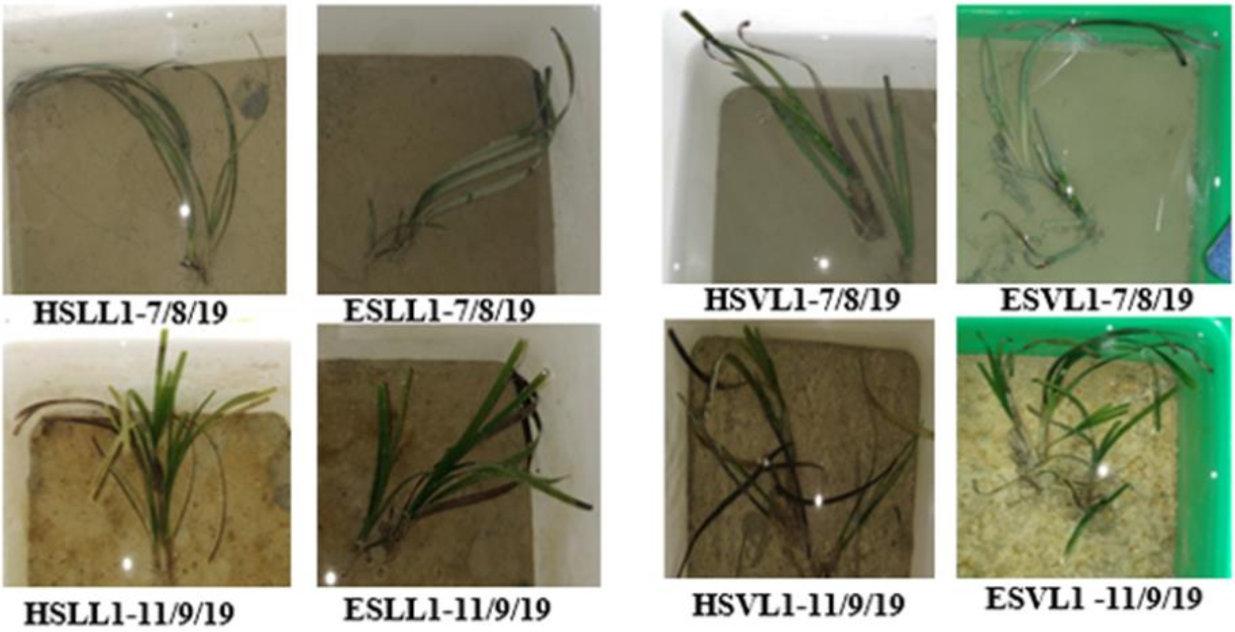


Figure 4.3. Seagrass traits at the end of the experiment: Shoot number increment, rhizome growth above ground biomass (AGB) and belowground biomass (BGB). Values are means (±SE) per treatment (n=40). Significant differences (ANOVA, Tukey HSD, $p<0.05$) among treatments are indicated by (*). Treatments are coded as: historical seagrass substrate under low light (HSLL), existing seagrass substrate under low light (ESLL), historical substrate under very low light (HSVL) and existing seagrass substrate under very low light (ESVL).


HSLL1-7/8/19 ESLL1-7/8/19 HSVL1-7/8/19 ESVL1-7/8/19

HSLL1-11/9/19 ESLL1-11/9/19 HSVL1-11/9/19 ESVL1 -11/9/19

Figure 4.4: Pictures show one replicate per treatment at the beginning of the experiment and immediately prior to harvesting. The four treatments are coded as: historical seagrass substrate under low light (HSLL), existing seagrass substrate under low light (ESLL), historical substrate under very low light (HSVL) and existing seagrass substrate under very low light (ESVL).

ANOVA (Table 4.3) showed that the increase in shoot number was significantly affected by irradiance ($p = 0.035$) and by the interaction between irradiance and substrate type ($p = 0.012$). Substrate muddiness did not significantly affect the increment of shoot number at the 5% confidence level but it was significant at 10% level ($p=0.09$). Rhizome growth was significantly influenced by both irradiance ($p = 0.001$) and substrate ($p<0.0001$), but not by their interaction. BGB and total biomass were significantly affected by substrate type ($p = 0.015$ and $p = 0.028$) but not by irradiance treatment. AGB and senescence ratios were not significantly affected by any treatment or interaction.

Table 4.3: 2-way ANOVA analysis for seagrass traits. Significant effects and interactions ($p<0.05$) between irradiance and substrate are shown in bold for each of the seagrass traits.

Trait	Factor	Df	Mean sq	F value	Pr (>F)
Increase in shoot number	Substrate	1	13.225	3.023	0.0906
	Irradiance	1	21.025	4.806	**0.0349***
	Interaction	1	30.625	7	**0.012***
	Residual	36	4.375		
Rhizome growth	**Substrate**	1	337.6	38.884	**3.37e-07***
	Irradiance	1	105.6	12.163	**0.0013***
	Interaction	1	2.3	0.269	0.6072
	Residual	36	8.7		
AGB	Substrate	1	7.747	0.958	0.334
	Irradiance	1	8,752	1.122	0.297
	Interaction	1	9.851	1.263	0.269
	Residual	36	7.801		
BGB	**Substrate**	1	4.516	6.519	**0.0151***
	Irradiance	1	0.139	0.201	0.6566
	Interaction	1	0.086	0.125	0.7259
	Residual	36	0.693		
Total Biomass m^{-2}	**Substrate**	1	3.813	5.228	**0.0282***
	Irradiance	1	0.001	0.001	0.978
	Interaction	1	0.08	0.11	0.7423
	Residual	36	0.729		
Senescence ratio	Substrate	1	2.814	0.766	0.388
	Irradiance	1	3.984	1.084	0.305
	Interaction	1	3.703	1.008	0.322
	Residual	34	3.674		

4.4.3 Substrate oxygen concentration profiles

The temporal evolution of sediment oxygen profiles from week 1 to week 6 shows a separation between light treatments for the first 4 weeks but there was a progressive convergence of profiles over time until by week 6 they were very similar (Figure 4.5). The PAR effect was larger than that of substrate type, although HS tended to have lower oxygen concentrations than ES. By six weeks all substrates showed anoxia at 4-5 mm depth.

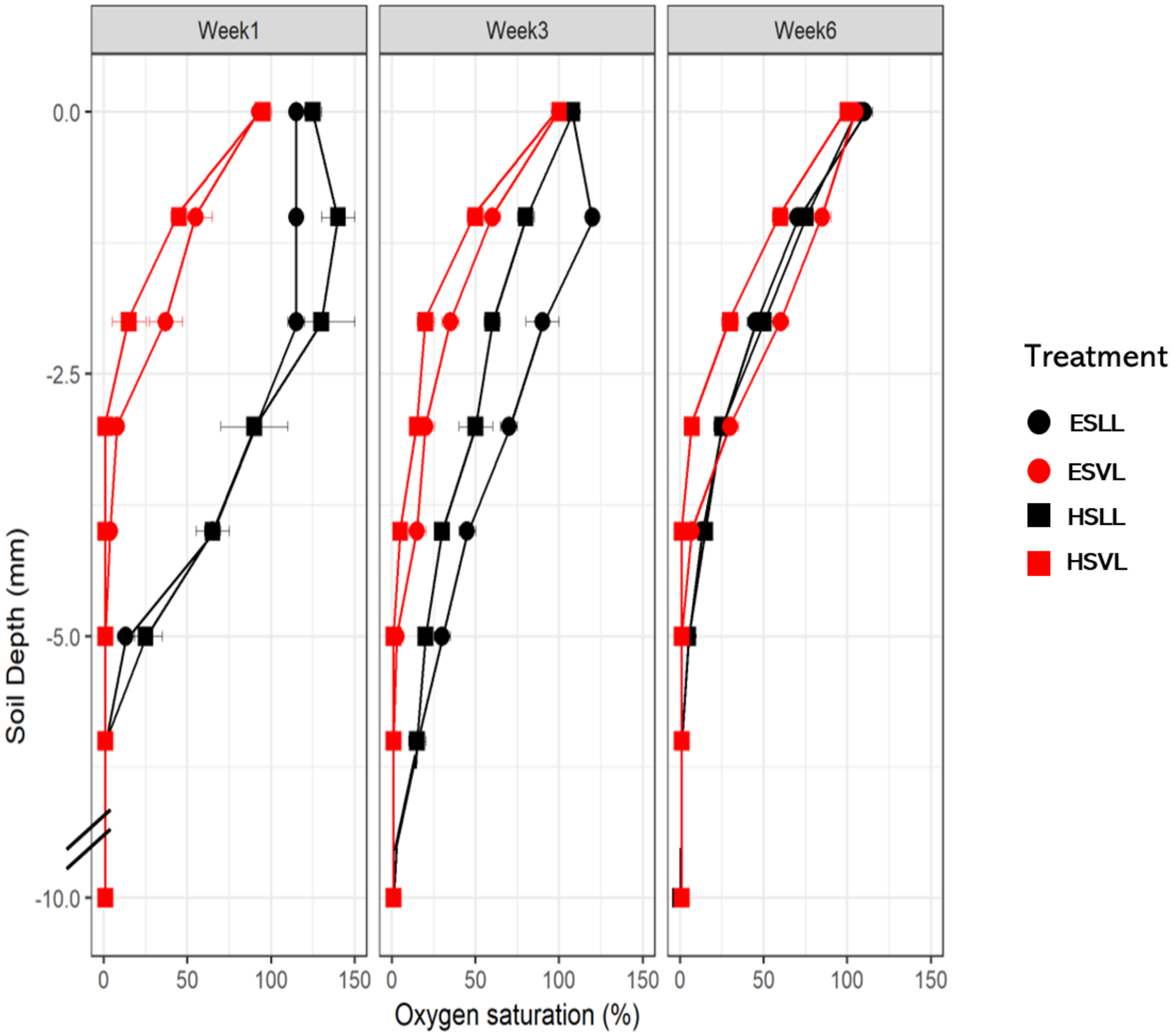


Figure 4.5: [O_2] depth profiles (means ±SE) among treatments and week of exposure. Figures truncate at 15 mm as profiles remain anoxic deeper than that substrate depth.

4.4.4 Substrate and bioaccumulated elemental concentration

Arsenic, chromium, cadmium, copper and lead concentrations exceeded recommended Sediment Quality Guidelines (SQG) thresholds (ANZECC, 2000) in one or both substrates (Figure 4.6). Arsenic and chromium concentrations exceeded SQG in the ES substrate but not HS substrate, and copper and lead exceeded SQG in both substrate types with no significant differences between substrates. Cadmium concentration was significantly higher in the HS substrate compared to ES substrate and both exceeded the guidelines. Nickel and zinc were below toxicity thresholds with the latter higher in HS than ES substrate (Figure 4.4).

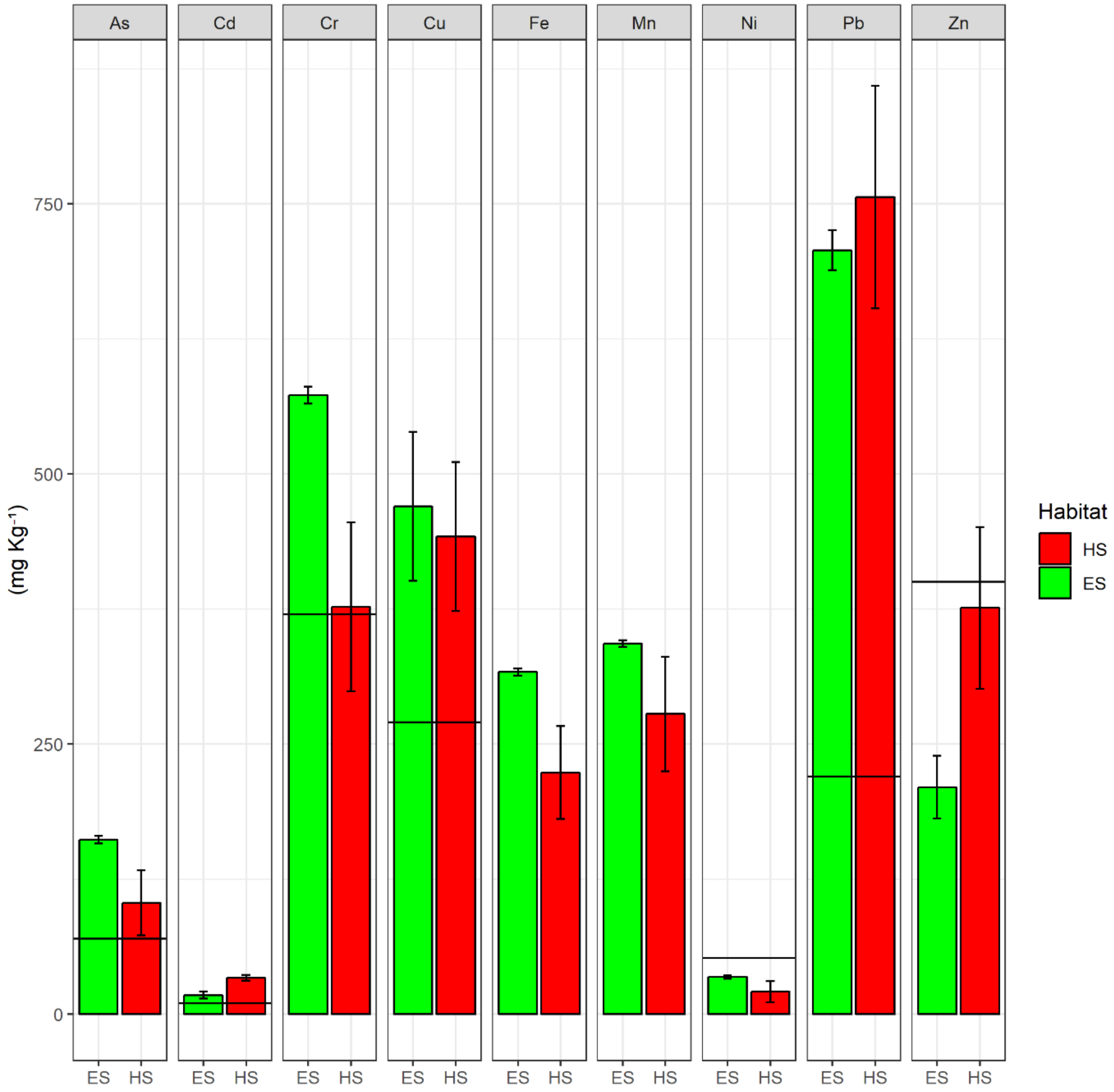


Figure 4.6: Elemental concentrations (means ±SE) on the two substrates. Horizontal black lines show the ANZECC recommended Sediment Quality Guidelines (SQG) threshold for each element.

Bioaccumulated copper and lead concentrations in both *Z. muelleri* AGB and BGB exceeded the reported toxicity thresholds in both HS and ES substrates, with no significant differences between substrates. Arsenic, cadmium, chromium and zinc were below toxicity thresholds reported in the literature (Figure 4.7).

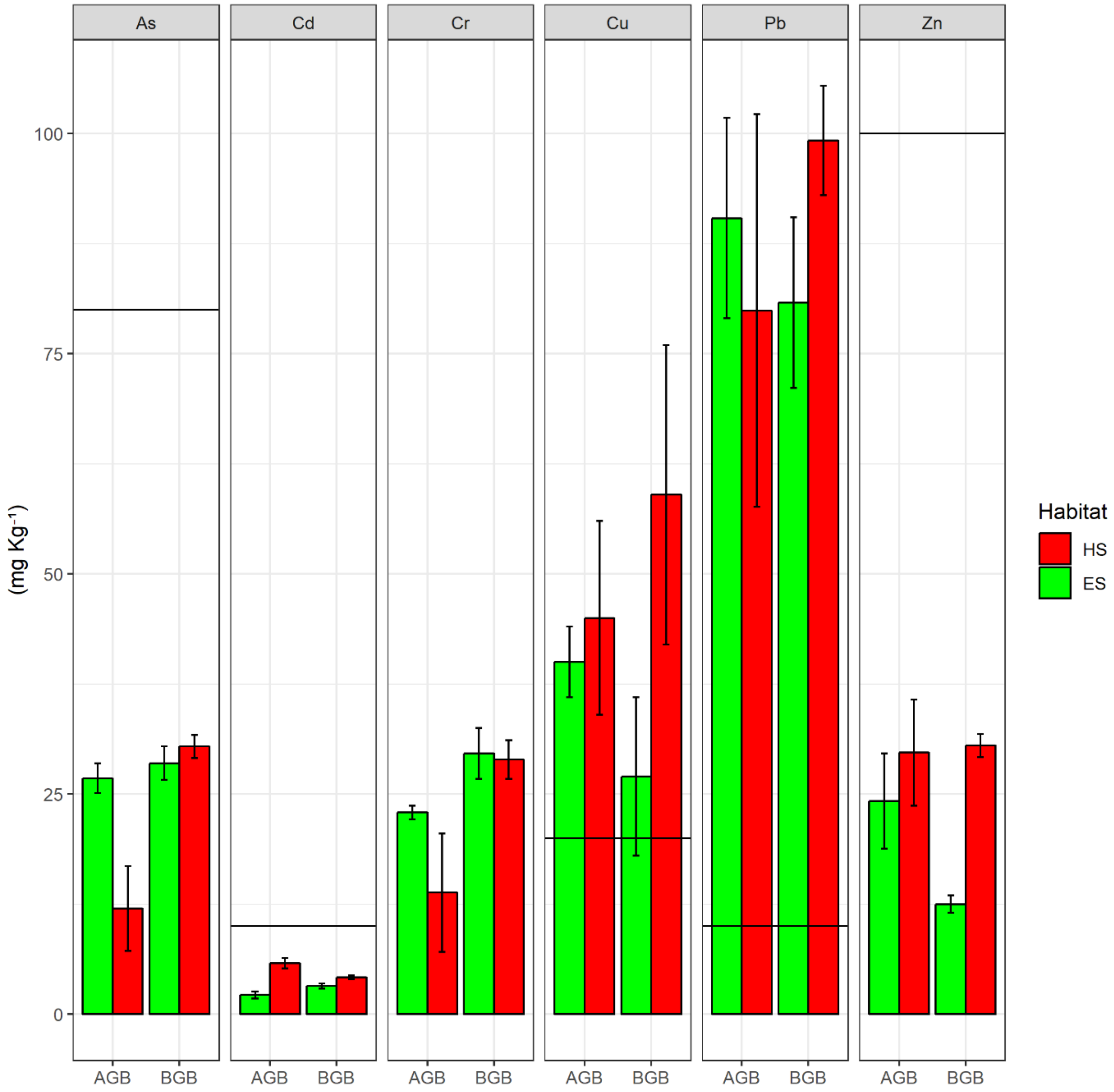


Figure 4.7: Elemental concentrations (means ± SE) of the sprigs aboveground biomass (AGB) and belowground biomass (BGB) on both substrates (historical substrate, HS) and (existing substrate, ES). Horizontal black lines show the thresholds reported in the literature (Table 4.5) for each element on *Zostera* genus species if available. Reported bioaccumulated element concentrations of other locations and species to provide context are shown in (Table 4.5).

4.5 Discussion

This study examined the combined effects of substrate quality and low irradiance on seagrass performance. Clear effects of the two variables were found, both separately and interactively. All plants growing in HS substrate had consistently lower rhizome growth and belowground biomass than those in ES substrate. There was no differential in belowground biomass between low and very low irradiances, regardless of sediment type, but shoot density was adversely affected by very low irradiance. There was an interaction between irradiance and substrate for shoot production such that the lowest shoot production was in the combination of HS and VLL.

While low, the irradiances used in this experiment were both sufficient to support biomass increase in terms of rhizome extension and new shoot generation at least in the short term ($\leqslant$ 6 weeks). Both were above the winter limiting PAR threshold of 2.1 mol $m^{-2}d^{-1}$, at 13 °C, estimated for *Z. muelleri* at nearby Kaipara Harbour, New Zealand (Bulmer *et al.*, 2016). Studies performed at a range of latitudes seem to be converging on a long-term overall daily minimum light requirement for *Z. muelleri* persistence of around 5 mol photons $m^{-2}d^{-1}$ which is lower than our LL treatment but higher than VL (Chapter 2, Table 2.4).

For *Z. muelleri* compensation irradiance (E_c, which is the irradiance at which oxygen production = respiration) for tropical individuals is 45 µmol $m^{-2}s^{-1}$ which translates (approximately) to a daily requirement of 1.9 mol $m^{-2}d^{-1}$ at 25 °C assuming a 12 hr photo-period (Flanigan & Critchley, 1996). Temperature plays an important role in determining PAR persistence thresholds (York *et al.*,2013), with required irradiance tending to increase with increasing temperature (Flanigan & Critchley, 1996; Longstaff *et al.*, 1999; Collier *et al.*, 2011; Collier *et al.*, 2012; Bulmer *et al.*, 2016; Chartrand *et al.*, 2016; Collier *et al.*, 2016). Therefore, the fact that, E_c of tropical *Z. muelleri* is higher than the VL treatment, in which positive growth occurred in ES substrate, and also higher than PAR at MDL determined for the winter time in Kaipara (Bulmer *et al.*, 2016) may be explained by the fact that temperature plays a strong role in the carbon balance of this species and moreover because respiration increases at higher temperatures so more photosynthesis is required to compensate for it as well as a higher substrate oxygen demand due to bacterial action on organic matter. Production irradiance (PI) curves for *Z. muelleri* which will provide context on the E_c determined by this project are shown in (Chapter 7).

In Chapter 3, the status of the HS substrate was documented, noting the low porosity and the tendency towards reduced chemistry. The low redox state of HS substrate was borne out further in this chapter by the tendency for a shallower oxycline. A shallow oxycline is consistent with a low porosity, and/or a high bulk density. Seagrasses have been shown to persist in hypoxic sediments through root oxygen release, a mechanism that requires adequate aboveground photosynthesis to oxygenate roots and their rhizosphere (Brodersen *et al.*, 2015; Brodersen *et al.*, 2017). The nature of HS may have exacerbated the effect of toxins such as hydrogen sulphide or heavy metals (Duarte *et al.*, 1997; Terrados *et al.*, 1999; Halun *et al.*, 2002; Brodersen *et al.*, 2015; Brodersen *et al.*, 2017; Brodersen *et al.*, 2018). Here, belowground biomass was negatively affected by the HS substrate conditions regardless of the irradiance, but rhizome growth was affected by both irradiance and substrate. HSVL treatments showed lower rhizome growth than HSLL, indeed HSVL was the only treatment in which rhizome growth was negative. This exemplifies the impact of muddification on growth and health of belowground structures. VL also reduced rhizome growth in ES sediments, which suggests a limit to allocation of resources to belowground growth when irradiance is very low, even if the sediment conditions are favourable (e.g. maximizing surface area for absorption of light vs increasing storage). Rhizome growth is an important trait as it is the main mechanism of recovery after disturbance (Larkum & West, 1983; Marbà & Duarte, 1998; Meehan & West, 2000; Jarvis & Moore, 2010). A disturbance/recovery experiment performed on *Z. muelleri* populations of Lake Macquarie (Macreadie *et al.*, 2014b) concluded that asexual, clonal growth and regeneration (via rhizome extension) is the only available mode of recovery from small-scale disturbances (e.g., anchor and boat damage, grazing and storms) (Macreadie *et al.*, 2014b). Seeds and seed dispersal play an important role in the recovery response of seagrass beds to larger scale disturbances such as wasting disease, eutrophication and sedimentation (Orth *et al.*, 2006).

The precise sediment chemical profile that makes HS less suited to seagrass growth than ES cannot be inferred from our data and indeed, it may not be a single factor but a multiplicity of factors all arising from increased muddification. While porewater ammonium ion and total sulphide levels were significantly higher in HS substrates compared to ES substrates, neither approaches known toxicity thresholds. The highest recorded ammonium ion concentration of 10.6 µM in the HS substrate is well below the toxicity thresholds of >1×10^5 µM, for *Z. muelleri* (Gladstone-Gallagher *et al.*, 2018; Li *et al.*) and 200-4000 µM for the related species *Z.noltii* (Govers *et al.*, 2014). Similarly, the highest total sulphide concentration of 2.6 µM is lower than thresholds of 10 µM and 13 µM determined for other Zostera genus species by Calleja *et al.* (2007); Krause-Jensen *et al.* (2011).

Substrate oxygen profiles did not show differences between HS and ES substrates. However, differences between light treatments occurred in the first three millimetres and these differences were attenuated from 3 to 5 mm deep in the substrate. Deeper than 5 mm no differences were observed between light treatments. During week 3 HSLL and ESLL showed slower oxygen consumption than HSVL and ESVL in the first 3 mm of substrate possibly caused by oxygen production from microphytobenthos (MPB) which is often a main contributor to oxygen production in estuaries (Barranguet *et al.*, 1998; Thrush *et al.*, 2012). Convergence of oxygen profiles during week 6 may be due to lower oxygen production rates during the last week of the experiment and equalization of the mineralization rates of the four treatments in the upper few millimetres of the substrate as MPB biomass was less effective in oxygenating surface sediments, perhaps through nutrient depletion (Jesus *et al.*, 2009).

While we found that all treatments were anoxic within the substrate depth of 5-50 mm, which is the seagrasses root zone, and thus could not conclusively demonstrate that anoxia is the main or only seagrass damage mechanism and therefore interactions between muddification and irradiance are suggested to be key as well as other toxicants such as heavy metals synergistically acting with substrate physical properties such as muddiness, bulk density and porosity.

Substrate arsenic, chromium, cadmium, copper and lead concentrations exceeded (ANZECC, 2000) recommended Sediment Quality Guidelines (SQG) thresholds at both habitats. These thresholds are generalized indicators of healthy sediment values, but they are not necessarily seagrass related and therefore only cautious conclusions are possible. In New Zealand, arsenic is naturally generated in geothermal regions, lead is probably residual from the use of leaded petrol and, since this was discontinued in 1996 in New Zealand, lead accumulation is not expected to grow through time. However, legacy lead is apparent in our results presumably arising from historic use of leaded petrol. Copper has previously been, and is still, used in horticultural sprays (Jeyakumar *et al.*, 2014). Cadmium as a trace contaminant of superphosphate fertiliser continues to be applied to pastureland. Heavy metals are potentially incorporated into seagrass leaves and vascular tissues both from the water column and substrate but mainly through uptake from the substrate (Korpinen *et al.*, 2010) and very few studies have assessed substrate heavy metal phytotoxicity to *Z. muelleri* seagrass. Moreover, limited information is available on the effect of heavy metal concentrations and toxicity thresholds for *Z. muelleri* to our knowledge.

Previous studies have underlined the importance of the leaching of toxic metals such as copper and lead from land into marine sediments (De Casabianca *et al.*, 2004) and suggested that most experiments testing seagrass response to contaminants are done using water-only leaf exposure (Macinnis-Ng & Ralph, 2004; Skillington *et al.*, 2020) or with the plants growing in terrestrial soil, rather than in natural sediment (Nielsen *et al.*, 2017). Our results of bioaccumulated element concentrations showed that copper and lead concentrations exceeded the toxicity thresholds applied to *Z. japonica* (which were derived from the average content in plant tissues and toxicity levels (Krämer, 2010)) of 20 mg kg^{-1} and 10 mg kg^{-1} levels respectively (Lin *et al.*, 2016). Our results exceeded these reported thresholds for copper and lead by 300-500 % for sprigs planted into *both* HS and ES substrates (Table 4.5), suggesting higher tolerance of *Z. muelleri* in comparison with values reported by (Lin *et al.*, 2016)

The bioaccumulation of metals may occur through uptake of inorganic salts or organo metals, the latter are readily bioavailable. In this study, we can attribute a higher bioaccumulation of lead and copper compared to cadmium to higher levels in the sediments, their greater solubility as inorganic salts as well as the historic input of readily bioavailable tetraethyllead, previously used in petrol.

Substrate and bioaccumulated elemental concentrations reported in other studies of *Zostera* genus are shown to provide meaningful interspecific and intraspecific comparisons (Table 4.5). However, more research towards the effect of heavy metals incorporated in the substrate and their effects on different seagrass species is needed in order to establish toxicity thresholds and for more robust comparisons to be made. Hu *et al.* (2019) reported in a comparative study, that *Z. marina* and *Z. muelleri* seem to have stronger heavy metal bioaccumulation capacity than other eelgrasses (*Z. japonica*, *Z. caespitosa* and *Z. noltii*), which is support by our data, but further research would be desirable.

Our results suggest that the concentrations measured in this study are not high enough to damage seagrass *Z. muelleri* in ES substrates but may contribute to a synergistic effect in HS substrates. However, we have no data on elemental concentration prior to harvesting the sprigs and there is no certainty that the concentration after 6 weeks is due to uptake from Pāuatahanui Inlet substrates or is inherent as a result of the Harbour environment from which the plants were sourced (Tauranga Harbour). This topic is definitely worth consideration for further studies. It is also acknowledged, the limitation of pseudoreplicated treatments and that experimental mesocosms results may or may not be transferred to real-life natural situations. Nevertheless, the experiment helped further understand seagrass substrate irradiance interactions and effects.

Table 4.4: Literature reported elemental concentrations values and ranges for *Zostera* spp biomass and substrates when available. Values are given in mg Kg^{-1} DW (dry weight). Values exceeding literature reported bioaccumulated thresholds in [] are identified with (*) in our and other studies as well as shown in bold.

Seagrass	**Tissue or substrate**	**Location**	**Element**							
			As [80] (mg Kg^{-1})	**Cu* [20]** (mg Kg^{-1})	**Zn* [100]** (mg Kg^{-1})	**Cd* [10]** (mg Kg^{-1})	**Cr** (mg Kg^{-1})	**Hg** (mg Kg^{-1})	**Mn** (mg Kg^{-1})	**Pb* [10]** (mg Kg^{-1})
Z. muelleri	**AGB HS**	**This study**	12.0	**45.0**	29.7	5.8	13.8	-	273.7	**32.2**
	BGB HS		30.4	**59.0**	30.5	4.2	28.9	-	340.7	**66.4**
	AGB ES		26.8	**40.0**	24.2	2.2	22.9	-	203.7	**56.9**
	BGB ES		25.8	**27.0**	12.5	3.2	29.6	-	263.7	**54.3**
	Substrate HS		**103.0**	**442.0**	376	**33.7**	377	-	278	**756**
	Substrate ES		**161.3**	**470.0**	210	**17.7**	573	-	343	**707**
Z. marina	AGB	Limfjord Sea (Denmark) [a]	-	1.9	41.0	0.1	-	-	-	0.5
	BGB			16.6	175.0	2.9				**37.5**
Z. marina	BGB	Thau Lagoon (France) [b]	-	9.0	44.0	-	2	-	-	2
	Substrate			18.7	36.1		21.8			13.8
Z. marina	Whole plant	Posyet Bay (Russia) [c]	-	2.1	12-64	1.6	0.01	-	-	Bd
Z. marina	Whole plant	Bosphorus Strait (Turkey) [d]	-	**23.4-39.8**	48.7-91.3	1.9-2.3	8.3-13.6	-	-	**26.1**
	Substrate			19.3-46.2	42.8-98.3	2.3-3.2	18.7-61.8			35.7-135.3
Z. marina	AGB	Sanggou Bay (China) [h]		18.3 ± 6.6	28.1 ± 13.7	5.12 ± 1.8	12.7 ± 3.2			4.90 ± 2.30
	BGB			10.5 ± 4.7	32.6 ± 14.4	1.6 ± 0.97	18.3			**16.67 ± 7.2**
Z. marina	AGB	Koje Bay (Korea) [i]	0.2-0.3	17-**20**	20-53	0.5-0.6				**20-53**
	BGB		0.2-0.4	9.9-14.4	12-25	0.16-0.2				0.7-1.5
	Substrate		6-19	16-148	171-424	0.3-1				75-165
Z. japonica	BGB	Yellow River Estuary (China) [e]	33.8	10.5	33.16	1.7	5.84	0.02	928.3	4.32
Z. muelleri	AGB	Port Curtis (Australia) [f]	-	12.3	74.7	-	30.6	-	-	-
	BGB			3.1	60.2		29.7			

Seagrass	**Tissue or substrate**	**Location**	**Element**							
			As [80] (mg Kg⁻¹)	**Cu* [20]** (mg Kg⁻¹)	**Zn* [100]** (mg Kg⁻¹)	**Cd* [10]** (mg Kg⁻¹)	**Cr** (mg Kg⁻¹)	**Hg** (mg Kg⁻¹)	**Mn** (mg Kg⁻¹)	**Pb* [10]** (mg Kg⁻¹)
Z. muelleri	Whole Plant	Lake Macquarie (Australia) [g]	-	9.4	133	**10**			1.7	

a) (Brix *et al.*, 1983); b) (De Casabianca *et al.*, 2004); c) (Chevnova *et al.*, 2002) d) (Güven *et al.*, 1993); e) (Lin *et al.*, 2016); f) (Prange & Dennison, 2000);

g) (Barwick & Maher, 2003); h) (Hu *et al.*, 2019); i) (Lee *et al.*, 2019)

Thresholds: (Lin *et al.*, 2016) → *Cu) (Kabata-Pendias, 2011); *Zn) (Krämer, 2010); *Pb) (Macinnis-Ng & Ralph, 2004); *Cd) (Macinnis-Ng & Ralph, 2004).

4.6 Conclusion

Research on estuary pollution with fine sediments and its effect on seagrass performance has been focused mainly on the reduction of the available PAR reaching the seabed. However, fine sediments may also stress seagrass by substrate muddification, which has been demonstrated to interact with irradiance in our mesocosm experiments. These interactions need to be further studied because seagrasses inhabiting muddified substrates appear to have increased light requirements for persistence due to reduced oxygen and/or other detrimental effects in the substrate such as increasing concentrations of sulphide or heavy metals. Therefore, PAR thresholds may not be fit for purpose if interactions with different substrates are not considered, leading to inaccurate assessment of habitat suitability.

Chapter 5

Examining the relative influence of substrate physicochemical condition versus smothering and light climate effects on seagrass growth: A transplanting experiment.

5.1 Abstract

A field experiment was carried out at Pāuatahanui Inlet with the aim of testing whether altered substrate conditions resulting from estuary siltation can be a primary driver of seagrass loss and failure to re-establish at former locations in New Zealand. The growth responses of seagrass following controlled exchanges of bare substrates among historical (HS) and existing seagrass habitats, (ES) were measured. Intact cores of substrate were transferred from HS habitat to ES habitat and vice versa at upper tidal and lower tidal fringes. Controls were established by extracting cores and replacing them at the same site. Healthy vegetative fragments (sprigs) of seagrass were collected from ES habitat. These were planted into bare substrates at both HS and ES habitats. In addition, a set of intact cores containing seagrass from ES habitat were transplanted at both HS and ES habitats which allowed inter-methodological comparison. As the experiment progressed, some challenges to its successful completion emerged. Firstly, it proved impossible to reliably relocate the sprigs transplanted into the ES habitat (both upper and lower meadows). The small size of *Zostera muelleri* plants makes them hard to mark without damaging them. Secondly, an incursion of the filamentous green alga *Chaetomorpha ligustica* smothered approximately half of the quadrats at the lower tidal ES habitat. Our experimental findings indicate that cores may be a more successful technique for transplantion of *Zostera muelleri* in intertidal areas. Given the difficulties relocating transplanted sprigs limits and gaps in data availability mean that few clear patterns related to the planned treatments emerge from results. However, it can be concluded that the cumulative effect of rhizosphere deterioration, lower irradiance and close location to a source of natural sediment input during events such as storms may underlie the inability for seagrass to re-establish at Pāuatahanui Inlet historical habitat.

5.2 Introduction

The ecological and economic values of seagrasses as well as fine sediment pollution effects on seagrasses are reviewed in Chapter 2. In response to widespread declines of seagrasses, restoration efforts have been carried out at locations across the globe, particularly in the USA, Australia, Europe and Japan (Larkum & West, 1983; Fonseca *et al.*, 1998; Peralta *et al.*, 2003; Paling *et al.*, 2009; Van Katwijk *et al.*, 2009; Leschen *et al.*, 2010; Fonseca, 2011; Domínguez *et al.*, 2012; Greiner *et al.*, 2013; Moksnes *et al.*, 2018). Worldwide, it is estimated that over 50% of the transplantation efforts have failed (Reed *et al.*, 2004; Orth *et al.*, 2006; Van Katwijk *et al.*, 2009). Nevertheless, important information gleaned from these projects, can guide future restoration attempts.

Past efforts have resulted in the development of five key recommendations to guide seagrass restoration (Van Katwijk *et al.*, 2009). These should be considered and adapted to site and species specificity before proceeding (Campbell & Paling, 2003; Van Katwijk *et al.*, 2009; Fonseca, 2011). Prior to restoration effort, causes of the decline should be known and reversed or alleviated (Cunha *et al.*, 2005; Paling *et al.*, 2009; Cunha *et al.*, 2012). Secondly, *site selection* for restoration effort needs to be appropriate, and several conceptual models and dichotomous trees have been published to guide this step (Campbell, 2002; Short *et al.*, 2002). Thirdly, selection of an appropriate donor population is required, which should be based upon plants that are adapted to the local environmental conditions (Calumpong & Fonseca, 2001) and are genetically diverse (Procaccini & Piazzi, 2001; Reusch *et al.*, 2005; Reusch & Hughes, 2006). The inevitable risk of plant losses needs to be considered and consequently replicate plantings are advisable especially where monitoring data is to be gathered to report of the effectiveness of the restoration efforts (Van Katwijk *et al.*, 2009). In environments with strong hydrodynamics, ameliorating physical disturbance of plantings through use of s*tabilizing devices* (e.g. staples, mesh, wires, artificial mats) may be warranted (Campbell, 2002; van der Heide *et al.*, 2007; Paling *et al.*, 2009; Van Katwijk *et al.*, 2009; Matheson *et al.*, 2017a).

In New Zealand to date, only a small number of seagrass restoration trials have been carried out and reported upon. These took place at Manukau Harbour 1995 (Turner, 1995; Morrisey & Turner, 1996), at Whangarei Harbour in 2008 and 2012 (Matheson *et al.*, 2016; Matheson *et al.*, 2017b) and at Porirua Harbour (Pāuatahanui Inlet) in 2015 (Matheson *et al.*, 2017a). Only the trials in Whangarei Harbour have successfully re-established seagrass to former sites in the longer term. The failure of the recent Pāuatahanui Inlet trials was attributed to a possible combination of low light availability, smothering by fine sediment and unfavourable substrate conditions. Since benthic light levels and sediment grain size measured at the Pāuatahanui Inlet restoration sites were above thresholds previously reported to support healthy growth of *Z. muelleri*, the possibilities exist that substrate conditions might play a critically important role (Matheson et al., 2017b).

The field experiment described in this Chapter was subsequently conceived to examine more closely the importance of substrate conditions to seagrass survival and re-establishment, in estuary locations affected by siltation. The objective of the experiment was to test the hypothesis that persistent alterations to substrate physico-chemistry (i.e. a siltation "legacy effect") is the primary factor contributing to failure of seagrass to *re-establish* at former sites impacted by fine sediment pollution.

With that aim, a field experiment was designed to disentangle substrate effects from other effects such as light climate and smothering. The underlying scientific question is whether substrate deterioration is the only factor impeding seagrass reestablishment at historical sites or whether other factors such as smothering, reduced irradiance when submerged and other environmental factors dictate this inability. To answer this, intact cores of substrate were transferred from historical habitat (HS) to existing seagrass (ES) habitat and vice versa, and substrates were also uplifted and replaced at both habitats to act as controls, then healthy sprigs of seagrass were transplanted into both types of substrate in both habitats. The healthy sprigs were sourced from the ES habitat. The premise of the experimental design was that if plants growing on HS substrate located in the ES habitat declined relative to ES control substrates this would suggest a strong influence of sediment physico-chemical conditions since light climate and smothering influences are presumably not compromised at this site. If the plants did not decline, then this would suggest that sediment physico-chemistry had little effect. Conversely, if plants grown on ES substrate at the HS habitat grow better than those on the HS substrate at this habitat then this also suggests a strong influence of sediment physico-chemical conditions. However, if plants were to decline on both types of substrate at this site this suggests a strong influence of sediment smothering and/or light climate.

5.3 Materials and Methods

5.3.1 Study location

The experiment was conducted at two habitat types in Pāuatahanui Inlet at two tidal levels (Figure 5.1). Firstly, an inner estuary habitat at a location where seagrass grew historically (pre-

1980) (HS) and which is strongly impacted by siltation. Secondly, an outer estuary habitat where seagrass beds continue to grow and have been present for at least 80 years (ES). Two transects were located at both habitats, one at an (upper intertidal position, (UT) and one at a lower intertidal, (LT) position (Figure 5.1A).

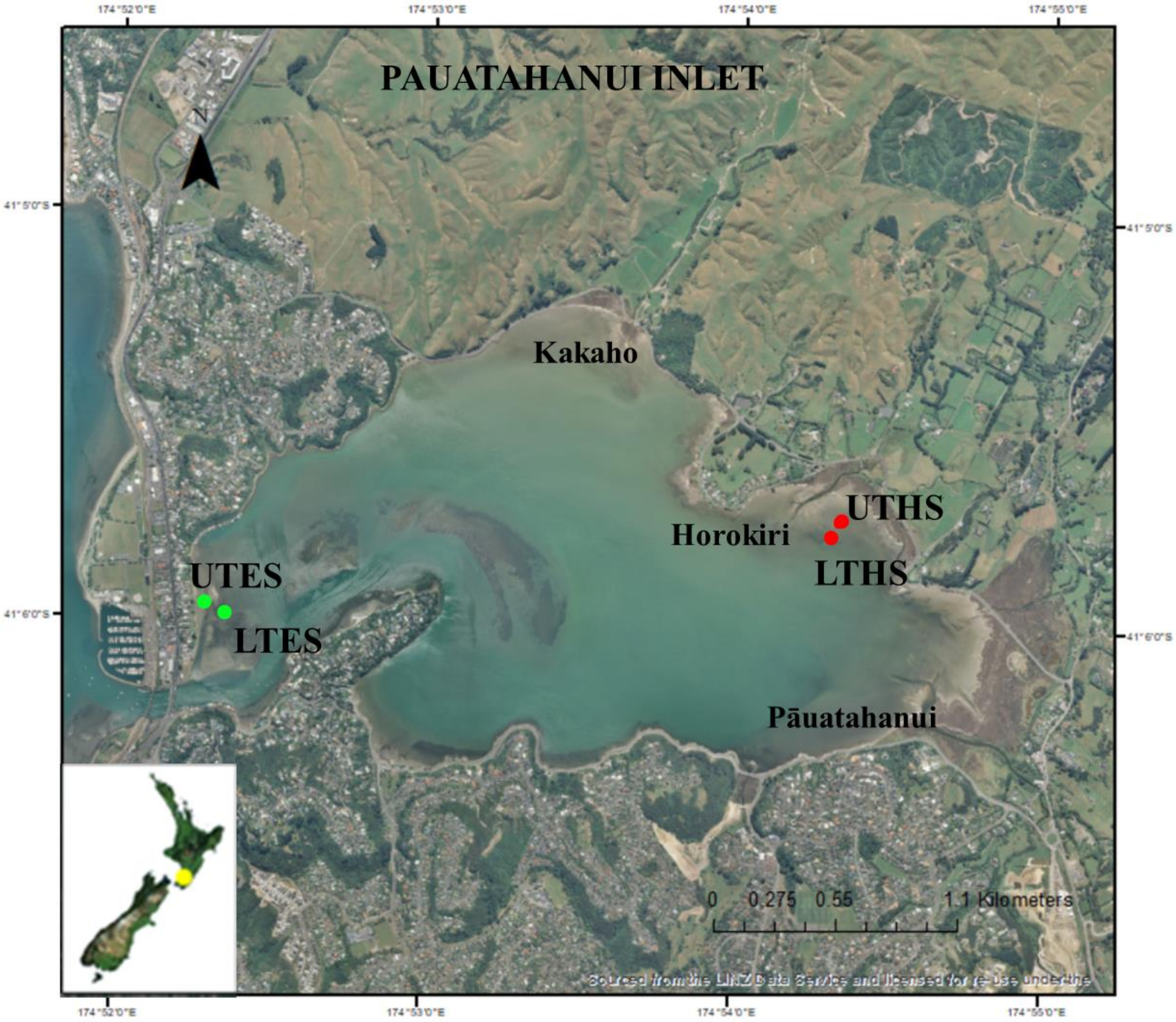


Figure 5.1: Map of Pāuatahanui Inlet (North Island, New Zealand) indicating the location of the four experimental transects: Two were located at a habitat in the inner estuary of Pāuatahanui Inlet where seagrass occurred historically, but no longer grows (HS, red dots), one at an upper intertidal position (UT/HS) and one at a lower intertidal position (LT/HS). A further two were located at a habitat in the outer estuary where seagrass continues to grow (ES, green dots) at upper and lower intertidal positions (UT/ES and LT/ES). Kakaho, Horokiri and Pāuatahanui are shown as the three major stream sources of sediment to the Inlet.

The locations of the four transects were sampled previously (Chapter 3). This sampling showed that HS substrate has significantly higher mud content and bulk density, and higher porewater sulphide, ammonium and phosphate concentrations than ES substrates (Table 5.1).

Table 5.1: Substrate physico-chemical characteristics at historical and existing seagrass habitats. Values are means (±SE). Significant differences ($p<0.05$, Tukey HSD test) among treatments are indicated by different alphabetic superscripts. ANOVA table can be found in (Chapter 5, Appendix).

Treatment	Substrate mud (%)	Bulk density (g cm^{-3})	Substrate porewater H_2S (µM)	Substrate porewater NH_4^+ (µM)	Substrate porewater PO_4^{3-} (µM)
Upper Tidal Historical Seagrass (UT/HS)	47.3 ± 0.1^d	1.6 ± 0.1^b	3.5 ± 1.3^b	31.6 ± 8.7^c	3.3 ± 0.6^d
Lower Tidal Historical Seagrass (LT/HS)	42.9 ± 1.7^c	1.5 ± 0.1^b	1.8 ± 1.2^b	10.6 ± 1.5^b	2.7 ± 0.1^c
Upper Tidal Existing Seagrass (UT/ES)	19.9 ± 0.9^b	1.0 ± 0.1^a	2.3 ± 1.4^b	5.4 ± 0.6^a	1.1 ± 0.1^b
Lower Tidal Existing Seagrass (LT/ES)	13.6 ± 1.1^a	1.2 ± 0.1^a	0.5 ± 0.1^a	4.8 ± 1.6^a	0.4 ± 0.1^a

5.3.2 Experimental design and setup

Cores of bare substrate from HS, cores of substrate containing seagrass plants from ES habitat, and sprigs of seagrass from ES habitat were collected at low tide. Core size was 15 cm diameter and 12cm depth. Sprigs and cores were selected to include an apical meristem with at least three shoots. Prior to transplanting the sprigs, their rhizome length was measured and the number of shoots and internodes were counted. Prior to transplanting, cover within the cores was estimated.

A single experimental transect of 10 m was laid out at each site parallel to the waterline with fifteen 0.5 m x 0.5 m (0.25 m²) quadrats equidistantly deployed along its length. At each quadrat a core of substrate was removed from each corner and discarded to enable the insertion of the cores gathered for the experiment. Only plants and substrates from the same tidal level were exchanged. In the top two corners of each quadrat a substrate core was inserted (one from ES habitat and one from HS habitat) and two sprigs (from the ES habitat at the same tidal level) were planted into each core. Each sprig was anchored with a tag. In the bottom two corners of each quadrat a substrate core containing seagrass plants (from the ES habitat at the same tidal level) was inserted (Figure 5.2). The intact cores of substrate containing plants were also extracted from ES habitat and transplanted to HS habitat enabling a comparison of the effectiveness of sprigs versus cores as a transplant method to former sites. The growth responses of plants as sprigs and in cores were monitored through time for 8 weeks and were harvested for traits analysis. The use of both transplanting techniques allowed an inter-methodological comparison.

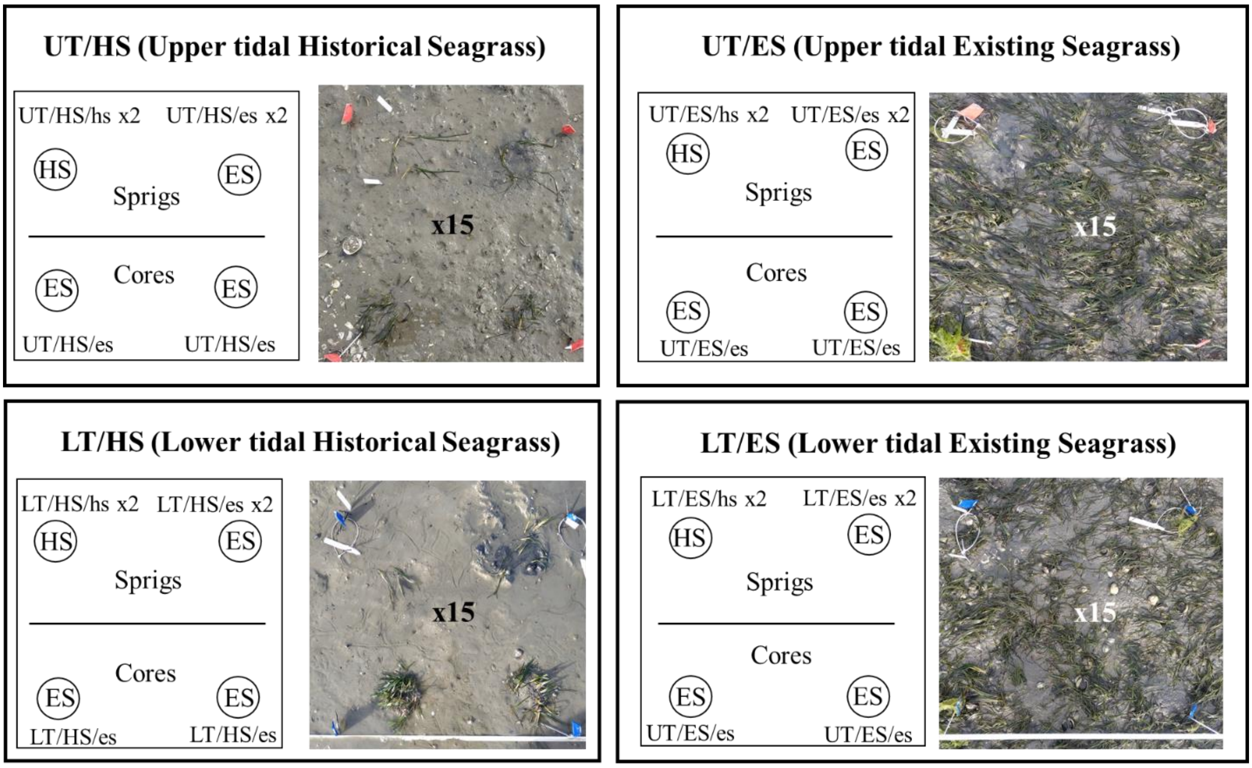


Figure 5.2: Diagram indicating the treatments within each of the four transects (UT/HS, LT/HS, UT/ES and LT/ES big black boxes). Within each transect 1 of 15 quadrats is shown as an example. In the top left corner of each quadrat 2 sprigs were planted into HS substrate. In the top right corner of each quadrat two sprigs were planted into ES substrate. In the bottom left and right corners of each quadrat two intact cores of substrate containing seagrass plants from the ES site were transplanted.

The experiment was conducted for 45 days from 28/10/19 to 12/12/19 (late spring to early summer). ECOPAR™ (http://www.seabird.com/ecopar) sensors were deployed for the duration of the experiment to measure photosynthetically available radiation (PAR) (mol m^{-2} d^{-1}) at 30 minutes intervals at all transects (one at the first quadrat of each transect). Temperature records were also obtained from one HOBO logger (http://www.onsetcomp.com/), deployed at each transect alongside the ECOPAR.

Finally, is noted that covarying factors – like temperature, salinity, currents, grazing rates, biofilms, shell deposits, drift algal accumulations technically are unreplicated test factors (Hurlbert, 1984). The aim here was primarily to test whether the effect of substrate and light on seagrasses are consistent and therefore (temperature, salinity, grazing rates, hydrodynamics…) are of less importance.

5.3.3 Monitoring and laboratory analysis

Six weeks after transplanting, the condition of seagrass sprigs and plants in cores in all quadrats was assessed. Seagrass cover in each core was estimated to the nearest 5% by visual inspection of photographs following the approach recommended by Short and Coles (2001). At the end of the experiment cores were re-extracted from each corner of the quadrats for biomass quantification.

In the laboratory plant material was extracted from each core, rinsed with water and separated into aboveground biomass (AGB – shoots and leaves) and belowground biomass (BGB – roots and rhizomes). Plants were photographed for later morphometric evaluation (see below). Plant biomass samples were dried at 60 ℃ to constant weight to determine biomass per unit area (g m^{-2}) (Short & Coles, 2001). Plant images were analysed for rhizome length using Image J software (https:/imagej.nih.gov/ij/) which allowed conversion of pixels to cm. Rhizome growth was calculated as, the difference between final rhizome length at the end of the experiment and the initial length prior to transplanting. The number of shoots were counted and shoot growth was calculated as the increase or decrease in shoot number over time and evidence of necrosis was determined by visual evaluation of leaf colour. Retention *in situ* of both cores and sprigs was determined through image analysis.

5.3.4 Data analysis

Daily irradiance (PAR, mol m^{-2} d^{-1}) was calculated by averaging the 48 half-hourly instantaneous PAR readings (in µmol m^{-2} d^{-1}) from the ECOPAR, and scaling to a daily rate (multiply by 86400). Statistical analysis of data was performed using the R statistical package (R Team 2016). If not stated differently, all mean values are presented with standard errors of the mean (mean ± SE). Light and sediment (fixed factors) effects were tested using a two way-ANOVA (Zar, 1984), followed by the post hoc Tukey test for multiple comparisons in case of a significant ANOVA result. In all cases, the significance level was set at 5 % probability.

In all the analyses, homogeneity of variance was examined using Cochran tests and when data was los-transformed.

5.4 Results

5.4.1 PAR

PAR was not significantly different between tidal levels at ES habitat, whereas at HS habitat, the upper tidal light was significantly higher than the lower tidal light. Overall, PAR was significantly higher at ES habitat than at HS habitat at equivalent tidal levels (Table 5.2).

Table 5.2: Photosynthetically available radiation (PAR) at historical and existing seagrass habitats. Values are means (±SE). Significant differences ($p<0.05$, Tukey HSD test) among treatments are indicated by different alphabetic superscripts.

Treatment	PAR (mol $m^{-2}d^{-1}$)
Upper Tidal Historical Seagrass (UT/HS)	33.8 ± 1.1^{b}
Lower Tidal Historical Seagrass (LT/HS)	22.4 ± 0.8^{a}
Upper Tidal Existing Seagrass (UT/ES)	37.4 ± 1.1^{c}
Lower Tidal Existing Seagrass (LT/ES)	36.1 ± 1^{c}

5.4.2 Seagrass trait responses to experimental treatments

5.4.2.1 Sprigs

Retention of the transplanted sprigs *in situ* ranged from 33% - 77% (Table 5.3). Retention was higher in the HS habitat compared to the ES habitat. Given the loss of sprigs resulting from the nuisance *C. ligustica* incursion at ES habitat, and difficulties relocating transplanted sprigs, also at the ES habitat, these limits and gaps in data availability mean that few clear patterns related to the planned treatments emerge from results.

At the HS habitat, sprigs transplanted into HS and ES substrates survived equally well over the course of the experiment in both the upper and lower tidal zones with no significant differences in rhizome growth, total plant biomass and shoot growth. Comparing sprig traits on the same HS substrate between the two habitats (HS and ES) and at both tidal levels, significant differences were observed in rhizome growth and aboveground biomass. Rhizome growth was more negative at the ES habitat yet aboveground biomass was higher.

Table 5.3: Percent retention *in situ* of sprigs, presence/absence of *C. ligustica*, rhizome growth (cm), aboveground biomass (AGB), belowground biomass (BGB) and total biomass per treatment for transplanted *sprigs*. Values are means (±SE). Significant differences ($p<0.05$, Tukey HSD test) among treatments are indicated by different alphabetic superscripts. ANOVA table can be found in (Chapter 5, Appendix).

Treatment	% Retention of transplanted sprigs	*C. ligustica* presence/ absence	Rhizome growth per sprig (cm)	AGB (g sprig^{-1})	BGB (g sprig^{-1})	Biomass (g sprig^{-1})	Shoot growth n^{-1}
LT/HS/hs	57	Absent	0.74 ± 0.47 ^{b}	0.01 ± 0.003 ^{b}	0.13 ± 0.04 ^{a}	0.13 ± 0.04 ^{a}	-0.55 ± 0.28 ^{a}
LT/HS/es	63	Absent	0.3 ± 0.78 ^{b}	0.01 ± 0.003 ^{b}	0.15 ± 0.03 ^{a}	0.15 ± 0.03 ^{a}	-1.3 ± 0.63 ^{a}
LT/ES/hs	40	Present	-1.73 ± 0.64 ^{a}	0.04 ± 0.008 ^{c}	0.09 ± 0.02 ^{a}	0.09 ± 0.02 ^{a}	0 ± 0.53 ^{a}
LT/ES/es	Could not relocate	**Present**	-	-	-	-	-
UT/HS/hs	73	Absent	-0.26 ± 0.44 ^{b}	0.003 ± 0.001 ^{a}	0.14 ± 0.02 ^{a}	0.14 ± 0.02 ^{a}	-0.41 ± 0.16 ^{a}
UT/HS/es	77	Absent	0.11 ± 0.53 ^{b}	0.0001 ± 0.0001 ^{a}	0.13 ± 0.02 ^{a}	0.13 ± 0.02 ^{a}	-0.8 ± 0.31 ^{a}
UT/ES/hs	33	Absent	-1.53 ± 2.01 ^{a}	0.05 ± 0.02 ^{c}	0.2 ± 0.13 ^{a}	0.2 ± 0.13 ^{a}	-1.14 ± 0.83 ^{a}
UT/ES/es	Could not relocate	Absent	-	-	-	-	-

5.4.2.2 Cores

Loss of plant cover for seagrass transplanted as cores was significantly higher in both tidal positions at the HS habitat compared to ES habitat, (Table 5.4). Loss of plant cover was also significantly higher in both habitats in the lower tidal position compared to the upper tidal position. Biomass data followed the same pattern as the cover data being significantly lower in the HS habitat than the ES habitat at both tidal positions. However in the HS habitat above ground biomass was higher in the lower tidal position than the upper tidal position, but belowground biomass and total biomass did not differ by tidal position. In the ES habitat aboveground biomass did not differ by tidal position, but belowground biomass and total biomass were significantly higher in the upper tidal position (Table 5.4).

Table 5.4: Initial cover, final cover and cover loss, presence versus absence of *C. ligustica*, aboveground biomass (AGB), belowground biomass (BGB) and total biomass per treatment for the core transplanting method. Values are means (±SE). Significant differences (p<0.05, Tukey HSD test) among treatments are indicated by different alphabetic superscripts. ANOVA table can be found in (Chapter 5, Appendix).

