Growth dynamics and drivers of deep-water seagrasses from the Great Barrier Reef lagoon

Thesis submitted by
Kathryn M Chartrand B.Sc., M.Sc.

in September 2021

for the degree of Doctor of Philosophy
Faculty of Science, Climate Change Cluster
University of Technology Sydney
Sydney, Australia

CERTIFICATE OF ORIGINAL AUTHORSHIP

I, Kathryn M. Chartrand declare that this thesis, is submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the Faculty of Science at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise referenced or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

This document has not been submitted for qualifications at any other academic institution.

This research is supported by the Australian Government Research Training Program.

Signature: Production Note:
Signature removed prior to publication.

Date: 31 March 2021

ACKNOWLEDGEMENTS

I am indebted first and foremost to my supervisors, Peter Ralph, Michael Rasheed and Mathieu Pernice. Michael and Peter helped craft this unique opportunity to carry out my candidature as a remote part-time student, affording me the space to deliver impactful research and grow my "toolbox" of skills. I truly appreciate the growth and doors you have both opened by persevering with me on this journey. Thank you especially to Peter for your support, patience and guidance, your expertise in applying innovative research approaches to classic ecological questions, the opportunity to engage with an amazing team in the Climate Change Cluster (C3) and the collaborations it continues to foster. I am also grateful for the support and space throughout this journey Michael has given me to carry it through to fruition despite my competing demands, and for providing thoughtful, constructive feedback and advice from decades of experience in applied marine research. Thank you to Mathieu for the dedicated time and energy you gave to expand my horizons in the world of 'omics' and for your encouragement during my studies from afar.

I could not have accomplished this thesis without the huge efforts of my TropWATER Cairns team who are more like family. Lloyd Shepherd, Paul Leeson, Catherine Bryant, Skye McKenna, Alex Carter, Carissa Reason, Jaclyn Wells, Emma Henry, Alysha Sozou, Mark Leith and many more volunteers and casual staff that assisted with countless hours in the laboratory and/or on the water. If nothing else, they have a new found appreciation (or loathing) for using spectral radiometers at sea. Thank you especially to Rob Coles for his wisdom, editorial wit, valuable perspective and inspiring

(yet satirical at times) science chats as the “godfather” of the Cairns Seagrass Group. You are a true mentor and friend.

I am also indebted to numerous staff of C3 who supported various portions of my thesis despite my remote student status: Milan Szabó, Sutinee Sinutok, Unnikrishnan Kuzhiumparambil, Nasim Shah Mohammadi, Verena Schrameyer, Louisa Norman, John Moore, Melissa Oey, Terence Li.

I thank BHP Billiton Mitsubishi Alliance and Queensland Gas Corporation who have provided funding for this postgraduate research and Richard Stump in particular who supported the proposal put forward to study deep-water seagrasses. I also thank the Great Barrier Reef Marine Park Authority who recognised the need to fill this gap in knowledge in order to better manage coastal development projects.

I could not have persevered this mental marathon without my immediate and extended family and friends who saw me through the roller coaster of juggling work, study and life as a mom. To Cath Bryant, Alex Carter, Skye McKenna, Nikki Vass, Naoko Cutler, Michelle Cohen, Juliette Wilkinson and many other countless friends who brought me joy and laughter when I needed it most. To Michelle Cohen and Cordel Scaife for sharing their beautiful home and family on my Sydney jaunts to UTS. Thank you especially to Margie Chartrand for her endless encouragement and reassuring tone only a mother can give even when an ocean apart. Thanks also to Michel Kibby for her steadfast support with the kids at the drop of a hat.

To my parents, Margie and Stephen Chartrand. Your boundless energy, strong work ethic, and passion for science and family set the foundation for me to succeed. I hope my actions can inspire Austin and Jonah as you have inspired me.

Above all, thank you to Ross, Austin and Jonah. Your love, support and patience during this PhD journey have meant the world to me. The joy and true happiness in my life stems from our family; with those cheeky grins, sparks of laughter, crazy adventures big and small…you have simply given me the best part of my world and helped me persevere on the research rollercoaster. I dedicate this thesis to you.

PREFACE

This thesis is written in the format of a thesis by compilation— a combination of published chapters and those unpublished but with the intention of publication in a peer-reviewed scientific journal in the near future. Citations and references have been formatted throughout using the style applied by the peer-reviewed journal *Frontiers of Marine Science*. Given that this thesis is presented as a series of ready to submit manuscripts, there is an element of repetition in the introduction of some chapters since they are each submitted as stand-alone manuscripts. At the time of thesis submission, Chapter 3 has been published in the peer-reviewed *Marine Environmental Research* (IF 2.73). Chapter 2 has been submitted to *Limnology and Oceanography* (IF 3.78), Chapter 4 to *Estuaries and Coasts* (IF 2.42) and Chapter 5 is being prepared for submission to *Frontiers of Marine Science* (IF 3.07).

Supervisory committee

Prof. Peter J. Ralph, Climate Change Cluster, University of Technology Sydney

Dr. Mathieu Pernice, Climate Change Cluster, University of Technology Sydney

A/Prof. Michael A. Rasheed, Centre for Tropical Water & Aquatic Ecosystem Research, James Cook University

STATEMENT OF CONTRIBUTION OF OTHERS

CHAPTER 2

Authors: Chartrand K.M., Bryant C.V., Ralph P.J., and Rasheed M.A.

KC and MR conceived the study and designed the sampling strategy. KC led and designed field data collection with CB leading field logistics and data collection during

KC's maternity leave. KC analysed the data, prepared all figures and tables, and wrote the draft manuscript. MR and PR supervised the study. All authors provided valuable feedback on the draft manuscript and contributed valuable insights in the discussion.

CHAPTER 3

Authors: Chartrand K.M., Szabó M., Sinutok S., Rasheed M.A., and Ralph P.J.

KC, MR and PR conceived the study and KC, MS, SS and PR designed the methodological approach and sampling strategy. KC led the collection and shipment of seagrass from Queensland to UTS for laboratory studies. SS maintained samples and performed weekly checks on samples in the laboratory while KC was based remotely in Cairns. KC conducted the laboratory studies with technical support from MS and SS. MS provided valuable contributions to data quality control measures and provided regular supervision through the data collection, analysis and interpretation. SS assisted with the use of oxygen optodes for oxygen determinations and fluorometric measurements. KC analysed the data, drafted the manuscript, prepared figures and tables and arranged the submission and approval for publication. MS and PR contributed to the intellectual content of the manuscript. PR supervised the study.

CHAPTER 4

Authors: Chartrand K.M., Bryant C.V., Ralph P.J., and Rasheed M.A.

KC and MR conceived the study and designed the sampling strategy. KC led and designed field data collection with CB leading field logistics and data collection during KC's maternity leave. KC analysed the data, prepared all figures and tables, and wrote

the draft manuscript. MR and PR supervised the study. All authors provided valuable feedback on the draft manuscript and contributed valuable insights in the discussion.

CHAPTER 5

Authors: Chartrand K.M., Kuzhiumparambil U., Pernice M., Ralph P.J.

KC, MP, and PR conceived the study. KC led the collection and shipment of seagrass samples from Queensland to UTS. KC organized laboratory standards for hormone analysis and worked alongside UK to extract hormones and prepare metabolome samples. UK ran all samples through mass spectrometry instrumentation. PR, UK, and MP provided advice and constructive feedback on test results and analysis and UK provided guidance on interpretation of the results. All authors contributed valuable feedback on the draft manuscript and contributed valuable insights in the discussion.

Additional Research Field & Laboratory Support

Field research was supported by a number of colleagues acting as part of dive teams, vessel operations, and laboratory handling over the four year period: TropWATER JCU — Catherine Bryant, Paul Leeson, Lloyd Shepherd, Alysha Sozou, Emma Henry, Mark Leith, Paul York, Skye McKenna, Carissa Reason, Jessie Jarvis, Jaclyn Wells, Tonia Sankey, Elizabeth Suarez Duque, David Clarke; UTS — Stacey Ong, Louisa Norman.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Seagrasses provide irreplaceable ecosystem services, yet in the Anthropocene, they are increasingly under threat from coastal development and climate impacts. Efforts to mitigate threats to seagrasses have led to investment and research into their distribution, ecological drivers and bioindicators of health. In the Great Barrier Reef (GBR), work continues to translate our mechanistic understanding of marine plants into impactful management of acute disturbances and chronic stressors. These applied outcomes have primarily focused on shallow seagrass communities, synthesising results and deriving relationships to be used by managers and regulators.

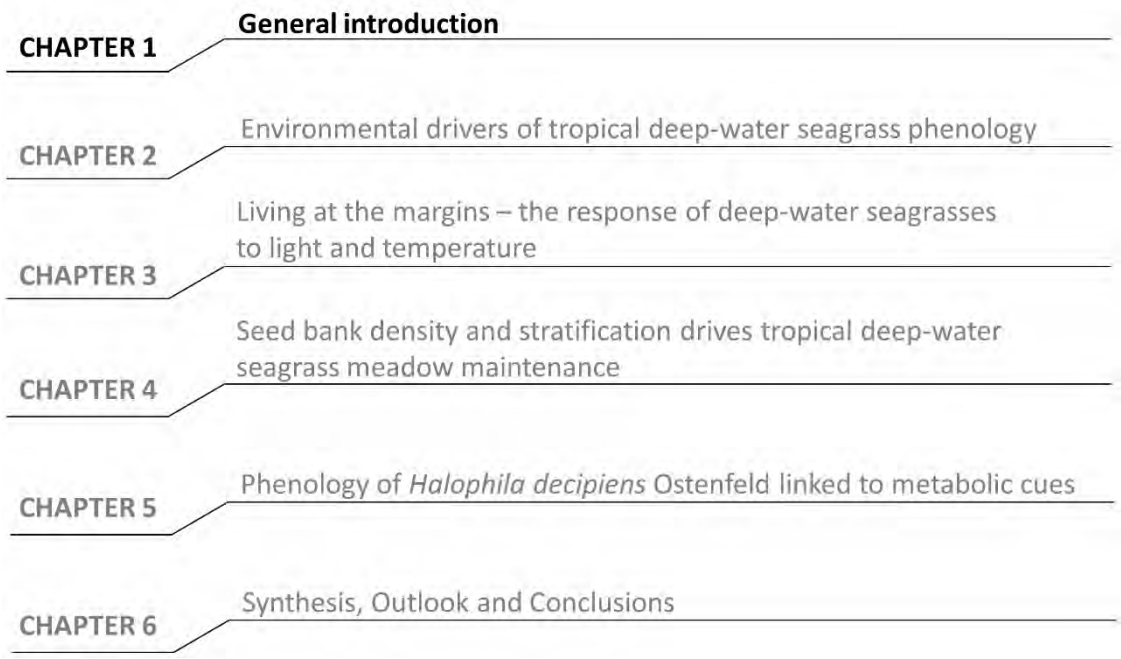
The goal of this thesis is to build our understanding of the dynamics and underlying drivers of GBR deep-water seagrasses for their better management and the communities they support. To achieve this, I (i) studied the seasonal patterns of deep-water seagrasses, characterising environmental parameters linked with growth and senescence; (ii) evaluated light and temperature as drivers of seagrass abundance and determined light thresholds for the dominant *Halophila* species; (iii) quantified seed banks over time and space, evaluating the role of seed stratification on germination; and (iv) investigated what role endogenous cues play in the phenology of a *Halophila* species.

Deep-water *Halophila* species did not all follow the same growth patterns. Only *Halophila decipiens* had a true annual pattern, completing its life cycle in one growing season and depositing seeds for the subsequent year's renewal. Deep-water GBR seagrasses grow near their physiological limits with small light reductions potentially

leading to meadow-scale loss, and yet their physiological limits also vary among species. Limiting light led to decreased shoot density for both *H. decipiens* and *H. spinulosa* over different timeframes, yet neither were affected by increases in temperature irrespective of compounding low light stress. Variations in meadow reproductive output and seed banks critically structure deep-water meadows and underscore species-specific responses to environmental perturbations. Endogenous cues responsible for life stage transitions in terrestrial plants had not been studied before in seagrasses. The metabolomic profile, including key hormones, within the life stages of the *H. decipiens* growing cycle provided the first study linking metabolomic regulation with seagrass growth and development and underpins the ecological findings in this thesis.

This thesis contributes critical information on growth strategies that drive spatial and seasonal dynamics of tropical deep-water *Halophila* communities. It provides new insights and a gateway to explore emerging lines of research including greater use of 'omics' technology and integrating terrestrial plant research to further improve deep-water seagrass management.

CHAPTER 1 General Introduction



In this chapter, I provide an outline of the rationale for my thesis, its objectives and structure. I provide a general introduction to the concepts of tropical seagrasses, the adaptations required to thrive in deep-water habitats and the known biology and life history traits of the seagrasses in the genus *Halophila* which shape these communities. I underscore the importance of understanding the ontogeny and strategies used by these plants to describe appropriate guidelines for managing their long-term health. In particular, I outline the value in understanding key drivers (e.g. light) of deep-water seagrass to ensure best practice management from known stressors. The above diagram will be repeated at the start of each chapter to orient the reader in relation to the wider scope of the thesis.

1.1 Tropical deep-water seagrass

The Great Barrier Reef World Heritage Area (GBRWHA) covers approximately 348,000 km^2 along the northeastern coast of Australia. It encompasses a wide range of tropical habitats and community assemblages from the outer-shelf coral reef ecosystems to the inshore estuaries and embayments. As a functional group, seagrasses are found across all of these habitats and recognised as a critical contributor to a wide range of ecosystem services (Costanza et al., 1997; Barbier et al., 2011; Cullen-Unsworth and Unsworth, 2013; Scott et al., 2018).

Seagrasses are vascular marine flowering plants with a true root-rhizome system anchoring them to the seabed and a leaf canopy responsible for oxygen and nutrient exchange as well as light capture (Borum et al., 2006; Kuo and den Hartog, 2006). They are clonal plants that re-invaded the marine biosphere around 100 million years ago (Les et al 1997) and are comprised of four independent taxonomic lineages (den Hartog, 1970; den Hartog and Kuo, 2006). The vast majority of species are localised to relatively shallow water habitats with favourable hydrodynamics and sediment chemistry and where light is sufficient to meet gross energy requirements (Hemminga and Duarte, 2000; Koch, 2001). Carruthers et al. (2002) classifies the 15 tropical seagrasses from northeast Australia into four broad habitat types: 1) river estuaries, 2) coastal, 3) reef and 4) deep-water, and describes the dominant factors controlling seagrasses in each category. The first three habitats include a diverse group of intertidal and subtidal seagrass communities. Field studies and long-term monitoring programs have produced a large body of knowledge on the distribution, seasonality, drivers, risks and threats to these specialised plant communities (Rasheed et al., 2008; Grech et al.,

2011; Collier et al., 2012a; McKenzie et al., 2012; Bryant et al., 2013; McKenna et al., 2015); however, information on deep-water seagrass communities is limited (Josselyn et al., 1986; Fonseca et al., 2008; Coles et al., 2009; York et al., 2015).

Tropical deep-water seagrasses are generally classified as growing at depths >10-15 m (Carruthers et al., 2002; Hammerstrom et al., 2006; Coles et al., 2009). These deeper meadows are primarily composed of species from the genus *Halophila* (Hydrocharitaceae). Within the GBR, *Halophila* spp. have been mapped down to 60 m and modelled to cover over 40,000 km^2 of the seafloor (Coles et al., 2009). In the Gulf of Mexico, an ephemeral *Halophila decipiens* meadow covers an estimated 20,000 km^2 of the west Florida shelf (Hammerstrom et al., 2006). Many other records of tropical deep-water meadows exist at less well-defined spatial scales (Buesa, 1975; Rodriguez and Simó, 1981; Jacobs and Dicks, 1985; Josselyn et al., 1986; Hovey et al., 2015). Other large-bladed tropical species have been recorded at depths >20 m (Taylor and Rasheed, 2010; Hays et al., 2018), nevertheless the ideal conditions required for those species to occur at such depths is rare.

Light availability is the primary driver of seagrass growth and distribution with change in the availability and quality of light with depth a major factor shaping the distribution of subtidal seagrasses (Dennison, 1987; Duarte, 1991; Ralph et al., 2007). Seagrasses are adapted to the variable conditions that occur in the marine environment, which create constantly shifting optical and metabolic challenges (de los Santos et al., 2010; Collier et al., 2011; Petrou et al., 2013). Marine plants respond to reductions in light through morphological, ecological and physiological mechanisms (Ralph et al., 2007),

yet larger strap-bladed species have limited capacity to adjust to chronic low light conditions that come with growing at greater depths (Larkum et al., 2006). However, strategies for seagrasses to tolerate temporary light reduction include: adjusting light harvesting capacity and the efficiency of light use (Abal et al., 1994; Enriquez, 2005); adjustments to rates of growth and plant turnover (Collier et al., 2009; Collier et al., 2012b); and drawing upon carbohydrate reserves to maintain a positive carbon balance (Burke et al., 1996; Touchette and Burkholder, 2000). Photosynthetic machinery and associated physiological pathways are constantly optimised to maintain a net positive carbon balance for plant growth, energy reserves and propagation of fruits and seeds to ensure long-term viability and resilience (Biber et al., 2005; Ralph et al., 2007).

Seagrasses in the genus *Halophila* have small fragile leaves, are oval or oblong in shape and occur in pairs that attach directly to either a vertical stem or rhizome via a petiole (Figure 1.1). Their size-associated characteristics likely play an important role in their dominance in deep-water seagrass meadows. Their stunted canopy height may increase risk of burial from sediment deposition; however, rapid leaf turnover and opportunistic growth can negate this issue (Duarte et al., 1997; Terrados et al., 1998; Cabaço et al., 2008). Leaves are extremely thin, only 2 cells thick with minimal lacunar space and contain densely packed chloroplasts (Roberts et al., 1984; Josselyn et al., 1986; Kenworthy et al., 1989; Cambridge and Lambers, 1998). Thin leaves allow for quick and efficient gas exchange with export of evolved oxygen from saturated epidermal cells and reciprocal uptake of CO_2 for fixation (Larkum et al., 2006). Comparatively, seagrasses with high standing biomass have higher diffusive boundary layers which could make living at depths with less wave action and water movement a challenge for

gas exchange and acquiring limited resources (i.e. light) (Enríquez and Rodríguez-Román, 2006).

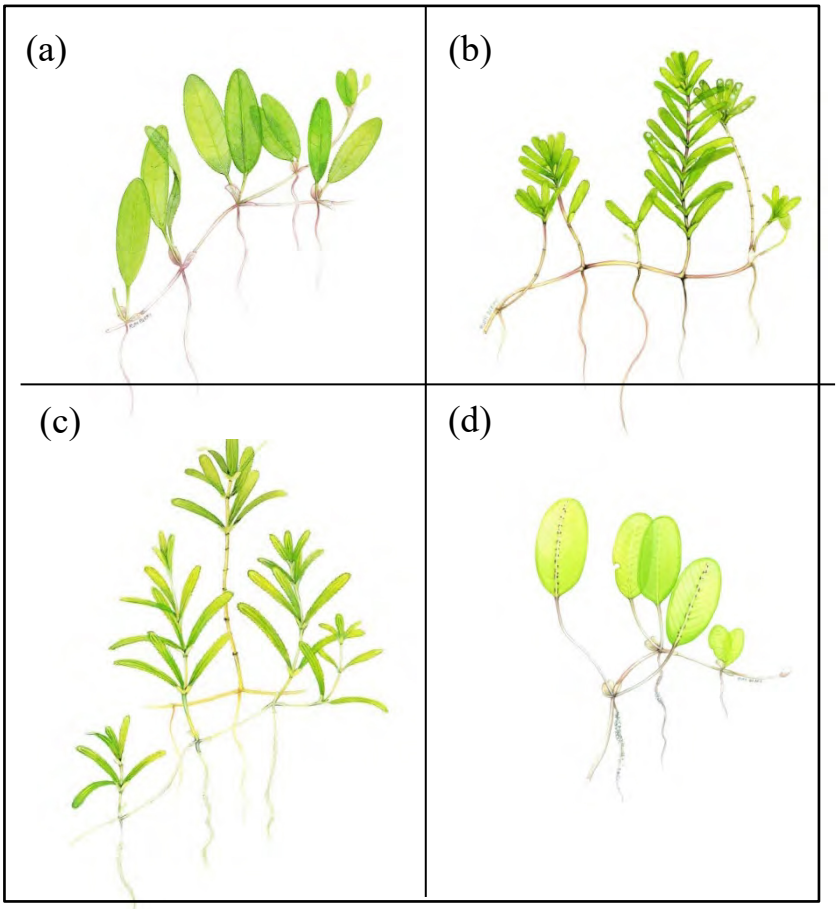


Figure 1.1 Seagrasses of the genus *Halophila* dominant in deepwater tropical meadows of the Great Barrier Reef. (a) *Halophila decipiens*, (b) *Halophila spinulosa*, (c) *Halophila tricostata* and (d) *Halophila ovalis*

Halophila spp. are well suited to grow at greater depths as well as in shallow turbid areas with chronic low light due to their minimal below-ground investment (Kuo and Kirkman, 1995; Durako et al., 2003). Non-photosynthetic below-ground tissues can act as a respiratory burden which may ultimately limit the compensation depth of seagrasses (Fourqurean and Zieman, 1991; Larkum et al., 2006). Large growth forms (e.g. *Thalassia* spp.) require much higher light levels to support considerable

investments in below ground resources (Duarte, 1991). The overall lower respiratory demands and efficient gas exchange in the leaves of *Halophila* spp. supports a positive carbon balance with lower irradiances, enabling colonisation in deeper zones.

Roots and rhizomes anchoring *Halophila* spp. in the sediment are small compared to other large-bodied seagrasses which maintain a significant proportion of their total biomass below ground (Duarte and Chiscano, 1999). This suggests a limited capacity to withstand strong wave energies. However, deep-water *Halophila* spp. may have an effect on sediment stabilisation in the GBR lagoon. Post-flood catchment-derived sediment loads in the GBR lagoon are partly dispersed by waves and currents into low energy environments and partly become buried into deeper benthic sediment of the lagoon (Lewis et al., 2014; Margvelashvili et al., 2018). Fonseca (1989) found *H. decipiens* increased the threshold velocity of sediment motion nearly akin to larger seagrasses making them important sediment stabilisers over vast areas despite their small stature. With this influence on stabilising sediments, *H. decipiens* or other deep-water *Halophila* spp. could be playing a more significant role, albeit seasonally, than previously thought in preventing sediment re-suspension of newly introduced catchment loads. Resuspended sediment is known to add pressure to regional water quality for the GBR (Fabricius et al., 2016).

Deep-water *Halophila* populations are reported to have high leaf turnover rates and active meristematic growth (Josselyn et al., 1986; Vermaat et al., 1995; Marba and Duarte, 1998; Hammerstrom et al., 2006), yet low daily carbon production attributable to the small stature of the plant form (Erftemeijer and Stapel, 1999; Hammerstrom et al.,

2006). Based on the projected widespread distribution within the GBR lagoon (Coles et al., 2009) and given fast rhizome elongation rates (Josselyn et al., 1986), deep-water *Halophila* meadows can contribute substantially to primary productivity at large spatial scales despite its small leaf morphology. Rapid leaf turnover by *Halophila* spp. offsets epiphyte loading and subsequent shading of older leaves (Josselyn et al., 1986), which in turn sustains meadow growth over such vast areas.

Species in the genus *Halophila* are functionally regarded as having a colonising growth form due to multiple life history traits that are effective in responding to disturbances (Kilminster et al., 2015). In addition to high leaf turnover rates, many colonising species have relatively high sexual reproductive effort (Orth et al., 2006). Abundant flowering, fruiting, seed production and retention in the surrounding sediment have been described globally in various *Halophila* spp. meadows (McMillan and Soong, 1989; Kuo and Kirkman, 1995; Kenworthy, 2000; Hammerstrom et al., 2006). Having the highest sexual fecundity of all seagrasses is likely responsible for the large seed banks often not found in larger persistent seagrass species where shoot retention and perseverance through unfavourable conditions is their critical strategy for long-term survival as opposed to die-off and restoring the meadow from seed (Kilminster et al., 2015). A substantial seed bank provides a means of recovery after a major disturbance or protracted period of unfavourable conditions (i.e. seasonal low light) (Kenworthy, 2000; Hammerstrom et al., 2006; Bell et al., 2008). Seeds can remain dormant for a period of time until conditions are favourable for germination and re-establishment of the meadow (Kenworthy, 2000; Hammerstrom et al., 2006). For example, Kuo and Kirkman (1995) found *Halophila decipiens* produced fruits every 3-4 days on each leaf

pair with over 25 seeds per fruit resulting in more than 176,000 seeds m^{-2} in a meadow. Species such as *H. tricostata* with erect stems, can produce fruits at each vertical node with 24-60 seeds per fruit, generating 70,000 seeds m^{-2} (Kuo et al., 1993). The West Florida Shelf deep-water *H. decipiens* meadow was estimated to have a seed bank up to 3,414 seeds m^{-2} (Hammerstrom et al., 2006), while a shallow Panama *H. decipiens* meadow had a maximum density at 13,500 seeds m^{-2} (McMillan and Soong, 1989). Some of these large seed banks have been responsible for documented recovery after cyclones and seasonal fluctuations in growing conditions (McMillan and Soong, 1989; Bell et al., 2008; Rasheed et al., 2014).

The large-scale mobilisation and re-distribution of deep-water *Halophila* seeds has been linked to physical disturbance of the sediment with major storm events such as cyclones and hurricanes (Bell et al., 2008; Fonseca et al., 2008). On smaller spatial and temporal scales, bioturbation may be instrumental in seed emergence. Fonseca et al. (2008) posited that bioturbation by callianassid shrimp was responsible for re-distributing the buried seed bank of a west Florida shelf meadow based on associated dense stands of *H. decipiens* around the opening of excavation mounds. The positive feedback of such bioturbation on seagrass densities is in contrast to studies measuring negative correlations between *Callianasa* and seagrass cover and productivity (Suchanek, 1983; DeWitt, 2009). Nevertheless, shrimp burrowing may actively contribute to deep-water *Halophila* seed emergence to facilitate germination success.

The ontogeny of deep-water *Halophila* spp. is largely described as following an annual life history strategy (Kenworthy et al., 1989; Hammerstrom et al., 2006; York et al.,

2015). Plant development progresses through distinct phases from germination, vegetative growth, reproductive output and eventual senescence. While environmental conditions are known to drive plant response, endogenous cues which control the genetic expression and transcription of these growth processes are poorly understood, particularly for seagrasses (Huijser and Schmid, 2011). Growth and differentiation at different life stages are controlled by cross-talk between environmental cues and endogenous signals (Figure 1.2). Hormones are well described as driving flowering, fruiting, seed set and final senescence (Davies and Gan, 2012). Studies of hormones in seagrasses have largely been limited to supplemental studies to enhance seagrass cultures as opposed to characterising internal plant concentrations (Zarranz et al., 2010; Glasby et al., 2015). Sugars have also been highlighted as important regulators of life stage transitions which interact with hormones to drive key development processes such as flowering (Smeekens, 2000; Yu et al., 2013). Identifying these molecular processes in seagrass will help untangle in-built life history transitions versus those driven by changes in their environment.

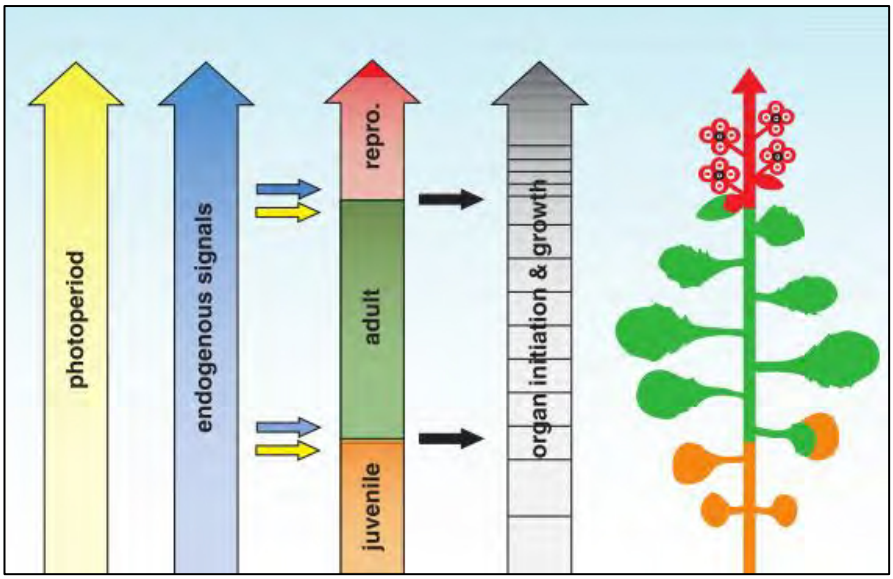


Figure 1.2 Model of plant development *(adapted from Poethig, 2003)*.

1.2 Response to shifting environmental conditions

While seagrasses adapted to deeper habitats may be tolerant of fluctuations in light intensity, they can also be acutely sensitive to reductions in light beyond the prevailing range of light conditions (Ralph et al., 2007). When irradiance drops below a critical level, seagrass productivity is compromised and significant physiological, biochemical and structural changes begin to take place (Abal et al., 1994; Goodman et al., 1995; Grice et al., 1996; Lee and Dunton, 1997; Alcoverro et al., 1999; Krause-Jensen et al., 2000; Ralph et al., 2007; Collier et al., 2012b). Within-plant changes eventually manifest into broader meadow-scale losses in productivity, density and distribution with downstream negative ecological implications (Preen and Marsh, 1995; Heck et al., 2008; Hughes et al., 2008).

At depth, fluctuations in the light climate are reduced compared to intertidal and shallow subtidal communities where tide cycles are the predominant driver of light flux. However, small adjustments to light availability for deeper growing plants still follows a definitive trajectory of response starting at the molecular and biochemical level, whereby explicit gene regulation may lead to changes in photosystems and pigment composition (Dennison and Alberte, 1986; Abal et al., 1994; Collier et al., 2012b; Mazzuca et al., 2013). Further changes in metabolism and growth rates will follow before effects on plant morphology or meadow scale reductions are apparent (Longstaff et al., 1999). While laboratory experiments have helped to resolve the fundamental timeline of these responses (Abal et al., 1994; Collier et al., 2012b; McMahon et al., 2013), the realised timeline of *in situ* seagrass growth dynamics is likely to be quite different due to additional extrinsic factors that cannot easily be replicated in laboratory

or mesocosm trials such as sediment biochemistry and hydrodynamics (Terrados et al., 1999; Peralta et al., 2006).

Light quality is also known to have a significant effect on photosynthetic output, pigment signatures and reflectance spectra in a variety of plants, both terrestrial and marine (Smith, 1982; Mercado et al., 2004; Kahn and Durako, 2009; Strydom et al., 2017a). Seagrasses have well described photosynthetic efficiency under blue (400-500 nm) and red (650-700 nm) wavelengths due to the absorption of light by chlorophyll pigments, while having lower efficiency in the green and yellow light regions (Kahn and Durako, 2009). However, deep-water seagrasses are exposed only to a narrow range of light qualities due to the natural attenuation of wavelengths beyond 500-600 nm at depths > 10 m in coastal waters (Kirk, 1994). One well-studied population of *Halophila* found in the Red Sea, *Halophila stipulacea*, has clear morphological and biochemical adaptations to growing at extreme depths of up to 50 m that sharpen its capacity to use a narrowed irradiance spectrum (Sharon, 2009; Sharon et al., 2011; Beca-Carretero et al., 2019). It is unclear what physiological or morphological changes are made by other, more widespread *Halophila* spp. to adapt to similar challenges at depth.

While the apparent strategies used by deep-water *Halophila* populations have been described (i.e. rapid growth, high reproductive effort, etc.) enabling them to thrive in deeper habitats even with regular disturbance events, we lack an empirical description of the environmental attributes that drive growth versus senescent phases. It is unclear what quantitative and qualitative characteristics of light are important to ensure proliferation versus conditions that trigger rapid die-off of meadows.

1.2 Management Implications

Australian state and federal management agencies have both recognised the importance of marine plants to ecosystem function in the GBRWHA over the last 25 years (Department of Agriculture and Fisheries, 2015; Great Barrier Reef Marine Park Authority, 2015). In the early 1990s, the state government enacted the Queensland *Fisheries Act 1994* protecting marine plants from damage or removal without prior authorisation in state waters, while the Great Barrier Reef Marine Park Authority (GBRMPA) incorporated large-scale seagrass and benthic community survey information into the multi-use plan for re-zoning of the marine park (Kenworthy et al., 2006). Long-term monitoring programs of tropical seagrass habitats at risk in major catchments and Queensland ports also began in the early 1990's and is continuing to assess trends in seagrass to decipher climate-driven versus human-driven effects, such as dredging and shipping, on the condition of coastal meadows (Coles et al., 2015; McKenna et al., 2015; York et al., 2015).

The GBRWHA is under pressure from a mixture of activities directed at maximising the agricultural, industrial and commercial value of the area's resources which have historically nurtured both regional and national economies (Productivity Commission, 2003; Stoeckl et al., 2011). Port expansions along the Great Barrier Reef Marine Park coastline continue to fulfil a greater demand for mineral- and gas exports to overseas markets (BREE, 2012). Large dredging campaigns and the associated risks to the light environment from dredge turbidity plumes have driven research to examine indicators of seagrass stress and light thresholds to be used by port authorities and government regulators (Grech et al., 2013; McMahon et al., 2013; Chartrand et al., 2016; Collier et

al., 2016b). While research has significantly advanced our understanding of sub-lethal stress and light requirements of some shallow-water species (Macreadie et al., 2014; Chartrand et al., 2016), the limited understanding of deep-water seagrass dynamics, their drivers and appropriate strategies to manage their life history traits have been largely overlooked. This gap in knowledge was recognised by GBRMPA as well as other stakeholders including BHP Billiton Mitsubishi Alliance and Queensland Gas Corporation who have provided funding for this PhD research. Understanding the drivers of tropical deep-water seagrass dynamics will not only provide the information to effectively manage acute stressors such as dredging and the impacts of associated turbidity plumes, but also will be valuable to long-term coastal development planning and managing seagrass health.

1.3 Thesis Objectives

The goal of this thesis is to address the lack of information on tropical deep-water seagrass phenology by examining growth strategies that drive spatial and seasonal dynamics of tropical deep-water *Halophila* spp. communities in the GBR lagoon. This includes determining the relationships between environmental conditions, namely light and temperature, and seagrass abundance, productivity and reproductive effort in a range of deep-water community types (integrating a latitudinal gradient from northern to more central GBR populations influenced by developed coastline activities) which will inform management to help protect these populations at risk from anthropogenic activities and stressors. To achieve this, my specific objectives were to:

1. Establish the seasonal dynamics of deep-water seagrasses across a latitudinal gradient including characterising key environmental parameters linked with growth and senescence (Chapter 2);

2. Evaluate light and temperature as drivers of deep-water seagrass abundance and determine light thresholds for the dominant *Halophila* species. (Chapter 3);

3. Quantify *Halophila* seed banks over time and space to evaluate the role of seed stratification on germination (Chapter 4);

4. Explore the phenology of the annual-like seagrass species *H. decipiens* by measuring the metabolomic profiles during development stages and compare the findings with patterns in terrestrial plants known to regulate growth and senescence (Chapter 5).