Treatment	**Initial % cover**	**Final % cover**	**% Cover loss**	***C. ligustica* presence/ absence**	**AGB (g core^{-1}) (g m^{-2})**	**BGB (g core^{-1}) (g m^{-2})**	**Total Biomass (g core^{-1}) (g m^{-2})**
LT/HS	88.9 ± 1.9 ^{b}	4.5 ± 1.6 ^{b}	80 ± 2.5^{d}	Absent	0.09 ± 0.02 ^{b} 5 ± 1.1 ^{b}	1.91 ± 0.19 ^{a} 107.9 ± 10.7 ^{a}	2.00 ± 0.20 ^{a} 113.0 ± 11.3 ^{a}
LT/ES	99.6 ± 0.2 ^{c}	45.8 ± 8.2 ^{c}	50 ± 3.3 ^{b}	**Present**	0.51 ± 0.09 ^{c} 28.8 ± 5.1 ^{c}	3.26 ± 0.53 ^{b} 184.2 ± 29.9 ^{b}	3.76 ± 0.61 ^{b} 173.8 ± 0.6 ^{b}
UT/HS	61.6 ± 4.6 ^{a}	0.9 ± 1.9 ^{a}	67 ± 8.2 ^{c}	Absent	0.02 ± 0.01 ^{a} 1.1 ± 0.6 ^{a}	2.19 ± 0.37 ^{a} 123.7 ± 20.9 ^{a}	2.22 ± 0.38 ^{a} 125.4 ± 21.5 ^{a}
UT/ES	99.8 ± 0.2 ^{c}	99.8 ± 0.2 ^{d}	0 ± 0 ^{a}	Absent	0.55 ± 0.05 ^{c} 31.1 ± 2.8 ^{c}	4.49 ± 0.28 ^{c} 253.7 ± 15.8 ^{c}	5.04 ± 0.28 ^{c} 284.7 ± 15.8 ^{c}

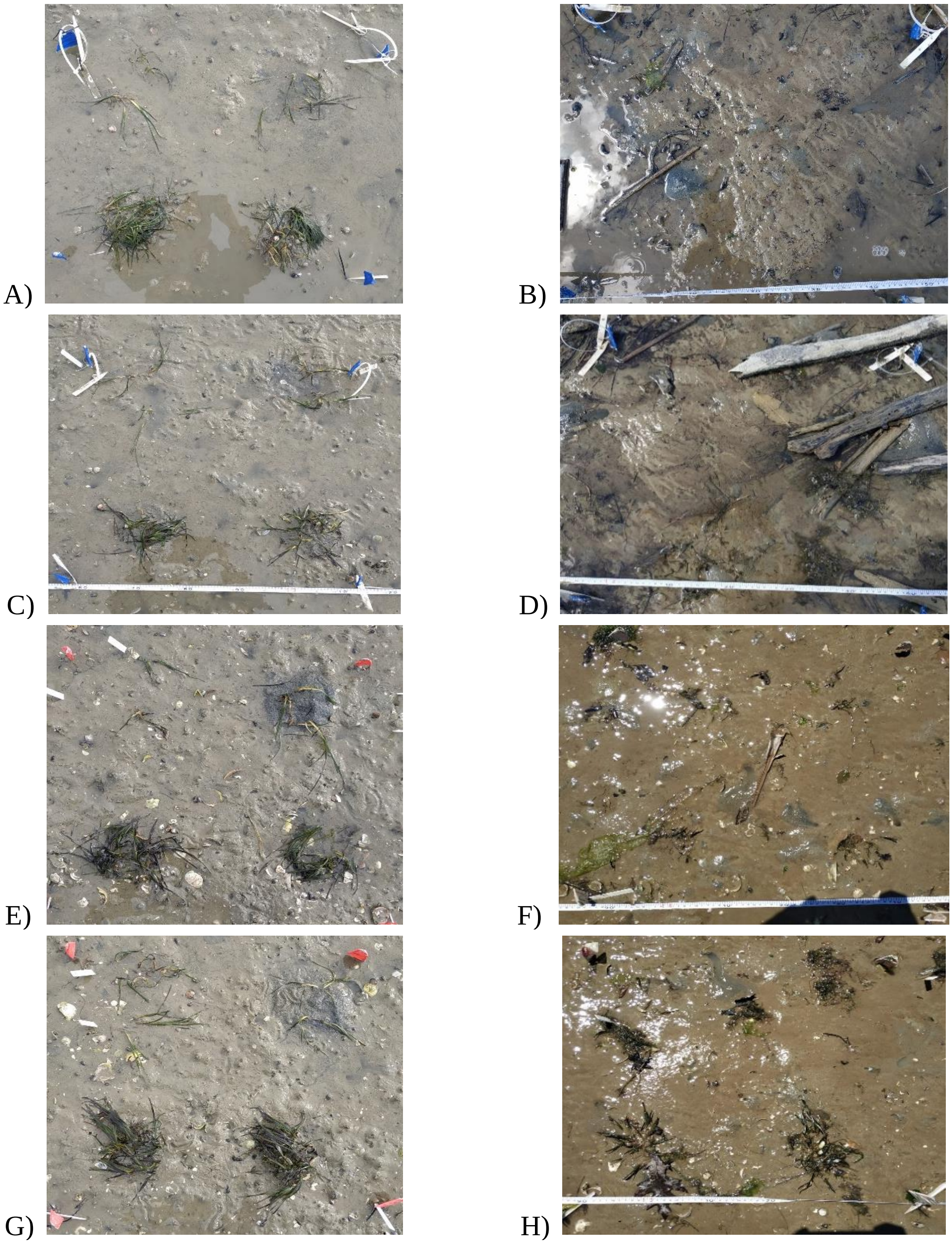
5.4.2.3 Sprigs vs Cores

Inter-methodological comparison when possible between sprigs and cores showed that retention for sprigs *in situ* ranged from 33 – 77 % across treatments and from 20 – 100 % for cores.

5.4.2.4 Qualitative assessment of storm effect

A storm hit the Inlet starting the 7/12/19 and lasting until the 9/12/19. It’s main characteristics were: 14 mm (NIWA weather station) of rainfall almost twice the averaged 8 mm daily rainfall for the month of December since 1981-2010 (NIWA weather station data), wind direction and maximum velocity 36 (N, km h^{-1}), mean Temperature (16°C), pressure

1010 mbar. The smothering effect of this storm on the LT/HS and UT/HS sites can be seen in Figure 5.3. A deposit of 2-3 mm of sediment covering the plants at HS habitat was quantified at harvesting, but not observed at ES habitat (Figure 5.3).



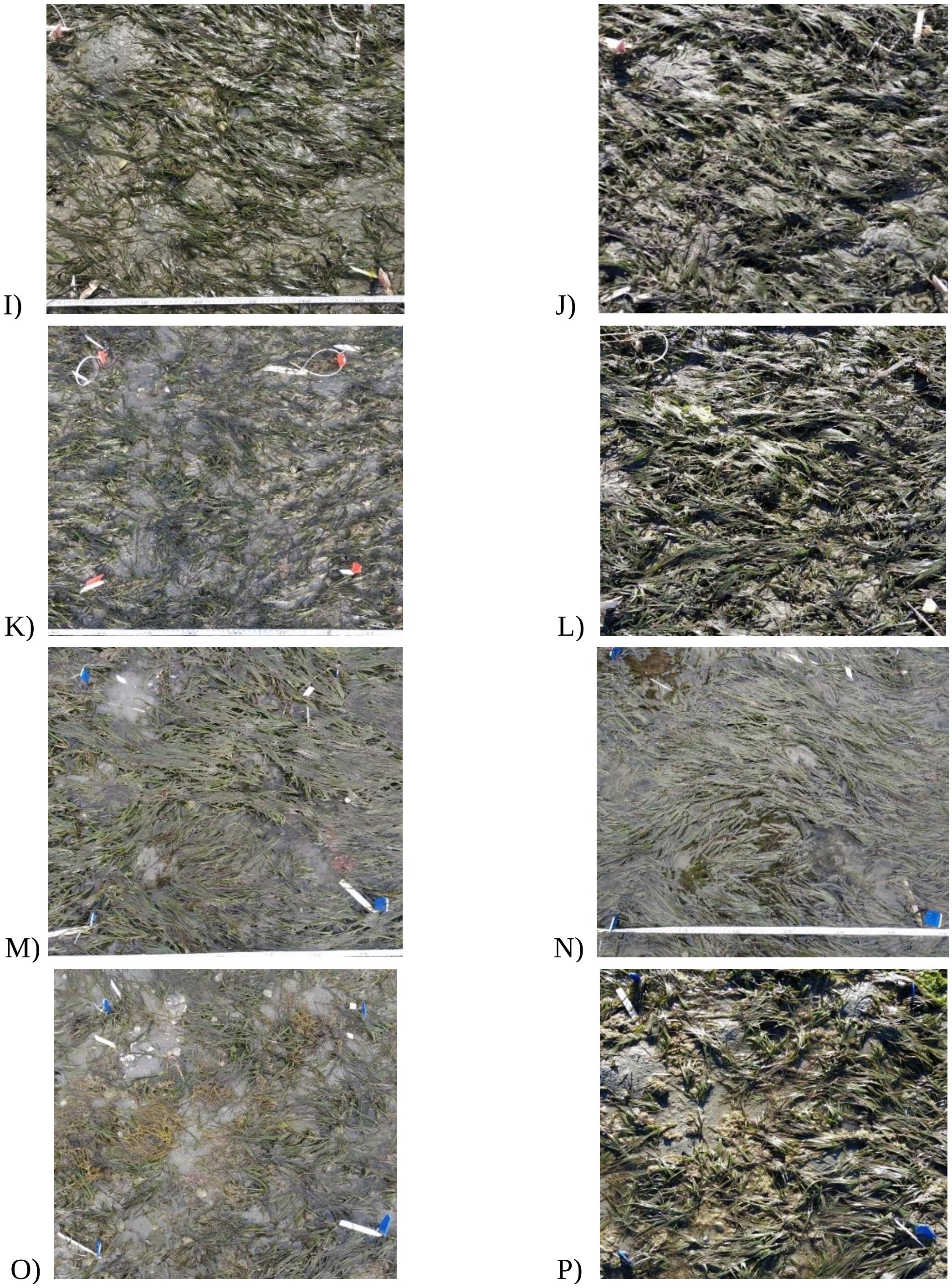


Figure 5.3: Visual impacts of a storm on the historical habitat during the experiment. Photos A & C are two example quadrats from the Lower Tidal, Historical Seagrass (LT/HS) habitat before the storm and B & D after the storm. Photos E & G are Upper Tidal, Historical Seagrass (UT/HS) habitat plots before the storm and F & H after the storm. Non-impact on existing seagrass habitat. Photos I & K are two example quadrats from the Lower Tidal, Existing (LT/ES) habitat before the storm and J & L after the storm. Photos M & O are Upper Tidal, Existing Seagrass (UT/ES) habitat plots before the storm and N & P after the storm.

5.5 Discussion

With this study, disentanglement of substrate effects on seagrass growth and re-establishment from light climate and smothering effects was attempted. However, not all of the planned sprig comparisons could be made given the failure to relocate transplanted units at ES habitat. Due to this limitation, we could not fully test the hypothesis that substrate physicochemical alterations caused by fine sediment intrusion are the main reason for the inability of seagrass to re-establish and grow in seagrass historical habitats. Nevertheless, some interesting results were found, and these are discussed below.

Firstly, we found that sprig retention was slightly higher on ES substrate compared to HS substrate at the HS habitat, providing some indication that the ES substrate is more favourable for growth. At the HS habitat, retention on both substrates was higher in the upper tidal than lower tidal zone suggesting that better benthic light availability may favour retention. However, at ES habitat retention on HS substrate was slightly higher in the lower tidal zone.

Secondly, comparing sprigs traits between the two substrates at HS habitat, there were no significant differences in rhizome growth, plant biomass and shoot growth.

Thirdly, comparing sprig traits on the same HS substrate between the two habitats (HS and ES) and at both tidal levels, significant differences were detected in rhizome growth and AGB, but not in BGB, total biomass and shoot growth. Rhizome growth was more negative at the ES habitat yet aboveground biomass was higher. A possible explanation for this is sprigs at the ES habitat may have used up carbohydrate reserves in their rhizomes to fuel aboveground biomass accrual stimulated by higher light availability at this habitat compared to the HS habitat (Macready *et al.,* 2014).

Results from the core technique experiments provided evidence for the historical habitat being a more challenging site for seagrass to persist even if planted in original existing healthy substrate. This presumably reflects the poorer growing conditions evident at the historical habitat, especially lower light availability and proximity to sources of fine sediment, capable of settlement, that can periodically smother the seagrass plants (Sorensen *et al.,* 2019). This suggests that the substrate legacy effects determined in previous chapters (physico-chemical alteration) are not the only factor impeding the re-establishment of seagrass at the historical habitat and suggests smothering and reduced light climate as other likely contributors to the decline of seagrass and its inability to re-stablish at the historical habitat. Despite the fact that the average light availability measured at transects at both HS and ES habitats during this experiment widely exceeded minimum daily PAR thresholds for this species previously reported in the literature (Collier *et al.*, 2011; Bulmer *et al.*, 2016; Chartrand *et al.*, 2016; Collier *et al.*, 2016), we argue that the significantly lower light availability at the HS habitat compared to the ES habitat is still important. Thus, rather than a single cause, smothering through partial burial and deposition, a lower receiving irradiance and substrate alteration must be viewed as cumulative effects contributing to the inability of seagrass to re-establish in historical habitats that are strongly affected by fine sediment pollution.

An inter-methodological comparison between sprig and core transplanting techniques in this six-week experiment at Pāuatahanui Inlet demonstrated that across treatments sprig retention ranged from 33-77% and core biomass retention ranged from 20-100%. The fact that these ranges overlap and that not all the paired comparisons could be made make it difficult to compare the efficacy of the two techniques for retaining *Zostera muelleri* plants *in situ*, in the intertidal zone. Nevertheless, the results do show that under growing conditions considered favourable at the ES habitat, and without *C. ligustica* interference, retention of seagrass in cores was very high (c. 100%), at least over the six-week period of the experiment. This demonstrates that this species is very tolerant of the disturbance created by transplanting using cores, provided that suitable growing conditions are provided at a transplant site. In contrast, only a third of seagrass sprigs were retained at the ES habitat, although more were retained at the HS habitat (57-77%) on both types of substrate.

In this study, despite using staples to hold transplanted sprigs in place and to aid in relocation of transplanted sprigs, it was not possible op recognise transplants amongst growing seagrass on ES substrate transplanted into ES habitat. This was attributed to the need to use small staples because of the small size of sprigs, combined with the highly dynamic, intertidal nature of the ES habitat, which dislodged both staples and sprigs. Furthermore, transplanted seagrass can break and fragment (i.e., still perform well but being split into multiple, unrecognizable smaller units) and that the surrounding seagrass can colonize the areas with sprigs and if their rhizomes break, they are impossible to separate from the initial transplants.

Although, previous studies have successfully employed and recommended the use of establishing techniques such as staples, when transplanting sprigs of *Zostera noltii*, another small *Zostera* species (Van Katwijk *et al.*, 2009), my experience is that it is much more challenging to use these types of techniques when working with small size seagrass species like *Z. muelleri* in the intertidal zone, compared with larger seagrasses such as *P. oceanica* in the less dynamic subtidal zone (I. Zabarte-Maeztu, personal observation). From the experiment conducted here, it is concluded that, cores with sufficient size to incorporate at least one apical meristem of *Z. muelleri* can be used to successfully transplant this species in the dynamic intertidal zone. Sprigs may also work successfully, but the results tend to suggest a lower efficacy. My experience also highlights potential challenges in the use of stabilising techniques, such as staples for marking transplanted sprigs of small seagrass species for monitoring purposes.

Unfortunately, the transplanting experiment coincided with an incursion of *C. ligustica* into the outer part of Pāuatahanui Inlet, where the two existing seagrass transects were located. The lower tidal transect was significantly affected by this nuisance growth and a seagrass loss of up to 50% is attributed to smothering caused by this mat-forming microalga (see Chapter 6 for further description of the incursion).

Restoration of seagrasses in the exposed intertidal zone faces special challenges, both logistical and environmental. Storms have been identified as a major threat to restoration of seagrass ecosystems (Calumpong & Fonseca, 2001; Paling *et al.*, 2001). The storm that affected Pāuatahanui Inlet during the experiment deposited an estimated 2-3 mm layer of fine sediments as well as larger woody debris onto the historical habitat transects. In contrast, less disturbance was observed in the existing seagrass habitat. Previous experiments have reported the damaging effects of storms on seagrass transplanting attempts in Western Australia (Lord *et al.*, 1999; Paling *et al.*, 2001; Campbell & Paling, 2003) and New Zealand (Turner, 1995). Mechanisms through which storms affect seagrass transplantation are partial burial (Cabaço *et al.*, 2008b; Campbell, 2016) and wave and surge action (Campbell & Paling, 2003). However, successful intertidal restoration in Australia and New Zealand is possible and is described in a recent review (Tan *et al.*, 2020).

Despite all the challenges encountered during the course of this experiment, it can be concluded that the cumulative effect of rhizosphere deterioration, lower irradiance and close proximity to a large source of fine sediment input and associated disturbance during storm events may be combined causes of the inability of seagrass to re-establish at Pāuatahanui Inlet historical habitat, and at similar vulnerable, inner estuary locations elsewhere. It strongly highlights, the difficulty of turning unvegetated habitats back to growing areas once again, despite these being suitable locations for flourishing habitats in the past (De Boer, 2007; Carr *et al.*, 2010; Adams *et al.*, 2016). Attempts to restore seagrass to such locations are likely to be unsuccessful unless the upstream sources of fine sediment can be reduced and the legacy sediments can be removed or remediated.

Chapter 6

First observations of *Chaetomorpha ligustica* (Cladophoraceae, Cladophorales) smothering the seagrass *Zostera muelleri* in a New Zealand estuary

Note: This Short notification has been prepared to be submitted to the New Zealand Journal of Freshwater and Marine Sciences under the title “First observations of *Chaetomorpha ligustica* (Cladophoraceae, Cladophorales) smothering the seagrass *Zostera muelleri* in a New Zealand estuary” by Zabarte-Maeztu, I., Matheson, F. E., D’Archino, R., Manley-Harris, M., Davies-Colley, R. J., & Hawes, I.

6.1 Abstract

The filamentous green alga *Chaetomorpha ligustica* (Cladophoraceae, Cladophorales) was recorded covering seagrass meadows at Pāuatahanui Inlet, New Zealand. Species of the genus *Chaetomorpha* are difficult to identify by thir morphological characters, and the identification of *C.ligustica* was confirmed by sequencing the 28S rRNA large subunit providing a high level of confidence in the naming. This species was previously recorded in New Zealand as *Lola tortuosa* or *Chaetomorpha capillaris* at Porirua, Leigh, Stewart Island and the Chatham Islands. In the 1970s at Pāuatahanui Inlet, *C. ligustica* was recorded forming “an abundant fleecy turf in salt marsh”. In November 2019, we found *Chaetomorpha ligustica* intertwined with *Ulva* spp. forming dense, heavy and sticky structures. Here we report, for the first time, negative impacts of this species upon meadows of the New Zealand seagrass *Zostera muelleri*. We observed significant loss of seagrass cover and evidence of anoxia under *Chaetomorpha ligustica* mats two weeks from the first sighting. *Chaetomorpha ligustica* can easily be misidentified in the field with other *Chaetomorpha* or *Rhizoclonium* species. This may lead to both over and under-reporting of species occurrence in previous surveys and we recommend the need for more careful identification of green macroalga blooms in future as well as further research on growth requirements and origins of strains.

6.2 Introduction

One of the most common symptoms of eutrophication in temperate estuaries is the proliferation of floating macroalgae (Lavery *et al.*, 1991; Kinney & Roman, 1998; Burkholder *et al.*, 2007). When these blooms reach high densities may settle in large aggregations over seagrasses where they contribute to declines of meadows (Ansell *et al.*, 1998; Cummins *et al.*, 2004). More often seaweed increase faunal biodiversity in seagrass beds through facilitation cascades (Thomsen 2010; Thomsen *et al.*, 2010; Thomsen *et al.*, 2012a; Thomsen *et al.*, 2013; Thomsen and Wernberg 2015; Thomsen *et al.*, 2016; Thomsen *et al.*, 2018, Gribben *et al.*, 2019; Siciliano *et al.*, 2019, Vieira *et al.*, 2020).

Seagrasses support abundant assemblages of fauna (Orth *et al.*, 2006; Waycott *et al.*, 2009) and macroalgal clumps have been reported to cause sizeable gaps in seagrass canopies (Holmquist, 1997; Hauxwell *et al.*, 2001; Hoeffle et al., 2011; Holmer *et al.*, 2011; Hoeffle *et al.*, 2012; Thomsen *et al.*, 2012a; Thomsen *et al.*, 2012b, Thomsen *et al.*, 2013; Siciliano *et al.*, 2019; Vieira *et al.*, 2020) with consequential negative effects upon associated fauna (Eggleston *et al.*, 1999; Boström & Bonsdorff, 2000; Cummins *et al.*, 2004). Some of the damage mechanisms are the anoxia generated through decomposition, production of hydrogen sulphide and alteration of the geochemistry of underlying sediments as well as shading, which affects production (Valiela *et al.*, 1997; Cummins *et al.*, 2004; Lyons *et al.*, 2014; Nelson *et al.*, 2015).

In New Zealand estuaries, species of three groups of macroalgae, Ulvophyceae, Phaeophyceae and Rhodophyceae are known to form nuisance macroalgal blooms, some at single sites and others widespread around New Zealand (Nelson *et al.*, 2015). The majority of these species are native to New Zealand, however, there are examples of non-natives such as *Solieria* spp. and *Gracilaria* spp. (Nelson *et al.*, 2015). The most commonly reported are *Ulva* species which bloom extensively in Tauranga Harbour (Hawes *et al.*, 1992; De Winton *et al.*, 1998; Park, 2011), Porirua Harbour (Stevens & Robertson, 2016) and in the Avon-Heathcote Estuary (the last with *Gracilaria* spp.) (Hawes & O'Brien, 2000). Declines of seagrass have been reported to accompany such blooms (Rasmussen *et al.*, 2012; Rowden *et al.*, 2012). These blooms are very often attributed to nutrient enrichment either natural through upwelling with El Niño conditions (Lanari & Copertino, 2017) or due to anthropogenic activities (Burkholder *et al.*, 2007).

In November- December 2019, during a study to characterise the effects of fine sediments on seagrass beds in Pāuatahanui Inlet, we observed a filamentous green alga, later identified as

Chaetomorpha ligustica (Kützing) Kützing forming dense heavy and sticky structures (intertwined filamentous), covering the seagrass. We are not aware of previous records of bloom formation by this alga within New Zealand.

Chateomorpha is a cosmopolitan genus that occurs in marine and brackish water and currently includes 74 species (Guiry & Guiry, 2010). The identification of *Chaetomorpha* to species level is challenging as thalli are simple, consisting of uniseriate, unbranched filaments without rhizoids and have few diagnostic characters e.g. filament diameter, type of growth, cell shape (Leliaert & Boedeker, 2007). The genus has been often confused with *Rhizoclonium,* though molecular studies have progressively disentangled the confusion around these genera, highlighting the need for molecular data to confirm species identification (Leliaert & Boedeker, 2007; Leliaert *et al.*, 2009; Leliaert *et al.*, 2011; Boedeker *et al.*, 2016). In New Zealand six species have been identified to date: *C. aerea* (Dillwyn) Kütz., *C. coliformis* (Mont.) Kütz., *C. elongata* V.J.Chapm., *C. ligustica* (Kütz.) Kütz., *C. linum* (O.F.Müll.) Kütz., and *C. valida* (Hook.f. & Harv.) Kütz. (Neill & Nelson, 2019).

Chaetomorpha ligustica had a complicated nomenclature history and in New Zealand, it has been recorded as *Lola tortuosa* (Dillwyn) Chapman (Chapman, 1956; Adams, 1972), *Lola capillaris* (Kütz) Hamel and *Chaetomorpha capillaris* (Kützing) Børgesen (Adams, 1994). Adams (1972) reported *C. ligustica* in Pāuatahanui Inlet, ‘forming a fleecy turf in salt marsh, abundant’. This alga has also been recorded in the Bay of Islands (Nelson & Adams, 1987) and in Whangarei harbour as *C. capillaris* (Neill *et al.*, 2012) and from Manukau Harbour, Great Barrier Island and Rangitoto Island (D'Archino, 2019).

The type locality of *C. ligustica* is Golfo di Genova in Italy and it is widely distributed globally with extensive representation throughout the coastal margins of the Atlantic and Pacific oceans (Guiry & Guiry 2021). Molecular data from Iceland, Norway, Ireland, Scotland, Netherlands (Boedeker *et al.*, 2016) and Japan (Ichihara *et al.*, 2013) have confirmed this broad distribution.

The aim of this study was to report and document for the first time the occurrence of *C. ligustica* smothering seagrass beds in Pāuatahanui Inlet, New Zealand.

Figure 6.1: *C. ligustica* bloom. A) photograph of *C.ligustica* smothering seagrass at low tide just prior to total exposure of the seafloor to emersion; B) Lower Tidal Existing Seagrass experimental transect (see Chapter 5) affected *by C. ligustica*; C & D) *C. ligustica* structures when submerged smothering seagrass. Photos are much like (Thomsen & Wernberg, 2009).

6.3 Materials and Methods

6.3.1 Study location

Pāuatahanui Inlet is a natural inlet and wildlife reserve on New Zealand's North Island southwestern coast (Figure 6.2). It is the eastern arm of Porirua Harbour which has a total catchment area of 199 km², comprising a land area of 185 km² and a harbour area of 14 km². Average annual rainfall is 1200 mm and the mean air temperature is 12.9 °C, with prevailing winds from the North and North-West (Blaschke *et al.*, 2010). The Pāuatahanui catchment is 109 km² (Milne & Warr, 2007), and has six sub-catchments. The Pāuatahanui, Horokiri and Kakaho streams are the major sources of sediment (Figure 6.2). Within these, predominantly pastoral sub-catchments, soil erosion and runoff has resulted in high downstream sedimentation rates and land use has driven moderate eutrophication (Stevens & Robertson, 2016).

Our observations were made since the bloom coincided with an experiment on seagrass conducted from 28/10/2019 to 12/12/2019 that involved repeated monitoring of four intertidal transects for which temperature and light records were obtained from HOBO loggers (http://www.onsetcomp.com/), deployed at each transect (see Chapter 5). Also, an ECOPAR™ (http://www.seabird.com/ecopar) sensor was deployed at each transect to directly measure photosynthetically available radiation (PAR) (mol m^{-2} d^{-1}). We collected two samples of *C. ligustica* on the 26/11/2019 randomly from a seagrass bed adjacent to the Lower Tidal Existing Seagrass (LTES) transect (Figure 6.2) which was later affected by the mat. Samples were cleaned to remove the less abundant *Ulva* spp. and promptly stored in an insulated container.

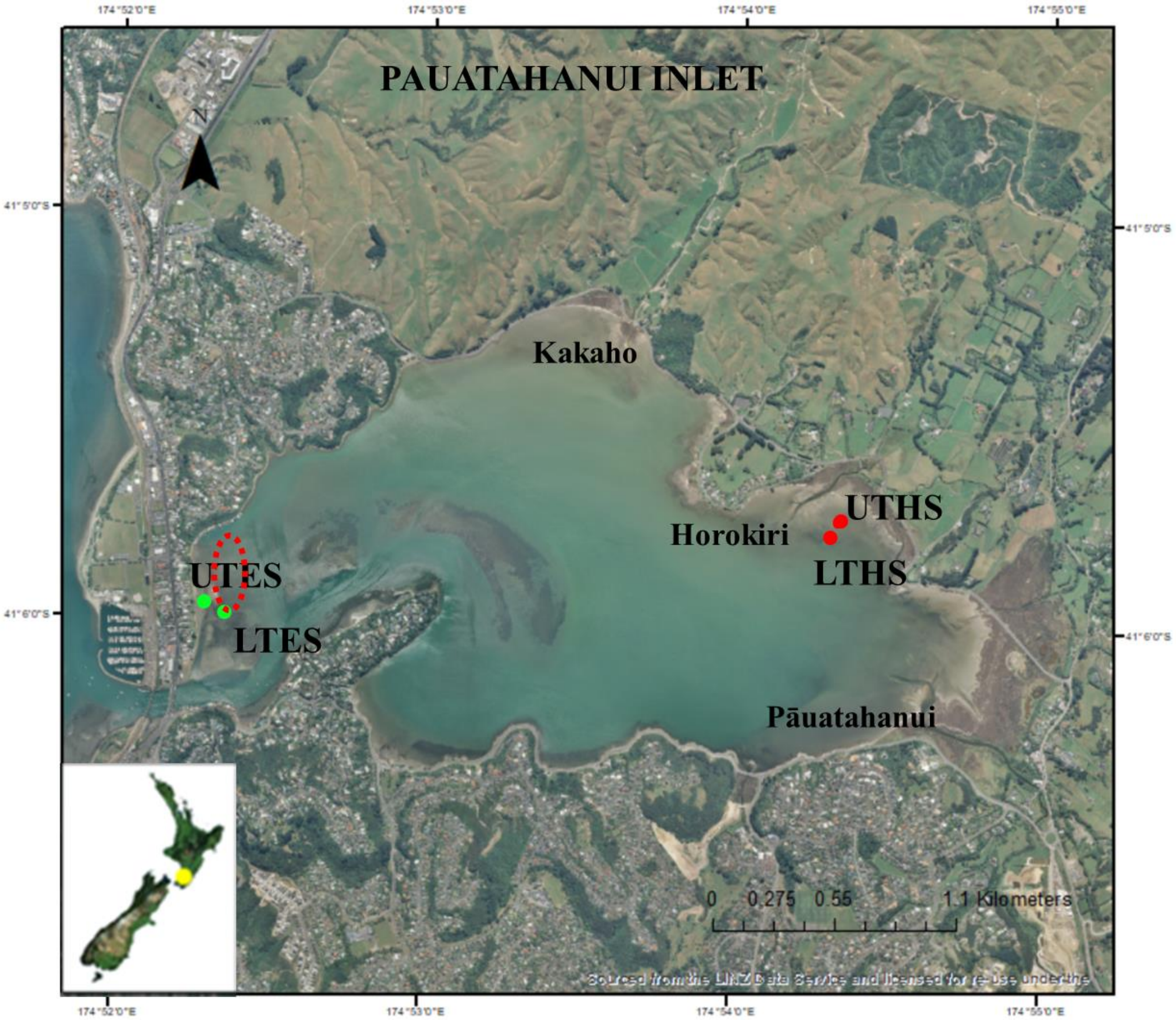


Figure 6.2: Map of Pāuatahanui Inlet (North Island, New Zealand) indicating the location of the four experimental transects. Kakaho, Horokiri and Pāuatahanui are the three major sources of nutrients and sediments. Red dashed line represents the area affected by *C. ligustica* mat.

6.3.2 Identification

On return to the laboratory, two samples were pressed as vouchers and deposited in the Museum of New Zealand *Te Papa* Tongarewa (Thiers, 2020). A small subsample was placed in silica gel desiccant for molecular analysis. Microscope observations and images were made on rehydrated material, using an Olympus BX53 microscope (Olympus, Tokyo, Japan) with an SC100 digital camera (Olympus, Münster, Germany).

DNA was extracted using the Chelex method of Goff and Moon (1993). Partial nuclear-encoded large subunit (LSU) of ribosomal RNA, was amplified using primers C1 forward and D2 reverse (Hassouna *et al.*, 1984; Leliaert *et al.*, 2003), at the annealing temperature of 48 ˚C. PCR products were cleaned using ExoSAP-IT (Affymetrix, Santa Clara, California, USA) and commercially sequenced (Macrogen, Seoul,Korea). The new sequences were deposited in GenBank (MW756935-MW756936).

6.4 Results

6.4.1 ID & Morphology

Thalli, forming large clumps, were bright green, and consisted of uniseriate, unbranched filaments, without rhizoids. Cells were 12-15 µm in diameter and 23-62 µm in length, aspect ratio 2-4. The chloroplast was reticulate, filling the cell and had multiple pyrenoids. The cell wall was 2-4 µm thick. Attachment and basal cells were not observed (Figure 6.3).

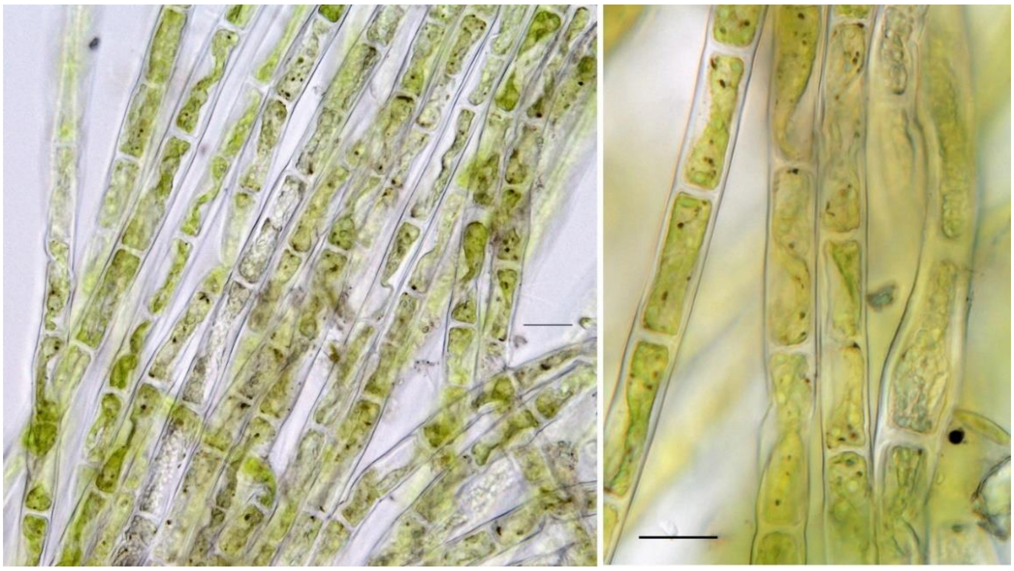


Figure 6.3: Microscopy images of *Chaetomorpha ligustica*, Scale bar 20 µm.

The identification of *Chaetomorpha ligustica* was confirmed by LSU sequence data. The New Zealand sequences (571 and 587 bp) were identical to sequences of *C. ligustica* from the Netherlands (LT607072) and from Japan (AB807611) and had two bp differences with sequences from France, Norway, Iceland, Ireland and Scotland. No other sequences from New Zealand were available.

6.4.2 Effects on seagrass

Between 26/11/2019 and 9/12/2019 the *C.ligustica* began to cover some plots within our transect (Figure 6.4). We measured a 50% decline in seagrass cover within two weeks of *C. ligustica* first being recorded on the 26/11/2019 (Figure 6.4). On removing the mats of algae from the seagrasses, sediments and seagrass plants were black and smelled of hydrogen sulphide, consistent with metal sulphide precipitation and excess sulphide (Figure 6.4). The other, nearby transect (Upper Tidal Existing Seagrass, UTES) was free of *C. ligustica* and no decline was observed. In fact, a small increase in seagrass cover was observed over the same period of time and both transects had a similar high cover of seagrass on 26/11/2019 (Figure 6.4). Comparative images of the same permanently marked seagrass plots before (26.11.19) and after (9.12.19) being affected by *C. ligustica* are shown in (Figure 6.5).

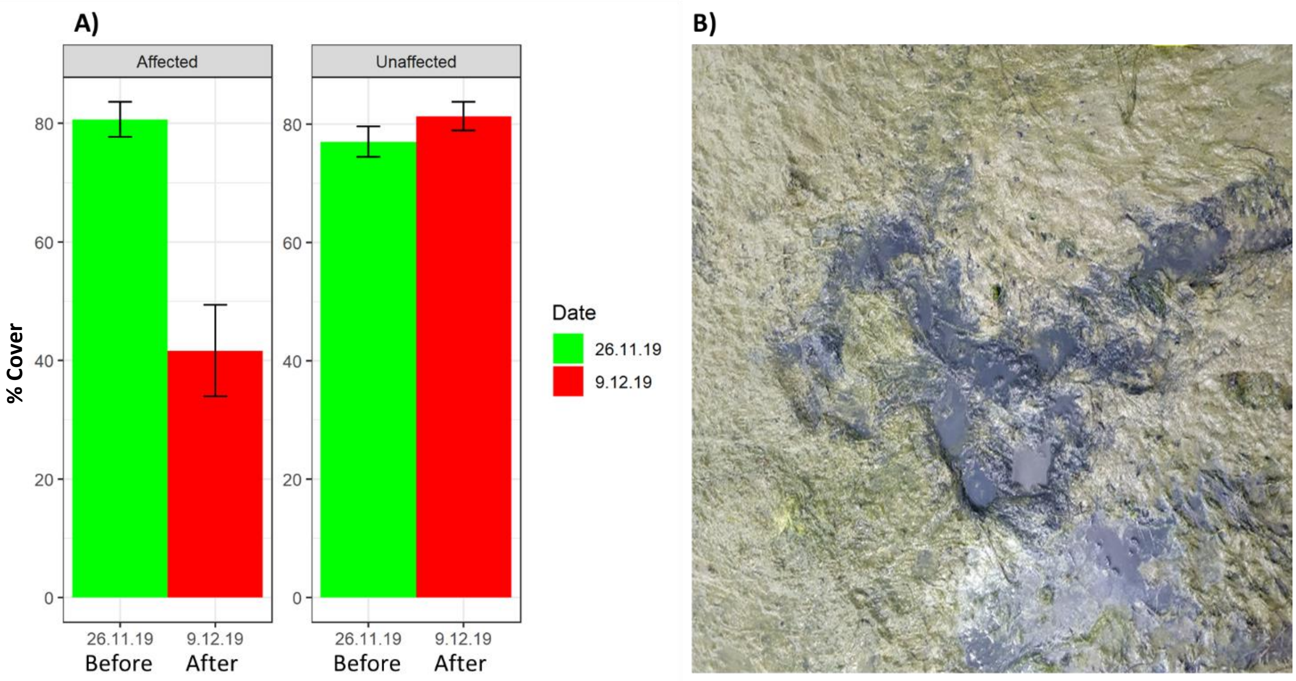


Figure 6.4: A) Seagrass percent cover in *C. ligustica* affected and unaffected transects. B) Removal of the algal mat suggested anoxia below.

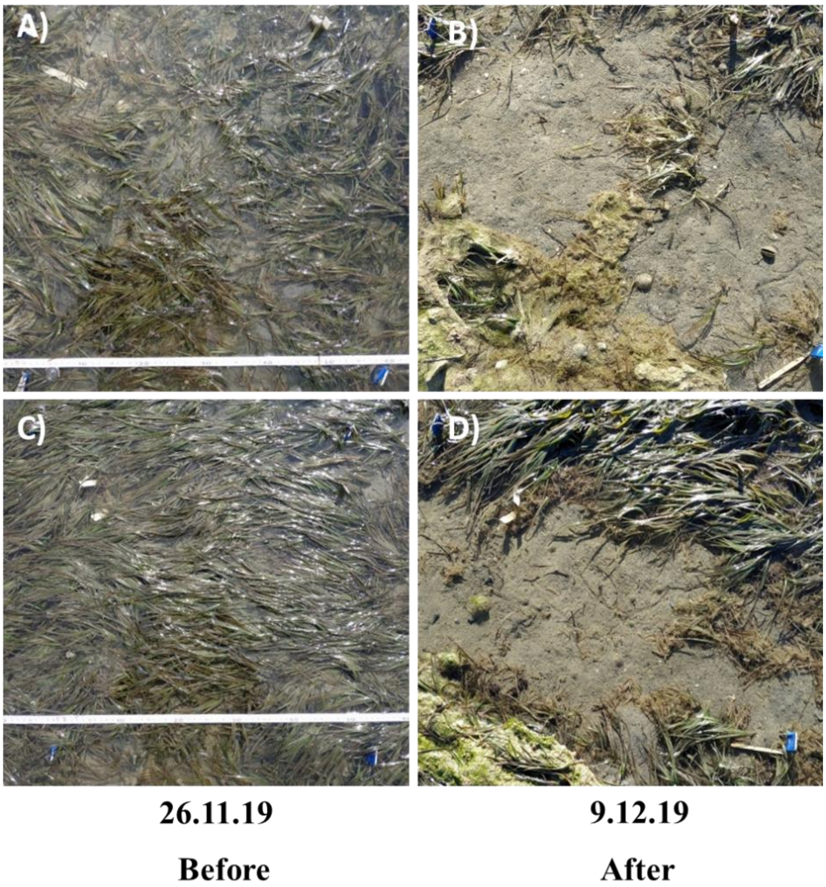


Figure 6.5: Images of the same permanently marked seagrass plots before (26.11.19) and after (9.12.19) being affected by *C. ligustica*.

6.4.3 Environmental data: PAR & Temperature

Proliferation of *C. ligustica* appears to correlate with a phase of high daily maximum temperatures driven by clear days with high irradiance with absence of rain and weak wind. Data from the closest HOBO and ECOPAR loggers is shown (Table 5.A.1).

Table 6.1: Daily mean Photosynthetically Available Radiation (PAR) and Temperature from the closest loggers are shown. (Means ±SE) and maximum values of *average* days are shown. Days after *C. ligustica* was reported are shown in bold.

Date	PAR (mol m^{-2} d^{-1})		Temperature (°C)	
	Mean	Max	Mean	Max
28/10/2019	36.6 ± 11.3	149.5	16 ± 0.3	23
7/11/2019	40.3 ± 7.9	195.9	17 ± 0.1	23
14/11/2019	38.6 ± 6.7	128.8	16 ± 0.4	22
25/11/2019	63.6 ± 2.3	231.2	18 ± 0.8	29
30/11/2019	**58.6 ± 8.8**	**156.3**	**18 ± 0.6**	**29**
10/12/2019	**60.7 ± 10.6**	**237.6**	**22 ± 1.1**	**34**

6.5 Discussion

Chaetomorpha ligustica has long been recognised as present in New Zealand coastal waters, however this is the first time that its identification has been confirmed by molecular data. The identification of *Chaetomorpha* species, based on morphological characters is challenging due to their plasticity and made even more complicated by a confusing taxonomic history. Adams (1970) annotated on the herbarium voucher WELT A730 “not the same as *Chaetomorpha capillaris* (A2418) or *Chaetomorpha capillaris* sensu Lindauer No. 305. Cells larger with several large pyrenoids, no rhizoids”. Further molecular data, obtained from historic herbarium specimens collected at Pauatahanui Inlet, would be necessary to determine if the past and recent samples belong to the same species. The fact that an exact homology exists with Japanese and Netherland strains raises questions around the possibility that this is a recent introduction to New Zealand. To date forty-six marine macroalgal especies have been recognized as introduced in New Zealand (Nelson *et al.*, 2019). The green genus *Ulva*, for example incudes seven introduced species (Nelson *et al.*, 2019), which are difficult to be identified by morphology and require molecular identification. *Ulva* species and filamentous green algae are commonly found as hull fouling and dominant inhabitants in ports, marinas and estuaries.

Previous experiences in New Zealand such as the “water net” *Hydrodictyon reticulatum* which was introduced through the aquarium trade, may provide a clue as to the origin of *C. ligustica* in New Zealand waters *H. reticulatum* was reported for the first time in the field in New Zealand 1988 and then successfully spread to localities which had not previously suffered filamentous algal problems (Hawes *et al.*, 1991). Like water net, *C. ligustica* may have arrived in New Zealand accidentally through marine transport or naturally. At least 12 species of waders are regular annual migrants from Asia (Falla *et al.*, 1979). This could potentially explain the homology with the Japanese strain as the intertwined filamentous mass could stick to birds and these may have acted as dispersal vectors, even though further research on the time that *C. ligustica* can survive out of the water is desirable.

Identification of *C. ligustica* requires microscopic examination as this species can easily be mistaken for other filamentous green algae in the field, however molecular data are necessary for a definitive identification. *C. ligustica* may have been a mat former before, which has gone unidentified and/or misidentified and raises the question of how long *C. ligustica* has been present in New Zealand.

This is the first report, of which we are aware, in which the negative effect of *C. ligustica* on keystone seagrass meadows is documented. Other macroalgal blooms may represent a worrying further stress on seagrass meadows inhabiting New Zealand estuaries (Nelson *et al.*, 2003). Many studies have noted negative effects of filamentous algae on *Zostera* species both in the field and in mesocosm experiments (Rasmussen *et al.*, 2012). *Ulvaria obscura* (Nelson *et al.*, 2003), *Cladophora* sp (Hauxwell *et al.*, 2001), *Enteromorpha* sp (Cummins *et al.*, 2004), *Ulva* sp (Sugimoto *et al.*, 2007) , *Chaetomorpha linum* (Holmer & Nielsen, 2007; Rasmussen *et al.*, 2012) and *Gracilaria vermiiculophylla* (Martínez-Lüscher & Holmer, 2010) have been reported to damage seagrass. It is likely that the presence of macroalgal blooms epiphytic on seagrass meadows may be affecting colonization and re-colonization processes of seagrasses compromising its ability to achieve a healthy ecosystem worldwide (Hauxwell *et al.*, 2001; Burkholder *et al.*, 2007; Collado-Vides *et al.*, 2013; Lyons *et al.*, 2014).

In order to find out whether blooms are likely to occur and affect estuarine ecosystems, further research on *C. ligustica*'s growth requirements is recommended. We hypothesize from our environmental data that high irradiance and high daily maximum temperatures and high nutrients as well as weak winds may facilitate *C. ligustica* blooms so it has been previously reported for other blooming species (Hallegraeff, 2003). Also, better monitoring to estimate the extent and frequency of occurrence of this species after identification via DNA-barcoding is recommended.

Chapter 7

Evaluating the effect of tidal exposure on *Zostera muelleri* photosynthesis combining gas exchange measurements and pulse amplitude-modulated (PAM) fluorometry

---

7.1 Abstract

New Zealand seagrass *Zostera muelleri* is almost exclusively intertidal due to loss of suitable subtidal habitat. The ability of the seagrass to photosynthesise both in air and in water is potentially important in determining its vulnerability to enhanced water turbidity. In this study, we compared photosynthetic rate measurements made using oxygen flux in water, CO_2 flux in air, and pulse amplitude modulated fluorometry in both. In water, light saturated "gross" photosynthesis (GPS), as oxygen exchange per unit leaf area, averaged 2.24 µmol O_2 $m^{-2}s^{-1}$, leaf respiration averaged 0.44 µmol O_2 $m^{-2}s^{-1}$ and saturation irradiance was 115 µmol photons $m^{-2}s^{-1}$. In air, plants showed light saturated gross photosynthesis of 2.26 µmol CO_2 $m^{-2}s^{-1}$, respiration of 0.7 µmol CO_2 $m^{-2}s^{-1}$ and saturation irradiance of 286 µmol photon $m^{-2}s^{-1}$. Compensation irradiance (E_c) was 22 µmol photons $m^{-2}s^{-1}$ and 140 µmol photons $m^{-2}s^{-1}$ when submerged and emerged, respectively. Potential production of intertidal seagrass under submerged and emerged conditions was modeled across tidal cycles using experimental gas exchange results and field measured irradiance, using two scenarios; a high tide scenario (1) when high tide coincided with midday and low tide scenario (2) when low tide did. Total GPS was higher in the high tide scenario during which emerged GPS was predicted to be 1.3 times greater than submerged GPS. Respiration rate differed little between scenarios, and approximately similar amounts of net photosynthesis were predicted for emerged and submerged periods. In contrast emerged net photosynthesis was 25 times greater than submerged in the low tide scenario. These results support previous studies that have reported emerged photosynthesis as a mechanism to mitigate degraded submerged light climate, and to contribute to seagrass production estimates.

7.2 Introduction

Seagrasses are a polyphyletic group of monocotyledons, closely related to freshwater plants, which are able to live in the marine environment (Drew, 1978). Seagrasses play a significant role in provision of estuarine ecosystem services (Orth *et al.*, 2006) and contribute to coastal marine productivity, which has placed them as a key group of organisms for "blue carbon" ecology (Duarte *et al.*, 2004b; Duarte *et al.*, 2010; Duarte *et al.*, 2013; Rohr *et al.*, 2016; Ferguson *et al.*, 2017). The number of physiological studies undertaken on this group reflects this importance (Beer, 1989; Beer & Björk, 2000; Beer *et al.*, 2001; Silva *et al.*, 2005; Silva *et al.*, 2009; Procaccini *et al.*, 2012; Rasmusson *et al.*, 2020).

New Zealand waters are inhabited by only one seagrass species, *Zostera muelleri*. *Z. muelleri* distribution is almost exclusively intertidal although subtidal beds have been reported on offshore islands (Grace & Whitten, 1974; Grace & Grace, 1976; Schwarz *et al.*, 2006; Matheson *et al.*, 2010). Historical existence of subtidal seagrass beds in some estuary locations implies that environmental conditions have deteriorated for growth of these marine angiosperms (Inglis, 2003; Matheson & Schwarz, 2007; Matheson & Wadhwa, 2012). It is suggested that survival of subtidal populations has been restricted by reduction in photosynthetically available radiation (PAR) due to increasing loads of sediments to estuaries as a result of catchment development (Hume & McGlone, 1986; Swales *et al.*, 2002). Sediment pollution has been reported as one of the main threats to seagrass in New Zealand estuaries (Matheson & Schwarz, 2007; Matheson & Wadhwa, 2012) (chapters 2,3,4,5).

Tides expose seagrass to changing photosynthetic environments in terms of both inorganic carbon and irradiance. In water, inorganic carbon (C_i) is present as gaseous $CO_2(g)$, dissolved $CO_2(aq)$, bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}), the proportions of which vary with pH (Millero, 1979; Dickson, 2010). At typical ocean pH, C_i is primarily as bicarbonate, whilst free CO_2 accounts for less than 1%. In contrast, when emerged, carbon dioxide is the primary form of C_i. The mechanisms by which seagrasses, use external C_i include, uptake of CO_2 formed spontaneously from HCO_3^-, extracellular carbonic anhydrase mediated conversion of HCO_3^- to CO_2 at normal seawater pH, and in acid zones created by H^+ extrusion, and H^+ driven utilization through direct uptake of HCO_3^-. The last mechanism has been indicated for *Zostera marina*, *Halophila stipulacea* and *Ruppia maritima* (Beer et al., 2002).

In clear water, the irradiance available for carbon fixation during immersion of intertidal taxa can be similar to that during emersion, and C_i may be the most important variable affecting photosynthesis. As water turbidity increases, irradiance differences between submerged and emerged phases may become more important. Seagrasses inhabiting turbid estuaries have been shown to exhibit positive net photosynthesis only during emersion (Vermaat & Verhagen, 1996). The utilization of high light availability during emersion has been described as a compensation mechanism to avoid the negative effect of high turbidity (Vermaat *et al.*, 1997; Drylie *et al.*, 2018). Other factors affecting photosynthesis, and also associated with emergence, must be considered (Schwarz, 2004); these include photoinhibition (Enríquez *et al.*, 2002), shelf shading, desiccation (Björk *et al.*, 1999), photorespiration (Buapet *et al.*, 2013) as well as the potential for limitation by low carbon availability (Björk *et al.*, 1997; Buapet *et al.*, 2013; Rasmusson *et al.*, 2020).

There is a considerable body of literature on seagrass photosynthesis as reviewed by (Silva *et al.*, 2009). Studies of photosynthesis of *Z. muelleri* in Australia (Clough & Attiwill, 1980; Ralph & Gademann, 2005; Ralph *et al.*, 2007; Brodersen *et al.*, 2017) and in New Zealand, using pulse amplitude modulated (PAM) fluorometry and oxygen exchange techniques (Schwarz, 2004) or community level production in chambers (Lohrer *et al.*, 2016; Drylie *et al.*, 2018) suggest that up to 50% of carbon accrual can be during the emerged period.

The most commonly used method to measure seagrass photosynthesis in the past was through incubation of leaf segments in water, in closed chambers and determining initial and end O_2 concentration in laboratory set ups (Silva *et al.*, 2009). This method is highly intrusive as plant detachment and manipulation is implied (Beer *et al.*, 2001). However, it has been useful to provide most of the fundamental understanding of responses of seagrasses to light, temperature and nutrients as reviewed by Lee *et al.* (2007). *In situ* determinations of photosynthetic activity were made possible after the development of a submersible PAM fluorometer which is commonly used in seagrass physiology studies (Björk *et al.*, 1997; Björk *et al.*, 1999; Ralph & Gademann, 2005; Silva *et al.*, 2009). Thus, studies involving laboratory situations, in water, or PAM fluorometry in air and water dominate the literature. Despite the existence of intertidal seagrass meadows worldwide, only a few studies, of which we are aware, have addressed photosynthesis in air (Leuschner & Rees, 1993; Leuschner *et al.*, 1998) and just one study has been done on *Z. muelleri* using dome enclosures (Clough & Attiwill, 1980). Therefore, the ability for in-air photosynthesis to compensate for low water clarity in this species is therefore incompletely understood. In Chapter 3 it was shown that intertidal seagrasses can receive more irradiance while emersed than immersed, and further research on the effects of tidal exposure upon photosynthetic rates, for temperate New Zealand *Z. muelleri* is appropriate.

In the current study, both *in situ* and laboratory experiments were performed to test the hypothesis that seagrass photosynthetic rates differed under submerged and emerged conditions (ie. *in* water and *out of* water). Gas exchange techniques following oxygen concentration in water and carbon dioxide in air were combined with the non-invasive PAM fluorometry. This study will provide further understanding of intertidal seagrass photosynthesis and its role in community production and will be an aid to managers for evidence-based management of sediment influx in estuaries.

7.3 Materials and Methods

7.3.1 Study location

This experiment was conducted over the months of December 2019, March 2020 and July – August 2020. Seagrass collection for laboratory measurements occurred at Sulphur Point, Tauranga Harbour (Figure 7.1A) and *in situ* fieldwork at Pāuatahanui Inlet (Figure 7.1B).

7.3.2 Experimental design

The experimental design rationale was to test the hypothesis that in seagrass inhabiting the intertidal environment photosynthetic performance differs according to the contrasting physical conditions (e.g., C_i availability and receiving irradiance) when submerged and emerged (*in* or *out* of water). With this aim, photosynthetic characteristics of intertidal *Zostera muelleri* were measured. Gas (O_2 and CO_2) exchange techniques were utilized in the laboratory for submerged and emerged conditions under controlled irradiances to construct Photosynthesis *vs* Irradiance (PI) curves PAM fluorometry was used both *in situ* and in the laboratory as a cross-over, non-invasive technique in both submerged and emerged conditions. The results thus obtained permitted meaningful physiological comparisons under contrasting conditions.

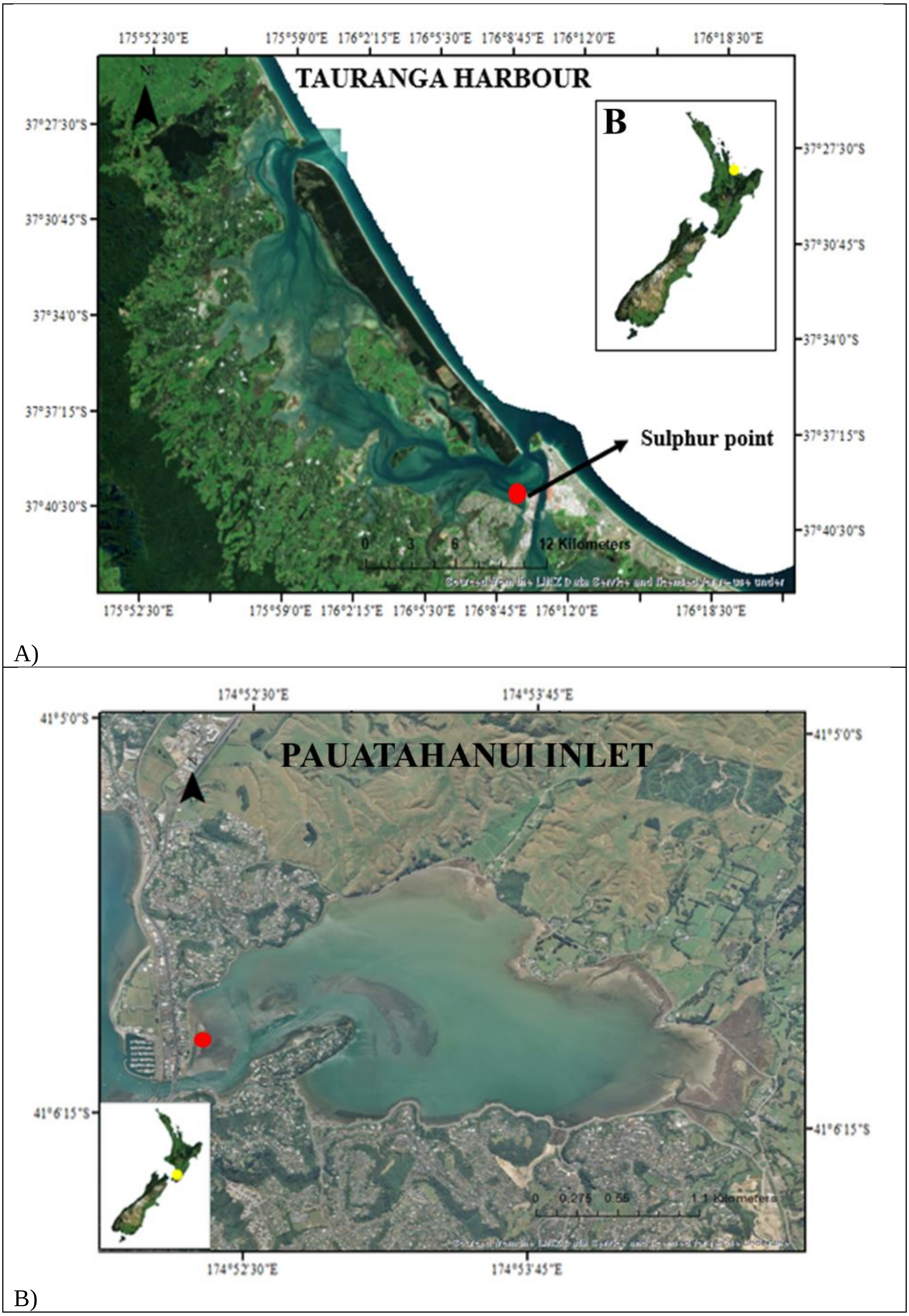


Figure 7.1: A) Map of Tauranga Harbour (North Island, New Zealand) indicating the sampling location (March 2020) (Red dot). B) Map of Pāuatahanui Inlet (North Island, New Zealand) indicating the location where the field PAM study was performed (December 2019) (Red dot).

7.3.3 Gas exchange techniques

7.3.3.1 Oxygen exchange technique

To establish PI curves for submerged plants, healthy shoots were incubated in 40 mL (2.5 x 10 cm) glass vials at a range of irradiances from dark to 720 µmol photons $m^{-2}s^{-1}$ and oxygen production rates were estimated. Darkness was provided by wrapping vials with aluminum foil. Irradiance was provided by two, 100W LED floodlights (colour temperature 4000K) that were placed about 20 cm above a temperature-controlled water bath (Figure 7.2). The irradiance was manipulated using layers of white translucent cloth below the LED lights and a LiCor Li-192 quantum sensor used to measure PAR. This arrangement provided a gradient of ten actinic irradiances: 0, 30, 65, 100, 170, 260, 320, 370, 450, 720 µmol photons $m^{-2}s^{-1}$. A circular area, 20 cm in diameter at the surface of the water bath, was illuminated almost homogenously, with variation less than 0.5% at each actinic light level. This area could accommodate up to 8 incubation vials on a rack 1cm below the water surface. Each element in the water bath (the water bath container, and the rack) was black to prevent reflections. The water bath was maintained between 17°C and 18°C and the water was cooled and circulated (23w, 1001 EHEIM, GmbH).

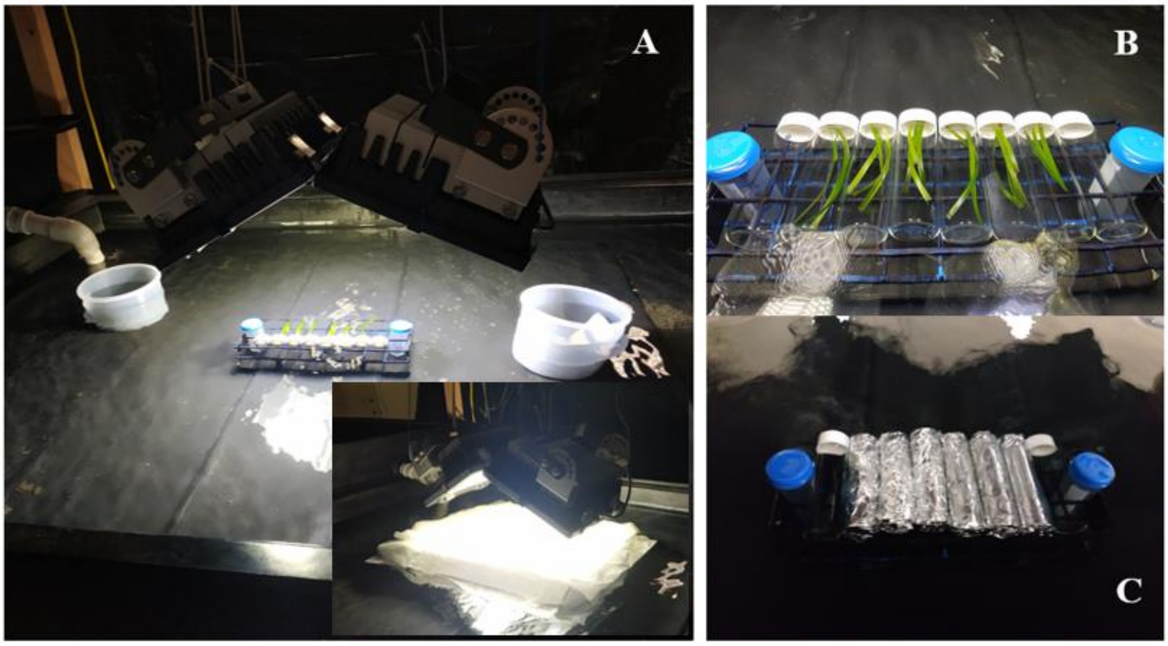


Figure 7.2: A) Shows the incubation arrangement in which seagrass shoots were exposed to different irradiances manipulated through white shade cloths. B) Shows a detailed picture of seagrass shoots under the irradiance and C) Shows a dark incubation which was used to estimate respiration.