1.4 Thesis Outline

The thesis is written in a format to enable publication of each research chapter (Chapters 2-5) in a peer reviewed journal. The approach to each research chapter is outlined below; however where there is overlap with data used across multiple chapters, clarification is provided. Chapter 3 has already been published in a peer reviewed journal.

In CHAPTER 2, I describe the four-year study that chronicled the drivers and fluctuations of three tropical deep-water seagrass communities from the Great Barrier

Reef (GBR) lagoon: 1) Lizard Island, 2) Green Island and 3) Mackay. *In situ* light and temperature data together with morphometrics, productivity and leaf chlorophyll fluorescence provide a detailed picture of growth and senescence linked to environmental and seasonal fluctuations in deep-water *Halophila* meadows. The patterns in reproductive output and seed banks assessed as part of this long-term field study is presented in Chapter 4.

In CHAPTER 3, I present the results of a controlled laboratory experiment testing the interactive effects of light intensity and temperature on the photobiology of two deep-water seagrasses, *H. decipiens* and *H. spinulosa*. The aim of this study was to define light and temperature regimes responsible for their growth requirements and potential thresholds of mortality. A number of metrics were tested including: shoot density; pigment concentrations; maximum and effective quantum yields of PSII; oxygen evolution and respiration; below-ground carbohydrates; and wavelength-dependent chlorophyll fluorescence parameters. This study has been published in *Marine Environmental Research*.

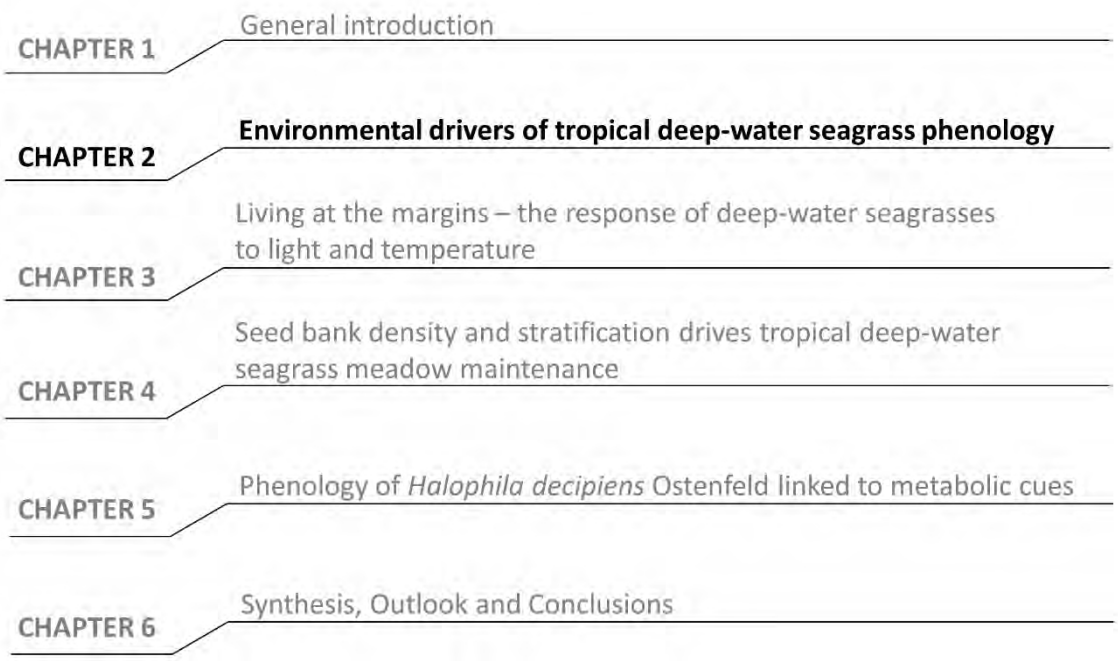
In CHAPTER 4, I assess the seed bank density and stratification of the deep-water seagrass meadows at the three long-term monitoring locations: 1) Lizard Island, 2) Green Island and 3) Mackay. Sediment cores were collected in conjunction with the long-term monitoring described in Chapter 2. This study aimed to provide empirical evidence of the relationship between seagrass abundance and seed bank status for deep-water populations that are described as relying heavily on seeds for long-term success in marginal habitats. Information on seed bank densities and stratification were analysed in

the context of germination and senescence patterns at each site to better understand seed bank dynamics.

In CHAPTER 5, I explore the mechanisms controlling life cycle transitions in *Halophila decipiens* by evaluating endogenous hormones and metabolomics. This study provides evidence of the endogenous control of plant development, flowering, fruiting and ultimate senescence programmed by metabolic circuits rather than driven explicitly by environmental cues. Findings re-inforce the importance of sexual reproductive success rather than maintaining a standing crop in this annual plant. Leaves were collected off Green Island at four time points over the growing season from August to December 2016. Samples were placed in liquid nitrogen for transport to UTS where they were stored before further processing using gas and liquid mass spectrometry to assess untargeted metabolome profiles in above- and below-ground tissues and fruits.

In CHAPTER 6, I provide a synthesis of the results of each of the data chapters and discuss how this thesis deepens our understanding of the ecological dynamics and underlying biological drivers of deep-water seagrasses from the GBR lagoon. I explore how the acquired knowledge can be applied in a meaningful way to support their management and the communities they support. I also highlight directions for further research emanating from my thesis study.

CHAPTER 2 Environmental drivers of tropical deep-water seagrass phenology



Chapter 2 has been submitted to the journal 'Limnology and Oceanography' in February 2021 as: Chartrand K.M.[*1,2], Bryant C.V. [1], Ralph P.J. [2], and Rasheed M.A. [1] In review. Environmental drivers of tropical deep-water seagrass phenology in the Great Barrier Reef lagoon. *Limnology and Oceanography*.

[1] Centre for Tropical Water & Aquatic Ecosystem Research, James Cook University, Cairns, Queensland, Australia.
[2] Climate Change Cluster, University of Technology Sydney, Broadway, New South Wales, Australia.

Abstract

An abundance of research into the environmental drivers and dynamic fluctuations of shallow-water seagrass communities has cemented the important role they play in maintaining a healthy marine ecosystem. Remarkably fewer studies have investigated their deep-water counterparts—a globally occurring subset of seagrasses that inhabit the photic zone seafloor greater than 10-15 m. Here we describe a four-year study that chronicled the drivers and fluctuations of three tropical deep-water seagrass communities from the Great Barrier Reef (GBR) lagoon. *In situ* light and temperature data together with morphometrics, productivity and leaf chlorophyll fluorescence provide a detailed picture of growth and senescence linked to environmental and seasonal fluctuations in deep-water *Halophila* meadows. The pan-tropical species *Halophila decipiens* significantly increased biomass in a central and northern meadow when mean 14-day total daily irradiance was greater than 2.0 and 2.7 mol photons m^{-2} d^{-1}, respectively. Time of year and maximum daily temperature—strongly collinear predictors—were also strongly correlated with *Halophila* biomass, driving peak abundance in October/November (26-27ºC) at both locations. This study also found a species-specific pattern for deep-water seagrasses, whereby *H. decipiens* had a distinct annual presence at both locations, while *Halophila ovalis* meadows would wax and wane but remain year-round. Direct impacts by two severe tropical cyclones at the northern site restructured the meadow during recovery with a shift in species dominance over the second half of the study. A shorter time series at the most southerly site fills a gap in the data deficient growth patterns and seasonality for *Halophila tricostata* and *Halophila spinulosa*. This study highlights differences among deep-water seagrass meadows in species composition, abundance and seasonal patterns of senescence and

recruitment coupled to location-specific environmental light and temperature regimes. As a whole, these outcomes underscore the need to adopt seagrass management strategies appropriate to the phenological pattern of these deep-water species or populations.

2.1 Introduction

Seagrasses are essential benthic primary producers found in coastal zones of tropical and temperate regions on all continents, excluding Antarctica. The vast majority of species are localised to relatively shallow water habitats with favourable hydrodynamics and sediment chemistry and where light is sufficient to meet their gross energy requirements (Hemminga and Duarte, 2000; Koch, 2001). However, on the global scale, seagrass distribution may be underestimated (Green and Short, 2003; Gattuso et al., 2006; Waycott et al., 2009), in large part due to limited survey efforts outside of highly populated urban coastlines and the immediate coastal zone—particularly in the tropical Indo-Pacific (Green and Short, 2003). Recent work has shown that coverage of deeper (>10-15 m) – low-light requiring seagrass populations has been underestimated despite their important role in delivering ecosystem services (Hays et al., 2018).

Along the northeastern coast of Australia, seagrasses are found throughout the 348,000 km^2 of the Great Barrier Reef (GBR) World Heritage Area from the outer-shelf coral reef systems to the inshore estuaries and embayments (Coles et al., 2015). Field studies and long-term monitoring programs have produced a large body of knowledge on the distribution, seasonality, environmental drivers, risks and threats to marine plant communities in intertidal and shallow subtidal areas (< 15 m) since the 1980s (Coles et

al., 1987; Rasheed et al., 2008; Grech et al., 2011; Collier et al., 2012a; McKenzie et al., 2012; Bryant et al., 2013; Coles et al., 2015; McKenna et al., 2015). In comparison, information on deep-water seagrass communities of the GBR lagoon, particularly their ecology, is still quite limited (Josselyn et al., 1986; Fonseca et al., 2008; Coles et al., 2009; York et al., 2015).

Tropical deep-water seagrasses are generally classified as growing at depths > 10-15 m (Carruthers et al., 2002; Hammerstrom et al., 2006; Coles et al., 2009). These deeper meadows are primarily composed of species from the genus *Halophila* (Hydrocharitaceae). Within the GBR, *Halophila* spp. have been mapped down to 60 m and modelled to cover over 40,000 km^2 of the seafloor (Coles et al., 2009). Their presence and distribution however is highly variable with large changes occurring as the result of major weather events (Rasheed et al., 2014) and some populations appearing to be annual in their growth pattern (York et al., 2015). In the Gulf of Mexico, an ephemeral *Halophila decipiens* meadow covers an estimated 20,000 km^2 of the west Florida shelf (Hammerstrom et al., 2006). Many other records of tropical deep-water meadows exist at less well-defined spatial scales (Buesa, 1975; Rodriguez and Simó, 1981; Jacobs and Dicks, 1985; Josselyn et al., 1986). In the Red Sea, *H. stipulacea* has adapted to conditions at depths up to 50 m (Sharon et al., 2011). Other large-bladed tropical species have been recorded at depths >20 m (Taylor and Rasheed, 2010; Esteban et al., 2018), nevertheless the ideal conditions required for these species to occur at such depths is thought to be rare.

Recent studies have underscored the ecosystem services these more cryptic seagrass populations may play (Esteban et al., 2018; York et al., 2018) as the recognised value of deeper photic zone communities grows (Loya et al., 2019). They are increasingly being recognised for their role in creating fish habitat and providing a food supply to marine megafauna (Esteban et al., 2018). Studies have also found smaller-bodied deep-water seagrasses are able to influence the threshold velocity of sediment motion nearly akin to larger seagrasses, making them important sediment stabilisers over vast areas of seafloor (Fonseca, 1989; Coles et al., 2009) and they may play a similar role in capturing carbon in the sediments as their shallow-water counterparts (York et al., 2018).

The apparent growth strategies enabling deep-water *Halophila* populations to thrive—even with regular disturbance events—have been described (i.e. rapid growth, high reproductive effort, etc.); however, we lack an empirical description of the environmental attributes that drive growth and senescence patterns. At the most fundamental level, seagrasses—like any photosynthesising marine organism—are limited foremost by the light environment in which they reside, but also factors such as temperature affect the plant's overall carbon balance (Ralph et al., 2007).

In this study, we establish the seasonal dynamics of three deep-water seagrass communities from the GBR, characterising key environmental parameters linked with growth and senescence. This work focused on a range of tropical deep-water community types (integrating a latitudinal gradient from northern to more central GBR populations influenced by developed coastline activities) to address information gaps on

growth strategies and effectively describe the drivers of seagrass condition at depth. This study also sought to inform best management practice of deep-water seagrasses at risk from acute coastal development impacts by delineating critical environmental thresholds, if they exist, and outlining key growing periods to maintain deep-water seagrass populations.

2.2 Methods

2.2.1 Study design

Deep-water monitoring sites were established at Green Island (16°45.12354'S, 145°59.5494'E; 17 m dbMSL) in December 2011, Lizard Island (14°39'10.072"S, 145°26'56.542"E; 15 m dbMSL in March 2012), and Keswick Island (20°54'49.74"S, 149°23'10.509"E; 13 m dbMSL) in November 2014 (Figure 2.1). These locations were selected to represent a variety of species known to occur throughout deep-water GBR habitats (Coles et al 2009) and provided a spatial gradient to examine potential latitudinal differences in seasonality and growth dynamics in deep-water seagrasses. Site depths ranged from 12-17 m with maximum tidal ranges of 2 m, 2.5 m and 5 m from north to south.

The Keswick Island site was added late to this research program (November 2014) after the original study location in the more inshore Mackay region was found to lack seagrass for two consecutive years following 2012 site establishment in an area where deep-water seagrasses were known to be present over consecutive years (York et al., 2015). Information on the light characteristics of this more inshore region are provided as context for the absence of seagrass during the study.

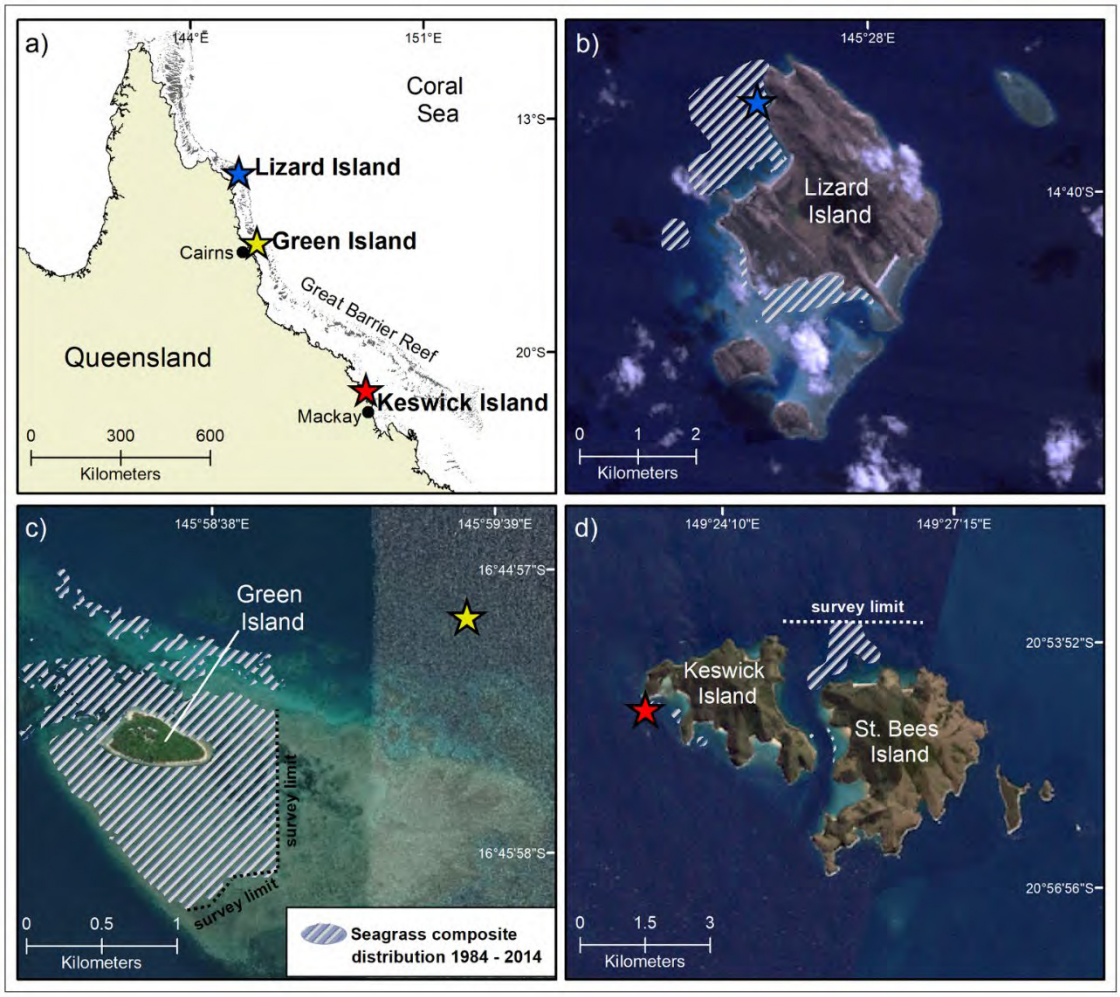


Figure 2.1 (a) Deep-water monitoring site locations along a north-south gradient of the Great Barrier Reef. (b) Lizard Island, (c) Green Island, and d) Keswick Island.

Seasonality for deep-water seagrass recruitment, growth, photophysiology and senescence in relation to spectral quality and total quantity of light, and temperature were investigated. Monthly sampling began at the Green Island site from December 2011 with quarterly sampling at both the Lizard Island and Mackay sites from March 2012 with a shift from Mackay to Keswick Island in November 2014. The three locations were monitored until January 2016.

All sites consisted of 27 replicate 0.25 m² quadrats marked out with pegs and flagging tape, spaced 0.5 – 1 m apart in a gridded 15 x 5 m area, to examine changes in seagrass abundance, species composition and the presence of reproductive structures.

At the Lizard Island site, three semi-permanent remote camera systems were established in the deep-water meadow for the entirety of the monitoring program. Each system consisted of a Canon 1100D SLR camera with intervalometer housed within a custom-built perspex housing atop a tripod frame with a battery operated wiper unit to maintain a clear field of view. Each camera was programmed to take three still images of a fixed 0.25 m^2 frame of seagrass each day at 09:00, 12:00 and 15:00. Images were downloaded quarterly and used for empirical evidence of changes in seagrass condition, sediment disturbance and bioturbation. Two severe tropical cyclones, Cyclone Ita and Cyclone Nathan, passed over the Lizard Island site in 2014 and 2015 provided a valuable opportunity to track cyclone impact and recovery at the site.

2.2.2 Seagrass abundance

Within each replicate quadrat, an observer made three separate measures of seagrass abundance— i. shoot density, ii. above-ground biomass, and iii. percent cover.

i. Shoot density of each species present was estimated by counting either (a) all shoots in the quadrat (0.25 m^{-2}) when the total was ≤ 40 shoots or (b) all shoots within a mini-quadrat (0.01 m²) randomly placed four times in each quadrat. The number of shoots in each quadrat was scaled up to 1 m² and mean number of shoots m^{-2} determined for each sampling event.

ii. Seagrass above-ground biomass was determined for each quadrat using a "visual estimates of biomass" technique (see Kirkman, 1978; Mellors, 1991) directly in the field. For each quadrat, an observer assigned a biomass rank made in reference to a series of quadrat photographs with biomass ranging from low to high. These quadrat photographs had previously been harvested from the deep-water meadow, the actual dry weight biomass determined in the laboratory and a rank value assigned. A regression of ranks and biomass from the calibration quadrats was generated and applied to each observer's ranks given in the field. Biomass ranks were converted into above-ground biomass estimates in grams dry weight per square metre (g DW m^{-2}).

iii. Percent cover for each quadrat was estimated using a guide sheet with a series of quadrat photographs of similar species composition and cover for which the percent seagrass cover had previously been assigned as per Seagrass-Watch subtidal percent cover standards (McKenzie et al., 2007). The mean seagrass percent cover was generated for each sampling event.

All observers were highly trained and collect similar quadrat-based metrics for seagrasses as part of the research group's seagrass monitoring programs. Inter-observer variance was checked amongst lab members assisting with data collection and negligible differences were found.

2.2.3 Asexual reproduction and productivity

Primary productivity of *H. decipiens* was measured at the Green Island and Lizard Island sites using rhizome tagging technique based on Short and Duarte (2001) to determine the total above-ground production, carbon production and meadow turnover rates. Briefly, rhizomes were tagged behind the first shoot after the basal meristem with a piece of wool. Tagged seagrasses were harvested 6-12 days after tagging and transported frozen to the laboratory for processing. Apical meristem counts were made on two occasions to account for seasonal differences.

In the laboratory, the first shoot was discarded and the remaining shoots counted as new shoot growth. Leaves were photographed and analysed by imaging software (ImageJ) to calculate leaf surface area. Leaves from a single time point were pooled to reduce the error associated with weighing very small and light-weight sections of individual leaves. Wet weights were taken prior to samples being placed in a drying oven at 80ºC for 48 hours to obtain dry weights. The dry weight biomass of an individual leaf section was calculated by dividing the surface area of the leaf by the pooled surface area of all leaves within the sample.

To calculate the total above-ground productivity at each time point, the density of apical meristems was multiplied by the average growth per shoot per day to arrive at daily rates of new leaf growth per meristem per square metre per day (mg DW m^{-2} d^{-1}). Turnover time was based on estimated above-ground biomass measured at the sampling site at the time of rhizome tagging together with the rate of productivity to calculate the number of days until complete turnover of shoots in the meadow. Carbon composition

of total above-ground dry weight was estimated based on a value of 29% for *Halophila* spp. studied in Northern Queensland and was the most geographically applicable (Atkinson and Smith, 1983). A more generalised value quoted for seagrass is slightly higher at 33.5% (Duarte, 1990), therefore our estimates are likely to underestimate rather than overestimate the carbon production capacity of *Halophila* seagrasses.

2.2.4 Chlorophyll *a* fluorescence

Chlorophyll (Chl) *a* fluorescence measurements were performed using a Pulse Amplitude Modulated fluorometer (Diving-PAM; Walz GmbH, Effeltrich, Germany) to assess the efficiency at which light is captured by photosynthetic pigments and passed through the photochemical pathway. Rapid light curves (RLCs) were measured on leaf blades using the in-built software routine of nine incrementing actinic illumination steps (0, 33, 70, 134, 206, 296, 453, 629 and 968 µmol photons m^{-2} s^{-1}) at 10 s intervals. A specialised leaf clip was used to position the fibre optic probe at a fixed distance from the leaf blade for each measurement. One leaf of a leaf pair was measured on six separate shoots for each *Halophila* spp. present at the site (n = 6). Relative electron transport rate was calculated as the product of effective quantum yield (ΦPSII) and irradiance (µmol photons m^{-2} s^{-1}). Data were fitted according to the double exponential function as in Ralph and Gademann (2005) and three photosynthetic parameters; maximum electron transport rate ($rETR_{max}$), light utilisation efficiency (α) and minimum saturating irradiance (E_k) were derived from these curves.

2.2.5 Below-ground carbohydrates

Below-ground carbohydrates were measured during all sampling events for each species present when sufficient root and rhizome material was available to meet minimum weight limits for laboratory analysis (>0.05 g DW). The percentage of soluble sugars and starch content was determined from 2012 – 2016 for *H. decipiens* at Green Island; *H. decipiens*, *H. ovalis*, and *H. spinulosa* at Lizard Island; and *H. tricostata* and *H. decipiens* from Keswick Island.

Samples were defrosted, scraped clean of epiphytes and separated into below- and above-ground structures. Root and rhizome material was dried (60ºC for 48 h) and ground into a fine powder in a bead mill for wet laboratory analysis.

Prepared samples were shipped to the University of Queensland where soluble and non-structural carbohydrates (i.e. starch) were extracted and quantified relative to total sample dry weight (Weir et al., 1977; Karkalas, 1985). Carbohydrates were extracted by placing each sample in 80% ethanol in an 80ºC water bath for 5 min prior to centrifuging (3,000 rpm for 5 min). The supernatant was diluted with 80% ethanol to 25 mL and kept for soluble carbohydrate determination. The pellet remaining was dissolved in 10 mL of deionized water and placed in a 95ºC water bath for a further 30 min while agitating samples at 10 min intervals to solubilize the starch. After reaching room temperature, samples were digested with amylase (Sigma-Aldrich Pty Ltd) and incubated at 55ºC for 30 min prior to dissolving the extracted sample in 20% trichloroacetic acid. Colorimetric determination of starch content was measured using a commercially available glucose oxidase/peroxidase testing kit (GOPOD, Megazyme)

with absorbance measured at 510 nm, and soluble carbohydrates colorimetrically determined with a potassium ferricyanide reagent measured at 420 nm.

2.2.6 Environmental parameters

Benthic irradiance was recorded at 15 minute intervals using submersible 2π cosine-corrected light loggers (Odyssey, Dataflow Systems Pty Ltd, Christchurch, New Zealand) calibrated using an underwater sensor (LI-190SA Li-Cor Inc., Lincoln, Nebraska USA) and fitted to an underwater wiper unit to minimise fouling of the light sensor. At each site, three replicate data loggers were deployed to ensure redundancy and minimize potential data loss due to equipment failure. Replicate temperature data loggers (Thermodata Pty Ltd, Melbourne, Australia) were also deployed at each site and recorded temperature at thirty minute intervals. Loggers were retrieved, data downloaded and loggers re-calibrated prior to re-deployment after each sampling event. Daily total rainfall from the closest known Australian Bureau of Meteorology weather station to each monitoring location was also assessed in relation to wet/dry season patterns of seagrass abundance and light.

To characterise the spectrally-resolved downwelling irradiance available to deep-water seagrasses, an integrated hyperspectral radiometer (RAMSES) was deployed during sampling at the three monitoring sites during regular monitoring trips. Changes in the spectral signature were analysed among and within sites to assess potential seasonal influences and spatial variation among locations. The RAMSES was lowered and profiles recorded at four meter increments until reaching the seafloor. A second

RAMSES was maintained on the surface from the vessel to provide a reference to measured downwelling irradiance relative to any changes in cloud cover.

A multi-spectral absorption and attenuation radiometer (AC-S, Wetlabs, USA) was deployed in tandem with the RAMSES at Green Island, Lizard Island, and the original inshore Mackay site at two time points during an initial 12-month phase, November 2012 and February 2013, to characterise particle scattering in the local water body and monitor changes seasonally and among locations. Depth-averaged absorption "a" and attenuation "c" spectra were recorded. Light scattering in the water column "b" was then assessed as the difference in attenuation and absorption ("a" – "c" = "b").

2.2.7 Statistical Analysis

Statistical analyses were conducted using R v.3.4.4 (R Core Team, 2018). All models included the quadrat as a random effect to account for heterogeneity and repeated measurements of all response variables. Above-ground biomass and presence-absence of seagrass were used as the key response variables for modelling temporal relationships with environmental data. The environmental parameters tested were mean total daily irradiance, and mean maximum daily water temperature.

i. Light Threshold Response to Seagrass Presence

A hinge threshold regression model was used to determine whether seagrass presence/absence and biomass was correlated with a critical level of irradiance at the sediment surface. A change point or threshold parameter provides an explicit way to

model a non-linear relationship between the outcome (i.e. above-ground biomass) and a predictor (i.e. environmental factor) (Fong et al., 2017). Mean total daily irradiance for 14 days prior to seagrass surveys (PAR_{14}) was assessed against above-ground biomass by species, based on previous work indicating low light impacts to deep-water *Halophila* spp. in ≤14 days (Chartrand et al., 2018). Biomass data were analysed using the chngpt package in R (Fong et al., 2017).

ii. Effect of Timing and Environmental Factors on Seagrass Presence and Biomass

The response variables, seagrass presence/absence data and biomass, were analysed against the predictors *Year* (June-May), *Day in Year*, PAR_{14}, and the 14 day mean maximum daily water temperature ($maxtemp_{14}$) to integrate the water temperature leading up to each sampling event. Based on the outcomes from the hinge regression analysis, PAR_{14} at each sampling time point was classified as either sufficient light to sustain seagrass (HL) or below the defined threshold (LL) and treated as a factor in all further analysis. Significant impacts on the Lizard Island site by a category 4 cyclone, Cyclone Ita, in April 2014 led to data in the annual 2014/15 growing cycle being considered as a cyclone effect (*CIta*) in all models. An exploratory analysis of response and predictor variables was completed prior to fitting models due to the non-linear relationship between the response (i.e. biomass) and predictor variables (Wood et al 2017).

Generalized additive mixed models (GAMMs) were fitted using the mgcv package in R (Wood 2017). All response variable data consisted of a large proportion of valid zeroes

due to the naturally patchy and variable growth and composition of deep-water seagrass meadows (i.e. “zero inflated”). The data were thus analysed with GAMMs in two forms: 1) presence-absence data with a binomial distribution (with logit link) and 2) positive only biomass data with a Gamma distribution (and log link).

To determine the optimal model, a global model was created for each response variable where all explanatory variables and interactions were considered. Sub-model sets of the global model were then generated using the dredge function in the MuMIn package (Bartoń, 2013). The best-fit models were considered to be those with the lowest Akaike's information criterion (AICc) and highest Akaike weight (w), which by definition contain the best set of explanatory factors for adequately predicting each response variable (Burnham and Anderson, 2002; Wagenmakers and Farrell, 2004). Models with AIC_c values within 2 of each other were considered strong models and are presented with the chosen model being the simplest of this sub-set which was further used for multiple comparison analysis (Burnham and Anderson, 2002). All models were validated by assessing Pearson residuals against fitted model values.

Standard model diagnostics including residuals of the best-fit models were inspected for heteroscedasticity and non-normality. *Halophila ovalis* biomass was square-root transformed to correct for heterogeneity. Pearson correlation coefficients were used to check for collinearity of predictor variables. Due to the strong collinearity of *Day in Year* and $maxtemp_{14}$ ($r = 0.9$), models were run using both covariates independently because these predictors are potentially interchangeable.

Biomass was also analysed for the period of the year when seagrass either first returned to the site following annual loss or was actively growing rather than senescing from the previous time point; data was binned based on an annual growth cycle (*Year*). Generalised linear mixed models (GLMMs) were performed separately on seagrass biomass to evaluate the effect of *Year.* Post-hoc comparisons were made on estimated marginal means using the Bonferonni adjustment method in the emmeans package (Lenth, 2016). Due to limited data at the Keswick Island site, model complexity had to be simplified and a linear mixed effects model was used to assess the effect of *Day in Year* alone on biomass. All models were again checked for residuals and the best-fit model selected based on AIC_c.

2.3 Results

2.3.1 Abundance and Growth Patterns

Green Island

The deep-water Green Island meadow was a patchy monospecific *Halophila decipiens* community with an annual pattern of germination, expansion and complete senescence of the meadow over the four-year study. Each year, shoots emerged between June and August (austral winter) and increased in abundance (above-ground biomass, percent cover and shoot density) through October/November before total seagrass senescence by the end of the year or early in the new calendar year (Figure 2.2). This cycle varied in magnitude with substantially smaller peak biomass in 2013 and 2015 compared to all other years ($F_3 = 18.6$, $p < 0.001$; Figure 2.3a).

Mean productivity rates for *H. decipiens* were generated from four sampling periods at Green Island (Table 2.1). Highest productivity rates paralleled trends in maximum above-ground biomass between September and November each year. Rhizome extension ranged from 4.69 ± 0.94 mm d^{-1} in October 2012 to 17.62 ± 2.06 mm d^{-1} in October 2015. New biomass production on newly generated rhizome during this period was 420 ± 10 to 690 ± 9 mg DW m^{-2} d^{-1}. In comparison, only a single tagged rhizome was retrievable in January 2012 with a measured extension rate of 1.43 mm d^{-1} and an estimated above-ground productivity of 0.10 mg DW m^{-2} d^{-1}; coinciding with the seasonal seagrass decline for the 2011/2012 growing year (Figure 2.2). A drop in apical meristem density underpinned the disparity between October 2012 growth and the previous January 2012 senescence (Table 2.1). Estimates of carbon production at Green Island were greatest in October 2015 at 200.3 mg C m^{-2} d^{-1} contrasting with 0.2 mg C m^{-2} d^{-1} in January 2012 (Table 2.1).

Lizard Island

Deep-water seagrass at Lizard Island followed a similar annual cyclic peak and trough pattern to Green Island for all morphometrics; greatest abundance was found each October and January with a marginal but persisting meadow in April (Figure 2.2). From April 2012 to April 2014, the deep-water Lizard Island meadow was dominated by *Halophila ovalis* year-round with a smaller presence of *H. decipiens*, *Halophila spinulosa* and *Halodule uninervis* (Figure 2.4). *Halophila decipiens* appeared during the October/November peak growing periods but in lower abundance during both 2012 and 2013 (Figure 2.3). Abundance of *H. ovalis* reached a maximum in late October 2012 (2.28 ± 0.49 gDW m^{-2}, 7.59 ± 0.83 percent cover and 606 ± 61 shoots m^{-2}; Figure 2.3)

and a minimum in the following April 2013 (0.25 ± 0.03 gDW m^{-2}, 0.75 ± 0.08 percent cover and 51 ± 7 shoots m^{-2}; Figure 2.3b).

Two category 4 cyclones crossed directly over the Lizard Island site in the following two years of the study which affected both the presence of seagrass at the site and species composition (Figure 2.4a). On 11 April 2014, Cyclone Ita buried the majority of the monitoring site with more than 10 cm of sand and led to complete loss of seagrass (Figure 2.2) when surveyed two weeks post cyclone. By June 2014, the first shoots of *H. decipiens* were found recruiting to the site before *H. ovalis* was recorded in September, five months following the cyclone (Figure 2.4a). *H. decipiens* dominated the meadow until January 2015 when it began senescing and *H. ovalis* reached its peak annual abundance; a delay from the October/November peaks recorded in 2012 and 2013 (Figure 2.4a). Cyclone Nathan (Category 4) subsequently passed over the site on the 20th of March 2015, but surveys two weeks post-cyclone showed minimal sediment shift, burial or erosion and a small number of *H. ovalis* shoots present; a contrast to impacts from Cyclone Ita. By January 2016, the end of this monitoring program, *H. ovalis* biomass remained depressed from pre-cyclone levels while in its place, biomass reached the highest ever recorded abundance at the site in November 2015 as a result of a proliferation of *H. decipiens* shoots (1.99 ± 0.25 gDW m^{-2}; $F_3 = 50.5$, $p < 0.001$; Figure 2.4a). Overall, *CIta* and *Day in Year* had a significant effect on seagrass biomass with the 2014/15 peak abundance during October surveys, which was depressed compared to all other years (Figure 2.3b). See Appendix A for time-lapse images capturing the impacts and recovery from these cyclone events.

The productivity estimates at Lizard Island in September 2014 when rhizome extension of *H. decipiens* was 11.49 ± 1.64 mm d^{-1}, was greater than rates for *H. decipiens* at Green Island for the same time of year. However, the new biomass produced was considerably less at 3.2 ± 0.5 mg DW m^{-2} d^{-1} (Table 2.1). In comparison, *H. ovalis* productivity of above-ground biomass over the same period was higher at 5.5 ± 0.9 mg DW m^{-2} d^{-1}, but with less extension of rhizome equating to a higher density of shoots compared to *H. decipiens*. This faster productivity equated to a faster turnover of *H. ovalis* compared to *H. decipiens;* 2.5 days versus 20.9 days, respectively.

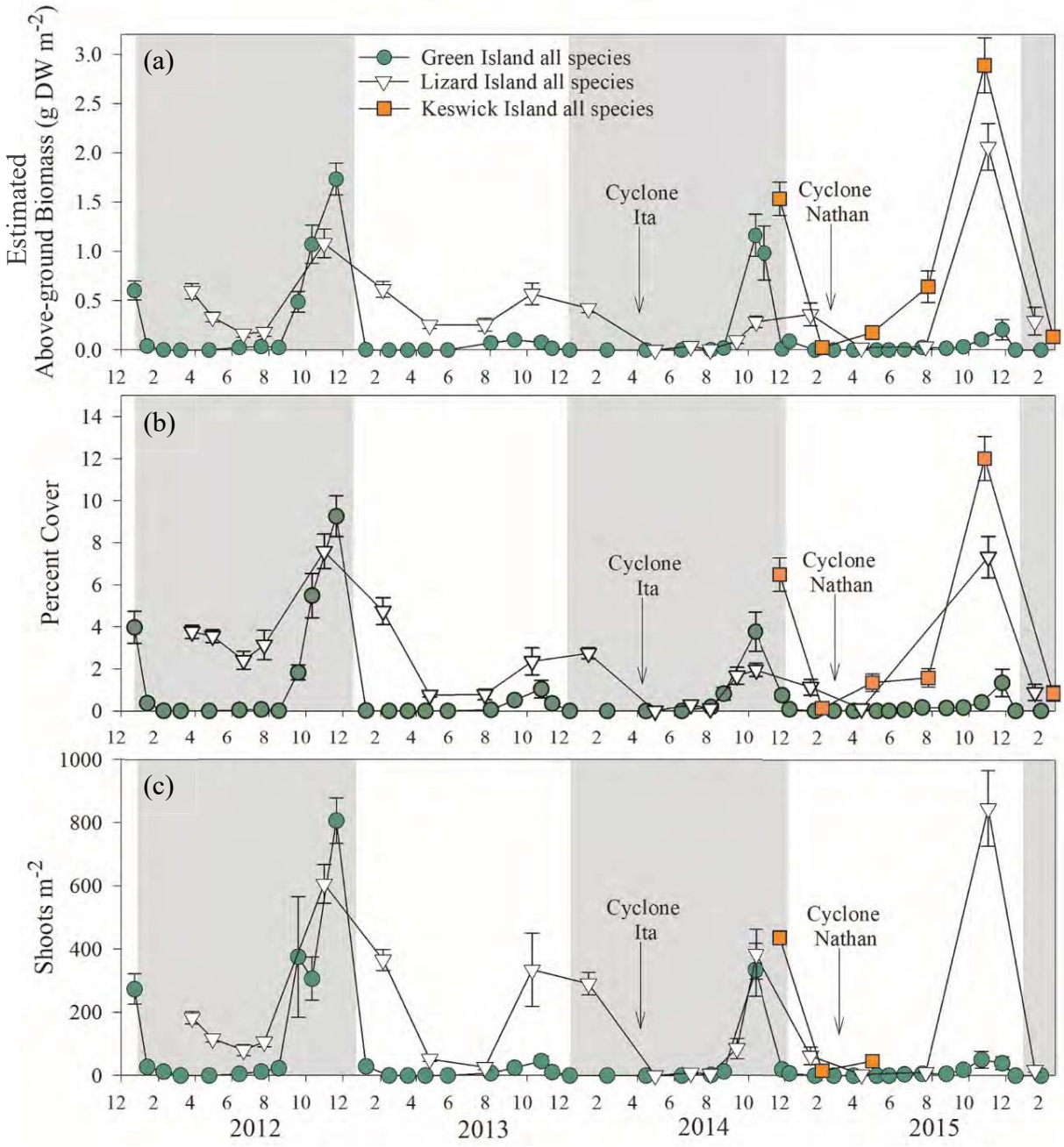


Figure 2.2 (a) Total species mean estimated above ground biomass (g DW m^{-2}), (b) percent cover (m^{-2}), and (c) shoot density (m^{-2}) for Green Island, Lizard Island, and Keswick Island deep-water monitoring sites (n = 27, ± SE). N*o data recorded due to inclement weather at Keswick Island in late 2015*.

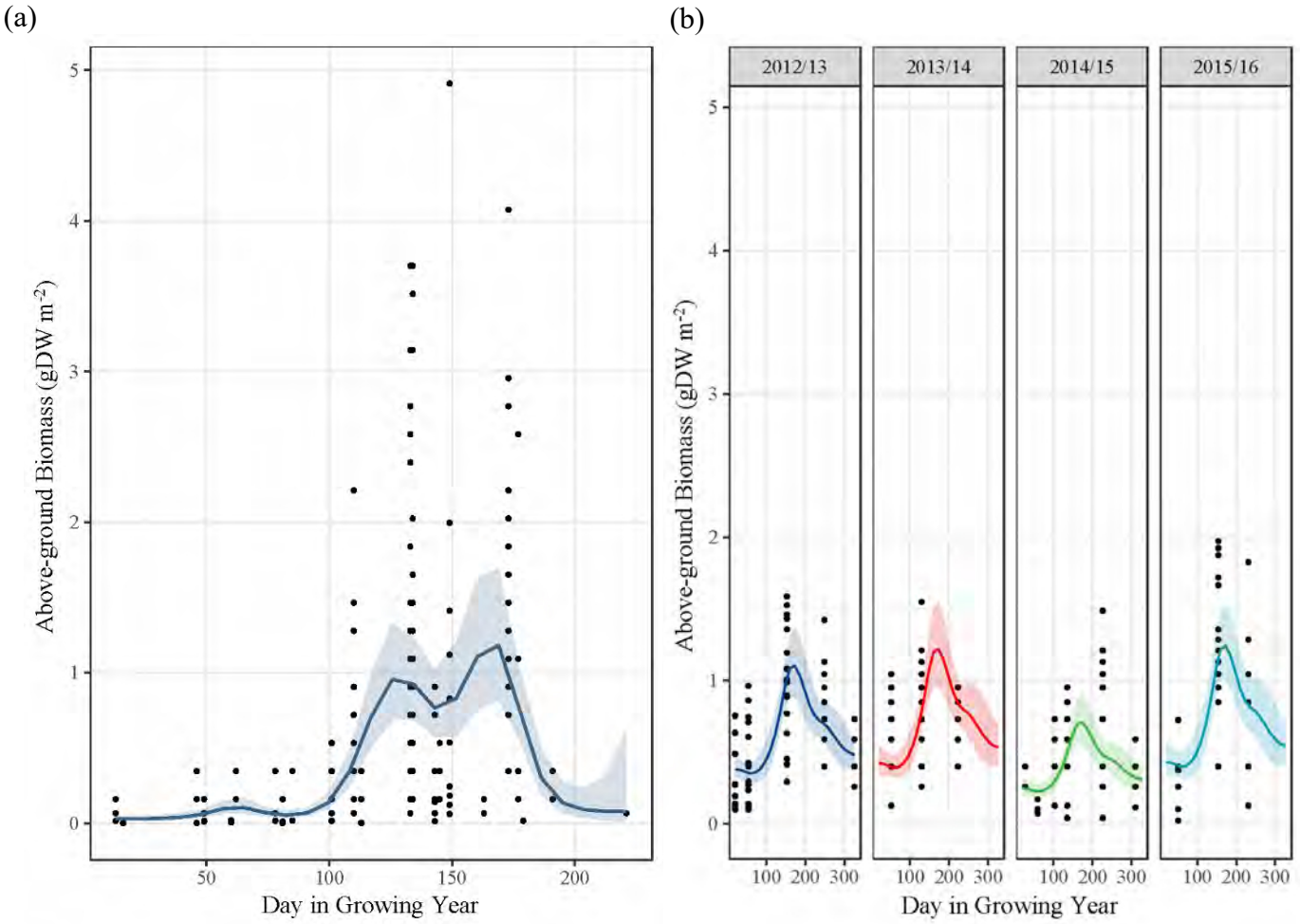


Figure 2.3 Predicted fit of (a) Green Island and (b) Lizard Island total site above-ground biomass as a function of *Day in Year*. Non-linear trends are the fit of gamma generalized additive mixed models with seagrass above-ground biomass as the response variable. Shaded areas are 95% confidence intervals.

Table 2.1 Productivity, rhizome extension rates and carbon production for *H. decipiens* at Green Island and Lizard Island and *H. ovalis* at Lizard Island. Comparative literature values are provided in grey for context.

Location	Date	Sample Size (n)	Apical Density (m^{-2})	Rhizome growth (mm d^{-1})	Above-ground production (mg DW m^{-2} d^{-1})	Above-ground carbon production (mg C m^{-2} d^{-1})	Turnover Time (days)
Green Island	Jan 2012	1	1.0	1.43 ± NA	0.1 ± NA	0.03	369
Green Island	Oct 2012	8	337.3	4.69 ± 0.94	420 ± 10	121.3	2.56
Green Island	Nov 2013	6	4.5	6.02 ± 1.38	10 ± 0	2.9	1.68
Green Island	Oct 2015	20	–*	17.62 ± 2.06	690 ± 9	200.3	0.15
Lizard Island *H. decipiens*	Sept 2014	15	15	11.49 ± 1.64	3.2 ± 0.5	0.9	20.9
Lizard Island *H. ovalis*	Sept 2014	12	15	7.59 ± 1.26	5.5 ± 0.9	1.6	2.5
Florida, USA *H. decipiens*	Jun 1999[1]	22	1370	–	68	23.0	23.3
US Virgin Islands *H. decipiens*	May 1985[2]	196	315.6	–	856	145	10.6
Indonesia *H. ovalis*	1999[3]	10	373	3.9 ± 4.4	–	830 - 1380	6.9

[1] Hammerstrom et al. (2006); [2] Kenworthy et al. (1989); [3] Erftemeijer and Stapel (1999);
*used October 2013 meristem data as a proxy for calculations due to unavailable data

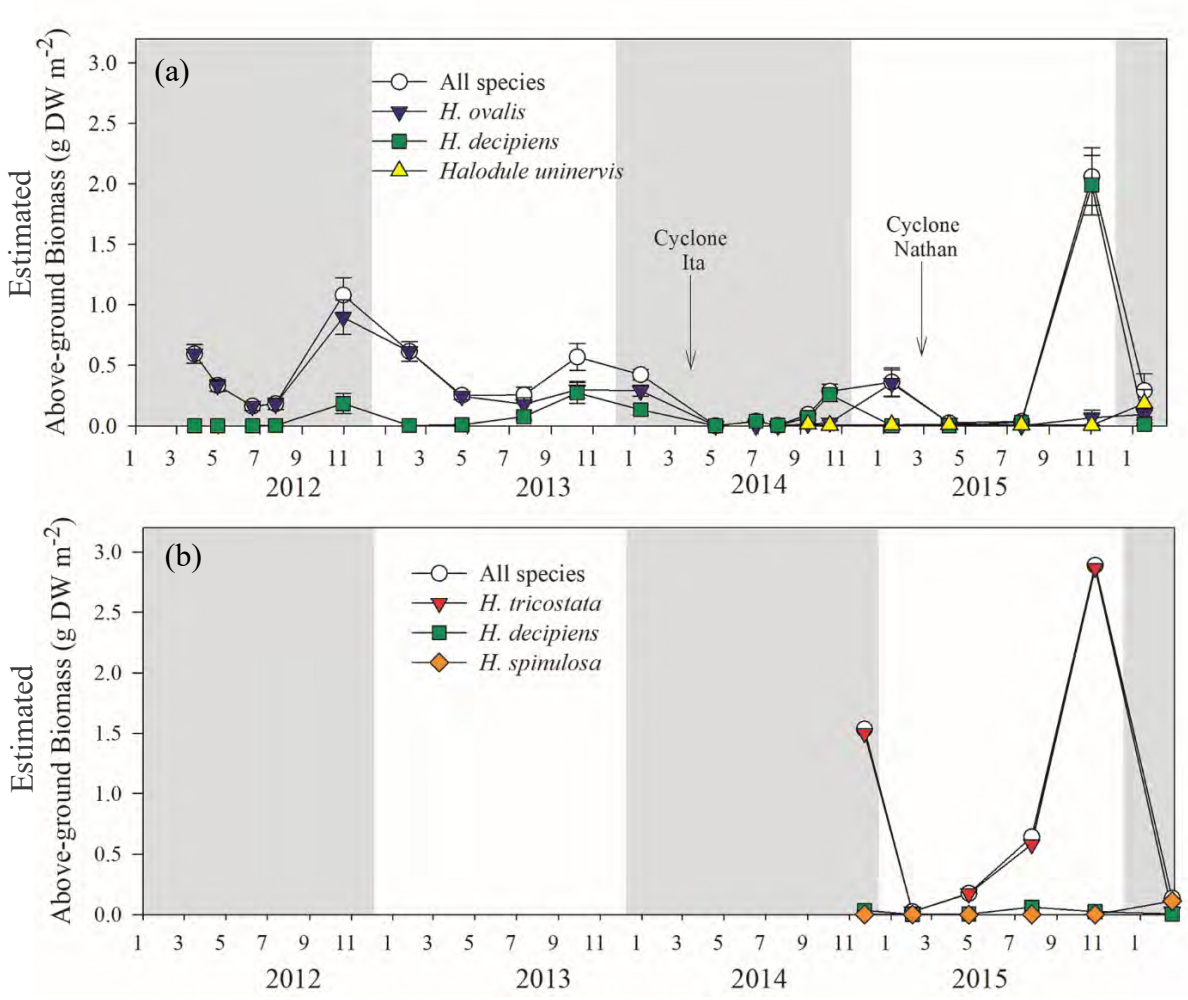


Figure 2.4 Estimated above-ground biomass by species at (a) Lizard Island and (b) Keswick Island. Error bars indicate ± SE (n = 27).

Keswick Island

The Keswick Island site was set up in November 2014 (Figure 2.2) where morphometrics were similar to the Green Island site (Figure 2.2). The site was in a mixed meadow dominated by *Halophila tricostata* with a lesser abundance of *H. spinulosa* and *H. decipiens* (Figure 2.4b). All species followed a similar cyclic growth pattern to Green Island and Lizard Island with peak abundance in November 2014 and October 2015 and a minimum in February 2015 ($F_5 = 58.8$, $p < 0.001$). While *H. decipiens* was absent at Green Island and Lizard Island by February each year and only recruiting in June/July, remnant shoots of *H. decipiens* and *H. tricostata* remained during February and April sampling at the Keswick Island site.

2.3.2 Environmental patterns and drivers

Green Island

Rainfall at the Green Island site followed a typical tropical wet-dry season pattern with highest rainfall totals from January to March and the lowest from June to September (Figure 2.5a). Overall, rainfall had a minimal effect on salinity due to the depth of the site with values stable at 35-36 PSU for the majority of the four-year program (pers. obs.). Exceptions were during peak rainfall events in the wet season when there was a drop to 32 PSU for a 24 to 48 hour period before returning to the characteristic range; likely triggered by flooding from local mainland rivers.

Available light at the Green Island site declined in line with wet season rainfall and ranged from 0 to 5.4 mol photons m^{-2} d^{-1} with an overall mean of 1.81 ± 0.02 mol photons m^{-2} d^{-1} (Table 2.2). Light increased over the drier months from July to

September, generally reaching 2 mol photons m^{-2} d^{-1} by August and remaining high through January followed by a gradual decline in line with the monsoon rains and associated decline in inshore water quality. Light from April to August routinely fell below 1 mol photons m^{-2} d^{-1}.

Maximum daily water temperatures at the seagrass canopy followed a similar annual pattern to light. While light levels peaked by late January, seasonal temperatures continued to increase until early March in all years reaching a maxima of 30˚C before dropping to a minima in mid-July at 22˚C (Figure 2.5a).

When seagrass was increasing in above-ground biomass, i.e. the growing season, the mean light level was 2.08 ± 0.02 mol photons m^{-2} d^{-1}. Mean growing season light level was consistent with the threshold regression model output which found a significant increase in above-ground biomass as a function of PAR_{14} greater than 2.0 mol photons m^{-2} d^{-1} (L = 80.7, p <0.001, [95% CI 1.7, 2.3], Figure 2.6a). This threshold response to light conditions also occurred for the likelihood of seagrass presence/absence as a function of PAR_{14} with a binomial hinge regression model (2.2 mol photons m^{-2} d^{-1}, L = 25.0, p <0.001, [95% CI 1.9, 2.4]).

At the end of each annual growing season, above-ground biomass declined before significant reductions in the ambient benthic light environment which began to decline with heavy rainfall and coastal runoff in late January and February (Figure 2.6a).

Seagrass was present at the Green Island site when temperatures ranged from 23˚C to 29˚C. When the PAR_{14} hinge threshold of 2.0 mol photons m^{-2} d^{-1} was met, seagrass biomass increased with $maxtemp_{14}$ up to a peak at 26.6ºC before declining at higher temperatures (Figure 2.7a, Table 2.3); a similar effect to the highly collinear predictor *Day in Year* (Figure 2.3a). The pattern between above-ground biomass and $maxemp_{14}$ did not hold true for seagrass presence/absence data; likely due to the extremely patchy nature of the meadow, even at the most abundant times of the year (Table 2.3).

Lizard Island

Daily rainfall was substantially lower at the Lizard Island site than at Green Island in both 2012 and 2013, while large totals were recorded in March 2014 and April 2015 when category 4 Cyclones Ita and Nathan tracked directly over Lizard Island (Figure 2.5b). Each year the monsoon rains occurred from January until late March when rainfall was greatest. As with Green Island, only slight fluctuations in salinity were recorded even during significant rain events, likely due to the depth at which the monitoring sites are located.

Light at the Lizard Island site ranged from 0.3 to 8.3 mol photons m^{-2} d^{-1} and followed the same seasonal pattern as the Green Island site (Figure 2.5b); however, with significantly higher light levels sustained over the course of the monitoring period (mean = 3.4 ± 0.04 mol photons m^{-2} d^{-1}) and specifically during the germination and peak growing season (mean = 3.8 mol photons m^{-2} d^{-1}; Table 2.2). Light was typically above 3 mol photons m^{-2} d^{-1} from September through to the onset of the wet season in late January when light was attenuated during seasonal storm events, increased cloud

cover and catchment runoff (Figure 2.5b). Despite these episodic drops in light levels and gradual decline in solar insolation from the Austral autumnal equinox in March, the mean light level at Lizard Island over the February to April period was relatively high, 2.9 ± 0.1 mol photons m^{-2} d^{-1}, compared to Green Island.

Maximum daily temperatures at the seagrass canopy ranged from 23°C in late July 2012 to 30°C in late February 2013 (Figure 2.5b). Maximum daily temperatures followed seasonal trends, much like Green Island, except minimum temperatures were reached, on average, a week later and annual maximum a week sooner at this more northern location.

Due to the impacts from the two category 4 cyclones, statistical relationships between seagrass biomass and species dynamics with environmental data were confounded. Analysis of seagrass presence or biomass by species with environmental parameters was ambiguous for relationships with *H. ovalis* but found the *H. decipiens* population had similar relationships to light and temperature as at the Green Island site (Table 2.3). The patchiness of *H. decipiens* resulted in no relationship between presence/absence and environmental variables (Table 2.3), but increases in Lizard Island *H. decipiens* biomass was significantly coupled to light above 2.7 mol photons m^{-2} d^{-1} (L = 56.8, $p < 0.001$, [95% CI 2.45, 2.90]; Figure 2.6b), higher than the *H. decipiens* light threshold at Green Island indicating seagrasses were likely acclimated to a higher light growing environment. When light was above 2.7 mol photons m^{-2} d^{-1}, *H. decipiens* had the highest likelihood of occurring when the mean maximum daily temperature was 25.6°C

for all years despite a reduced peak in biomass following Cyclone Ita (Figure 2.7b; Table 2.4).

Halophila ovalis was persistent year round prior to cyclone impacts signifying light was not limiting its' presence at Lizard Island. There was also no clear threshold relationship between light and *H. ovalis* presence (Table 2.4). The best model fit of *H. ovalis* above-ground biomass was driven by PAR_{14} although the model fit was relatively poor (Figure 8a; Table 2.4). Deep-water seagrass presence as a whole was not explained by any of the tested covariates, while total site biomass was correlated with $maxtemp_{14}$. (Figure 8b; Table 2.4).

Keswick Island

Overall mean light at Keswick Island was 2.0 ± 0.1 mol photons m^{-2} d^{-1} with an increase in above-ground biomass during the germination/growing season when light was on average 1.5 ± 0.1 mol photons m^{-2} d^{-1} (Figure 2.5c; Table 2.2). Substantial wet season rainfall in 2014 and a more prolonged wet season through the Austral winter months in 2015 pushed light levels below 1.0 mol photons m^{-2} d^{-1} for short, albeit regular intervals, owing to the oceanic flushing at this location (Waltham et al., 2019). Due to the short time period of monitoring at this location, the dataset was too limited for GAMM or hinge regression analysis.

Maximum daily temperatures during the winter reached 21°C in late July 2015 and a high of 29°C in February each year.

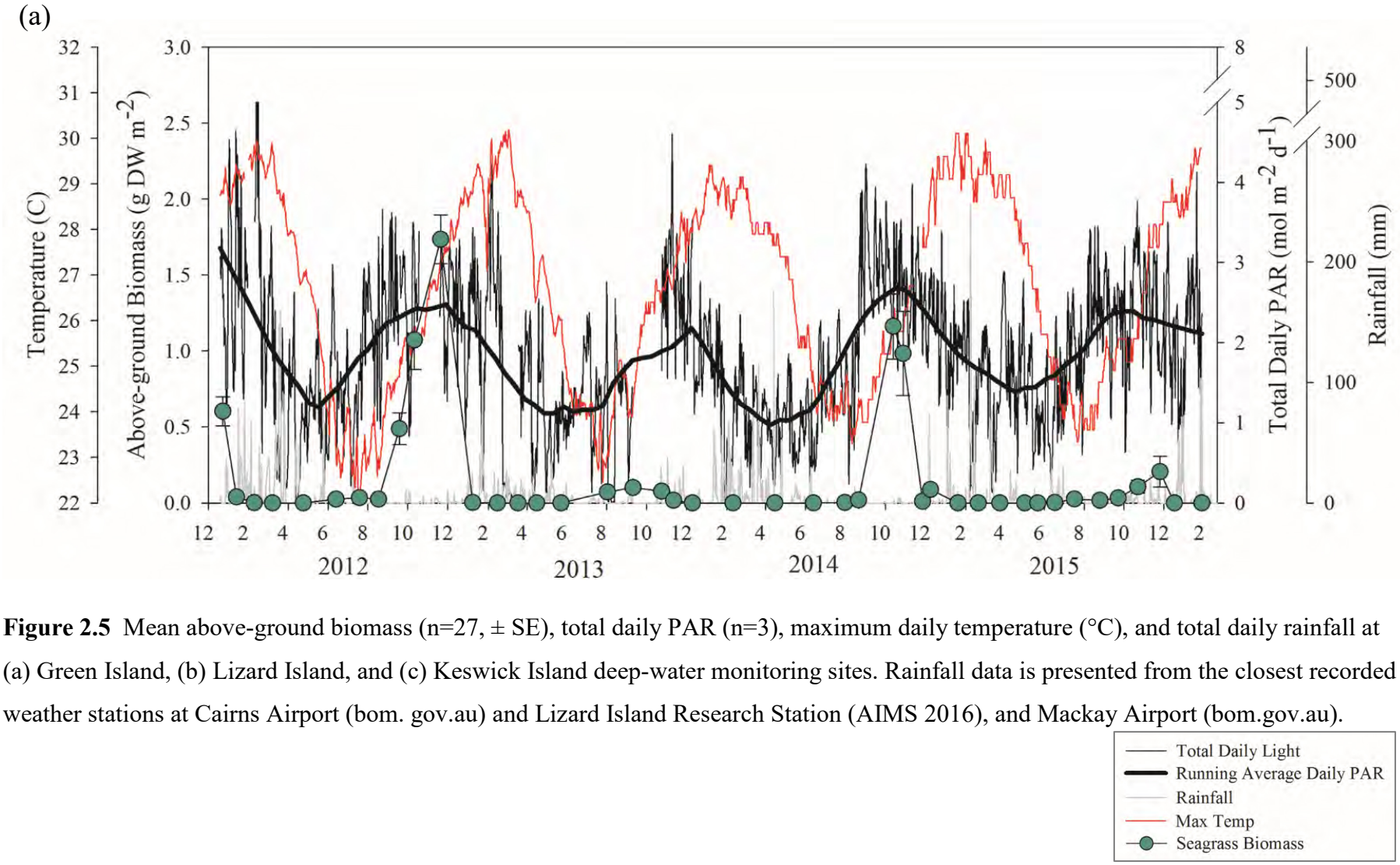


Figure 2.5 Mean above-ground biomass (n=27, ± SE), total daily PAR (n=3), maximum daily temperature (°C), and total daily rainfall at (a) Green Island, (b) Lizard Island, and (c) Keswick Island deep-water monitoring sites. Rainfall data is presented from the closest recorded weather stations at Cairns Airport (bom. gov.au) and Lizard Island Research Station (AIMS 2016), and Mackay Airport (bom.gov.au).

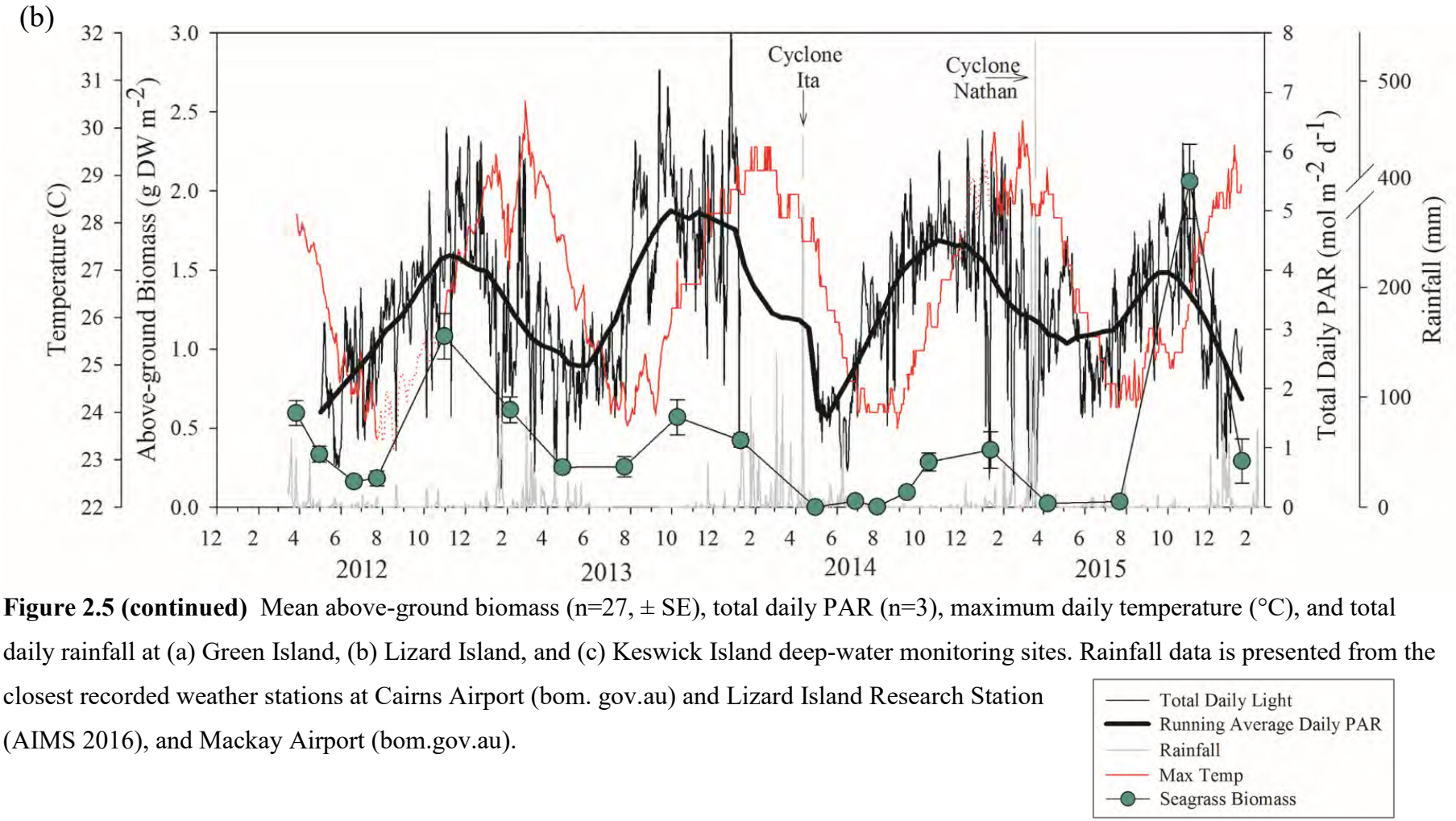


Figure 2.5 (continued) Mean above-ground biomass (n=27, ± SE), total daily PAR (n=3), maximum daily temperature (°C), and total daily rainfall at (a) Green Island, (b) Lizard Island, and (c) Keswick Island deep-water monitoring sites. Rainfall data is presented from the closest recorded weather stations at Cairns Airport (bom. gov.au) and Lizard Island Research Station (AIMS 2016), and Mackay Airport (bom.gov.au).

(c)

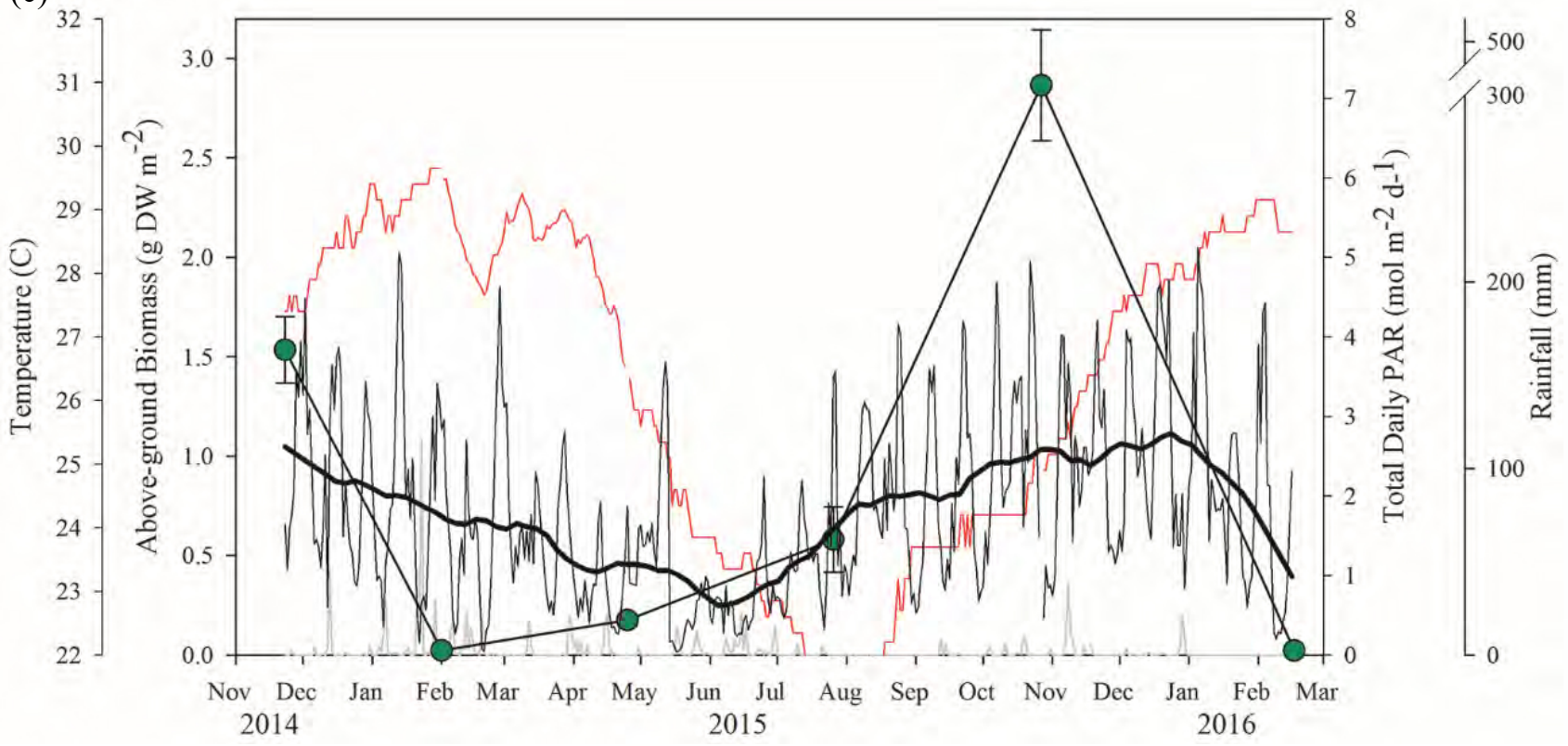


Figure 2.5 (continued) Mean above-ground biomass (n=27, ± SE), total daily PAR (n=3), maximum daily temperature (°C), and total daily rainfall at (a) Green Island, (b) Lizard Island, and (c) Keswick Island deep-water monitoring sites. Rainfall data is presented from the closest recorded weather stations at Cairns Airport (bom. gov.au) and Lizard Island Research Station (AIMS 2016), and Mackay Airport (bom.gov.au).

Table 2.2 Light (*I*) (mol photons m^{-2} d^{-1}) and temperature conditions at the three monitoring sites from April 2012 – January 2016 for Green and Lizard Island sites and November 2014 – January 2016 for Keswick Island. Growing season site median and mean values incorporate days when seagrass was increasing since the previous survey (i.e. ~July – November).

Location	Depth (m)	Median Total Daily *I*	Mean Total Daily *I* (± S.E.)	Median Daily Max Temp (°C)	Mean (± S.E.) Daily Max Temp (°C)
Overall					
Lizard Island	15	3.43	3.43 (0.04)	26.7	26.7 (0.05)
Green Island	17	1.78	1.81 (0.02)	26.6	26.5 (0.05)
Mackay*	12	0.65	1.08 (0.05)		
Keswick Island	13	1.78	2.01 (0.06)	27.4	26.2 (0.12)
Germination/ Growing Phase					
Lizard Island		3.80	3.73 (0.05)1.21	25.2	25.3 (0.04)
Green Island		2.11	2.08(0.04)0.86	25.3	25.6 (0.03)
Keswick Island		1.17	1.51 (0.07)1.08	24.2	24.7 (0.13)

* Only monitored from July 2012 - March 2014; no seagrass present at site to generate growing season values

Table 2.3 Summary of generalized additive mixed models (GAMMs) and the best model selected (in bold) based on an $AIC_C < 2$ for predicting *H. decipiens* seagrass presence/absence and above-ground biomass at Green Island and Lizard Island. All models had a random effect (β) of quadrat and random error term *ε*. *Y* is *Year*; *CIta* is a fixed factor for the 12 months post-Cyclone Ita data*; maxtemp*$_{14}$ is mean maximum daily temperature for the 14 days prior to sampling; and PAR_{14} is mean daily light for the 14 days prior to sampling.