Shoots were incubated in seawater enriched with bicarbonate ion (10 mM) to prevent inorganic carbon limitation in the sealed vials. Two glass vials with only incubation medium were incubated as controls. The glass vials were incubated incrementally under sequentially increasing actinic irradiance. The incubation time was varied by irradiance from 20 to 60 minutes to avoid ebullition but allow measurable changes in oxygen concentration. Incubation medium was replaced for each irradiance. At the end of each incubation, the vials were mixed and oxygen concentrations were measured in the same temperature bath using a PreSens Oxygen Microsensor connected to a Microx 4 control unit (PreSens GmbH, Germany). Oxygen production was determined as the difference from the average of the two control vials, corrected for incubation time, and normalized to leaf surface area, yielding units of μmol O_2 $m^{-2}s^{-1}$. Leaf surface area was measured from a 3D digital image of each shoot (flattened) using Image J software (https://imagej.nih.gov/ij/).

Rates were pooled across replicates and plotted against actinic irradiances. PI curves were fitted following the Platt, Gallegos & Harrison (1980) "PGH" model, which describes the photosynthetic response as a single continuous function of irradiance, covering both the initial linear response, as well as the photoinhibited region at high light (Platt *et al.*, 1981) amended to include respiration term. Curves were fitted using a Marquardt–Levenberg regression algorithm: in the "phytotools package" within R studio (https://cran.r-project.org/package=phytotools).

$$P = (P_s\,(1-e^{-(\alpha\frac{Ed}{Ps})})xe^{-(\frac{\beta Ed}{Ps})}) - R \qquad (1)$$

Where:

P_s is a scaling factor defined as the maximum potential photosynthetic capacity,

P_m is the photosynthetic capacity at saturating irradiance,

α is the initial, near linear slope of the PI relationship before the onset of saturation,

E_d is the downwelling irradiance (400–700 nm),

ß characterizes the slope of the PI curve where photosynthesis declines (Henley, 1993),

R is dark respiration.

In the absence of photoinhibition ($\beta = 0$), the function becomes a standard rectangular hyperbola, with an asymptotic maximum P value (Harrison & Platt, 1986), and Eq. (1) can be simplified to Eq. (2).

$$P = P_m\, (1 - e^{-(\alpha \frac{Ed}{Pm})}) - R \qquad (2)$$

The parameters P_{max} (light saturated rate of photosynthesis), E_k (saturating irradiance for photosynthesis) and E_c (compensation irradiance where net photosynthesis equals zero) were estimated after Platt *et al.* (1981) using the following equations:

$$P_{max} = P_s\, (\alpha / [\alpha + \beta])\, (\beta / [\alpha + \beta])\, \beta/\alpha \qquad (3)$$

$$E_k = P_{max} / \alpha \qquad (4)$$

$$E_c = R / \alpha \qquad (5)$$

12 replicates/shoots per irradiance were grouped to fit the model, and, in addition, each shoot was fitted individually.

7.3.3.2 Gaseous CO_2 fluxes.

Infra-red gas analysis (IRGA) has long been used to measure, with high accuracy, the evolution of CO_2 exchanged in either the photosynthetic or respiratory process in terrestrial plants (Field *et al.*, 2000; Douthe *et al.*, 2018). An IRGA CO_2 analyzer (Qubit, Model No. S151X, range 0-2000 ppm) was utilized to measure CO_2 uptake by individual leaves (five to ten leaves), which were enclosed in a mini cuvette, with irradiance, temperature and humidity control, and connected to the instrument using a high precision pump with a mass flow controller, configured in open circuit (Figure 7.3). CO_2 differentials between air entering and leaving the enclosed cuvette were measured, and PI curves generated by ramping the cuvette LED irradiance (A113) from zero to 1100 µmol photons $m^{-2}s^{-1}$. The PGH model was again used to derive PI parameters, using the same approach as for oxygen-based curves.

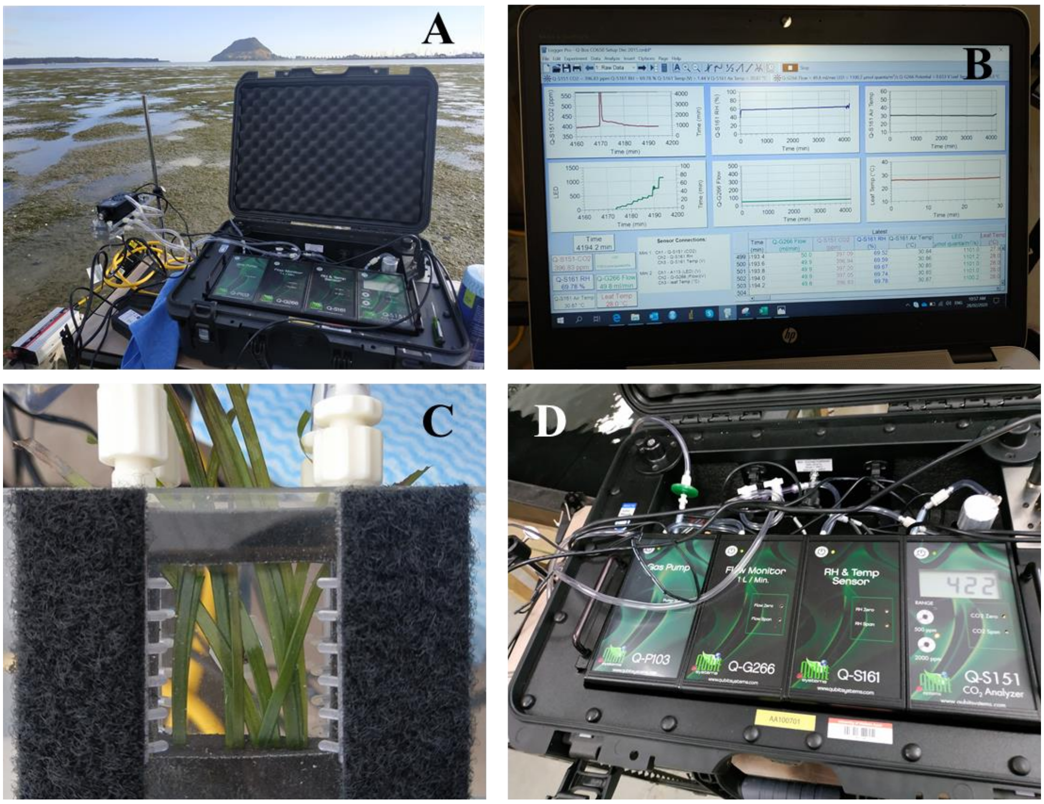


Figure 7.3: A) IRGA CO_2 analyzer (Qubit, Model No. S151X, range 0-2000 ppm) instrument. B) detail of the software in which data is logged, C) detail of the cuvette where leaves are incubated and D) set up of the Qbox 650 interior with all the channels (Temperature, Relative Humidity, Pump and Flow Monitor) set.

7.3.4 Chlorophyll variable fluorescence: PAM fluorometry

In the laboratory, photosynthetic activity at a range of irradiances was also investigated using a Moni-DA PAM fluorometer (Walz, Germany). In the field , the same PAM instrument was deployed in logging mode, together with ECOPAR™ (http://www.seabird.com/ecopar) PAR sensors and chlorophyll fluorescence was monitored over tidal cycles (Figure 7.4).

PAM fluorometry is a non-invasive technique, which allows instantaneous measurement of variable fluorescence of Photosystem II (PSII) *in situ*, with centimeter spatial resolution. In logging mode, the instrument can make repeated measurements over time, allowing insights into PI relationships under natural conditions. Variable fluorescence of PSII can be used to estimate and infer aspects of photosynthetic activity (Schreiber *et al.*, 1986). The system measures the fluorescence of chlorophyll under ambient irradiance (F), and during application of a short pulse of saturating white light (maximum fluorescence F_m'). The difference in fluorescence ($F_m' - F$) is called the variable fluorescence (ΔF). The ratio of variable fluorescence to maximum fluorescence ($\Delta F / F_m'$) is the effective quantum yield (YII) of the plant under the prevailing irradiance condition (Hanelt *et al.*, 1993). YII is normally at its highest in non-photoinhibited material under dark-acclimated conditions and tends to decline proportionately with the extent to which photosynthesis is light saturated (Hawes *et al.*, 2003).

Figure 7.4: Moni-DA Diving PAM (Walz, Germany) along with ECOPARs deployment in the field. B & C) underwater detail of the deployment of the leaves in the clips of the instrument.

As a metric of photosynthetic activity, we estimated electron transport ratio (ETR) (Beer *et al.*, 2001). YII was multiplied by the incident irradiance, multiplied by 0.5 (assuming that half of the incident photons were absorbed by PSII and half by PSI), and by the leaf absorption factor (AF) (Genty *et al.*, 1989). AF was derived from the attenuation of LED actinic lights by one layer of seagrass leaves, measured using a LiCor Li-192 PAR sensor (Eq.6).

$$AF = (I_1 - I_2) / I_1 \qquad (6)$$

Where:

I_1 is the PAR with no leaf present

I_2 is the PAR when the sensor was covered by a seagrass leaf

Laboratory experiments were conducted under the same conditions and lamps as the oxygen evolution experiment. Shoots were acclimated to each successive actinic irradiance for 30 minutes before a saturation pulse was applied to measure YII. ETR was calculated as described above and plotted against actinic irradiances and the PGH curve (with no respiration term) again fitted to determine parameters comparable to the gas flux PI curves (E_k, ETR_{max} and α).

Field observations with the Moni-DA PAM fluorometer (Moni-DA Diving-PAM, Walz) were undertaken during a daytime low tide in Pāuatahanui Inlet from 4:00 pm (11/12/19) to 7:00 am (13/12/19). A seagrass shoot was fastened in a leaf clip attached to each of three sensing heads connected to the Moni-DA. The Moni-DA PAM was programmed to take one YII measurement, under ambient irradiance, every 15 min during the deployment time. Readings with YII < 0.1 were removed to reduce noise. Irradiance incident at the time of the YII measurement was recorded concurrently, and adjacent to the leaf, using ECOPAR™ loggers (http://www.seabird.com/ecopar) deployed in orientations mimicking those of the leaves, either parallel to the substrate when emerged or near perpendicular to the substrate when submerged. YII was converted to ETR and ETR- irradiance curves generated and the PGH model fitted to derive parameters. Measurements were repeated for three different leaves on three occasions (n=9) and (Equations 1 - 4).

7.3.5 Modeling potential *in situ* photosynthesis

Potential rates of photosynthesis over a 24 hour period for intertidal seagrass was modelled under two scenarios: Scenario 1 when high tide coincided with mid-day and Scenario 2 when low tide coincided with mid-day. Oxygen based PI models developed above were used to predict the photosynthesis when submerged, and carbon dioxide-based models for emerged photosynthesis. A photosynthetic quotient (moles of O_2 evolved per moles of C fixed) of 1.25 was assumed to convert O_2 measurements to carbon values (McRoy & McMillan, 1977).

The model used measured irradiance at Pāuatahanui Inlet, as described in Chapter 3, as input, on dates selected as those that fitted the two scenarios and had similar incident irradiance, and assumed temperature was constant, and similar to that used in incubations from which PI parameters were derived. As such, the model is not intended as a true production model, but simply to allow comparison of the potential accumulation of carbon during immersion and emersion, and the importance of the timing of the tidal cycle. In order to get a true model production and irradiance data should be coupled to real exposure through water level sondes and integrated for at least a full tidal cycle or a month. The model was based on the optical model developed by Zimmerman (2003), which estimates irradiance at the top of and through the seagrass canopy based on canopy architecture, leaf orientation and water clarity under immersed and emerged conditions. It provides a robust irradiance modelling tool for investigating photosynthetic performance of seagrass canopies. All parameters needed for the model were estimated on site during the experiments, and it was linked to photosynthetic parameters derived from the oxygen and carbon dioxide flux experiments.

7.3.6 Statistical analysis

Key PI curve parameters of leaves, dark respirations, and light compensation irradiance were compared between submerged and emerged condition using analysis of variance (ANOVA). Prior to analysis normality was tested using the Shapiro-Wilk test (Shapiro & Wilk, 1965) and homogeneity of variances using the Cochran test. All tests were performed using the R statistical package (v 3.6.2).

7.4 Results

7.4.1 Gas exchange techniques

Photosynthesis in submerged leaf oxygen exchange experiments followed expected saturation characteristics, with a rapid, near linear rise in photosynthetic rate at low irradiance plateauing at high irradiance. Dark respiration rate was 0.44 µmol O_2 $m^{-2}s^{-1}$, net photosynthesis exceeded zero at 22 µmol photons $m^{-2}s^{-1}$ and was constant above 150-200 µmol photons $m^{-2}s^{-1}$ (Figure 7.5, Table 7.1). Gross photosynthesis at saturating irradiance was 1.6 µmol O_2 $m^{-2}s^{-1}$. The derived PI curve parameters, obtained using equation 2 (no photoinhibition) are shown in (Table 7.1).

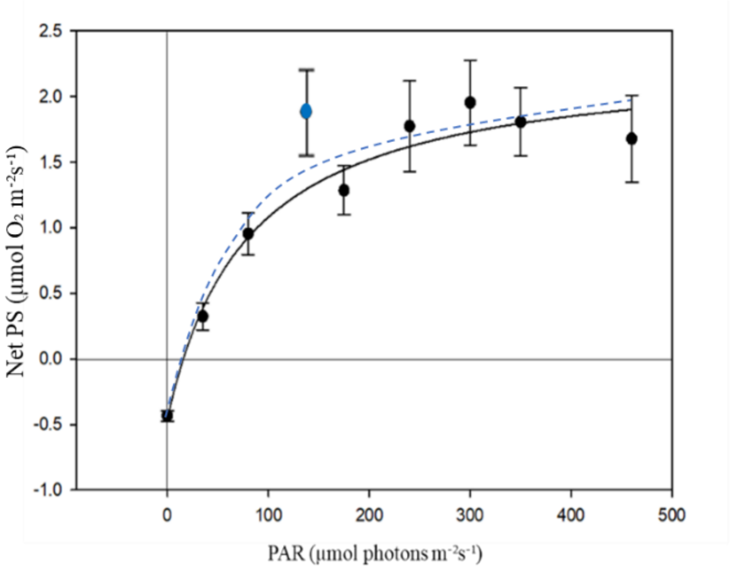


Figure 7.5: Submerged Photosynthesis Irradiance (PI) curve showing net photosynthesis (NPS) in (µmol O_2 $m^{-2}s^{-1}$) vs photosynthetically available radiation (PAR) in (µmol photons $m^{-2}s^{-1}$). Values are means (±SE) of 6 replicates per irradiance (n=54). The dashed blue curve includes all of the experimental readings and its parameters are **R^2 = 0.91; E_k = 103 ± 1.4; P_{max} = 2.1 ± 0.0; α = 0.018 ± 00**; E_c =24. The black curve is the same data after removal of irradiance of 140 µmol photons $m^{-2}s^{-1}$ for the best fit and its parameters values are **R^2 = 0.98**; **E_k = 115± 2; P_{max} = 2.2 ± 0.1**; **α = 0.016 ± 0.0**; E_c = 22. Normality Shapiro-Wilk **0.96.**

The emerged CO_2 uptake PI curve, also fitted to equation 2, showed a similar shape to that of the submerged oxygen experiment (Figure 7.6). A good curve fit was obtained (r^2 = 0.97), and parameters are shown in Table 7.1.

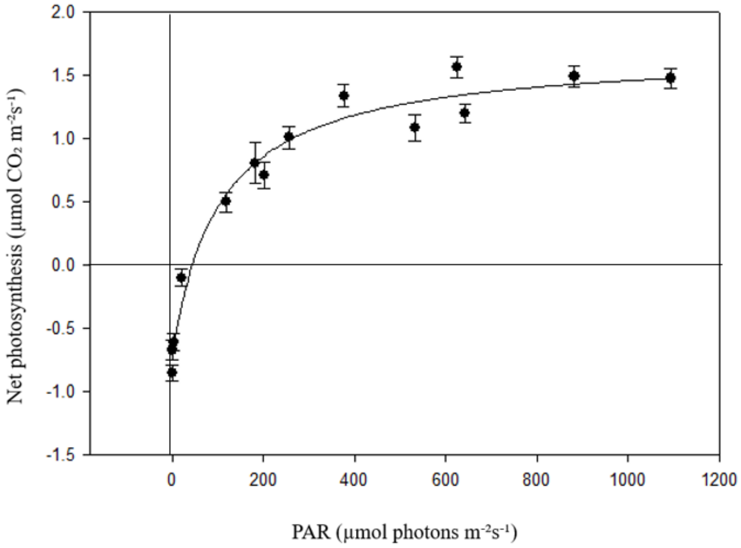


Figure 7.6: Emerged Photosynthesis irradiance (PI) curve showing net photosynthesis (µmol CO_2 $m^{-2}s^{-1}$) vs irradiance in (µmol photons $m^{-2}s^{-1}$). Values are means (±SE) of 35 replicates per irradiance (n=350) at (18.2 °C ± 0.32). **R^2** = **0.97** ± 0.2; **E_k = 286 ± 10 P_{max} = 2.3 ± 0.03; α = 0.005 ± 0.0;** E_c = 140 Normality Shapiro-Wilk **0.97.**

Both in air and submerged net and gross PI curves combined in one plot are shown for comparison purposes in (Figure 7.7), with the carbon data converted to oxygen using a molar photosynthetic quotient of 1.25 mol oxygen per mol carbon dioxide (McRoy & McMillan, 1977). The curves are similar both quantitatively and qualitatively, with submerged plants showing overall higher rates of oxygen evolution in the light, particularly at low irradiance, and lower dark respiration.

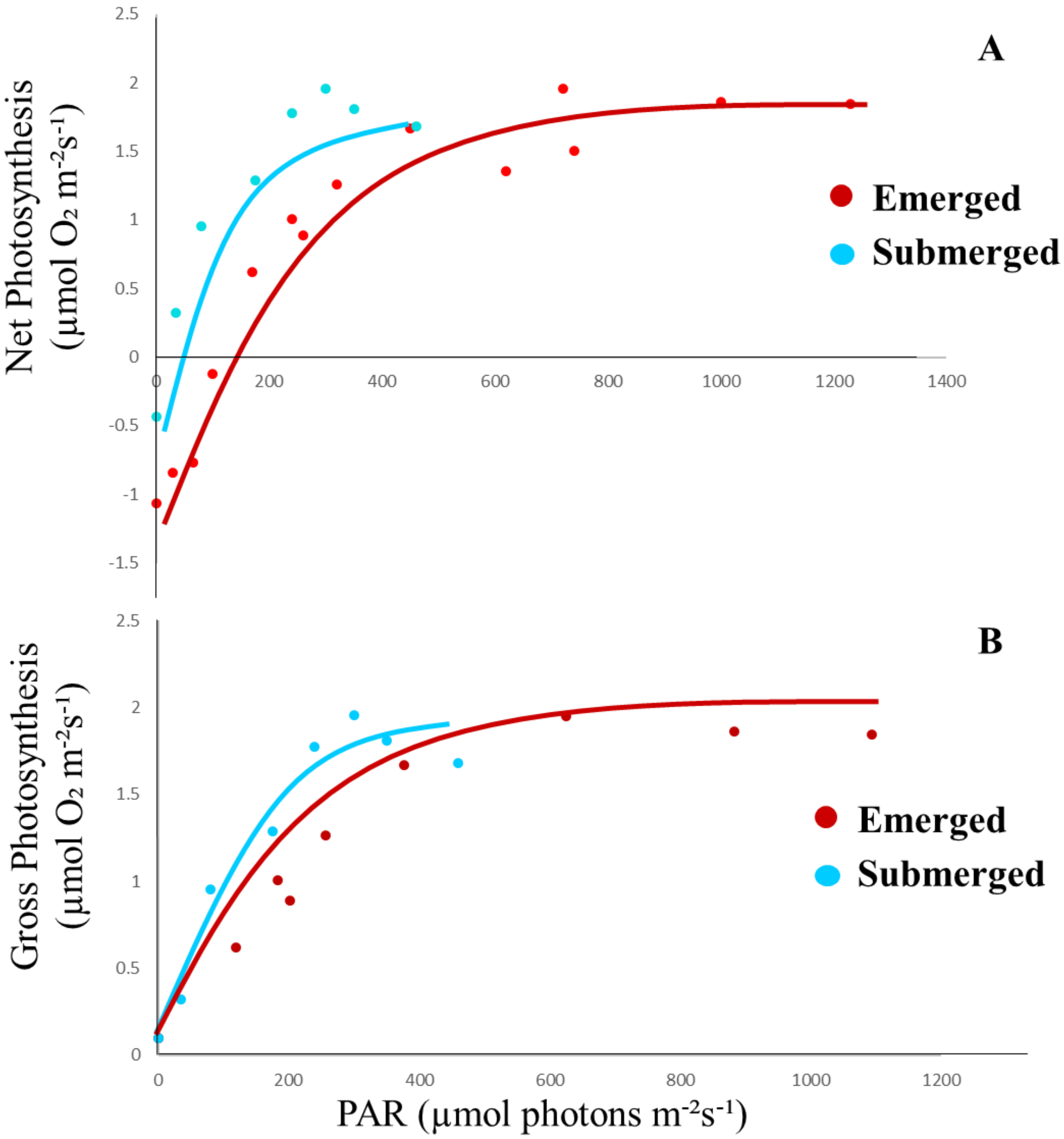


Figure 7.7: Submerged and emerged net (A) and gross (B) Photosynthesis Irradiance (PI) curves showing photosynthesis vs photosynthetically available radiation (PAR) in (μmol photons $m^{-2}s^{-1}$).

Table 7.1: Photosynthesis irradiance (PI) curve parameters for gas exchange measurements are shown for each of the exposures. Irradiances are shown in μmol photons $m^{-2}s^{-1}$ and production and respiration in μmol O_2 $m^{-2}s^{-1}$ for submerged and in μmol CO_2 $m^{-2}s^{-1}$ for emerged exposures. Parameters are derived by fitting equations 1 and 2 and are net photosynthesis.

Method	**Exposure**	**Exp**	**PI parameters**						
			P_{max}	**E_k**	**ETR_{max}**	**α**	**E_c**	**R**	**r^2**
Oxygen	Submerged	Lab	2.2 ± 0.1	115 ± 2	-	0.02 ± 0.0	22	0.44 ± 0.1	**0.97**
IRGA	Emerged	Lab	2.3 ± 0.1	286 ± 10	-	0.005 ± 0.0	140	0.7 ± 0.1	**0.97**
PAM	Submerged	Lab	-	208 ± 15	64 ± 2	0.33 ± 3.5	-	-	**0.95**
PAM	Emerged	Lab	-	161 ± 30	30 ± 3	0.2 ± 0.1	-	-	0.85

7.4.2 PAM fluorometry

The average AF for seagrass leaves was 0.79 ± 0.04 (n = 20) and this was used throughout when calculating ETR.

In the laboratory, the ETR vs PAR curve saturated at a lower irradiance and showed lower ETR_{max} value when emerged than when submerged (Figure 7.6). Emerged and submerged curves showed no differences from 0-120 μmol photons $m^{-2}s^{-1}$ but the emerged plants began to show saturation of ETR above this irradiance, whereas, ETR in submerged plants continued to increase up to 400 μmol photons $m^{-2}s^{-1}$. ETR_{max} and E_k were both higher submerged than out of water (Table 7.1).

When the Moni-DA PAM (Walz, Germany) was recovered at the end of the deployment the battery was found to be very low, which may have affected the later results.

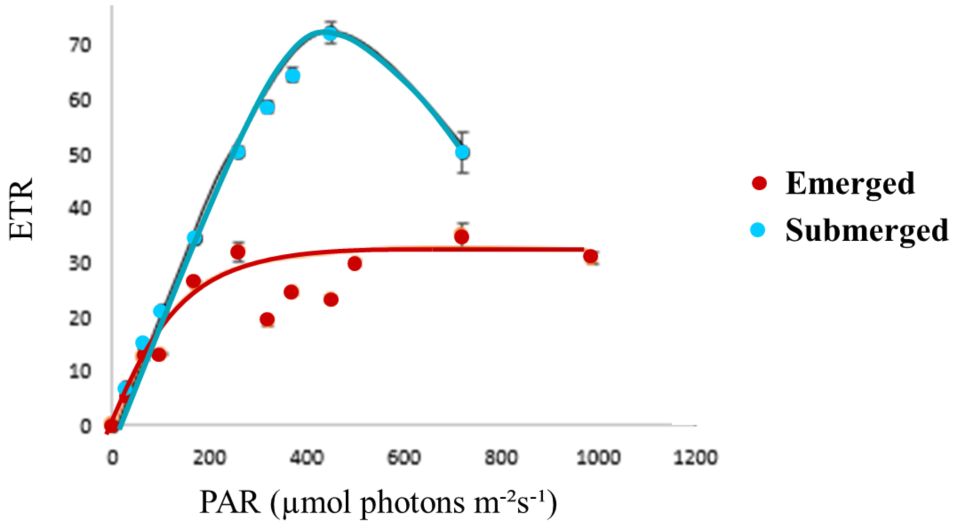


Figure 7.8: Photosynthesis irradiance (PI) curve showing electron transport ratio (ETR) vs irradiance in (μmol photons $m^{-2}s^{-1}$). Values are means (±SE) of 5 replicates per irradiance and per submerged or emerged condition (n=100). **Submerged PGH R^2 = 0.88 ± 9.8; ETR_{max} = 64 ± 2; E_k = 208 ± 15;** α = 0.33 ± 3.5 Normality Shapiro-Wilk W = **0.96. Emerged HTM R^2**= 0.85 ±4.7; ETR_{max}= 30.5 ± 3.1; **E_k = 161 ± 30; α = 0.2 ± 0.1.** Normality Shapiro-Wilk W = **0.98.**

In situ PAM measurements were used to generate an ETR vs Irradiance curve under ambient conditions (Figure 7.9). Rather than a saturation curve, a linear relationship emerged up to high irradiance, for which virtually identical equations were derived for emerged ($y = 0.49x - 15.3$; $r^2 = 0.8$) and submerged ($y = 0.44x + 1.2$; $r^2 = 0.94$) treatments. With the exception of a cluster of data points with low ETR under emerged conditions, there was little difference between these two modes.

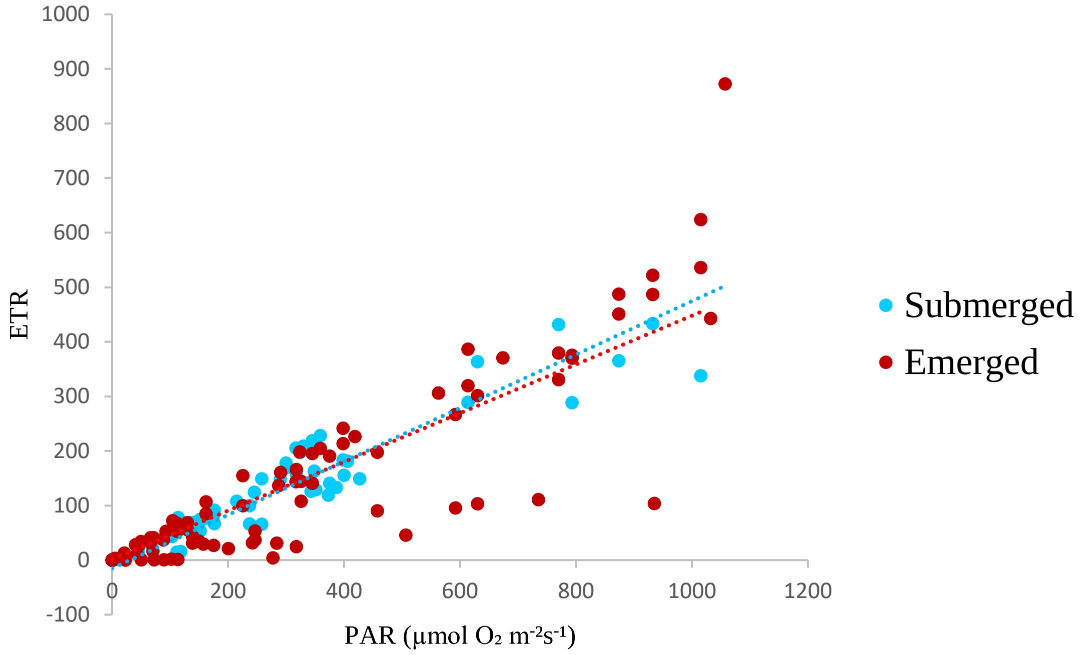


Figure 7.9: Electron transport ratio (ETR) vs Irradiance (I). Yield (YII) values < 0.1 have been removed for ETR calculations to avoid PAM fluorimeter noise.

7.4.3 Production models

Over a 24-hour period, total GPS and total NPS per unit leaf area under scenario 1 (mid-day high tide) were predicted as 770 and 152 mmol O_2 $m^{-2}d^{-1}$, the difference reflecting respiration. Of total GPS, a little over one half was produced when emerged and a little less than a half when submerged, resulting in NPS being evenly spread over the tide (Table 7.2, Figure 7.10).

Under scenario 2 (mid-day low tide) total GPS was slightly lower than scenario 1, 757 mmol O_2 $m^{-2}d^{-1}$ and of that 2/3rd was produced when emerged and 1/3rd when submerged. Respiration was similar in the two scenarios, resulting in overall lower NPS. While submerged, gross photosynthesis barely exceeded respiration, and effectively NPS was only occurring when plants were emerged (Table 7.2, Figure 7.10).

Gross Emerged photosynthesis was consistently greater than submerged production for both scenarios. Net emerged production was 1.3 and 25 times greater than submerged for midday high tide day and midday low tide day respectively (Table 7.2 or Figure 7.10). Because of the model characteristics, with respiration essentially constant at either submerged or emerged rate, it was expected that respiration would be predicted to be similar for the two scenarios.

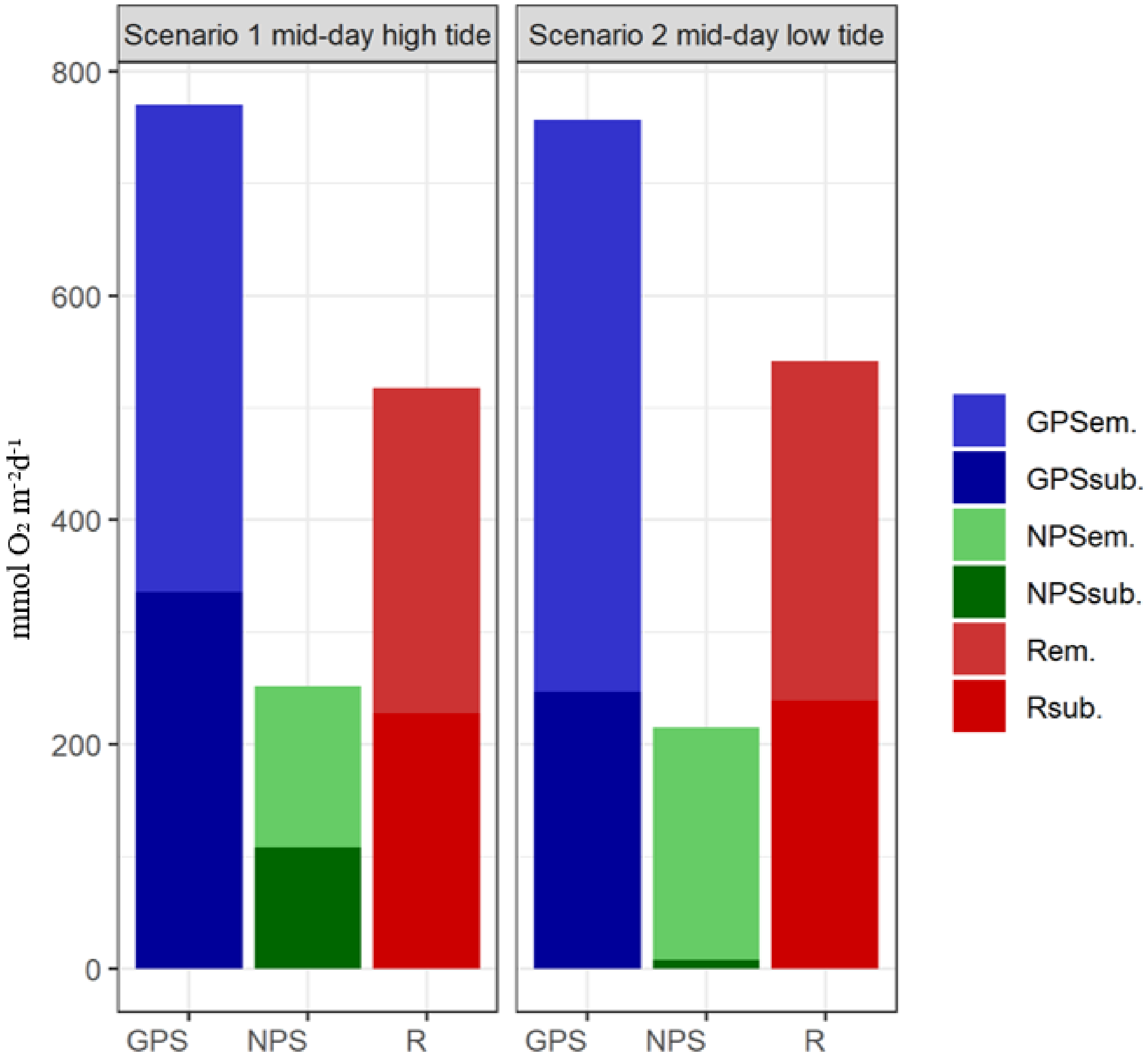


Figure 7.10: 24 hour Gross Photosynthesis (GPS) and Net Photosynthesis in (mmol O_2 $m^{-2}d^{-1}$) for two scenarios: a day in which submerged period fits with mid-day high tide (scenario 1) and for a day in which emerged period fits with mid-day low tide (Scenario 2); and at two canopy levels top and within. Production is divided in submerged and emerged for comparison purposes.

Table 7.2: Modelled Gross Photosynthesis (GPS) and Net Photosynthesis (NPS) outcomes for a day in which submerged period fits with mid-day high tide (scenario 1) and for a day in which emerged period fits with mid-day low tide (Scenario 2). A photosynthetic quotient (moles of O_2 evolved per moles of C fixed) of 1.25 was assumed to convert carbon measurements to O_2 values (McRoy & McMillan, 1977).

Modelled Photosynthesis	**Scenario 1 mid-day high tide** (mmol O_2 $m^{-2}d^{-1}$)	**Scenario 2 mid-day low tide** (mmol O_2 $m^{-2}d^{-1}$)
GPS_{total}	770	757
$GPS_{em.}$	434	510
$GPS_{sub.}$	336	247
NPS_{total}	252	215
$NPS_{em.}$	144	207
$NPS_{sub.}$	108	8
R_{total}	518	542
$R_{em.}$	290	303
$R_{sub.}$	228	239

7.5 Discussion

Intertidal *Z. muelleri* was capable of photosynthesis in both air and water, though rates and PI curve shapes differed slightly. This was evident in the results obtained through the three techniques which were utilized to estimate seagrass photosynthesis performance. In water, light saturated gross photosynthetic O_2 evolution averaged 2.24 µmol O_2 $m^{-2}s^{-1}$, leaf respiration 0.44 µmol O_2 $m^{-2}s^{-1}$ and saturation irradiance 115 µmol photons $m^{-2}s^{-1}$. In air, light saturated gross photosynthesis was 2.26 µmol CO_2 $m^{-2}s^{-1}$, which would marginally exceed the oxygen-based rate if PQ is 1.25, but respiration rate at 0.85 µmol CO_2 $m^{-2}s^{-1}$ and saturating irradiance 286 µmol photon $m^{-2}s^{-1}$, were higher. Superficially, when plotted together and corrected to similar units, it appeared that the PI curve for air was moved downwards relative to that in water. Comparing data from in air and in water highlights how failing to achieve the same range of irradiances in the two sets of experiments meant that the apparent differences between key fitted parameters of P_{max} and E_k need to be treated with a degree of caution. It is clear, however, from the gas exchange results that leaves are similarly capable of using light in air and water, at least at low and moderate irradiance.

There are few existing measurements of rates of photosynthesis on *Z. muelleri*, and the variety of techniques used makes direct comparison difficult. The most robust comparisons can be made for E_k, since this is largely technique agnostic, and here the values obtained in air and water are similar to those from other studies (Clough & Attiwill, 1980; Vermaat *et al.*, 1997; Schwarz, 2004). Where comparisons of in air and in water activity have been made, as in this study, broadly similar respiration and maximum rates of photosynthesis are reported, as here (Table 7.3).

Modeled scenarios were intended to provide some insight into the importance of submerged and emerged photosynthesis in a realistic irradiance regime. Models predicted that gross emerged photosynthesis was consistently greater than submerged production for both high and low tide scenarios. Under scenario 1 (high tide at noon) submerged GPS was higher than scenario 2, because the irradiance is higher when submerged. In contrast emerged GPS is lower under scenario 1 than in scenario 2 due to high irradiances received at noon low tide in scenario 2.

Net emerged photosynthesis under scenario 2 was also significantly higher than under scenario 1 due to the high irradiances received at noon in scenario 2. However, submerged NPS was over 10-fold times higher in scenario 1 compared to scenario 2 (Table 7.2, Figure 7.10). The fact that NPS is higher for scenario 1 is explained because the irradiance when submerged at noon allowed seagrass to photosynthesise at higher rates than at scenario 2 in which the submerged periods are morning and evening and hence irradiances are lower to those at scenario 1.

Under both scenarios, the respiration over the day is similar, as expected, as this is independent of light, and the ratio of time submerged to time emerged is similar for the two scenarios. Under both, photosynthesis while emerged is shown to be critical to maintaining a positive daily carbon balance.

Z. muelleri GPS during emergence in the simulations can far exceed that during submergence, as previously inferred using PAM and oxygen exchange techniques (Schwarz, 2004) and community production chambers (Drylie *et al.*, 2018). This is driven by the very high receiving irradiances when emerged compared to submerged periods. Previous researchers have determined emerged production to make a very important contribution to total production (Vermaat & Verhagen, 1996; Vermaat *et al.*, 1997), consistent with the results presented here. This reinforces the important role of emerged production to mitigate for when submerged irradiance falls below survival thresholds, as previously reported by Drylie *et al.* (2018). Previous studies performed in *Z. noltii* showed a similar pattern of in air and in water production (Silva *et al.*, 2005).

However, photosynthetic gains are not restricted to periods of emergence if sufficient irradiance reaches the seabed when submerged. Moreover, our results showed that E_c was lower and α higher when submerged than emerged, suggesting that, when submerged, seagrasses utilize low PAR more efficiently, or that when emerged the light-dependent respiration rate increases. Ultimately, *Zostera muelleri* is more efficient when submerged. This may relate to the alternate sources of inorganic carbon available in air and in water. In water, carbon is available as HCO_3^- through carbon-concentrating mechanisms (CCM). These can involve extracellular carbonic anhydrase (CA) mediated conversion of HCO_3^- to CO_2 at normal seawater pH, chemical conversion in acid zones created by active H^+ extrusion, and through direct uptake of HCO_3^- (Beer *et al.*, 2002) followed by a carbon-concentrating mechanism in the cytoplasm and/or chloroplasts (Larkum *et al.*, 2017). For *Zostera muelleri* in air CO_2 uptake relies on diffusion, which can result in high ratios of O_2 to CO_2 within cells, which favours photorespiration and reduces overall carbon fixation. Additional challenging environmental conditions include photoinhibiting irradiance and desiccation (Björk *et al.*, 1997; Schwarz, 2004; Buapet *et al.*, 2013; Rasmusson *et al.*, 2020).

Gas exchange techniques results were utilized to estimate production despite some limitations, which were dealt with as best as possible. The oxygen exchange technique is a highly intrusive method as plant samples are detached and have difficulty in maintaining homogenization of the medium during incubations which can result in underestimation of photosynthetic rates (Koch, 1994; Koch *et al.*, 2007a). To avoid potential inorganic carbon depletion which, at high O_2 saturation can result in photosynthetic inhibition through photorespiration (Beer, 1989) and carbon limitation itself, sea water was enriched with bicarbonate (10 mM) and short incubation times were used throughout (Silva *et al.*, 2009). In spite of these limitations, this technique remains in wide use as it still provides oxygen exchange measurements under highly controlled conditions as well as making possible the manipulation of incubation medium.

The short-term incubation method IRGA based on CO_2 fluxes and used in this work has previously proven to be a powerful tool for field measurements of intertidal seagrass productivity (Leuschner & Rees, 1993; Leuschner *et al.*, 1998). IRGA provides fast and precise values of leaf CO_2 fixation in air-exposed conditions. An important feature in this approach is the use of small chambers and short incubation periods so that the temperature and humidity conditions in the chamber remain fairly constant.

PAM fluorometry, measures electron flow through PSII rather than oxygen evolution or carbon accrual, and it is often difficult, if not impossible to compare such measurements. However, the most suitable questions to ask using PAM fluorometry are those concerning the photosynthetic light responses to the whole range of ambient parameters such as exposure to tides in this study. Our PAM results seem contradictory as PI curves generated with laboratory experiment data showed saturation kinetics, whereas the PI curves generated with field data showed very similar linear relationships for both submerged and emerged plants. This apparent discrepancy may at least in part be explained by the extreme sensitivity of estimated ETR to measured irradiance. Field collected irradiance data used ECOPAR loggers, which are large, disrupt the local meadow structure and are likely to be recording different PAR to the leaves in the canopy, and even more so the leaves in the PAM fluorometer clip. This is due to the size of the logger itself and its cosine-corrected measuring head, but more importantly by the orientation of the leaf within the canopy.

The complexity of leaf orientation under both submerged and emerged conditions has been addressed (Krause - Jensen & Sand - Jensen, 1998; Hawes *et al.*, 2003) , but reliable methods to collect such data are still lacking, this is definitely a research gap that requires further consideration. Schwarz (2004), demonstrated in *Z. muelleri* beds in New Zealand that only 25% of incident irradiance filtered through to the bottom leaves when seagrasses were flat on the sediment surface, thus leaves beneath the upper layer may receive more optimum irradiance to maintain high photosynthetic efficiency.

The model approach to understand the relative photosynthesis when immersed and emerged is indicative at best, since it ignores other stressors on the plants during the exposed period. We might expect that photosynthetic rates and efficiency would decrease with increasing duration of emergence due to elevated desiccation stress, as has previously been demonstrated in other *Zostera* species (Leuschner & Rees, 1993; Leuschner *et al.*, 1998),. Additionally, morphological characteristics may change in response to desiccation stress, with higher seagrass shoot densities and broader leaves conveying desiccation tolerance (Park *et al.*, 2016; Manassa *et al.*, 2017). The self-shading effect of seagrasses during emergence also means that whilst the uppermost/outer leaves may have experienced irradiance >286 µmol photon $m^{-2}s^{-1}$, most of the seagrass bed would have received only a fraction of this (Schwarz, 2004; Clavier *et al.*, 2011). Whether, when allowance has been made for leaf orientation when submerged and emerged, the relative importance of each would be as projected in the model is not known (Zimmerman, 2003).

Whilst it is hard to determine the exact values for *in situ* production in such a complex system as a seagrass community, this study highlights the potential importance of emerged photosynthesis to maintain a positive carbon balance.

Table 7.3: Light-saturated net photosynthesis rates under submersed and exposed conditions according to different authors for different intertidal species. A photosynthetic quotient (moles of O_2 evolved per moles of C fixed) of 1.25 was assumed to convert O_2 measurements to carbon values (McRoy & McMillan, 1977). Results from this study are shown in bold. Irradiance units are in µmol photons $m^{-2}s^{-1}$. * showing relative electron transport ratio (rETR).

Technique	Exposure	R	P_{max}	ETR_{max}	E_k	α	E_c	Species	Reference
Oxygen	**Submerged**	**0.44 µmol O_2 $m^{-2}s^{-1}$**	**2.24 µmol O_2 $m^{-2}s^{-1}$**	**-**	**115**	**0.02**	**22**	***Z.muelleri***	**This study**
IRGA	**Emerged**	**0.7 µmol CO_2 $m^{-2}s^{-1}$**	**2.26 µmol O_2 $m^{-2}s^{-1}$**	**-**	**286**	**0.005**	**140**	***Z.muelleri***	**This study**
PAM	**Emerged**	**-**	**-**	**31**	**161**	**0.2**	**-**	***Z.muelleri***	**This study**
PAM	**Submerged**	**-**	**-**	**64**	**208**	**0.3**	**-**	***Z.muelleri***	**This study**
Oxygen	Submerged	0.8 µmol O_2 $m^{-2}s^{-1}$	4.2 µmol O_2 $m^{-2}s^{-1}$		182	0.02	45	*Z.muelleri*	(Flanigan & Critchley, 1996)
Oxygen	Emerged	0.21 µmol O_2 g^{-1} s^{-1}	1 µmol O_2 g^{-1} s^{-1}	-	-	-	-	*Z.muelleri*	(Schwarz, 2004)
Oxygen	Submerged	0.24 µmol O_2 g^{-1} s^{-1}	0.93 µmol O_2 g^{-1} s^{-1}	-	-	-	-	*Z.muelleri*	(Schwarz, 2004)
Oxygen	Emerged	-	0.19 µmol O_2 g^{-1} s^{-1}	-	80	-	-	*Z.marina*	(Vermaat *et al.*, 1997)
Oxygen	Submerged	-	0.9 µmol O_2 g^{-1} s^{-1}	-	140	-	-	*Z.marina*	(Vermaat *et al.*, 1997)
Oxygen	Emerged	-	3.20 mg O_2 g^{-1} h^{-1} ^{1}	-	340	-	-	*Z.noltii*	(Vermaat *et al.*, 1997)
Oxygen	Submerged	-	1.81 µmol O_2 g^{-1} s^{-1}	-	255	-	-	*Z.noltii*	(Vermaat *et al.*, 1997)
PAM	Emerged	-	4.98 µmol O_2 $m^{-2}s^{-1}$	182* (summer) 127* (winter)	242 195	-	-	*Z.muelleri*	(Schwarz, 2004)
PAM	Submerged	-	3,98 µmol O_2 $m^{-2}s^{-1}$	160* (summer) 155* (winter)	223 242	-	-	*Z.muelleri*	(Schwarz, 2004)
IRGA	Submerged chamber	-	2.9 µmol CO_2 m-² s^{-1}	-	90			*Z.muelleri*	(Clough & Attiwill, 1980)
IRGA	Emerged chamber	0.7 µmol CO_2 $m^{-2}s^{-1}$	5.2 µmol CO_2 m-² s^{-1}	-	120	0.048	106	*Z muelleri*	(Clough & Attiwill, 1980)

Technique	**Exposure**	**R**	**P_{max}**	**ETR_{max}**	**E_k**	**α**	**E_c**	**Species**	**Reference**
IRGA	Emerged Cuvette	-	56 nmol CO_2 g^{-1} DM s^{-1}	8.08 mg O_2 g^{-1} DW h^{-1}	863	-	78	*Z.marina*	(Leuschner & Rees, 1993)
IRGA	Emerged Cuvette	-	92 nmol CO_2 g^{-1} DM s^{-1}	13.24 mg O_2 g^{-1} DW h^{-1}	1831	-	6	*Z.noltii*	(Leuschner & Rees, 1993)

Chapter 8

Sediment effects on New Zealand seagrass *Zostera muelleri*: a synthesis

---

8.1 State of knowledge prior to this thesis

Seagrass meadows are one of the most important, and threatened, ecosystems on the planet (Waycott *et al.*, 2009). They have immense ecological and socio-economic value (Orth *et al.*, 2006; Burkholder *et al.*, 2007; Waycott *et al.*, 2009; Cunha *et al.*, 2012). However, as a result of human activities these ecosystems are in decline (Short & Coles, 2001).

Natural factors such as extreme climatic events (e.g., hurricanes, storms, typhoons) and biotic influences (e.g., plant diseases, avian grazing and invasive species) may contribute to the permanent or temporary loss of seagrass beds (Waycott *et al.*, 2009). However, human-related activities are thought to be the major contributors to seagrass decline globally. World-wide, approximately a billion or more people live within 50 km of the coast (Cunha *et al.*, 2012). Due to their shallow coastal habitat, seagrasses are exposed to a range of human disturbances.

It is estimated that seagrasses have disappeared at a rate of 110 km^2 yr^{-1} since 1980 and that 29% of the known areal extent of seagrasses has disappeared since they were initially recorded in 1879 (Waycott *et al.*, 2009). Furthermore, rates of decline have accelerated from a median of 0.9% $year^{-1}$ before 1940 to 7% $year^{-1}$ since 1990. These high rates of loss place seagrass meadows among the most threatened ecosystems on earth (Waycott *et al.*, 2009).

In New Zealand, fine sediment is considered to be the most pervasive contaminant affecting estuaries and sheltered coastal embayments (Green & Short, 2003; Matheson & Schwarz, 2007; Morrison *et al.*, 2009) and it is thought to have contributed substantially to documented losses of seagrass meadows in a number of New Zealand estuaries (Inglis, 2003; Matheson *et al.*, 2011). Several losses of seagrass linked to human activities have been documented for Avon-Heathcote estuary (Inglis, 2003), Manukau Harbour (Turner, 1995), Tauranga Harbour (Park, 1999), Waitemata (Hayward *et al.*, 1999), Whangarei Harbour (Reed *et al.*, 2004), and Porirua Harbour (Matheson & Wadhwa, 2012). In Tauranga Harbour, approximately one-third of intertidal seagrass beds and 90% of sub-tidal seagrass beds were lost in the period from 1954 to 1996 and this has been linked to increased siltation of the estuary (Park, 1999). In Porirua

Harbour, approximately 40% of seagrass beds have been lost since 1980 (Matheson & Wadhwa, 2012). The largest loss (c. 32 ha) is from the head of the Pāuatahanui arm where there is strong evidence for siltation effects.

In Chapter 2, an extensive literature review and quantitative synthesis led to the development of the overarching issues addressed through this thesis. I hypothesised that excessive sediment inputs to estuaries affects the seagrass growing environment in three main ways: 1) by affecting light climate, 2) by coating and smothering plants; and 3) by altering physicochemical conditions in the rhizosphere and that these effects interact to cause seagrass loss.

In this chapter, I draw together information gained throughout the thesis of the effects of sediment on the New Zealand seagrass *Z. muelleri*, including:

(i) a seasonal **field survey**, designed to test potential sediment effects on seagrass and comparing habitats with and without seagrass within an evident estuary gradient;

(ii) **manipulative mesocosm experiments** to elucidate how irradiance and substrate condition affect seagrass performance.

(iii) a **transplanting field experiment** to test if persistent alterations to sediment physico-chemistry can be the primary factor driving seagrass loss and failure to re-establish; and

(iv) a field and laboratory experiment measuring differences in **seagrass photosynthesis** when submerged versus emerged (Figure 8.1).

This new knowledge is then used to improve understanding of the interactions between sediment, receiving irradiance and seagrasses to inform future management and restoration efforts.

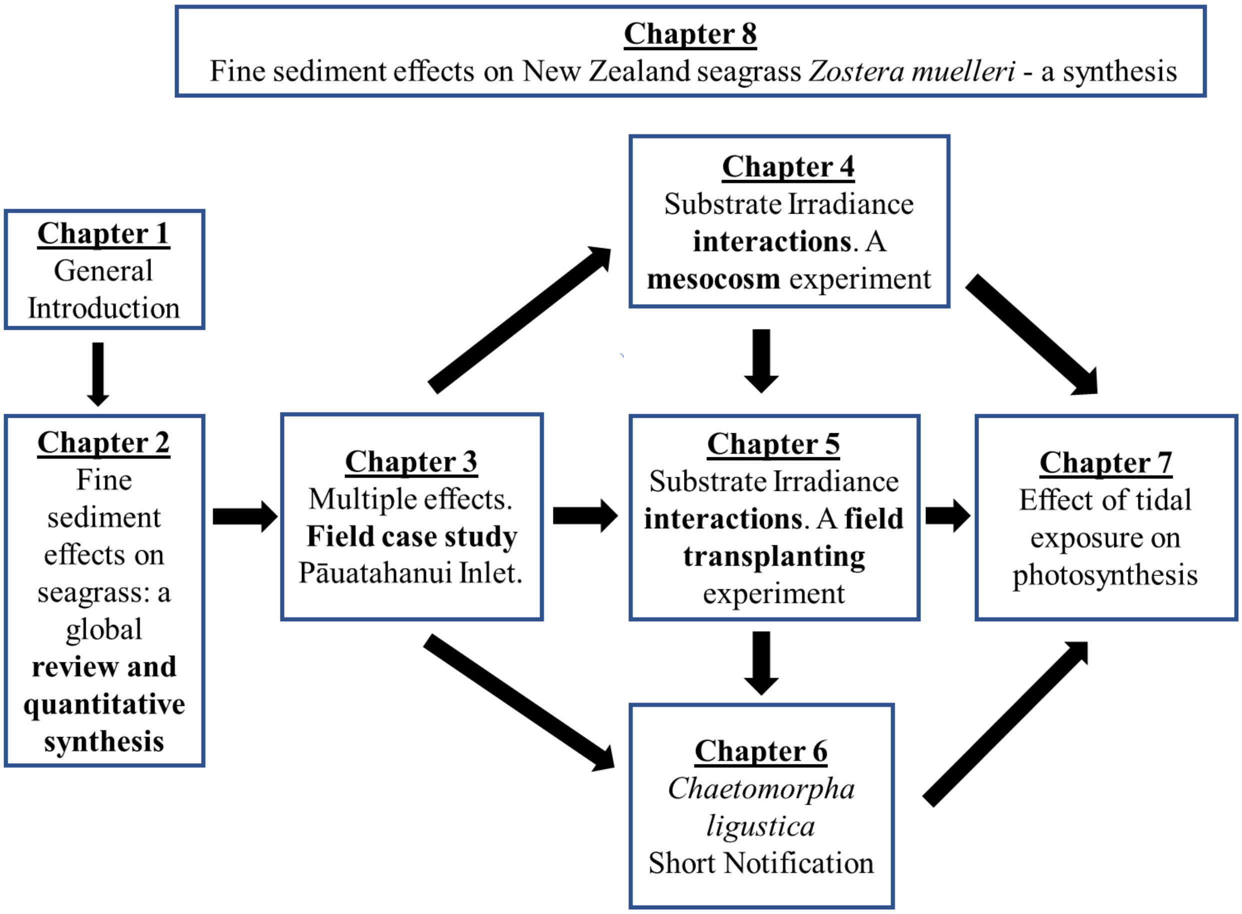


Figure 8.1: PhD thesis structural diagram showing the logical order and relationship between the developed chapters.

This PhD research project thus aimed to examine closely the mechanisms by which sediment pollution affects the condition and resilience of seagrass in New Zealand estuaries. It has contributed to the study of interactive sediment effects and the identification of thresholds in terms of substrate physico-chemistry and photosynthesis irradiance parameters which can be used by resource managers to protect and restore seagrass meadows by limiting catchment fine-sediment loads in the future.

If we are to maintain these important ecosystems, then it is essential that we understand the factors that have caused their demise.

8.2 Multiple and interactive effects of sediment on seagrasses

In Chapter 3, I tested two non-exclusive hypotheses, that mud particles (<63 µm) impact seagrasses through both (1) the light climate and (2) changes in substrate physico-chemistry. Results suggested that failure of seagrass to recolonize Historical substrate (HS) habitat reflects substrate muddiness and resultant unfavorable rhizosphere conditions, or an inability to compensate for high water column turbidity by photosynthesis in air. This chapter, introduced evidence for the multi-stressor effects of sediment on seagrasses, with both substrate suitability and submerged light climate for seagrass having detrimental effects.

In Chapter 4, using a 2 x 2 factorial mesocosm experiment, I examined the combined effect of substrate “muddiness” and irradiance dose on seagrass growth and survival over a six-week period. Belowground biomass and rhizome growth were significantly reduced by substrate muddiness but unaffected by irradiance. However, shoot growth was significantly reduced by *both* reduced Photosynthetic Available Radiation (PAR) and increased substrate muddiness with a clear interaction effect. These results suggested that *Z. muelleri* inhabiting muddy substrates has an increased PAR demand to deal with adverse rhizosphere conditions and specifically to oxygenate the rhizosphere, suggesting a mechanism to underpin the concept of multiple stressor effects. This chapter added to the previous chapter by identifying interactions between substrate and light climate. Both are affected by fine sediment pollution and should be interactively considered when determining light thresholds for seagrass survival and planning rehabilitation.

To confirm the previous conclusions, in Chapter 5, a field experiment is described, which was carried out at Pāuatahanui Inlet with the aim of testing if altered substrate conditions as a result of estuary siltation can be a primary driver of seagrass loss and failure to re-establish at former locations in New Zealand. As the experiment progressed, some challenges to its successful completion emerged. Firstly, it proved impossible to reliably relocate sprigs transplanted into the ES habitat (both upper and lower meadows) because sprigs transplanted in amongst existing plants quickly became incorporated into the turf. Secondly, an incursion of the filamentous green algae *Chaetomorpha ligustica* smothered approximately half of the quadrats at the lower tidal ES habitat complicating interpretation of results. It was concluded that the cumulative effect of previously reported rhizosphere deterioration and lower irradiance plus close location to a source of natural sediment input and disturbance during storm events, that mobilise sediment and debris from the catchment, may cause the inability of seagrass to re-establish at Pāuatahanui Inlet historical habitat. The same may apply in similar inner estuary habitats elsewhere. Findings confirm previous recommendations that cores rather than sprigs may be a more successful technique for transplanting of *Zostera muelleri* in intertidal areas.

During chapter 5’s main experiment, the filamentous green alga *Chaetomorpha ligustica* (Cladophoraceae, Cladophorales) was recorded covering seagrass meadows at Pāuatahanui

Inlet, New Zealand. This genus is difficult to speciate, but microscopic identification was confirmed by sequencing of the 18S rRNA large providing a high level of confidence in the naming. In November 2019, we found *Chaetomorpha ligustica* intertwined with *Ulva* spp. forming dense heavy and sticky structures. Here we report, for the first time, negative impacts of this species upon meadows of the New Zealand seagrass *Zostera muelleri*. While outside of the planned experiments in this thesis, it emphasises how seagrasses are vulnerable to a range of other stressors that may or may not be linked to anthropogenic activities.

In Chapter 7 we investigated photosynthetic characteristics of intertidal *Zostera muelleri* exposed to two natural contrasting physical environments, emerged and submerged. The intent was to address questions arising from Chapter 4 as to whether plants are able to mitigate the effects of low water clarity on irradiance received while submerged by photosynthesis while emersed at low tide. In particular, whether emerged photosynthesis could offset the impact of high turbidity on carbon acquisition. With that purpose, photosynthetic rates (emerged and submerged) were measured combining: oxygen (O_2) electrode techniques, infrared gas analysis (IRGA) and pulse amplitude modulated (PAM) fluorometry. Potential production of intertidal seagrass under submerged and emerged conditions was modelled across tidal cycles using experimental gas exchange results and field measured irradiance, using two scenarios in both cases. Results support previous studies that have reported emerged photosynthesis as a mechanism to mitigate degraded submerged light climate, and to contribute to seagrass production estimates. This final result reinforces the importance of removal or remediation of substrate and rhizosphere conditions of intertidal seagrass habitats.