Location/ Species		Models	df	Log Lik	AIC_c	ΔAIC_c	*w*
Green Island	**Response Variable**						
H. decipiens	Presence/ Absence	$\mathbf{Hd_{presence} = 1 + \beta_{quadrat} + \varepsilon}$	2	-852.7	1709.4	0.00	1
		$Hd_{presence} = Y + \beta_{quadrat} + \varepsilon$	5	-906.66	1823.5	114.05	0
	Biomass	$\mathbf{Hd_{biomass} = s(maxemp14) + \beta_{quadrat} + \varepsilon}$	**5**	**-280.67**	**571.7**	**0.00**	**0.81**
		$Hd_{biomass} = s(maxemp14) + Y + \beta_{quadrat} + \varepsilon$	8	-278.86	574.5	2.87	0.19
Lizard Island							
H. decipiens	Presence/ Absence	$\mathbf{Hd_{presence} = 1 + \beta_{quadrat} + \varepsilon}$	**2**	**-628.71**	**1261.5**	**0.00**	**0.76**
		$Hd_{presence} = CIta + \beta_{quadrat} + \varepsilon$	3	-628.87	1263.8	2.35	0.24

Table 2.3 (Continued)

Location/ Species		Models	df	Log Lik	AIC_c	ΔAIC_c	*w*
		$Hd_{presence} = s(maxemp14) + CIta + \beta_{quadrat} + \varepsilon$	5	-676.67	1363.6	102.1	0.00
	Biomass	$\boldsymbol{Hd_{biomass} = s(maxemp14) + CIta + \beta_{quadrat} + \varepsilon}$	**7**	**-272.93**	**560.6**	**0.00**	**0.97**
		$Hd_{biomass} = s(maxemp14) + \beta_{quadrat} + \varepsilon$	6	-278.22	569.0	8.39	0.01
H. ovalis	Presence/ Absence	$\boldsymbol{Ho_{presence} = 1 + \beta_{quadrat} + \varepsilon}$	**2**	**-971.11**	**1946.2**	**0.00**	**1**
		$Ho_{presence} = s(maxemp14) + \beta_{quadrat} + \varepsilon$	4	-1010.0	2028.1	81.84	0
	Biomass*	$\boldsymbol{Sqrt\ (Ho) = 1 + s(par14) + \beta_{quadrat} + \varepsilon}$	**3**	**-245.57**	**501.4**	**0.00**	**0.89**
		$Sqrt\ (Ho) = s(maxemp14) + s(par14) + \beta_{quadrat} + \varepsilon$	5	-246.00	505.6	4.14	0.11
All species	Presence/ Absence	$\boldsymbol{Bmass_{presence} = 1 + \beta_{quadrat} + \varepsilon}$	**3**	**-459.92**	**925.9**	**0.00**	**1**
		$Bmass_{presence} = s(maxemp14) + \beta_{quadrat} + \varepsilon$	5	-472.24	954.6	28.71	0
	Biomass*	$\boldsymbol{Sqrt\ (Bmass) = s(maxemp14) + \beta_{quadrat} + \varepsilon}$	**5**	**-649.06**	**1308.2**	**0.00**	**0.97**
		$Sqrt\ (Bmass) = s(maxemp14) + s(par14) + \beta_{quadrat} + \varepsilon$	7	-650.59	1315.4	7.17	0.03

* Square root transformed due to skewness of data

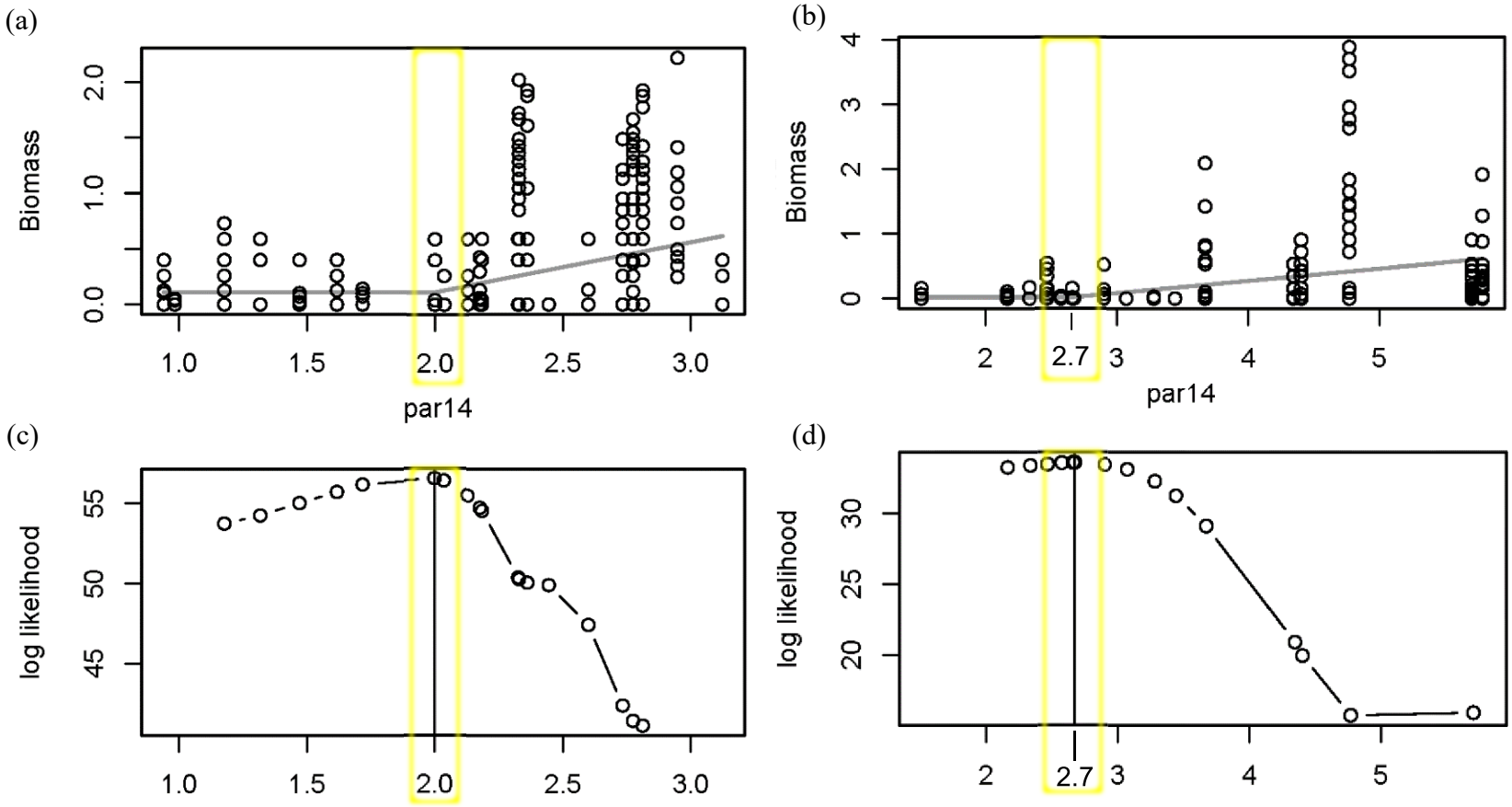


Figure 2.6 Hinge regression model (a) of predicted Green Island and (b) Lizard Island *H. decipiens* above-ground biomass as a function of 14 day average light (par14; mol photons m^{-2} d^{-1}); and (c) Green Island and (d) Lizard Island likelihoods of the restricted regression models with fixed change points (highlighted in yellow) versus candidate change points of 14 day mean light. Note varying scales.

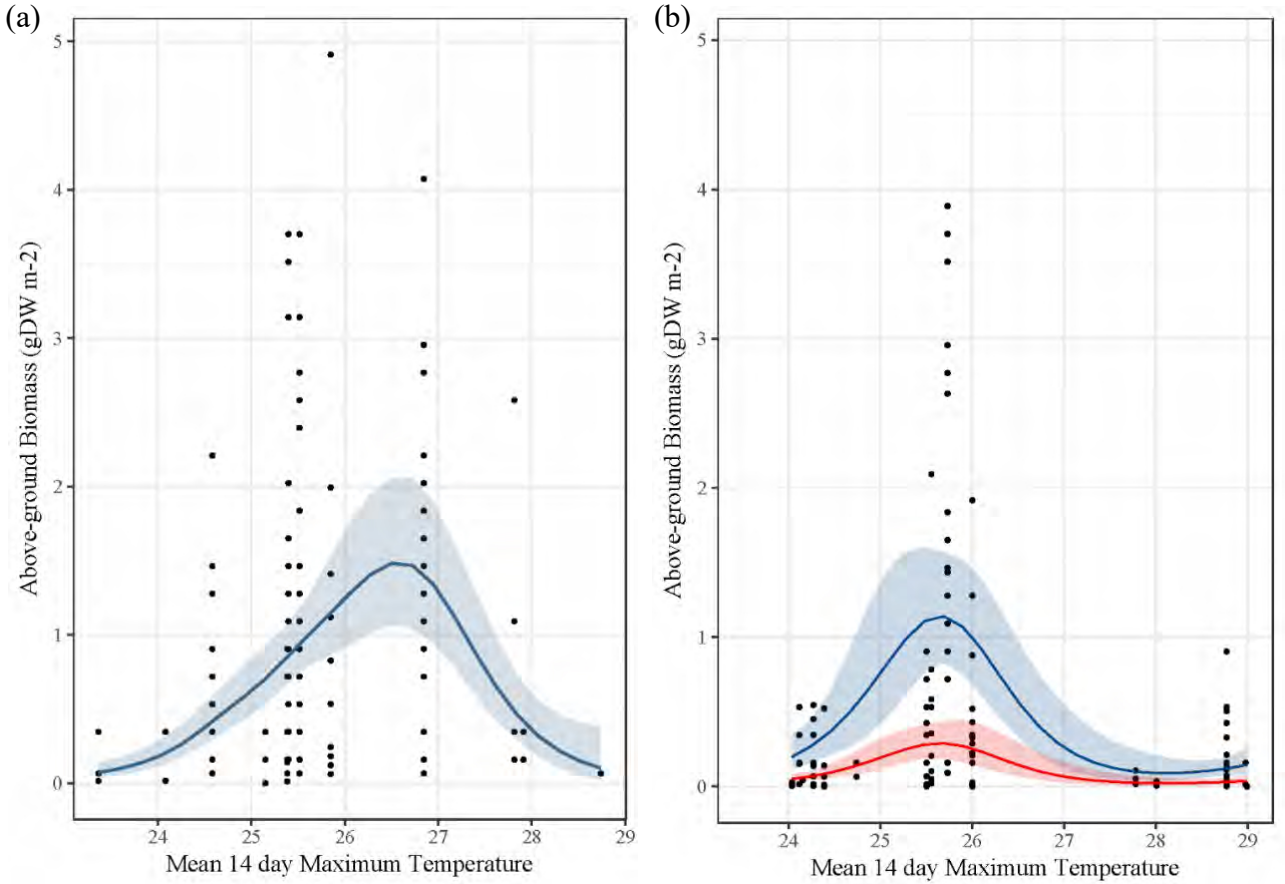


Figure 2.7 Predicted *H. decipiens* above-ground biomass as a function of the 14 day mean maximum daily temperature at (a) Green Island when PAR_{14} was greater than 2.0 mol photons m^{-2} d^{-1} and at (b) Lizard Island when PAR_{14} was greater than 2.7 mol photons m^{-2} d^{-1} (see Fig. 6). Non-linear trends are the fit of gamma generalized additive mixed models with seagrass above-ground biomass as the response variable. The red line represents the 12 month period following Cyclone Ita at Lizard Island. Shaded areas are 95% confidence intervals.

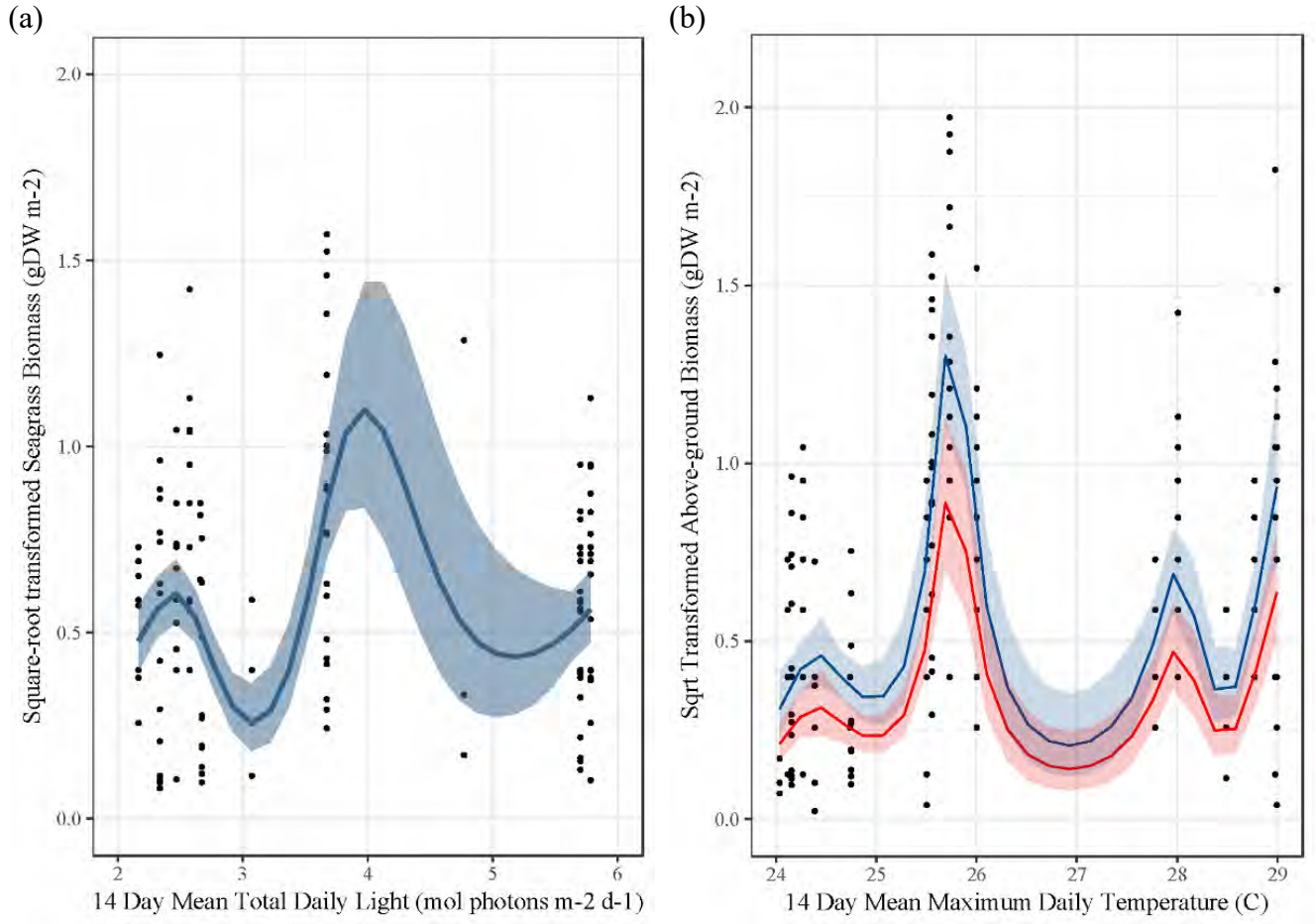


Figure 2.8 Predicted fit of Lizard Island square-root transformed (a) *H. ovalis* and (b) all species above-ground biomass as a function of PAR_{14} and $maxtemp_{14}$ respectively. Non-linear trends are the fit of gamma generalized additive mixed models with seagrass above-ground biomass as the response variable. Shaded areas are 95% confidence intervals.

2.3.3 Spectral quality of light

The percent distribution of spectrally resolved downwelling irradiance showed differences between sites and time of year. While sampling timelines at each location were variable due to inclement weather and logistical constraints, seasonal information to compare among sites was available and on a few occasions sampling among locations only varied over a week or two. For example, in January 2015, Green Island had a slightly more blue-shifted spectra with more downwelling irradiance from ~420 – 500 nm and total loss of light by ~580 nm compared to either Lizard Island or Keswick Island sites which had total loss of light above 600 nm (Figure 2.9). This sharp attenuation of light above 600 nm at all locations is typical for deeper waters (Kirk, 1994). Keswick had the lowest proportion of irradiance in the equivalent blue wavelength range but the greatest proportion of light amongst the three locations in the 500 – 580 nm range (Figure 2.9). For comparison, a previously monitored deep-water seagrass meadow inshore from Keswick Island near Mackay was absent when surveyed in October 2013 and site spectral irradiance measurements found a significant loss of light from 400-520 nm leaving a very small window of available light for seagrass photosynthesis (Figure 2.9). This also coincided with the lowest bandwidth of leaf specific absorptance measured and the lowest total site irradiance amongst all monitored locations. While overall patterns of qualitative light were somewhat similar, total quantity differed by location (Figure 2.10). Lizard Island had the highest proportion of surface irradiance at all wavelengths, whereas the Green Island and Keswick Island sites recorded nearly half the intensity at many time points.

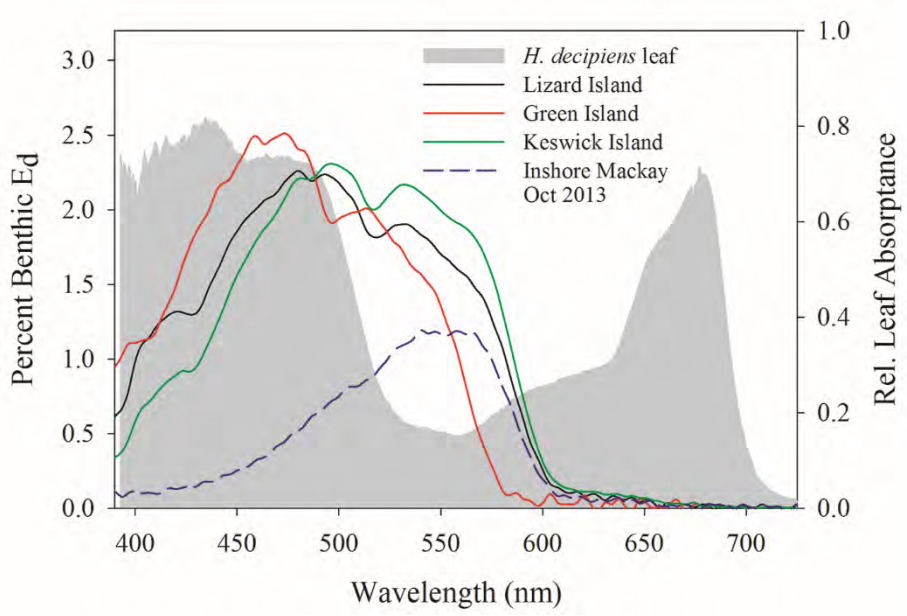


Figure 2.9 Spectrally-resolved downwelling irradiance as a percentage of surface irradiance at Green Island, Lizard Island, and Keswick Island monitoring locations in January 2015. Spectra from October 2013 in a previously monitored Mackay inshore deep-water seagrass meadow is provided for reference. Grey shaded area (right y-axis) is the relative leaf specific absorptance of a representative *H. decipiens* leaf as measured in the laboratory.

In the wet season, patterns among the locations were similar but with more pronounced differences when high rainfall and runoff appeared to influence the southern sites greater than Lizard Island (Figure 2.10). For example, light spectra profiles in April 2012 and April 2013 at Green Island showed a pronounced loss of light at the benthos between 400-500 nm compared to all other months (Figure 2.10a). In contrast, the Lizard Island and Keswick Island sites had a fairly constant spectral signature with overall light quantity fluctuating up to 20% among surveys (Figure 2.10c-f). Profiles from 2012 to 2014 at the inshore Mackay site did exhibit some spectral shifts which varied with season (Appendix A).

In November 2012, there were small differences in depth-averaged attenuation “c” with virtually no variation in absorption among sites (Appendix A). The slightly elevated attenuation at Green Island and Mackay in November 2012 is therefore due to scattering by particulate matter or suspended solids in the water column rather than organic, photosynthetically absorbing particulates such as phytoplankton. A small increase in attenuation was recorded at the Lizard Island site in February 2013, with no concurrent change in absorption from November 2012 measurements (Appendix A). Again, this indicates a small increase in scattering from particulates which may be linked with wet season runoff events in the region. Green Island did not show a measurable change from November to February sampling, while Mackay had the most substantial increase in both attenuation and absorption during the wet season sampling (Appendix A). While absorption increased, the greater component of attenuation can be attributed to scattering by particulate matter across all wavelengths rather than by phytoplankton.

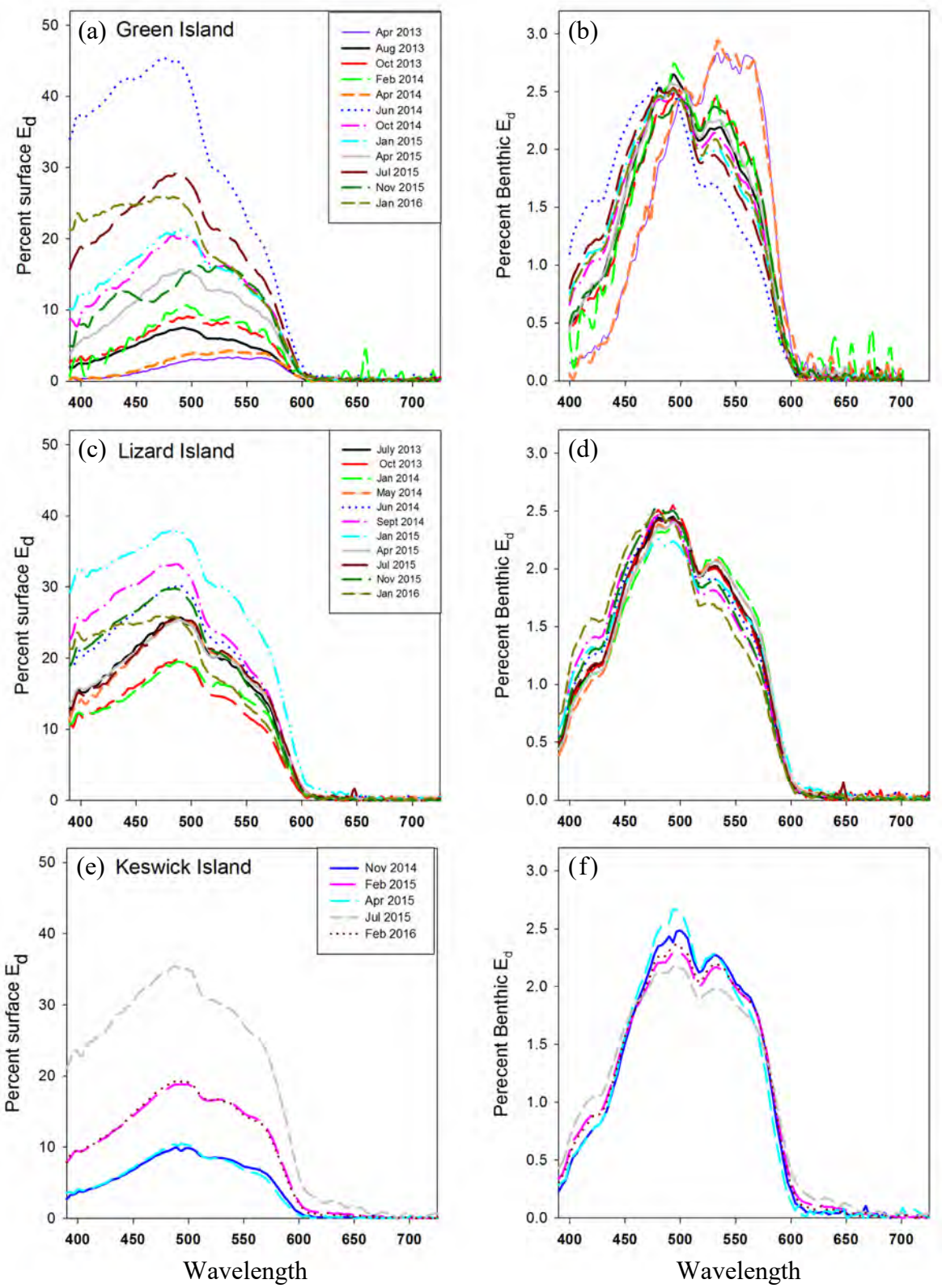


Figure 2.10 Green Island (a,b), Lizard Island (c,d), and Keswick Island (e,f) spectrally-resolved downwelling irradiance as a percentage of surface irradiance (a, c, e) and as a percentage of total benthic irradiance (b, d, f) over time. Each line represents an average of four spectral recordings.

2.3.4 Chlorophyll *a* fluorescence

Light utilisation efficiency (α) for *H. decipiens* was relatively stable over time both at Green Island and Lizard Island sites despite variability in irradiance at the time of sampling (Figure 2.11). Conversely, mean saturating irradiances (E_k) and $rETR_{max}$ were more variable, but they paralleled shifts in irradiance at the time of sampling (Figure 2.11). E_k ranged from 22 - 66 µmol photons m^{-2} s^{-1} at Green Island and 31 – 59 µmol photons m^{-2} s^{-1} at Lizard Island. Overall, E_k was greater than incident irradiance at the time of sampling indicating plants were light-limited. The exception is December 2013 at Green Island when light and E_k were similar at 26.0 and 22.0 ± 2.1 µmol photons m^{-2} s^{-1} respectively indicating a near optimum rate of photochemistry to electron transport was achieved (Figure 2.11). However, December 2013 was also a time when leaves were senescing despite a photochemical balance being reached during measurements.

The only analysis of *H. decipiens* leaf pigment concentrations in October 2013 found light harvesting complex (LHC) pigments were higher at Green Island compared to Lizard Island corresponding with the overall lower light environment at the former location despite higher instantaneous light levels at the time of sampling (Figure 2.11).

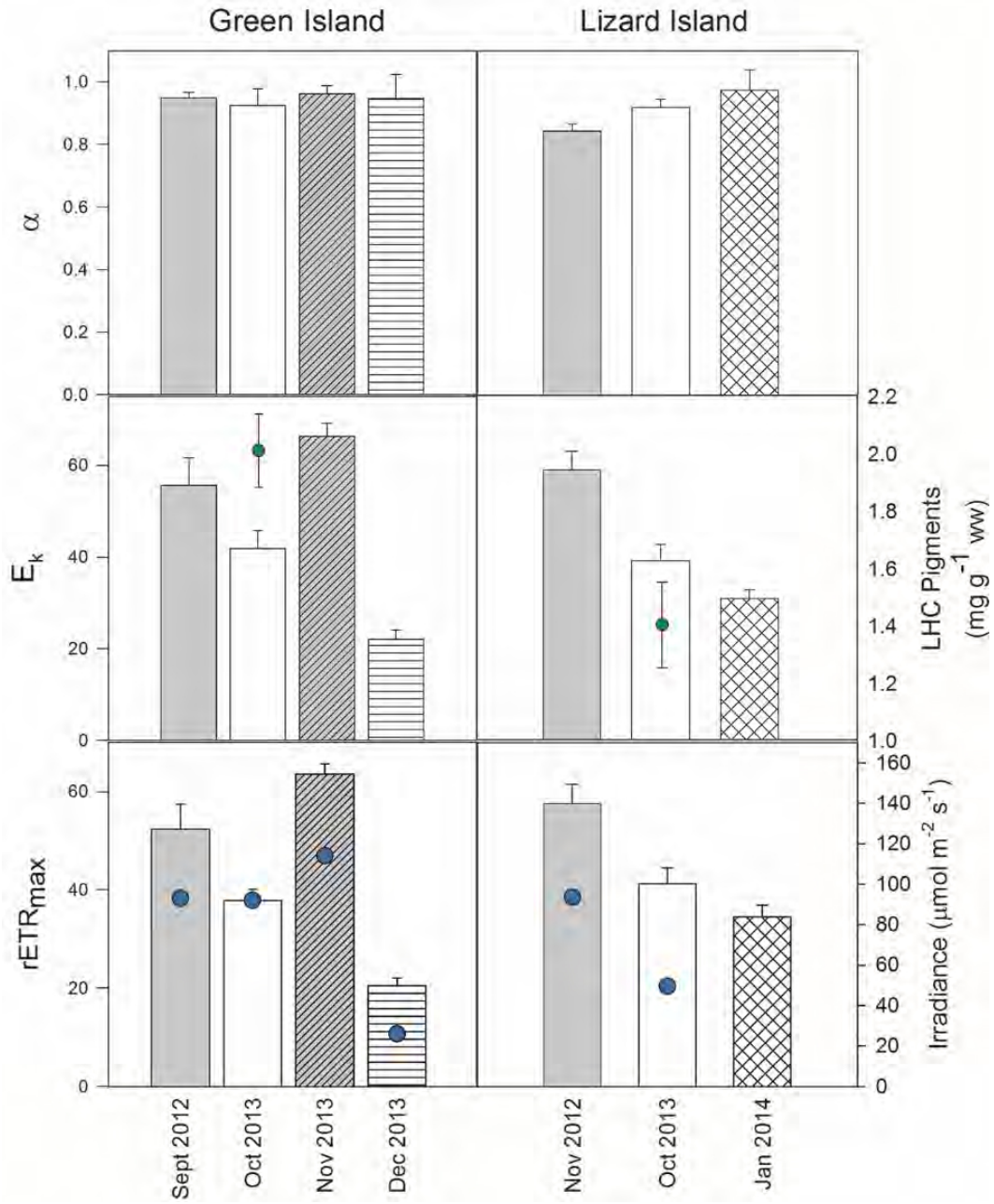


Figure 2.11 *H. decipiens* photosynthetic parameters: light utilisation efficiency (α), minimum saturating irradiance (E_k) and maximum electron transport rate ($rETR_{max}$) calculated from RLCs. Light harvesting pigments (LHCs) are represented by green circles and irradiance at the time of RLCs are shown with blue dots. Error bars indicate ± SE (n = 6).

2.3.5 Below-ground carbohydrates

The small size of rhizomes and roots in both *H. decipiens* and *H. ovalis* limited the available biomass at some sampling time points to reach detectable limits for laboratory analysis where a minimum of 0.05 g dry biomass was required. Overall, soluble carbohydrates were a greater proportion of below-ground structures relative to starch content. Substantial variations over time, however, did not maintain a clear seasonal trend with biomass or growing pattern for any species or location (Appendix A). The only periods in which starch was greater than soluble carbohydrates was for *H. ovalis* and *H. spinulosa* during February and April, wet season months when both species were declining in biomass (Appendix A).

2.4 Discussion

This study detailed the environmental drivers and dynamic fluctuations of tropical deep-water seagrasses in Australia. The study has highlighted differences among deep-water seagrass meadows in species composition, abundance, and seasonal patterns of senescence as well as recruitment coupled to location-specific environmental light and temperature regimes. Overall, seagrass above-ground biomass adhered to a cyclic growth pattern across locations in relation to measured annual light and temperature cycles (McKenzie, 1994; Chartrand et al., 2016). Extent of seasonality for deep-water seagrasses is species-specific with a persistent seagrass presence throughout the year around Lizard Island and Keswick Island where other *Halophila* spp. also occurred in the meadows, while Green Island was clearly annual with a short growing season.

In this study, we found *H. decipiens* above-ground biomass showed a threshold response with significantly increasing biomass when the mean 14-day daily light was > 2.0 mol photons m^{-2} d^{-1} and 2.7 mol photons m^{-2} d^{-1} at Green Island and Lizard Island, respectively. The negligible amount of *H. decipiens* recorded at Keswick Island did not allow for a comparative light threshold to be considered at this site. The small variation among populations is likely a reflection of acclimation to location-specific light regimes.

Seagrass abundance and cycles of recruitment and senescence are likely driven by changes in a suite of environmental factors, of which light availability and temperature are fundamental (Hemminga and Duarte, 2000; Ralph et al., 2007; Collier et al., 2016b). Deep-water seagrasses occupy the margins of the photic zone where the structural complexity and associated energetic demands of a vascular plant can just be sustained. This marginality can equate to reduced tolerance of a fluctuating light intensity and acute sensitivity to reductions in light beyond the lower bounds of the plant's normal growing environment (Ralph et al., 2007). When irradiance drops below a critical level, seagrass productivity is compromised and significant physiological, biochemical and structural changes begin to take place (Abal et al., 1994; Goodman et al., 1995; Grice et al., 1996; Lee and Dunton, 1997; Alcoverro et al., 1999; Krause-Jensen et al., 2000; Ralph et al., 2007; Collier et al., 2012b). Within-plant changes eventually manifest into broader meadow-scale losses in productivity, density and distribution with downstream negative ecological implications (Preen and Marsh, 1995; Heck et al., 2008; Hughes et al., 2008).

Our work's threshold values reinforce previous research into the light requirements of similar deep-water *Halophila* populations (Dennison et al., 1993; Erftemeijer and Stapel, 1999; Hammerstrom et al., 2006; Chartrand et al., 2018). A laboratory study (see Chapter 3) of deep-water *H. decipiens* collected from the Green Island site found a significant loss of shoots within two weeks when plants were grown under 1.1 mol photons m^{-2} d^{-1} (Chartrand et al., 2018). Conversely, shoots were able to be sustained under 3.2 mol photons m^{-2} d^{-1} over the same period (Chartrand et al., 2018). Work by Hammerstrom et al. (2006) recorded a contiguous deep-water *H. decipiens* meadow off the west coast of Florida growing year-round at 20 m under 1.8 mol photons m^{-2} d^{-1}. Research in Cuba and St. Croix found deep-water *H. decipiens* required 4.4 and 8.8% of surface irradiances, respectively (Williams and Dennison, 1990; Duarte, 1991); our values equate to 6% at Green Island and 8% at Lizard Island of light recorded at the surface.

Despite the similar morphology to *H. decipiens*, *H. ovalis* did not show a clear threshold response to light levels. Its' persistence year-round—fluctuating seasonally in biomass—suggests light was sufficient for this species at Lizard Island prior to cyclone impacts. The best model fit did show increasing biomass correlated with increasing light, albeit a poor one. *Halophila ovalis* is known for its' plasticity to grow in both intertidal and subtidal habitats and across a broad range of light environments and sediment types (Erftemeijer and Stapel, 1999; Beer et al., 2006; Bité et al., 2007). Most studies evaluating the response of *H. ovalis* to light conditions have investigated populations growing in shallow water, high light environments (Beer et al., 2006; Bité et al., 2007; Collier et al., 2016a). Erftemeijer and Stapel (1999) investigated a

population in 14 – 16 m depth off South Sulawesi, Indonesia and found similar growing conditions and growth characteristics to the Lizard Island population. The presence of *H. ovalis* at only the Lizard Island site in this study—a substantially higher overall light environment as well as less spectral attenuation than Green Island or Keswick Island—suggests it may have higher minimum light requirements than other *Halophila* spp.; however, further work would be needed to confirm this and other environmental drivers not measured in this study should also be considered.

At Green Island, monthly sampling captured the specific timing of annual recruitment and senescence for *H. decipiens*. Germination from a local seed bank occurred over winter months from June/July before peak biomass was reached in October/November each year. While there was a strong relationship between light and the increase in biomass, the patchiness and sparse nature of seagrass during germination months makes it difficult to define a specific threshold of light linked to germination, as opposed to increases in biomass following germination. Only a few studies have explored extrinsic factors that drive germination in *Halophila* species (Birch, 1981; McMillan, 1988a; Kuo and Kirkman, 1992) with only one such study indicating light increased germination success (Kuo et al., 1993). Further understanding of the drivers of germination for the most widespread deep-water species, *H. decipiens,* would be valuable to ensure reduced light from coastal development activities does not impact upon key germination cues.

The Keswick Island site added valuable insights into the growth dynamics of *H. tricostata*, a fourth species found in deep-water seagrass communities in the GBR lagoon (Coles et al., 2009). *H. tricostata* followed a similar cyclic growth pattern with

negligible biomass in February 2015 despite the brief one-year monitoring at this location. While a few sparse shoots remained in February, the limited 12 month dataset and quarterly surveys at Keswick Island may have missed the brief periods when the species was lost at this site. The only other study investigating *H. tricostata*, off Fitzroy Island near Cairns, found the species had a similar annual growth pattern with complete loss from February to August each year (Kuo et al., 1993). The limited dataset at Keswick Island also prevented any modelled relationship with light levels. Overall, the light environment during the growing period at Keswick Island was less than Green Island and therefore the threshold for local *Halophila* species could be expected to be somewhat lower due to photoacclimation to local conditions as was observed with differences between the Lizard Island and Green Island *H. decipiens* populations.

Inshore from the Keswick Island site, Mackay deep-water seagrasses have been monitored over multiple years (York and Rasheed, 2018) close to the mouth of the Pioneer River, which delivers turbid water and nutrients during heavy rainfall and flood events (Kroon et al., 2012). Large tidal flux and chronic low light together with catchment effects likely drives the extremely patchy and variable seagrass cover in the area. A previous study found that seagrasses at nearby Hay Point were naturally highly variable with peak abundances and distribution occurring in winter and spring before seasonal declines over summer with seagrass absent from December to June each year (York et al., 2015). The effects from coastal runoff and sediment re-suspension in this more inshore area has likely resulted in an exceptionally sparse meadow that was absent for a number of years.

Seagrasses regulate light-harvesting efficiency (e.g. internal structure, sclerenchyma fibers and chlorophyll contents) under changes in light and/or temperature (Olesen et al., 2002; Durako, 2007). Naturally, the minimum saturating irradiance, E_k, for *H. decipiens* was much lower than E_k measured for other seagrasses growing under much higher ambient light conditions (Bité et al., 2007; Campbell et al., 2007; Petrou et al., 2013) but comparable to other studies on deep-water *Halophila* species (Josselyn et al., 1986; Erftemeijer and Stapel, 1999). Between populations, significantly greater LHC pigments at Green Island compared to Lizard Island may be a result of half the available light over the previous few days at the former, despite the relatively high instantaneous light at the time of sampling in October 2013. An increase in leaf pigments in a reduced light environment is one of many ways to regulate light harvesting and maintain photochemical efficiency (Ralph et al., 2007). Despite the difference in LHC content between the populations, the saturating irradiance (E_k) and relative electron transport rate ($rETR_{max}$) were quite similar; a reflection of potential package effect whereby self-shading of pigments and chloroplasts counteracts potential gains in light harvesting efficiency (Enriquez, 2005). These results suggest deep-water seagrasses can regulate pigment composition to some extent allowing them to acclimate and take advantage of available windows of increased irradiance for growth and to maintain a continued presence, albeit in a narrower range of conditions than their shallow-water counterparts. Negligible below-ground structures for *H. decipiens* means efforts to cope with any additional low light stress are largely limited to altering its' bio-optical capacity and efficiency of light use, rather than mobilising stored carbohydrates like other larger bodied seagrasses (O'Brien et al., 2018).

Unique light quality of each experimental site may drive differences observed among the three studied seagrass communities. The overall higher light environment at Lizard Island compared to the other two sites supported a year-round mixed species meadow. The spectral quality of light at Lizard Island was consistent both among and within years, providing a stable spectral light environment for photophysiological adaptation by leaf pigments and photosynthetic machinery (Smith, 1982; Zimmerman, 2007). Lizard Island occurs on an undeveloped region of the Queensland coastline with reduced runoff loads from major catchments (Devlin et al., 2012). Green Island had both a lower overall light environment and also was likely more impacted by coastal runoff from nearby catchments driving shifts in spectral quality during wet season months. Lower and more variable quality of light may in part explain the lack of seagrass during these times of year. Light quality at Keswick Island was similar to Lizard Island despite being along a more developed part of the coastline with historically greater impacts from wet season flooding and coastal runoff (Devlin et al., 2012). Rainfall and associated flooding in the Keswick Island region was well below the long-term average rainfall in 2015 and 2016 (Bureau of Meteorology; www.bom.gov.au); the brief period during which the site was monitored for this study. A reduced wet season may have tempered any spectral shifts that would likely occur with these significant runoff events. The inshore Mackay site, monitored for the first 1.5 years of the study, situated near the mouth of the Pioneer River, was strongly impacted by turbid waters with high light-scattering particulate matter that attenuated disproportionately portions of the blue spectrum. This, in conjunction with lower total irradiance, helps explain the relative lack of seagrasses in the Mackay site compared to other locations.

There has been limited research into what physiological or morphological changes are made by *Halophila* spp. to adjust to spectral attenuation at depth outside of a *H. stipulacea* population in the Red Sea growing at 50 m (Sharon, 2009; Sharon et al., 2011). Some studies have found negligible effect of spectral quality on whole plant condition in *Halophila* spp. (Kahn and Durako, 2009; Strydom et al., 2017b); however, shifts in the available light spectrum can drive changes in pigment composition and photochemistry (Strydom et al., 2017b; Chartrand et al., 2018), which are at a cost to the plant trying to acclimate to a variable spectral qualitative light environment. A further exploration of how spectral attenuation modulates deep-water seagrass growth patterns would be valuable to better address water quality impacts on these GBR populations.

In addition to light, temperature underpins seasonal growth patterns for temperate and tropical seagrasses and an optimal temperature range for a species usually coincides with peak productivity (Lee et al., 2007). However, temperatures exceeding this optimal range have been shown to negatively affect production due to growth inhibition (Marsh Jr et al., 1986; Collier and Waycott, 2014). While temperatures were relatively higher when seagrasses died back each year, it is unlikely temperatures alone led to observed seagrass declines. Temperatures were not in a range typically considered stressful for a wide range of tropical seagrass species (Lee et al., 2007). Furthermore, the effect of temperature on productivity has an interactive role with light on the net carbon balance of a plant (Collier et al., 2016a). Plants at higher temperatures have higher metabolic rates and therefore likely need more light to maintain positive carbon balances than

those at lower temperatures. Photosynthetic production imbalance in seagrasses is therefore more susceptible to higher water temperatures at reduced light conditions. At the Green Island site, seagrass loss during the senescent phase coincided with the highest light levels and higher, although not extreme temperatures. Further exploration of the light-temperature relationship and the effect of temperature on a light threshold response in deep-water seagrass were investigated in associated laboratory experiments (see Chapter 3, Chartrand et al., 2018). According to the laboratory findings, it is unlikely that temperature is a major driver of seasonal decline; there was no effect from temperatures under controlled conditions at the maximum daily temperatures recorded at any of monitoring sites (Chartrand et al., 2018).

The complete annual die-off of *H. decipiens* at Green Island and Lizard Island (outside of cyclone related impacts) from light fluctuations may not be the driver of the annual cycle observed for these communities. Available total daily light noticeably dropped off at the site in January/February each year; however, seagrass abundance often declined prior to seasonal light attenuation by late November or December. In fact leading up to annual seagrass loss, light levels were closer to those recorded during the early spring recruitment of new shoots. Small relative increases in late summer water temperatures with equitable light environments have driven seagrass dieback in studies of temperate seagrasses (Moore et al., 2012) highlighting the importance of temperature-dependent light requirements which can affect populations growing close to their physiological limits.

The ontogeny of *H. decipiens*, the most prolific deep-water species, follows an annual life history strategy as demonstrated in this study and in the literature (Kenworthy et al., 1989; Hammerstrom et al., 2006; Hovey et al., 2015; York et al., 2015). The plant develops through distinct phases of germination, vegetative growth, reproductive output and eventual senescence. While environmental conditions are known to drive plant response, endogenous cues which control the genetic and metabolic expression of these growth processes are poorly understood, particularly for seagrasses and may play a role in determining annual growth habits independently of light or other abiotic environmental cues (Huijser and Schmid, 2011). Further work is needed to better understand molecular level responses to critical environmental cues for recruitment and senescence in these deep-water seagrass species.

Deep-water *Halophila* populations are reported to have high turnover rates of leaves and active meristematic growth (Josselyn et al., 1986; Vermaat et al., 1995; Marba and Duarte, 1998; Hammerstrom et al., 2006) yet low daily carbon production attributable to the small stature of the plant form (Erftemeijer and Stapel, 1999; Hammerstrom et al., 2006). In this study, *H. decipiens* and *H. ovalis* measured even faster rates of turnover during peak growing months of October and November than has been documented elsewhere (Kenworthy et al., 1989; Erftemeijer and Stapel, 1999; Hammerstrom et al., 2006). Based on the projected widespread distribution within the GBR lagoon (Coles et al., 2009) and fast rhizome elongation rates (Josselyn et al., 1986), deep-water *Halophila* meadows can contribute substantially to primary productivity at large spatial scales despite its small leaf morphology. More recent studies have shown that the vast coverage of these sparse and small-bodied seagrasses on the photic zone seafloor could

play a substantial role in locking away carbon (York et al., 2018), making studies such as this one important for better managing this ecosystem service.

Abundant flowering, fruiting, seed production and seed retention in the surrounding sediment have been described globally in various *Halophila* spp. meadows (McMillan and Soong, 1989; Kuo and Kirkman, 1995; Kenworthy, 2000; Hammerstrom et al., 2006). On the Kimberly coastline of northwest Australia, Hovey et al. (2015) found similar phenological patterns of annual growth related to monsoon conditions. To expand on the patterns described in this study, an analysis of the seed bank from the three monitoring sites will consider how sexual reproduction structures deep-water seagrass meadows both in time and space in relation to the seasonality of meadow abundance on the GBR (see Chapter 4).

The two category 4 cyclones, Cyclone Ita and Cyclone Nathan, at Lizard Island in 2014 and 2015 provided a valuable opportunity to track cyclone impact and recovery on a deep-water seagrass meadow. One of the most striking results was how each cyclone had a distinctive effect on the local meadow despite both passing directly over Lizard Island at a similar time of year. Cyclone Ita in March 2014 led to complete loss of above-ground biomass at the site and resulted in 5 – 10 cm of sand burial over the monitoring plots. The overall above-ground biomass in 2014 was likely much smaller than would be expected under typical seasonal cycles due to the recovery from Cyclone Ita and progression from a *H. decipiens* to *H. ovalis* dominated meadow. Cyclone Nathan did not drive the same local impacts from sediment burial but did appear to depress recovery of *H. ovalis*, allowing *H. decipiens* to proliferate.

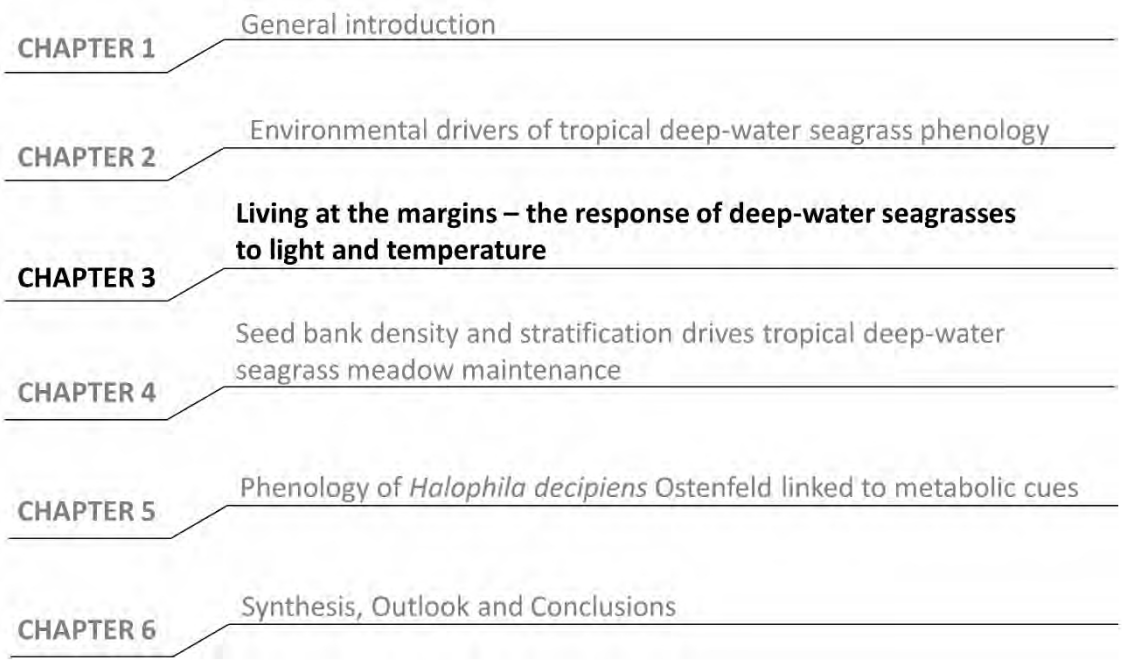
The clear shift from a *H. ovalis* to a *H. decipiens* dominated meadow may be linked to differences in recovery strategy following local disturbance. *Halophila* spp. are generally thought to rely on a seed bank (Hammerstrom et al., 2006), but connectivity of the meadow to surrounding seagrass beds via dispersal of vegetative fragments may assist or drive recovery by one species over another (Grech et al., 2016). Because *Halophila* spp. are generally grouped as having a single ontogenetic growth strategy, no evidence has been found to show whether there are species-specific differences in reproductive effort among this genus. Greater understanding of the species-specific differences in strategies used to recover from disturbances may help explain why there was a substantial shift in species dominance, at Lizard Island.

2.5 Conclusion

This study presents a detailed look at multi-year drivers and dynamic fluctuations of tropical deep-water seagrasses in Australia. The study has highlighted several differences among deep-water seagrass meadows with regards to species composition, abundance and seasonal patterns of senescence and recruitment. Extent of seasonality for deep-water seagrasses is species-specific with a persistent seagrass presence throughout the year around Lizard Island and Keswick Island, while Green Island was clearly annual with a short growing season. This study also provided an opportunity to explore the impact and recovery from severe tropical cyclones on these deep-water communities. It is clear that while these communities are adapted to quick recovery, the impacts on species composition and community structure which may be less apparent could alter longer term dynamics in these habitats.

From a management perspective, this study has provided a strong foundation to understand the light requirements and population dynamics of multiple *Halophila* spp. within the GBR lagoon. Light thresholds can be valuable tools in order to manage impacts from both acute and chronic impacts to the light environment (Chartrand et al., 2016; Collier et al., 2016b). This study indicates *H. decipiens*, the most prevalent deep-water seagrass species in the GBR lagoon, requires at least 2.0 mol photons m^{-2} d^{-1} on average during the key growing period from ~July – November to maintain growth and function. This study supports the recommendation of Hovey et al. (2015) that stress the importance of adapting appropriate management strategies that fit the annual life history strategy of this species.

CHAPTER 3 Living at the margins – the response of deep-water seagrasses to light and temperature



Chapter 3 has been published as: Chartrand K.M.*[1,2], Szabó M.[2,3], Sinutok S.[3,4,5], Rasheed M.A.[1] and Ralph P.J.[2]. 2018. Living at the margins – the response of deep-water seagrasses to light and temperature renders them susceptible to acute impacts. *Marine Environmental Research*, 136, 126-138. https://doi.org/10.1016/j.marenvres.2018.02.006

[1] Centre for Tropical Water & Aquatic Ecosystem Research, James Cook University, Cairns, Queensland, Australia.
[2] Climate Change Cluster, University of Technology Sydney, Broadway, New South Wales, Australia.
[3] Institute of Plant Biology, Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary
[4] Faculty of Environmental Management, Prince of Songkla University, Hat Yai, Thailand
[5] Coastal Oceanography and Climate Change Research Center, Prince of Songkla University, Hat Yai, Thailand

Abstract

Seagrasses inhabit environments where light varies at different timescales, nonetheless are acutely sensitive to reductions in light beyond some conditional bounds. Two tropical deep-water seagrasses, *Halophila decipiens* and *Halophila spinulosa*, from the Great Barrier Reef were tested for their response to defined light and temperature regimes to identify their growth requirements and potential thresholds of mortality. Species were exposed to two light intensities, saturating (75 µmol photons m^{-2} s^{-1}) and limiting (25 µmol photons m^{-2} s^{-1}) light and two temperature treatments (26°C and 30°C) over a four-week period. Wavelength-specific parameters of PSII photochemistry were evaluated for seagrass leaves, as well as shoot density, gas exchange, and pigment content. Both species were sustained under saturating light levels (3.2 mol photons m^{-2} d^{-1}) while limiting light led to decreased shoot density for *H. decipiens* and *H. spinulosa* after two and four weeks, respectively. Wavelength-specific photochemistry was also affected under light-limiting treatments for both species while the functional absorption cross section was highly conserved. Photoacclimation and physiological adjustments by either species was not adequate to compensate for reduced irradiance suggesting these plants reside at the margins of their functional limits. As such, relatively short periods of light attenuating events, like dredging or flood plumes, may be detrimental to deep-water seagrass populations.

3.1 Introduction

It is widely accepted that seagrasses are critical to the health and ecosystem function of the coastal marine environment. They provide key inter-habitat connectivity for migrating fauna, feeding grounds for globally threatened turtles and dugong, habitat for

commercially important fisheries, sediment trapping and stabilisation, effective nutrient filtration from coastal inputs and carbon sequestration (Hemminga and Duarte, 2000; Jackson et al., 2001; Orth et al., 2006; Heck et al., 2008; Duarte et al., 2010). Despite being highly valued globally for their contribution to these ecosystem services, seagrass habitats are threatened by a range of anthropogenic activities including coastal development and declining water quality from poor catchment management practices (Waycott et al., 2009; Grech et al., 2012; Costanza et al., 2014), and compounded by natural events such as severe storms and flooding that can accentuate seagrass decline (Rasheed et al., 2014).

The vast majority of seagrass species are located in relatively shallow water habitats with quiescent conditions, favourable sediment chemistry, and where light is adequate to meet gross energy requirements (Hemminga and Duarte, 2000; Koch, 2001). In the Great Barrier Reef World Heritage Area (GBRWHA), research and monitoring programs have detailed the distribution, seasonality, environmental drivers, risks and threats to these specialised plant communities (Grech et al., 2011; Collier et al., 2012a; Bryant et al., 2013; McKenna et al., 2015). However, information on deep-water tropical seagrass communities — generally classified as growing at depths >10-15 m — is limited (Josselyn et al., 1986; Carruthers et al., 2002; Hammerstrom et al., 2006; Fonseca et al., 2008). These deeper meadows are primarily composed of species from the genus *Halophila* (Hydrocharitaceae) and within the GBRWHA have been mapped down to 60 m and modelled to cover over 40,000 km^2 of the seafloor (Coles et al., 2009).

Halophila spp. have size-associated characteristics that are likely to play an important role in their dominance of deep-water seagrass meadows. Small delicate leaves, oval or oblong in shape, occur in pairs that attach directly to either a vertical stem or rhizome via a petiole. Their short canopy height may increase risk of burial from sediment deposition; however, rapid leaf turnover and opportunistic growth can negate this issue (Duarte et al. 1997, Terrados et al. 1998, Cabaço et al. 2008). With leaves only two-cells thick, they have minimal lacunar space and contain densely packed chloroplasts in the epidermal layer (Roberts et al. 1984, Josselyn et al. 1986, Kenworthy et al. 1989, Cambridge & Lambers 1998). Thin leaves allow for quick and efficient gas exchange of evolved oxygen from saturated epidermal cells and reciprocal carbon uptake for fixation (Larkum et al. 2006). Comparatively, seagrasses with high standing biomass (such as *Posidonia* and *Zostera*) have higher diffusive boundary layers which could make living at depth with less wave action and water movement a challenge for gas exchange and acquiring limited resources (Enríquez and Rodríguez-Román, 2006). Minimal below-ground biomass also makes *Halophila* spp. well suited to grow at greater depths and in shallow turbid areas with chronic low light (Kuo & Kirkman 1995, Durako et al. 2003). Non-photosynthetic below-ground tissues can act as a respiratory burden which may ultimately limit the compensation depth of structurally larger species (Fourqurean & Zieman 1991, Larkum et al. 2006).

The capacity to cope with both a quantitatively low-light environment and a narrowed spectral window of light is critical to living at depth (Duarte, 1991; Ralph et al., 2007); yet little is known about the spectral tuning of deep-water seagrasses. Deep-water seagrasses likely have a reduced threshold of tolerance to low-light because they are

growing under lower ambient light intensities, a smaller range of intensities, and a significantly narrowed spectral window from which to harvest light energy (Larkum et al., 2006). Strategies for deep-water seagrasses to maintain a positive carbon balance likely involve the same mechanisms observed in their shallow water counterparts to cope under low-light conditions (Collier et al., 2012b): modifying light harvesting capacity and the efficient use of light (Abal et al., 1994; Enriquez, 2005); adjustments to rates of growth and plant turnover (Collier et al., 2012b); and drawing upon carbohydrate reserves to maintain a positive carbon balance (Burke et al., 1996; Touchette and Burkholder, 2000). Temperature, which directly affects metabolic rates of carbon fixation and respiration in plants, influences whether photophysiological adjustments to a low-light environment are sufficient to maintain a net positive carbon balance, as opposed to a net negative; the latter of which would lead to plant- or meadow-scale losses (Bulthuis, 1987; Lee et al., 2007).

Some deep-water *Halophila* populations are annual or ephemeral in their above-ground presence, likely in response to seasonally unfavourable conditions to support positive growth and carbon balance (York et al., 2015). In these circumstances the plants may rely on high investment in the production of seeds and a seed bank on which recovery and population maintenance depend (Rasheed et al., 2014; Hovey et al., 2015).

Photoacclimation to low-light environments by seagrasses is similar to that seen in other higher plants (Smith, 1982; Ralph et al., 2007). Changes in accessory pigment content can increase light capture efficiency and its' relative use in the photochemical pathways (Falkowski and Raven, 2007). However, a point can be reached beyond which the self-

shading of pigments reduces the effectiveness of this strategy (i.e. the package effect; Cummings and Zimmerman, 2003). While the capacity for photoacclimation to total light reduction is well documented in higher plants including seagrasses, qualitative shifts in the spectral distribution of available light at depth is largely undescribed for seagrass with an exception by Sharon et al. (2011) on *H. stipulacea* growing at 48 m in the Red Sea. While the spectral distribution of light with depth varies according to the absorption properties of the water, total spectral attenuation of light >600 nm occurs in the GBRWHA at ≥10 m (see Figure 2.9). Descriptions of seagrass pigment signatures is somewhat typical of a green higher plant with chlorophyll and a suite of accessory pigments that absorb light largely in the blue (400-500 nm) and red bands (650-680 nm) (Costa et al., 2014). How a spectrally-attenuated light climate affects the photosynthetic properties, pigment composition, or light capture efficiency of *Halophila* spp. growing in >10 m is largely undescribed.

Measuring photosynthetic capacity to understand plant condition under varying light intensities with pulse-amplitude modulated (PAM) chlorophyll fluorometry is commonplace in recent years, whether in the laboratory or field (Schreiber, 2004; Cosgrove and Borowitzka, 2010). A PAM fluorometer typically measures variable chlorophyll fluorescence parameters, from which photochemical efficiency of PSII can be calculated, energy transfer efficiency can be measured, and energy dissipation quantified. However, these measurements do not account for variable absorption by PSII across the PAR spectrum which can vary widely by wavelength depending on the pigment composition and its' ambient growing conditions (Schreiber et al., 2012; Szabó et al., 2014).

3.2 Methods

3.2.1 Sample Collection

Halophila decipiens Ostenfeld was collected adjacent to a long-term monitoring site off Green Island (16°45.12354'S, 145°59.5494'E) at approximately 17 m depth below MSL. *H. spinulosa* (R. Brown) Ascherson, was collected approximately 400 km to the south, at a location near Bowen at 10 m depth below MSL (19° 54.4061 'S, 148° 11.0841'E). Plants were harvested in October and November 2013 on SCUBA using a large metal scoop to place transplants and ~7 cm of sediment depth into 26 x 21 x 10 cm plastic tubs in order to minimise disruption to their growing environment. Tubs were transferred overnight to the University of Technology Sydney with enough water to keep shoots wet but not fully submerged, fastened with lids, and kept in the dark during the approximately 18 hour transfer period. On arrival, tubs with samples were maintained in 10-L aquaria with aerated natural seawater (26 °C, pH 8.1, 35 PSU) for one month prior to the start of the experiment. Plants were illuminated using 150W four-channel LED lights (Cidly, China) programmed to simulate an incident deep-water spectral profile with an intensity of 75 µmol photons m^{-2} s^{-1} over a diel light-dark cycle (ramping from 0 to 75 µmol photons m^{-2} s^{-1} from 0500 h to 0700 h and from 75 to 0 µmol photons m^{-2} s^{-1} from 1800 h to 2000 h). The light, temperature, and salinity conditions in the tanks were based on a two year record of water quality monitoring at the collection sites with in situ loggers (Chartrand pers. obs.). The tank conditions mimic the mean maximum daily light intensity, mean daily temperature, and salinity during the October/November period when plants were harvested.

3.2.2 Experimental design

Each tub of *H. decipiens* and *H. spinulosa* were randomly allocated to one of four treatments (4 tubs per treatment) manipulating light intensity (*LI*) and temperature (*T*): (1) control (75 µmol photons m^{-2} s^{-1}, 26 °C; equivalent to mean daily irradiance at depth and mean ambient temperature at field sites), (2) elevated temperature only (75 µmol photons m^{-2} s^{-1}, 30 °C), (3) reduced light only (25 µmol photons m^{-2} s^{-1}, 26 °C) and (4) a combination of both reduced light and high temperature (25 µmol photons m^{-2} s^{-1}, 30 °C). The reduced *LI* and elevated *T* levels were chosen to reflect conditions beyond those recorded when plants were actively growing but still found to occur at the collection site at times of the year when seagrasses were absent (Chartrand pers. obs), and therefore realistic as a level of plant stress within their environment. Temperature was controlled using submersible heaters (Aqua One, Australia). Water quality was the same as the holding aquaria, with weekly 30% water changes to maintain salinity within 1 PSU and availability of trace nutrients. Tubs were rotated every other day within tanks in order to remove an effect of location within the tank in relation to the light source or water flow. The experiment was performed over 4 weeks.

The number of seagrass shoots (i.e. *H. decipiens* number of leaf pairs or *H. spinulosa* vertical shoots) in each tub (0.05 m^2) was recorded weekly during the study.

3.2.3 Oxygen determinations

Oxygen production and respiration rates of both species were measured at the start (T_0) and end (T_f) of the experiment using oxygen optodes (OXSP5-OI, Pyroscience

Germany) connected to a O_2 sensor unit (FireStingO2 Fiber-Optic Oxygen Meter, Pyroscience, Germany). Samples were placed in 10 mL glass bottles filled with 0.45 µm filtered seawater from treatment tanks with a magnetic stirrer and connected to an oxygen sensor. Oxygen production and respiration were determined under treatment irradiance and darkness, respectively and rates were calculated as oxygen differentials over the time of incubation and normalised to leaf area.

3.2.4 Variable fluorescence measurements – wavelength-dependent parameters

The wavelength-dependent functional absorption cross-section of PSII, $\sigma_{II}(\lambda)$, was recorded according to Schreiber and Klughammer (2013; and see Szabó et al., 2014) using a multi-colour pulse amplitude modulated fluorometer (further referred as MC-PAM; Walz GmbH, Germany). Briefly, $\sigma_{II}(\lambda)$ calculations are based on the so-called O-I_1 (or O-J, Strasser and Govindjee, 1992) fluorescence rise kinetics, which corresponds to the photochemical phase of the polyphasic fluorescence rise upon the onset of strong actinic illumination. These measurements were recorded using an automated measuring routine in PamWin v3.2 (Walz GmbH, Germany). At 500 µs after the start of actinic illumination, i.e., before the secondary thermal rise phases contribute significantly to the fluorescence rise, a saturating single-turnover flash (ST) was given to estimate the I_1-level, which represents the fluorescence yield of the fully reduced primary electron acceptor Q_A, with the PQ-pool being oxidized.

Consecutive measurements with the same leaf sample using 440, 480, 540, 590, and 625 nm measuring light (ML) and actinic light (AL) were pre-programmed in a script-file with 10 s dark-time between measurements. For each wavelength, ML intensity and

gain settings were programed to give approximately equal F_0 values (designated as 'O' in the O-I_1 terminology). The AL and multiple turnover (MT) flash intensity settings were programmed to obtain similar slopes of the O-I_1 curves for all wavelengths. When kinetics of the O-I_1 fluorescence rise are identical among wavelengths, PAR is directly proportional to changes in $\sigma_{II}(\lambda)$. Values of $\sigma_{II}(\lambda)$ were analysed using a dedicated fitting routine in the PamWin-3 software to determine τ, the time-constant of light-driven Q_A^- reduction (ms) and used in the following equation:

$$\sigma_{II}(\lambda) = \frac{1}{\tau \cdot N_A \cdot E_d}$$

where τ is the time-constant of light-driven Q_A^- reduction (ms), N_A is Avogadro's constant ($3.03 \cdot 10^{23}$ mol photons^{-1}) and E_d is the incident downwelling irradiance (see Schreiber et al. 2012 and Schreiber and Klughammer 2013).

Wavelength-dependent chlorophyll fluorescence parameters, i.e. the effective quantum yield of PSII (Y(II)), relative electron transport rate (rETR(II)) and non-photochemical quenching (NPQ) were determined by using custom-made scripts to record steady-state light curves (SSLC) across the wavelengths provided by the LEDs of the MC-PAM at a fixed intensity. Essentially, scripts were designed to start with the wavelength with the lowest measured $\sigma_{II}(\lambda)$ then progressing towards greater theoretical wavelength-specific PSII excitation pressure; this equated to a sequence order of 540, 590, 625, 480 and 440 nm. Two scripts were used to determine the chlorophyll fluorescence parameters described above: a sub-saturating (25 µmol photons m^{-2} s^{-1}) and a supra-saturating (150 µmol photons m^{-2} s^{-1}) AL at each wavelength. The F_V/F_M values were calculated upon dark-adaptation for ~5 min, followed by a white saturating pulse (SP). At each of the

five wavelengths, AL was set for 3 min at the end of which a SP pulse was given with the same wavelength as the AL to determine F_M'.

3.2.5 Pigment Characterisation

Seagrass leaves used in MC-PAM measurements were photographed and analysed by imaging software (ImageJ) prior to the extraction and quantification of chlorophyll content. Each leaf sample was ground in 5 mL ice-cold 90% acetone using a mortar and pestle. Chlorophyll *a* (Chl *a*) and chlorophyll *b* (Chl *b)* concentrations were determined spectrophotometrically using the equations and extinction coefficients of Ritchie (2006) and normalised to surface area of the leaf.

3.2.6 Below ground carbohydrates

Additional samples of *H. spinulosa* only were collected at the beginning and end of the experiment for below-ground carbohydrate analysis and were placed in liquid nitrogen to await further processing. Samples were later defrosted, scraped clean and separated into below and above-ground structures. Root and rhizome material was dried at 40˚C for 48 hours when a constant dry weight was obtained. Dried material was ground to a fine powder in a bead mill (Mini-Beadbeater, Biospec) for wet laboratory analysis. Prepared samples were further processed at the University of Queensland where soluble and non-structural carbohydrates (i.e. starch) were extracted and quantified relative to total sample dry weight according to Weir et al. (1977) and Karkalas (1985). Briefly, carbohydrates were extracted by placing each sample in 80% ethanol in an 80°C water bath for 5 min prior to centrifuging (3000 rpm for 5 min). The supernatant was diluted with 80% ethanol to 25 mL and kept for soluble carbohydrate determination. The pellet

remaining was dissolved in 10 mL of deionized water and placed in a 95°C water bath for a further 30 min while agitating samples at 10 min intervals to solubilize the starch. After coming to room temperature, samples were digested with amylase and incubated at 55°C for 30 min prior to dissolving the extracted sample in 20% trichloroacetic acid. Colorimetric determination of starch content was determined using a commercially available glucose oxidase/peroxidase testing kit (GOPOD, Megazyme) with absorbance measured at 510 nm, and soluble carbohydrates colorimetrically determined with a potassium ferricyanide reagent measured at 420 nm.

H. decipiens was not tested for below-ground carbohydrate concentrations due to extremely low below-ground biomass insufficient to perform laboratory analyses.

3.2.7 Data analysis

Generalized linear mixed-effect models (GLMM) were used to examine the fixed effects of light intensity (*LI*), temperature (*T*), and week (*W*) on shoot count (*SC*) and chlorophyll fluorescence parameters (F_V/F_M, $\Delta F/F_M'$, Q_m) for each species. Each factor was modelled as an additive term and as an interaction with other factors. The factor tub was also included as a random effect in all models to eliminate potential bias resulting from the non-independence of measurements taken from the same tub over the four-week study. Three-way interactions between *LI*, *T* and *W* were considered in the analyses. The response variable *SC* was modelled with a Poisson distribution with logit link function. To assess differences in response variables between species, separate models were run on the effect of species (*SPP*) as an additive and interaction term to avoid over-parameterization of the models.