8.3 A conceptual multi-stressor model

According to the findings of the previous chapters, a conceptual model was developed (Figure 8.2). Sediment may act as a pollutant for seagrass in many ways and these may be interacting causing both interactive and synergistic effects to cause seagrass demise in New Zealand and worldwide. Sediment effects are not simple and need to be addressed with a holistic and **interactive** perspective as sediment has been shown to be acting as a cumulative stressor.

In Chapter 3 substrate muddiness and resultant unfavorable rhizosphere conditions, or an inability to compensate for high water column turbidity by photosynthesis in air were suggested as the main sediment related stressors. Further investigation in detail of this primary conclusion leaded to Chapter 4 in which low irradiance and poor substrate diffusivity were demonstrated to be significantly **interacting** to cause seagrass demise. An increased oxygen demand in very reducing muddified substrate was suggested to compromise seagrass along with low receiving irradiance when submerged. However, not only substrate muddiness and low irradiance related issues were addressed but other chemical stressors such as nutrients, hydrogen sulphide and heavy metals were evaluated and compared with reported toxicity thresholds. None of these was demonstrated to be significantly over thresholds for seagrass survival however presence of these phytotoxins in addition to muddified substrate and low submerged irradiance related stress were suggested to act as multi-stressors (Figure 8.2)

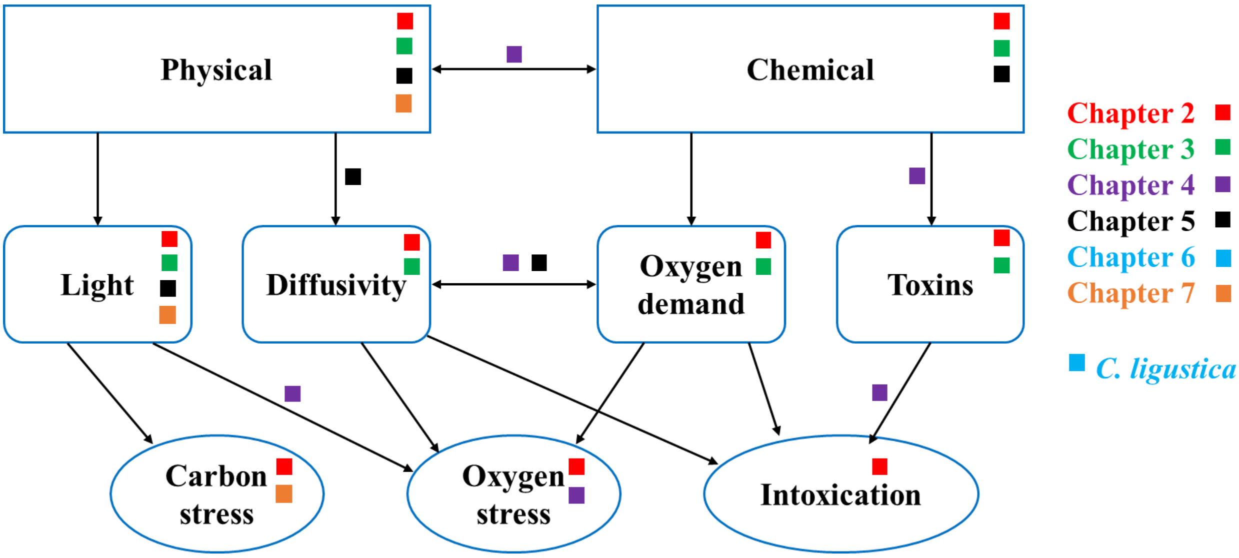


Figure 8.2: Conceptual model of sediment physical and chemical interactive effects on seagrasses and the near shore sedimentary environment. The colors indicate processes that were the main focus of the experimental chapters.

8.4 Management and restoration implications of the findings

In this thesis, I have improved knowledge of how sediment influences seagrasses and provided new insights into *Z. muelleri* ecology, which may assist in understanding seagrass decline and improve restoration efforts. It was anticipated that this research would assist in providing management and restoration strategies, however, it has also revealed another layer of complexity in understanding seagrass, that is the interactions reported between substrate and receiving light.

A reoccurring theme of this thesis is the mechanisms by which sediments damage seagrass. It is likely that there is a different hierarchy of importance of various physicochemical factors and their effect upon seagrass decline between sites. These relationships are not only spatially dynamic but also vary temporally as both substrate and light undergo continuous changes. This highlights the difficulty of generalizing across a wide range of estuaries. This fact also represents a challenge from a management perspective as it suggests that management and restoration options may need to be estuary - or even site-specific or at least estuary type specific.

Possible management options likely to lessen the decline of seagrass and improve the likelihood of successful restoration in the near future could include:

1) Reduce the input of sediments into rivers feeding into shallow, sheltered coastal waters where seagrasses would normally grow.
    - This could be achieved through better agricultural and urban land water management practices and riparian planting.
    - Improve transplantation guidelines and restoration efforts *sensu* Campbell (2002) and Van Katwijk *et al.* (2009)
    - Improve and build on existing site-specific guidelines for successful **site selection.** Discussed further below.

2) Key parameters are related to tidal position and hence receiving irradiance, hydrodynamics, *substrate physico-chemistry*, salinity and temperature. These were thoroughly reviewed for potential restoration of *Zostera* spp at Whangarei Harbour by (Schwarz *et al.*, 2005) and are updated here for Pāuatahanui Inlet with data from chapters 3 & 4 (Table 8.1).

3) Removal or remediation of legacy sediments. To improve restoration at large scale, we may need to tackle these effects with innovative interventions. One possibility might be chemical intervention to alter the substrate prior to transplanting. However, in some cases it may be sufficient for natural remediation to occur gradually over a long period of time, as possibly occurred in Whangarei Harbour (Matheson *et al.*, 2017a).

4) Support future research endeavors. Further investigations are required to gain a deeper understanding of sediment effects/interactions on seagrass decline (see section 2.8 and 8.5 below for recommendation).

Table 8.1: Key environmental parameters requiring consideration when selecting seagrass *Zostera muelleri* restoration sites in New Zealand by location, updated from (Schwarz *et al.*, 2005). The updates to previous research with results from this project for Pāuatahanui Inlet sites are shown in bold.

Location	Parameter	Too little	Suggested optimum	Too much	Reference
Manukau Harbour	Storm			-Onset of Autumn storm	(Turner, 1995)
Whangarei Harbour	Depth/Elevation/ Emersion (h)	exposed 6 h desiccation stress	0.5-2 m Moderate 2-5 h	Insufficient light 0-1 h	(Turner & Schwarz, 2006)
	Photosynthetically available radiation (PAR) (mol $m^{-2}d^{-1}$)	<2.1	10-50 25	Unlikely	(Schwarz *et al.*, 2005) (Matheson *et al.*, 2017a)
	Wave/current exposure	-	Current speed < 0.5 m s^{-1}	Exposure = uprooting of plants and/or excessive sediment movement. Storms potentially uproot seagrass	(Fonseca *et al.*, 1998)
	Water column Nutrients	Limiting	Ammonium 3-62 µg L^{-1} Nitrate 0.5-12 µg L^{-1} Phosphate 3-13 µg L^{-1}	>25 µM ammonium	(Matheson *et al.*, 2017a)
	Grain size of substrate	Clay/silt	Silt/Sand	Sand	(Turner & Schwarz, 2006)
	Salinity	Fresh water 15 ppt	Wide range of salinity 29 ppt	Fully sea water will not pose a problem 36 ppt	(Hemminga & Duarte, 2000) (Matheson *et al.*, 2017a)
	Temperature	Unlikely for frost damage	Harbour Temperature	Extreme temperature can affect growth through photosynthesis, respiration and nutrient uptake	(Schwarz *et al.*, 2005)
	Organic matter	< 0.5%	0.5-6%	> 16.5	(Erftemeijer & Koch, 2001)
Kaipara Harbour	PAR (mol $m^{-2}d^{-1}$)	<2.1	10-50	Unlikely	(Bulmer *et al.*, 2016)
Tauranga Harbour	Dredging	Reduction of Surface Irradiance (SI) by 36%	-	-	(Cussioli *et al.*, 2019)
	Spectral differences	Orange and white	< 500 nm < 700 nm	-	(Cussioli *et al.*, 2020)
Pāuatahanui Inlet	**Substrate mud (%)**	**<5 (%mud)**	**5-22 (% mud)**	**>23 (% mud)**	**(Zabarte-Maeztu *et al.*, 2020)**
	Porewater Nutrients	**Limiting**	**Ammonium < 30 µM**	**> 70 µM Ammonium**	
	Substrate Organic Matter	**< 0.5%**	**0.5-3%**	**> 5**	
	Hydrogen sulphide	**-**	**0-9 µM**	**10 µM**	
	Sedimentation rates	**-**	**1-2 mm yr^{-1}**	**> 3 mm yr^{-1}**	
	PAR (mol $m^{-2}d^{-1}$)	**<2.4**	**10-35**	**>70**	(Chapter 7)
	Algal blooms	-	-	*C. ligustica*	(Chapter 6)
	Storm	-	-	> 14 mm rainfall	(Chapter 5)

8.5 Suggestion for future research

The knowledge described in this thesis on the processes by which sediment damages seagrass could be further explored. A number of suggestions are outlined below that follow directly from the results of this work described in the preceding chapters.

5) In Chapter 2 the literature review suggested research gaps on the effects of both acute and chronic effects of sediments and species-specific responses.

6) In Chapters 3 and 4, I was unable to confidently quantify or attribute the exact mechanisms causing seagrass demise at historical seagrass sites and therefore interactive, synergistic or multiple stressors were argued. These may be studied and improved through further controlled mesocosm experiments to try and disentangle the mechanistic effect by which sediment damages seagrass under different environmental conditions (i.e. light and temperature regimes). These may include substrate mud gradient experiments, substrate-controlled anoxia treatments, and the study of substrate-associated toxins such as heavy metals, pesticides and nutrients under different irradiance levels.

7) In Chapter 5 the results indicated that the cumulative effect of rhizosphere deterioration, lower irradiance and close location to a source of natural sediment input during events such as storms may be the cause of seagrasses inability to re-establish in upper Pāuatahanui Inlet. This could be further evaluated by assessing the short-term effects of sedimentation events in the upper part of estuaries and the effect of natural burial and smothering degree.

8) Further investigation on sediment origin and transport dynamics to better understand how this may vary in different estuaries will build upon recommendations from Chapter 5. Previous burial experiments on intertidal Zostera muelleri at Tauranga Harbour have shown that plants can unbury relatively quickly (Soerensen, 2020) so further investigation of burial dynamics is warranted.

9) New Zealand is an appropriate location to fit seagrass restoration with ongoing shellfish restoration efforts which are a major focus of Māori coastal research (Paul-Burke et al., 2018). This will be benefited by the international collaboration with

seagrass-sulphide-bivalves feedback project run by Dr. Jim De Fouw (University of Radboud, The Netherlands).

10) In Chapter 6, proliferation of Chaetomorpha ligustica was found and identified. The difficulties of identification of this species must be dealt with by future use of genetic tests. As significant loss of seagrass by smothering was reported, it seems important to further study C. ligustica blooms, the species growth requirements and extent of spread.

11) In Chapter 7, Z. muelleri's photosynthetic performance under submerged and emerged conditions was modelled using experimental gas exchange data and field irradiance to the best of our abilities. Results showed the importance of emerged production as a mechanism to mitigate degraded submerged light climate. Many of the factors influencing photosynthesis could not be controlled in this experiment, most importantly: leaf orientation, self-shading, desiccation, and pigmentation, and therefore further research should be carried out on the effect of sediment on seagrass photosynthesis both in the intertidal and in the subtidal. Further understanding of effects such as within canopy light attenuation, photoinhibition, down regulation leaf orientation and photorespiration would improve our estimates and understanding of seagrass production in New Zealand. The significant contribution of emerged production revealed in this chapter may explain why subtidal seagrass has apparently proven more vulnerable to sedimentation than intertidal populations.

12) During fieldwork I came across seagrass flowering in different estuaries around the country and I quantified densities in some of them (Short Note, In preparation). It may not be strictly linked to the sediment issue but as an aid to restoration of seagrasses, which was one of the higher goals of the project, it would be useful to create a seedbank.

13) Incorporate identified thresholds of substrate muddiness, anoxia and photosynthetic limiting irradiance levels into source-to-sea hydrodynamic models to enable robust determination of the permitted sediment loading rates to receiving environments, required for seagrass to grow or persist, which will provide for the protection and restoration of estuarine seagrass meadows.

References

Abal, E., & Dennison, W. (1996). Seagrass depth range and water quality in southern Moreton Bay, Queensland, Australia. *Marine and Freshwater Research, 47*(6), 763-771.

Abal, E., Loneragan, N., Bowen, P., Perry, C., Udy, J., & Dennison, W. (1994). Physiological and morphological responses of the seagrass *Zostera capricorni* Aschers, to light intensity. *Journal of Experimental Marine Biology and Ecology, 178*(1), 113-129.

Adams, M. P., Hovey, R. K., Hipsey, M. R., Bruce, L. C., Ghisalberti, M., Lowe, R. J., Gruber, R. K., Ruiz‐Montoya, L., Maxwell, P. S., & Callaghan, D. P. (2016). Feedback between sediment and light for seagrass: Where is it important? *Limnology and Oceanography, 61*(6), 1937-1955.

Adams, N. M. (1972). The marine algae of the Wellington area. A list of species.

Adams, N. M. (1994). *Seaweeds of New Zealand*. Christchurch, New Zealand: Canterbury University Press.

Alcoverro, T., Zimmerman, R. C., Kohrs, D. G., & Alberte, R. S. (1999). Resource allocation and sucrose mobilization in light-limited eelgrass *Zostera marina*. *Marine Ecology Progress Series*, 121-131.

Ansell, A., Gibson, R., Barnes, M., & Press, U. (1998). Ecological impact of green macroalgal blooms. *Oceanography and Marine Biology: an annual review, 36*, 97-125.

ANZECC. (2000). *Australian and New Zealand guidelines for fresh and marine water quality.* Australian and New Zealand Environment and Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand, Canberra, Australia. 103p.

Bainbridge, Z., Lewis, S., Bartley, R., Fabricius, K., Collier, C., Waterhouse, J., Garzon-Garcia, A., Robson, B., Burton, J., & Wenger, A. (2018). Fine sediment and particulate organic matter: a review and case study on ridge-to-reef transport, transformations, fates, and impacts on marine ecosystems. *Marine Pollution Bulletin, 135*, 1205-1220.

Barranguet, C., Kromkamp, J., & Peene, J. (1998). Factors controlling primary production and photosynthetic characteristics of intertidal microphytobenthos. *Marine Ecology Progress Series, 173*, 117-126.

Barwick, M., & Maher, W. (2003). Biotransference and biomagnification of selenium copper, cadmium, zinc, arsenic and lead in a temperate seagrass ecosystem from Lake Macquarie Estuary, NSW, Australia. *Marine Environmental Research, 56*(4), 471-502.

Batley, G. (1987). Heavy metal speciation in waters, sediments and biota from Lake Macquarie, New South Wales. *Marine and Freshwater Research, 38*(5), 591-606.

Battley, P., Melville, D., Schuckard, R., & Ballance, P. (2011). *Zostera muelleri* as a structuring agent of benthic communities in a large intertidal sandflat in New Zealand. *Journal of Sea Research, 65*(1), 19-27.

Beer, S. (1989). Photosynthesis and photorespiration of marine angiosperms. *Aquatic Botany, 34*(1-3), 153-166.

Beer, S., & Björk, M. (2000). Measuring rates of photosynthesis of two tropical seagrasses by pulse amplitude modulated (PAM) fluorometry. *Aquatic Botany, 66*(1), 69-76.

Beer, S., Björk, M., Gademann, R., & Ralph, P. (2001). Measurements of photosynthetic rates in seagrasses. In *Global seagrass research methods* (pp. 183-198). Elsevier.

Beer, S., Bjork, M., Hellblom, F., & Axelsson, L. (2002). Inorganic carbon utilization in marine angiosperms (seagrasses). *Functional Plant Biology, 29*(3), 349-354.

Benham, C. F., Beavis, S. G., & Jackson, E. L. (2019). Tolerance of tropical seagrasses *Zostera muelleri* and *Halophila ovalis* to burial: Toward an understanding of threshold effects. *Estuarine, Coastal and Shelf Science, 218*, 131-138.

Bertelli, C. M., & Unsworth, R. K. (2014). Protecting the hand that feeds us: Seagrass (*Zostera marina*) serves as commercial juvenile fish habitat. *Marine Pollution Bulletin, 83*(2), 425-429.

Birch, G., O'Donnell, M., & McCready, S. (2017). Complex relationships between shallow muddy benthic assemblages, sediment chemistry and toxicity in estuaries in southern New South Wales, Australia. *Marine Pollution Bulletin*.

Björk, M., Uku, J., Weil, A., & Beer, S. (1999). Photosynthetic tolerances to desiccation of tropical intertidal seagrasses. *Marine Ecology Progress Series, 191*, 121-126.

Björk, M., Weil, A., Semesi, S., & Beer, S. (1997). Photosynthetic utilisation of inorganic carbon by seagrasses from Zanzibar, East Africa. *Marine Biology, 129*(2), 363-366.

Blaschke, P., Woods, J., & Forsyth, F. (2010). *The Porirua Harbour and its catchment: a literature summary and review.* Prepared for Porirua City Council and Wellington City Council. Blaschke and Rutherford Environmental Consultants, Wellington, New Zealand.

Boedeker, C., Leliaert, F., & Zuccarello, G. C. (2016). Molecular phylogeny of the Cladophoraceae (Cladophorales, Ulvophyceae), with the resurrection of *Acrocladus* Nägeli and *Willeella* Børgesen, and the description of *Lurbica* gen. nov. and *Pseudorhizoclonium* gen. nov. *Journal of Phycology, 52*(6), 905-928.

Bonanno, G., & Orlando-Bonaca, M. (2018). Trace elements in Mediterranean seagrasses and macroalgae. A review. *Science of the Total Environment, 618*, 1152-1159.

Booth, J. D. (2019). Recent (post-1930) changes in the extent of subtidal seagrass (*Zostera muelleri*) beds of the eastern Bay of Islands, New Zealand. *New Zealand Journal of Marine and Freshwater Research, 53*(1), 113-127.

Borum, J., Pedersen, O., Greve, T., Frankovich, T., Zieman, J., Fourqurean, J. W., & Madden, C. (2005a). The potential role of plant oxygen and sulphide dynamics in die‐off events of the tropical seagrass, *Thalassia testudinum*. *Journal of Ecology, 93*(1), 148-158.

Borum, J., Pedersen, O., Greve, T. M., Frankovich, T. A., Zieman, J. C., Fourqurean, J. W., & Madden, C. J. (2005b). The potential role of plant oxygen and sulphide dynamics in die-off events of the tropical seagrass, *Thalassia testudinum*. *Journal of Ecology, 93*(1), 148-158.

Bos, A. R., Bouma, T. J., de Kort, G. L., & van Katwijk, M. M. (2007). Ecosystem engineering by annual intertidal seagrass beds: sediment accretion and modification. *Estuarine, Coastal and Shelf Science, 74*(1-2), 344-348.

Boström, C., & Bonsdorff, E. (2000). Zoobenthic community establishment and habitat complexity the importance of seagrass shoot-density, morphology and physical disturbance for faunal recruitment. *Marine Ecology Progress Series, 205*, 123-138.

Brix, H., Lyngby, E., & Schierup, H.-H. (1983). Eelgrass (*Zostera marina* L.) as an indicator organism of trace metals in the Limfjord, Denmark. *Marine Environmental Research. London, 8*(3), 165-181.

Brodersen, K. E., Hammer, K. J., Schrameyer, V., Floytrup, A., Rasheed, M. A., Ralph, P. J., Kühl, M., & Pedersen, O. (2017). Sediment resuspension and deposition on seagrass leaves impedes internal plant aeration and promotes phytotoxic H_2S intrusion. *Frontiers in Plant Science, 8*, 657.

Brodersen, K. E., Kühl, M., Nielsen, D. A., Pedersen, O., & Larkum, A. W. (2018). Rhizome, Root/Sediment Interactions, Aerenchyma and Internal Pressure Changes in Seagrasses. In *Seagrasses of Australia* (pp. 393-418). Springer.

Brodersen, K. E., Nielsen, D. A., Ralph, P. J., & Kühl, M. (2015). Oxic microshield and local pH enhancement protects *Zostera muelleri* from sediment derived hydrogen sulphide. *New Phytologist, 205*(3), 1264-1276.

Brun, F. G., Hernández, I., Vergara, J. J., Peralta, G., & Pérez-Lloréns, J. L. (2002). Assessing the toxicity of ammonium pulses to the survival and growth of *Zostera noltii*. *Marine Ecology Progress Series, 225*, 177-187.

Buapet, P., Rasmusson, L. M., Gullström, M., & Björk, M. (2013). Photorespiration and carbon limitation determine productivity in temperate seagrasses. *PLoS One, 8*(12), e83804.

Bulmer, R., Kelly, S., & Jeffs, A. (2016). Light requirements of the seagrass, *Zostera muelleri*, determined by observations at the maximum depth limit in a temperate estuary, New Zealand. *New Zealand Journal of Marine and Freshwater Research, 50*(2), 183-194.

Bulthuis, D. A. (1987). Effects of temperature on photosynthesis and growth of seagrasses. *Aquatic Botany, 27*(1), 27-40.

Burkholder, J. M., Tomasko, D. A., & Touchette, B. W. (2007). Seagrasses and eutrophication. *Journal of Experimental Marine Biology and Ecology, 350*(1-2), 46-72.

Cabaço, S., Machás, R., Vieira, V., & Santos, R. (2008a). Impacts of urban wastewater discharge on seagrass meadows (*Zostera noltii*). *Estuarine, Coastal and Shelf Science, 78*(1), 1-13.

Cabaço, S., & Santos, R. (2007). Effects of burial and erosion on the seagrass *Zostera noltii*. *Journal of Experimental Marine Biology and Ecology, 340*(2), 204-212.

Cabaço, S., Santos, R., & Duarte, C. M. (2008b). The impact of sediment burial and erosion on seagrasses: a review. *Estuarine, Coastal and Shelf Science, 79*(3), 354-366.

Cabello-Pasini, A., Lara-Turrent, C., & Zimmerman, R. C. (2002). Effect of storms on photosynthesis, carbohydrate content and survival of eelgrass populations from a coastal lagoon and the adjacent open ocean. *Aquatic Botany, 74*(2), 149-164.

Calleja, M. L., Marbà, N., & Duarte, C. M. (2007). The relationship between seagrass (*Posidonia oceanica*) decline and sulfide porewater concentration in carbonate sediments. *Estuarine, Coastal and Shelf Science, 73*(3-4), 583-588.

Calumpong, H. P., & Fonseca, M. S. (2001). Seagrass transplantation and other seagrass restoration methods. In F. T. Short & R. G. Coles (Eds.), *Global Seagrass Research Methods* (pp. 425-443). Amsterdam, The Netherlands: Elsevier.

Campbell, M. L. (2002). Getting the foundation right: a scientifically based management framework to aid in the planning and implementation of seagrass transplant efforts. *Bulletin of Marine Science, 71*(3), 1405-1414.

Campbell, M. L. (2016). Burial Duration and Frequency Influences Resilience of Differing Propagule Types in a Subtidal Seagrass, *Posidonia australis*. *PLoS One, 11*(8), Article no. e0161309.

Campbell, M. L., & Paling, E. I. (2003). Evaluating vegetative transplant success in *Posidonia australis:* a field trial with habitat enhancement. *Marine Pollution Bulletin, 46*(7), 828-834.

Carlson Jr, P. R., Yarbro, L. A., & Barber, T. R. (1994). Relationship of sediment sulfide to mortality of *Thalassia testudinum* in Florida Bay. *Bulletin of Marine Science, 54*(3), 733-746.

Carr, J., D'Odorico, P., McGlathery, K., & Wiberg, P. (2010). Stability and bistability of seagrass ecosystems in shallow coastal lagoons: Role of feedbacks with sediment resuspension and light attenuation. *Journal of Geophysical Research: Biogeosciences, 115*(G3).

Chapman, V. J. (1956). The marine algae of New Zealand. Part I. Myxophyceae and Chlorophyceae. *Botanical Journal of the Linnean Society, 55*(360), 333-501.

Chartrand, K. M., Bryant, C. V., Carter, A. B., Ralph, P. J., & Rasheed, M. A. (2016). Light thresholds to prevent dredging impacts on the Great Barrier Reef seagrass, *Zostera muelleri* ssp. *capricorni*. *Frontiers in Marine Science, 3*, 106.

Chevnova, E., Khristoforova, N., & Vyshkvartsev, D. (2002). Heavy metals in seagrasses and algae of Posyet Bay, the Sea of Japan. *Biologiya Morya, 28*, 425-430.

Clavier, J., Chauvaud, L., Carlier, A., Amice, E., Van der Geest, M., Labrosse, P., Diagne, A., & Hily, C. (2011). Aerial and underwater carbon metabolism of a *Zostera noltii* seagrass bed in the Banc d'Arguin, Mauritania. *Aquatic Botany, 95*(1), 24-30.

Clough, B., & Attiwill, P. (1980). Primary productivity of *Zostera muelleri* Irmisch ex Aschers. in Westernport Bay (Victoria, Australia). *Aquatic Botany, 9,* 1-13.

Coles, R., Long, W. L., Squire, B., Squire, L., & Bibby, J. (1987). Distribution of seagrasses and associated juvenile commercial penaeid prawns in north-eastern Queensland waters. *Marine and Freshwater Research, 38*(1), 103-119.

Collado-Vides, L., Avila, C., Blair, S., Leliaert, F., Rodriguez, D., Thyberg, T., Schneider, S., Rojas, J., Sweeney, P., & Drury, C. (2013). A persistent bloom of Anadyomene JV Lamouroux (Anadyomenaceae, Chlorophyta) in Biscayne Bay, Florida. *Aquatic Botany, 111*, 95-103.

Collier, C., Adams, M., Langlois, L., Waycott, M., O'Brien, K., Maxwell, P., & McKenzie, L. (2016). Thresholds for morphological response to light reduction for four tropical seagrass species. *Ecological Indicators, 67,* 358-366.

Collier, C. J., Uthicke, S., & Waycott, M. (2011). Thermal tolerance of two seagrass species at contrasting light levels: implications for future distribution in the Great Barrier Reef. *Limnology and Oceanography, 56*(6), 2200-2210.

Collier, C. J., Waycott, M., & Ospina, A. G. (2012). Responses of four Indo-West Pacific seagrass species to shading. *Marine Pollution Bulletin, 65*(4-9), 342-354.

Costanza, R., d'Arge, R., De Groot, R., Farber, S., Grasso, M., Hannon, B., Limburg, K., Naeem, S., O'neill, R. V., & Paruelo, J. (1997). The value of the world's ecosystem services and natural capital. *Nature, 387*(6630), 253.

Cummins, S., Roberts, D., & Zimmerman, K. (2004). Effects of the green macroalga *Enteromorpha intestinalis* on macrobenthic and seagrass assemblages in a shallow coastal estuary. *Marine Ecology Progress Series, 266,* 77-87.

Cunha, A., Santos, R., Gaspar, A., & Bairros, M. (2005). Seagrass landscape-scale changes in response to disturbance created by the dynamics of barrier-islands: a case study from Ria Formosa (Southern Portugal). *Estuarine, Coastal and Shelf Science, 64*(4), 636-644.

Cunha, A. H., Marbá, N. N., van Katwijk, M. M., Pickerell, C., Henriques, M., Bernard, G., Ferreira, M. A., Garcia, S., Garmendia, J. M., & Manent, P. (2012). Changing Paradigms in Seagrass Restoration. *Restoration Ecology, 20*(4), 427-430.

Cussioli, M. C., Bryan, K. R., Pilditch, C. A., de Lange, W. P., & Bischof, K. (2019). Light penetration in a temperate meso-tidal lagoon: Implications for seagrass growth and dredging in Tauranga Harbour, New Zealand. *Ocean & Coastal Management, 174*, 25-37.

Cussioli, M. C., Seeger, D., Pratt, D. R., Bryan, K. R., Bischof, K., de Lange, W. P., Bornman, J. F., & Pilditch, C. A. (2020). Spectral differences in the underwater light regime caused by sediment types in New Zealand estuaries: implications for seagrass photosynthesis. *Geo-Marine Letters*, 1-9.

D'Archino, R. (2019). Seaweeds of Auckland. *New Zealand Journal of Botany, 57*(3), 193-194.

Daughton, C. G. (2005). " Emerging" chemicals as pollutants in the environment: a 21st century perspective. *Renewable Resources Journal, 23*(4), 6.

Davies-Colley, R. J., Vant, W. N., & Smith, D. G. (2003). *Colour and Clarity of Natural Waters*. New York: Blackburn Press.

Davies‐Colley, R., & Smith, D. (2001). Turbidity suspended sediment, and water clarity: a review *JAWRA Journal of the American Water Resources Association, 37*(5), 1085-1101.

De Boer, W. (2007). Seagrass–sediment interactions, positive feedbacks and critical thresholds for occurrence: a review. *Hydrobiologia, 591*(1), 5-24.

De Casabianca, M., Tari, P., Gauchet, R., Raynaud, C., & Rigollet, V. (2004). Relationships between heavy metal concentrations in sediments and eelgrass and environmental variables (*Zostera marina,* Thau Lagoon, France). *Vie et Milieu, 54*(4), 231-238.

De Winton, M., Hawes, I., Clayton, J., Champion, P., & Smith, R. (1998). Sea lettuce dynamics and ecophysiology in Tauranga Harbour, Bay of Plenty. *NIWA Client Report No. BPR802. National Institute of Water and Atmospheric Research Ltd, Hamilton, New Zealand*.

Dennison, W. C., Orth, R. J., Moore, K. A., Stevenson, J. C., Carter, V., Kollar, S., Bergstrom, P. W., & Batiuk, R. A. (1993). Assessing water quality with submersed aquatic vegetation. *BioScience, 43*(2), 86-94.

Dickson, A. G. (2010). The carbon dioxide system in seawater: equilibrium chemistry and measurements. *Guide to best practices for ocean acidification research and data reporting, 1*, 17-40.

Domínguez, M., Celdrán, D., Muñoz‐Vera, A., Infantes, E., Martinez‐Baños, P., Marín, A., & Terrados, J. (2012). Experimental Evaluation of the Restoration Capacity of a Fish‐Farm Impacted Area with *Posidonia oceanica* (L.) Delile Seedlings. *Restoration Ecology, 20*(2), 180-187.

Dos Santos, V. (2011). *Impact of black swan grazing and anthropogenic contaminants on New Zealand seagrass meadows*. PhD thesis, University of Waikato, Hamilton, New Zealand.

Dos Santos, V. M., & Matheson, F. E. (2017). Higher seagrass cover and biomass increases sexual reproductive effort: A rare case study of *Zostera muelleri* in New Zealand. *Aquatic Botany, 138*, 29-36.

Douthe, C., Gago, J., Ribas-Carbó, M., Núñez, R., Pedrol, N., & Flexas, J. (2018). Measuring photosynthesis and respiration with infrared gas analysers. In *Advances in Plant Ecophysiology Techniques* (pp. 51-75). Springer.

Drew, E. A. (1978). Factors affecting photosynthesis and its seasonal variation in the seagrasses *Cymodocea nodosa* (Ucria) Aschers, and *Posidonia oceanica* (L.) Delile in the Mediterranean. *Journal of Experimental Marine Biology and Ecology, 31*(2), 173-194.

Drylie, T. P., Lohrer, A. M., Needham, H. R., Bulmer, R. H., & Pilditch, C. A. (2018). Benthic primary production in emerged intertidal habitats provides resilience to high water column turbidity. *Journal of Sea Research, 142*, 101-112.

Duarte, C. M. (1991). Seagrass depth limits. *Aquatic Botany, 40*(4), 363-377.

Duarte, C. M. (1995). Submerged aquatic vegetation in relation to different nutrient regimes. *Ophelia, 41*(1), 87-112.

Duarte, C. M., & Chiscano, C. L. (1999). Seagrass biomass and production: a reassessment. *Aquatic Botany, 65*(1-4), 159-174.

Duarte, C. M., Kennedy, H., Marbà, N., & Hendriks, I. (2013). Assessing the capacity of seagrass meadows for carbon burial: current limitations and future strategies. *Ocean & coastal management, 83*, 32-38.

Duarte, C. M., Marbá, N., Agawin, N., Cebrián, J., Enriquez, S., Fortes, M. D., Gallegos, M. E., Merino, M., Olesen, B., & Sand-Jensen, K. (1994). Reconstruction of seagrass dynamics: age determinations and associated tools for the seagrass ecologist. *Marine ecology Progress series*, 195-209.

Duarte, C. M., Marbà, N., Gacia, E., Fourqurean, J. W., Beggins, J., Barrón, C., & Apostolaki, E. T. (2010). Seagrass community metabolism: Assessing the carbon sink capacity of seagrass meadows. *Global Biogeochemical Cycles, 24*(4).

Duarte, C. M., Marbà, N., & Santos, R. (2004a). What may cause loss of seagrasses. *European seagrasses: an introduction to monitoring and management*, 24.

Duarte, C. M., Middelburg, J. J., & Caraco, N. (2004b). Major role of marine vegetation on the oceanic carbon cycle. *Biogeosciences discussions, 1*(1), 659-679.

Duarte, C. M., Terrados, J., Agawin, N. S., Fortes, M. D., Bach, S., & Kenworthy, W. J. (1997). Response of a mixed Philippine seagrass meadow to experimental burial. *Marine Ecology Progress Series, 147*, 285-294.

Dudley, B. D., R. Burge, O., Plew, D., & Zeldis, J. (2020). Effects of agricultural and urban land cover on New Zealand's estuarine water quality. *New Zealand Journal of Marine and Freshwater Research*, 1-21.

Dumbauld, B. R., & Wyllie-Echeverria, S. (2003). The influence of burrowing thalassinid shrimps on the distribution of intertidal seagrasses in Willapa Bay, Washington, USA. *Aquatic Botany, 77*(1), 27-42.

Eggleston, D. B., Elis, W. E., Etherington, L. L., Dahlgren, C. P., & Posey, M. H. (1999). Organism responses to habitat fragmentation and diversity: habitat colonization by estuarine macrofauna. *Journal of Experimental Marine Biology and Ecology, 236*(1), 107-132.

Eldridge, P. M., & Morse, J. W. (2008). Origins and temporal scales of hypoxia on the Louisiana shelf: Importance of benthic and sub-pycnocline water metabolism. *Marine Chemistry, 108*(3-4), 159-171.

Enríquez, S., Marbà, N., Duarte, C., Van Tussenbroek, B., & Reyes-Zavala, G. (2001). Effects of seagrass *Thalassia testudinum* on sediment redox. *Marine Ecology Progress Series, 219*, 149-158.

Enríquez, S., Merino, M., & Iglesias-Prieto, R. (2002). Variations in the photosynthetic performance along the leaves of the tropical seagrass *Thalassia testudinum*. *Marine Biology, 140*(5), 891-900.

Erftemeijer, P. L., & Koch, E. W. (2001). *Sediment geology methods for seagrass habitat*. Amsterdam, the Netherlands: Elsevier Science.

Erftemeijer, P. L., & Lewis III, R. R. R. (2006). Environmental impacts of dredging on seagrasses: a review. *Marine Pollution Bulletin, 52*(12), 1553-1572.

Falla, R. A., Sibson, R. B., & Turbott, E. G. (1979). *The new guide to the birds of New Zealand and outlying islands*. Collins Publishers.

Ferguson, A. J., Gruber, R., Potts, J., Wright, A., Welsh, D. T., & Scanes, P. (2017). Oxygen and carbon metabolism of *Zostera muelleri* across a depth gradient–Implications for resilience and blue carbon. *Estuarine, Coastal and Shelf Science, 187*, 216-230.

Fernandes, M. B., Daly, R., van Gils, J., Kildea, T., Caires, S., & Erftemeijer, P. L. (2017). Parameterization of an optical model to refine seagrass habitat requirements in an urbanized coastline. *Estuarine, Coastal and Shelf Science*.

Field, C. B., Ball, J. T., & Berry, J. A. (2000). Photosynthesis: principles and field techniques. In *Plant Physiological Ecology* (pp. 209-253). Springer.

Flanigan, Y. S., & Critchley, C. (1996). Light response of D1 turnover and photosystem II efficiency in the seagrass *Zostera capricorni*. *Planta, 198*(3), 319-323.

Folk, R. L. (1968). Petrology of sedimentary rocks: Hemphill's. *Austin, Texas, 170*, 85.

Fonseca, M. S. (2011). Addy revisited: what has changed with seagrass restoration in 64 years? *Ecological Restoration, 29*(1-2), 73-81.

Fonseca, M. S., Kenworthy, W. J., & Thayer, G. W. (1998). Guidelines for the conservation and restoration of seagrasses in the United States and adjacent waters.

Fourqurean, J. W., Duarte, C. M., Kennedy, H., Marbà, N., Holmer, M., Mateo, M. A., Apostolaki, E. T., Kendrick, G. A., Krause-Jensen, D., & McGlathery, K. J. (2012). Seagrass ecosystems as a globally significant carbon stock. *Nature Geoscience, 5*(7), 505.

Fourqurean, J. W., & Rutten, L. M. (2004). The impact of Hurricane Georges on soft-bottom, back reef communities: Site-and species-specific effects in south Florida seagrass beds. *Bulletin of Marine Science, 75*(2), 239-257.

Gacia, E., & Duarte, C. M. (2001). Sediment retention by a Mediterranean *Posidonia oceanica* meadow: the balance between deposition and resuspension. *Estuarine, Coastal and Shelf Science, 52*(4), 505-514.

Gacia, E., Duarte, C. M., Marbà, N., Terrados, J., Kennedy, H., Fortes, M. D., & Tri, N. H. (2003). Sediment deposition and production in SE-Asia seagrass meadows. *Estuarine, Coastal and Shelf Science, 56*(5-6), 909-919.

Gall, M., & Davies‐Colley, R. (2020). A portable underway flow‐through sampler for rapid survey of contaminated river plumes in coastal waters. *Limnology and Oceanography: Methods*.

Genty, B., Briantais, J.-M., & Baker, N. R. (1989). The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochimica et Biophysica Acta (BBA)-General Subjects, 990*(1), 87-92.

Gibb, J. G., & Cox, G. J. (2009). *Patterns and rates of sedimentation within Porirua Harbour*. Porirua City Council.

Gladstone-Gallagher, R. V., Hughes, R. W., Douglas, E. J., & Pilditch, C. A. (2018). Biomass-dependent seagrass resilience to sediment eutrophication. *Journal of Experimental Marine Biology and Ecology, 501*, 54-64.

Glasby, G., Moss, R., & Stoppers, P. (1990). Heavy‐metal pollution in Porirua Harbour, New Zealand.

Goff, L. J., & Moon, D. A. (1993). PCR amplification of nuclear and plastid genes from algal herbarium specimens and algal spores 1. *Journal of Phycology, 29*(3), 381-384.

Govers, L. L., de Brouwer, J. H., Suykerbuyk, W., Bouma, T. J., Lamers, L. P., Smolders, A. J., & van Katwijk, M. M. (2014). Toxic effects of increased sediment nutrient and organic matter loading on the seagrass *Zostera noltii*. *Aquatic Toxicology, 155*, 253-260.

Grace, R. V., & Grace, A. (1976). Benthic communities west of Great Mercury Island, north-eastern New Zealand. *Tane, 22*, 85-101.

Grace, R. V., & Whitten, R. F. (1974). Benthic communities west of Slipper Island, north-eastern New Zealand. *Tane, 20*, 4-20.

Green, E. P., & Short, F. T. (Eds.). (2003). *World Atlas of Seagrasses*. Berkeley, CA: University of California Press.

Greiner, J. T., McGlathery, K. J., Gunnell, J., & McKee, B. A. (2013). Seagrass restoration enhances “blue carbon” sequestration in coastal waters. *PloS one*, *8*(8), e72469.

Gribben, P. E., C. Angelini, A. H. Altieri, M. J. Bishop, M. S. Thomsen, and F. Bulleri. (2019). Facilitation cascades in marine ecosystems: a synthesis and future directions. *Oceanography and Marine Biology: An Annual Review 57*:95-136.

Guiry, M., & Guiry, G. (2010). *AlgaeBase. World-wide electronic publication*. Retrieved February, 2021, from http://www. algaebase. org/.

Güven, K., Saygı, N., & Öztürk, B. (1993). Survey of metal contents of Bosphorus algae, *Zostera marina* and sediments. *Botanica Marina, 36*(3), 175-178.

Hallegraeff, G. (2003). Harmful algal blooms: a global overview. *Manual on Harmful Marine Microalgae*, *33*, 1-22.

Halun, Z., Terrados, J., Borum, J., Kamp-Nielsen, L., Duarte, C. M., & Fortes, M. D. (2002). Experimental evaluation of the effects of siltation-derived changes in sediment conditions on the Philippine seagrass *Cymodocea rotundata*. *Journal of Experimental Marine Biology and Ecology, 279*(1-2), 73-87.

Hanelt, D., Huppertz, K., & Nultsch, W. (1993). Daily course of photosynthesis and photoinhibition in marine macroalgae investigated in the laboratory and field. *Marine Ecology Progress Series. Oldendorf, 97*(1), 31-37.

Harrison, W., & Platt, T. (1986). Photosynthesis-irradiance relationships in polar and temperate phytoplankton populations. *Polar Biology, 5*(3), 153-164.

Hassouna, N., Mithot, B., & Bachellerie, J.-P. (1984). The complete nucleotide sequence of mouse 28S rRNA gene. Implications for the process of size increase of the large subunit rRNA in higher eukaryotes. *Nucleic Acids Research, 12*(8), 3563-3583.

Hauxwell, J., Cebrián, J., Furlong, C., & Valiela, I. (2001). Macroalgal canopies contribute to eelgrass (*Zostera marina*) decline in temperate estuarine ecosystems. *Ecology, 82*(4), 1007-1022.

Hawes, I., Hall, J., & Pridmore, R. (1992). Research directed towards the management of sea lettuce in Bay of Plenty Coastal waters. *Unpublished Contract Report prepared for Environment Bay of Plenty*.

Hawes, I., Howard ‐ Williams, C., Wells, R., & Clayton, J. (1991). Invasion of water net, *Hydrodictyon reticulatum*: the surprising success of an aquatic plant new to our flora. *New Zealand Journal of Marine and Freshwater Research, 25*, 227-229.

Hawes, I., & O'Brien, R. (2000). Contribution to the assessment of the effects of the Bromley oxidation ponds effluent on the Avon-Heathcote Estuary: modelling the growth of sea lettuce in the estuary. *NIWA client report CHC00/19. Christchurch, NIWA*.

Hawes, I., Sutherland, D., & Hanelt, D. (2003). The use of pulse amplitude modulated fluorometry to determine fine-scale temporal and spatial variation of *in situ* photosynthetic activity within an Isoetes-dominated canopy. *Aquatic Botany, 77*(1), 1-15.

Haynes, D., Müller, J., & Carter, S. (2000a). Pesticide and herbicide residues in sediments and seagrasses from the Great Barrier Reef World Heritage Area and Queensland coast. *Marine Pollution Bulletin, 41*(7-12), 279-287.

Haynes, D., Ralph, P., Prange, J., & Dennison, B. (2000b). The impact of the herbicide diuron on photosynthesis in three species of tropical seagrass. *Marine Pollution Bulletin, 41*(7-12), 288-293.

Hayward, B. W., Morley, M. S., Stephenson, A. B., Blom, W. M., Grenfell, H. R., Prasad, R., Rogan, D., Thompson, F., Cheetham, J., & Webb, M. (1999). Intertidal and subtidal biota and habitats of the central Waitemata Harbour. *Auckland Regional Council Technical Publication, 127*, 40.

Healy, W. (1980). *Pauatahanui Inlet: an environmental study*. Science Information Division, DSIR.

Hemminga, M. A., & Duarte, C. M. (2000). *Seagrass Ecology*. Cambridge, U.K.: Cambridge University Press.

Henley, W. J. (1993). Measurement and interpretation of photosynthetic light‐response curves in algae in the context of photoinhibition and diel changes. *Journal of Phycology, 29*(6), 729-739.

Hoeffle, H., M. S. Thomsen, and M. Holmer. (2011). High mortality of *Zostera marina* under high temperature regimes but minor effects of the invasive macroalgae *Gracilaria vermiculophylla*. *Estuarine, Coastal and Shelf Science 92*:35-46.

Hoeffle, H., T. Wernberg, M. S. Thomsen, and M. Holmer. (2012). Drift algae, an invasive snail and elevated temperature reduces the ecological performance of a warm-temperate seagrass via additive effects. *Marine Ecology Progress Series 450*:67-80.

Holmer, M., Andersen, F. Ø., Nielsen, S. L., & Boschker, H. T. (2001). The importance of mineralization based on sulfate reduction for nutrient regeneration in tropical seagrass sediments. *Aquatic Botany, 71*(1), 1-17.

Holmer, M., Marbà, N., Lamote, M., & Duarte, C. M. (2009). Deterioration of sediment quality in seagrass meadows (*Posidonia oceanica*) invaded by macroalgae (*Caulerpa* sp.). *Estuaries and Coasts, 32*(3), 456-466.

Holmer, M., & Nielsen, R. M. (2007). Effects of filamentous algal mats on sulfide invasion in eelgrass (*Zostera marina*). *Journal of Experimental Marine Biology and Ecology, 353*(2), 245-252.

Holmer, M., P. Wirachwong, and M. S. Thomsen. (2011). Negative effects of stress-resistant drift algae and high temperature on a small ephemeral seagrass species. *Marine Biology 158*:297-309.

Holmquist, J. G. (1997). Disturbance and gap formation in a marine benthic mosaic: influence of shifting macroalgal patches on seagrass structure and mobile invertebrates. *Marine Ecology Progress Series, 158*, 121-130.

Hooper, K. L. (2002). *The impact of stormwater discharges on freshwater, marine water and marine sediments and the implications for environmental management of the Pauatahanui Inlet, Porirua, New Zealand.* MSc thesis, Massey University, Palmerston North, New Zealand.

Hoven, H., Gaudette, H., & Short, F. (1999). Isotope ratios of ^{206}Pb/^{207}Pb in eelgrass, *Zostera marina*, indicate sources of Pb in an estuary. *Marine Environmental Research, 48*(4-5), 377-387.

Hu, C., Yang, X., Gao, L., Zhang, P., Li, W., Dong, J., Li, C., & Zhang, X. (2019). Comparative analysis of heavy metal accumulation and bioindication in three seagrasses: Which species is more suitable as a bioindicator? *Science of the Total Environment, 669*, 41-48.

Hume, T., & McGlone, M. (1986). Sedimentation patterns and catchment use change recorded in the sediments of a shallow tidal creek, Lucas Creek, Upper Waitemata Harbour, New Zealand. *New Zealand Journal of Marine and Freshwater Research, 20*(4), 677-687.

Ichihara, K., Shimada, S., & Miyaji, K. (2013). Systematics of *Rhizoclonium*-like algae (Cladophorales, Chlorophyta) from Japanese brackish waters, based on molecular phylogenetic and morphological analyses. *Phycologia, 52*(5), 398-410.

Infantes, E., Terrados, J., & Orfila, A. (2011). Assessment of substratum effect on the distribution of two invasive Caulerpa (*Chlorophyta*) species. *Estuarine, Coastal and Shelf Science, 91*(3), 434-441.

Inglis, G. (2003). Seagrasses of New Zealand. In E. P. Green & F. T. Short (Eds.), *World Atlas of Seagrasses* (pp. 148-157). Berkeley, CA: University of California Press.

Isaksen, M. F., & Finster, K. (1996). Sulphate reduction in the root zone of the seagrass *Zostera noltii* on the intertidal flats of a coastal lagoon (Arcachon, France). *Marine Ecology Progress Series, 137,* 187-194.

Jackson, E. L., Rowden, A. A., Attrill, M. J., Bossey, S. J., & Jones, M. B. (2001). The importance of seagrass beds as a habitat for fishery species. *Oceanography and Marine Biology, 39*, 269-304.

Jacobs, S. W., Les, D. H., & Moody, M. L. (2006). New combinations in Australasian *Zostera* (*Zosteraceae*). *Telopea, 11*(2), 127-128.

Jarvis, J. C., & Moore, K. A. (2010). The role of seedlings and seed bank viability in the recovery of Chesapeake Bay, USA, *Zostera marina* populations following a large-scale decline. *Hydrobiologia, 649*(1), 55-68.

Jensen, H. F., Holmer, M., & Dahllöf, I. (2004). Effects of tributyltin (TBT) on the seagrass *Ruppia maritima*. *Marine Pollution Bulletin, 49*(7-8), 564-573.

Jesus, B., Brotas, V., Ribeiro, L., Mendes, C., Cartaxana, P., & Paterson, D. (2009). Adaptations of microphytobenthos assemblages to sediment type and tidal position. *Continental Shelf Research, 29*(13), 1624-1634.

Jeyakumar, P., Anderson, C., Holmes, A., & Miller, S. (2014). Optimising Copper Sprays on Kiwifruit: a Review. *Palmerston North*.

Jones, T. C., Gemmill, C. E., & Pilditch, C. A. (2008). Genetic variability of New Zealand seagrass (*Zostera muelleri*) assessed at multiple spatial scales. *Aquatic Botany, 88*(1), 39-46.

Kabata-Pendias, A. (2011). Trace Elements in Soils and Plants. CRC Press, Taylor and Francis Group.

Kemp, W. M., Batleson, R., Bergstrom, P., Carter, V., Gallegos, C. L., Hunley, W., Karrh, L., Koch, E. W., Landwehr, J. M., & Moore, K. A. (2004). Habitat requirements for submerged aquatic vegetation in Chesapeake Bay: Water quality, light regime, and physical-chemical factors. *Estuaries, 27*(3), 363-377.

Kenworthy, W. J., & Fonseca, M. (1977). Reciprocal transplant of the seagrass *Zostera marina* L. Effect of substrate on growth. *Aquaculture, 12*(3), 197-213.

Kinney, E. H., & Roman, C. T. (1998). Response of primary producers to nutrient enrichment in a shallow estuary. *Marine Ecology Progress Series, 163*, 89-98.

Kirk, J. T. (1985). Effects of suspensoids (turbidity) on penetration of solar radiation in aquatic ecosystems. *Hydrobiologia, 125*(1), 195-208.

Kirk, J. T. (1994). *Light and photosynthesis in aquatic ecosystems*. Cambridge university press.

Kirkman, H., & Kuo, J. (1990). Pattern and process in southern Western Australian seagrasses. *Aquatic Botany, 37*(4), 367-382.

Koch, E. (1994). Hydrodynamics, diffusion-boundary layers and photosynthesis of the seagrasses *Thalassia testudinum* and *Cymodocea nodosa*. *Marine Biology, 118*(4), 767-776.

Koch, E. W. (2001). Beyond light: physical, geological, and geochemical parameters as possible submersed aquatic vegetation habitat requirements. *Estuaries, 24*(1), 1-17.

Koch, E. W., Ackerman, J. D., Verduin, J., & van Keulen, M. (2007a). Fluid dynamics in seagrass ecology—From molecules to ecosystems. In A. W. D. Larkum, *et al.* (Eds.), *Seagrasses: Biology, Ecology and Conservation* (pp. 193-225). Dordrecht, Netherlands: Springer.

Koch, M., Schopmeyer, S., Kyhn-Hansen, C., & Madden, C. (2007b). Synergistic effects of high temperature and sulfide on tropical seagrass. *Journal of Experimental Marine Biology and Ecology, 341*(1), 91-101.

Korpinen, S., Laamanen, M., Andersen, J. H., Asplund, L., Berger, U., Bignert, A., Boalt, E., Broeg, K., Brzozowska, A., & Cato, I. (2010). Hazardous substances in the Baltic Sea: an integrated thematic assessment of hazardous substances in the Baltic Sea.

Krämer, U. (2010). Metal hyperaccumulation in plants. *Annual Review of Plant Biology, 61*, 517-534.

Krause-Jensen, D., Carstensen, J., Nielsen, S. L., Dalsgaard, T., Christensen, P. B., Fossing, H., & Rasmussen, M. B. (2011). Sea bottom characteristics affect depth limits of eelgrass *Zostera marina*. *Marine Ecology Progress Series, 425*, 91-102.

Krause‐Jensen, D., & Sand‐Jensen, K. (1998). Light attenuation and photosynthesis of aquatic plant communities. *Limnology and Oceanography, 43*(3), 396-407.

Kuo, J., & Den Hartog, C. (2000). Seagrasses: a profile of an ecological group. *Biologia Marina Mediterranea, 7*(2), 3-17.

Lanari, M., & Copertino, M. (2017). Drift macroalgae in the Patos Lagoon Estuary (southern Brazil): effects of climate, hydrology and wind action on the onset and magnitude of blooms. *Marine Biology Research, 13*(1), 36-47.

Larkum, A., & West, R. (1983). Stability, depletion and restoration of seagrass beds. In *Proc. Linn. Soc. NSW* (Vol. 106, pp. 201-212).

Larkum, A. W., Orth, R. J., & Duarte, C. M. (2006). *Seagrasses*. Springer.

Larkum, A. W. D., Davey, P. A., Kuo, J., Ralph, P. J., & Raven, J. A. (2017). Carbon-concentrating mechanisms in seagrasses. *Journal of Experimental Botany, 68*(14), 3773-3784.

Lavery, P. S., Lukatelich, R., & McComb, A. (1991). Changes in the biomass and species composition of macroalgae in a eutrophic estuary. *Estuarine, Coastal and Shelf Science, 33*(1), 1-22.

Lee, G., Suonan, Z., Kim, S. H., Hwang, D.-W., & Lee, K.-S. (2019). Heavy metal accumulation and phytoremediation potential by transplants of the seagrass *Zostera marina* in the polluted bay systems. *Marine Pollution Bulletin, 149*, 110509.

Lee, K.-S., Park, S. R., & Kim, Y. K. (2007). Effects of irradiance, temperature, and nutrients on growth dynamics of seagrasses: a review. *Journal of Experimental Marine Biology and Ecology, 350*(1-2), 144-175.

Leliaert, F., & Boedeker, C. (2007). Cladophorales. In *Green seaweeds of Britain and Ireland* (pp. 131-183). The British Phycological Society.

Leliaert, F., Rousseau, F., De Reviers, B., & Coppejans, E. (2003). Phylogeny of the Cladophorophyceae (Chlorophyta) inferred from partial LSU rRNA gene sequences: is the recognition of a separate order Siphonocladales justified? *European Journal of Phycology, 38*(3), 233-246.

Leliaert, F., Verbruggen, H., Wysor, B., & De Clerck, O. (2009). DNA taxonomy in morphologically plastic taxa: algorithmic species delimitation in the Boodlea complex (Chlorophyta: Cladophorales). *Molecular Phylogenetics and Evolution, 53*(1), 122-133.

Leliaert, F., Verbruggen, H., & Zechman, F. W. (2011). Into the deep: new discoveries at the base of the green plant phylogeny. *Bioessays, 33*(9), 683-692.

Leschen, A. S., Ford, K. H., & Evans, N. T. (2010). Successful eelgrass (*Zostera marina*) restoration in a formerly eutrophic estuary (Boston Harbor) supports the use of a multifaceted watershed approach to mitigating eelgrass loss. *Estuaries and Coasts, 33*(6), 1340-1354.

Leuschner, C., Landwehr, S., & Mehlig, U. (1998). Limitation of carbon assimilation of intertidal *Zostera noltii* and *Z. marina* by desiccation at low tide. *Aquatic Botany, 62*(3), 171-176.

Leuschner, C., & Rees, U. (1993). CO2 gas exchange of two intertidal seagrass species, *Zostera marina* L. and *Zostera noltii* Hornem., during emersion. *Aquatic Botany, 45*(1), 53-62.

Li, M., Lundquist, C. J., Pilditch, C. A., Rees, T., & Ellis, J. (2019). Implications of nutrient enrichment for the conservation and management of seagrass *Zostera muelleri* meadows. *Aquatic Conservation: Marine and Freshwater Ecosystems, 29*, 1484-1502.

Lin, H., Sun, T., Xue, S., & Jiang, X. (2016). Heavy metal spatial variation, bioaccumulation, and risk assessment of *Zostera japonica* habitat in the Yellow River Estuary, China. *Science of the Total Environment, 541*, 435-443.

Lohrer, A., Halliday, N., Thrush, S., Hewitt, J., & Rodil, I. (2010). Ecosystem functioning in a disturbance-recovery context: contribution of macrofauna to primary production and nutrient release on intertidal sandflats. *Journal of Experimental Marine Biology and Ecology, 390*(1), 6-13.

Lohrer, A. M., Hewitt, J. E., & Thrush, S. F. (2006). Assessing far-field effects of terrigenous sediment loading in the coastal marine environment. *Marine Ecology Progress Series, 315*, 13-18.

Lohrer, A. M., Thrush, S. F., & Gibbs, M. M. (2004). Bioturbators enhance ecosystem function through complex biogeochemical interactions. *Nature, 431*(7012), 1092-1095.

Lohrer, A. M., Townsend, M., Hailes, S. F., Rodil, I. F., Cartner, K., Pratt, D. R., & Hewitt, J. E. (2016). Influence of New Zealand cockles (*Austrovenus stutchburyi*) on primary productivity in sandflat-seagrass (*Zostera muelleri*) ecotones. *Estuarine, Coastal and Shelf Science, 181*, 238-248.

Longstaff, B., Loneragan, N., O'donohue, M., & Dennison, W. (1999). Effects of light deprivation on the survival and recovery of the seagrass *Halophila ovalis* (R. Br.) Hook. *Journal of Experimental Marine Biology and Ecology, 234*(1), 1-27.

Longstaff, B. J. (2003). *Investigations into the Light Requirements of Seagrasses in Northeast Australia.* PhD thesis, University of Queensland, Brisbane, Australia.

Lord, D., Paling, E., & Gordon, D. (1999). Review of seagrass rehabilitation and restoration programmes in Australia. *Chapter, 3*, 65-115.

Lyons, D. A., Arvanitidis, C., Blight, A. J., Chatzinikolaou, E., Guy‐Haim, T., Kotta, J., Orav‐Kotta, H., Queirós, A. M., Rilov, G., & Somerfield, P. J. (2014). Macroalgal blooms alter community structure and primary productivity in marine ecosystems. *Global Change Biology, 20*(9), 2712-2724.

Macinnis-Ng, C. M., & Ralph, P. J. (2004). In situ impact of multiple pulses of metal and herbicide on the seagrass, *Zostera capricorni*. *Aquatic Toxicology, 67*(3), 227-237.

Macreadie, P., Schliep, M., Rasheed, M., Chartrand, K., & Ralph, P. (2014a). Molecular indicators of chronic seagrass stress: A new era in the management of seagrass ecosystems? *Ecological Indicators, 38*, 279-281.

Macreadie, P. I., York, P. H., & Sherman, C. D. (2014b). Resilience of *Zostera muelleri* seagrass to small‐scale disturbances: the relative importance of asexual versus sexual recovery. *Ecology and Evolution, 4*(4), 450-461.

Manassa, R., Smith, T. M., Beardall, J., Keough, M., & Cook, P. (2017). Capacity of a temperate intertidal seagrass species to tolerate changing environmental conditions: Significance of light and tidal exposure. *Ecological Indicators, 81*, 578-586.

Manzanera, M., Pérez, M., & Romero, J. (1998). Seagrass mortality due to oversedimentation: an experimental approach. *Journal of Coastal Conservation, 4*(1), 67-70.

Marba, N., & Duarte, C. M. (1994). Growth response of the seagrass *Cymodocea nodosa* to experimental burial and erosion. *Marine Ecology Progress Series. Oldendorf, 107*(3), 307-311.

Marbà, N., & Duarte, C. M. (1998). Rhizome elongation and seagrass clonal growth. *Marine Ecology Progress Series*, *174*, 269-280.