To determine the optimal model, a global model was created for each response variable where all explanatory variables up to 3-way interactions were considered. Sub-model sets of the global model were then generated using the dredge function in the MuMIn package (Bartoń, 2013). The best-fit models were considered to be those with the lowest Akaike's information criterion (AICc) and highest Akaike weight (w), which by definition contain the best set of explanatory factors for adequately predicting each response variable (Burnham and Anderson, 2002; Wagenmakers and Farrell, 2004). Models with AICc values within 2 of each other were considered strong models and are presented with the chosen model being the simplest of this sub-set which was further used for multiple comparison analysis (Burnham and Anderson, 2002). All models were validated by assessing Pearson residuals against fitted model values. The best model for *H. decipiens* shoot count data did show some heterogeneity in the residuals versus fitted values. Two influential tubs were identified using standardized measures of influential data for the point estimates of generalized mixed effects models (Nieuwenhuis et al., 2012) and were removed, which greatly improved the residual patterns while not changing the model selection or significance of the fixed effects in the model output. Multiple comparison procedures using a Bonferroni adjustment were run on least square means when significant overall effects were generated from all best fit models (lsmeans package, Lenth, 2016).

A similar GLMM approach was used to examine the fixed effects of light level (*LI*), temperature (*T*), and the random effect of tub, on oxygen production, respiration rates, P:R ratios, pigment content and all wavelength-dependent fluorescence measurements

(Y(II), F, F_m, rETR, NPQ, and $\sigma_{II}(\lambda)$), which were also tested against wavelength (*WV*). Since these response variables were only measured at the start (T_0) and end (T_F) of the study, a separate t-test was performed between T_0 and T_F "control" conditions to test whether the tub conditions affected the status of leaves in addition to the treatment design. Model selection for wavelength-dependent fluorescence measurements was also done using the dredge function, but with a Gaussian or gamma distribution (with logit link) applied for continuous and positive data (Zuur et al., 2009). Model selection for all other response variables (oxygen production, respiration rates, P:R ratios, pigment content) was made using the drop1 command from the lmerTest package (Kuznetsova et al., 2015). Validation steps were the same; Pearson residuals were assessed against fitted model values, and multiple comparison procedures using a Bonferroni adjustment were run on least square means when significant overall effects were generated from all best fit models.

All GLMMs were performed in R using lme, glmmADMB and mgcv packages (Wood, 2006; Bates et al., 2012; Fournier et al., 2012).

In this study, we investigate the effects of light intensity and temperature on the two most prevalent deep-water seagrasses in the GBRWHA (Coles et al., 2009), *Halophila decipiens* and *Halophila spinulosa*. *H. decipiens* has a pan-tropical distribution and has a small stature in both above and below-ground tissues (Waycott et al., 2004). *H. spinulosa* is limited to the Indo-Pacific region, has similar oblong leaf pairs, but grows upright on a vertical stem, creating a much larger canopy-forming habitat.

Both species were expressly sourced from deep-water meadows, whereby depth creates unique inherent challenges to the biology and physiology of the plant from that of a turbid shallow water habitat: 1) a unique spectral signature in which light >600nm is absent; 2) lower variation in water quality related to tidal effects, coastal runoff, and sediment re-suspension due to wind and wave activity; and 3) a unique pressure environment which may impact leaf diffusion and plant physiology (Beer and Waisel, 1982). We assessed morphological, optical and physiological adjustments to both plants when they were exposed to peak growing season light (spectrally-adjusted) and temperature conditions (Chartrand in prep) versus reduced light levels and elevated temperatures. We measured wavelength-dependent photochemical efficiencies, oxygen production, pigment composition, carbohydrate reserves and shoot densities over a four-week period to assess changes in the plants. The aim of this experiment was to i) describe the changes in optical, photochemical, physiological, and physical characteristics used to cope with light and temperature stress events, ii) evaluate wavelength-specific characteristics of light capture in response to the light/temperature treatments, iii) identify the time to detect any such significant changes, and iv) establish an indicative minimum light threshold for *H. decipiens* and *H. spinulosa* to help guide environmental management of tropical deep-water seagrass communities. In addition, we aimed to describe potential differences in physiological responses between two species from the *Halophila* genus.

3.3 Results

3.3.1 Shoot density

Light deprivation had a negative effect on *Halophila decipiens* and *Halophila spinulosa* shoot density after two and four weeks, respectively (Figure 3.1). Change in shoot counts of *H. decipiens* were driven by *LI* and *W* with no effect of *T* (Table 3.1). *H. decipiens* shoots under low *LI* were significantly lower by week 2 with approximately 40% (26˚C) and 60% (30˚C) loss of total shoots and this declined further over the subsequent two weeks of the study (Figure 3.1a). Overall, shoots under low *LI* decreased from 1960 ± 826 to 130 ± 52 shoots m^{-2} and 2540 ± 915 to 785 ± 350 shoots m^{-2} from week 0 to week 4, for low and high temperature treatments, respectively.

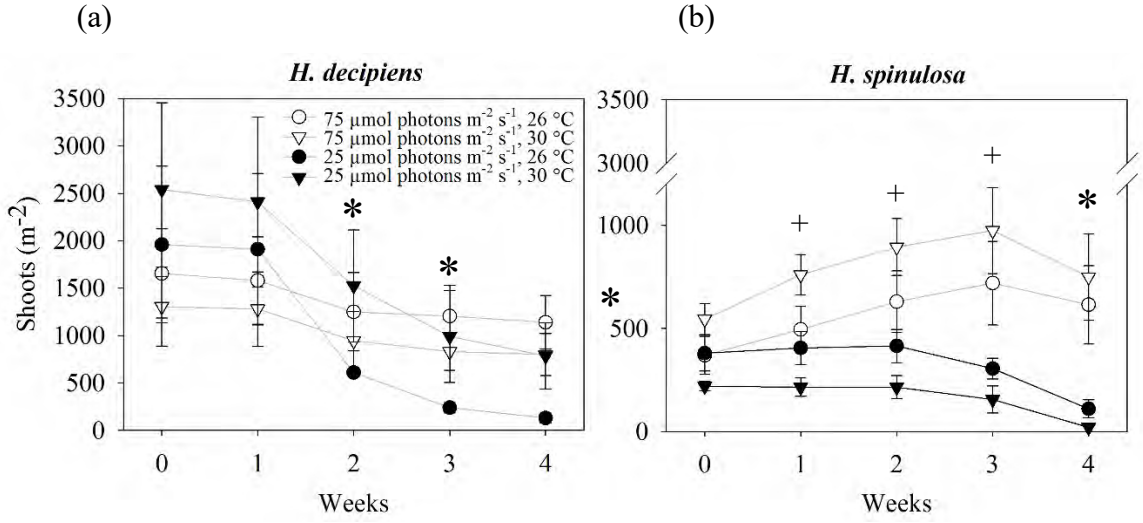


Figure 3.1 Shoot density (shoots m^{-2}) for *H. decipiens* (a) and *H. spinulosa* (b) over a four-week study. * indicate significant declines in low *LI* treatments from *T*0 while + indicate significant gains in high *LI* from *T*0. Data symbols and error bars represents mean ± SE. (n = 4).

Shoots of *H. spinulosa* were also driven by *LI* and *W* with no effect of *T* (Table 3.1). In total, *H. spinulosa* shoots declined under low *LI* from 350 ± 88 to 110 ± 44 shoot m^{-2} (26˚C) and 220 ± 23 to 20 ± 20 shoot m^{-2} (30˚C) from week 0 to week 4. Shoot loss did not begin until after week 2 and was only significant at week 4 (Figure 3.1b). Conversely, under high *LI* there was a significant gain in shoots from week 1 to week 3 (Figure 3.2b).

Table 3.1 *Halophila decipiens* and *H. spinulosa* parameter estimates where significant effects of covariates were found with generalized linear mixed-effects models (GLMM). Effects of light intensity (*LI*), temperature (T*)* and week (*W*) on shoot counts (SC), and wavelength-dependent fluorescence parameters are presented. Wavelength (*WV)* was also included in all models for wavelength-dependent fluorescence parameters. Models with interaction terms also include main effects. Shoot count was modelled with a negative binomial distribution, chlorophyll fluorescence parameters with a beta distribution and oxygen/respiration rates with a gamma distribution (all with logit link function). β_{tub} is the random effect of tub and ε is the error term. The best model selected for each parameter is in bold.

Halophila decipiens					*Halophila spinulosa*				
Model	df	AIC_C	ΔAIC_C	w	Model	df	AIC_C	ΔAIC_C	w
Shoot Count (SC)					Shoot Count (SC)				
LI * W + β_{tub} + ε	**12**	**745.3**	**0.00**	**0.703**	*LI * W + LI * T + β_{tub} + ε*	13	511.8	0.00	0.472
					LI * W + β_{tub} + ε	**11**	**512.2**	**0.34**	**0.398**
$\sigma_{II}(\lambda)$					$\sigma_{II}(\lambda)$				
T + WV + β_{tub} + ε	8	71.6	0.00	0.288	*LI * T + WV + β_{tub} + ε*	8	239.6	0.00	0.386
*LI * T + WV + β_{tub} + ε*	9	71.8	0.22	0.259	*LI + T + WV + β_{tub} + ε*	9	240.1	0.50	0.300
WV + β_{tub} + ε	10	72.6	0.99	0.176	***WV + β_{tub} + ε***	**7**	**240.9**	**1.31**	**0.200**
WV + β_{tub} + ε	**7**	**72.8**	**1.20**	**0.158**					
$YII_{(LL)}$					$YII_{(LL)}$				
LI + WV + β_{tub} + ε	**9**	**-194.8**	**0.00**	**0.956**	***WV + β_{tub} + ε***	**8**	**-290.2**	**0.00**	**0.773**
$YII_{(HL)}$					$YII_{(HL)}$				
WV + β_{tub} + ε	**8**	**-298.9**	**0.00**	**0.930**	***LI + WV + β_{tub} + ε***	**9**	**-434.9**	**0.00**	**0.418**
					*LI * T + WV + β_{tub} + ε*	11	-433.7	1.19	0.231
					LI + T + WV + β_{tub} + ε	10	-433.0	1.92	0.160

Table. 3.1 (Continued).

Halophila decipiens					***Halophila spinulosa***				
rETRII$_{(LL)}$					rETRII$_{(LL)}$				
$\boldsymbol{LI + WV + \beta_{tub} + \varepsilon}$	**8**	**173.3**	**0.00**	**0.539**	$LI + WV + \beta_{tub} + \varepsilon$	8	146.2	0.00	0.436
					$\boldsymbol{WV + \beta_{tub} + \varepsilon}$	**7**	**147.6**	**1.36**	**0.221**
					$LI + T + WV + \beta_{tub} + \varepsilon$	9	148.2	1.99	0.161
rETRII$_{(HL)}$					rETRII$_{(HL)}$				
$\boldsymbol{WV + \beta_{tub} + \varepsilon}$	**7**	**211.7**	**0.00**	**0.477**	$LI * WV * T + \beta_{tub} + \varepsilon$	22	277.3	0.00	0.250
$WV + T + \beta_{tub} + \varepsilon$	8	213.4	1.66	0.208	$LI * WV + LI * T + \beta_{tub} + \varepsilon$	14	277.7	0.32	0.213
$LI + WV + \beta_{tub} + \varepsilon$	8	213.4	1.72	0.202	$LI * WV + LI * T + LI * WV + \beta_{tub} + \varepsilon$	18	278.3	1.00	0.152
					$LI * WV + T + \beta_{tub} + \varepsilon$	13	278.5	1.18	0.138
					$\boldsymbol{LI * WV + \beta_{tub} + \varepsilon}$	**12**	**278.7**	**1.36**	**0.127**
					$LI * T + WV + \beta_{tub} + \varepsilon$	17	278.8	1.46	0.120
NPQII$_{(LL)}$					NPQII$_{(LL)}$				
$\boldsymbol{WV + \beta_{tub} + \varepsilon}$	**7**	**-65.1**	**0.00**	**0.616**	$\boldsymbol{WV + \beta_{tub} + \varepsilon}$	**7**	**-87.0**	**0.00**	**0.900**
$T + WV + \beta_{tub} + \varepsilon$	8	-63.8	1.37	0.310					
NPQII$_{(HL)}$					NPQII$_{(HL)}$				
$\boldsymbol{LI + WV + \beta_{tub} + \varepsilon}$	**8**	**-95.8**	**0.00**	**0.599**	$\boldsymbol{LI + WV + \beta_{tub} + \varepsilon}$	**8**	**-49.5**	**0.00**	**0.676**
Below-ground Soluble Sugars					Below-ground Soluble Sugars				
–					***LI***	**3**	**65.9**	**0.00**	**0.865**
Below-ground Starch					Below-ground Starch				
–					***Null***	**2**	**39.7**	**0.00**	**0.645**

3.3.2 Below-ground carbohydrates

Below-ground tissues of *H. spinulosa* were significantly affected by *LI* (Table 3.1) with measurable increases of soluble sugar concentrations under high *LI* treatments compared to the start of the study and low *LI* tubs (Figure 3.2). In contrast, below-ground starch concentrations were unchanged over time and among treatments.

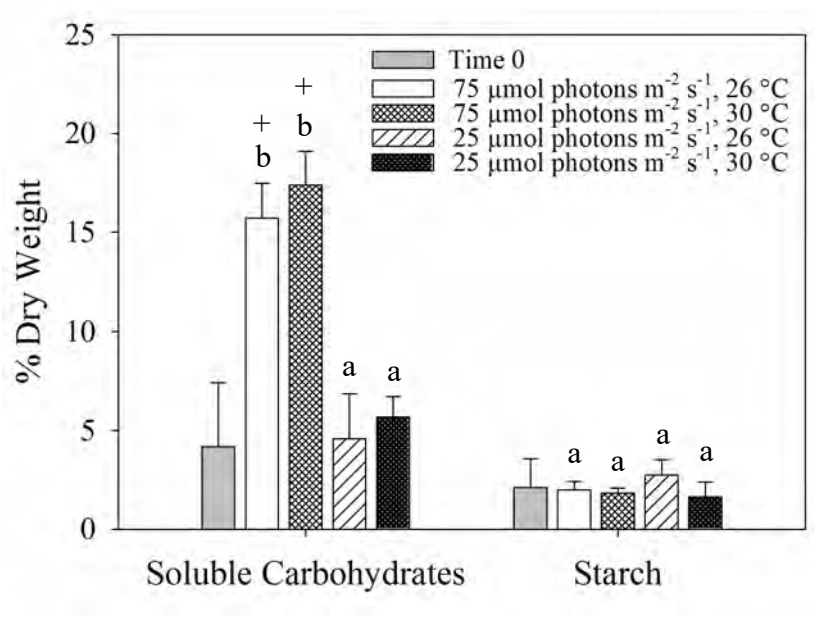


Figure 3.2 Percent soluble carbohydrates and percent starch in below ground roots and rhizomes at the start (Time 0) and end of the experiment (4 weeks) for *H. spinulosa* under each treatment. Differing letters indicate significant differences among treatments at the end of the study; + indicate difference from time 0 measurements. Data symbols and error bars represents mean ± S.E.M. (n = 3).

3.3.3 O_2 gas exchange determinations

Overall, oxygen and respiration measurements of *H. decipiens* leaves were highly variable both within and among treatments (Figure 3.3). Gas exchange in leaves from the start to the end of the study was not statistically significant despite the appearance of a large increase in both oxygen production and respiration under control conditions at

the end of the study due to the measured variance. For *H. decipiens* leaves, significant declines in oxygen production ($F = 6.8$, $p = 0.02$) and respiration ($F = 5.8$, $p = 0.03$) were related to high *T* alone, while there were no differences in the P:R ratios among treatments at the end of the study (Figure 3.3).

In *H. spinulosa* leaves, oxygen production and respiration were correlated with *LI*; relatively higher oxygen ($F = 7.1$, $p = 0.02$) and respiration ($F = 6.56$, $p = 0.02$) under low *LI* with no measurable patterns in P:R measurements (Figure 3.3). Oxygen and P:R for *H. spinulosa* was significantly lower at the end of the study compared to the start in control treatments (75 µmol photons m^{-2} s^{-1}, 26 °C; $F = 23.0$, $p = 0.005$ and $F = 10.1$, $p = 0.02$ respectively).

3.3.4 Wavelength-dependent variable chlorophyll fluorescence

Wavelength-dependent functional absorption cross-section of PSII ($\sigma_{II}(\lambda)$) of both *H. decipiens* and *H. spinulosa* significantly differed by *WV*, but *LI* and *T* had no effect on $\sigma_{II}(\lambda)$ for either species (Figure 3.4, Table 3.1). Wavelength (*WV*) also significantly affected effective quantum yield (Y(II)), relative electron transport rate (rETR(II)), and non-photochemical quenching (NPQ) in both *H. decipiens* and *H. spinulosa* in all treatments when exposed to sub- and supra-saturating AL (Figures 3.5 and 3.6; Table 3.1). These results are in agreement with the $\sigma_{II}(\lambda)$ spectrum (Figure 3.4); the largest differences among wavelengths were between those most absorbed (440 and 480 nm), whereas smaller differences were recorded between less absorbed wavelengths (540 and 590 nm) for both species.

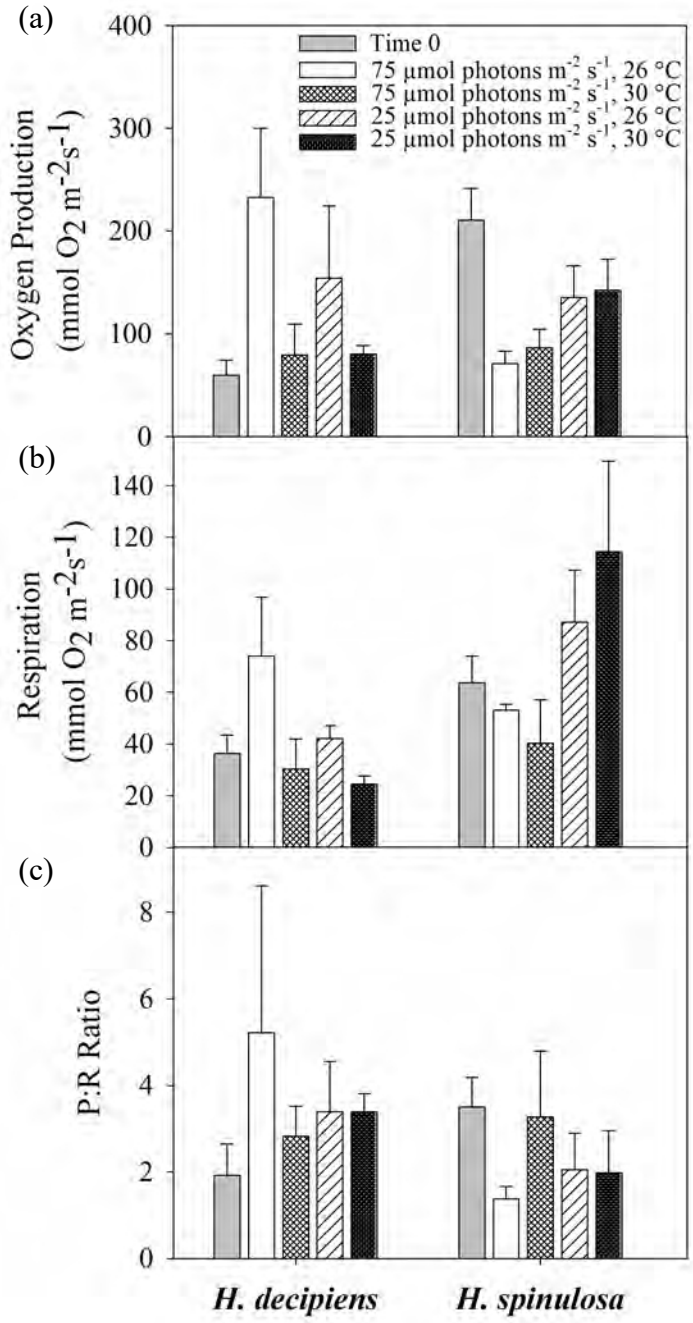


Figure 3.3 (a) Oxygen production (µmol O_2 m^{-2} s^{-1}, (b) respiration (µmol O_2 m^{-2} s^{-1}, and (c) P:R ratios of *H. decipiens* and *H. spinulosa* measured at the start (Time 0; 75 µmol m^{-2} s^{-1}, 26°C) and the end of the experiment. Data symbols and error bars represents mean ± S.E.M. (n = 4).

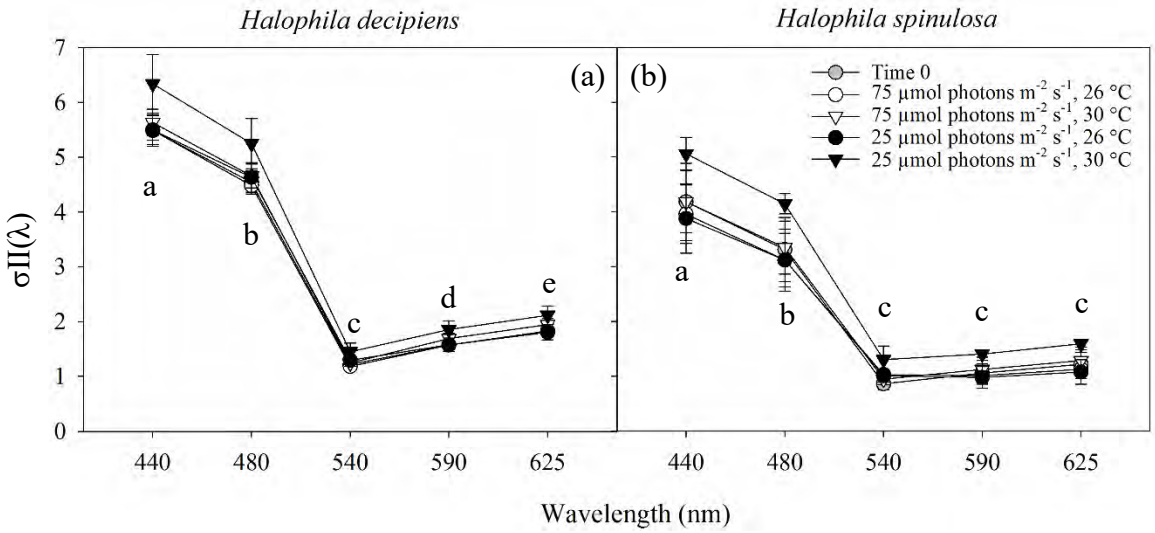


Figure 3.4 Wavelength-dependent functional absorption cross-section of PSII, σII(λ), for *H. decipiens* (a) and *H. spinulosa* (b) measured across five wavelengths at the start (Time 0) and the end of the study. Data symbols and error bars represents mean ± S.E.M (n = 4).

In addition to a clear separation among wavelengths, a pattern of low *LI* affected both species, but under opposing AL conditions and regardless of *T* (Table 3.1). For *H. decipiens*, leaves from low *LI* treatments had lower overall Y(II) and rETR(II) across all wavelengths under the sub-saturating AL, while *H. spinulosa* leaves did not have measurable differences under the same sub-saturating AL (Figure 3.5). Under the supra-saturating AL, leaves of *H. spinulosa* from low *LI* treatments had lower overall Y(II) and rETR(II) across all wavelengths, while *H. decipiens* did not measurably differ among treatments (Figure 3.6). NPQ for both species was significantly reduced under low *LI,* but only when exposed to supra-saturating AL (Figures 3.5 and 3.6; Table 3.1).

The overall magnitude of wavelength-dependent photochemical parameters differed between the sub- and supra-saturating AL measurements (Figures 3.5 and 3.6). Y(II)

values, as expected, were depressed under the supra-saturating AL ranging from a mean of 0.05 – 0.2 (Figures 3.5) compared to 0.3 – 0.6 (Figures 3.6) under sub-saturating conditions. rETR(II) and NPQ concomitantly increased under the same conditions.

Y(II) and rETR(II) for *H. decipiens* and *H. spinulosa* under both sub- and supra-saturating AL measurements were significantly lower at 440 and 480 nm compared to all other wavelengths (Figures 3.5 and 3.6). The lowest quantum yields of PSII and relative electron transport rates were followed by measurements at 625 nm which were significantly lower than both 540 and 590 nm, the least absorbed wavelengths according to $\sigma_{II}(\lambda)$ measurements. An inverse relationship found for non-photochemical quenching (NPQ), a measure of the plant's capacity to dissipate excess light energy, meant the highest values were recorded at 440 nm in *H. decipiens* and 440 and 480 nm for *H. spinulosa* where light was most absorbed and relative electron transport rates were lowest (Figures 3.5 and 3.6).

Overall species differences in wavelength-dependent parameters, irrespective of treatment, were found with both sub- and supra-saturating AL measurements (Table 3.2); however, stronger patterns were under supra-saturating AL. *H. decipiens* had higher overall $\sigma_{II}(\lambda)$ than *H. spinulosa* (Figure 3.4; Table 3.2) but lower Y(II), rETR(II), and NPQ than *H. spinulosa* irrespective of AL (Figures 3.5 and 3.6).

Table 3.2 GLMM model fit for species comparison (*SPP*) of wavelength-dependent variable fluorescence parameters. n =4 ± SE. * p < 0.05, ** p< 0.01, *** p< 0.001

Model	**MS**	***F***	**p**
$\sigma_{II}(\lambda)$	55.98	19.30	***
$YII_{(LL)}$	0.04	4.60	*
$YII_{(HL)}$	0.07	23.59	***
$rETRII_{(LL)}$	13.71	5.97	*
$rETRII_{(HL)}$	396.88	23.05	***
$NPQII_{(LL)}$	0.51	10.87	**
$NPQII_{(HL)}$	2.18	42.48	***

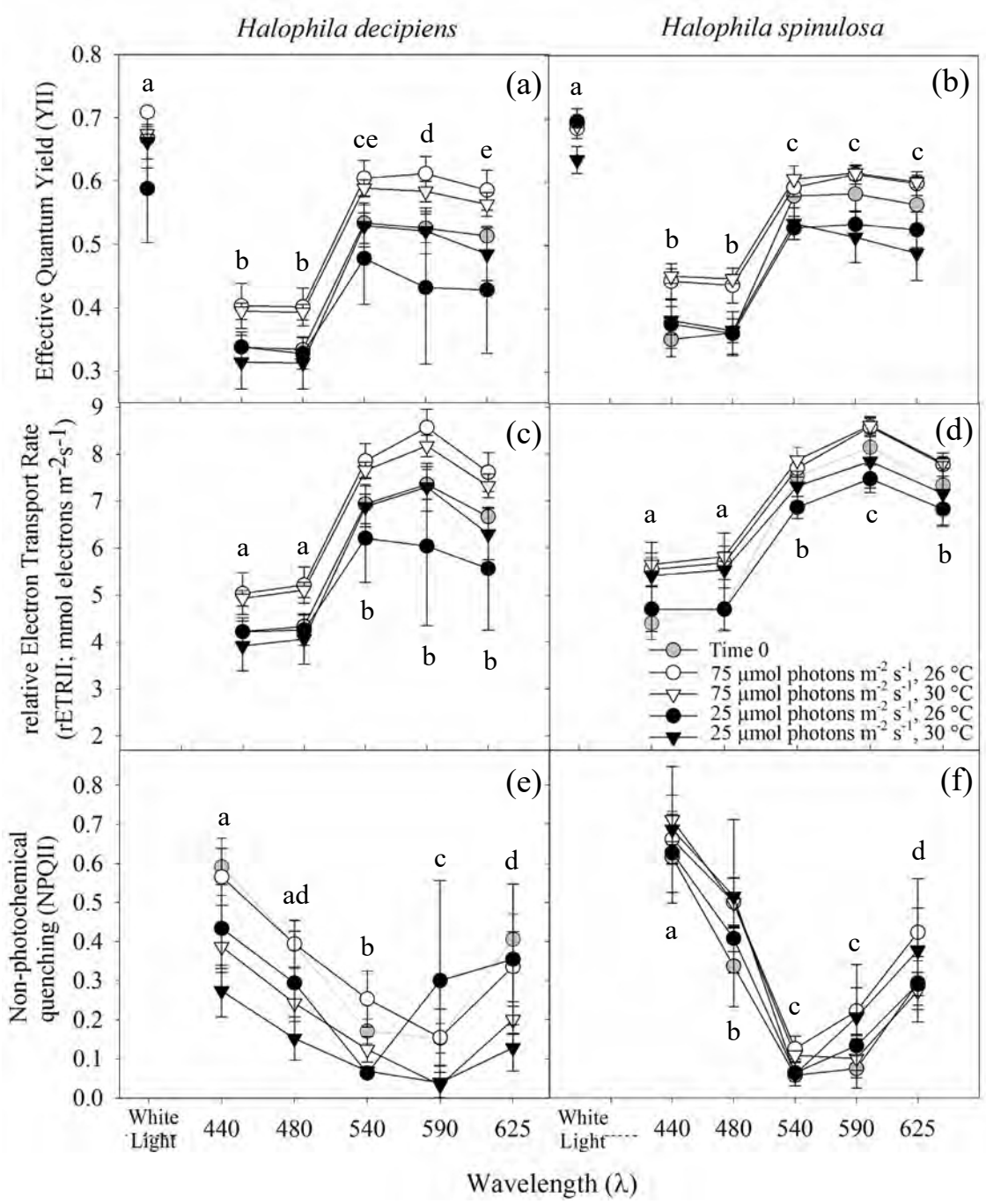


Figure 3.5 Effective quantum yield (YII; (a, b)), relative electron transport rate (rETRII; (c, d)), and non-photochemical quenching (NPQII; (e, f)) for *H. decipiens* (a, c, e) and *H. spinulosa* (b, d, f) measured under sub-saturating AL at five wavelengths at start (Time 0) and the end of the experiment. Differing letters indicate significant differences among wavelengths at the end of the study based on a Bonferroni correction. Data symbols and error bars represents mean ± S.E.M. (n = 4).

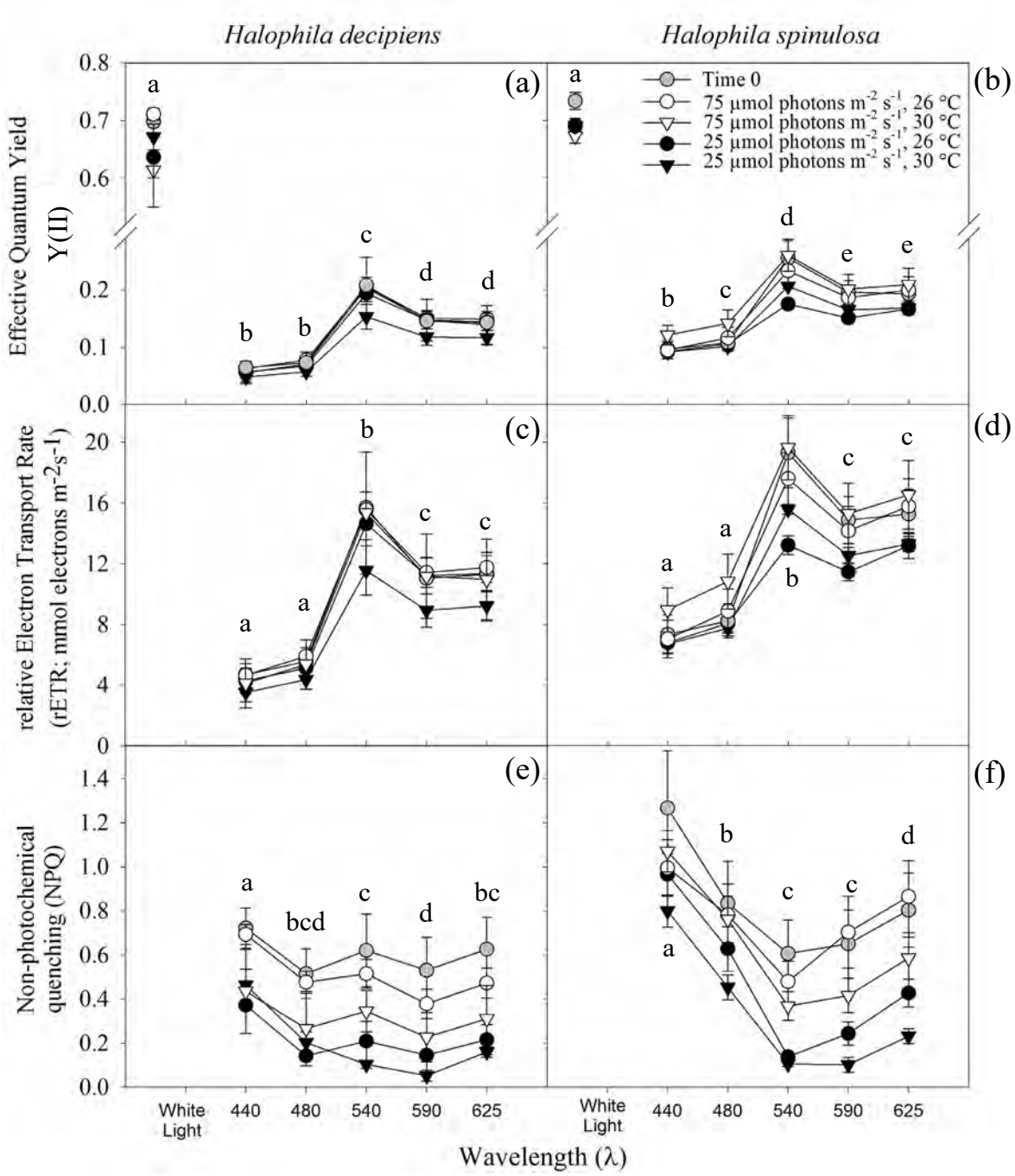


Figure 3.6 Effective quantum yield (YII; (a, b)), relative electron transport rate (rETRII; (c ,d)), and non-photochemical quenching (NPQ; e, f for *H. decipiens* (a, c, e) and *H. spinulosa* (b, d, f) measured under supra-saturating AL at five wavelengths at start (Time 0) and the end of the experiment. Differing letters indicate significant differences among wavelengths at the end of the study. Data symbols and error bars represents mean ± S.E.M. (n = 4).

3.3.5 Pigment Characterisation

Effect of light and temperature treatments on chlorophyll content differed between the two species (Table 3.3). *H. decipiens* total chlorophyll, Chl *a,* and Chl *b* were unaffected by treatment, while Chl *b* increased somewhat under low *LI* ($F = 6.1$, $p = 0.02$). Chl *a*:*b* was significantly affected by *LI,* but dependent on *T* with significantly lower Chl *a*:*b* only under low *LI* and low *T* ($F = 7.0$, $p = 0.01$; Table 3.3).

All measures of chlorophyll content relative to leaf area for *H. spinulosa* leaves were affected in some way by *LI* and *T* treatments (Table 3.3). Total chlorophyll, Chl *a*, and Chl *b* were highest under low *LI* but with Chl *a* only when under low *T* ($F = 5.65$, $p = 0.03$). Chl *b* was also significantly higher under high *LI* when combined with high *T*. Chl *a*:*b* was significantly lower under high *T* irrespective of *LI* ($F = 16.2$, $p = 0.001$).

Table 3.3 Chlorophyll composition of MC-PAM leaves under two light treatments and two temperature treatments (n =4). Pigment concentrations units are µg cm^{-2}. Differing letters indicate significant differences among treatments for each species when the null model was rejected (Bonferroni correction method).