Marbà, N., Duarte, C. M., Terrados, J., Halun, Z., Gacia, E., & Fortes, M. D. (2010). Effects of seagrass rhizospheres on sediment redox conditions in SE Asian coastal ecosystems. *Estuaries and Coasts, 33*(1), 107-117.

Martínez-Lüscher, J., & Holmer, M. (2010). Potential effects of the invasive species *Gracilaria vermiculophylla* on *Zostera marina* metabolism and survival. *Marine Environmental Research, 69*(5), 345-349.

Matheson, F., Reed, J., Dos Santos, V., Mackay, G., & Cummings, V. (2017a). Seagrass rehabilitation: successful transplants and evaluation of methods at different spatial scales. *New Zealand Journal of Marine and Freshwater Research, 51*(1), 96-109.

Matheson, F., Wadhwa, S., Taumoepeau, A., & Smith, J. (2010). *Seagrass in the eastern Bay of Islands: past and present abundance, threats and management options.* Client Report: HAM2010-043, Consultancy Report for Northland Regional Council. NIWA, Hamilton, New Zealand. 24p. https://www.scribd.com/document/43914816/Seagrass-in-the-Eastern-Bay-of-Islands.

Matheson, F. E., Lundquist, C. J., Gemmill, C. E., & Pilditch, C. A. (2011). New Zealand seagrass–More threatened than IUCN review indicates. *Biological Conservation, 144*(12), 2749-2750.

Matheson, F. E., Mackay, G., Middleton, C., Griffiths, R., Eyre, R., & Smith, J. (2016). *Using transplants to restore seagrass: success of a second trial in Whāngārei Harbour, NZ*. Presented at the Proceedings of the Joint Conference of the Society for Ecological Restoration Australasia (SERA) and the New Zealand Ecological Society.

Matheson, F. E., Oliver, M., Mackay, G., & Griffiths, R. (2017b). *Minimum light thresholds for seagrass indicated by continuous data from multi-year rehabilitation trials.* Presented at the Proceedings of the New Zealand Marine Sciences Society 56th Annual Conference.

Matheson, F. E., & Schwarz, A. M. (2007). Growth responses of *Zostera capricorni* to estuarine sediment conditions. *Aquatic Botany, 87*(4), 299-306.

Matheson, F. E., & Wadhwa, S. (2012). *Seagrass in Porirua Harbour.: Preliminary Assessment of Restoration Potential.* Report No. HAM2012-037; Prepared for Greater Wellington Regional Council. National Institute of Water and Atmospheric Research, Hamilton, New Zealand. 35p. http://www.gw.govt.nz/assets/Seagrass-restoration-in-Porirua-Harbour.pdf.

McGlathery, K. J., Berg, P., & Marino, R. (2001). Using porewater profiles to assess nutrient availability in seagrass-vegetated carbonate sediments. *Biogeochemistry, 56*(3), 239-263.

McMahon, K., Collier, C., & Lavery, P. S. (2013). Identifying robust bioindicators of light stress in seagrasses: a meta-analysis. *Ecological Indicators*, *30*, 7-15.

McRoy, C., & McMillan, C. (1977). Production ecology and physiology of seagrasses. *Seagrass Ecosystems: a scientific perspective,* 53-81.

Meehan, A. J., & West, R. J. (2000). Recovery times for a damaged *Posidonia australis* bed in south eastern Australia. *Aquatic Botany, 67*(2), 161-167.

Meinesz, A., Lefevre, J., & Astier, J. (1991). Impact of coastal development on the infralittoral zone along the southeastern Mediterranean shore of continental France. *Marine Pollution Bulletin, 23,* 343-347.

Millero, F. J. (1979). The thermodynamics of the carbonate system in seawater. *Geochimica et Cosmochimica Acta*, *43*(10), 1651-1661.

Mills, K. E., & Fonseca, M. S. (2003). Mortality and productivity of eelgrass *Zostera marina* under conditions of experimental burial with two sediment types. *Marine Ecology Progress Series*, *255*, 127-134.

Milne, J. R., & Warr, S. (2007). Annual coastal monitoring report for the Wellington region, 2007/08.

Moksnes, P.-O., Eriander, L., Infantes, E., & Holmer, M. (2018). Local regime shifts prevent natural recovery and restoration of lost eelgrass beds along the Swedish west coast. *Estuaries and Coasts, 41*(6), 1712-1731.

Morrisey, D. J., & Turner, S. J. (1996). Experimental Restoration of seagrass beds. *Conservation Science Newsletter, No. 20*.

Morrison, M., Jones, E. G., Consalvey, M., & Berkenbusch, K. (2014). *Linking marine fisheries species to biogenic habitats in New Zealand: a review and synthesis of knowledge*. Ministry for Primary Industries.

Morrison, M. A., Lowe, M., Parsons, D., Usmar, N., & McLeod, I. (2009). *A Review of Land-Based Effects on Coastal Fisheries and Supporting Biodiversity in New Zealand.* New Zealand Aquatic Environment and Biodiversity Report No. 37 Ministry of Fisheries, Wellington, New Zealand. 100p. http://fs.fish.govt.nz/Page.aspx?pk=113&dk=22003.

Munkes, B., Schubert, P. R., Karez, R., & Reusch, T. B. (2015). Experimental assessment of critical anthropogenic sediment burial in eelgrass *Zostera marina*. *Marine Pollution Bulletin, 100*(1), 144-153.

Neill, K., D'Archino, R., Farr, T., & Nelson, W. (2012). Macroalgal diversity associated with soft sediment habitats in New Zealand. *New Zealand Aquatic Environment and Biodiversity Report, 87*, 127.

Neill, K., & Nelson, W. (2019). *New Zealand Marine Macroalgae Species Checklist.* NIWA, Wellington, New Zealand. https://nzobisipt.niwa.co.nz/resource?r=nzmma_2018&v=1.0.

Nelson, T. A., Lee, D. J., & Smith, B. C. (2003). Are "green tides" harmful algal blooms? Toxic properties of water‐soluble extracts from two bloom‐forming macroalgae, *Ulva fenestrata* and *Ulvaria obscura* (Ulvophyceae). *Journal of Phycology, 39*(5), 874-879.

Nelson, W., Neill, K., & D'Archino, R. (2015). When seaweeds go bad: an overview of outbreaks of nuisance quantities of marine macroalgae in New Zealand. *New Zealand Journal of Marine and Freshwater Research, 49*(4), 472-491.

Nelson, W. A., & Adams, N. M. (1987). *Marine algae of the Bay of Islands area.* National Museum of New Zealand. Wellington, New Zealand.

Nelson, W. A., Sutherland, J. E., Ringham, S., & Murupaenga, H. (2019). *Dictyota korowai* sp. nov.(Dictyotales, Phaeophyceae) from Manawatāwhi/Three Kings Islands, northern New Zealand, previously confused with *Dictyota intermedia*. *Phycologia, 58*(4), 433-442.

Nielsen, S. L., Banta, G. T., Khan, F. R., & Palmqvist, A. (2017). Copper in the sediment: a major stressor for eelgrass, *Zostera marina* L. *Hydrobiologia, 788*(1), 143-155.

Orth, R. J., Carruthers, T. J., Dennison, W. C., Duarte, C. M., Fourqurean, J. W., Heck, K. L., Hughes, A. R., Kendrick, G. A., Kenworthy, W. J., & Olyarnik, S. (2006). A global crisis for seagrass ecosystems. *Bioscience, 56*(12), 987-996.

Paling, E. I., Fonseca, M., van Katwijk, M. M., & van Keulen, M. (2009). Seagrass restoration. *Coastal wetlands: an integrated ecosystem approach*, 687-713.

Paling, E. I., van Keulen, M., Wheeler, K., Phillips, J., & Dyhrberg, R. (2001). Mechanical seagrass transplantation in Western Australia. *Ecological Engineering, 16*(3), 331-339.

Park, S. G. (1999). *Changes in Abundance of Seagrass (Zostera spp.) in Tauranga Harbour from 1959-96.* Environmental Report 99/30. Environment BOP, Whakatane, New Zealand. 19p. https://www.boprc.govt.nz/media/362713/changes-in-abundance-of-seagrass-zostera-spp-in-tauranga-harbour-from-1959-96.pdf.

Park, S. G. (2011). *Sea lettuce and nutrient monitoring in Tauranga harbour 1991-2010*. Bay of Plenty Regional Council.

Park, S. G. (2016). *Extent of seagrass in the Bay of Plenty in 2011*. Bay of Plenty Regional Council.

Park, S. R., Kim, S., Kim, Y. K., Kang, C.-K., & Lee, K.-S. (2016). Photoacclimatory responses of *Zostera marina* in the intertidal and subtidal zones. *PLoS One, 11*(5), e0156214.

Paul-Burke, K., Burke, J., Team, T. Ū. R. M., Bluett, C., & Senior, T. (2018). Using Māori knowledge to assist understandings and management of shellfish populations in Ōhiwa harbour, Aotearoa New Zealand. *New Zealand Journal of Marine and Freshwater Research, 52*(4), 542-556.

Pedersen, O., Binzer, T., & Borum, J. (2004). Sulphide intrusion in eelgrass (*Zostera marina* L.). *Plant, Cell & Environment, 27*(5), 595-602.

Pedersen, T. M., Gallegos, C. L., & Nielsen, S. L. (2012). Influence of near-bottom re-suspended sediment on benthic light availability. *Estuarine, Coastal and Shelf Science, 106*, 93-101.

Peralta, G., Bouma, T. J., van Soelen, J., Pérez-Lloréns, J. L., & Hernández, I. (2003). On the use of sediment fertilization for seagrass restoration: a mesocosm study on *Zostera marina* L. *Aquatic Botany, 75*(2), 95-110.

Pérez, M., Invers, O., Ruiz, J. M., Frederiksen, M. S., & Holmer, M. (2007). Physiological responses of the seagrass *Posidonia oceanica* to elevated organic matter content in sediments: an experimental assessment. *Journal of Experimental Marine Biology and Ecology, 344*(2), 149-160.

Philippart, C. J. (1994). Interactions between *Arenicola marina* and *Zostera noltii* on a tidal flat in the Wadden Sea. *Marine Ecology Progress Series*, 251-257.

Platt, T., Gallegos, C., & Harrison, W. G. (1981). Photoinhibition of photosynthesis in natural assemblages of marine phytoplankton. *Instituto del Mar del Peru, Boletin*.

Prange, J., & Dennison, W. (2000). Physiological responses of five seagrass species to trace metals. *Marine Pollution Bulletin, 41*(7-12), 327-336.

Procaccini, G., Beer, S., Björk, M., Olsen, J., Mazzuca, S., & Santos, R. (2012). Seagrass ecophysiology meets ecological genomics: are we ready? *Marine Ecology, 33*(4), 522-527.

Procaccini, G., Dattolo, E., Innocenti, A., Mazzuca, S., Cavallini Natali, A., & Gu, J. (2010). Differential gene expression profiling under different light conditions in *Posidonia oceanica* (L.) Delile by SSH analysis. *Rapp. Commite International Mer Méditerraneo, 39*, 641-642.

Procaccini, G., & Piazzi, L. (2001). Genetic polymorphism and transplantation success in the Mediterranean seagrass *Posidonia oceanica*. *Restoration Ecology, 9*(3), 332-338.

Ralph, P., Durako, M. J., Enriquez, S., Collier, C., & Doblin, M. (2007). Impact of light limitation on seagrasses. *Journal of Experimental Marine Biology and Ecology, 350*(1-2), 176-193.

Ralph, P. J., & Gademann, R. (2005). Rapid light curves: a powerful tool to assess photosynthetic activity. *Aquatic Botany, 82*(3), 222-237.

Ramage, D., & Schiel, D. (1998). Reproduction in the seagrass *Zostera novazelandica* on intertidal platforms in southern New Zealand. *Marine Biology, 130*(3), 479-489.

Rasheed, M. A., & Unsworth, R. K. (2011). Long-term climate-associated dynamics of a tropical seagrass meadow: implications for the future. *Marine Ecology Progress Series, 422*, 93-103.

Rasmussen, J. R., Olesen, B., & Krause-Jensen, D. (2012). Effects of filamentous macroalgae mats on growth and survival of eelgrass, *Zostera marina*, seedlings. *Aquatic Botany, 99*, 41-48.

Rasmusson, L. M., Buapet, P., George, R., Gullström, M., Gunnarsson, P. C., & Björk, M. (2020). Effects of temperature and hypoxia on respiration, photorespiration, and photosynthesis of seagrass leaves from contrasting temperature regimes. *ICES Journal of Marine Science, 77*(6), 2056-2065.

Reed, J., Schwarz, A., Gosai, A., & Morrison, M. (2004). *Feasibility Study to Investigate The Replenishment/Reinstatement of Seagrass Beds in Whangarei Harbour–Phase 1.* Client Report: AKL2004-33, Prepared for Northland Regional Council. NIWA, Auckland, New Zealand. 24p.

Reusch, T. B., Ehlers, A., Hämmerli, A., & Worm, B. (2005). Ecosystem recovery after climatic extremes enhanced by genotypic diversity. *Proceedings of the National Academy of Sciences, 102*(8), 2826-2831.

Reusch, T. B., & Hughes, A. R. (2006). The emerging role of genetic diversity for ecosystem functioning: estuarine macrophytes as models. *Estuaries and Coasts, 29*(1), 159-164.

Robertson, B. P., Gardner, J. P., & Savage, C. (2015). Macrobenthic–mud relations strengthen the foundation for benthic index development: a case study from shallow, temperate New Zealand estuaries. *Ecological Indicators, 58*, 161-174.

Rohr, M. E., Bostrom, C., Canal-Vergés, P., & Holmer, M. (2016). Blue carbon stocks in Baltic Sea eelgrass (*Zostera marina*) meadows. *Biogeosciences, 13*(22), 6139-6153.

Romero, J., Pérez, M., Mateo, M., & Sala, E. (1994). The belowground organs of the Mediterranean seagrass *Posidonia oceanica* as a biogeochemical sink. *Aquatic Botany, 47*(1), 13-19.

Rowden, A., Berkenbusch, K., Brewin, P., Dalen, J., Neill, K., Nelson, W., Oliver, M., & Probert, P. (2012). A review of the marine soft-sediment assemblages of New Zealand. *NZ Aquatic Environment and Biodiversity Report*.

Ruiz-Halpern, S., Macko, S. A., & Fourqurean, J. W. (2008). The effects of manipulation of sedimentary iron and organic matter on sediment biogeochemistry and seagrasses in a subtropical carbonate environment. *Biogeochemistry, 87*(2), 113-126.

Ruiz, J., & Romero, J. (2003). Effects of disturbances caused by coastal constructions on spatial structure, growth dynamics and photosynthesis of the seagrass *Posidonia oceanica*. *Marine Pollution Bulletin, 46*(12), 1523-1533.

Schreiber, U., Schliwa, U., & Bilger, W. (1986). Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. *Photosynthesis Research, 10*(1), 51-62.

Schwarz, A.-M., Morrison, M., Hawes, I., & Halliday, J. (2006). Physical and biological characteristics of a rare marine habitat: sub-tidal seagrass beds of offshore islands. *Science for Conservation, 269*, 39.

Schwarz, A. M. (2004). Contribution of photosynthetic gains during tidal emersion to production of *Zostera capricorni* in a North Island, New Zealand estuary. *New Zealand Journal of Marine and Freshwater Research, 38*(5), 809-818.

Schwarz, A. M., Reed, J., & Morrison, M. (2005). *Decision making document. Prepared for Sustainable Management Fund and Northland Regional Council.* NIWA client report Auckland, New Zealand.

Shapiro, S. S., & Wilk, M. B. (1965). An analysis of variance test for normality (complete samples). *Biometrika, 52*(3/4), 591-611.

Short, F. T. (1987). Effects of sediment nutrients on seagrasses: literature review and mesocosm experiment. *Aquatic Botany, 27*(1), 41-57.

Short, F. T., & Coles, R. G. (Eds.). (2001). *Global Seagrass Research Methods*. Amsterdam, Netherlands: Elsevier.

Short, F. T., Davis, R. C., Kopp, B., Short, C. A., & Burdick, D. M. (2002). Site-selection model for optimal transplantation of eelgrass *Zostera marina* in the northeastern US. *Marine Ecology Progress Series, 227*, 253-267.

Short, F. T., & Wyllie-Echeverria, S. (1996). Natural and human-induced disturbance of seagrasses. *Environmental Conservation, 23*(1), 17-27.

Siciliano, A., D. R. Schiel, and M. S. Thomsen. (2019). Effects of local anthropogenic stressors on a habitat cascade in an estuarine seagrass system. *Marine and Freshwater Research 70*:1129- 1142.

Silva, J., Santos, R., Calleja, M. L., & Duarte, C. M. (2005). Submerged versus air-exposed intertidal macrophyte productivity: from physiological to community-level assessments. *Journal of Experimental Marine Biology and Ecology, 317*(1), 87-95.

Silva, J., Sharon, Y., Santos, R., & Beer, S. (2009). Measuring seagrass photosynthesis: methods and applications. *Aquatic Biology, 7*(1-2), 127-141.

Singer, J., Anderson, J., Ledbetter, M., McCave, I., Jones, K., & Wright, R. (1988). An assessment of analytical techniques for the size analysis of fine-grained sediments. *Journal of Sedimentary Research, 58*(3), 534-543.

Skillington, A., Flint, N., Anastasi, A., Dwane, G., & Jackson, E. (2020). Influence of variable salinity and low light on copper accumulation in the potential seagrass bioindicator, *Zostera muelleri*. *Marine Pollution Bulletin, 160*, 111602.

Smale, D. A., T. Wernberg, E. C. Oliver, M. Thomsen, B. P. Harvey, S. C. Straub, M. T. Burrows, L. V. Alexander, J. A. Benthuysen, and M. G. Donat. (2019). Marine heatwaves threaten global biodiversity and the provision of ecosystem services. *Nature Climate Change 9*:306-312.

Smith, N. M., & Walker, D. I. (2002). Canopy structure and pollination biology of the seagrasses *Posidonia australis* and *P. sinuosa* (Posidoneaceae). *Aquatic Botany, 74*(1), 57-70.

Soerensen, S. T. (2020). *New Zealand seagrass (Zostera muelleri) response to acute sedimentation: Linking non-structural carbohydrate reserves to resilience.* PhD thesis, The University of Waikato, Hamilton, New Zealand.

Sørensen, S. T., Campbell, M. L., Duke, E., & Manley-Harris, M. (2018). A standard, analytical protocol for the quantitation of non-structural carbohydrates in seagrasses that permits inter-laboratory comparison. *Aquatic Botany, 151*, 71-79.

Stevens, L., & Robertson, B. (2016). *Porirua Harbour; Sediment Plate Monitoring 2015/16.* Report prepared for Greater Wellington Regional Council. Wriggle Coastal Management, Nelson, New Zealand. 16p. http://www.gw.govt.nz/assets/council-publications/Porirua-Harbour-Sediment-plate-monitoring-2016.pdf.

Stoffers, P., Glasby, G., Plueger, W., & Walter, P. (1983). Reconnaissance survey of the mineralogy and geochemistry of some New Zealand lake and nearshore sediments. *New Zealand Journal of Marine and Freshwater Research, 17*(4), 461-480.

Sugimoto, K., Hiraoka, K., Ohta, S., Niimura, Y., Terawaki, T., & Okada, M. (2007). Effects of ulvoid (*Ulva* spp.) accumulation on the structure and function of eelgrass (*Zostera marina* L.) bed. *Marine Pollution Bulletin, 54*(10), 1582-1585.

Suykerbuyk, W., Bouma, T. J., Govers, L. L., Giesen, K., de Jong, D. J., Herman, P., Hendriks, J., & van Katwijk, M. M. (2016). Surviving in changing seascapes: Sediment dynamics as bottleneck for long-term seagrass presence. *Ecosystems, 19*(2), 296-310.

Swales, A., Bentley, S., McGlone, M., Ovenden, R., Hermanspahn, N., Budd, R., Hill, A., Pickmere, S., Haskew, R., & Okey, M. (2005). Pauatahanui estuary: effects of historical catchment landcover changes on estuary sedimentation. *National Institute of Water & Atmospheric Research Ltd*.

Swales, A., Williamson, R. B., Van Dam, L. F., Stroud, M. J., & McGlone, M. S. (2002). Reconstruction of urban stormwater contamination of an estuary using catchment history and sediment profile dating. *Estuaries, 25*(1), 43-56.

Tan, Y. M., Dalby, O., Kendrick, G. A., Statton, J., Sinclair, E. A., Fraser, M. W., Macreadie, P. I., Gillies, C. L., Coleman, R. A., Waycott, M., van Dijk, K.-j., Verges, A., Ross, J. D., Campbell, M. L., Matheson, F., Jackson, E., Irving, A. D., Govers, L. L., Connollt, R., McLeod, I., Rasheed, M., Kirkman, H., Flindt, M., Lange, T., Miller, A., & Sherman, C. D. (2020). Seagrass restoration is possible: Insights and lessons from Australia and New Zealand. *Frontiers in Marine Science, 7*, 617.

Terrados, J., Duarte, C. M., Kamp-Nielsen, L., Agawin, N., Gacia, E., Lacap, D., Fortes, M., Borum, J., Lubanski, M., & Greve, T. (1999). Are seagrass growth and survival constrained by the reducing conditions of the sediment? *Aquatic Botany, 65*(1-4), 175-197.

Thiers, B. (2020). *Index Herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium [continuously updated].* Retrieved 20 June, 2020, from http://sweetgum.nybg.org/science/ih/.

Thimijan, R. W., & Heins, R. D. (1983). Photometric, radiometric, and quantum light units of measure: a review of procedures for interconversion. *HortScience, 18*(6), 818-822.

Thomsen, M. S. (2010). Experimental evidence for positive effects of invasive seaweed on native invertebrates via habitat-formation in a seagrass bed. *Aquatic Invasions 5*:341-346.

Thomsen, M. S., A. H. Altieri, C. Angelini, M. J. Bishop, P. E. Gribben, G. Lear, Q. He, D. R. Schiel, B. R. Silliman, and P. M. South. (2018). Secondary foundation species enhance biodiversity. *Nature Ecology & Evolution 2*:634.

Thomsen, M. S., T. de Bettignies, T. Wernberg, M. Holmer, and B. Debeuf. (2012a). Harmful algae are not harmful to everyone. *Harmful Algae 16*:74-80.

Thomsen, M. S., T. Hildebrand, P. M. South, T. Foster, A. Siciliano, E. Oldach, and D. R. Schiel. (2016). A sixth-level habitat cascade increases biodiversity in an intertidal estuary. *Ecology and Evolution*:1-13.

Thomsen, M. S., P. A. Staehr, L. Nejrup, and D. R. Schiel. (2013). Effects of the invasive macroalgae *Gracilaria vermiculophylla* on two co-occurring foundation species and associated invertebrates. *Aquatic Invasions 8*:133-145.

Thomsen, M. S., and T. Wernberg. (2009). Drift algae, invasive snails and seagrass health in the Swan River: patterns, impacts and linkages. Report no. CMER-2009-02 from the Centre for Marine Ecosystems Research

Thomsen, M. S., and T. Wernberg. (2015). The devil in the detail: harmful seaweeds are not harmful to everyone. *Global Change Biology 21*:1381-1382.

Thomsen, M. S., T. Wernberg, A. H. Altieri, F. Tuya, D. Gulbransen, K. J. McGlathery, M. Holmer, and B. R. Silliman. (2010). Habitat cascades: The conceptual context and global relevance of facilitation cascades via habitat formation and modification. *Integrative and Comparative Biology 50*:158-175.

Thomsen, M. S., T. Wernberg, A. H. Engelen, F. Tuya, M. A. Vanderklift, M. Holmer, K. J. McGlathery, F. Arenas, J. Kotta, and B. R. Silliman. (2012b). A meta-analysis of seaweed impacts on seagrasses: generalities and knowledge gaps. *PLOS one 7*:e28595.

Thrush, S., Hewitt, J., Cummings, V., Ellis, J., Hatton, C., Lohrer, A., & Norkko, A. (2004). Muddy waters: elevating sediment input to coastal and estuarine habitats. *Frontiers in Ecology and the Environment, 2*(6), 299-306.

Thrush, S., Hewitt, J., & Lohrer, A. (2012). Interaction networks in coastal soft‐sediments highlight the potential for change in ecological resilience. *Ecological Applications, 22*(4), 1213-1223.

Touchette, B. W., & Burkholder, J. M. (2000). Overview of the physiological ecology of carbon metabolism in seagrasses. *Journal of Experimental Marine Biology and Ecology, 250*(1-2), 169-205.

Townsend, M., & Lohrer, D. (2015). ANZECC guidance for estuary sedimentation. *Prepared for the Ministry for the environment by NIWA. New Zealand: Hamilton*.

Tukey, J. W. (1977). *Exploratory data analysis*. (Vol. 2). Reading, Mass.

Turner, S. (1995). Restoring seagrass systems in New Zealand. *Water and Atmosphere, 3*(2), 9-11.

Turner, S., & Schwarz, A.-M. (2006). *Management and Conservation of Seagrass in New Zealand: An Introduction.* Science for Conservation 264. Science & Technical Publishing, Wellington, New Zealand. 90p. http://www.doc.govt.nz/documents/science-and-technical/sfc264.pdf.

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.

Valiela, I., McClelland, J., Hauxwell, J., Behr, P. J., Hersh, D., & Foreman, K. (1997). Macroalgal blooms in shallow estuaries: controls and ecophysiological and ecosystem consequences. *Limnology and Oceanography, 42*(5part2), 1105-1118.

van der Heide, T., van Nes, E. H., Geerling, G. W., Smolders, A. J., Bouma, T. J., & van Katwijk, M. M. (2007). Positive feedbacks in seagrass ecosystems: implications for success in conservation and restoration. *Ecosystems, 10*(8), 1311-1322.

van der Heide, T., van Nes, E. H., van Katwijk, M. M., Olff, H., & Smolders, A. J. (2011). Positive feedbacks in seagrass ecosystems–evidence from large-scale empirical data. *PloS one, 6*(1), e16504.

Van Katwijk, M., Bos, A., De Jonge, V., Hanssen, L., Hermus, D., & De Jong, D. (2009). Guidelines for seagrass restoration: importance of habitat selection and donor population, spreading of risks, and ecosystem engineering effects. *Marine Pollution Bulletin, 58*(2), 179-188.

Van Katwijk, M., Bos, A., Hermus, D., & Suykerbuyk, W. (2010). Sediment modification by seagrass beds: Muddification and sandification induced by plant cover and environmental conditions. *Estuarine, Coastal and Shelf Science, 89*(2), 175-181.

Van Katwijk, M., Van der Welle, M., Lucassen, E., Vonk, J., Christianen, M., Kiswara, W., al Hakim, I. I., Arifin, A., Bouma, T., & Roelofs, J. (2011). Early warning indicators for river nutrient and sediment loads in tropical seagrass beds: a benchmark from a near-pristine archipelago in Indonesia. *Marine Pollution Bulletin, 62*(7), 1512-1520.

Vermaat, J. E., Agawin, N., Fortes, M., Uri, J., Duarte, C., Marba, N., Enriquez, S., & Van Vierssen, W. (1997). The capacity of seagrasses to survive increased turbidity and siltation: the significance of growth form and light use. *Ambio, 26*(8), 499-504.

Vermaat, J. E., & Verhagen, F. C. (1996). Seasonal variation in the intertidal seagrass *Zostera noltii* Hornem.: coupling demographic and physiological patterns. *Aquatic Botany, 52*(4), 259-281.

Vieira, R., A. Martin, A. H. Engelen, M. S. Thomsen, and F. Arenas. (2020). Interactive effects of cooccurring anthropogenic stressors on the seagrass, *Zostera noltei*. *Ecological Indicators 109*:105780.

Walker, D., & McComb, A. (1992). Seagrass degradation in Australian coastal waters. *Marine Pollution Bulletin, 25*(5-8), 191-195.

Walker, D. I., Kendrick, G. A., & McComb, A. J. (2007). Decline and recovery of seagrass ecosystems—the dynamics of change. In *Seagrasses: Biology, Ecologyand Conservation* (pp. 551-565). Springer.

Waycott, M., Duarte, C. M., Carruthers, T. J., Orth, R. J., Dennison, W. C., Olyarnik, S., Calladine, A., Fourqurean, J. W., Heck, K. L., Jr., Hughes, A. R., Kendrick, G. A., Kenworthy, W. J., Short, F. T., & Williams, S. L. (2009). Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Proceedings of the National Academy of Sciences U S A, 106*(30), 12377-81.

Widdows, J., Pope, N. D., Brinsley, M. D., Asmus, H., & Asmus, R. M. (2008). Effects of seagrass beds (*Zostera noltii* and *Z. marina*) on near-bed hydrodynamics and sediment resuspension. *Marine Ecology Progress Series, 358*, 125-136.

Woods, C. M., & Schiel, D. R. (1997). Use of seagrass *Zostera novazelandica* (Setchell, 1933) as habitat and food by the crab *Macrophthalmus hirtipes* (Heller, 1862)(*Brachyura: Ocypodidae*) on rocky intertidal platforms in southern New Zealand. *Journal of Experimental Marine Biology and Ecology*, *214*(1-2), 49-65.

Zabarte-Maeztu, I., Matheson, F. E., Manley-Harris, M., Davies-Colley, R. J., Oliver, M., & Hawes, I. (2020). Effects of Fine Sediment on Seagrass Meadows: A Case Study of *Zostera muelleri* in Pāuatahanui Inlet, New Zealand. *Journal of Marine Science and Engineering, 8*(9), 645.

Zar, J. (1984). Biostatistical Analysis. 2nd edPrentice Hall. *Englewood Cliffs, NJ*.

Zimmerman, R. C. (2003). A biooptical model of irradiance distribution and photosynthesis in seagrass canopies. *Limnology and Oceanography, 48*(1part2), 568-585.

Appendices

Chapter 2 Appendix: Equilibria

*The first dissociation constant of H_2S is 8.9×10^{-8} that is the pKa is 7.05 and the second dissociation constant is 1×10^{-19} that is the pKa is 19. This means that at normal environmental pH values there is virtually **no free sulphide S^{2-} ion** present. Thus, we need to consider only the equilibrium between H_2S and HS^-:

$$K_a = 8.9 \times 10^{-8} = \frac{[H_3O^+][HS^-]}{[H_2S]}$$

Rearranging this gives:

$$\frac{[H_2S]}{[HS^-]} = \frac{[H_3O^+]}{8.9 \times 10^{-8}}$$

So at pH =7.05, that is $[H^+]= 8.9 \text{ x} 10^{-8}$M the ratio of H_2S to HS^- is 1 that is 50:50 of the two forms; as pH increases above 7.05 the proportion of HS- increases and so at pH 8.5 the ratio is 0.03 and it is predominantly in the form HS^-. Similarly, at pH 6.0 the ratio is 11 and it is predominantly in the form H_2S. Thus, the toxicity of the sulphide present depends upon pH, since only the molecular form is gaseous and toxic.

The APHA methylene blue method for quantitative analysis of sulphides depends upon the ability of hydrogen sulphide and acid-soluble metallic sulphides to convert *N*, dimethyl-*p*-phenylenediamine to methylene blue in the presence of a mild oxidizing agent (acidified ferric chloride). The colour intensity of the methylene blue is directly proportional to the amount of hydrogen sulphide and acid-soluble metallic sulphides in the pore water. Iron II sulphide is acid-insoluble so not measured by this assay. Thus, when the term sulphide is used in this work indicates the total of H_2S, HS^- plus any acid-soluble sulphides.

The dissociation constant of NH_3 is 1.8× 10^{-5} that is the pKb is 4.75. We need to consider the equilibrium between NH_3 and NH_4^+.

$$K_b = 1.8 \times 10^{-5} = \frac{[NH_4{}^+][OH^-]}{[NH_3]}$$

Rearranging this gives:

$$\frac{[NH_4{}^+]}{[NH_3]} = \frac{1.8 \times 10^{-5}}{[OH^-]}$$

So at pOH =4.75 that is pH=9.25, $[OH^-]$= 1.8 x10^{-5} M the ratio of $NH_4^+$to NH_3 is 1 that is 50:50 of the two forms; as pH decreases below 9.25 the proportion of NH_3 decreases and so at seawater pH 8.2 it is predominantly in the form NH_4^+. At pH ≤ 6.5 it is only in the form NH_4^+. At pH ≥ 11 the molecular form NH_3 predominates. Thus, the ratio of NH_4^+ NH_3 present depends upon pH too.

In this case, the term **Ammonium ion NH_4^+** will be used hereunder as it is the predominant fraction at seawater pH.

Chapter 3 Appendix

Table A.3.1. 2-way ANOVA analysis for substrate properties. Significant effects and interactions ($p<0.05$) between Habitat and time are shown in bold for each of the substrate properties.

Trait	Factor	Df	Mean sq	F value	Pr (>F)
% Mud	**Habitat**	2	9387	114.461	2e-16
	Time	1	662	8.077	0.00513
	Residual	146	82		
% Silt	**Habitat**	2	6532	134.336	2e-16
	Time	1	237	4.869	0.0289
	Residual	146	49		
% Clay	**Habitat**	2	281.15	38.06	4.97e-14
	Time	1	107.12	14.5	0.000206
	Residual	146	7.39		
% Sand	**Habitat**	2	9017	108.864	2e-16
	Time	1	388	4.682	0.0321
	Residual	146	83		
% Fine sand	**Habitat**	2	11814	107.5	**2e-16**
	Time	1	27081	246.4	2e-16
	Residual	146	110		
% Coarse sand	**Habitat**	2	221	5.919	0.00337
	Time	1	5673	152.230	2e-16
	Residual	146	37		
Bulk density	**Habitat**	2	1.6516	23.11	1.91e-09
	Time	1	2.3407	32.75	5.74e-08
	Residual	146	0.0715		
Organic matter	Habitat	2	78895967	0.998	0.371
	Time	1	78876259	0.998	0.319
	Residual	146	7902888		
$PO_4]^{3-}$ µM (0-5 cm)	**Habitat**	2	32.27	3.845	0.025149
	Time	1	136.57	16.274	0.000118
	Residual	86	8.39		
$[PO_4]^{3-}$ µM (5-10 cm)	**Habitat**	2	0.15060	4.093	0.0201
	Time	1	0.00177	0.048	0.8269
	Residual	86	0.03680		
$[NH_4]^+$ µM (0-5 cm)	**Habitat**	2	7491	13.57	7.55e-6
	Time	1	7228	13.09	0.000499
	Residual	86	552		
$[NH_4]^+$ µM (5-10 cm)	**Habitat**	2	14306	13.346	8.95e-6
	Time	1	3871	3.611	0.067
	Residual	86	1072		
$[H_2S]$ µM (0-5 cm)	Habitat	2	2.17	1.92	0.153
	Time	1	24.56	21.73	1.18e-05
	Residual	84	1.13		
$[H_2S]$ µM (5-10 cm)	**Habitat**	2	19.103	6.857	0.00174
	Time	1	0.174	0.062	0.80332
	Residual	84	2.786		
PAR	**Habitat**	2	1544.8	23.727	3.05E-09
	Time	1	188.9	2.901	0.0914
	Residual	106	65.1		

Table A.3.2. ANOVA analysis for seagrass traits properties. Significant effects (p<0.05) are shown in bold for each of the seagrass traits.

Trait	Factor	Df	Mean sq	F value	Pr (>F)
% Cover	**Time**	1	5832	6.025	0.0178
	Residual	48	968		
Shoot density	**Time**	1	634855	10.18	0.0025
	Residual	48	62353		
AGB	Time	1	8.5	0.193	0.662
	Residual	48	43.96		
BGB	**Time**	1	49507	4.724	0.0347
	Residual	48	10480		
AGB/BGB	Time	1	47.7	0.15	0.7
	Residual	48	317.2		
Total biomass	**Time**	1	50813	4.544	0.0382
	Residual	48	11183		

Table A.3.3. ANOVA analysis for modelled data. Significant effects (p<0.05) are shown in bold for each of the environmental variables.

Trait	Factor	Df	Mean sq	F value	Pr (>F)
Current velocity	**Habitat**	2	0.013786	69.95	2.43e-07
	Residual	12	0.000197		
Wave period	**Habitat**	2	2383.3	13.86	0.000759
	Residual	12	171.9		
Salinity	**Habitat**	2	85.25	81.17	1.06e-7
	Residual	12	1.05		
SSC	**Habitat**	2	119.59	216.6	3.84e-10
	Residual	12	0.55		
Deposition	**Habitat**	2	34.18	3.771	0.0536
	Residual	12	9.06		

Journal of
*Marine Science
and Engineering*

Article

Effects of Fine Sediment on Seagrass Meadows: A Case Study of *Zostera muelleri* in Pāuatahanui Inlet, New Zealand

Iñigo Zabarte-Maeztu [1,2,*], Fleur E. Matheson [1], Merilyn Manley-Harris [2], Robert J. Davies-Colley [1], Megan Oliver [3] and Ian Hawes [4]

[1] National Institute of Water & Atmospheric Research, Hamilton 3216, New Zealand; Fleur.Matheson@niwa.co.nz (F.E.M.); Rob.Davies-Colley@niwa.co.nz (R.J.D.-C.)
[2] School of Science, University of Waikato, Hamilton 3260, New Zealand; merilyn.manley-harris@waikato.ac.nz
[3] Greater Wellington Regional Council, PO Box 11646, Wellington 6142, New Zealand; Megan.Oliver@gw.govt.nz
[4] Coastal Marine Field Station, University of Waikato, Tauranga 3110, New Zealand; ian.hawes@waikato.ac.nz
* Correspondence: izm1@students.waikato.ac.nz or Inigo.Zabarte-Maeztu@niwa.co.nz; Tel.: +64-21-122-3805

Received: 17 June 2020; Accepted: 19 August 2020; Published: 21 August 2020

Abstract: Seagrass meadows are vulnerable to fine sediment (mud) pollution, with impacts usually attributed to reduction in submerged light. Here we tested two non-exclusive hypotheses, that mud particles (<63 µm) impact seagrasses through both (1) the light climate and (2) changes in substrate physico-chemistry. We tested these hypotheses in Pāuatahanui Inlet, New Zealand, by comparing seagrass presence, abundance, and health, together with light climate and substrate physico-chemistry at contrasting habitats where (1) seagrass used to thrive but no longer grows (historical seagrass), (2) seagrass still persists (existing seagrass) and (3) seagrass has been present recently, but not currently (potential seagrass). Historical seagrass substrate had significantly higher mud (35% average), bulk density (1.5 g cm^{-3}), porewater ammonium concentration (65 µM), and a more reduced redox profile (negative redox at only 2 cm soil depth) as well as a lower light availability when submerged compared to other habitats, while total daily light exposure differed little between habitats. This suggests that failure of seagrass to recolonize historical seagrass habitat reflects substrate muddiness and consequent unfavorable rhizosphere conditions. Our results provide evidence for the multi-stressor effects of fine sediment on seagrasses, with substrate suitability for seagrass being detrimentally affected even where light exposure seems sufficient.

Keywords: sedimentation; pollution; fine sediment; mud; photosynthetically available radiation (PAR); rhizosphere; redox potential; sulfide

1. Introduction

Seagrass meadows are one of the most important and threatened ecosystems on the planet [1]. They have immense ecological and socio-economic value [1–4], yet as a result of human activities, these ecosystems are increasingly threatened [5].

A major contributor to global anthropogenic stress on seagrass is the reduction of available light, particularly photosynthetically active radiation (PAR), the primary driver of seagrass growth [6–12]. The usual cause of underwater PAR reduction is increased suspended sediment loads to coastal waters. Natural events, such as runoff and windstorms, can provoke sedimentary movement to and within coastal systems leading to acute, large-scale alteration of seagrass habitat [13–15]. However, acute and chronic human-induced impacts, resulting from land clearance and other activities that

J. Mar. Sci. Eng. **2020**, *8*, 645; doi:10.3390/jmse8090645 www.mdpi.com/journal/jmse

increase fine sediment concentrations, can result in complete and perhaps irreversible, extinction of seagrass meadows [16–19].

While impacts of suspended fine sediments on seagrasses via reduced underwater PAR exposure are widely reported [20,21], sediments may also affect seagrasses after they settle. Fine sediment, initially settled as nepheloid layers, continue to shade seagrasses [22,23] and may also restrict solute flux by reducing substrate porosity. This may exacerbate hypoxia [24] and drive changes to substrate biogeochemistry. Seagrass substrate typically becomes anaerobic a few millimeters or centimeters below the bed surface as a result of slow oxygen diffusion rates and a high microbial oxygen demand associated with mineralization of organic matter (OM) within the rhizosphere [25]. Anoxia may lead to high porewater concentrations of phytotoxic compounds such as sulfide and metals [26–28].

In New Zealand, fine sediments are considered to be the most pervasive contaminant affecting estuaries and sheltered coastal embayments [29–32]. Fine sediment is thought to have contributed substantially to documented losses of seagrass meadows in a number of New Zealand estuaries [33,34]. In Pāuatahanui Inlet, ca. 39 ha of seagrass in the inner estuary in 1980 [35] and earlier, has been subsequently lost. This loss has been tentatively, but plausibly, attributed to anthropogenic catchment development and increased sedimentation and eutrophication of the estuary [36]. Nevertheless, in the better-flushed, outer part of this estuary, seagrass beds still persist and are a prominent feature of the intertidal zone. For this study, we investigated potential causes of seagrass decline and failure to recover by comparing light climate and substrate physical and chemical characteristics at habitats with and without seagrass. We hypothesized that high fine sediment affects seagrass through multiple stresses caused by deposited sediment in addition to PAR attenuation by suspended and re-suspended sediment. To test our hypothesis, we quantified and compared conditions (PAR, substrate grain size, and biogeochemistry) at habitats with and without seagrass in Pāuatahanui Inlet. Specifically we investigated (1) sites in the inner estuary where seagrass used to thrive but no longer grows (historical seagrass), (2) sites where seagrass still persists in the outer estuary (existing seagrass), and (3) sites in the outer estuary, adjacent to existing seagrass beds, where seagrass has been present intermittently for the past decade, but where seagrass is not currently growing (potential seagrass). Comparisons among these sites potentially would allow disentanglement of the effect of anthropogenic sediments on biogeochemistry in the absence of seagrass, the impacts of seagrass on biogeochemistry in the absence of anthropogenic sedimentation, and the effects of habitat on light regime.

2. Materials and Methods

2.1. Study Location

Pāuatahanui Inlet is a natural inlet and wildlife reserve on New Zealand's North Island south-western coast (Figure 1). It is the eastern arm of Porirua Harbour which has a total catchment area of 199 km^2, comprising a land area of 185 km^2 and a harbour area of 14 km^2. The maximum elevation of the catchment is 530 m at the head of the Horokiri sub-catchment and mean altitude is 150 m. Average annual rainfall is 1200 mm and the mean air temperature is 12.9 °C, with prevailing winds from the North and North-West [37].

The Pāuatahanui catchment is 109 km^2 [38] and has six sub-catchments. The Pāuatahanui, Horokiri, and Kakaho streams are the major sources of sediment (Figure 1). Within these, predominantly pastoral sub-catchments, soil erosion and runoff has resulted in high downstream sedimentation rates and land use has driven moderate eutrophication [39]. These inputs have almost certainly contributed to estuary infilling and have likely contributed to the loss of seagrass meadows from inner parts of the Inlet (historical seagrass, HS), which has occurred since 1980 [36]. From 1974 to 2009 the mean sedimentation rate for Pāuatahanui Inlet was 9.1 mm yr^{-1} [40] which is high compared to rates of <1 mm yr^{-1} expected in natural, undeveloped catchments in this region and elsewhere [41,42]. In the better-flushed, outer part of this estuary, seagrass beds still persist and are a prominent feature of the intertidal zone (existing seagrass, ES). Seagrasses are very dynamic plants [43,44] and have been present in the southern (potential seagrass, PS) areas in the recent past, but were not observed there during this study (Supplementary materials, Figure S1). From 2012, Greater Wellington Regional Council (GWRC) implemented a local catchment management plan to reduce sediment loads with a target average sedimentation rate of 1 mm yr^{-1} for Porirua Harbour. The latest data suggests that this rate was close to being achieved in 2016 [39]. However, while this strategy may limit further deterioration in estuary conditions, it does not address the legacy effects of fine sediment previously washed into the Inlet which may be re-suspended.

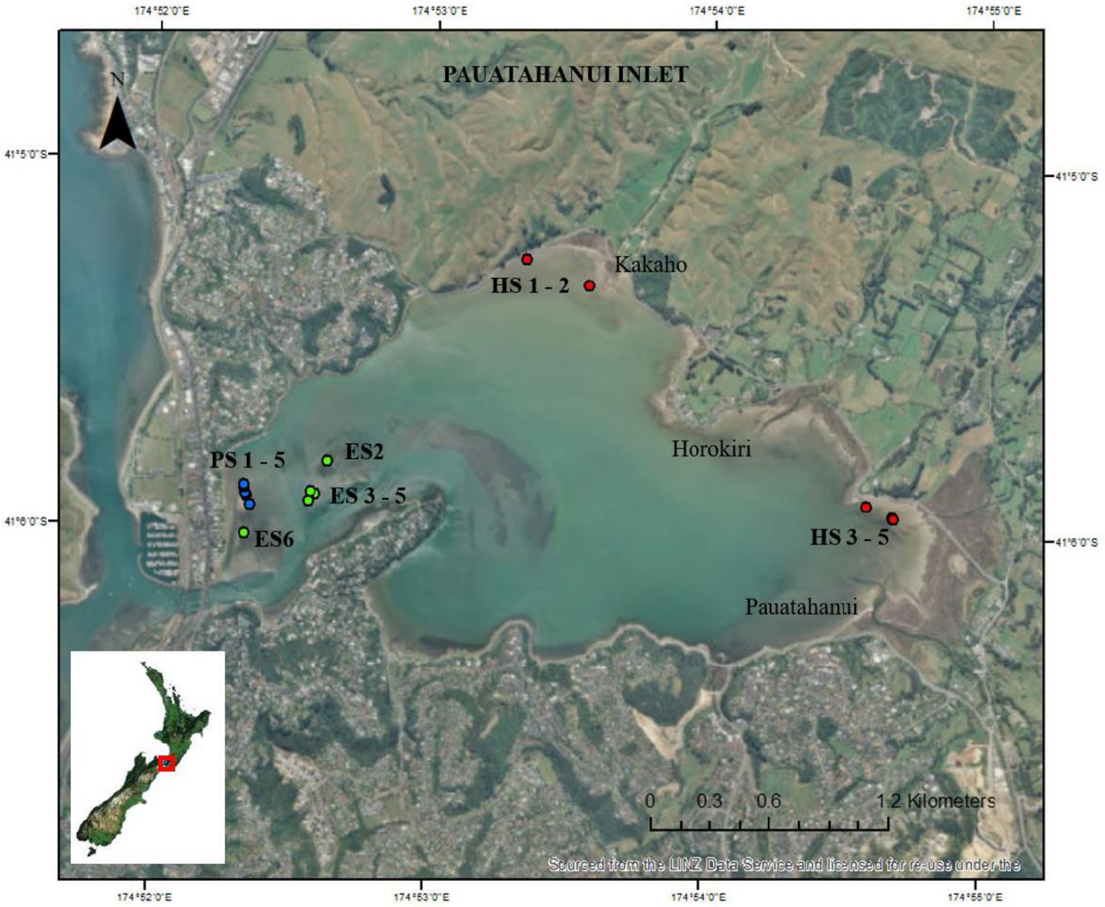


Figure 1. Locations of the 15 sampling sites are shown. Sites in the inner Pāuatahanui Inlet are where seagrass occurred historically but no longer grows (red, HS 1–5), sites in the outer estuary are where seagrass continues to persist (green, ES 2–6) and sites in the outer estuary where seagrass declined recently but the environment is sufficiently similar to ES to be considered potential seagrass habitat (blue, PS 1–5). Kakaho, Horokiri, and Pāuatahanui streams are major sources of water and sediment to the Inlet. Bathymetry and site coordinates are tabulated in Supplementary materials, Table S1.

2.2. *Field Sampling*

Five sites in each of historical, existing, and potential seagrass habitats (HS, ES, PS, respectively) were sampled in southern hemisphere winter (23–31 August 2018) and again in summer (8–15 February 2019). At each site substrate condition, light climate and seagrass traits were measured. A 10 m transect was laid out parallel to shore along an elevation contour in the intertidal zone (0.2–0.4 m below mean sea level), along which five equidistant 0.5 m × 0.5 m (0.25 m^2) sampling plots were located. At each plot, a photograph was taken for subsequent determination of seagrass percent cover and two 12 cm diameter cores, each 10 cm deep, were randomly collected within the plot for laboratory analysis of substrate properties and seagrass traits. In the first, third, and fifth plot of each transect, porewater was extracted at two soil depth ranges (0–5 and 5–10 cm) using a hollow, 10 cm stainless steel cannula drilled with 1 mm diameter pores at intervals of 3 mm [45]. Extracted porewater was passed promptly through a 0.45 µm pore filter on site and then stored in a vial out of sunlight at 0 °C (chilled by slush ice), before freezing on return to shore base within 3 h. One 50 mL vial and one 15 mL vial of porewater was extracted per plot; the first for nutrient analysis and the second for hydrogen sulfide analysis. Two drops of zinc acetate were added to 15 mL vials prior to sampling to capture free sulfides as ZnS precipitate (APHA 4500-S2-D). Adjacent to each transect at least two redox potential (Eh) profiles were measured with readings taken at 0, 2, 4, 6, and 8 cm depths. A platinum redox electrode was used to measure potential versus an Ag/AgCl reference electrode filled with 3M KCl solution. Field observations showed that most seagrass roots and rhizomes were confined to the upper 4 cm of the substrate, and we define this as the root zone (RZ). Average RZ Eh was calculated as the mean of 0, 2, and 4 cm values.

Sensors were deployed for long term (months) monitoring of PAR, temperature, and water level. An ECOPAR™ sensor by Sea-Bird Scientific, Bellevue, WA, USA, (http://www.seabird.com/ecopar) was deployed at one site in each of HS, PS, and ES habitats to directly measure PAR (mol m^{-2} d^{-1}). To address variability within habitats, HOBO loggers by Onset Computer Corporation, Bourne, MA, USA (http://www.onsetcomp.com/), measuring luminous flux (lux) and temperature, were deployed adjacent to the first plot at each site. Luminous flux was converted to PAR following [46] as validated by running each Hobo logger alongside a calibrated ECO-PAR sensor [47]. A U20L water level data logger (http://www.onsetcomp.com/) was deployed at one site in each habitat to record water level.

Loggers were deployed from 23 August 2018 to 3 October 2018 (winter) and from 8 February 2019 to 21 March 2019 (summer), in both cases covering a complete lunar cycle with the aim of characterizing every possible tidal exposure. Water level data were used to calculate the periods of immersion and emersion and the received PAR was calculated separately for these two periods using the different in-water and out of water calibration factors supplied by the manufacturer for each ECOPAR instrument. We also calculated mean daily PAR. Unfortunately, HOBO loggers were lost from sites HS2, PS4, and PS5 in winter and PS3, PS5, and HS1 in summer, potentially due to exposure to public during spring tides.

2.3. Laboratory Analysis

In the laboratory we homogenized each substrate sample over the 0–10 cm depth range and subsamples were used for analysis. Substrate organic matter content (% OM) was estimated as weight loss-on-ignition through combustion (450 °C for 4 h) [48] and bulk density (g m^{-3}) was determined as weight of dry solids in a known pre-determined substrate volume. Grain size was measured using a laser diffraction particle size analyzer (Malvern Mastersizer 2000) over the particle size range 0.05–2000 µm [49]. We used grain size categories as defined by [50], and defined mud (silt + clay) as <63 µm particles. Porewater samples were analyzed for dissolved inorganic nutrients: nitrate NO_3^-, nitrite NO_2^-, ammonium NH_4^+, and phosphate PO_4^{3-} ion concentrations using standard colorimetric techniques on a Lachat Quick Chem 8000 series flow injection analyzer (FIA) + (Zellweger Analytics Inc., Milwaukee, WI 53218, USA).

Seagrass cover was estimated to the nearest 5% by visual inspection of photographs following the approach recommended by [5]. Shoot density was determined by counting the number of seagrass shoots in each core. Plant material was extracted from each core, rinsed with water, and separated into above-ground biomass (AGB—shoots and leaves) and below-ground biomass (BGB—roots and rhizomes), respectively. Plant samples were dried at 80 °C to constant weight to determine biomass per unit area (g m^{-2}) [5].

2.4. Hydrodynamic Model Results and Background Information for Study Sites

We used an existing hydrodynamic model for Porirua Harbour [51] to characterize hydrodynamic condition. The model predicts current velocity, wave period, wave height, salinity, and suspended sediment concentration (SSC) across both arms of the harbour, at 30 min intervals and was validated with field monitoring [51]. The model uses wind, precipitation, and insolation as meteorological drivers, and we summarized predictions for 2010, which is considered a typical year, with existing bathymetry. Predictions were averaged to provide annual mean (± SE) values for each modelled characteristic.

Current velocity, wave period, and salinity were predicted to be similar at ES and PS, and higher in comparison with HS. In contrast, SSC and sediment deposition rates were predicted to be significantly lower at ES and PS compared to HS (Table 1).

Table 1. Predicted hydrodynamics parameters and salinity for historical seagrass (HS), existing seagrass (ES), and potential seagrass (PS) sites using the model of [51]. Values are means (±SE). Significant differences ($p < 0.05$, Tukey HSD (honestly significant difference)) are indicated by different alphabetic superscripts.

Habitat	Current Velocity ($m\ s^{-1}$)	Wave Period (s)	Salinity (PSU)	SSC ($mg\ L^{-1}$)	Deposition ($mm\ yr^{-1}$)
HS	0.05 ± 0.003 ^{a}	0.59 ± 0.010 ^{a}	18.67 ± 0.76 ^{a}	10.72 ± 0.570 ^{b}	6.4 ± 2.10 ^{c}
ES	0.15 ± 0.010 ^{c}	0.66 ± 0.010 ^{c}	25.63 ± 0.24 ^{b}	2.35 ± 0.095 ^{a}	3.6 ± 0.90 ^{b}
PS	0.08 ± 0.005 ^{b}	0.61 ± 0.001 ^{b}	25.99 ± 0.01 ^{b}	2.17 ± 0.020 ^{a}	1.22 ± 0.02 ^{a}

2.5. Data Analysis

Statistical analysis of data was performed using the R statistical package (RStudio Team 2015) Boston, MA, USA. Normality of data was tested, and parameters were log-transformed to reduce skewness if appropriate. Mean values are presented with standard errors of the mean (mean ± SE). Two way-analysis of variance (ANOVA) (with post-hoc Tukey HSD tests) [52] was used to detect significant differences in seagrass traits, PAR, and substrate properties between habitats and seasons. Pearson's product-moment correlation coefficients were calculated to indicate associations between habitat and seagrass variables, and, where nonlinear relationships were apparent, we applied logistic or logarithmic (power-law) models, and report goodness of fit (R^2).

3. Results

3.1. Substrate Conditions

Substrate grain size composition showed no summer–winter differences, but significant differences between HS and both ES and PS habitats. These last two did not differ significantly from each other (Table 2). HS sites had high average substrate mud contents (33.8–38.8%), compared to ES and PS (average values ranging from 11.1–15.3% and 10.3–13.6%, respectively) (Table 2). Sand was the dominant size fraction at ES and PS, whereas HS has significantly lower fine sand content and more coarse sand, particularly in winter (Figure 2).

A principal component analysis (PCA) of grain size category distribution across sites separates HS from ES and PS primarily along Axis-1 which correlates with mud vs. fine sand. Axis-2 correlates with coarse sand, which was, on average, higher and more variable at HS than other habitats. Amongst HS sites, HS2 was notably higher for coarse sand, reflecting proximity to an inflowing stream. Overall the PCA showed greater heterogeneity of grain size among HS than either PS or ES, (which were not significantly different). HS had a strong tendency for a high proportion of very fine particles, and relatively low fractions of intermediate-sized particles (sand and fine sand) (Figure 2).

Table 2. Substrate properties at historical seagrass (HS), existing seagrass (ES), and potential seagrass (PS) sites. Values are means (±SE). Significant differences ($p < 0.05$, Tukey HSD test) among habitats and seasons for each substrate property are indicated by different alphabetic superscripts.

Habitat	Season	Clay (%)	Silt (%)	Mud (%)	Fine Sand (%)	Coarse Sand (%)	Sand (%)	Bulk Density ($g\ cm^{-3}$)	% OM [1]	Eh [2] (mV) Mean (0–10 cm) RZ [3] (0–4 cm)
HS	Winter	6.3 ± 0.8^{e}	27.5 ± 2.5^{c}	33.7 ± 5.8^{b}	48.1 ± 4.1^{a}	17.8 ± 2.7^{d}	65.9 ± 3.2^{a}	1.32 ± 0.05^{b}	1.79 ± 0.13^{d}	-79.8 ± 13.1^{a} Eh (RZ) $-35.7^{a'}$
	Summer	8.0 ± 0.9^{e}	30.8 ± 1.9^{c}	38.8 ± 1.9^{b}	49.9 ± 3.2^{a}	11.3 ± 0.9^{b}	61.2 ± 2.6^{a}	1.63 ± 0.07^{c}	1.60 ± 0.09^{c}	-71.6 ± 17.3^{a} Eh (RZ) $8.9^{b'}$
ES	Winter	2.0 ± 0.2^{a}	9.1 ± 0.7^{a}	11.1 ± 2.1^{a}	78.5 ± 1.8^{b}	10.1 ± 0.9^{b}	88.7 ± 0.9^{c}	1.01 ± 0.03^{a}	1.53 ± 0.14^{c}	-33.8 ± 11.4^{b} Eh (RZ) $22.6^{c'}$
	Summer	3.3 ± 0.1^{c}	12.1 ± 0.7^{b}	15.3 ± 1.7^{a}	73.7 ± 1.4^{b}	10.5 ± 0.4^{b}	84.7 ± 0.8^{b}	1.28 ± 0.05^{b}	1.71 ± 0.09^{d}	-22.3 ± 21.5^{b} Eh (RZ) $13.6^{c'}$
PS	Winter	2.6 ± 0.2^{b}	7.7 ± 0.7^{a}	10.3 ± 0.8^{a}	74.8 ± 1.2^{b}	12.6 ± 0.4^{c}	87.4 ± 1^{c}	1.09 ± 0.05^{a}	1.39 ± 0.04^{b}	-14.4 ± 14.8^{c} Eh (RZ) $40.6^{d'}$
	Summer	4.7 ± 0.4^{d}	8.9 ± 0.8^{a}	13.6 ± 1.0^{a}	76.9 ± 0.8^{b}	9.5 ± 0.3^{a}	86.4 ± 0.7^{c}	1.16 ± 0.05^{a}	1.14 ± 0.02^{a}	-41.6 ± 22.2^{b} Eh (RZ) $5.6^{b'}$

[1] Organic matter (OM); [2] redox potential (Eh); [3] root zone (RZ).

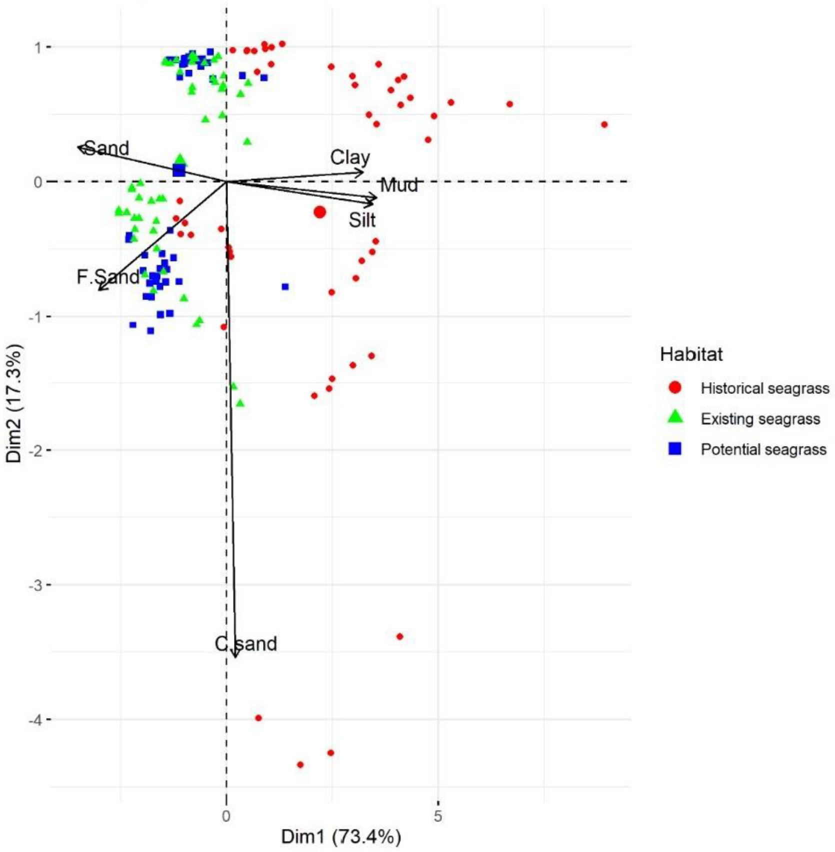


Figure 2. Principal component analysis (PCA) of the grain size categories for historical seagrass (HS), existing seagrass (ES), and potential seagrass (PS) habitats. Fine sand (F. sand) and coarse sand (C. sand). Dimension 1 (Dim1) and dimension 2 (Dim2) together explain 90.7% of the variation on measured grain sizes. Dim1 explains 73.4% of the variability and Dim2 17.3%. Larger symbols show averages for HS, ES, and PS.

3.2. Substrate Bulk Density, Organic Matter and Redox Potential

Substrate bulk density was higher at HS than PS and ES (the last two were similar) and substrates at all sites were somewhat denser in summer than in winter (Table 2). The latter could indicate compaction of sediments under drier summer conditions. Substrate organic content was significantly higher at HS and ES than PS. HS had significantly higher organic content compared to ES during winter and significantly lower during summer (Table 2). These patterns suggest organic content at ES is related to seagrass productivity during summer, whereas organic content at HS is linked to catchment runoff and fine sediment (and organic) inputs during generally wetter winters [37].

Redox (Eh) profiles all had similar gradients but were more negative at HS than at PS and ES during winter (Figure 3). Profiles were very similar for all habitats in summer (Figure 3). Eh values became negative between 0 and 2 cm depth for HS in winter, while in summer, and in both seasons for PS and ES, negative values commenced at greater depth, between 2 and 4 cm (Figure 3). ES and PS habitats root zone's Eh values showed a range from −45 to −20 mV whereas all HS root zone readings were below −45 mV.

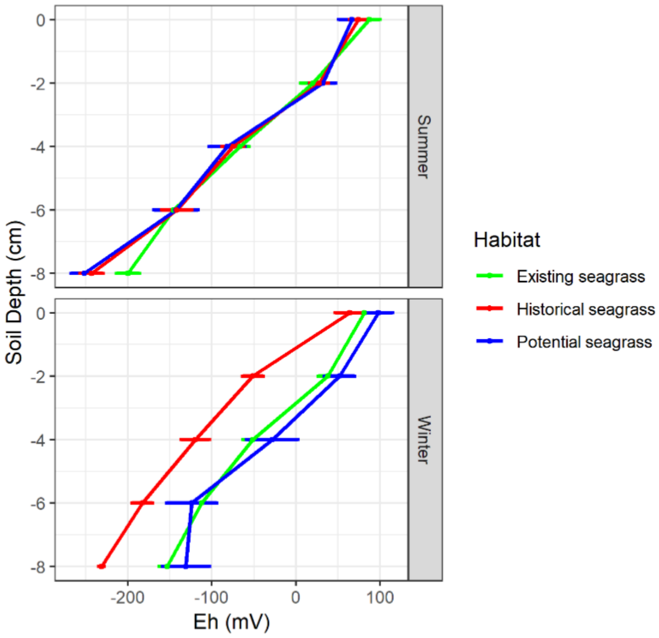


Figure 3. Redox potential (Eh) profiles (means ±SE) for historical seagrass (HS), existing seagrass (ES), and potential seagrass (PS) habitats in summer and in winter.

3.3. Porewater Chemistry

Porewater analyses for NO_3^- and NO_2^- were below detection limits (<0.01 µM) at all sites, in both seasons and at both depth ranges. Overall, PO_4^{3-} was lower in PS than either HS or ES, which were similar. In winter PO_4^{3-} was lower near the surface in all habitats. Deeper (5–10 cm) PO_4^{3-} concentrations varied little from winter to summer, and were similar to near-surface (0–5 cm) values in summer. A similar seasonal depth pattern emerged for both NH_4^+ and sulfide, with concentrations at depth similar in both winter and summer, but with near-surface (0–5 cm) values significantly lower in winter. NH_4^+ was similar at ES and PS but was considerably higher for all date/depth combinations in the HS habitat. Hydrogen sulfide tended to have lower concentrations at PS than either HS or ES habitats, which were similar (Table 3).

Table 3. Substrate porewater nutrients and sulfide concentrations at historical seagrass (HS), existing seagrass (ES), and potential seagrass (PS) for two sampling depths. Values are means (±SE). Significant differences ($p < 0.05$, Tukey HSD test) among habitats and seasons for a particular substrate property are indicated by different alphabetic superscripts.

Habitat	Season	Sampling Depth	PO_4^{3-} (µM)	NH_4^+ (µM)	H_2S (µM)
HS	Winter	(0–5 cm)	0.04 ± 0.004 ^{a}	12.47 ± 3.49 ^{b}	1.11± 0.29 ^{b}
		(5–10 cm)	0.27 ± 0.03 ^{d}	61.43 ± 5.93 ^{c}	2.69 ± 0.53 ^{d}
	Summer	(0–5 cm)	0.40 ± 0.09 ^{e}	51.72 ± 21.23 ^{c}	2.89 ± 0.18 ^{d}
		(5–10 cm)	0.24 ± 0.07 ^{d}	71.52 ± 15.14 ^{c}	3.22 ± 0.12 ^{e}
ES	Winter	(0–5 cm)	0.05 ± 0.01 ^{b}	5.71 ± 1.89 ^{a}	0.83 ± 0.22 ^{a}
		(5–10 cm)	0.29 ± 0.06 ^{d}	22.12 ± 4.93 ^{b}	3.23 ± 0.71 ^{e}
	Summer	(0–5 cm)	0.29 ± 0.04 ^{d}	12.70 ± 3.05 ^{b}	2.10 ± 0.19 ^{c}
		(5–10 cm)	0.25 ± 0.04 ^{d}	14.97 ± 5.21 ^{b}	2.61 ± 0.23 ^{d}
PS	Winter	(0–5 cm)	0.07 ± 0.01 ^{b}	8.76 ± 1.33 ^{a}	1.55 ± 0.41 ^{b}
		(5–10 cm)	0.12 ± 0.01 ^{c}	21.84 ± 1.44 ^{b}	1.62 ± 0.47 ^{b}
	Summer	(0–5 cm)	0.17 ± 0.04 ^{c}	14.41 ± 2.77 ^{b}	1.58 ± 0.21 ^{b}
		(5–10 cm)	0.16 ± 0.01 ^{c}	14.53 ± 3.16 ^{b}	1.46 ± 0.14 ^{b}

3.4. Photosynthetically Available Radiation (PAR)

The PAR data shows that all habitats receive >90% of their daily PAR dose while emerged (Table 4) reflecting high irradiance attenuation in the (often muddy) water during submersion under higher than mid-tide water levels. During winter, total PAR (submerged + emerged) increased from ES, through PS to HS, whereas in summer this order was reversed (Table 4). The last is surprising, and the summer PAR values might be biased (low) because of shading of sensors due to fouling by drifting macroalgae (*Ulva* sp.) that were frequently observed during field work in this season. Data as a timeseries plot and the number of days below a daily average compensation irradiance level (CI) are shown in Supplementary materials, Table S2.

HS also experienced a higher number of days in winter and in summer when *total* PAR availability was on average below a CI of 3.9 mol m^{-2} d^{-1} for *Zostera muelleri* based on the reported value of 45 µmol m^{-2} s^{-1} [53] (Table 4).

Table 4. Photosynthetically available radiation (PAR) at historical seagrass (HS), existing seagrass (ES), and potential seagrass (PS) during winter (from 23 August 2018 to 3 October 2018) and summer (from 8 February 2019 to 21 March 2019) deployments. Values are daily mean PAR (±SE). PAR doses when submerged and emerged are shown as well as the number of days each habitat was below a compensation irradiance for *Zostera muelleri* (CI, 3.9 mol m^{-2} d^{-1}) during deployments. The value given is the *total* number of days and the figure in parentheses is the longest number of *consecutive* days below CI. Significantly different mean values (ANOVA, Tukey HSD, $p < 0.05$) among habitats and seasons are indicated by different alphabetic superscripts and (*), respectively.

Habitat	Season	PAR When Submerged (mol m^{-2} d^{-1})	PAR When Emerged (mol m^{-2} d^{-1})	Number of Days Where Total Mean PAR Was Below Compensation Irradiance (Days)
HS	Winter	2.2 ± 0.2 ^{a}	30.9 ± 2 ^{a}	6 (2) ^{b}
	Summer	5.2 ± 0.2 ^{d*}	60 ± 2.5 ^{d*}	3 (1) ^{b*}
ES	Winter	4.4 ± 0.3 ^{c}	44.3 ± 2 ^{b}	3 (1) ^{a}
	Summer	4.0 ± 0.2 ^{c}	46.1 ± 2 ^{b}	1 (0) ^{a*}
PS	Winter	3.5 ± 0.2 ^{b}	33.0 ± 2 ^{a}	5 (0) ^{a}
	Summer	4.6 ± 0.2 ^{c*}	53.1 ± 2.4 ^{c*}	0 (0) ^{a*}

3.5. Relationship of Habitat to Substrate Characteristics and PAR

A PCA ordination of all independent variables effectively segregates the three habitats along PCA axis 1, which is linked to % mud, total suspended solids (TSS), *total* PAR, and Eh (Figure 4). HS sites had high muddiness and suspended sediment concentrations (and low Eh and PAR) whereas sites in PS and ES habitat had lower muddiness and higher PAR and redox potential. PCA Axis 2 separates mostly within habitat, and is driven by variation in porewater chemistry. The degree of scatter along this axis indicates more variability in porewater chemistry within ES and HS than PS.

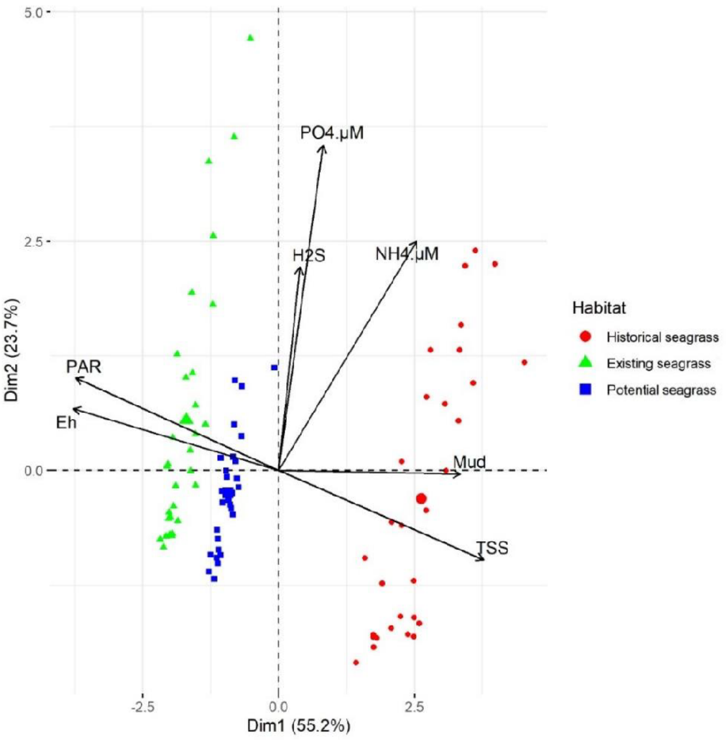


Figure 4. Principal component analysis (PCA) of porewater nutrients, sulfide, total photosynthetically available radiation (PAR), Redox (Eh), and % Mud from historical seagrass (HS), existing seagrass (ES), and potential seagrass (PS). Dimension 1 (Dim1) and dimension 2 (Dim2) of the PCA explain 55.2% and 23.7% of the variation, respectively. Larger symbols show averages for HS, ES, and PS.

3.6. Seagrass Traits

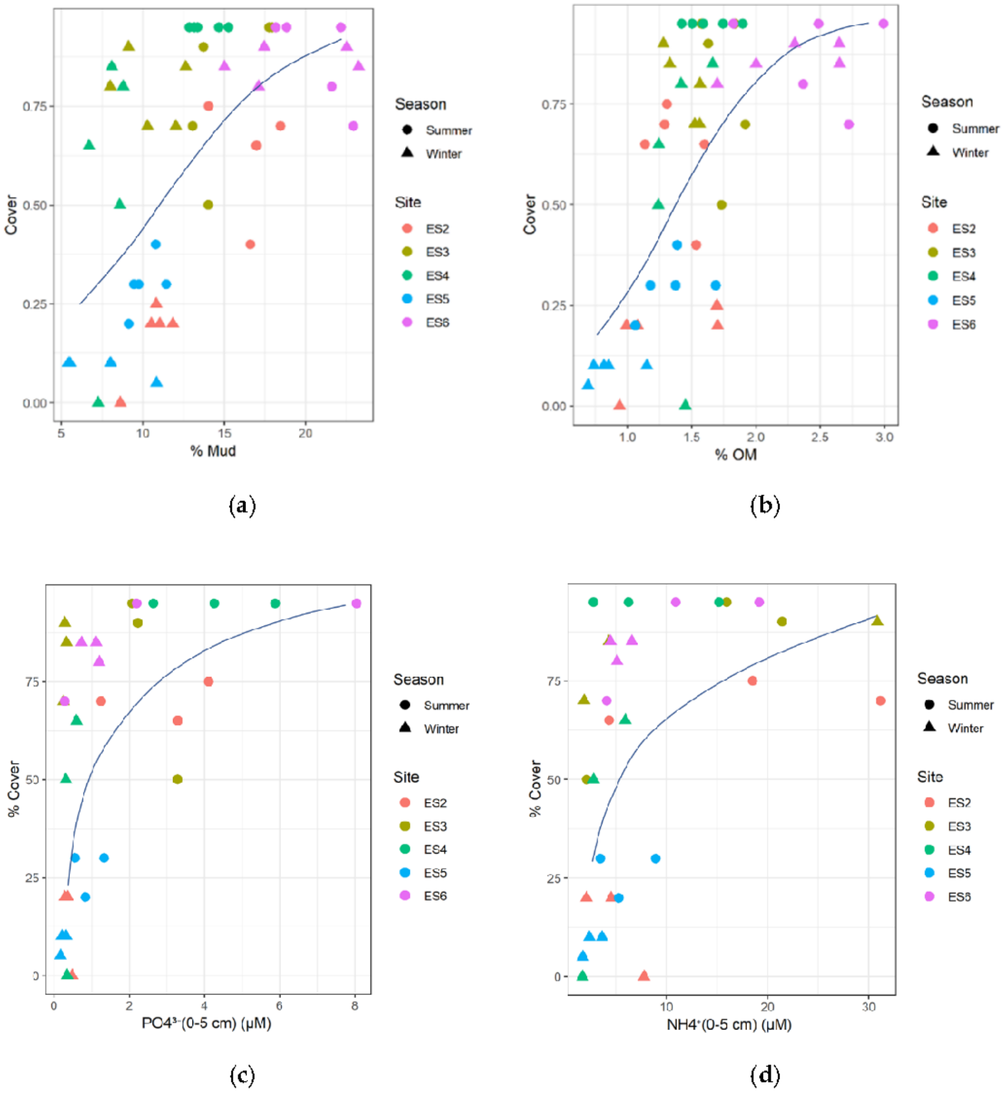
At ES sites seagrass cover ranged from 10% to 95%, shoot density from 172 to 700 shoots m^{-2}, and total biomass from 33 to 243 g m^{-2} (Table 5). Below-ground biomass was always higher than above-ground biomass with the average summer and winter BGB:AGB ratio varying slightly (15.9 versus 17.9, differences not significant). BGB and total biomass were significantly higher in summer (average 150.7 g m^{-2}) than in winter (87.8 g m^{-2}), whereas AGB was not significantly higher in summer than in winter.

Table 5. Seagrass traits at existing seagrass habitat (ES). Statistical parameters are mean (±SE), maximum, minimum, and median. Significant seasonality (ANOVA, Tukey HSD, $p < 0.05$) in traits is indicated by different alphabetic superscripts.

Trait	Season	Mean ± SE	Max	Min	Median
% Cover	Winter	49.4 ± 7.1 ^{a}	90	0	65
	Summer	71.0 ± 5.2 ^{b}	95	0	75
Shoot density (m^{-2})	Winter	444 ± 51.7 ^{a}	828	0	414
	Summer	670 ± 51.7 ^{b}	1401	159	700
AGB (g m^{-2})	Winter	9.4 ± 1.6 ^{a}	22.7	0.13	6.1
	Summer	10.2 ± 1.0 ^{a}	22.7	2.1	11.3
BGB (g m^{-2})	Winter	87.8 ± 15.9 ^{a}	302.5	1.9	46.9
	Summer	150.7 ± 24.2 ^{b}	488.8	4.6	111.5
BGB/AGB	Winter	17.9 ± 4.5 ^{a}	95.1	2.04	11.5
	Summer	15.9 ±2.3 ^{a}	44.8	0.7	13.4
Biomass (g m^{-2})	Winter	97.2 ± 17 ^{a}	321.6	2.5	64.5
	Summer	161 ± 24.6 ^{b}	500	10.9	122.9

3.7. Seagrass Relationships with Substrate Conditions

Seagrass cover increase was associated with an increase in percent mud, percent OM, and recycled dissolved inorganic nutrients. Percentage of mud and percent OM followed a logistic relationship (Figure 5a,b) whereas a saturation relationship was the best fit for porewater PO_4^{3-} and NH_4^+ (Figure 5d). Equations are shown in Supplementary materials. Maximum seagrass cover occurred where substrate mud content was in the range 13–23% and organic content 1.3–3% with optimums (maximum slope) of 13% mud and 1.3% OM. Porewater recycled nutrients followed saturation relationships with saturation concentrations of 2 and 8 μM for PO_4^{3-} and NH_4^+, respectively. None of the seagrass traits followed a significant relationship with PAR presumably because no ES sites were strongly light-limited. Mutual scatter plots between different biomass indices and substrate conditions, porewater chemistry are shown in the Supplementary materials, Table S4.



(**a**) (**b**)

(**c**) (**d**)

Figure 5. Scatter plots of seagrass cover versus substrate conditions at (existing seagrass, ES). (**a**) Seagrass cover versus substrate % mud; (**b**) seagrass cover versus substrate % organic matter; (**c**) seagrass cover vs. porewater PO_4^{3-}; (**d**) seagrass cover versus porewater NH_4^+.

4. Discussion

Our study addresses relationships between seagrasses, light climate, fine sediment, and substrate conditions by comparing three habitats, HS where seagrass was present many years ago and has never recovered, ES where seagrass is currently present, and PS where seagrass has been present in recent years but is currently absent. We used these data to address two non-exclusive, hypothetical mechanisms for seagrass decline; light attenuation and substrate degradation.

HS substrates had significantly higher mud content (34–39%), bulk density (1.3–1.6 g cm^{-3}), porewater ammonium concentration (13–72 μM), a more reduced redox profile (negative redox at 2 cm soil depth), and higher (modelled) sedimentation rates (6 mm yr^{-1}) than both PS and ES (which were broadly similar). Differences in substrate organic content were less clear, and while HS had significantly higher organic content than ES during winter the opposite pattern occurred during summer. PS substrate organic content was consistently lower than the other two sites, but again slightly, but significantly, lower in summer than in winter. We infer that during the warmer summer months the mineralization of organic matter in the two seagrass-free sites temporarily exceeds accumulation, while the summer growth in seagrass cover in ES habitat causes an increase in substrate OM. This summer observation of incremented OM in ES habitat concurred with a study performed at Puerto Morelos Mexico on the effects of *Thalassia testudinum* seagrass on substrate biogeochemistry [54]. Accumulation of phosphate and ammonium ions in the upper substrate at all sites during summer is consistent with increased mineralization of OM in this season.

That the presence of seagrass is a significant source of organic material is supported by the higher substrate OM concentrations at ES than PS, and this is well established in the literature [55–60]. That the organic content at ES exceeds that at HS at times, suggests that the amount of organic material per se does not exclude seagrass at HS. The simplest explanation of the more reduced substrate at HS, and the accumulation of reduced chemical species, is reduced diffusivity accompanying the higher proportion of mud (and greater bulk density implying lower pore space) at HS compared to the ES substrate. Under seagrass, root oxygen release [24–26,28,61], together with enhanced burrowing by invertebrates, mainly bivalves such as *Austrovenus stutchburyi* and *Macocoma liliana* [62–64] are processes expected to make redox potential less negative.

Unfavorable alterations to substrate chemistry as a result of fine sediment deposition is a likely cause of the failure of seagrass to colonize at HS while persisting at ES. Our results suggest that the significantly higher mud content, bulk density, and more reduced conditions of the substrate at HS, is likely to be associated with a lower availability of interstitial oxygen compared to substrates at ES and PS. A poor substrate oxygen status, linked to denser substrates and higher oxygen-demanding organic matter content, can affect the capability of seagrasses to grow and is a potential cause of seagrass demise [65]. Nevertheless, there appear to be strong differences between species in their ability to tolerate anoxia [25]. A Mediterranean species *Cymodocea nodosa* did not die after sucrose additions to generate anoxia, in a manipulative experiment, and temperate *Zostera marina* did not show leaf growth reduction until 2 months post treatment. However, *Thalassia hemprichii* suffered mortality in a multi-specific meadow under a similar experimental regime [25]. Substrate redox values previously measured in substrates under seagrass ranged from −108 to 55 mV in three New Zealand estuaries [31]. At HS in Pāuatahanui Inlet we measured values ranging from −230 to 70 mV and −50 mV at a substrate depth of just 2 cm below the surface indicating very reducing conditions. The redox values are also lower than the typical range reported for seagrass substrates of −100 and 200 mV in the first 10 cm of the substrate by [25].

Despite the more reducing conditions in HS substrates in winter we did not find consistently increased concentrations of hydrogen sulfide and phosphate, which tend to accumulate under these conditions [26–28]. However, ammonium ion concentrations were higher in HS than other habitats in both seasons. In marine substrates, sulphate reduction is a major pathway for the mineralization of the organic matter leading to production of hydrogen sulfide [8,57]. However, our results for porewater hydrogen sulfide analysis showed values under 4 μM at all sites, which is below the 10 and

13 µM thresholds considered to trigger decline in seagrasses [66,67]. This may be explained by high concentration of iron II (Fe^{2+}), which reacts with sulfide to form stable precipitates [68], and iron-rich substrates have low hydrogen sulfide, hence low toxicity for seagrass [69]. HS substrate samples were noticeably dark, suggesting the presence of iron sulfide. Iron solubilization ($Fe^{3+} \rightarrow Fe^{2+}$) occurs at −47 mV redox potential which seems consistent with the values of Eh at PS and ES. Levels of iron in Pāuatahanui Inlet were typical of other New Zealand estuaries [37,70,71]. However, further research into iron–sulphide interactions seems desirable. Potentially phytotoxic heavy metals such as zinc, lead, ferrous iron, and copper bound to ferric oxy-hydroxides are released when Fe (III) is reduced and may also be implicated in sediment toxicity [72]. Chromium, copper, lead, and zinc were determined to have maximum values of 1.68, 1.1, 3.3, 9.7 mg L^{-1}, respectively, at other sites in Pāuatahanui Inlet [73]. These copper and lead concentrations exceed the reported thresholds of 1 mg L^{-1} reported to have incipient effects on seagrass physiological traits [72].

For the ammonium ion, which is the most common form of N in pore waters of New Zealand estuaries [74], concentrations at HS (up to 71 µM) were higher than at ES and PS and no seagrass was found to thrive at sites over 30.8 µM. However, concentration at all sites were below levels considered phytotoxic for the related species Z. *nolti* 200–4000 µM; [75,76]. Therefore, direct hydrogen sulfide and/or ammonium ion phytotoxicity appears unlikely to have contributed to seagrass loss and failure to re-establish at HS in Pāuatahanui Inlet. That lack of apparent influence of sulfide and ammonia is consistent with the PCA plot for different sites showing strong separation of habitat along 'mud' gradients, but separation along pore-water chemistry only within habitats.

Nevertheless, the reducing HS substrates implies that, for seagrasses to recolonize, high release of oxygen into the rhizosphere would be required to oxygenate it which, in turn, implies an increased light demand. This effect may be preventing the establishment of different seagrass species in other coastal locations around the world such as the Mediterranean Sea, Southeast Asia, the North Sea [25], and other areas which may be under duress of fine sediment pollution. Degradation of the oxic-microshield protecting the vital basal meristems may be the initial external chemical mechanism behind seagrass die-off events in highly reduced marine substrates [24], such as HS in Pāuatahanui Inlet.

Reduced underwater PAR availability is often considered the leading cause of seagrass decline in estuaries suffering high sediment pollution. Increased suspended sediment loads to coastal waters have an indirect negative effect on seagrasses by reducing the available PAR penetrating through the water to the seabed [6,17]. The Pāuatahanui Inlet study sites were located in the intertidal zone, whereas many studies that have implicated light limitation have been carried out at subtidal locations or with specimens cultivated in permanently submerged conditions [11,12,77,78]. In intertidal locations, the irradiance during the emerged periods is much higher than when submerged (by ca. 10-fold in winter and 12-fold in summer, in our study) which can compensate for poor light penetration of muddy water during immersion. Being at the same tidal height, we found no significant differences in emerged irradiance between the three habitats in either summer or winter. Any effect of sediment mediated through attenuation of downwelling irradiance therefore must be expressed in the submerged irradiance, which was lowest at HS habitats during winter (but not summer), and highest in the ES habitat, perhaps reflecting both proximity of sediment sources (to HS) and the capacity of seagrasses (at ES) to enhance sedimentation and thus generate a clearer overhead environment [79]. The efficiency of PAR use under submerged and emerged conditions becomes critical in evaluating the role of PAR limitation, since the effect of the elevated fines on seagrass light climate at HS sites appears to be relatively small and seasonal.

Daily emerged PAR dose has been proven to be key to providing resilience to inter-tidal seagrasses [80–82]. However, at HS PAR during emersion was high and yet seagrass has not re-stablished there suggesting that emerged production cannot sustain the plants or, more likely, that other factors are dominant. Considering the submerged irradiance, the average winter value for HS is close to the PAR determined at maximum depth limit for subtidal specimens of this species, in winter, in Kaipara Harbour (2.1 ± 0.19 mol $m^{-2}d^{-1}$) [78]. However, our sites in Pāuatahanui Inlet, including HS, experience on average at least this amount of PAR when submerged and a higher amount when emerged, thus leading to an expectation of total net photosynthetic gains, year round [81]. Furthermore, total PAR at HS, ES and PS exceeds the CI value of 3.9 mol $m^{-2}d^{-1}$ reported by [53], (by at least ca. 12-fold at both seasons). Light availability alone therefore does not appear to explain the failure of seagrass to re-establish, at HS in Pāuatahanui Inlet.

In addition to alteration to substrate physico-chemistry and low winter submerged PAR availability, periodic smothering of seagrass plants by sedimentation events may have also contributed to seagrass loss, and failure to recover, at HS. Recently settled fine sediment within nepheloid layers not only shades seagrass [22] but may also exert an oxygen demand and inhibit oxygen transfer so contributing to deoxygenation. Seagrass was present at HS around 1980 [35] but disappeared sometime afterwards [36]. A large sedimentation event occurred in 1981 which delivered a sediment load of about 40,000 tons to the inlet [51]. It was linked to a particularly wet spring and winter and to a large rainfall event during May (150 mm in a week). It is plausible that this event may have caused an acute and catastrophic loss of seagrass from the inner part of the inlet, where plants have not subsequently re-established. However, it is also apparent that HS continues to experience chronically elevated fine sediment. The rate of sediment accumulation in most NZ estuaries under natural conditions is below 1 mm yr^{-1} [42]. However, HS is predicted to experience fine sediment deposition of about 6 mm yr^{-1} compared to rates of 3.6 and 1.2 mm yr^{-1} at ES and PS, respectively. Moreover, forcing might be highly nonlinear such that extreme windstorms or large floods may be disproportionally important as regards consequent suspended sediment 'climate' and therefore must be further considered [83].

Intertidal flats of relatively undeveloped estuaries with healthy seagrass meadows are typically characterized by sandy substrates with minimal mud content [31]. We showed here that multi stressor effects of mud are strong determinants of seagrass growth and persistence. Moreover, fine sediment often interacts with other factors such as nutrient enrichment leading to increased organic matter which may interact with mud loading by both increased shading by phytoplankton and epiphytes and extra oxygen demand, further complicating seagrass response [84,85].

We also showed that seagrass grew at ES sites throughout the year, but plant cover, shoot density, and biomass were 1.4-fold, 1.5-fold, and 1.7-fold higher, in summer than in winter. For ES sites maximum seagrass cover occurred in an intermediate range of substrate mud content ranging from 13% to 23%. Substrate grain size has been proposed as an influence on seagrass growth [67,86–92]. The substrate mud range (13–23%) in which seagrass grows in Pāuatahanui Inlet is higher than the silt ($3.9 < 63$ μm) threshold determined for this species in another New Zealand estuary, Tauranga Harbour, of 13% [92]. However, we note that our measurements are for a substrate depth of 0–10 cm whereas samples taken in Tauranga Harbour are for the 0–2 cm depth range (Table 6) so results are not directly comparable. Nevertheless, this suggest that *Zostera muelleri* may have a broader tolerance for mud than we thought, initially, based on the Tauranga experience. This suitable substrate muddiness range is local and may vary for different estuaries depending on the *nature* of the mud and *interaction* with other stressors, for example presence of other particle sizes, percent OM, oxygen demand, and pore water condition. Studies in other countries report a wide range of substrate muddiness thresholds ranging from 13% to 70% (Table 5), but note differences in depth of substrate sampled. Furthermore, tolerances of seagrasses to substrate muddiness are likely to vary between species and between geographical locations due to interactions with other biogeochemical characteristics of the substrates [67].

Table 6. Substrate percent mud ranges for *Zostera* genus occurrence and transplanting success (if available) reported in the literature.

Study Location	Seagrass Species	Depth of Substrate Sampled	Means of Determination	Substrate Mud (%) Where Seagrass Thrives or Recommended for Transplanting	References
New Bedford Harbour, USA	*Z. marina*	0–10 cm	x	<70%	[87]
Multiple locations, Review	*Z. marina*	x	x	2.3–56.3% <20%	[88]
Boston Harbour, USA	*Z. marina*	0–10 cm	x	<35% At >57% transplant failed	[89]
NW coast, Sweden	*Z. marina*	0–9 cm	Wet sieving	Survival at 35% no survival where >60%	[90]
Western Port, Australia	*Z. muelleri*	x	x	0.5–72%	[91]
Tauranga Harbour, New Zealand	*Z. muelleri*	0–2 cm	x	<13 % silt threshold	[92]
Porirua Harbour, New Zealand	*Z. muelleri*	0–10 cm	Laser analyzer	8–23%	This study

x Not defined.

5. Conclusions

We attribute loss of the seagrass, *Zostera muelleri*, from some habitats in Pāuatahanui Inlet to pollution with fine sediment, which exerts multiple stresses, particularly deoxygenation of the root zone. Light exposure does not appear to be an important factor limiting inter-tidal seagrass—which get sufficient PAR during emergence even where very muddy waters attenuate light strongly during submerged periods. Nor do toxic materials such as sulfide in porewaters appear to be implicated. However, further research combined with determination of concentration of potentially phytotoxic heavy metals is desirable. We recommend further experiments to isolate mechanisms of mud damage to seagrass and to improve our understanding of its interactions with other factors such as light deprivation and eutrophication. This will assist policy makers to better manage this pollutant for seagrass protection and restoration when considering mitigation and remediation strategies. The results of this study highlight the critical importance of catchment strategies that reduce fine sediments to estuaries.

Supplementary Materials: The following are available online at http://www.mdpi.com/2077-1312/8/9/645/s1, included with this manuscript submission.

Author Contributions: Conceptualization, I.Z.-M.; F.E.M.; M.M.-H. and I.H.; methodology, I.Z.-M.; validation, F.E.M.; M.M.-H.; I.H. and R.J.D.-C.; formal analysis, I.Z.-M.; investigation, I.Z.-M.; resources, F.E.M.; R.J.D.-C.; data curation, I.Z.-M.; writing—original draft preparation, I.Z.-M.; writing—review and editing, F.E.M.; M.M.-H.; I.H.; R.J.D.-C. and M.O.; supervision, I.H.; F.E.M. and M.M.-H. project administration, F.E.M. and R.J.D.-C.; funding acquisition, I.H.; F.E.M. and R.J.D.-C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Strategic Science Investment programme Managing Mud from National Institute of Water and Atmospheric Research NIWA, grant number FWCE2011 and the APC was funded by NIWA and University of Waikato. We also acknowledge the Greater Wellington Regional Council for supporting this work with its Science and Research Fund.

Acknowledgments: We thank Andrew Swales NIWA's Managing Mud programme leader. NIWA-Wellington field staff: Peter Notman, Owen Anderson, and Warrick Lyon for field assistance. Margaret McMonagle (NIWA-Hamilton, Water Quality Laboratory staff) is thanked for helping with assays of sulfide on estuarine porewater samples. Rebecca Gibson (University of Waikato) for the help provided with nutrient analysis with FIA. We thank NIWA-Hamilton field surveyor Ron Ovenden for measuring elevation of study sites and John Oldman from DHI New Zealand for the aid with modelled hydrodynamics data. We extend our appreciation to four anonymous reviewers for their thoughtful comments and suggestions to improve our manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Waycott, M.; Duarte, C.M.; Carruthers, T.J.B.; Orth, R.J.; Dennison, W.C.; Olyarnik, S.; Calladine, A.; Fourqurean, J.W.; Heck, K.L.; Hughes, A.R.; et al. Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 12377–12381. [CrossRef]
2. Orth, R.J.; Carruthers, T.J.B.; Dennison, W.C.; Duarte, C.M.; Fourqurean, J.W.; Heck, K.L.; Hughes, A.R.; Kendrick, G.A.; Kenworthy, W.J.; Olyarnik, S.; et al. A Global Crisis for Seagrass Ecosystems. *Bioscience* **2006**, *56*, 987–996. [CrossRef]
3. Burkholder, J.M.; Tomasko, D.A.; Touchette, B.W. Seagrasses and eutrophication. *J. Exp. Mar. Biol. Ecol.* **2007**, *350*, 46–72. [CrossRef]
4. Cunha, A.H.; Marbà, N.; Van Katwijk, M.M.; Pickerell, C.; Henriques, M.; Bernard, G.; Ferreira, M.A.; García, S.; Garmendia, J.M.; Manent, P. Changing Paradigms in Seagrass Restoration. *Restor. Ecol.* **2012**, *20*, 427–430. [CrossRef]
5. Short, F.T.; Coles, R.G. *Global Seagrass Research Methods*; Elsevier: Amsterdam, The Netherlands, 2001.
6. Duarte, C.M. Seagrass depth limits. *Aquat. Bot.* **1991**, *40*, 363–377. [CrossRef]
7. Dennison, W.C.; Orth, R.J.; Moore, K.A.; Stevenson, J.C.; Carter, V.; Kollar, S.; Bergstrom, P.W.; Batiuk, R.A. Assessing Water Quality with Submersed Aquatic Vegetation. *Bioscience* **1993**, *43*, 86–94. [CrossRef]
8. Duarte, C.M.; Marbà, N.; Santos, R. What may cause loss of seagrasses. In *European Seagrasses: An Introduction to Monitoring and Management*; Borum, J., Duarte, C.M., Krause-Jensen, D., Greve, T.M., Eds.; The M&MS Project: Copenhagen, Denmark, 2004. Available online: http://www.seagrasses.org (accessed on 18 May 2020).
9. Ralph, P.J.; Gademann, R. Rapid light curves: A powerful tool to assess photosynthetic activity. *Aquat. Bot.* **2005**, *82*, 222–237. [CrossRef]
10. Adams, M.P.; Hovey, R.K.; Hipsey, M.R.; Bruce, L.C.; Ghisalberti, M.; Lowe, R.; Gruber, R.K.; Ruiz-Montoya, L.; Maxwell, P.S.; Callaghan, D.P.; et al. Feedback between sediment and light for seagrass: Where is it important? *Limnol. Oceanogr.* **2016**, *61*, 1937–1955. [CrossRef]
11. Chartrand, K.M.; Bryant, C.; Carter, A.B.; Ralph, P.J.; Rasheed, M.A. Light Thresholds to Prevent Dredging Impacts on the Great Barrier Reef Seagrass, *Zostera muelleri* ssp. capricorni. *Front. Mar. Sci.* **2016**, *3*, 106. [CrossRef]
12. Collier, C.J.; Adams, M.P.; Langlois, L.; Waycott, M.; O'Brien, K.R.; Maxwell, P.; McKenzie, L.J. Thresholds for morphological response to light reduction for four tropical seagrass species. *Ecol. Indic.* **2016**, *67*, 358–366. [CrossRef]
13. Kirkman, H.; Kuo, J. Pattern and process in southern Western Australian seagrasses. *Aquat. Bot.* **1990**, *37*, 367–382. [CrossRef]
14. Fourqurean, J.W.; Rutten, L.M. The impact of Hurricane Georges on soft-bottom, back reef communities: Site-and species-specific effects in south Florida seagrass beds. *Bull. Mar. Sci.* **2004**, *75*, 239–257.
15. Suykerbuyk, W.; Bouma, T.J.; Govers, L.L.; Giesen, K.; De Jong, D.J.; Herman, P.; Hendriks, A.J.; Van Katwijk, M.M. Surviving in Changing Seascapes: Sediment Dynamics as Bottleneck for Long-Term Seagrass Presence. *Ecosystems* **2015**, *19*, 296–310. [CrossRef]
16. Short, F.T.; Wyllie-Echeverria, S. Natural and human-induced disturbance of seagrasses. *Environ. Conserv.* **1996**, *23*, 17–27. [CrossRef]
17. Erftemeijer, P.L.A.; Lewis, R.R.R. Environmental impacts of dredging on seagrasses: A review. *Mar. Pollut. Bull.* **2006**, *52*, 1553–1572. [CrossRef] [PubMed]
18. Cabaco, S.; Santos, R.; Duarte, C.M. The impact of sediment burial and erosion on seagrasses: A review. *Estuar. Coast. Shelf Sci.* **2008**, *79*, 354–366. [CrossRef]
19. Benham, C.F.; Jackson, E.L.; Beavis, S.G. Tolerance of tropical seagrasses *Zostera muelleri* and *Halophila ovalis* to burial: Toward an understanding of threshold effects. *Estuar. Coast. Shelf Sci.* **2019**, *218*, 131–138. [CrossRef]
20. Ralph, P.; Durako, M.; Enríquez, S.; Collier, C.J.; Doblin, M.A. Impact of light limitation on seagrasses. *J. Exp. Mar. Biol. Ecol.* **2007**, *350*, 176–193. [CrossRef]
21. Moore, K.A.; Wetzel, R.L.; Orth, R.J. Seasonal pulses of turbidity and their relations to eelgrass (*Zostera marina* L.) survival in an estuary. *J. Exp. Mar. Biol. Ecol.* **1997**, *215*, 115–134. [CrossRef]
22. Pedersen, T.M.; Gallegos, C.; Nielsen, S.L. Influence of near-bottom re-suspended sediment on benthic light availability. *Estuar. Coast. Shelf Sci.* **2012**, *106*, 93–101. [CrossRef]

23. Bainbridge, Z.; Lewis, S.; Bartley, R.; Fabricius, K.; Collier, C.J.; Waterhouse, J.; Garzon-Garcia, A.; Robson, B.; Burton, J.; Wenger, A.S.; et al. Fine sediment and particulate organic matter: A review and case study on ridge-to-reef transport, transformations, fates, and impacts on marine ecosystems. *Mar. Pollut. Bull.* **2018**, *135*, 1205–1220. [CrossRef] [PubMed]
24. Brodersen, K.E.; Hammer, K.J.; Schrameyer, V.; Floytrup, A.; Rasheed, M.A.; Ralph, P.J.; Kühl, M.; Pedersen, O. Sediment Resuspension and Deposition on Seagrass Leaves Impedes Internal Plant Aeration and Promotes Phytotoxic H2S Intrusion. *Front. Plant Sci.* **2017**, *8*, 657. [CrossRef] [PubMed]
25. Terrados, J.; Duarte, C.M.; Kamp-Nielsen, L.; Agawin, N.; Gacia, E.; Lacap, D.; Fortes, M.; Borum, J.; Lubanski, M.; Greve, T. Are seagrass growth and survival constrained by the reducing conditions of the sediment? *Aquat. Bot.* **1999**, *65*, 175–197.
26. Borum, J.; Pedersen, O.; Greve, T.M.; Frankovich, T.A.; Zieman, J.C.; Fourqurean, J.W.; Madden, C.J. The potential role of plant oxygen and sulphide dynamics in die-off events of the tropical seagrass, Thalassia testudinum. *J. Ecol.* **2005**, *93*, 148–158. [CrossRef]
27. Koch, M.; Schopmeyer, S.; Kyhn-Hansen, C.; Madden, C. Synergistic effects of high temperature and sulfide on tropical seagrass. *J. Exp. Mar. Biol. Ecol.* **2007**, *341*, 91–101. [CrossRef]
28. Brodersen, K.E.; Nielsen, D.A.; Ralph, P.J.; Kühl, M. Oxic microshield and local pH enhancement protects *Zostera muelleri* from sediment derived hydrogen sulphide. *New Phytol.* **2014**, *205*, 1264–1276. [CrossRef]
29. Green, E.P.; Short, F.T. *World Atlas of Seagrasses*; University of California Press: Berkeley, CA, USA, 2003.
30. Thrush, S.; Hewitt, J.; Cummings, V.; Ellis, J.; Hatton, C.; Lohrer, A.; Norkko, A. Muddy waters: Elevating sediment input to coastal and estuarine habitats. *Front. Ecol. Environ.* **2004**, *2*, 299–306. [CrossRef]
31. Matheson, F.E.; Schwarz, A.-M. Growth responses of *Zostera capricorni* to estuarine sediment conditions. *Aquat. Bot.* **2007**, *87*, 299–306. [CrossRef]
32. Morrison, M.A.; Lowe, M.; Parsons, D.; Usmar, N.; McLeod, I. *A Review of Land-Based Effects on Coastal Fisheries and Supporting Biodiversity in New Zealand*; New Zealand Aquatic Environment and Biodiversity Report No. 37; Ministry of Fisheries: Wellington, New Zealand, 2009; p. 100. Available online: http://fs.fish.govt.nz/Page.aspx?pk=113&dk=22003 (accessed on 6 July 2020).
33. Inglis, G. Seagrasses of New Zealand. In *World Atlas of Seagrasses*; Green, E.P., Short, F.T., Eds.; University of California Press: Berkeley, CA, USA, 2003; pp. 148–157.
34. Matheson, F.E.; Lundquist, C.; Gemmill, C.E.; Pilditch, C. New Zealand seagrass—More threatened than IUCN review indicates. *Biol. Conserv.* **2011**, *144*, 2749–2750. [CrossRef]
35. Healy, W.B. *Pāuatahanui Inlet: An environmental study*; DSIR Information Series 141; Science Information Division: Wellington, New Zealand, 1980.
36. Matheson, F.E.; Wadhwa, S. Seagrass in Porirua Harbour. In *Preliminary Assessment of Restoration Potential. Report No. HAM2012-037*; Prepared for Greater Wellington Regional Council; National Institute of Water and Atmospheric Research: Hamilton, New Zealand, 2012; p. 35. Available online: http://www.gw.govt.nz/assets/Seagrass-restoration-in-Porirua-Harbour.pdf (accessed on 8 March 2019).
37. Blaschke, P.; Woods, J.; Forsyth, F. *The Porirua Harbour and Its Catchment: A Literature Summary and Review*; Porirua City Council: Porirua, New Zealand, 2010; p. 99.
38. Milne, J.R.; Warr, S. *Annual Coastal Monitoring Report for the Wellington Region*; Report No GW/EMI-T-07/224; Environmental Monitoring and Investigation Department, Greater Wellington Regional Council: Wellington, New Zealand, 2007.
39. Stevens, L.; Robertson, B. Porirua Harbour: Sediment Plate Monitoring 2015/16. In *Report Prepared for Greater Wellington Regional Council*; Wriggle Coastal Management: Nelson, New Zealand, 2016; p. 16. Available online: http://www.gw.govt.nz/assets/council-publications/Porirua-Harbour-Sediment-plate-monitoring-2016.pdf (accessed on 8 March 2018).
40. Gibb, J.G.; Cox, G.J. *Patterns and Rates of Sedimentation within Porirua Harbour*; Porirua City Council: Porirua, New Zealand, 2009.
41. Swales, A.; Bentley, S.; McGlone, M.; Ovenden, R.; Hermanspahn, N.; Budd, R.; Hill, A.; Pickmere, S.; Haskew, R.; Okey, M. *Pauatahanui Estuary: Effects of Historical Catchment Landcover Changes on Estuary Sedimentation*; National Institute of Water & Atmospheric Research Ltd.: Auckland, New Zealand, 2005.
42. Townsend, M.; Lohrer, D. *ANZECC Guidance for Estuary Sedimentation*; Prepared for the Ministry for the Environment by NIWA; NIWA: Hamilton, New Zealand, 2015.

43. Waycott, M.; Longstaff, B.J.; Mellors, J. Seagrass population dynamics and water quality in the Great Barrier Reef region: A review and future research directions. *Mar. Pollut. Bull.* **2005**, *51*, 343–350. [CrossRef]
44. Duarte, C.M.; Fourqurean, J.W.; Krause-Jensen, D.; Olesen, B. Dynamics of Seagrass Stability and Change. In *Seagrasses: Biology, Ecologyand Conservation*; Larkum, A.W., Orth, R.J., Duarte, C.M., Eds.; Springer: Dordrecht, The Netherlands, 2007; pp. 271–294. Available online: https://doi.org/10.1007/978-1-4020-2983-7_11 (accessed on 1 May 2019).
45. McGlathery, K.J.; Berg, P.; Marino, R. Using porewater profiles to assess nutrient availability in seagrass-vegetated carbonate sediments. *Biogeochemistry* **2001**, *56*, 239–263. [CrossRef]
46. Thimijan, R.W.; Heins, R.D. Photometric, radiometric, and quantum light units of measure: A review of procedures for interconversion. *HortScience* **1993**, *18*, 818–822.
47. Matheson, F.E.; Zabarte-Maeztu, I.; Mackay, G.; Middleton, C.; Oliver, M.; Griffiths, R.; Ovenden, R.; Davies-Colley, R.J. Estuary muddiness and seasonally low light constrains seagrass restoration. *Mar. Pollut. Bull.* (Under Review).
48. Mook, D.H.; Hoskin, C.M. Organic determinations by ignition: Caution advised. *Estuar. Coast. Shelf Sci.* **1982**, *15*, 697–699. [CrossRef]
49. Singer, J.; Anderson, J.; Ledbetter, M.; McCave, I.; Jones, K.; Wright, R. An assessment of analytical techniques for the size analysis of fine-grained sediments. *J. Sediment. Res.* **1988**, *58*, 534–543.
50. Folk, R.L. *Petrology of Sedimentary Rocks*; Hemphill's: Austin, TX, USA, 1968; p. 85.
51. Porirua Harbour-Modelling for Whaitua Collaborative Modelling Group. *Report 44800943/01 Prepared for GWRC for the Development of the Te Awarua-o'Porirua Whaitua Implementation Programme*; DHI: Wellington, New Zealand, 2019.
52. Zar, J.H. *Biostatistical Analysis*; Prentice-Hall: Englewood Cliffs, NY, USA, 1984.
53. Flanigan, Y.S.; Critchley, C. Light response of D1 turnover and photosystem II efficiency in the seagrass *Zostera capricorni*. *Planta* **1996**, *198*, 319–323. [CrossRef]
54. Enríquez, S.; Marbà, N.; Duarte, C.M.; Van Tussenbroek, B.I.; Reyes-Zavala, G. Effects of seagrass *Thalassia testudinum* on sediment redox. *Mar. Ecol. Prog. Ser.* **2001**, *219*, 149–158. [CrossRef]
55. Romero, J.; Perez, M.; Mateo-Pérez, M.A.; Sala, E. The belowground organs of the Mediterranean seagrass *Posidonia oceanica* as a biogeochemical sink. *Aquat. Bot.* **1994**, *47*, 13–19. [CrossRef]
56. Gacia, E.; Duarte, C.M. Sediment Retention by a Mediterranean *Posidonia oceanica* Meadow: The Balance between Deposition and Resuspension. *Estuar. Coast. Shelf Sci.* **2001**, *52*, 505–514. [CrossRef]
57. Holmer, M.; Andersen, F.Ø.; Nielsen, S.L.; Boschker, H.T. The importance of mineralization based on sulfate reduction for nutrient regeneration in tropical seagrass sediments. *Aquat. Bot.* **2001**, *71*, 1–17. [CrossRef]
58. Gacia, E.; Duarte, C.M.; Marbà, N.; Terrados, J.; Kennedy, H.; Fortes, M.; Tri, N. Sediment deposition and production in SE-Asia seagrass meadows. *Estuar. Coast. Shelf Sci.* **2003**, *56*, 909–919. [CrossRef]
59. Larkum, A.W.; Orth, R.J.; Duarte, C.M. *Seagrasses*; Springer: Dordrecht, The Netherlands, 2006; p. 691.
60. Fourqurean, J.W.; Duarte, C.M.; Kennedy, H.; Marbà, N.; Holmer, M.; Mateo-Pérez, M.A.; Apostolaki, E.T.; Kendrick, G.A.; Krause-Jensen, D.; McGlathery, K.J.; et al. Seagrass ecosystems as a globally significant carbon stock. *Nat. Geosci.* **2012**, *5*, 505–509. [CrossRef]
61. Brodersen, K.E.; Kühl, M.; Nielsen, D.A.; Pedersen, O.; Larkum, A.W.D. Rhizome, Root/Sediment Interactions, Aerenchyma and Internal Pressure Changes in Seagrasses. In *Seagrasses of Australia*; Larkum, A.W.D., Kendrick., G.A., Ralph, P.J., Eds.; Springer Cham: Dordrecht, The Netherlands, 2018; pp. 393–418.
62. Lohrer, A.M.; Thrush, S.F.; Gibbs, M.M. Bioturbators enhance ecosystem function through complex biogeochemical interactions. *Nature* **2004**, *431*, 1092–1095. [CrossRef] [PubMed]
63. Lohrer, A.M.; Halliday, N.; Thrush, S.F.; Hewitt, J.E.; Rodil, I.F. Ecosystem functioning in a disturbance-recovery context: Contribution of macrofauna to primary production and nutrient release on intertidal sandflats. *J. Exp. Mar. Biol. Ecol.* **2010**, *390*, 6–13. [CrossRef]
64. Lohrer, A.M.; Townsend, M.; Hailes, S.F.; Rodil, I.F.; Cartner, K.; Pratt, D.; Hewitt, J.E. Influence of New Zealand cockles (*Austrovenus stutchburyi*) on primary productivity in sandflat-seagrass (*Zostera muelleri*) ecotones. *Estuar. Coast. Shelf Sci.* **2016**, *181*, 238–248. [CrossRef]
65. Pérez, M.; Invers, O.; Ruiz, J.M.; Frederiksen, M.S.; Holmer, M. Physiological responses of the seagrass *Posidonia oceanica* to elevated organic matter content in sediments: An experimental assessment. *J. Exp. Mar. Biol. Ecol.* **2007**, *344*, 149–160. [CrossRef]

66. Calleja, M.L.; Marbà, N.; Duarte, C.M. The relationship between seagrass (*Posidonia oceanica*) decline and sulfide porewater concentration in carbonate sediments. *Estuar. Coast. Shelf Sci.* **2007**, *73*, 583–588. [CrossRef]
67. Krause-Jensen, D.; Carstensen, J.; Nielsen, S.L.; Dalsgaard, T.; Christensen, P.; Fossing, H.; Rasmussen, M. Sea bottom characteristics affect depth limits of eelgrass *Zostera marina*. *Mar. Ecol. Prog. Ser.* **2011**, *425*, 91–102. [CrossRef]
68. Nielsen, A.H.; Lens, P.N.L.; Vollertsen, J.; Hvitvedjacobsen, T. Sulfide–iron interactions in domestic wastewater from a gravity sewer. *Water Res.* **2005**, *39*, 2747–2755. [CrossRef]
69. Ruiz-Halpern, S.; Macko, S.A.; Fourqurean, J.W. The effects of manipulation of sedimentary iron and organic matter on sediment biogeochemistry and seagrasses in a subtropical carbonate environment. *Biogeochemistry* **2008**, *87*, 113–126. [CrossRef]
70. Stoffers, P.; Glasby, G.P.; Plüger, W.L.; Walter, P. Reconnaissance survey of the mineralogy and geochemistry of some New Zealand lake and nearshore sediments. *N. Z. J. Mar. Freshw. Res.* **1983**, *17*, 461–480. [CrossRef]
71. Glasby, G.P.; Moss, R.L.; Stoppers, P. Heavy metal pollution in Porirua Harbour, New Zealand. *N. Z. J. Mar. Freshw. Res.* **1990**, *24*, 233–237. [CrossRef]
72. Macinnis-Ng, C.M.; Ralph, P.J. In situ impact of multiple pulses of metal and herbicide on the seagrass, *Zostera capricorni*. *Aquat. Toxicol.* **2004**, *67*, 227–237. [CrossRef] [PubMed]
73. Hooper, K.L. The Impact of Stormwater Discharges on Freshwater, Marine Water and Marine Sediments and the Implications for Environmental Management of the Pauatahanui Inlet, Porirua, New Zealand: A Thesis Prepared in Partial Fulfilment of Master of Applied Science (Natural Resource Management). Master's Thesis, Massey University, Palmerston North, New Zealand, 2002.
74. Tay, H.; Bryan, K.R.; De Lange, W.; Pilditch, C.; De Lange, W. The hydrodynamics of the southern basin of Tauranga Harbour. *N. Z. J. Mar. Freshw. Res.* **2013**, *47*, 249–274. [CrossRef]
75. Brun, F.G.; Hernández, I.; Vergara, J.; Peralta, G.; Perez-Llorens, J.L. Assessing the toxicity of ammonium pulses to the survival and growth of *Zostera noltii*. *Mar. Ecol. Prog. Ser.* **2002**, *225*, 177–187. [CrossRef]
76. Govers, L.L.; De Brouwer, J.H.; Suykerbuyk, W.; Bouma, T.J.; Lamers, L.P.; Smolders, A.J.; Van Katwijk, M.M. Toxic effects of increased sediment nutrient and organic matter loading on the seagrass *Zostera noltii*. *Aquat. Toxicol.* **2014**, *155*, 253–260. [CrossRef] [PubMed]
77. Longstaff, B.J. Investigations into the Light Requirements of Seagrasses in Northeast Australia. Ph.D. Thesis, University of Queensland, Brisbane, Australia, 2003.
78. Bulmer, R.H.; Kelly, S.; Jeffs, A.G. Light requirements of the seagrass, *Zostera muelleri*, determined by observations at the maximum depth limit in a temperate estuary, New Zealand. *N. Z. J. Mar. Freshw. Res.* **2016**, *50*, 183–194. [CrossRef]
79. De Boer, W.F. Seagrass–sediment interactions, positive feedbacks and critical thresholds for occurrence: A review. *Hydrobiologia* **2007**, *591*, 5–24. [CrossRef]
80. Vermaat, J.E.; Agawin, N.; Fortes, M.; Uri, J.; Duarte, C.; Marba, N.; Enriquez, S.; Van Vierssen, W. The capacity of seagrasses to survive increased turbidity and siltation: The significance of growth form and light use. *Ambio* **1997**, *26*, 499–504.
81. Schwarz, A.-M. Contribution of photosynthetic gains during tidal emersion to production of *Zostera capricorni* in a North Island, New Zealand estuary. *N. Z. J. Mar. Freshw. Res.* **2004**, *38*, 809–818. [CrossRef]
82. Drylie, T.; Lohrer, A.M.; Needham, H.R.; Bulmer, R.H.; Pilditch, C. Benthic primary production in emerged intertidal habitats provides resilience to high water column turbidity. *J. Sea Res.* **2018**, *142*, 101–112. [CrossRef]
83. Marbà, N.; Cebrián, J.; Enríquez, S.; Duarte, C.M. Growth patterns of Western Mediterranean seagrasses: Species-specific responses to seasonal forcing. *Mar. Ecol. Prog. Ser.* **1996**, *133*, 203–215. [CrossRef]
84. Walker, D.; McComb, A. Seagrass degradation in Australian coastal waters. *Mar. Pollut. Bull.* **1992**, *25*, 191–195. [CrossRef]
85. Gladstone-Gallagher, R.V.; Hughes, R.W.; Douglas, E.J.; Pilditch, C. Biomass-dependent seagrass resilience to sediment eutrophication. *J. Exp. Mar. Biol. Ecol.* **2018**, *501*, 54–64. [CrossRef]
86. Kenworthy, W.; Fonseca, M. Reciprocal transplant of the seagrass *Zostera marina* L. Effect of substrate on growth. *Aquaculture* **1977**, *12*, 197–213. [CrossRef]
87. Short, F.T. Effects of sediment nutrients on seagrasses: Literature review and mesocosm experiment. *Aquat. Bot.* **1987**, *27*, 41–57. [CrossRef]
88. Koch, E.W. Beyond Light: Physical, Geological, and Geochemical Parameters as Possible Submersed Aquatic Vegetation Habitat Requirements. *Estuaries* **2001**, *24*, 1–17. [CrossRef]

89. Leschen, A.S.; Ford, K.H.; Evans, N.T. Successful Eelgrass (*Zostera marina*) Restoration in a Formerly Eutrophic Estuary (Boston Harbor) Supports the Use of a Multifaceted Watershed Approach to Mitigating Eelgrass Loss. *Chesap. Sci.* **2010**, *33*, 1340–1354. [CrossRef]
90. Moksnes, P.-O.; Eriander, L.; Infantes, E.; Holmer, M. Local Regime Shifts Prevent Natural Recovery and Restoration of Lost Eelgrass Beds Along the Swedish West Coast. *Chesap. Sci.* **2018**, *41*, 1712–1731. [CrossRef]
91. Edgar, G.; Shaw, C. The production and trophic ecology of shallow-water fish assemblages in southern Australia III. General relationships between sediments, seagrasses, invertebrates and fishes. *J. Exp. Mar. Biol. Ecol.* **1995**, *194*, 107–131. [CrossRef]
92. Park, S.; Donald, R. *Environment Bay of Plenty Tauranga Harbour Regional Plan Environmental Investigations*; Ecology of Tauranga Harbour. Environment Bay of Plenty Environmental Report 94/8; Environment BOP: Whakatane, New Zealand, 1994; p. 177.

Supplementary Materials

1. Study Site Coordinates and Bathymetry

Our sampling site coordinates and mean elevation relative to mean sea level (MSL) are shown in Table S1.

Table S1. Longitude, latitude, and elevation of study sites.

Habitat	Site ID	Latitude	Longitude	Elevation (m)
Historical Seagrass HS	HS1	-41.08765	174.8888	-0.3
	HS2	-41.08881	174.8926	x
	HS3	-41.09862	174.9096	-0.2
	HS4	-41.09912	174.9111	-0.2
	HS5	-41.09918	174.9112	-0.2
Existing Seagrass ES	ES2	-41.09702	174.8771	-0.3
	ES3	-41.09851	174.8763	-0.4
	ES4	-41.09842	174.8761	-0.4
	ES5	-41.09883	174.8759	-0.2
	ES6	-41.10039	174.8721	-0.3
Potential Seagrass PS	PS1	-41.09908	174.8725	-0.4
	PS2	-41.09866	174.8723	-0.4
	PS3	-41.09851	174.8722	-0.4
	PS4	-41.09827	174.8721	-0.4
	PS5	-41.09814	174.8721	-0.4

* ES1 was discarded due to field difficulties and depth. HS2 could not be surveyed.