	Halophila decipiens				*Halophila spinulosa*			
Treatment	Total Chlorophyll	Chl *a*	Chl *b*	Chl *a*:*b*	Total Chlorophyll	Chl *a*	Chl *b*	Chl *a*:*b*
Time 0 (75 µmol m^{-2} s^{-1}; 26°C)	0.54 ± 0.04	0.34 ± 0.02	0.20 ± 0.01[a]	1.68 ± 0.02[a]	0.71± 0.05[a]	0.47 ± 0.03[a]	0.25 ± 0.02[a]	1.89 ± 0.05[a]
75 µmol m^{-2} s^{-1}; 26°C	0.53 ± 0.04	0.33 ± 0.02	0.20 ± 0.01[a]	1.68 ± 0.06[a]	0.64 ± 0.04[a]	0.43 ± 0.03[a]	0.22 ± 0.02[a]	1.98 ± 0.03[a]
75 µmol m^{-2} s^{-1}; 30°C	0.61 ± 0.05	0.36 ± 0.03	0.24 ± 0.02[a]	1.52 ± 0.05[a]	0.86 ± 0.08[a]	0.55 ± 0.05[a]	0.30 ± 0.02[b]	1.81 ± 0.04[b]
25 µmol m^{-2} s^{-1}; 26°C	0.58 ± 0.05	0.34 ± 0.03	0.24 ± 0.02[b]	1.38 ± 0.05[b]	0.98 ± 0.03[b]	0.64 ± 0.02[b]	0.34 ± 0.01[b]	1.90 ± 0.01[a]
25 µmol m^{-2} s^{-1}; 30°C	0.76 ± 0.08	0.46 ± 0.05	0.30 ± 0.03[b]	1.55 ± 0.03[a]	0.87 ± 0.06[b]	0.54 ± 0.04[a]	0.32 ± 0.02[b]	1.67 0.04[b]

3.4 Discussion

This is the first study to assess two deep-water seagrasses of which neither species' physiology, optical characteristics or morphological response to light and temperature stress has been previously described. Under light spectrally adjusted to mimic natural deep-water conditions, our study showed that a 66% reduction in light availability from 75 to 25 µmol photons m^{-2} s^{-1} caused a decrease in shoots for both *H. decipiens* and *H. spinulosa* after two and four weeks respectively. Surprisingly, temperature did not further affect *H. decipiens* or *H. spinulosa* shoot density under low light. A reduction in light led to characteristic optical changes in the leaves such as increases in Chl *b* concentrations and lower electron transport rates; however, both species lacked the capacity to withstand shoot loss over the 4-week study. The effect of light stress on both *H. decipiens* and *H. spinulosa* followed a characteristic response that has been well documented in studies on other seagrass species (Longstaff et al., 1999; Collier et al., 2012b; Chartrand et al., 2016).

Minimum light requirement and optimal temperature for growth and photosynthesis vary among species due to unique physiological and morphological adaptation (Lee et al., 2007). Previous studies showed light requirements for *H. decipiens* in Cuba and St. Croix, were 4.4 and 8.8% of surface irradiances, respectively (Dennison et al., 1993). Additional work by Erftemeijer and Stapel (1999) off South Sulawesi, Indonesia on similar deep-water *H. ovalis* beds found a light compensation point (i.e. when productivity equals respiration and net carbon balance is zero) of 33 µmol photons m^{-2} s^{-1}, equivalent to approximately 1.4 mol photons m^{-2} d^{-1}. A contiguous deep-water *H. decipiens* meadow off the west coast of Florida was recorded growing year-round at 20

m under light as low as 1.8 mol photons m^{-2} d^{-1} (Hammerstrom et al., 2006). Our study showed that an average irradiance at 75 µmol photons m^{-2} s^{-1} (equal to 3.2 mol photons m^{-2} d^{-1}) which is approximately 4% of surface irradiance (based on a typical midday measurement of 2000 µmol photons m^{-2} s^{-1} at the surface) is an adequate light regime for both *H. decipiens* and *H. spinulosa*. A 66% reduction in light (25 µmol photons m^{-2} s^{-1}, equal to 1.1 mol photons m^{-2} d^{-1}) had a significant effect on shoot density and optical properties of both *H. decipiens* and *H. spinulosa* within 4 weeks.

Elevated temperature had little overall effect on optical and physiological responses and no consequence to shoot loss in either species. Seagrasses respond to light reduction in various ways e.g. including changes in light absorption properties of the leaves, altering morphology and modifying carbon storage (Abal et al., 1994; Gordon et al., 1994; Campbell and Miller, 2002; Ralph et al., 2007), however temperature is known to have a direct effect on seagrass metabolism, nutrient uptake and enzyme activities (Short and Neckles, 1999; Lee et al., 2007). The optimal temperature for seagrass growth is dependent upon irradiance (Bulthuis, 1987) and an increase in temperature to some optimal level promotes photosynthesis and higher growth rates (Lee et al., 2007). If temperatures increase further without a concomitant increase in light levels to support photosynthesis, metabolic demand will outstrip supply and seagrass condition will deteriorate (Masini et al., 1995; Lee et al., 2007; Collier et al., 2011). In the current study, neither species appeared to be adversely affected – metabolically nor physically — by elevated temperature under either light treatment. Seagrasses are actively growing from July to October in waters of 24-28˚C each year in the meadows where plants were collected. Seagrass die-back occurs rapidly by the following January each austral

summer when *in situ* temperatures reach a maximum of 29˚C (Chartrand pers. comm). The 30˚C treatment in this study therefore reflects a biologically-meaningful elevated condition for tropical deep-water seagrasses under a warming climate. Our study showed no changes in P:R ratio in either species suggesting that the high temperature (30˚C) is not beyond some optima for these species and suggests both species are tolerant to minor temperature increases irrespective of the light climate. Testing more extreme temperatures would likely negatively affect plant metabolism and productivity and establish an upper thermal limit for these populations as measured for other shallow water tropical seagrasses (Lee et al., 2007; Collier et al., 2011; York et al., 2013; Adams et al., 2017).

The decrease in shoot density under low light that we measured is consistent with other studies (Longstaff et al., 1999; Collier et al., 2012b), and yet the interactive effect with high temperature seen with strap-bladed species did not occur (York et al., 2013). This may be a response of small-bodied opportunistic species that are expected to exhibit fast growth to exploit resources under high light conditions and disappear when light levels deteriorate (Ralph et al., 2007; Kilminster et al., 2015). Strap-bladed species may decrease leaf area with loss of light which will reduce the respiratory demand of the shoot and reduce the photosynthetic capacity of the leaves (Campbell and Miller, 2002). Instead of modifying leaf size, the reduction in shoot density we observed may be a strategy used to restore carbon balance by reducing above-ground tissues, which have higher respiratory demands than below-ground tissues (Alcoverro et al., 2001; York et al., 2013).

Halophila spp. have meager below-ground architecture compared to other morphologically large and long-lived species which rely on below-ground reserves to compensate for poor water quality over short durations (Collier et al., 2009; Collier et al., 2012b). A lack of below-ground tissues may further encourage a reduction in shoot density as a quick strategy to restore an energy balance in the whole plant. While diminutive size is an attribute of the *Halophila* family, some species do have greater structural complexity and size than others. The disparity in morphological characteristics of *H. decipiens* and *H. spinulosa* may explain the time differential to impact each species under low-light treatments in our study. We found *H. spinulosa*, the larger of the two species, was able to increase its below-ground sugars if given saturating light (Figure 3.2). The controlled mobilization and oxidation of stored carbohydrates can release free energy in the form of NADPH and ATP (Touchette and Burkholder, 2000). This energy supply can be used to support cellular and metabolic processes in the absence of sufficient light (Touchette and Burkholder, 2000). The larger physical form of *H. spinulosa* may also provide energy to endure short durations of poor light, whereas the diminutive form of *H. decipiens* has little capacity to draw from structural reserves. While we were not able to measure below-ground carbohydrates in *H. decipiens*, the delayed decline in shoot density by 2 weeks under low light in *H. spinulosa* compared to *H. decipiens* may be linked to relatively higher starting carbohydrate reserves in the former species which allowed for it to thrive longer before losing shoots. The lack of appreciable below-ground biomass to perform such tests in *H. decipiens* is in itself an indication of scarce carbohydrate reserves.

Seagrasses, as with other higher plants, possess an array of optical strategies to enhance light harvesting and photosynthetic efficiency, which include increasing chlorophyll content and decreasing Chl *a:b* ratio under low light in order to enhance the capabilities of PSII reaction centres (Abal et al., 1994; Walters, 2005). We found both *H. decipiens* and *H. spinulosa* modified their chlorophyll content somewhat in response to light; Chl *b* was increased to a greater extent for both species under reduced light, but this did not always alter the overall Chl *a:b*. As an important accessory pigment in the light harvesting complexes, Chl *b* is known to enhance light absorption and capture via increased thylakoid grana stacking, particularly under low-light growing conditions (Leong and Anderson, 1984; Voitsekhovskaja and Tyutereva, 2015). Although, the small changes to Chl *a:b* and no significant effect on the functional absorption cross section by treatment suggests that no net effect of enhanced light capture was observed.

Overall, both species had somewhat lower Chl *a:b* — independent of treatment — than found in other higher plants and strap-bladed seagrasses which typically range in values from 2-3 (Abal et al., 1994; Czerny and Dunton, 1995; Zivcak et al., 2014). Lower Chl *a:b* is consistent with other *Halophila* spp. studies (Williams and Dennison, 1990; Ralph and Burchett, 1998; Longstaff and Dennison, 1999) and suggests an enriched Chl *b* light-harvesting antenna independent of experimental conditions. *Halophila* spp. may have adapted their photosynthetic machinery to capitalise on low-light habitats such as deep-water or turbid inshore areas where their light harvesting capabilities maximise their chances of success in these extreme conditions to greater extent than other shade-adapted land plants (Kitajima and Hogan, 2003).

The wavelength-dependent pattern of $\sigma_{II}(\lambda)$ measured in both seagrass species was similar to that measured in other green phototrophs including *Chlorella* suspensions and terrestrial leaves (Schreiber and Klughammer, 2013). However, overall $\sigma_{II}(\lambda)$ values measured on the adaxial surface of a dandelion leaf by Schreiber and Klughammer (2013) were much lower than the algal suspensions. Authors of both studies believe these reductions in overall $\sigma_{II}(\lambda)$ are likely due to the apparent gradient of light absorption within optically-dense samples compared to those seen in optically thin algal suspensions (Evans, 2009; Schreiber and Klughammer, 2013). Plant leaves are well known to have in-built light gradients affecting absorption by different layers within the leaf tissue (Vogelmann and Evans, 2002; Evans, 2009), limiting the accuracy of such intrinsic $\sigma_{II}(\lambda)$ measurements (Schreiber and Klughammer, 2013; Osmond et al., 2017). However, the apparent differences in $\sigma_{II}(\lambda)$ can be assessed based on inherent properties of PSII and the assumption that average leaf measurements are equivalent to average conditions within the leaf itself and therefore acceptable to measure relative changes (Osmond et al., 2017). These relative changes in $\sigma_{II}(\lambda)$ have been used in studies as a metric to assess the acclimation capacity of $\sigma_{II}(\lambda)$ in a number of genotypes, genetic mutants, algae and higher plants (Szabó et al., 2014; Ware et al., 2015; Osmond et al., 2017). The optically thin nature of *Halophila* leaves (2 cells-thick) and the location of chlorophyll pigments exclusively in the outer epidermal layer of seagrass leaves (Kuo and den Hartog, 2006; Ferreira et al., 2015) correspond with the relatively high levels of $\sigma_{II}(\lambda)$ for a leaf sample in our study. Beyond $\sigma_{II}(\lambda)$, the morphological framework of the seagrass blades allowed us to explore other spectrally-resolved measurements; wavelength-specific electron transport rates (rETR(II)) and energy dissipation (NPQ).

Despite no effect of light or temperature on $\sigma_{II}(\lambda)$ in deep-water *Halophila* spp. there was a classic wavelength-specific response of photochemical reactions of PSII. Both species absorbed the highest proportion of light from the blue (440 and 480 nm) and secondarily from red wavebands (625 nm), characteristic of a typical leaf (Vogelmann and Evans, 2002; Schreiber and Klughammer, 2013). The greater absorption in the blue region in turn increases the potential of a photoinhibitory/damaging response. More efficient photoprotective mechanisms are evolved and are evident in the higher NPQ measured at 440 and 480 nm (Figure 3.5, 3.6). Plants adapted to higher irradiance can regulate photosynthesis with a larger range of NPQ, where-as 'shade-adapted' plants tend to have lower range and NPQ values. A 'shade-adapted' plant, under low-light intensities has lower excitation pressure on its' light harvesting antennae and therefore NPQ does not need to compete with energy delivery to the reaction centres (Ruban, 2014). On the other hand, a 'sun-adapted' plant effectively relies upon NPQ to cope with excess light above and beyond the state where closed reaction centres have been saturated under high light. This effect was clear with NPQ measurements in this study when plants were exposed to the supra-saturating light; HL treated plants had significantly greater NPQ than those grown in LL treatments (Figure 3.6e, f). The wavelength-specific differences in NPQ follow the same relationship; adaptation to greater absorption at 440, 480, and 625 nm correlate with greater NPQ capacity to protect the productive light harvesting pigments at the corresponding wavebands. The higher NPQ at 440 and 480 nm created the expected concomitant reduction in photochemical efficiency and relative electron transport rates at these wavelengths for both *H. decipiens* and *H. spinulosa*.

Both seagrass species in this study grow at depths that create similar light challenges to that of a forest floor where the niche is filled by shade-loving plants. *H. decipiens* has almost exclusively been described as growing in turbid shallow waters or in deep-water habitats whereas *H. spinulosa* was mainly found in subtidal and turbid water habitats (Walker et al., 1988; Kenworthy et al., 1989; Kuo and Kirkman, 1995; Coles et al., 2009) where chronic low-light intensities would seem to reduce the need for high NPQ to protect light harvesting and PSII reaction centre proteins from photodamage. Investigations by Dawes et al. (1989) and a reciprocal transplant experiment by Durako et al. (2003) concluded *H. decipiens* is actually intolerant of higher light intensities. Despite the lesser need for high light photoprotective processes in a naturally low-light environment, the machinery and pathways are highly conserved across many higher plants found in low-light habitats (Ruban, 2014). At what capacity or for how long photoprotection via NPQ is sustained in both species under high light conditions, would be a valuable extension of research. It would provide a better understanding of how flexible or rigid these plants are to acclimate to various light regimes and by extension define the potential habitats each species could, hypothetically, inhabit. In particular, there has been little research into the photobiology or metabolic tolerances of *H. spinulosa* outside of this study.

The wavelength-specific photochemistry did differ somewhat between species. For *H. decipiens*, the sub-saturating AL measurements used to assess wavelength-specific parameters resulted in significantly higher Y(II) and rETR(II) in high light treatment leaves, regardless of temperature, despite no measurable difference in NPQ. Under supra-saturating AL, only wavelength-specific differences (no treatment effects) were

found in these parameters for *H. decipiens*. While NPQ was operational under supra-saturating AL for both HL and LL treated plants, photodamage may have occurred in this species irrespective of growing treatment conditions. Plants typically grown under lower light intensities produce greater amount of light harvesting accessory pigments in the antennae, namely Chl *b*, than high light grown plants (Lichtenthaler et al., 1981; Ruban, 2014). The larger antenna serves to increase light harvesting and therefore increased photochemical reactions in shaded conditions. However, Ware et al. (2015) point out the enhanced antenna size would actually increase excitation pressure unnecessarily under rare high light conditions, which is suggested to be related to uncoupling of the antenna structure in LL grown plants having poor connectivity to reaction centres. Their research found LL plants had high NPQ under high light intensities, but with poor efficacy of dissipating excess energy and protecting PSII reaction centres. Therefore, measured NPQ was accounting for both connected and disassociated antenna complexes and exaggerating the effect of NPQ on bound antenna involved in the electron transport chain. This would explain the lack of treatment effect in our study on Y(II) and rETR(II) measurements despite differences in NPQ between LL and HL treatments under the supra-saturating light conditions for *H. decipiens*. It is also further photophysiological support that *H. decipiens* is an obligate low-light adapted species compared to *H. spinulosa,* which is tolerant of a wider range of irradiance. For *H. spinulosa*, the opposite was true. Higher Y(II) and rETR(II) values under supra-saturating AL condition were measured in leaves from the high-light treatments, whereas only wavelength was correlated with sub-saturating AL measurements. This outcome supports a greater inherent capacity to maintain

connectivity between antennae and reaction centres when exposed to supra-saturating conditions.

The pronounced wavelength-dependent patterns in these deep-water seagrasses are a reflection of biochemical pathways used to maximise photochemical efficiencies. In order to integrate these patterns in photo-physiology with the underlying molecular processes, techniques such as transcriptomics and metabolomics would invaluably enhance our understanding of the observations in this study. Efforts to innovate and fuse classical ecological studies with molecular approaches has been established and used as an important path forward to enhance multidisciplinary seagrass research (Mazzuca et al., 2013; Macreadie et al., 2014).

Both species investigated did not show dramatic changes in photochemistry or metabolism due to light stress, yet there was a significant decline in shoot density for *H. decipiens* and *H. spinulosa* over the four-week period. The compensatory mechanisms and photoacclimation that did occur in low-light treated plants are either not sufficient to counteract light limitation or the physiology is impacted downstream of photochemistry in other metabolic pathways. An alternative explanation of shoot loss in our study is a sacrificial approach whereby changes are made at the ramet scale instead of the leaf scale. This strategy would place efforts on repartitioning resources away from new shoots and directing energy into a few remaining leaves while sacrificing all others. While we were not able to reconcile this in the current study, identifying regulatory pathways and resource allocation signalling through a gene expression and

bioinformatics approach could be correlated with threshold responses to light stress as we tested here.

Acute light stress to deep-water seagrasses has implications for the larger context of deep-water seagrass meadow maintenance. Despite not directly investigating the effects of light on flowering and seed banks in this study, the net effects of light stress on sexual reproductive effort by seagrasses has been described (Cabaço and Santos, 2012). Seed production is likely vital in deep-water population in order to regenerate annually or following natural disturbances such as storms or cyclones (Kenworthy, 2000; Hammerstrom et al., 2006) and any impact on fruit production and seed recruitment into the sediment could have significant impacts on the subsequent year's seedling recruitment. Ensuring suitable light levels to ensure sufficient energy requirements to be put into reproductive output during key growing phases may be the most important factor for long-term viability and deep-water seagrass' success.

3.5 Conclusion

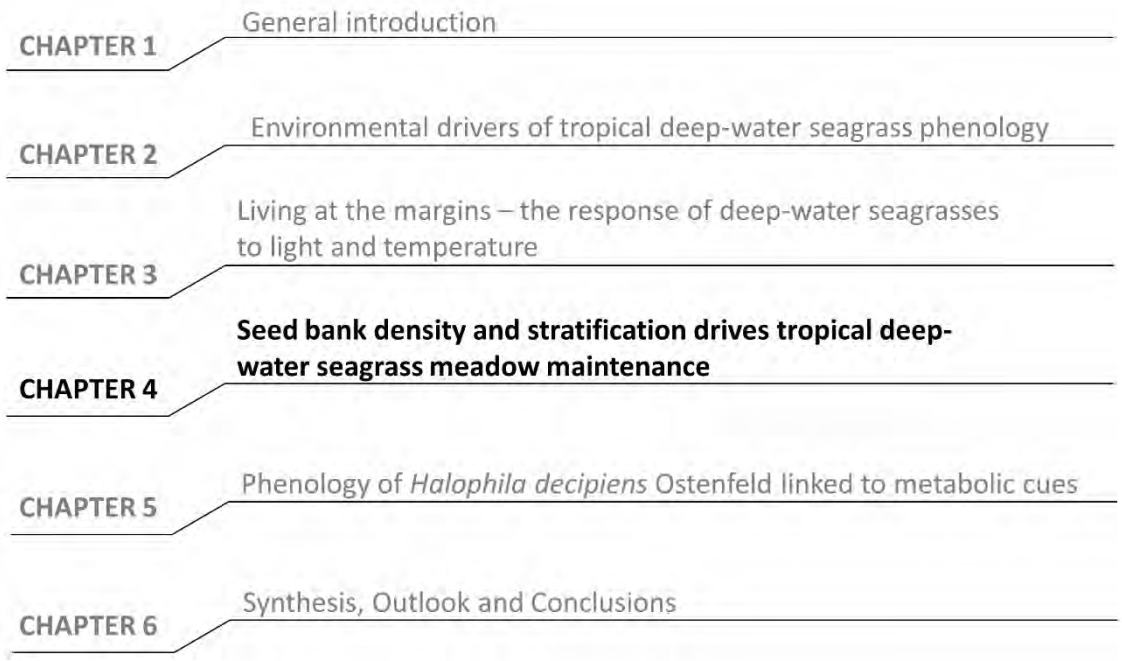
This study has established a clear, negative effect of relatively small quantitative reductions in growing season light on deep-water *Halophila decipiens* and *Halophila spinulosa* communities from the Great Barrier Reef lagoon. It further highlights hitherto undetected differences of closely related species to light and temperature conditions. This could have implications for making broad-based decisions using tools such as form-function models (Walker et al., 1999; Kilminster et al., 2015) of seagrass to infer light requirements and the associated response of seagrasses with limited understanding of their specific energetic needs. Such approaches to kerb seagrass loss could overlook

the species-specific adaptations to the local environment and lead to unintended negative outcomes for local seagrass communities. In spite of inter-species differences, *H. decipiens* and *H. spinulosa* did show classic higher plant responses to low light and significant shoot loss as a response to the same quantitative light levels over a short time-span. Some generalised decision tools to mitigate impacts to deep-water *Halophila* spp. could therefore still facilitate best-practice management of these mixed species seagrass communities (Wu et al., 2018).

For the first time, wavelength-specific parameters of PSII photochemistry were evaluated for seagrass leaves. While there was no effect of light or temperature on $\sigma_{II}(\lambda)$ in deep-water *Halophila* spp. in this study, there was a wavelength-specific response of photochemical reactions of PSII. The effect of low-light acclimation was apparent in non-photochemical quenching patterns including differences in tolerance between species to supra-saturating intensities, which likely reflects their inherent adaptations to their natural light environments. A valuable next step would be to integrate the measured patterns in photo-physiology with the underlying biochemical processes through an interdisciplinary bioinformatics approach.

With measurable impacts to *H. decipiens* and *H. spinulosa* after 2 weeks and 4 weeks respectively, even relatively short periods of increased light attenuation can affect key life history strategies used to ensure long-term meadow maintenance such as flowering and seed bank generation.

CHAPTER 4 Seed bank density and stratification drives tropical deep-water seagrass meadow maintenance



Chapter 4 has been submitted to the journal 'Estuarine, Coastal and Shelf Science' in February 2021 as: Chartrand K.M.[*1,2], Bryant C.V.[1], Ralph P.J.[2], and Rasheed M.A.[1] In review. Seed bank density and stratification drives tropical deep-water seagrass meadow maintenance: a multi-year case study from the Great Barrier Reef. *Estuarine, Coastal and Shelf Science*.

[1] Centre for Tropical Water & Aquatic Ecosystem Research, James Cook University, Cairns, Queensland, Australia.
[2] Climate Change Cluster, University of Technology Sydney, Broadway, New South Wales, Australia.

Abstract

The life history characteristics of a seagrass population shapes their capacity to persist or collapse when impacted by natural or anthropogenic disturbances. Tropical deep-water seagrass populations are often rapidly lost by even minor fluctuations in growing conditions because they are found living so close to their physiological limits. They rely heavily on sexual reproductive output, namely seed banks, as a maintenance strategy to ensure their capacity to recover and therefore long-term resilience. Despite this maintenance strategy, multi-year studies of these critical storage reserves (i.e. seeds) are rare and uncertainty exists as to the magnitude of seed burial versus export from the meadow. This study quantifies the intra- and inter-annual changes in species-specific seed bank densities and sediment stratification from three Great Barrier Reef deep-water seagrass meadows, the relationship to localised seed production (i.e. fruiting) and retention following senescence, as well as an opportunistic case study in how seed bank capacity drives recovery after a localised natural disturbance event (e.g. cyclone). Seeds were present at all three locations and were most dense directly after the peak in annual above-ground biomass before gradually declining to a minima at the time of broad meadow germination and rapid rhizome expansion. The predominant species of seed found at all locations was *Halophila decipiens*, the most widespread deep-water seagrass species of the GBR. No detectable *Halophila ovalis* seed bank was found at one of the three locations (Lizard Island meadow) despite the species dominating the meadow prior to the site being impacted from two severe tropical cyclones. Seasonal germination of the Lizard Island *H. decipiens* seed bank and delayed *H. ovalis* recruitment led to opportunistic gains by the former during meadow recovery. Two out of three meadows studied had their seed bank stratified with sediment depth, with the

greatest proportion of seeds consistently in the deepest fraction (5-10 cm) over the annual seagrass growing cycle. This study found the presence of a persistent seed bank likely ensures deep-water GBR seagrasses can re-populate areas when suitable germination conditions return. If critical growing conditions required to generate sexual reproductive output (i.e. seeds) is impaired, these seagrasses may be susceptible to losing their ability for annual recovery.

4.2 Introduction

Seagrasses are true flowering plants that returned to the sea and colonised the coastal zone in temperate to tropical environments around the world. Within tropical waters of the Great Barrier Reef, fifteen species across three seagrass families occupy estuarine, coastal, reef, and deep-water habitats (Carruthers et al. 2002). In deeper tropical waters, depths > 10-15 m, seagrass communities are dominated by *Halophila* spp. and are considered colonising species that form transitory meadows (Kilminster et al. 2015). However, these more cryptic deep-water seagrasses are increasingly recognised for their ecosystem service roles: creating fish habitat, providing a food supply to marine megafauna, carbon sequestration and sediment stabilisers over vast areas (Fonseca, 1989; Coles et al., 2009; Esteban et al., 2018; York et al., 2018; Hayes et al., 2020).

The diminutive structure of *Halophila* spp.,—oval or oblong shaped leaves that attach in pairs directly to either a vertical stem or rhizome via a petiole—produce a relatively large number of fruits at the base of each node (Orth et al., 2006). Abundant flowering, fruiting, seed production and retention in the surrounding sediment have been described globally for various *Halophila* spp. meadows (McMillan and Soong, 1989; Kuo and

Kirkman, 1995; Kenworthy, 2000; Hammerstrom et al., 2006). This high sexual fecundity resulting in large seed banks is a long-term maintenance strategy, whereby total meadow die-off is succeeded by re-establishment from seed. This contrasts with larger, more persistent seagrass species where shoot retention and perseverance through unfavourable conditions is the preferred strategy for long-term survival (Kilminster et al., 2015). Other mechanisms that support meadow resilience and recovery include transport of waterborne vegetative fragments if seeds are not present, seed viability is poor, or seeds become buried to a depth at which they are no longer an effective mechanism for recovery (Jarvis et al., 2014). A substantial seed bank, however, provides a direct means of recovery after a major disturbance or protracted period of unfavourable germination conditions (i.e. seasonal low light) (Kenworthy, 2000; Hammerstrom et al., 2006; Bell et al., 2008). There is research showing some seed banks do not persist throughout the year and rather, are seasonal or transient leaving questions as to the export versus retention of seeds to generate a local recovery (Hovey et al., 2015).

The majority of seagrass seed bank studies rely on opportunistic or short-term sampling to describe the density of seeds as a measure of meadow resilience (Kuo and Kirkman, 1995; Cabaço and Santos, 2010; Hovey et al., 2015). Less is known regarding how these seed banks change over time or vary year to year. Characterising these seed bank temporal dynamics is likely to provide a better understanding of their longevity, net retention versus export from the immediate habitat, and how frequently they are renewed to maintain a viable cohort in the event of a disturbance or opportunity for meadow expansion.

Deep-water *Halophila* spp. are known for producing copious number of seeds that remain close to where they were released from the plant and function in restoring the immediate meadows following major disturbances (Kenworthy, 2000; Hammerstrom et al., 2006), yet information on species variation in seed bank status is limited. Multi-year monitoring of tropical deep-water seagrasses (see Chapter 2) has found some species such as *Halophila ovalis* can persist year-round similar to the seasonality found in shallow-water meadows (see Chapter 2). *H. decipiens*—the most dominant deep-water species in the Great Barrier Reef and with a pan-tropical distribution—however, it adheres to a more definitive annual pattern of rapid germination and growth prior to total senescence (see Chapter 2, Kenworthy, 2000; York et al., 2015).

This study aimed to provide empirical evidence of the relationship between seagrass abundance and seed bank status for deep-water populations that are described as relying heavily on seeds for long-term success in marginal habitats. We measured the seasonal and inter-annual patterns of species-specific seed banks and their sediment stratification within three deep-water seagrass communities from northern and central GBR populations. We also describe how seeds are spatially distributed within the sediment profile and how these patterns may relate to germination success and recovery of meadows whether from acute impacts or from seasonal loss.

4.3 Methods

4.3.1 Study design

Deep-water monitoring sites were established at Green Island (16°45.12354'S, 145°59.5494'E; 17 m dbMSL) in December 2011, Lizard Island (14°39'10.072"S, 145°26'56.542"E; 15 m dbMSL) in March 2012, and Keswick Island (20°54'49.74"S, 149°23'10.509"E; 13 m dbMSL) in November 2014 (Figure 2.1). These locations were selected to represent a variety of species known to occur throughout deep-water GBR habitats (Coles et al 2009) and provided a spatial gradient to examine potential latitudinal differences in seasonality and growth dynamics in seagrasses. See Chapter 2 for the full methods and description of the broader monitoring and data recorded at each site over the four year program.

4.3.2 Sexual reproduction and seed bank assessments

Fifteen sediment cores (50 mm x 100 mm) were collected at each experimental site at least quarterly and frozen for transportation to the laboratory to quantify seeds. Cores were thawed and run through a series of test sieves to separate out seagrass seeds from the sediment. The 250 and 710 μm fractions were inspected using a dissecting microscope to identify *Halophila* spp. seeds. *H. decipiens* produces the smallest known seagrass seeds in the GBR region and are found in the 250 μm fraction, while *H. ovalis* and *H. tricostata* are both found in the 710 μm (Kuo et al., 1993; Waycott et al., 2004). Further differentiation between *H. ovalis* and *H. tricostata* is based on distinct seed coat differences between these species (Kuo and Kirkman, 1992; Kuo et al., 1993).

Each size fraction was aliquoted in 20-30 mL increments into a custom designed 3D printed plastic disc with a series of undulating rings engraved into the base. The sediment was spread in the outer most ring and using an additional 40 mL of tap water to evenly distribute the sediments. The disc was placed on an orbital shaker for 90 s, with a set amplitude of 5 mm, and at a frequency of 60-80 rpm depending on aliquot size and sediment type. The lighter density of the seeds relative to the sediment grains resulted in their migration to the centre two rings of the disc, while the majority of sediments remained in the outer rings. The inner two rings were checked closely under a dissecting microscope with the third ring also assessed to confirm seeds were not overlooked.

Halophila spp. seeds were distinguished to species level using both a dissecting microscope and at times scanned electron microscopy for ultra-magnification to confirm seed coat characteristics and seed size. The number of seeds for each species in each core was scaled up to 1 m^2 using the surface area of the core to determine the mean number of seeds in the sediment at each sampling event.

Based on field observations of high sediment movement from physical forcings and infaunal bioturbation, sediment cores from 2014 onwards were sectioned into depth categories (0-20 mm, 20-50 mm and 50-100 mm) prior to being frozen for transport back to the laboratory and analysed for presence of seeds in each depth category.

Due to the location of flowers and fruits at the base of each node, they were often partially or fully buried under surface sediments making any formal observational data

prone to error without disturbing rhizomes. Instead, observational notes of flowering shoots and fruits included the presence, frequency of occurrence, and maturity of flowers and/or fruits at each time point, both in the field and on samples returned to the laboratory for further processing (see Chapter 2 for sediment cores, carbohydrate samples, and rhizome-tagged plants). A sample of mature fruits were collected in November 2014 (n=18) at Green Island to estimate number of seeds produced per fruit. This was only studied at Green Island where the proximity of the site to our laboratory enabled monthly sampling to capture mature fruits before seeds were released from the plant.

4.3.3 Statistical Analysis

Statistical analyses were conducted using R v.3.4.4 (R Core Team, 2018). Total number of seeds per core and number of seeds per sediment depth were used as the key response variables for modelling temporal trends and relationships with environmental data. Due to the extreme patchiness inherent in the seed bank, seed analysis was not scaled up to per m^2 because of the skewness this created in the model output and residuals. The parameters tested for total seeds and number of seeds per sediment depth included *Depth* (0-2, 2-5, and 5-10 cm), *Year* (June-May; *Y*) and *Day in Year (DY)*; the latter two were modelled with smoothing splines (*s*) after preliminary analysis indicated non-linear effects on seed counts. Significant impacts on the Lizard Island site by a category 4 cyclone, Cyclone Ita, in April 2014 led to data in the annual 2014/15 growing cycle being considered as a cyclone effect (*CIta*) in all Lizard Island models. Generalized additive models (GAMs; total seeds per core) and mixed models (GAMMs; seeds per sediment depth) with a negative binomial distribution (with log link) were fitted using

the mgcv package in R (Wood 2017). Limited sediment seed depth data at the Keswick Island site inhibited additive modelling (i.e. smoothers) and model complexity had to be simplified to generalised linear mixed models (GLMMs) performed with month (*M*) in place of *DY*. Seed stratification models included the sediment core as a random effect to account for heterogeneity and repeated measurements of the response variable. The global models applied to each meadow for total seeds and sediment stratification analysis are presented in Table 4.1.

An exploratory analysis of response and predictor variables was done prior to fitting GAM and GAMMs due to the non-linear relationship between the response (i.e. number of seeds) and predictor variables (Wood et al 2017).

To determine the optimal model, a global model was created for each response variable where all explanatory variables and interactions were considered (Table 4.1). Sub-model sets of the global model were then generated using the dredge function in the MuMIn package (Bartoń, 2013). The best-fit models were considered to be those with the lowest Akaike's information criterion (AICc) and highest Akaike weight (w), which by definition contain the best set of explanatory factors for adequately predicting each response variable (Burnham and Anderson, 2002; Wagenmakers and Farrell, 2004). Models with AIC_c values within 2 of each other were considered strong models and are presented with the chosen model being the simplest of this sub-set which was further used for multiple comparison analysis (Burnham and Anderson, 2002). All models were validated by assessing Pearson residuals against fitted model values. Model comparisons can be found in the supplementary material (Appendix B).

Standard model diagnostics including residuals of the best-fit models were inspected for heteroscedasticity and non-normality. Pearson correlation coefficients were used to check for collinearity of predictor variables. Post-hoc comparisons were done on estimated marginal means using the Bonferonni adjustment method in the emmeans package (Lenth, 2016).

Biomass models are also presented for comparison with seed model output, but full methodology for these analyses is presented in Chapter 2.