2. Google Earth Imagery of Pāuatahanui Inlet, Potential Habitat (PS) (2002–2019)

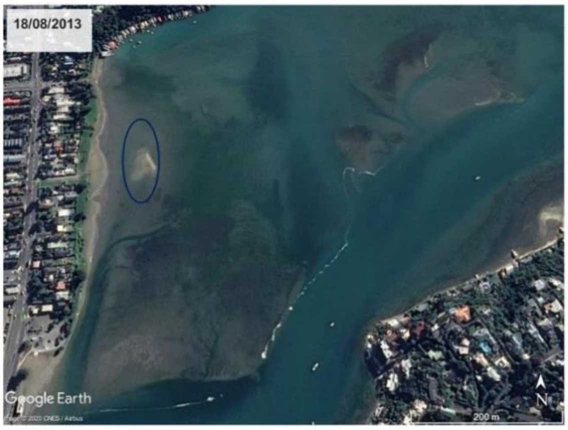
The objective of this timeseries is to show the dynamism of the seagrass patches nearby and at potential seagrass habitat (PS) since 2002–2019. South PS habitat has been intermittently colonized by seagrasses during some years (2016, 2017, 2018, and 2019) but this did not happen previously (2002–2014) (Figure S1).

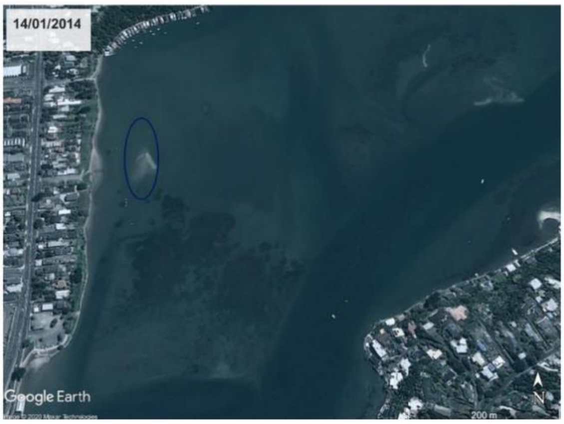

11/03/2005
Google Earth
200 m
N

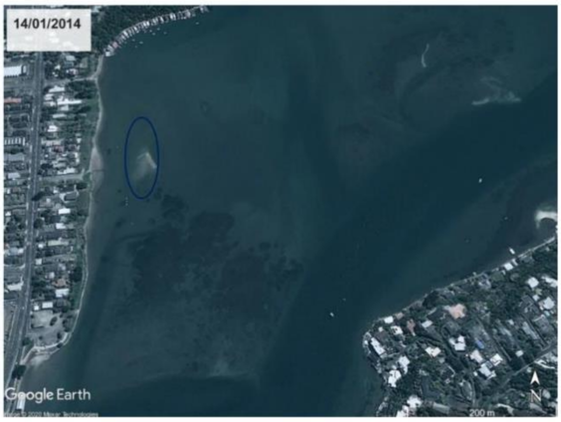

06/02/2006
Google Earth
200 m
N


25/11/2009
Google Earth
200 m
N


12/12/2012
Google Earth
200 m
N


18/08/2013
Google Earth
200 m
N


14/01/2014
Google Earth
200 m
N


14/01/2014
Google Earth
200 m
N


11/05/2015
Google Earth
200 m
N

Figure S1. Timeseries imagery of potential seagrass (PS) habitat (blue circle) since 2002–2019.

3. Light Monitoring

Row light monitoring and water level data from 23/8/18 to 3/10/18 (winter) and from 8/2/19 to 21/3/19 (summer) are shown in Figures S2–7. Calculations for each site are shown in Table S2.

Table S2. Light availability at historical seagrass (HS), existing seagrass (ES), and potential seagrass (PS) sites during winter and summer deployments. Values are daily mean photosynthetically available radiation (PAR) (±SE). Daily total PAR dose and PAR dose when submerged and emerged are shown as well as the number of days each site was under compensation irradiance (CI) during deployments (x = loss of device).

Site	**Season**	**PAR When Submerged (mol m^{-2} d^{-1})**	**PAR When Emerged (mol m^{-2} d^{-1})**	**Total PAR (mol m^{-2} d^{-1})**	**Number of Days Where Total PAR Was below Compensation Irradiance (Days)**
HS1	Winter	2.3 ± 0.2	25.2 ± 2.3	27.5	6 (2)
	Summer	x	x	x	x
HS2	Winter	x	x	x	x
	Summer	5.1 ± 0.2	59.6 ± 2.4	64.7	3 (0)
HS3	Winter	1.8 ± 0.2	46 ± 1.5	47.8	0 (0)
	Summer	4.8 ± 0.2	56.1± 2.8	60.9	3 (0)
HS4	Winter	0.8 ± 0.1	27.3 ± 3.2	28.1	11 (4)
	Summer	5.8 ± 0.2	66.6 ± 2.6	72.4	3 (0)
HS5	Winter	3.9 ± 0.2	25.2 ± 1	29.2	7 (2)
	Summer	5 ± 0.2	57.9 ± 2.2	62.9	5 (2)
ES2	Winter	4 ± 0.3	52.7 ± 2.4	56.7	3 (0)
	Summer	4.4 ± 0.2	51.4 ± 2	55.8	1 (0)
ES3	Winter	x	x	x	x
	Summer	5.1 ± 0.2	58.5 ± 2.7	63.6	1 (0)
ES4	Winter	4.5 ± 0.3	30 ± 1.5	34.5	4 (2)
	Summer	5 ± 0.2	57.9 ± 2.3	62.9	2 (0)
ES5	Winter	4.8 ± 0.3	47.8 ± 2.3	52.6	4 (2)
	Summer	3 ± 0.2	34.4 ± 1.8	37.4	2 (0)
ES6	Winter	4.3 ± 0.3	46.8 ± 2.1	51.1	3 (0)
	Summer	2.5 ± 0.1	28.5 ± 1.5	31	2 (0)
PS1	Winter	5.1 ± 0.3	36.7 ± 2	41.8	4 (0)
	Summer	5.5 ± 0.2	63.3 ± 2.6	68.8	1 (0)
PS2	Winter	2 ± 0.2	30 ± 2	32	5 (0)
	Summer	x	x	x	x
PS3	Winter	x	x	x	x
	Summer	4.6 ± 0.2	53.4 ± 2.9	58	0 (0)
PS4	Winter	x	x	x	x
	Summer	3.7 ± 0.2	42.6 ± 1.8	46.3	0 (0)
PS5	Winter	x	x	x	x
	Summer	x	x	x	x

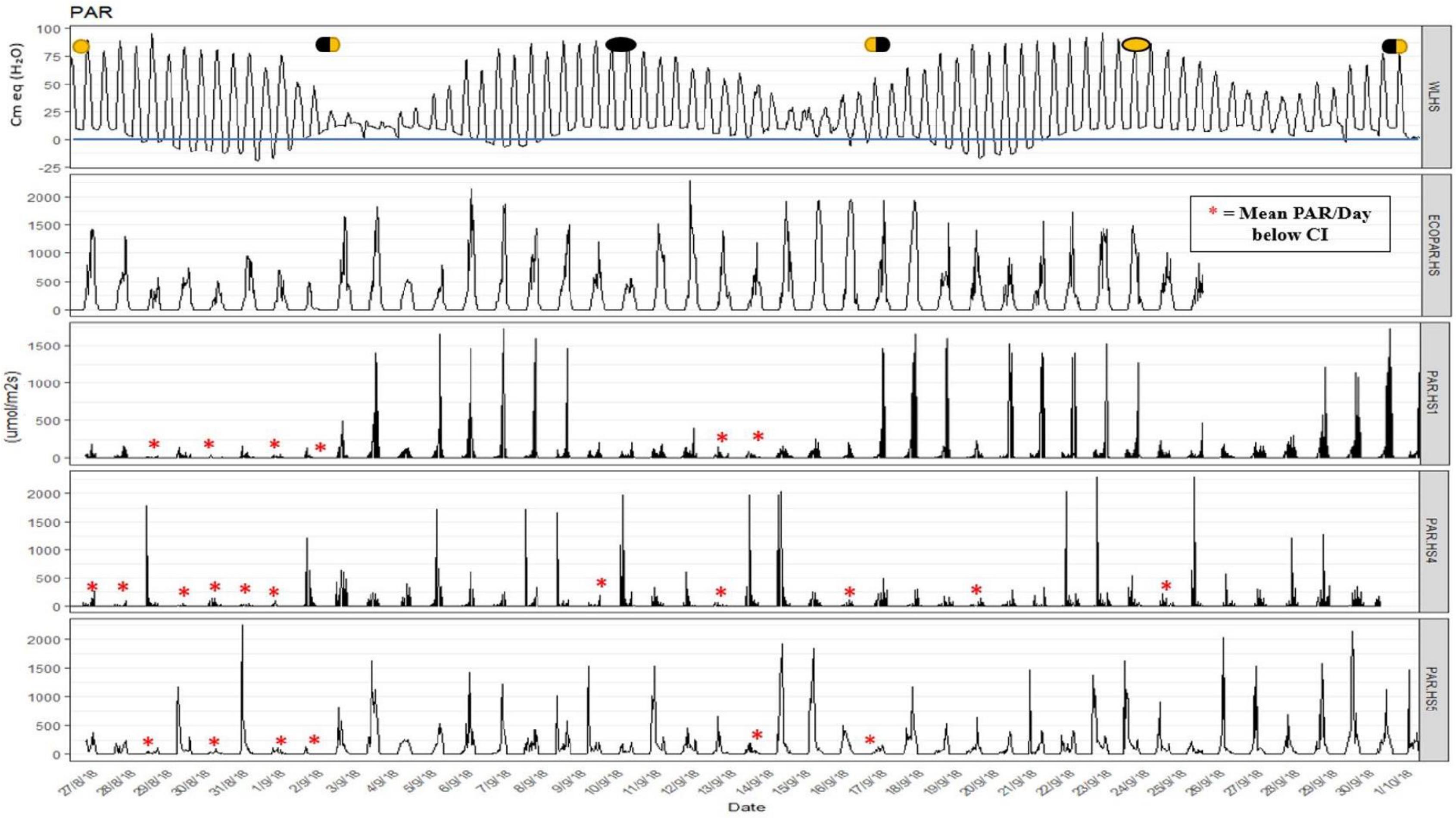


Figure S2. PAR across a complete tidal cycle in winter at historical seagrass (HS) sites. The top panel indicates relative water level and the second PAR collected with ECOPAR (http://www.seabird.com/ecopar) loggers is indicated. The other series show data collected with HOBO loggers.

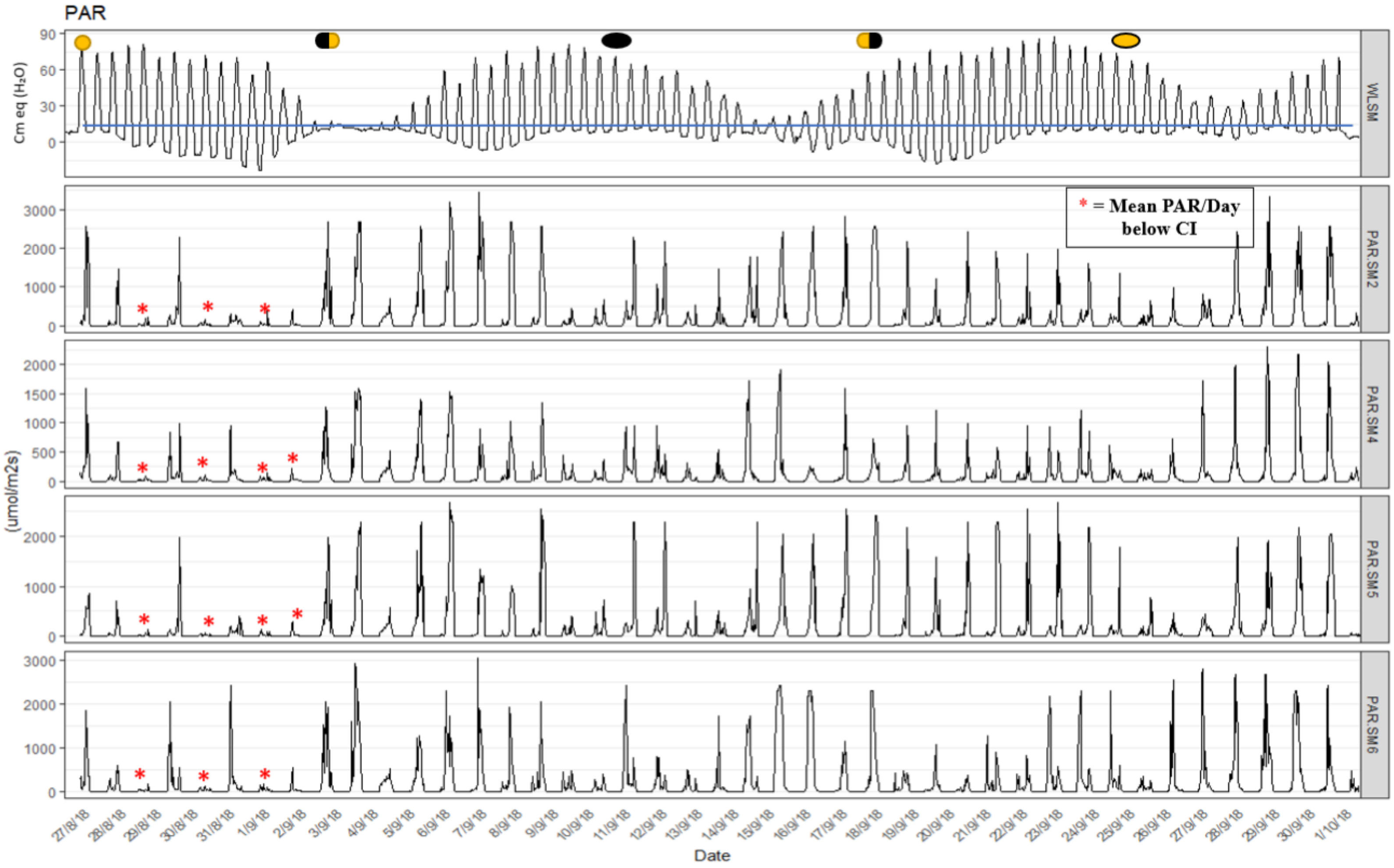


Figure S3. PAR across a complete tidal cycle in winter at existing seagrass (ES) sites. The top panel indicates relative water level. The other series show data collected with HOBO loggers.

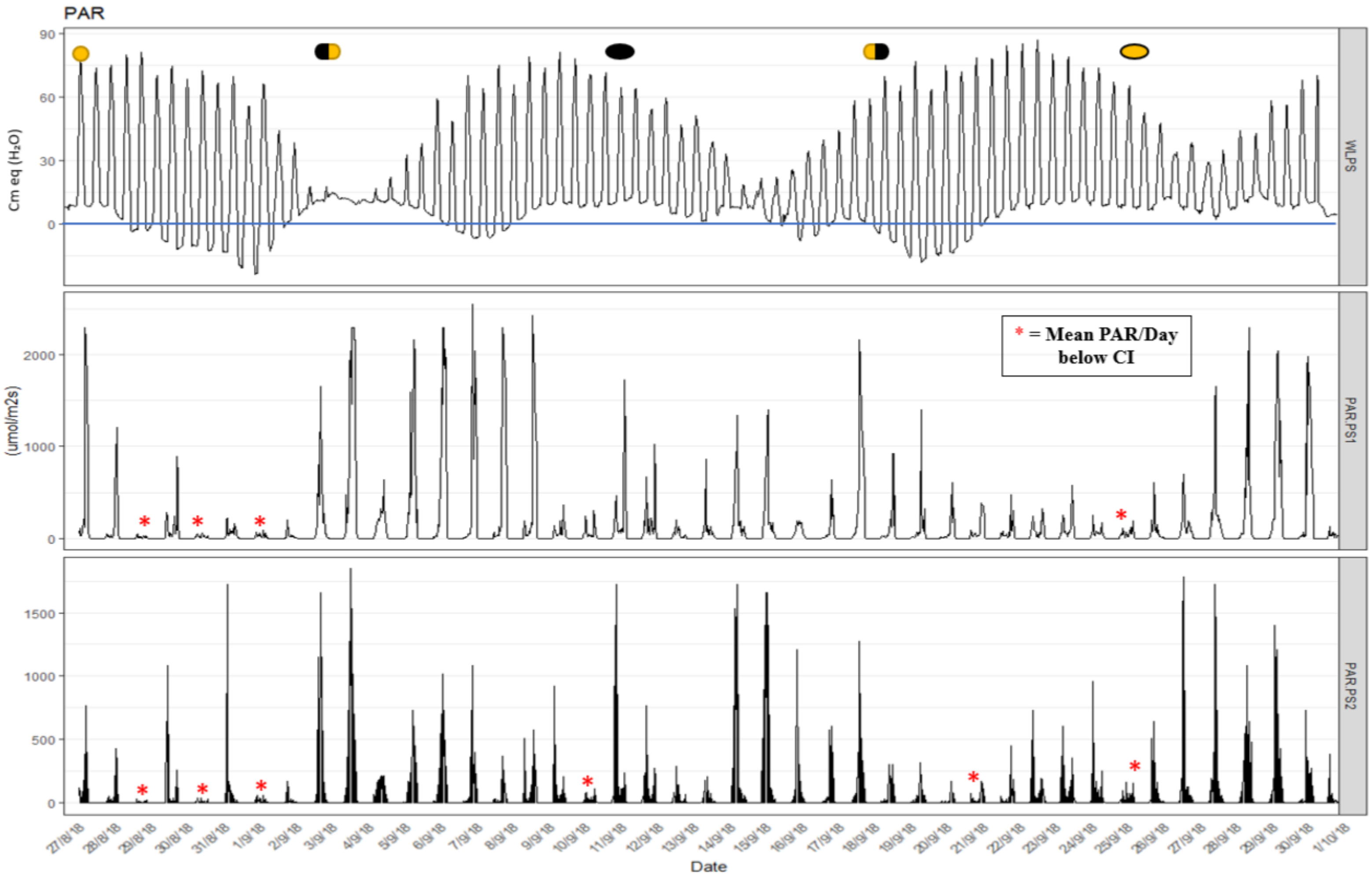


Figure S4. PAR across a complete tidal cycle in winter at potential seagrass (PS) sites. The top panel indicates relative water level. The other series show data collected with HOBO loggers.

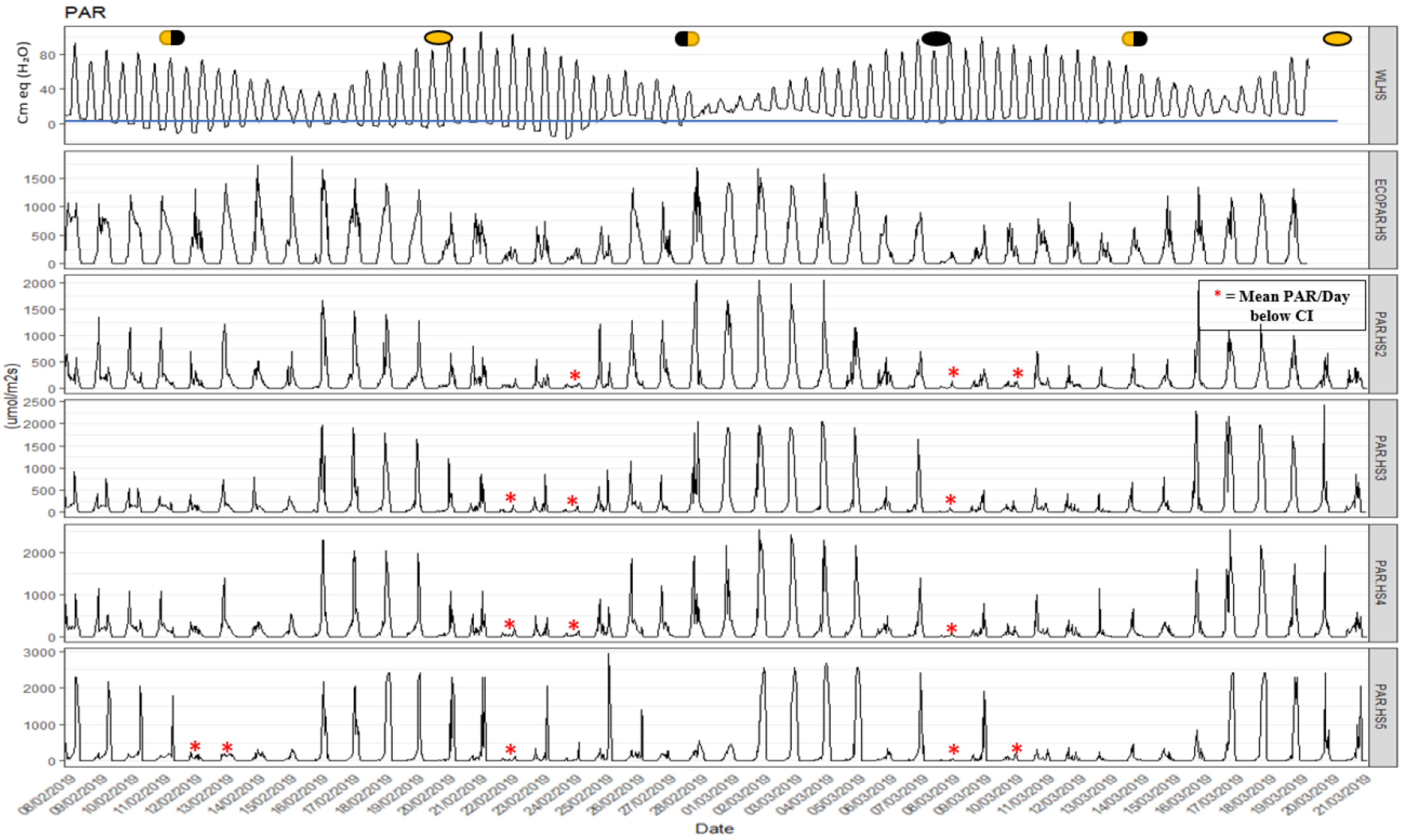


Figure S5. PAR across a complete tidal cycle in summer at historical seagrass (HS) sites. The top panel indicates relative water level, and the second PAR collected with ECOPAR (http://www.seabird.com/ecopar) loggers is indicated. The other series show data collected with HOBO loggers.

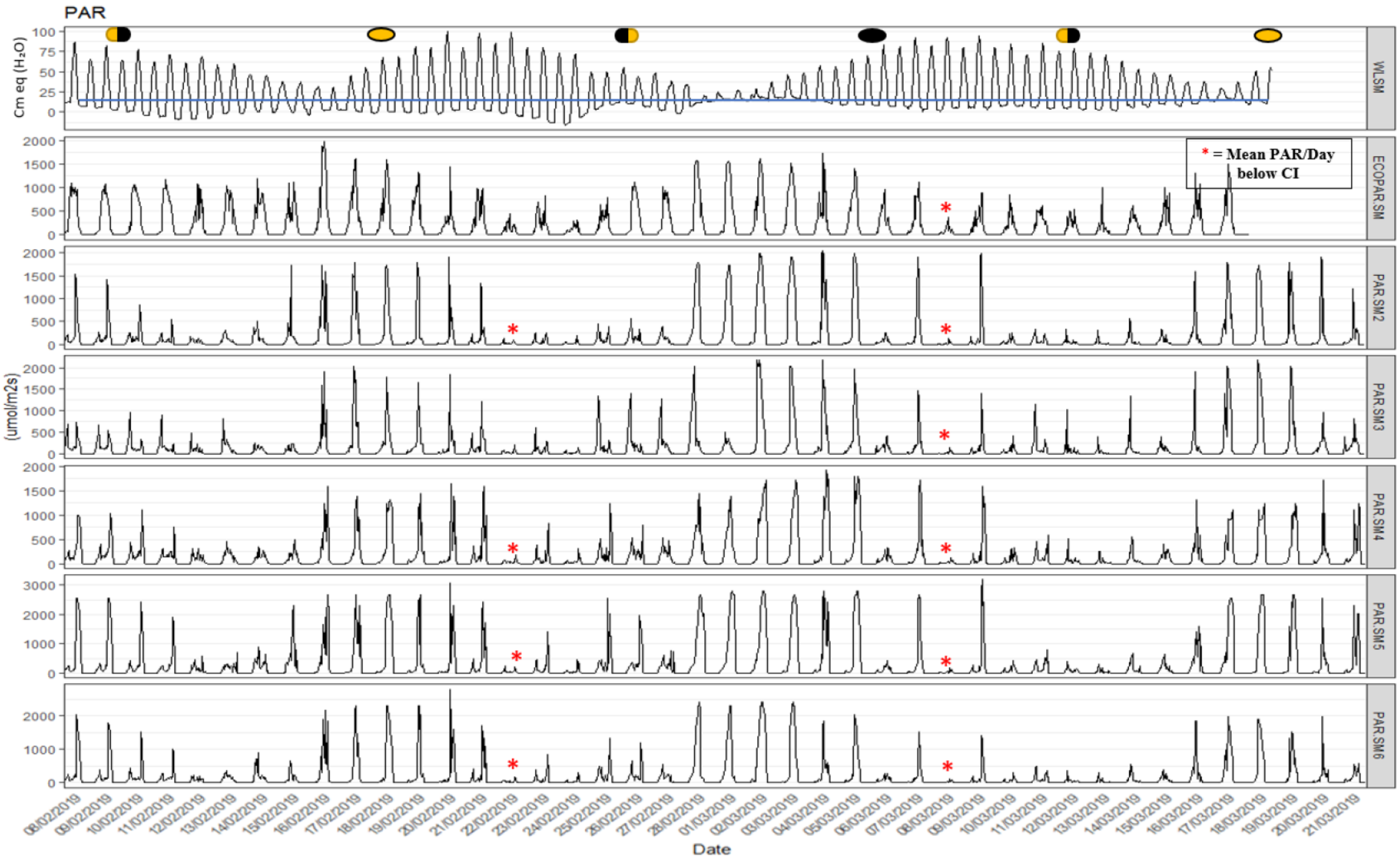


Figure S6. PAR across a complete tidal cycle in summer at existing seagrass (ES) sites. The top panel indicates relative water level, and the second PAR collected with ECOPAR (http://www.seabird.com/ecopar) loggers is indicated. The other series show data collected with HOBO loggers.

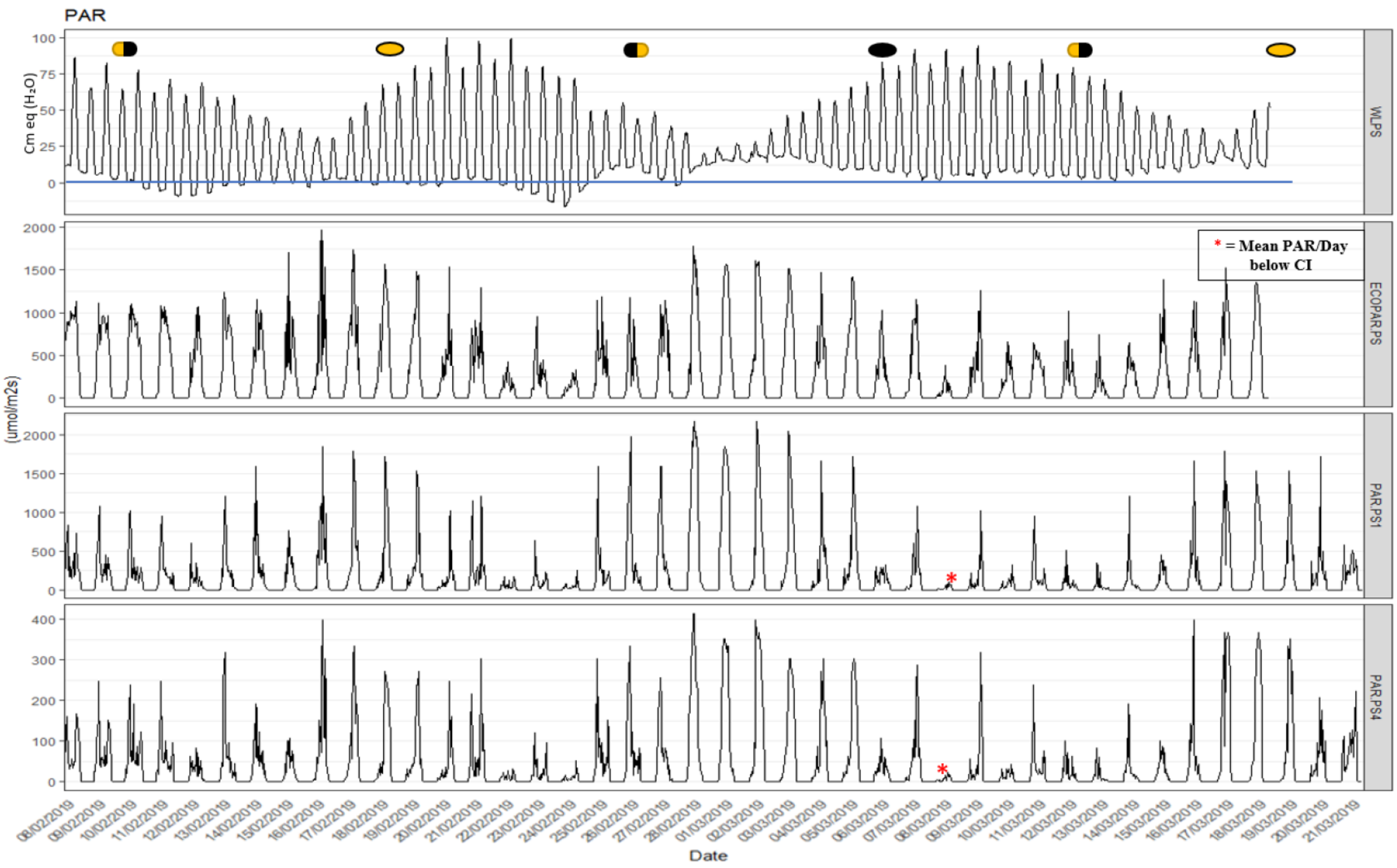


Figure S7. PAR across a complete tidal cycle in summer at potential seagrass (PS) sites. The top panel indicates relative water level, and the second PAR collected with ECOPAR (http://www.seabird.com/ecopar) loggers is indicated. The other series show data collected with HOBO loggers.

4. Seagrass substrate and porewater correlations

Seagrass and substrate Pearson correlation matrix is shown in Table S3. Seagrass % cover relationship with % mud, % organic matter, porewater PO_4^{3-}, and NH_4^+ from 0 to 5 cm are shown in (Equations 1,2,3,4). Relationship of other seagrass traits with substrate are also shown in Figure S8.

$$\%\ \text{Cover} = \frac{89.8184}{1+e^{(-(-3.0134+0.307Mud))}} \tag{1}$$

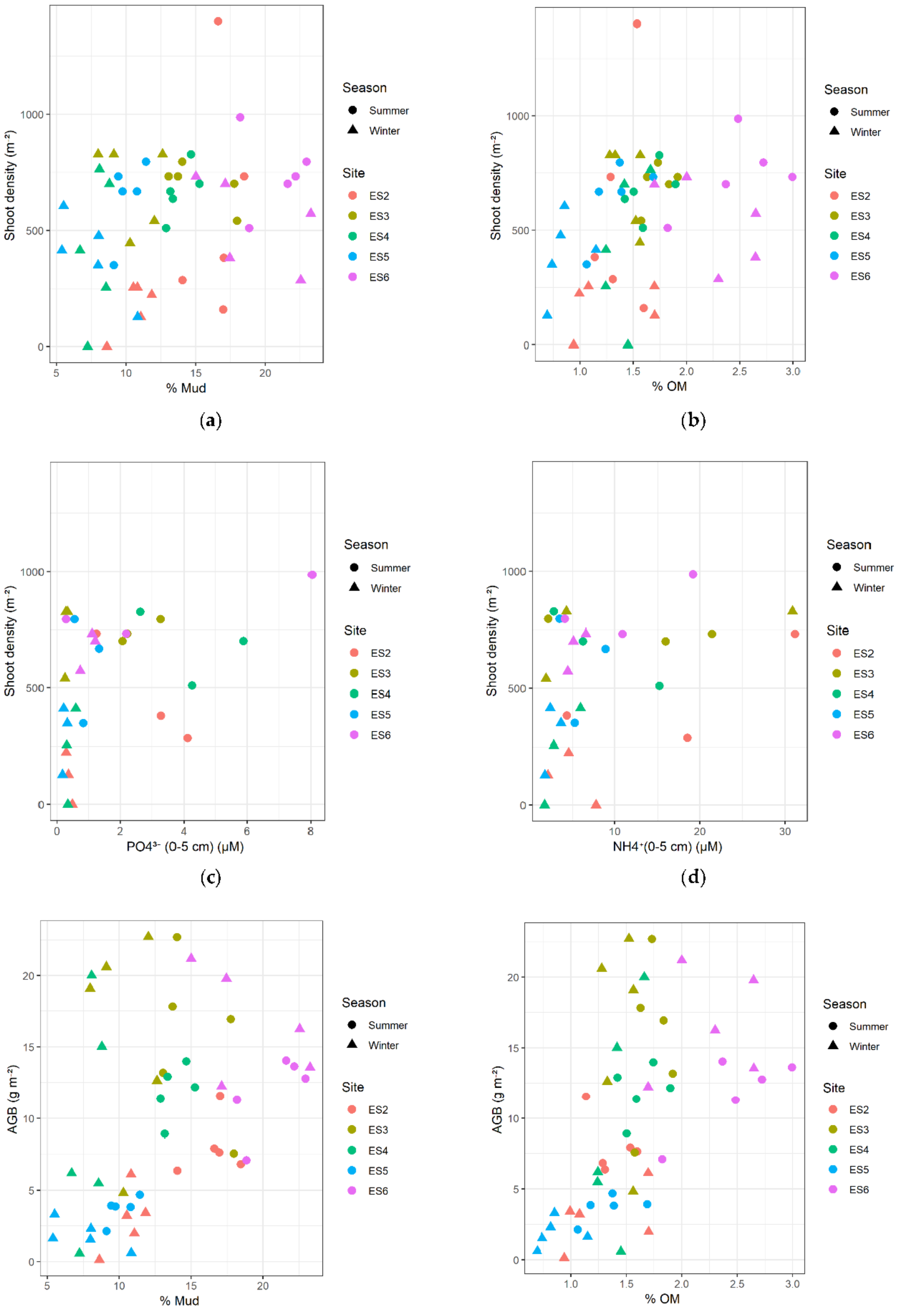
$$\%\ \text{Cover} = \frac{77.647}{1+e^{(-(-7.668+6.683Sed.OM))}} \tag{2}$$

$$\%\ \text{Cover} = 18.241\ \ln(PO_4^{3-}) + 60.184;\ R^2 = 0.3619 \tag{3}$$

$$\%\ \text{Cover} = 21.451\ \ln(NH_4^+) + 20.475;\ R^2 = 0.2964 \tag{4}$$

Table S3. A variable by variable matrix of Pearson correlation coefficients based on observations from Pāuatahanui Inlet, New Zealand. Significant correlations are indicated in bold type, (probability > |r| under H_o: Rho = 0; *n* = 30 observations).

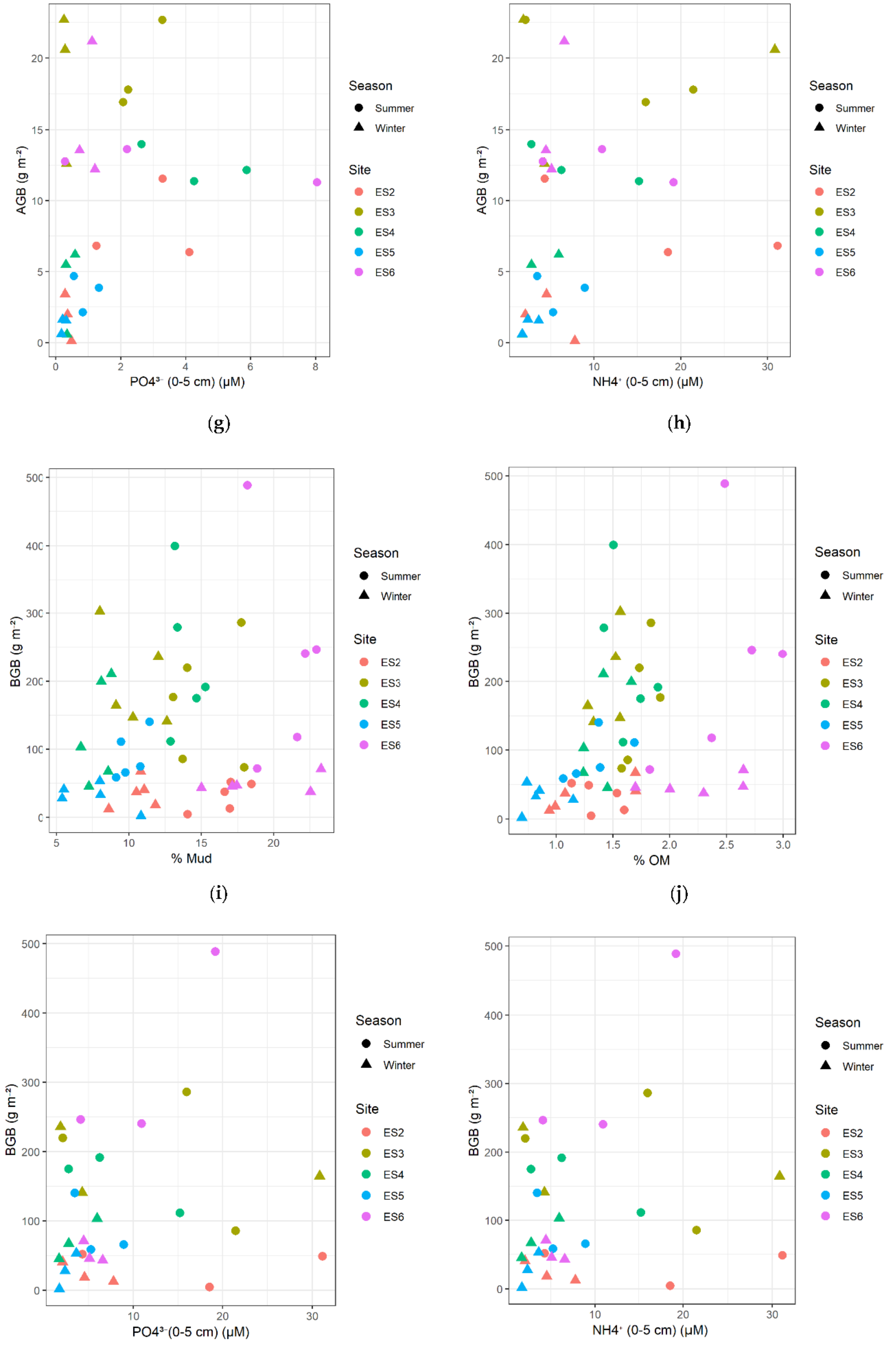
	%Cover	**Shoot d.**	**AGB**	**BGB**	**Biomass**	**Mud**	**Sand**	**Bulk d.**	**OM**	**PAR**	**Eh**	**$H_2S0.5$**	**$H_2S5.10$**	**PO_4^{3-}**	**$PO_4^{3-}510$**	**NH_4^+05**	**NH_4^+510**
%Cover	1	**0.73**	**0.77**	**0.54**	**0.58**	**0.65**	**-0.65**	0.18	**0.62**	-0.00	**0.64**	0.20	0.22	**0.52**	0.26	**0.46**	-0.11
Shoot d.	**0.73**	1	**0.70**	**0.67**	**0.70**	**0.54**	**-0.52**	0.06	**0.55**	0.22	**0.58**	0.23	0.18	**0.39**	0.18	**0.37**	-0.01
AGB	**0.77**	**0.70**	1	**0.53**	**0.57**	**0.49**	**-0.49**	0.19	**0.52**	0.16	**0.55**	0.08	0.23	0.27	**0.27**	0.26	**0.29**
BGB	**0.54**	**0.67**	**0.53**	1	**0.99**	**0.44**	**-0.43**	0.03	**0.62**	0.07	**0.39**	0.28	0.17	**0.55**	0.14	0.20	0.00
Biomass	**0.58**	**0.70**	**0.57**	**0.99**	1	**0.46**	**-0.45**	0.04	**0.63**	0.07	**0.41**	0.28	0.18	**0.55**	0.16	0.21	0.02
Mud	**0.65**	**0.54**	**0.49**	**0.44**	**0.46**	1	**-0.99**	0.03	**0.79**	-0.16	**0.64**	0.33	0.09	**0.37**	**0.51**	0.21	0.18
Sand	**-0.65**	**-0.52**	**-0.49**	**-0.43**	**-0.45**	**-0.99**	1	-0.03	**-0.81**	0.19	**-0.64**	-0.33	-0.1	-0.36	**-0.53**	-0.19	-0.19
Bulk d.	0.18	0.06	0.19	0.03	0.04	0.03	-0.03	1	-0.02	-0.18	0.34	**0.57**	-0.01	0.31	0.09	0.26	-0.20
OM	**0.62**	**0.55**	**0.52**	**0.62**	**0.63**	**0.79**	**-0.81**	-0.02	1	-0.14	**0.51**	0.22	-0.02	**0.36**	**0.58**	0.08	0.21
PAR	-0.00	0.22	0.13	0.07	0.07	-0.16	0.19	-0.18	-0.14	1	-0.06	-0.29	0.12	0.22	-0.27	-0.02	-0.08
Eh	**0.64**	**0.58**	**0.55**	**0.39**	**0.41**	**0.64**	**-0.64**	0.34	**0.51**	-0.06	1	0.5	0.17	**0.54**	0.27	**0.38**	0.09
$H_2S0.5$	0.20	0.23	0.08	0.28	0.28	0.33	-0.33	**0.57**	0.22	-0.29	**0.50**	1	0.22	**0.47**	-0.05	0.33	-0.17
$H_2S5.10$	0.22	0.18	0.23	0.17	0.18	0.09	-0.10	-0.01	-0.02	0.12	0.17	0.22	1	0.1	-0.23	0.25	-0.14
PO_4^{3-}	**0.52**	**0.39**	0.27	**0.55**	**0.55**	**0.37**	-0.36	0.31	**0.36**	-0.22	**0.54**	**0.47**	0.10	1	0.03	0.32	-0.11
$PO_4^{3-}510$	0.26	0.18	**0.27**	**0.14**	0.16	**0.51**	**-0.53**	-0.09	**0.58**	-0.27	0.27	-0.05	-0.23	0.03	1	-0.00	**0.66**
NH_4^+05	**0.46**	**0.37**	0.26	0.20	0.21	0.21	-0.19	0.26	0.08	-0.02	**0.38**	0.33	0.25	0.32	-0.00	1	-0.21
NH_4^+510	-0.11	0.01	**0.29**	0.00	0.02	0.18	-0.19	-0.20	0.21	-0.08	0.09	-0.17	-0.14	-0.11	**0.66**	-0.21	1


Shoot density (m⁻²)
0
500
1000
5
10
15
20
% Mud
% OM
1.0
1.5
2.0
2.5
3.0
PO4³⁻ (0-5 cm) (μM)
0
2
4
6
8
NH4⁺(0-5 cm) (μM)
10
20
30
AGB (g m⁻²)
Season
● Summer
▲ Winter
Site
● ES2
● ES3
● ES4
● ES5
● ES6

(**a**) (**b**)

(**c**) (**d**)

(**e**) (**f**)


AGB (g m⁻²)
PO4³⁻ (0-5 cm) (µM)
NH4⁺ (0-5 cm) (µM)
BGB (g m⁻²)
% Mud
% OM
Season
Summer
Winter
Site
ES2
ES3
ES4
ES5
ES6

(g) (h)

(i) (j)

(k) (l)

Figure S8. Scatter plots of seagrass biomass versus substrate conditions at existing seagrass (ES). (**a**) Seagrass shoot density vs. substrate % mud; (**b**) seagrass shoot density vs. substrate % organic matter; (**c**) seagrass shoot density vs. porewater PO_4^{3-}; (**d**) seagrass shoot density vs. porewater NH_4^+; (**e**) seagrass above ground biomass (AGB) vs. substrate % mud; (**f**) seagrass AGB vs. substrate % organic matter; (**g**) seagrass AGB vs. porewater PO_4^{3-}; (**h**) seagrass AGB vs. porewater NH_4^+; (**i**) seagrass below ground biomass (BGB) vs. porewater % mud; (**j**) seagrass BGB vs. porewater % organic matter; (**k**) seagrass BGB vs. porewater PO_4^{3-}; and (**l**) seagrass AGB vs. porewater NH_4^+.

Chapter 4 Appendix

Table A.4.1. Seagrass traits at the end of the experiment. Values are means (±SE) per treatment. Significant differences (ANOVA, Tukey HSD, p<0.05) among treatments are indicated by alphabetic superscripts.

Treatment	**Increase in shoot number (m^{-2})**	**Rhizome growth (cm)**	**AGB ($g\ m^{-2}$)**	**BGB ($g\ m^{-2}$)**	**Total Biomass ($g\ m^{-2}$)**	**Senescence ratio**
ESLL	20.6 ± 3.8^{b}	6.5 ± 0.9^{d}	2.063 ± 0.31^{a}	10.85 ± 1.63^{b}	12.913 ± 1.5^{b}	0.25 ± 0.03^{a}
HSLL	23.4 ± 5.6^{b}	1.1 ± 0.9^{b}	3.544 ± 1.13^{a}	6.069 ± 2^{a}	9.613 ± 2.11^{a}	0.30 ± 0.05^{a}
ESVL	22.5 ± 3.1^{b}	3.7 ± 0.8^{c}	2.419 ± 0.75^{a}	11.006 ± 1.44^{b}	13.425 ± 1.27^{b}	0.22 ± 0.06^{a}
HSVL	4.4 ± 2.5^{a}	-2.6 ± 1.1^{a}	1.619 ± 0.38^{a}	7.39 ± 1.63^{a}	9.006 ± 1.76^{a}	0.33 ± 0.12^{a}

Table A.4.2. Element concentration on the substrate and bioaccumulated on seagrasses aboveground biomass (AGB) and belowground biomass (BGB). Significant differences (p<0.05, Tukey HSD test) among seagrass biomass fraction and treatments are indicated by different alphabetic superscripts and * correspondingly. Elements reported exceeding (ANZECC, 2000) recommended Sediment Quality Guidelines (SQG) are shown in bold.

Habitat	**Biomass**	**Element**	**Mean (mg Kg^{-1})**	**max**	**min**	**se**	**SQG-High (mg Kg^{-1})**
Historical seagrass	AGB	Ag	101.6 ^{a}	184	57	41.2	
	BGB		86.3 ^{a*}	91	79	3.7	
	Substrate		140 ^{a}	207	60	42.9	-
Existing seagrass	AGB	Ag	125.7 ^{a}	215	72	45	
	BGB		209.7 ^{b*}	221	190	9.9	
	Substrate		301.3 ^{b}	318	269	16.2	-
Historical seagrass	AGB	B	206.3 ^{a}	260	100	53.2	
	BGB		152 ^{a*}	198	122	23.4	
	Substrate		312.3 ^{b}	364	284	25.9	-
Existing seagrass	AGB	B	271.3 ^{a}	365	101	85.3	
	BGB		217.7 ^{a*}	383	124	82.9	
	Substrate		262.7 ^{a}	293	232	17.6	-
Historical seagrass	AGB	Cd	2.2 ^{a*}	3.1	1.8	0.4	
	BGB		3.2 ^{b*}	3.8	2.8	0.3	
	Substrate		**33.7** ^{b}	**38**	**29**	**2.6**	**10**
Existing seagrass	AGB	Cd	5.8 ^{b*}	7.1	5.0	0.6	
	BGB		4.2 ^{a*}	4.5	3.7	0.2	
	Substrate		**17.7** ^{a}	**22**	**11**	**3.4**	**10**
Historical seagrass	AGB	Ba	318.3 ^{b*}	364	292	22.9	
	BGB		264 ^{a*}	291	231	17.6	
	Substrate		358.7 ^{b}	402	334	21.7	-
Existing seagrass	AGB	Ba	218 ^{b*}	300	141	46	
	BGB		117 ^{a*}	134	104	8.9	
	Substrate		276 ^{a}	392	54	111	-

Habitat	**Biomass**	**Element**	**Mean (mg Kg^{-1})**	**max**	**min**	**se**	**SQG-High (mg Kg^{-1})**
Historical seagrass	AGB	Pb	79.9 ^{a*}	92.8	25.4	22.3	
	BGB		99.2 ^{b*}	111.8	31.4	6.2	
	Substrate		**556.7** ^{a}	**936**	**580**	**101.4**	**220**
Existing seagrass	AGB	Pb	90.4 ^{a*}	105.8	42.8	11.4	
	BGB		80.8 ^{a*}	108.9	32.6	9.7	
	Substrate		**718.4** ^{a}	**742**	**678**	**18.6**	**220**
Historical seagrass	AGB	U	45 ^{a*}	61	35	8.1	
	BGB		127.3 ^{b*}	217	77	45	
	Substrate		112.33 ^{a}	150	46	33.27	-
Existing seagrass	AGB	U	61.3 ^{a*}	69	56	3.9	
	BGB		72.7 ^{b*}	81	63	5.2	
	Substrate		141.67 ^{a}	143	140	0.88	-
Historical seagrass	AGB	Na	211.7 ^{a*}	263	172	27	
	BGB		258.3 ^{b*}	295	238	18.4	
	Substrate		277.33 ^{a}	307	228	24.84	-
Existing seagrass	AGB	Na	278.3 ^{a*}	350	139	69.7	
	BGB		317.7 ^{a*}	340	289	15.1	
	Substrate		302.33 ^{a}	325	266	18.35	-
Historical seagrass	AGB	Mg	209.7 ^{a}	168	99	55.4	
	BGB		212 ^{a}	378	98	84.9	
	Substrate		86.33 ^{a}	136	59	24.88	-
Existing seagrass	AGB	Mg	165.3 ^{a}	193	147	14.1	
	BGB		152.3 ^{a}	169	140	8.6	
	Substrate		166 ^{b}	171	157	4.51	-
Historical seagrass	AGB	Al	305.3 ^{b*}	349	226	39.7	
	BGB		256.7 ^{a}	290	239	16.7	
	Substrate		147.33 ^{a}	229	98	41.13	-
Existing seagrass	AGB	Al	178 ^{a*}	195	166	8.7	
	BGB		276.7 ^{b}	328	236	27.1	
	Substrate		198.33 ^{b}	201	194	2.19	-
Historical seagrass	AGB	P	139 ^{a*}	170	119	15.7	
	BGB		165.3 ^{b*}	178	155	6.7	
	Substrate		285 ^{b}	391	108	89.07	-
Existing seagrass	AGB	P	249.7 ^{b*}	261	243	5.7	
	BGB		181 ^{a*}	194	174	6.5	
	Substrate		130 ^{a}	134	124	3.06	-
Historical seagrass	AGB	S	297.3 ^{b*}	360	265	31.33	
	BGB		201.3 ^{a}	361	105	8.0	
	Substrate		378.67 ^{a}	405	361	13.42	-
Existing seagrass	AGB	S	163 ^{a*}	171	150	6.6	
	BGB		210 ^{b}	382	121	8.6	
	Substrate		371.33 ^{a}	381	365	4.91	-
Historical seagrass	AGB	K	125.3 ^{a*}	164	97	20	
	BGB		278 ^{b*}	366	123	77.7	
	Substrate		309 ^{a}	352	273	23.07	-
Existing seagrass	AGB	K	247 ^{b*}	264	233	9.1	
	BGB		162.7 ^{a*}	192	129	18.3	
	Substrate		334 ^{a}	339	326	4.04	-
Historical seagrass	AGB	Ca	205.3 ^{b}	237	145	30.2	
	BGB		143.3 ^{a}	162	126	10.4	
	Substrate		164 ^{b}	202	95	34.56	-
Existing seagrass	AGB	Ca	290 ^{b}	380	116	87.3	
	BGB		167 ^{a}	207	137	20.8	
	Substrate		61 ^{a}	65	55	3.06	-

Habitat	Biomass	Element	Mean (mg Kg^{-1})	max	min	se	SQG-High (mg Kg^{-1})
Historical seagrass	AGB	V	218.7 ^{a*}	250	158	30.3	
	BGB		215 ^{a}	235	202	10.1	
	Substrate		308.33 ^{a}	384	250	39.64	-
Existing seagrass	AGB	V	114 ^{a*}	151	95	18.5	
	BGB		210 ^{b}	230	191	11.3	
	Substrate		400.67 ^{b}	406	397	2.73	-
Historical seagrass	AGB	Cr	27.4 ^{a*}	54.2	13.2	13.4	
	BGB		57.8 ^{c*}	66.4	51.6	4.4	
	Substrate		378.0 ^{a}	523	157	77.6	**370**
Existing seagrass	AGB	Cr	45.8 ^{b*}	49.2	44	1.6	
	BGB		59.2 ^{c*}	70.4	51	3.2	
	Substrate		574 ^{b}	585	559	7.7	**370**
Historical seagrass	AGB	Mn	273.7 ^{a}	342	152	61	
	BGB		340.7 ^{b*}	356	323	9.6	
	Substrate		343 ^{b}	349	338	3.21	-
Existing seagrass	AGB	Mn	203.7 ^{a}	210	197	3.8	
	BGB		263.7 ^{b*}	358	102	81.2	
	Substrate		278.33 ^{a}	379	196	53.62	-
Historical seagrass	AGB	Fe	168 ^{a*}	269	117	51	
	BGB		338.3 ^{c*}	363	325	12.3	
	Substrate		223.67 ^{a}	309	169	43.23	-
Existing seagrass	AGB	Fe	221.7 ^{b*}	240	211	9.2	
	BGB		331.7 ^{c*}	362	294	20	
	Substrate		316.67 ^{b}	323	313	3.18	-
Historical seagrass	AGB	Co	97.3 ^{a*}	180	55	41.3	
	BGB		121 ^{a}	186	85	32.6	
	Substrate		86.33 ^{a}	120	53	19.34	-
Existing seagrass	AGB	Co	187.3 ^{a*}	189	185	1.2	
	BGB		153.3 ^{a}	184	93	30.2	
	Substrate		114.67 ^{b}	121	111	3.18	-
Historical seagrass	AGB	Ni	1.3 ^{a*}	2.47	7.6	0.5	
	BGB		2.3 ^{b*}	2.57	2.2	0.12	
	Substrate		20.9 ^{a}	39.8	5.8	9.9	**52**
Existing seagrass	AGB	Ni	2.9 ^{a}	3.2	2.6	0.2	
	BGB		2.6 ^{a}	2.8	2.5	0.9	
	Substrate		34.5 ^{b}	37.4	32.9	1.5	**52**
Historical seagrass	AGB	Cu	44.5 ^{b*}	56.5	22.2	11.2	
	BGB		55.4 ^{c*}	73.9	21.5	17	
	Substrate		**445.4 ^{a}**	**562**	**321**	**69.6**	**270**
Existing seagrass	AGB	Cu	39.5 ^{b*}	45.4	32.4	3.8	
	BGB		27.1 ^{a*}	28.7	26.2	8	
	Substrate		**471 ^{a}**	**594**	**352**	**69.9**	**270**
Historical seagrass	AGB	Zn	59.3 ^{c}	75.6	31.5	13.1	
	BGB		60.8 ^{c}	64.8	56.4	2.6	
	Substrate		376.3 ^{b}	518	264	74.8	**400**
Existing seagrass	AGB	Zn	47.5 ^{b*}	58.6	26	10.8	
	BGB		34 ^{a*}	53.3	21.7	9.48	
	Substrate		210.3 ^{a}	250	150	30.8	**400**
Historical seagrass	AGB	As	12.0 ^{a*}	21.6	7.0	4.8	
	BGB		30.4 ^{c*}	31.8	27.7	1.3	
	Substrate		**103 ^{a}**	**156**	**52**	**30.0**	**70**
Existing seagrass	AGB	As	26.8 ^{b}	30	24.9	1.7	
	BGB		28.5 ^{b}	31.9	25.2	1.9	
	Substrate		**161.33 ^{b}**	**167**	**155**	**3.5**	**70**

Habitat	Biomass	Element	Mean (mg Kg^{-1})	max	min	se	SQG-High (mg Kg^{-1})
Historical seagrass	AGB	Se	40.3 ^{a*}	89	23	20.4	
	BGB		88.6 ^{b*}	94	84	3	
	Substrate		362.3 ^{b}	585	213	121.3	-
Existing seagrass	AGB	Se	52.0 ^{a*}	46	40	1.8	
	BGB		88.9 ^{b*}	96	81	4.3	
	Substrate		406.3 ^{a}	432	389	13.2	-
Historical seagrass	AGB	Sr	289.3 ^{b*}	347	177	56.2	
	BGB		202.3 ^{a}	232	176	16.2	
	Substrate		131.33 ^{a}	182	82	28.9	-
Existing seagrass	AGB	Sr	208.7 ^{a*}	227	196	9.4	
	BGB		215 ^{a}	228	205	6.8	
	Substrate		401.67 ^{b}	404	400	1.2	-

Aquatic Botany 175 (2021) 103435

Contents lists available at ScienceDirect

Aquatic Botany

journal homepage: www.elsevier.com/locate/aquabot

Interaction of substrate muddiness and low irradiance on seagrass: A mesocosm study of *Zostera muelleri*

Iñigo Zabarte-Maeztu[a,b,*], Fleur E. Matheson[a], Merilyn Manley-Harris[b], Robert J. Davies-Colley[a], Ian Hawes[c]

[a] *National Institute of Water & Atmospheric Research, Hamilton, 3216, New Zealand*
[b] *School of Science, University of Waikato, Hamilton, 3260, New Zealand*
[c] *Coastal Marine Field Station, University of Waikato, Tauranga, 3110, New Zealand*

ARTICLE INFO

Keywords:
Sedimentation
Pollution
Fine sediment
Muddification
Photosynthetically available radiation (PAR)
Rhizosphere
Anoxia
Seagrass

ABSTRACT

Seagrass meadows are important estuarine habitats, and in recent decades, have suffered global declines. Fine sediment pollution is recognised as a major cause of decline, usually attributed to the combined effects of reduction of photosynthetically available radiation (PAR) and burial. However, intruded fine sediment affects the seagrass rhizosphere and this interacts with reduced irradiance to affect seagrass performance. We undertook a 2 × 2 factorial mesocosm experiment, to examine the interaction between substrate muddiness and irradiance on seagrass growth and survival over a six-week period. The seagrass *Zostera muelleri* was grown on two substrates from the same estuary: (1) an inner estuary substrate with high mud content (42 %) from a location where seagrass formerly grew; and (2) an outer estuary substrate with moderate mud content (20 %) from a location at which seagrass persists. Two irradiance levels were used: (1) low (6.3 mol quanta m^{-2} d^{-1}) and (2) very low (2.3 mol quanta m^{-2} d^{-1}) both above a published compensation irradiance (E_c) of 1.9 mol quanta m^{-2} d^{-1}. Belowground biomass and rhizome growth were significantly reduced by substrate muddiness but not detectably affected by irradiance. Shoot growth, was reduced by both reduced irradiance and increased muddiness, with a significant interaction. We conclude that muddification of substrates imposes an increased irradiance requirement for *Z. muelleri* to cope with adverse rhizosphere conditions, which should be taken into account when planning seagrass conservation and restoration interventions.

1. Introduction

Seagrass meadows have a worldwide distribution in tropical and temperate coastal waters, (Waycott et al., 2009), where they support a wide range of ecosystem services and have socio-economic value estimated at $1.9 trillion per year (Orth et al., 2006; Waycott et al., 2009). These aquatic angiosperms act as 'ecological engineers' (*sensu* Jones et al., 1994), modifying their environment to create critical intertidal and subtidal habitat for many marine organisms, providing food and structural habitat (Jackson et al., 2001; Orth et al., 2006; Bertelli and Unsworth, 2014; Morrison et al., 2014). Seagrasses dampen wave action and reduce sediment erosion (Bos et al., 2007; Battley et al., 2011), oxygenate their rhizospheres and modify sediment biogeochemistry (Terrados et al., 1999; Enríquez et al., 2001; Borum et al., 2005; Marbà et al., 2010; Brodersen et al., 2015).

Seagrasses are, however, in decline across their range. It is estimated that 29 % of the known areal extent of seagrass has disappeared globally since 1879 (Waycott et al., 2009). Rates of decline have accelerated from a median of 0.9 % $year^{-1}$ before 1940 to 7% $year^{-1}$ since 1990, placing seagrass meadows among the most threatened ecosystems on earth (Cullen-Unsworth and Unsworth, 2018). Natural effects such as extreme climatic events, for example cyclonic storms, and biotic influences, such as plant diseases, avian grazing and invasive species, may contribute to the permanent or temporary loss of seagrass beds (Walker et al., 2007; Infantes et al., 2011). However, human-related activities that enhance supply of fine sediments to estuaries and coastal waters, such as soil disturbance and land clearing in catchments and dredging activities in harbours are often implicated in long-lasting seagrass declines (Costanza et al., 1997; Erftemeijer and Lewis, 2006; Burkholder et al., 2007; Cunha et al., 2012; Bainbridge et al., 2018).

In New Zealand, fine sediment is considered to be the most pervasive contaminant affecting estuaries and sheltered coastal embayments

---

* Corresponding author at: National Institute of Water & Atmospheric Research, Hamilton, 3216, New Zealand.
E-mail address: Inigo.Zabarte-Maeztu@niwa.co.nz (I. Zabarte-Maeztu).

https://doi.org/10.1016/j.aquabot.2021.103435
Received 24 April 2021; Received in revised form 4 August 2021; Accepted 11 August 2021

(Inglis, 2003; Thrush et al., 2004; Lohrer et al., 2006; Matheson and Schwarz, 2007; Matheson et al., 2010; Drylie et al., 2018; Gladstone-Gallagher et al., 2018; Dudley et al., 2020) and is thought to have contributed substantially to documented losses of the seagrass *Zostera muelleri* (Inglis, 2003; Matheson and Schwarz, 2007). Losses have been reported for Manukau Harbour (Turner, 1995), Waitemata Harbour (Hayward et al., 1999), Avon-Heathcote Estuary (Inglis, 2003), Whangarei Harbour (Reed et al., 2004), Porirua Harbour (Matheson and Wadhwa, 2012) and Tauranga Harbour (Park, 1999, 2016). In Tauranga Harbour, approximately one-third of intertidal seagrass and 90 % of sub-tidal seagrass were lost in the period from 1954 to 1996 and this has been linked to increased sediment loading of the estuary (Park, 2016; Ha et al., 2021).

Fine sediment increases the rate of light attenuation in the water column by increased light scattering, which increases the photon path length and therefore the probability of extinction (Kirk, 1985, 1994; Davies-Colley and Smith, 2001). Reduced photosynthetically available radiation (PAR) for photosynthesis impacts directly on the growth of seagrasses and other benthic primary producers (Erftemeijer and Lewis, 2006; De Boer, 2007; Adams et al., 2016; Bainbridge et al., 2018). For *Z. muelleri* recent studies have provided minimum mean daily irradiance dose (mol quanta m^{-2} d^{-1}) and compensation irradiances (E_c) (Table 1) which were used as reference values for the experimental design. Fine sediment deposition may also cause seagrass decline through whole or partial burial (Cabaço et al., 2008; Bainbridge et al., 2018). Acute ‘dumps’ of fine sediment in coastal waters resulting from storm events particularly where soils are exposed by construction, have been widely reported to cause large-scale alteration of seagrass habitat and catastrophic decline (Kirkman and Kuo, 1990; Fourqurean and Rutten, 2004; Suykerbuyk et al., 2016). Studies indicate a range of tolerance to burial in different seagrass taxa from 1 to 20 cm (Cabaço and Santos, 2007; Brodersen et al., 2017; Benham et al., 2019), largely correlated with plant size, with smaller plants generally more sensitive to burial than larger plants (Duarte et al., 1997; Cabaço et al., 2008). *Zostera muelleri* is a small species and as little as 0.75 cm of sediment deposition has been reported to cause damage (Benham et al., 2019).

Less is known about the effects of fine sediments entrained into the substrate, which can potentially modify rhizosphere physico-chemistry. “Muddification” is a term used to describe the increase in fine sediments and organic content of the substrate at the expense of sand (Van Katwijk et al., 2010), usually as a consequence of estuaries infilling with terrigenous sediments. Muddified substrates have low diffusivity due to small pore spaces and high bulk densities. Combined with the increased oxygen demand associated with organic content of fine sediments (Bainbridge et al., 2018) this usually results in a poorly oxygenated or anoxic substrate (Brodersen et al., 2017) and elevated concentration of phytotoxins such as heavy metals (Bonanno and Orlando-Bonaca, 2018) and sulphide (Borum et al., 2005; Pedersen et al., 2012). While seagrass can oxygenate their immediate rhizosphere though root oxygen release, this is dependent on high rates of photosynthesis (Brodersen et al., 2015, 2017; Brodersen et al., 2018).

While there is a considerable body of literature describing the effect upon seagrass of either fine sediment-driven light attenuation or burial, few studies have examined the interactions between them. Benham et al. (2019) demonstrated that these effects can independently or interactively cause significant damage to *Z. muelleri*, but other interactions among various effects of fine sediments on habitat quality have received little attention. The multiple effects of fine sediment on the estuarine environment make the likelihood of additive or interactive effects to be relevant to understanding the needs of restoration interventions. The link between the light-dependence of photosynthesis-driven rhizosphere oxygenation and the tolerance of highly reducing substrates points towards a potential interaction between irradiance and muddification stresses. In this contribution we test the hypothesis that low irradiance and substrate muddification may interact to reduce seagrass growth and persistence. To explore this, we undertook a factorial (2 × 2) mesocosm experiment, in which *Z. muelleri* sprigs were planted into two natural substrates, sourced from a single New Zealand estuary but differing in muddiness, and exposed to two low irradiance levels, the lowest just above known minimum persistence irradiance to increase the chance of detecting a light-substrate quality interaction.

Table 1
Minimum light requirements reported as photosynthetically active radiation (PAR) measured at maximum depth limit (MDL) or derived by experimentation for *Zostera muelleri* or synonyms.

Study location	Biogeography	Experiment type and minimum PAR threshold determination criteria	Minimum PAR threshold (mol quanta m^{-2} d^{-1})	Substrate composition	Reference
Gladstone Harbour, QLD, Australia	Tropical	Laboratory experiment. Minimum irradiance required for protection levels below at cool (c = 23 °C) and warm (w = 28 °C) water temperatures *Protection level:* 80 % shoot density 80 % growth rate 50 % shoot density 50 % growth rate	7.5 (c)*, 10.4 (w)* 4.8 (c), 7.2 (w) 3.4 (c), 5.7 (w) 2.2 (c), 5.0 (w)	Minimized substrate anoxia by using orchid pots and filter sock	(Collier et al., 2016)
Moreton Bay, QLD, Australia	Tropical	Reported irradiance at MDL	5–10	<20 % mud	(Longstaff, 2003)
Gladstone Harbour, QLD, Australia	Tropical	Field shading experiment. Minimum PAR derived from light history over a 4-year period to prevent measurable loss of seagrass	6.0	Semi-firm substrate composition	(Chartrand et al., 2016)
Kaipara Harbour, North Island, NZ	Temperate	Reported irradiance at MDL	4.9 (summer) 2.1 (winter)	Not reported	(Bulmer et al., 2016)
Tauranga Harbour, North Island, NZ	Temperate	Laboratory experiment. Five irradiance levels at 20 °C. Minimum required PAR to maintain biomass	2.9	Predominantly sand. Mud 2.7 %	Matheson pers com unpublished data
Tauranga Harbour, North Island, NZ	Temperate	Laboratory experiment. Compensation Irradiance (E_c). Irradiance where NPS = 0.	Emerged 12.1 Submerged 1.9	Substrate free	Zabarte-Maeztu unpublished data
Moreton Bay, QLD, Australia. Dunwich	Tropical	Laboratory experiment. Compensation Irradiance (E_c). Irradiance where NPS = 0.	1.95	Not reported	(Flanigan and Critchley, 1996)

* NPS Net photosynthesis.

2. Materials and methods

2.1. Substrate and plant collection, and incubations

Two substrate types were collected from Pāuatahanui Inlet, North Island, New Zealand: (1) an inner estuary site known to have high mud content (HM, Table 2) where seagrass formerly grew; and (2) an outer estuary substrate with a lower mud content (LM, Table 2) from a location at which seagrass persists (Fig. 1A). Previous research has associated fine sediment pollution with seagrass loss in this inner part of this inlet after 1980 (Matheson and Wadhwa, 2012). Substrates were transferred to the mesocosms at the University of Waikato field laboratory in Tauranga, New Zealand for incubations.

Substrates were sieved (2 mm) to remove infauna and left for 5 days for stabilization of oxygen profiles prior to planting the seagrass at average ambient temperature of 14.8 °C. Two irradiance levels were used: 6.3 mol quanta m ^{2}d ^{1} (LL- low light) and 2.3 mol quanta m ^{2}d ^{1} (VL- very low light); both above reported thresholds for temperate *Z. muelleri* (Table 1). Water temperature and irradiance were monitored at 30 min intervals using HOBO loggers (http://www.onsetcomp.com/) calibrated in lux, and PAR was measured on 3 occasions (beginning, middle and end of experiment) with a Li-Cor Li 192 Quantum Sensor (Li-Cor, Inc., US) for local calibration of the HOBO loggers. Dissolved oxygen in water and sediment was measured three times per week using a Needle Type oxygen microsensor, NTH-PSt7 (http://www.presense.com/) with three replicates per treatment and three profiles performed per tank. Sediment oxygen concentration was profiled at mm intervals from 0 to 40 mm depth, which corresponded to the seagrass root zone.

Vegetative fragments (sprigs) comprising a rhizome with apical meristem and three leaf shoots were collected at low tide from the intertidal close to the Tauranga field station (Fig. 1B), and immediately transported to the laboratory in seawater. Prior to planting, sprig rhizome length, and the numbers of shoots and internodes were recorded. Sprigs were transplanted individually into each tank and allowed to grow for 6 weeks before being harvested.

2.2. Experimental design and mesocosms setup

The mesocosm experiment was conducted in an indoor, recirculating mesocosm system using filtered seawater from Tauranga Harbour. The water content of each tank was renewed every two to three days in ‘batch-mode’ by siphoning off water and adding new water. The mesocosm system comprised 40 tanks arranged in two sub-systems, each supporting 20 × 1.8 l tanks. Treatments were randomised within each sub-system. A 16:8 h light:dark cycle was applied using J Series Cyanosis 1200 mm tubes (clear 4000 K–4500 K AC220−240 V CRI90 http://www.ecopoint.co.nz) positioned above each subsystem. PAR was averaged over the full 24 h (mol quanta m ^{2} d^{-1}) and irradiance was adjusted with different numbers of lighting tubes per subsystem to provide the required PAR. Under each irradiance, ten replicates of each of HM and LM sediment were used to give a total of four treatments. One sprig of freshly collected seagrass was transplanted into each mesocosm.

2.3. Monitoring and laboratory analyses

2.3.1. Seagrass condition

At the end of the 6-week incubation, plant material was extracted from each tank, rinsed with water, and separated into aboveground biomass (AGB - shoots and leaves) and belowground biomass (BGB - roots and rhizomes), respectively. Plant samples were dried at 80 °C to determine biomass per unit area (g m ^{2}) (Short and Coles, 2001).

Plant morphometric parameters were measured from digital images of each harvested plant using Image J software (https:/imagej.nih.gov/ij/). Images were calibrated using scale bars included in each image, and estimates of rhizome length, leaf length and leaf width made to +/- 0.1 mm. Increase in shoot number was calculated as the difference between the number of shoots at the end of the experiment and the number of shoots when transplanted. Rhizome length was used as the primary measure of initial plant size because of its importance in determining growth responses to disturbance in *Z. muelleri* (Macreadie et al., 2014). Senescent leaves were defined as those with > 50 % of leaf length lacking any green coloration and a “senescence ratio” was calculated for each plant as the ratio of senescent to total leaves.

2.3.2. Substrate condition

Substrate organic matter content (% OM) was estimated by loss-on-ignition after combustion at 450 °C for 4 h and bulk density (g m ^{3}) was determined as weight of dry solids in a known volume of substrate (Grove and Bilotta, 2014). Grain size was measured using a laser diffraction particle size analyser (Malvern Mastersizer 2000) and % mud particles (<63 μm) calculated. Porewater samples were extracted using a syringe to draw water through a hollow, 10 cm long and 4 mm diameter stainless steel cannula drilled with 1 mm diameter pores at intervals of 3 mm (McGlathery et al., 2001). Porewater samples were filtered through membrane filters (0.45 μm) for subsequent analysis of dissolved inorganic nutrients: nitrate, nitrite, ammonium and phosphate concentrations using a Lachat Quick Chem 8000 series flow injection analyser (FIA) + (Zellweger Analytics Inc. Milwaukee, Wisconsin, 53218, USA). Total sulphide was determined using the Methylene Blue APHA method (APHA 4500-S2-D).

Trace metal analysis was performed on transplanted sprigs and substrate samples to evaluate potential phytotoxicity, using an Agilent 8900 Inductively Coupled Plasma – Mass Spectrometer (ICP-MS; Agilent Technologies, Santa Clara, California, USA) controlled by a MassHunter Workstation (version 4.5). Substrate concentrations were evaluated for toxicity following ANZECC (2000) which are Australasian guidelines for general estuary health, rather than being specific to seagrass.

2.4. Data analysis

Statistical analysis of data was performed using the R statistical package (v 3.6.2) in R Studio. If not stated otherwise, all mean values are presented with standard errors of the mean (mean ± SE). Irradiance and substrate effects were tested using a two way-ANOVA after testing for normality and homogeneity of variance (Zar, 1984), followed by post hoc Tukey tests for multiple comparisons (Tukey, 1977) in the case of a significant ANOVA result (with significance level set, conventionally, at 5% probability of a Type I error).

Table 2
Physico chemical characteristics of the two substrates. Values are means (±SE). Significant differences ($p<0.05$, Tukey HSD test) between HM and LM substrates are indicated by different alphabetic superscripts.

Treatment	Sediment mud (%)	Sediment organic matter (%)	Sediment bulk density (g cm ^{3})	Sediment porewater [S^{2}] (μM)	Sediment porewater [NH_4^{+}] (μM)	Sediment porewater [PO_4^{3}] (μM)
High mud substrate HM	42 ± 1.7 ^{b}	2.3 ± 0.2 ^{a}	1.6 ± 0.1 ^{b}	2.6 ± 0.6 ^{b}	10.6 ± 1.5^{b}	3.1 ± 1.7 ^{a}
Low mud substrate LS	20 ± 0.9 ^{a}	2.4 ± 0.1 ^{a}	1.0 ± 0.04 ^{a}	1.4 ± 0.4 ^{a}	5.4 ± 0.6 ^{a}	2.3 ± 1.2 ^{a}

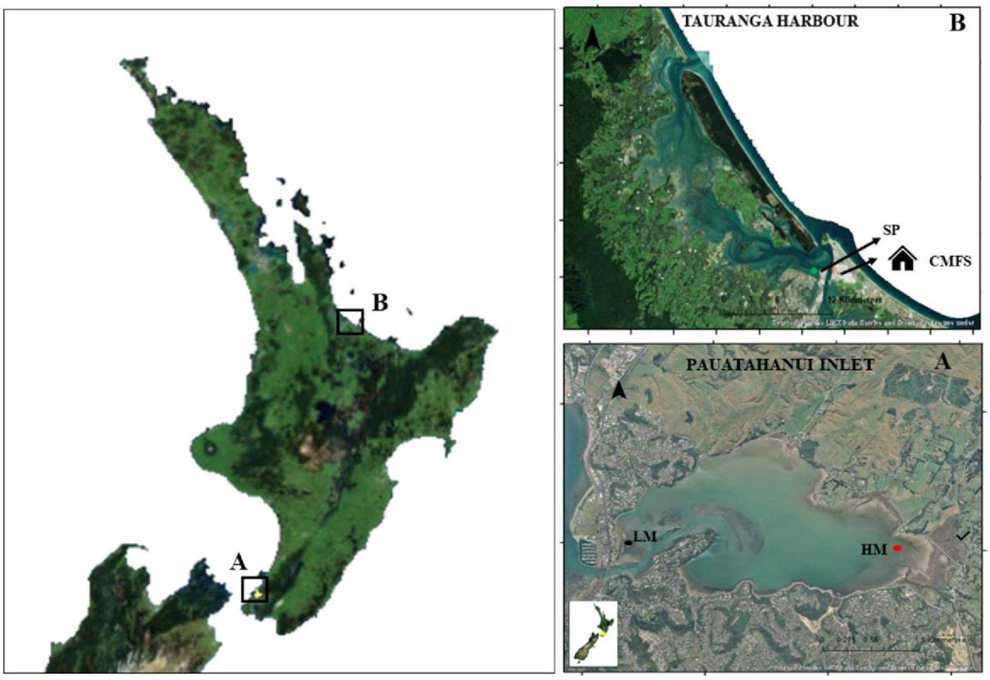


Fig. 1. Map of North Island, New Zealand indicating the locations of (A) Pāuatahanui Inlet where the two substrate types were collected: site in the inner estuary of Pāuatahanui Inlet where seagrass occurred historically but no longer grows with high mud content (HM), and site in the outer estuary where seagrass continues to persist with low mud content (LM). (B) Tauranga Harbour indicating the location of the seagrass meadow from which sprigs were collected Sulphur Point (SP). This location is close to Tauranga Coastal Marine Field Station of University of Waikato (CMFS), where the mesocosm experiment was conducted.

3. Results

3.1. *Substrate and PAR*

Substrate quality parameters and PAR per treatment as well as substrate trace metal concentrations are shown in Table 2, Fig. 2 & Supplementary Materials (Table 1). These confirm that HM substrate was muddier and denser, with lower pore space, than LM substrate, which concurs with the findings in (Zabarte-Maeztu et al., 2020). HM substrates also had higher concentrations of S^{2} and NH_4^+ in porewater, but not PO_4^{3}. Nitrate was not detectable.

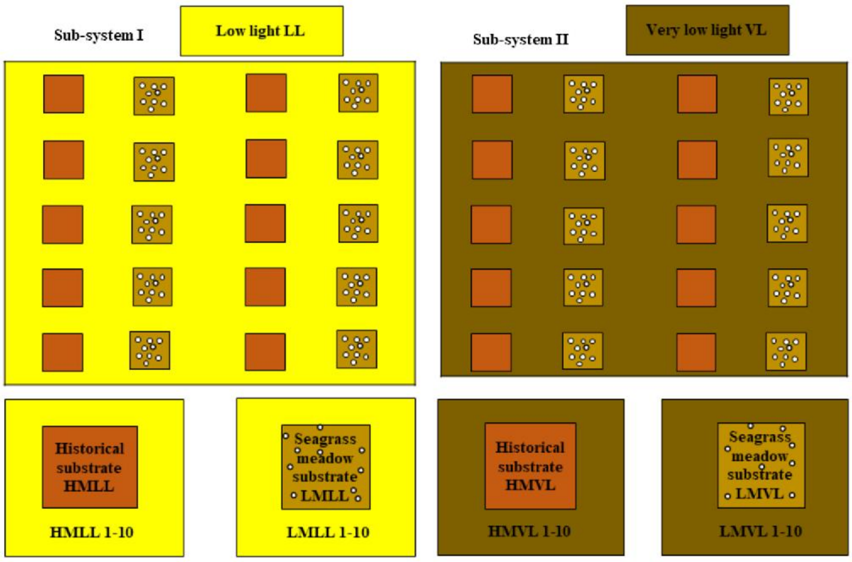


Fig. 2. Diagram showing the distribution of the two substrate types and the two PAR levels within the mesocosms sub-systems. Tanks are set out regularly for diagrammatic clarity, but, for the experiment, their position was randomized. The four treatments are coded as: high mud content substrate under low light (HMLL), low mud content substrate under low light (LMLL), high mud content substrate under very low light (HMVL) and low mud content substrate under very low light (LMVL). One sprig per tank was transplanted (10 replicates per treatment). Low light (LL) was 6.3 mol quanta m^{-2} d^{-1}; Very low light (VL) was 2.3 mol quanta m^{-2} d^{-1}. White points symbolize the greater porosity of LM substrate.

3.2. Seagrass response to mesocosm treatments

At harvest, the treatment combinations LMLL, LMVL and HMLL had similar shoot production (>20 m ^{2}) whereas HMVL had much lower shoot production averaging just 0.7 shoots tank ^{1} (Fig. 3A). Rhizome growth was ordered LMLL > LMVL>HMLL > HMVL with significant change at each step in the progression (Fig. 3B) suggesting that the substrate effect dominated irradiance for rhizome growth but that two stressors interacted. HMVL was the only treatment with negative rhizome growth (i.e. loss of rhizome biomass). As expected, aboveground biomass (AGB) was lowest in HMVL, but there was high variance and no significant differences among treatments (Fig. 3C). Belowground biomass (BGB) was significantly lower in HM treatments than in LM regardless of irradiance, suggesting an effect of substrate. Total biomass (Table SM1) followed BGB and was significantly lower in HM compared to LM treatments, with no significant effect of irradiance. AGB:BGB ratio showed no significant differences between treatments (Table SM1). Senescence was observed during the experiment, but senescence ratio was not significantly different among the treatments (Table SM1) (Fig. 4).

ANOVA (Table 3) showed that the increase in shoot number was significantly affected by irradiance ($p = 0.035$) and by the interaction between irradiance and substrate type ($p = 0.012$). Substrate muddiness alone did not significantly affect shoot number at the 5% level but it was significant at the 10 % level ($p = 0.09$). Rhizome growth was significantly influenced by both irradiance ($p = 0.001$) and substrate ($p <$ n0.0001), but not by their interaction. BGB and total biomass were significantly affected by substrate type ($p = 0.015$ and $p = 0.028$) but not by irradiance treatment. AGB and senescence indices were not significantly affected by any treatment or interaction.

3.3. Substrate oxygen concentration profiles

The temporal evolution of sediment oxygen profiles from week 1 to week 6 shows an initial separation between light treatments, with oxygen depletion with depth more rapid in VL treatments. There was a progressive convergence of profiles over time until by week 6 all were similar (Fig. 5). The PAR effect was larger than that of substrate type, although HM tended to have lower oxygen concentrations than LM. By six weeks, at the completion of the incubation, all substrates showed anoxia below 4–5 mm depth.

3.4. Substrate and bioaccumulated elemental concentration

Elemental concentrations are given in (Table SM2) for the substrate, BGB and AGB. Copper and lead concentrations in biomass exceeded reported toxicity thresholds, but other elements were below toxicity levels.

4. Discussion

This study examined the combined effects of substrate quality and low irradiance on seagrass performance. Clear effects of the two variables were found, both separately and interactively. All plants growing in muddified (HM) substrate had consistently lower rhizome growth and belowground biomass than those in less muddified substrate. There was no difference in belowground biomass between low and very low irradiances, regardless of sediment type, but shoot density was adversely affected by very low irradiance. There was an interaction between irradiance and substrate for shoot production such that the lowest shoot production was in the combination of HM and VL.

While low, the LL and VL irradiances used in this experiment were both sufficient to support biomass increase in terms of rhizome extension and new shoot generation at least in substrates with relatively low mud. Both experimental irradiances were above the winter limiting PAR threshold of 2.1 mol quanta m ^{2}d ^{1}, at 13 °C, estimated for *Z. muelleri* in Kaipara Harbour, New Zealand (Table 1) (Bulmer et al., 2016). Studies performed at a range of latitudes seem to be converging on a long-term overall daily minimum light requirement for *Z. muelleri* persistence of around 5 mol quanta m ^{2}d ^{1} which is lower than the LL but higher than the VL treatment (Table 1).

For tropical *Z. muelleri*, E_c (the irradiance at which oxygen production = respiration) is 45 μmol m ^{2}s ^{1}, which translates to a daily requirement of 1.9 mol quanta m ^{2}d ^{1} at 25 °C (Flanigan and Critchley,

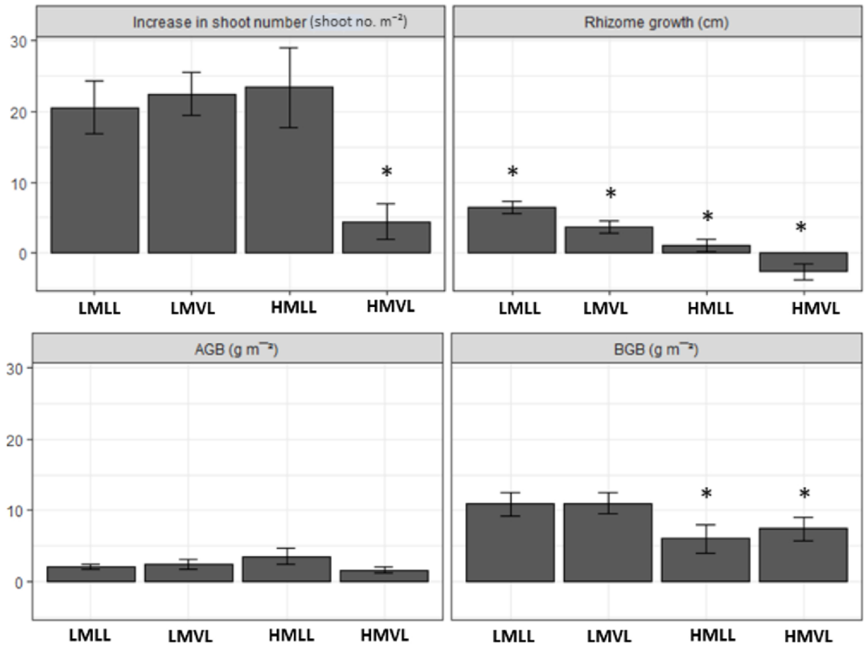


Fig. 3. Seagrass traits at the end of the experiment: Panels A-D show: A shoot number increment, B. rhizome growth, C. aboveground biomass (AGB) and D. belowground biomass (BGB). Values are means (±SE) per treatment (10 replicates per each treatment). Significant differences (ANOVA, Tukey HSD, $p < 0.05$) among treatments are indicated by (*). Treatments are coded as: low mud substrate under low light (LMLL), low mud substrate under very low light (LMVL) and high mud substrate under low light (HMLL), high mud substrate under very low light (HMVL).

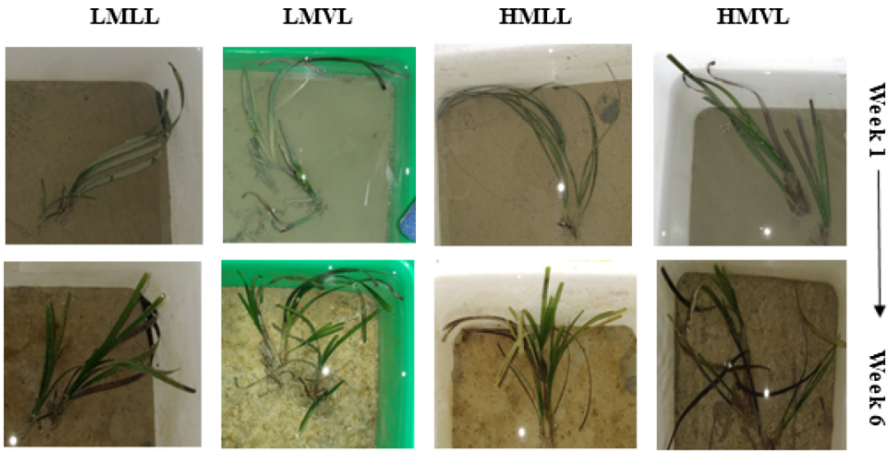


Fig. 4. Pictures show one representative replicate per treatment at the beginning of the experiment (top line) and (the same replicate) at the end of 6-weeks incubation (bottom line) immediately prior to harvesting. The four treatments are coded as: low mud substrate under low light (LMLL), low mud substrate under very low light (LMVL) and high mud substrate under low light (HMLL), high mud substrate under very low light (HMVL).

Table 3
2-way ANOVA results for seagrass traits. Significant effects and interactions ($p < 0.05$) between irradiance and substrate are shown in bold for each of the seagrass traits.

Trait	Factor	Df	Sum Sq	Mean sq	F value	Pr (>F)
Increase in shoot number	Substrate	1	13.23	13.225	3.023	0.0906
	Irradiance	1	21.02	21.025	4.806	**0.0349***
	Interaction	1	30.62	30.625	7	**0.012***
	Residual	36	157.50	4.375		
Rhizome growth	**Substrate**	1	337.6	337.6	38.884	**3.37e-07***
	Irradiance	1	105.6	105.6	12.163	**0.0013***
	Interaction	1	2.3	2.3	0.269	0.6072
	Residual	36	312.6	8.7		
AGB	Substrate	1	7.47	7.747	0.958	0.334
	Irradiance	1	8.75	8752	1.122	0.297
	Interaction	1	9.85	9.851	1.263	0.269
	Residual	36	280.83	7.801		
BGB	**Substrate**	1	4.516	4.516	6.519	**0.0151***
	Irradiance	1	0.139	0.139	0.201	0.6566
	Interaction	1	0.086	0.086	0.125	0.7259
	Residual	36	24.939	0.693		
Total Biomass	**Substrate**	1	3.813	3.813	5.228	**0.0282***
	Irradiance	1	0.001	0.001	0.001	0.978
	Interaction	1	0.08	0.08	0.11	0.7423
	Residual	36	26.256	0.729		
Senescence ratio	Substrate	1	2.81	2.814	0.766	0.388
	Irradiance	1	3.98	3.984	1.084	0.305
	Interaction	1	3.7	3.703	1.008	0.322
	Residual	34	124.91	3.674		

1996). Temperature plays an important role in determining PAR thresholds for seagrass persistence (York et al.,2013), with required irradiance tending to increase with increasing temperature (Table 1) (Flanigan and Critchley, 1996; Longstaff et al., 1999; Collier et al., 2011, 2012; Bulmer et al., 2016; Chartrand et al., 2016; Collier et al., 2016). Therefore, the fact that, E_c of tropical *Z. muelleri* is higher than the VL treatment, in which positive growth occurred in LM substrate, and also higher than PAR at the maximum depth limit determined for the winter season in Kaipara (Bulmer et al., 2016), may be explained by temperature effects. More photosynthesis is required at the compensation point to balance increased respiration at higher temperature as well as a higher substrate oxygen demand due to more rapid organic matter decomposition.

The HM substrate has a lower porosity and reduced chemistry compared to LM, consistent with the shallower oxycline, and high bulk density (c.f. Zabarte-Maeztu et al., 2020). Seagrasses have been shown to persist in hypoxic sediments through root oxygen release, a mechanism that requires adequate aboveground photosynthesis to oxygenate roots and their rhizosphere (Brodersen et al., 2015, 2017). Muddification may also have increased the prevalence of toxins such as hydrogen sulphide or heavy metals (Duarte et al., 1997; Terrados et al., 1999; Halun et al., 2002; Brodersen et al., 2015, 2017; Brodersen et al., 2018). In our experiment, belowground biomass was negatively affected by muddification, regardless of the irradiance, and only rhizome growth was affected by both irradiance and substrate. HMVL was the only treatment in which rhizome biomass declined, which clearly indicates the stress of muddification on growth and health of belowground structures. VL also reduced rhizome growth in non-muddified sediments, which suggests a reduced allocation of resources to belowground growth when irradiance is very low, even if the sediment conditions are favourable. Rhizomes are important, being the most persistent part of the plant and the main source of recovery after disturbance (Larkum and West, 1983; Marbà and Duarte, 1998; Meehan and West, 2000; Jarvis

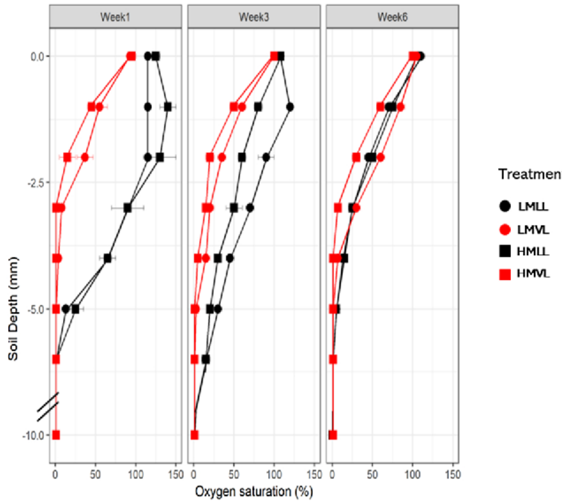


Fig. 5. Convergence over time of [O₂] depth profiles (means ± SE) among treatments. Figures truncate at 10 mm as profiles remain anoxic deeper than that substrate depth.

and Moore, 2010). A disturbance/recovery experiment performed on *Z. muelleri* populations at Lake Macquarie (Macreadie et al., 2014) concluded that asexual, clonal growth and regeneration (through rhizome extension) is the only available mode of recovery from small-scale disturbances (e.g., anchor, boat damage, grazing and wave-action in storms). Seeds and seed dispersal appear to be more important in the recovery response of seagrass beds to larger scale disturbances such as wasting disease, eutrophication and sediment loading (Orth et al., 2006).

The precise sediment chemical profile that makes HM less suited to seagrass growth than LM is challenging to infer from our data and indeed it may reflect a multiplicity of factors all arising from increased muddification. While porewater ammonium ion and total sulphide levels were significantly higher in HM substrates compared to LM, neither approached known toxicity thresholds. The highest recorded ammonium concentration of 10.6 µM in HM is well below the toxicity thresholds of $>1 \times 10^5$ µM, for *Z. muelleri* (Gladstone-Gallagher et al., 2018; Li et al., 2019) and 200–4000 µM for related species *Z. noltii* (Govers et al., 2014). Similarly, the highest total sulphide concentration of 2.6 µM is lower than thresholds of 10 µM and 13 µM determined by (Calleja et al., 2007; Krause-Jensen et al., 2011).

Substrate oxygen profiles were primarily related to irradiance and converged over time. Differences between light treatments, but not by muddiness, occurred in week one in the upper five millimetres of the substrate. During week 3, LL and VL treatments began to converge and this was complete by week 6. This effect is possibly caused by oxygen production by microphytobenthos (MPB) which is often a major contributor to oxygen production of estuaries (Barranguet et al., 1998; Thrush et al., 2012). Convergence of oxygen profiles during week 6 likely reflects lower MPB production rates during the last week of the experiment as MPB biomass apparently became progressively less effective in oxygenating surface sediments, perhaps through nutrient depletion (Jesus et al., 2009).

While we found clear evidence of significant interactions between sediment muddiness and irradiance on seagrass growth, we found that all treatments were largely anoxic within the 5−50 mm, seagrasses root zone, and thus could not conclusively demonstrate that root anoxia is the main or only seagrass inhibition mechanism that is being alleviated through increased irradiance. Our measures of sediment oxygen were, however, bulk measures and may not reflect immediate rhizosphere at the microscale. Toxicants related to anoxia, sulphide and ammonium ion were elevated in the muddified sediment, but not to known toxic concentrations, while other toxicants (arsenic, chromium, cadmium, copper and lead) concentrations exceeded (ANZECC, 2000) recommended Sediment Quality Guidelines (SQG) thresholds in both HM and LM sediments (Fig. SM1). These thresholds are generalized indicators of healthy sediment values, but are not necessarily seagrass related and therefore only cautious interpretation of their relevance to our experiment are possible. We suggest that interactions between muddification and irradiance in our study system are indicative of a complex of stressors including toxicants potentially acting at sublethal concentrations, physical properties such as porosity, partially offset by increased net photosynthesis. Nonetheless we suggest that they show how the combined effects of irradiance and sediment quality need to be included in other New Zealand and international restoration and conservation endeavours where degraded sediment and water columns are present.

5. Conclusion

Research on estuary pollution with fine sediments and its effect on seagrass performance has been focused mainly on the reduction of the PAR reaching the seabed. However, fine sediments may also stress seagrass by substrate muddification, which has been demonstrated to interact with irradiance in our mesocosm experiments. These interactions need to be further studied because seagrasses inhabiting muddified substrates appear to have increased light requirements for persistence due to reduced oxygen and/or other detrimental effects in the substrate such as increasing concentrations of sulphide or heavy metals. Therefore, use of PAR thresholds alone may not be sufficient for assessment of habitat suitability, and interactions with substrate condition may need to be considered. These findings are available to aid worldwide conservation and restoration of seagrasses undergoing sedimentation exerted multi stresses.

CRediT authorship contribution statement

Iñigo Zabarte-Maeztu: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft. **Fleur E. Matheson**: Conceptualization, Supervision, Review & editing, Funding acquisition. **Merilyn Manley-Harris**: Supervision, Methodology, Review & editing. **Robert J. Davies-Colley**: Conceptualization, Funding acquisition, Review & editing. **Ian Hawes**: Conceptualization, Validation, Resources, Writing-review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We thank Andrew Swales NIWA's Catchments-to-Estuaries Portfolio leader. NIWA-Wellington field staff: Peter Notman, Warrick Lyon and Dean Stotter assisted in the field. Margaret McMonagle (NIWA-Hamilton, Water Quality Laboratory staff) is thanked for helping with assays of sulphide on estuarine porewater samples. Rebecca Gibson (University of Waikato) assisted with nutrient analysis with FIA. We thank Megan Oliver from Greater Wellington Regional Council (GWRC) for the aid and support to this study. We thank Anne-Maree Schwarz for helpful comments on the manuscript and two anonymous reviewers for very useful comments on a previous version of this paper. This research was funded by the Strategic Science Investment programme Managing Mud from National Institute of Water and Atmospheric Research NIWA, grant number FWCE2011.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.aquabot.2021.103435.

References

Adams, M.P., Hovey, R.K., Hipsey, M.R., Bruce, L.C., Ghisalberti, M., Lowe, R.J., Gruber, R.K., Ruiz-Montoya, L., Maxwell, P.S., Callaghan, D.P., 2016. Feedback between sediment and light for seagrass: Where is it important? Limnol. Oceanogr. 61 (6), 1937–1955.

ANZECC, 2000. Australian and New Zealand Guidelines for Fresh and Marine Water Quality. Australian and New Zealand Environment and Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand. Canberra, Australia. 103p..

Bainbridge, Z., Lewis, S., Bartley, R., Fabricius, K., Collier, C., Waterhouse, J., Garzon-Garcia, A., Robson, B., Burton, J., Wenger, A., 2018. Fine sediment and particulate organic matter: a review and case study on ridge-to-reef transport, transformations, fates, and impacts on marine ecosystems. Mar. Pollut. Bull. 135, 1205–1220.

Barranguet, C., Kromkamp, J., Peene, J., 1998. Factors controlling primary production and photosynthetic characteristics of intertidal microphytobenthos. Mar. Ecol. Prog. Ser. 173, 117–126.

Battley, P., Melville, D., Schuckard, R., Ballance, P., 2011. *Zostera muelleri* as a structuring agent of benthic communities in a large intertidal sandflat in New Zealand. J. Sea Res. 65 (1), 19–27.

Benham, C.F., Beavis, S.G., Jackson, E.L., 2019. Tolerance of tropical seagrasses *Zostera muelleri* and *Halophila ovalis* to burial: toward an understanding of threshold effects. Estuar. Coast. Shelf Sci. 218, 131–138.

Bertelli, C.M., Unsworth, R.K., 2014. Protecting the hand that feeds us: seagrass (*Zostera marina*) serves as commercial juvenile fish habitat. Mar. Pollut. Bull. 83 (2), 425–429.

Bonanno, G., Orlando-Bonaca, M., 2018. Trace elements in Mediterranean seagrasses and macroalgae. A review. Sci. Total Environ. 618, 1152–1159.

Borum, J., Pedersen, O., Greve, T.M., Frankovich, T.A., Zieman, J.C., Fourqurean, J.W., Madden, C.J., 2005. The potential role of plant oxygen and sulphide dynamics in die-off events of the tropical seagrass, *Thalassia testudinum*. J. Ecol. 93 (1), 148–158.

Bos, A.R., Bouma, T.J., de Kort, G.L., van Katwijk, M.M., 2007. Ecosystem engineering by annual intertidal seagrass beds: sediment accretion and modification. Estuar. Coast. Shelf Sci. 74 (1–2), 344–348.

Brodersen, K.E., Nielsen, D.A., Ralph, P.J., Kühl, M., 2015. Oxic microshield and local pH enhancement protects *Zostera muelleri* from sediment derived hydrogen sulphide. New Phytol. 205 (3), 1264–1276.

Brodersen, K.E., Hammer, K.J., Schrameyer, V., Floytrup, A., Rasheed, M.A., Ralph, P.J., Kühl, M., Pedersen, O., 2017. Sediment resuspension and deposition on seagrass leaves impedes internal plant aeration and promotes phytotoxic H_2S intrusion. Front. Plant Sci. 8, 657.

Brodersen, K.E., Kühl, M., Nielsen, D.A., Pedersen, O., Larkum, A.W., 2018. Rhizome, Root/Sediment interactions, aerenchyma and internal pressure changes in seagrasses. Seagrasses of Australia. Springer, pp. 393–418.

Bulmer, R., Kelly, S., Jeffs, A., 2016. Light requirements of the seagrass, *Zostera muelleri*, determined by observations at the maximum depth limit in a temperate estuary, New Zealand. N. Z. J. Mar. Freshwater Res. 50 (2), 183–194.

Burkholder, J.M., Tomasko, D.A., Touchette, B.W., 2007. Seagrasses and eutrophication. J. Exp. Mar. Biol. Ecol. 350 (1–2), 46–72.

Cabaço, S., Santos, R., 2007. Effects of burial and erosion on the seagrass *Zostera noltii*. J. Exp. Mar. Biol. Ecol. 340 (2), 204–212.

Cabaço, S., Santos, R., Duarte, C.M., 2008. The impact of sediment burial and erosion on seagrasses: a review. Estuar. Coast. Shelf Sci. 79 (3), 354–366.

Calleja, M.L., Marbà, N., Duarte, C.M., 2007. The relationship between seagrass (*Posidonia oceanica*) decline and sulfide porewater concentration in carbonate sediments. Estuarine. Coastal and Shelf Science 73 (3-4), 583–588.

Chartrand, K.M., Bryant, C.V., Carter, A.B., Ralph, P.J., Rasheed, M.A., 2016. Light thresholds to prevent dredging impacts on the Great Barrier Reef seagrass, *Zostera muelleri* ssp. *Capricorni*. Front. Mar. Sci. 3, 106.

Collier, C.J., Uthicke, S., Waycott, M., 2011. Thermal tolerance of two seagrass species at contrasting light levels: implications for future distribution in the Great Barrier Reef. Limnol. Oceanogr. 56 (6), 2200–2210.

Collier, C.J., Waycott, M., Ospina, A.G., 2012. Responses of four Indo-West Pacific seagrass species to shading. Mar. Pollut. Bull. 65 (4–9), 342–354.

Collier, C., Adams, M., Langlois, L., Waycott, M., O'Brien, K., Maxwell, P., McKenzie, L., 2016. Thresholds for morphological response to light reduction for four tropical seagrass species. Ecol. Indic. 67, 358–366.

Costanza, R., d'Arge, R., De Groot, R., Farber, S., Grasso, M., Hannon, B., Limburg, K., Naeem, S., O'neill, R.V., Paruelo, J., 1997. The value of the world's ecosystem services and natural capital. Nature 387 (6630), 253.

Cullen-Unsworth, L.C., Unsworth, R.F.K., 2018. A call for seagrass protection. Science 361, 446–448.

Cunha, A.H., Marbá, N.N., van Katwijk, M.M., Pickerell, C., Henriques, M., Bernard, G., Ferreira, M.A., Garcia, S., Garmendia, J.M., Manent, P., 2012. Changing paradigms in seagrass restoration. Restor. Ecol. 20 (4), 427–430.

Davies-Colley, R., Smith, D., 2001. Turbidity suspended sediment, and water clarity: a review. JAWRA J. Am. Water Resou. Assoc. 37 (5), 1085–1101.

De Boer, W., 2007. Seagrass–sediment interactions, positive feedbacks and critical thresholds for occurrence: a review. Hydrobiologia 591 (1), 5–24.

Drylie, T.P., Lohrer, A.M., Needham, H.R., Bulmer, R.H., Pilditch, C.A., 2018. Benthic primary production in emerged intertidal habitats provides resilience to high water column turbidity. J. Sea Res. 142, 101–112.

Duarte, C.M., Terrados, J., Agawin, N.S., Fortes, M.D., Bach, S., Kenworthy, W.J., 1997. Response of a mixed Philippine seagrass meadow to experimental burial. Mar. Ecol. Prog. Ser. 147, 285–294.

Dudley, B.D.R., Burge, O., Plew, D., Zeldis, J., 2020. Effects of agricultural and urban land cover on New Zealand's estuarine water quality. N. Z. J. Mar. Freshwater Res. 1–21.

Enríquez, S., Marbà, N., Duarte, C., Van Tussenbroek, B., Reyes-Zavala, G., 2001. Effects of seagrass *Thalassia testudinum* on sediment redox. Mar. Ecol. Prog. Ser. 219, 149–158.

Erftemeijer, P.L., Lewis I.I.I., R.R.R., 2006. Environmental impacts of dredging on seagrasses: a review. Mar. Pollut. Bull. 52 (12), 1553–1572.

Flanigan, Y.S., Critchley, C., 1996. Light response of D1 turnover and photosystem II efficiency in the seagrass *Zostera capricorni*. Planta 198 (3), 319–323.

Fourqurean, J.W., Rutten, L.M., 2004. The impact of Hurricane Georges on soft-bottom, back reef communities: site-and species-specific effects in south Florida seagrass beds. Bull. Mar. Sci. 75 (2), 239–257.

Gladstone-Gallagher, R.V., Hughes, R.W., Douglas, E.J., Pilditch, C.A., 2018. Biomass-dependent seagrass resilience to sediment eutrophication. J. Exp. Mar. Biol. Ecol. 501, 54–64.

Govers, L.L., de Brouwer, J.H., Suykerbuyk, W., Bouma, T.J., Lamers, L.P., Smolders, A. J., van Katwijk, M.M., 2014. Toxic effects of increased sediment nutrient and organic matter loading on the seagrass *Zostera noltii*. Aquat. Toxicol. 155, 253–260.

Grove, M.K., Bilotta, G.S., 2014. On the use of loss-on-ignition techniques to quantify fluvial particulate organic carbon. Earth Surf. Process. Landf. 39 (9), 1146–1152.

Ha, N.T., Manley-Harris, M., Pham, T.D., Hawes, I., 2021. Detecting multi-decadal changes in seagrass cover in Tauranga Harbour, New Zealand, using landsat imagery and boosting ensemble classification techniques. ISPRS Int. J. Geoinf. 10 (6), 371.

Halun, Z., Terrados, J., Borum, J., Kamp-Nielsen, L., Duarte, C.M., Fortes, M.D., 2002. Experimental evaluation of the effects of siltation-derived changes in sediment conditions on the Philippine seagrass *Cymodocea rotundata*. J. Exp. Mar. Biol. Ecol. 279 (1–2), 73–87.

Hayward, B.W., Morley, M.S., Stephenson, A.B., Blom, W.M., Grenfell, H.R., Prasad, R., Rogan, D., Thompson, F., Cheetham, J., Webb, M., 1999. Intertidal and Subtidal Biota and Habitats of the Central Waitemata Harbour. Auckland Regional Council Technical Publication, p. 40, 127.

Infantes, E., Terrados, J., Orfila, A., 2011. Assessment of substratum effect on the distribution of two invasive Caulerpa (*Chlorophyta*) species. Estuar. Coast. Shelf Sci. 91 (3), 434–441.

Inglis, G., 2003. Seagrasses of New Zealand. In: Green, E.P., Short, F.T. (Eds.), World Atlas of Seagrasses. University of California Press, Berkeley, CA, pp. 148–157.

Jackson, E.L., Rowden, A.A., Attrill, M.J., Bossey, S.J., Jones, M.B., 2001. The importance of seagrass beds as a habitat for fishery species. Oceanography and Marine Biology 39, 269–304.

Jarvis, J.C., Moore, K.A., 2010. The role of seedlings and seed bank viability in the recovery of Chesapeake Bay, USA, *Zostera marina* populations following a large-scale decline. Hydrobiologia 649 (1), 55–68.

Jesus, B., Brotas, V., Ribeiro, L., Mendes, C., Cartaxana, P., Paterson, D., 2009. Adaptations of microphytobenthos assemblages to sediment type and tidal position. Cont. Shelf Res. 29 (13), 1624–1634.

Jones, C.G., Lawton, J.H., Shachak, M., 1994. Organisms as ecosystem engineers. Ecosystem Management. Springer, New York, NY, pp. 130–147.

Kirk, J.T., 1985. Effects of suspensoids (turbidity) on penetration of solar radiation in aquatic ecosystems. Hydrobiologia 125 (1), 195–208.

Kirk, J.T., 1994. Light and Photosynthesis in Aquatic Ecosystems. Cambridge university press.

Kirkman, H., Kuo, J., 1990. Pattern and process in southern Western Australian seagrasses. Aquat. Bot. 37 (4), 367–382.

Krause-Jensen, D., Carstensen, J., Nielsen, S.L., Dalsgaard, T., Christensen, P.B., Fossing, H., Rasmussen, M.B., 2011. Sea bottom characteristics affect depth limits of eelgrass *Zostera marina*. Mar. Ecol. Prog. Ser. 425, 91–102.

Larkum, A., West, R., 1983. Stability, depletion and restoration of seagrass beds. Proc. Linn. Soc. NSW 106, 201–212.

Li, M., Lundquist, C.J., Pilditch, C.A., Rees, T., Ellis, J., 2019. Implications of nutrient enrichment for the conservation and management of seagrass *Zostera muelleri* meadows. Aquat. Conserv. Mar. Freshw. Ecosyst. 29, 1484–1502.

Lohrer, A.M., Hewitt, J.E., Thrush, S.F., 2006. Assessing far-field effects of terrigenous sediment loading in the coastal marine environment. Mar. Ecol. Prog. Ser. 315, 13–18.

Longstaff, B., Loneragan, N., O'donohue, M., Dennison, W., 1999. Effects of light deprivation on the survival and recovery of the seagrass *Halophila ovalis* (R. Br.) Hook. J. Exp. Mar. Biol. Ecol. 234 (1), 1–27.

Macreadie, P.I., York, P.H., Sherman, C.D., 2014. Resilience of *Zostera muelleri* seagrass to small-scale disturbances: the relative importance of asexual versus sexual recovery. Ecol. Evol. 4 (4), 450–461.

Marbà, N., Duarte, C.M., 1998. Rhizome elongation and seagrass clonal growth. Mar. Ecol. Prog. Ser. 174, 269–280.

Marbà, N., Duarte, C.M., Terrados, J., Halun, Z., Gacia, E., Fortes, M.D., 2010. Effects of seagrass rhizospheres on sediment redox conditions in SE Asian coastal ecosystems. Estuaries Coasts 33 (1), 107–117.

Matheson, F.E., Schwarz, A.M., 2007. Growth responses of *Zostera capricorni* to estuarine sediment conditions. Aquat. Bot. 87 (4), 299–306.

Matheson, F.E., Wadhwa, S., 2012. *Seagrass in Porirua Harbour.: Preliminary Assessment of Restoration Potential.* Report No. HAM2012-037; Prepared for Greater Wellington Regional Council, 35p. National Institute of Water and Atmospheric Research, Hamilton, New Zealand. http://www.gw.govt.nz/assets/Seagrass-restoration-in-Porirua-Harbour.pdf.

Matheson, F., Wadhwa, S., Taumoepeau, A., Smith, J., 2010. *Seagrass in the Eastern Bay of Islands: Past and Present Abundance, Threats and Management Options*. Client Report: HAM2010-043, Consultancy Report for Northland Regional Council. NIWA, Hamilton, New Zealand. 24p. https://www.scribd.com/document/43914816/Seagrass-in-the-Eastern-Bay-of-Islands.

McGlathery, K.J., Berg, P., Marino, R., 2001. Using porewater profiles to assess nutrient availability in seagrass-vegetated carbonate sediments. Biogeochemistry 56 (3), 239–263.

Meehan, A.J., West, R.J., 2000. Recovery times for a damaged *Posidonia australis* bed in south eastern Australia. Aquat. Bot. 67 (2), 161–167.

Morrison, M., Jones, E.G., Consalvey, M., Berkenbusch, K., 2014. Linking Marine Fisheries Species to Biogenic Habitats in New Zealand: a Review and Synthesis of Knowledge. Ministry for Primary Industries.

Orth, R.J., Carruthers, T.J., Dennison, W.C., Duarte, C.M., Fourqurean, J.W., Heck, K.L., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Olyarnik, S., 2006. A global crisis for seagrass ecosystems. Bioscience 56 (12), 987–996.

Park, S.G., 1999. Changes in Abundance of Seagrass (Zostera spp.) in Tauranga Harbour from 1959-1996. Environmental Report 99/30. Environment BOP, Whakatane, New Zealand, 19p. https://www.boprc.govt.nz/media/362713/changes-in-abundance-of-seagrass-zostera-spp-in-tauranga-harbour-from-1959-96.pdf.

Park, S.G., 2016. Extent of Seagrass in the Bay of Plenty in 2011. Bay of Plenty Regional Council.

Pedersen, T.M., Gallegos, C.L., Nielsen, S.L., 2012. Influence of near-bottom re-suspended sediment on benthic light availability. Estuar. Coast. Shelf Sci. 106, 93–101.

Reed, J., Schwarz, A., Gosai, A., Morrison, M., 2004. Feasibility Study to Investigate the replenishment/reinstatement of Seagrass Beds in Whangarei Harbour–Phase 1. NIWA Client Report to Northland Regional Council.

Short, F.T., Coles, R.G. (Eds.), 2001. Global Seagrass Research Methods. Elsevier, Amsterdam, Netherlands.

Suykerbuyk, W., Bouma, T.J., Govers, L.L., Giesen, K., de Jong, D.J., Herman, P., Hendriks, J., van Katwijk, M.M., 2016. Surviving in changing seascapes: sediment dynamics as bottleneck for long-term seagrass presence. Ecosystems 19 (2), 296–310.

Terrados, J., Duarte, C.M., Kamp-Nielsen, L., Agawin, N., Gacia, E., Lacap, D., Fortes, M., Borum, J., Lubanski, M., Greve, T., 1999. Are seagrass growth and survival constrained by the reducing conditions of the sediment? Aquat. Bot. 65 (1–4), 175–197.

Thrush, S., Hewitt, J., Cummings, V., Ellis, J., Hatton, C., Lohrer, A., Norkko, A., 2004. Muddy waters: elevating sediment input to coastal and estuarine habitats. Front. Ecol. Environ. 2 (6), 299–306.

Thrush, S., Hewitt, J., Lohrer, A., 2012. Interaction networks in coastal soft-sediments highlight the potential for change in ecological resilience. Ecol. Appl. 22 (4), 1213–1223.

Tukey, J.W., 1977. Exploratory Data Analysis, Vol. 2. Reading, Mass.

Turner, S., 1995. Restoring seagrass systems in New Zealand. Water and Atmosphere 3 (2), 9–11.

Van Katwijk, M., Bos, A., Hermus, D., Suykerbuyk, W., 2010. Sediment modification by seagrass beds: muddification and sandification induced by plant cover and environmental conditions. Estuar. Coast. Shelf Sci. 89 (2), 175–181.

Walker, D.I., Kendrick, G.A., McComb, A.J., 2007. Decline and recovery of seagrass ecosystems—the dynamics of change. Seagrasses: Biology, Ecologyand Conservation. Springer, pp. 551–565.

Waycott, M., Duarte, C.M., Carruthers, T.J., Orth, R.J., Dennison, W.C., Olyarnik, S., Calladine, A., Fourqurean, J.W., Heck Jr., K.L., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Short, F.T., Williams, S.L., 2009. Accelerating loss of seagrasses across the globe threatens coastal ecosystems. Proc. Nat. Acad. Sci. U. S. A. 106 (30), 12377–12381.

Zabarte-Maeztu, I., Matheson, F.E., Manley-Harris, M., Davies-Colley, R.J., Oliver, M., Hawes, I., 2020. Effects of fine sediment on seagrass meadows: a case study of *Zostera muelleri* in Pauatahanui Inlet, New Zealand. J. Mar. Sci. Eng. 8 (9), 645.

Zar, J., 1984. Biostatistical Analysis., 2nd ed. Prentice Hall. Englewood Cliffs, NJ.

Chapter 5 Appendix

Table A.5.1. 2-way ANOVA analysis for substrate properties. Significant effects and interactions ($p<0.05$) between Habitat and time are shown in bold for each of the substrate properties.

Trait	Factor	Df	Mean sq	F value	Pr (>F)
% Mud	**Habitat**	2	9387	114.461	2e-16
	Time	1	662	8.077	0.00513
	Residual	146	82		
% Silt	**Habitat**	2	6532	134.336	2e-16
	Time	1	237	4.869	0.0289
	Residual	146	49		
% Clay	**Habitat**	2	281.15	38.06	4.97e-14
	Time	1	107.12	14.5	0.000206
	Residual	146	7.39		
% Sand	**Habitat**	2	9017	108.864	2e-16
	Time	1	388	4.682	0.0321
	Residual	146	83		
% Fine sand	**Habitat**	2	11814	107.5	**2e-16**
	Time	1	27081	246.4	2e-16
	Residual	146	110		
% Coarse sand	**Habitat**	2	221	5.919	0.00337
	Time	1	5673	152.230	2e-16
	Residual	146	37		
Bulk density	**Habitat**	2	1.6516	23.11	1.91e-09
	Time	1	2.3407	32.75	5.74e-08
	Residual	146	0.0715		
Organic matter	Habitat	2	78895967	0.998	0.371
	Time	1	78876259	0.998	0.319
	Residual	146	7902888		
$PO_4]^{3-}$ µM (0-5 cm)	**Habitat**	2	32.27	3.845	0.025149
	Time	1	136.57	16.274	0.000118
	Residual	86	8.39		
$[PO_4]^{3-}$ µM (5-10 cm)	**Habitat**	2	0.15060	4.093	0.0201
	Time	1	0.00177	0.048	0.8269
	Residual	86	0.03680		
$[NH_4]^+$ µM (0-5 cm)	**Habitat**	2	7491	13.57	7.55e-6
	Time	1	7228	13.09	0.000499
	Residual	86	552		
$[NH_4]^+$ µM (5-10 cm)	**Habitat**	2	14306	13.346	8.95e-6
	Time	1	3871	3.611	0.067
	Residual	86	1072		
$[H_2S]$ µM (0-5 cm)	Habitat	2	2.17	1.92	0.153
	Time	1	24.56	21.73	1.18e-05
	Residual	84	1.13		
$[H_2S]$ µM (5-10 cm)	**Habitat**	2	19.103	6.857	0.00174
	Time	1	0.174	0.062	0.80332
	Residual	84	2.786		
PAR	**Habitat**	2	1544.8	23.727	3.05E-09
	Time	1	188.9	2.901	0.0914
	Residual	106	65.1		

Table A.5.2. 2-way ANOVA analysis for Core's transplants seagrass traits. Significant effects and interactions ($p<0.05$) between Habitat and time are shown in bold for each of the substrate properties.

Trait	Factor	Df	Mean sq	F value	Pr (>F)
% Lost cover	**Habitat**	1	101210	150.735	2e-16
	Light	1	13975	20.814	1.27e-5
	Interaction	1	775	1.155	0.285
	Residual	116	671		
AGB	**Habitat**	1	6.539	85.648	1.51e-15
	Light	1	0.004	0.058	0.81
	Interaction	1	0.076	0.99	0.322
	Residual	114	0.076		
BGB	**Habitat**	1	96.57	24.492	2.63e-6
	Light	1	17.2	4.366	0.0389
	Interaction	1	6.56	1.665	0.1996
	Residual	113	3.94		
Total Biomass	**Habitat**	1	157.82	32.632	8.75e-8
	Light	1	10.84	2.241	0.137
	Interaction	1	8.23	1.701	0.195
	Residual	116	4.84		