4.4 Results

4.4.1 Total seed bank

Green Island

H. decipiens seeds were detected in the sediment at Green Island every month that seeds were sampled for over the course of the monitoring program between December 2011 and January 2016 (Figure 4.1a). Despite no above-ground shoots occurring during the monitoring program, *Halophila tricostata* seeds were found each quarter over a one year period from December 2014 to October 2015 at very low numbers (Figure 4.1a).

Green Island seed density reached an annual peak in December-January with an overall average of 7,585 ± 846 seeds m^{-2} each year followed by a steady decline to an average minimum in October/November of 2,480 ± 390 seeds m^{-2} (Figure 4.1a). This annual depression in the local seed density repeatedly occurred at the same time as above-ground biomass began to rapidly increase with peak productivity and growth (see

Chapter 2). *H. decipiens* flowers and fruits were observed at the Green Island site from October to January each year. Flowers began to appear by early October and were most abundant by late October when a small number of immature fruits were recorded. Fruits occurred at the base of most leaf pairs along a rhizome with more mature fruits the further away from the apical meristem. Fruit density and maturity peaked in November and by December/January, fruits were either fully ripened or fruit casings remained where seeds had been dropped into the sediment. Often, shoots had entirely senesced along the rhizome where only ripened fruits or fruit casings remained. The November 2014 sample of mature fruits from senescing *H. decipiens* at Green Island found an average of 35.1 ± 1.1 seeds fruit^{-1}. Seed concentration in the sediment reached a maximum directly after this above-ground senescent phase, indicating the new production and immediate burial of a substantial seed bank.

Trends were verified by model output. The overall Green Island seed bank was affected by *Day in Year* but varied by *Year* with no effect of *Day in Year* in the 2013/14 growing year or in 2015/16 (Figure 4.2a, Table 4.2), years in which above-ground biomass was significantly low compared to other years (Chapter 1).

Lizard Island

At Lizard Island, *H. ovalis* seeds were absent from the seed bank over the monitoring program despite *H. ovalis* being the dominant species in the meadow prior to Cyclone Ita which impacted in early 2014 (Figure 4.1b). *H. decipiens* seeds were present in the sediment in all quarterly surveys since April 2012, yet at a much lower density than at Green Island (Figure 4.1b).

Lizard Island, like Green Island, had a substantial peak in *H. decipiens* seeds in the January/February survey each year with an average of 2,236 ± 475 seeds m^{-2}, decreasing to a minimum each October/November (153 ± 44 seeds m^{-2}) when above-ground biomass was highest (Figure 4.1b). Flowers and fruits at the Lizard Island site were present in a similar cyclic pattern to Green Island with flowering and fruiting observed during Austral spring to early summer sampling, varying from September – November each year. Due to the less frequent visits to this location, the peaks in seeds, flowers and fruits may not have always been observed during our surveys, but the overall pattern was consistent. Like the Green Island site, the seed bank was likely replenished by local fruiting shoots in the Austral summer leading to the peak in seeds recorded in February.

The quantity of seeds in the sediment at the Lizard Island site was affected by *Day in Year* with a measured and predicted peak each February (Figure 4.2b, Table 4.2). Total seeds estimated in the sediment also varied by *Year* with 2012/13 and 2014/15 being significantly lower than 2015/16 (Figure 4.2b, Table 4.2). Like Green Island, the annual peak seed bank estimates from the best fit model corresponded to the overall peak *H. decipiens* above-ground biomass measured in the meadow (see Chapter 2).

The effect of TC Ita was a significant predictor of total seeds at Lizard Island, but the simpler model with *CIta* was the best fit based on residuals and a very close AIC_c score (Table 4.2). Very few seeds were found in sediments directly after TC Ita in May 2014 and remained low through November 2014 (Figure 4.1b). However, germination by *H.*

decipiens seeds over the post-TC Ita growing period (August – November 2014) likely led to the significant increase in the seed bank recorded in January 2015. In contrast to TC Ita, there was negligible sediment movement at the site during TC Nathan in March 2015. Seeds declined from January to August before increasing in the Austral summer when seed densities peaked with the production and release of fruits (Figure 4.2b).

Keswick Island

At the Keswick Island site, the one-year of sediment core profiles exhibited similar patterns to Green and Lizard Island with peak seed bank density during the January/February survey following flowering/fruiting in late October (Figure 4.1c). The seed bank consisted of mainly *H. tricostata* except for February 2015 when only *H. decipiens* seeds were present; a month when high seed densities of *H. decipiens* were recorded at all three locations (Figure 4.1c). *H. decipiens* seed density was on average 5,094 ± 801 seeds m^{-2} during the seasonal peak and at a minimum in July with 356 ± 171 seeds m^{-2} (Figure 4.1c). Overall densities were similar to Green Island compared to the relatively sparser seed bank at Lizard Island (Figure 4.1).

The Keswick Island *H. decipiens* seed bank was also best modelled by the predictor *Day in Year* (Table 4.2). Trends in the seed bank were consistent with seasonal patterns recorded in the multi-year datasets at Green Island and Lizard Island locations despite the limited timeframe that seeds were assessed at Keswick Island.

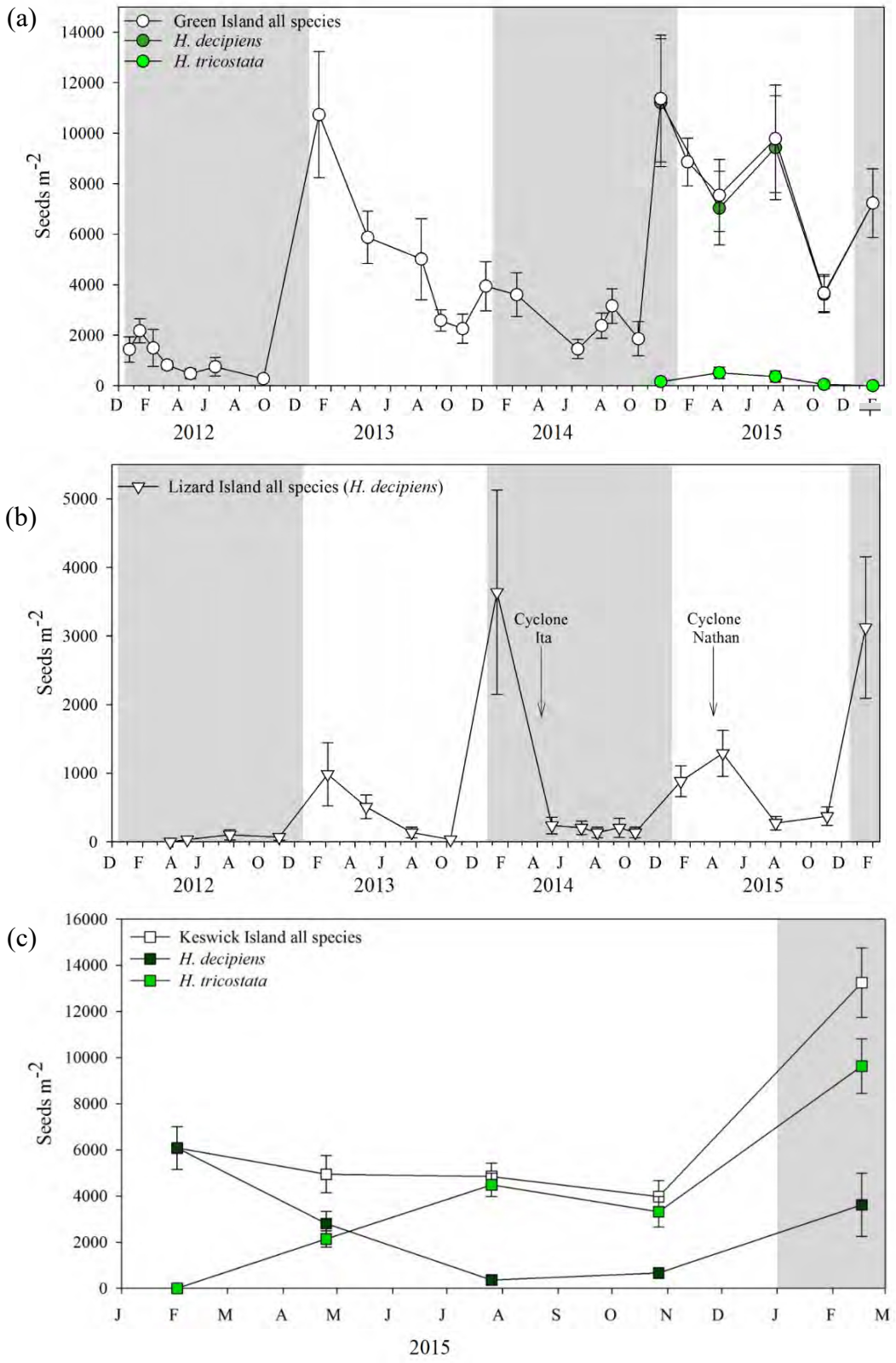


Figure 4.1 Seed densities (m^{-2}) recorded in local sediments at (a) Green Island, (b) Lizard Island, and (c) Keswick Island. Note shorter timescale at Keswick Island site. Data symbols and error bars represents mean ± S.E.M. (n = 12).

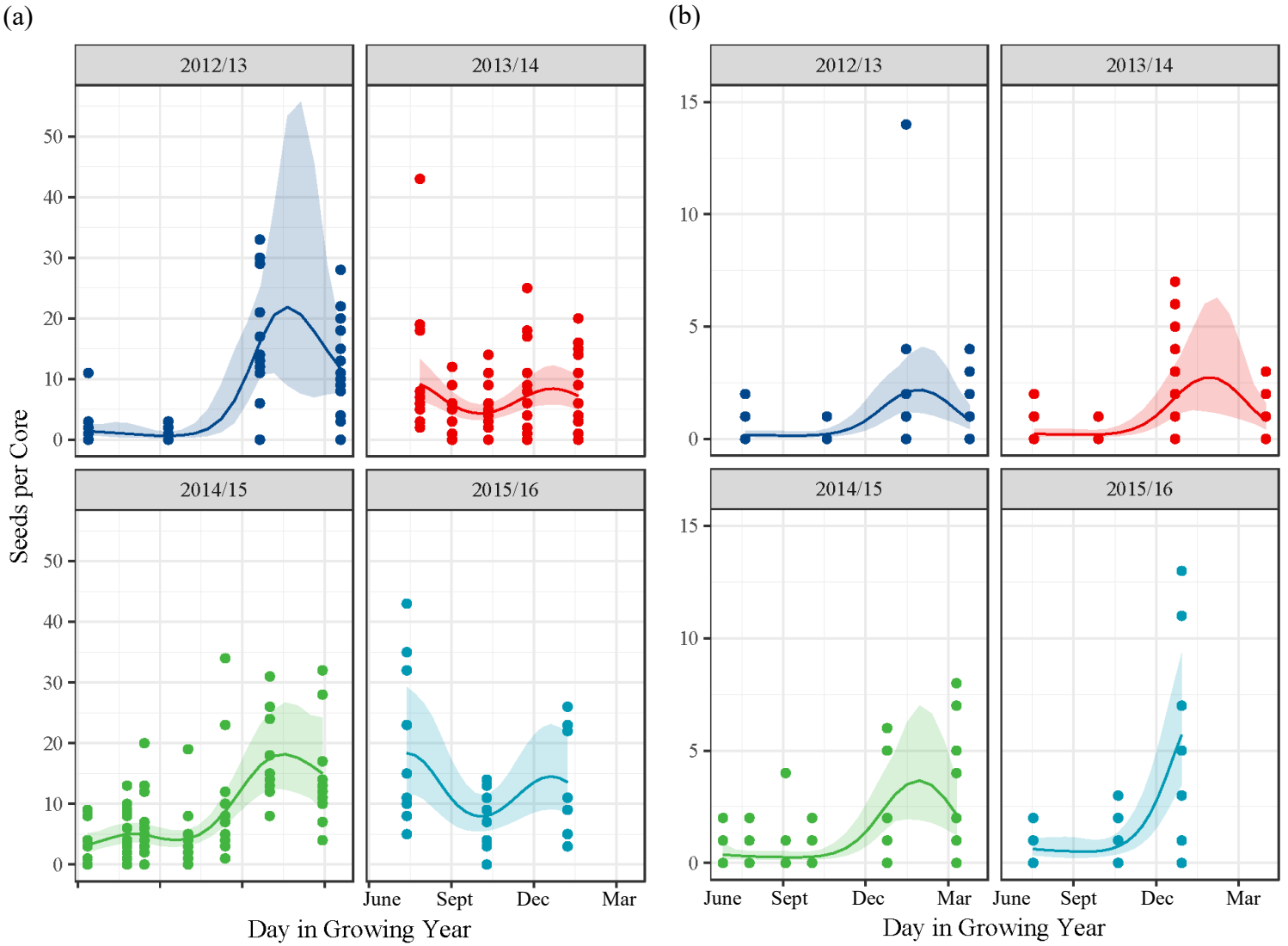


Figure 4.2 Modelled seeds per sediment core over the growing year (June – May) by annual seagrass growth cycle at (a) Green Island and (b) Lizard Island. *Note:* Day 0 is 1 June and Day 365 is 31 May.

Table 4.1 Overall fit of selected best models of the total *H. decipiens* seed bank and stratification of seeds at Green Island, Lizard Island, and Keswick Island. All models included a random error term ε. edf is the estimated degrees of freedom, X^2 is chi-square statistic, *F* is the F-statistic. *Y* is *Year; DY* is the day in growing year (June – July); *CIta* is a fixed factor for the 12 months post-Cyclone Ita data at Lizard Island; *M* is a fixed factor for sampling month at Keswick Island; and *Depth* is a fixed factor for the three sediment layers seeds were counted in the stratification models. All stratified seed models had a random effect (β) of core.

Response Variable	Location	Model Terms	(e)df	X^2	p-value
Total Seeds	Green Island	$s(DY*Y_{12/13})$	2.79	36.7	< 0.0001
		$s(DY*Y_{13/14})$	1.0	0.07	0.79
		$s(DY*Y_{14/15})$	1.0	13.9	< 0.001
		$s(DY*Y_{15/16})$	0.00	0.00	1.00
		s(DY)	4.68	22.0	< 0.001
		Y	3	30.68	< 0.001
	Lizard Island	*s(DY)*	4.49	98.4	< 0.0001
		Y	3	8.74	< 0.05
	Keswick Island	*s(DY)*	3.30	53.48	< 0.0001
Seed Stratification	Green Island	*s(DY)*	8.12	76.7	< 0.0001
		Depth	2	70	< 0.0001
	Lizard Island	*s(DY)*	4.34	22.32	< 0.0001
	Keswick Island	*Depth*	2	9.97	< 0.01
		M	3	85.6	< 0.0001

4.4.2 Seed stratification in sediment

Green Island

From June 2014 to February 2016, sectioned sediment cores indicated the seed bank was stratified (Figure 4.3a). As with the total seed bank analysis, all three sediment sections were significantly affected by *Day in Year* with a predicted peak in January/February and with an overall effect of sediment *Depth* ($F_8 = 23.7$, $p < 0.001$ and $F_2 = 70.0$, $p < 0.001$ respectively; Figure 4.3a, Table 4.2). A significantly greater proportion of seeds were found in the deepest sediment fraction, 5-10 cm and the least in the surface 0-2 cm layer ($F_2 = 70.0$, $p < 0.001$; Figure 4.3a; Table 4.2).

Lizard Island

No significant depth stratification of seeds could be modelled for the Lizard Island meadow. Considerable burrowing and mounding activity by alpheid shrimps observed across the site throughout the sampling conducted between January 2014 and February 2016 suggests turnover in the sediments may have impacted seed distribution. In addition, large deposits and scouring of sediment observed at the site in the weeks following Tropical Cyclone Ita likely lead to the complete absence of seeds, either lost or buried, from the top two sediment fractions; a significant drop from the annual peak in seeds which were largely from these top two layers in January 2014 (Figure 4.1b).

Keswick Island

Seed bank stratification was similar to Green Island with a significant effect of sediment *Depth* ($X^2 = 9.97$, $p < 0.01$) and month ($X^2 = 85.6$, $p < 0.001$; Figure 4.3b, Table 4.2). The highest number of seeds was in the 5-10 cm sediment fraction in all months and

with greater seed numbers in February with fewer seeds in July and October; Figure 4.3b, Table 4.2). No mounding was observed at this location, indicating that bioturbation activity was lower compared to the more northern sites.

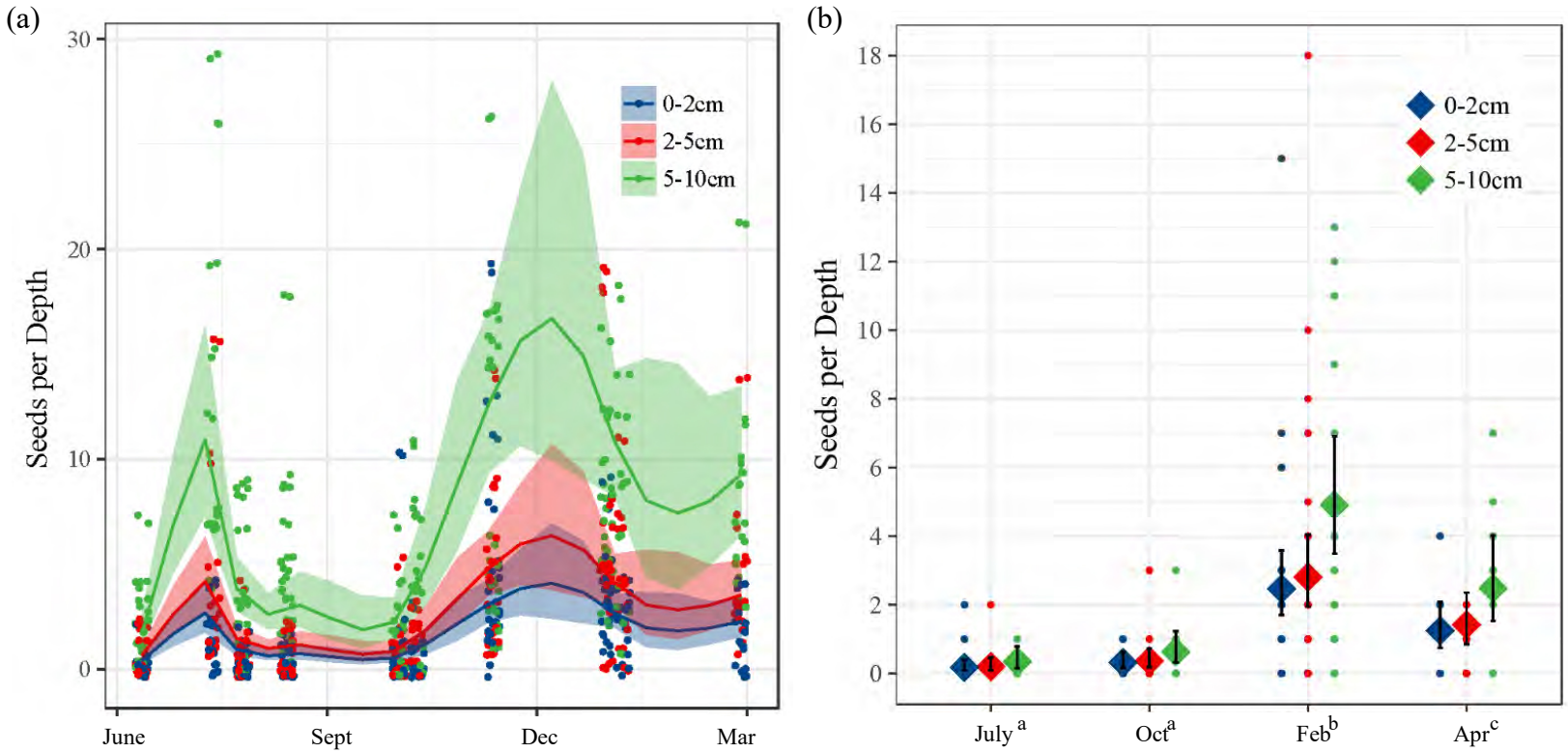


Figure 4.3 Modelled seed stratification by sectioned depth at (a) Green Island over the growing year (June – May), and (b) Keswick Island by sampling month (*M*). Dots represent raw data, smoothers and diamonds represent predicted model output ± 95% confidence intervals at Green Island and Keswick Island respectively. Superscript letters indicate post-hoc bonferroni comparisons among depths (Green Island and Keswick Island) and months (Keswick Island).

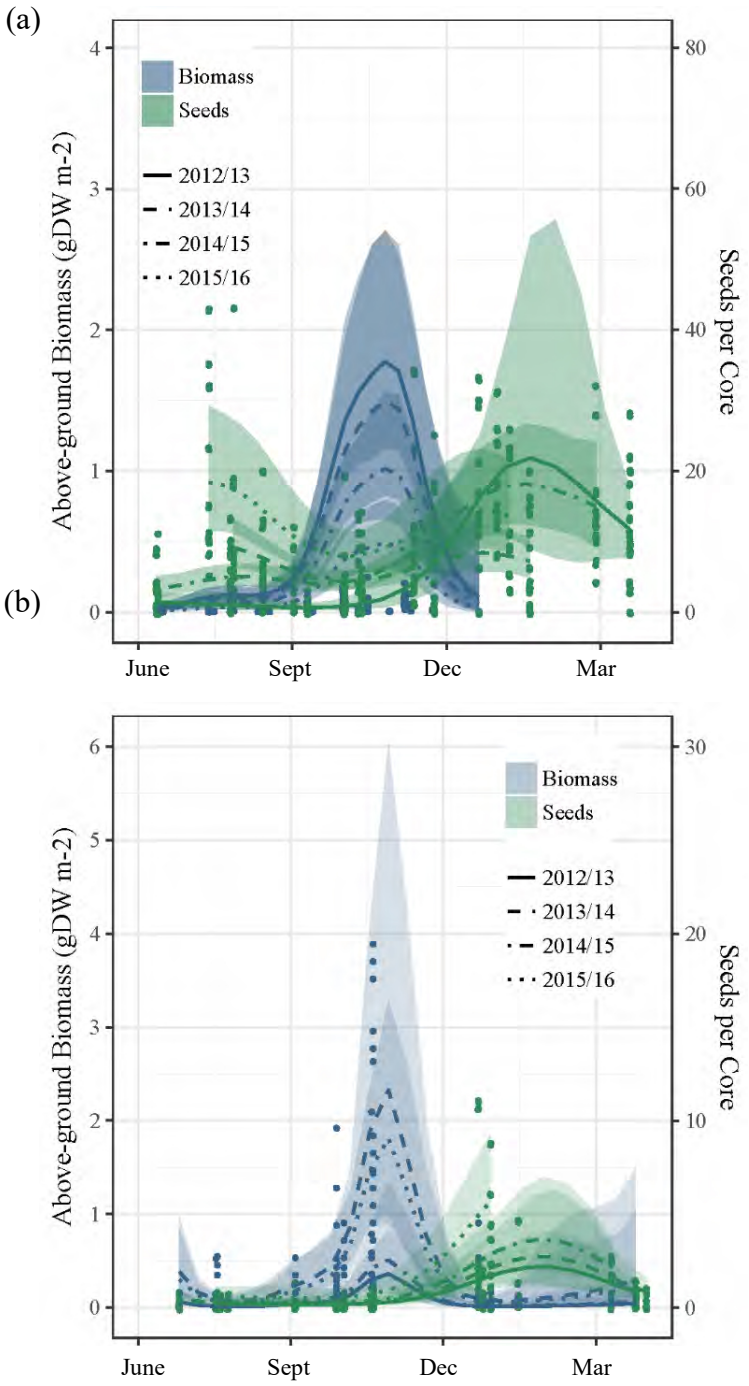


Figure 4.4 Predicted fit of *H. decipiens* seeds and above-ground biomass at (a) Green Island, (b) Lizard Island, and (c) Keswick Island over the growing year (June – May). Non-linear trends are the fit of gamma generalized additive mixed models with seagrass above-ground biomass as the response variable. Shaded areas are 95% confidence intervals. The small dataset at Keswick Island restricted biomass model output to a categorical response by sampling date rather than as a mixed effect model; however trends of seasonal peak biomass and seeds is similar (diamonds represent predicted mean biomass ± SE, n = 12) . Note varying y axes scales.

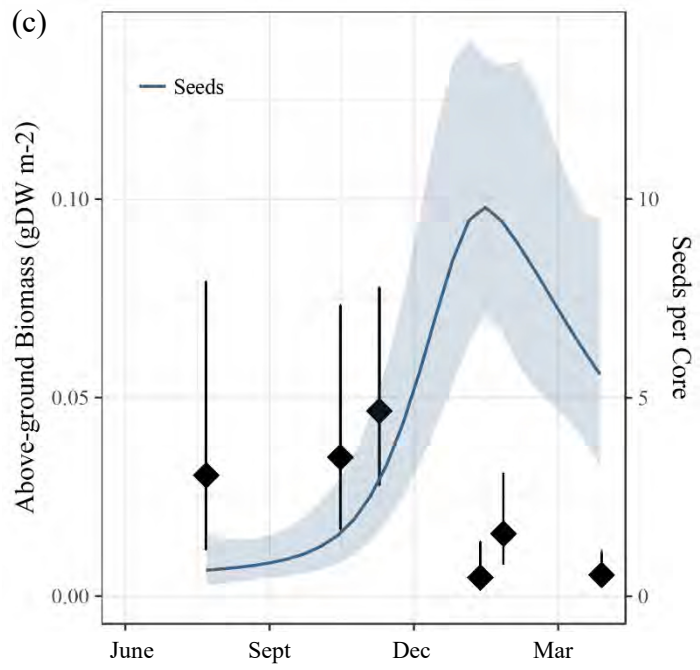


Figure 4.4 (continued) Predicted fit of *H. decipiens* seeds and above-ground biomass at (a) Green Island, (b) Lizard Island, and (c) Keswick Island over the growing year (June – May). Non-linear trends are the fit of gamma generalized additive mixed models with seagrass above-ground biomass as the response variable. Shaded areas are 95% confidence intervals. The small dataset at Keswick Island restricted biomass model output to a categorical response by sampling date rather than as a mixed effect model; however trends of seasonal peak biomass and seeds is similar (diamonds represent predicted mean biomass ± SE, n = 12) . Note varying y axes scales.

Table 4.2 Summary results of *H. decipiens* in sampled GBR meadows and a comparative study from Western Australia. Data is mean ± S.E.M. (n=12).

	Peak Annual Biomass$_{AG}$ (g DW m^{-2})	**Peak Annual Seeds (m^{-2})**	**Seeds:Biomass$_{AG}$ (g^{-1} DW)**
Green Island			
2012	1.73 ± 0.16	10,734 ± 2,496	6,205
2013	0.10 ± 0.03	3,939 ± 972	39,390
2014	1.16 ± 0.21	11,366 ± 2,513	9,798
2015	0.21 ± 0.10	7,233 ± 1358	34,442
Lizard Island			
2012	0.18 ± 0.08	985 ± 459	5,472
2013	0.30 ± 0.08	3,639 ± 1,491	12,130
2014	0.26 ± 0.06	1,290 ± 337	4,961
2015	1.99 ± 0.25	3,124 ± 1031	1,570
Keswick Island			
2014	<0.1	5,094 ± 801	50,940
2015	0.28 ± 0.02	13,245 ± 1503	47,304
Hovey et al (2015)	~0.75*	2,800	3,733

***** Estimate based on 1:1 above:below ground biomass (Duarte and Chiscano, 1999) and 85% water loss from wet:dry weights (Kenworthy et al., 1989)

4.5 Discussion

Seed banks are a valuable trait for plants living in seasonally fluctuating environments (McMillan and Soong, 1989; Kenworthy, 2000; Hammerstrom et al., 2006; Hovey et al., 2015) and may be the only mechanism for recruitment in some more isolated seagrass communities (Kendrick et al., 2012).

This multi-year study of seed bank dynamics in deep-water seagrass meadows found a substantial *H. decipiens* seed reserve present throughout the year in all three GBR meadows monitored with a strong relationship to the annual life cycle of the plant. Monthly and quarterly sampling across sites captured the specific timing of recruitment and senescence in relation to trends in the seed bank. Seed densities peaked from January to March following the annual summer maximum in *H. decipiens* above-ground biomass at each site (Figure 4.4). Shoots rapidly senesced over a period of 1-2 months during which metabolic resources were shifted into the production and ripening of fruits before their release into the sediments to replenish the local seed bank (see Chapter 2). The magnitude of peak above-ground biomass each year appeared to influence the subsequent peak, or lack thereof, of seeds present locally in the sediment. Seed densities steadily declined to a minimum early in the growing season which coincided with germination and the start of rapid meadow growth and rhizome expansion (Figure 4.4). Flowers and fruits were observed during each October/November period when above-ground biomass peaked (pers. obs). Similar studies have also found *Halophila* flowers and fruits to be more prevalent during summer months (McMillan & Soong 1989, Hammerstrom et al. 2006). While the overall patterns have been well described for *H. decipiens* in both the GBR and around the world (McMillan and Soong, 1989;

Hammerstrom et al., 2006; Hovey et al., 2015; York et al., 2015), this is the first multi-year study of temporal and spatial (i.e. depth stratified) trends from three disparate deep-water meadows where multiple species contribute to the meadows' standing biomass.

Annual maximum seed bank densities were comparable with the range found in previous studies of the same species at similar depths (Hammerstrom et al., 2006; Hovey et al., 2015). For example, the West Florida Shelf deep-water *H. decipiens* meadow was estimated to have a seed bank up to 3,414 seeds m^{-2} (Hammerstrom et al., 2006), while a shallow Panama *H. decipiens* meadow had a maximum density of 13,500 seeds m^{-2} (McMillan and Soong, 1989). In this study, we found seed banks at their annual peak ranged on average from 2,236 - 7,585 seed m^{-2} across the three GBR meadows. In northwest Australia, Hovey et al. (2015) measured the *H. decipiens* seed bank to peak at a similar time of year with an average of 2,800 seeds m^{-2}.

Accurate estimates of seed bank densities for *Halophila* dominated meadows is challenging due to the inherent patchiness in shoot growth and dispersal of seeds in these habitats. The multi-year and regular sampling described here provides strong evidence for the trends and relative patterns that exist in these communities and is in line with other studies (Birch, 1981; Kuo et al., 1993; Hammerstrom et al., 2006; Kenworthy et al., 2006; Hovey et al., 2015). However, observations of fruits while monitoring in the field were significantly lower than what was measured from rhizomes returned to the lab; a likely product of most fruits and even many shoots growing below a shallow layer of surface sediments. This direct burial of fruits results in a very

localised pocket of seeds being released that may or may not be detected from cores. Only centimetres away from a dense pocket of seeds can be a bare patch of sediment with no seeds. It is therefore important that sampling intensity accounts for this intrinsic variability and clustering in seed density in naturally patchy meadows.

The seed production for *Halophila* spp. is remarkable given the small stature of these plants and the extreme patchiness by which these deep-water seagrasses occupy the seafloor. Despite this, the number of seeds generated per fruit at the base of most leaf pairs along the rhizome results in high seed densities in the sediment seed bank. Kuo and Kirkman (1995) found *Halophila decipiens* in southwestern Australia produced fruits on each leaf pair with over 25 seeds per fruit, resulting in over 176,000 seeds m^{-2} in meadow sediments if fully buried. Species such as *H. tricostata* with erect stems, can produce fruits at each vertical node with 24-60 seeds per fruit, generating 70,000 seeds m^{-2} (Kuo et al., 1993).

Across all three GBR meadows in this study, the period of peak seed density tended to follow closely the periods of peak above-ground biomass (Table 4.3). One of the highest above-ground biomass estimates, 1.16 ± 0.21 g DW m^{-2}, was recorded in the monospecific Green Island meadow in November 2014 (Chapter 2). Seed densities at this time reached 11,366 ± 2513 seeds m^{-2}, equating to an estimated 9,798 seeds g^{-1} DW of above-ground biomass. In comparison, Hovey et al (2015) had a mean maximum of 2,800 seeds m^{-2} and an estimated 0.75 g DW m^{-2} above-ground biomass, equating to 3,733 seeds g^{-1} DW or a 68% lower proportion of seeds to shoots than measured in the Green Island population. The significantly lower estimate in the Western Australia

meadow may be due to be a much lower production of seeds, low localised seed retention, or difference in biomass approximation between their grab samples and the methods applied here.

Recent reviews have highlighted the lack of knowledge around seagrass seed movement; to what degree are seeds retained versus exported out of a meadow and in turn driving seagrass connectivity, versus maintaining localised meadow resilience (Kendrick et al., 2012; Grech et al., 2016; Kendrick et al., 2017). At the Green Island meadow, mature fruits dissected in the laboratory produced on average 35.1 seeds per fruit^{-1} and peak season shoot densities reached 400 m^{-2} (Chapter 2). Assuming every *H. decipiens* shoot produces a fruit (Waycott et al., 2004), an estimated 14,040 seeds m^{-2} were generated. This production versus measured seed density equates to an 81% retention rate within the local seed bank. In the Western Australia study, they measured 2,800 seeds m^{-2} in local sediments equating to a 37% retention rate of what could be generated from density estimates (Hovey et al., 2015). The disparity from seed retention in our study may be due to sediment movement and shear stress driving bed load transport in this Kimberley Region known for large tidal fluxes (>12 m) (Kendrick et al., 2017).

In contrast to the direct burial of fruits and seeds at the base of *H. decipiens* shoots, the erect shoots of *H. tricostata* produce fruits developing amongst the rosette of leaves and has been hypothesised to help with seed dispersal (Kuo et al., 1993). Buoyant fruits may float in the water column for a period of time before fruit dehiscence and seeds fall into the sediments. This may explain the less dense seed bank in the mixed species *H.*

tricostata meadow at Keswick Island and for the presence of a small number of seeds showing up at Green Island despite no shoots being observed at this site during the monitoring program.

In the Lizard Island meadow, there were far fewer *H. decipiens* seeds in the sediment than at Green Island which reflects the patchy nature and lower seasonal above-ground biomass of *H. decipiens* at the site. Somewhat surprisingly we did not identify *H. ovalis* seeds in the sediment at Lizard Island, indicating this population is either perennial and expands mainly through clonal growth with little to no seed production, exports seeds to nearby meadows rather than maintaining a local seed bank, or germinates newly dispersed seeds to immediately replenish the standing crop. The limited flowering and fruiting of *H. ovalis* observed at our Lizard Island site suggests it is living close to its' light limits and may require higher light conditions in order to have sufficient energy supply to trigger sexual reproduction. *H. ovalis* flowers and fruits prolifically in shallow coastal meadows along the GBR coast (C. Reason pers. comm.) and work on this species elsewhere has found substantial seed banks are an important mechanism for long-term resilience of this species (McMillan, 1988c). Kuo and Kirkman (1992) measured a very small seed bank in a southwest Australia *H. ovalis* meadow, and thus they also hypothesised an external supply of vegetative propagules or rapid colonisation by the locally few, rare seeds from this species. Shallower populations of *H. ovalis* occur around Lizard Island (Saunders et al., 2015) and these may be providing the source material to supply propagules into the nearby deep-water meadow.

Large-scale mobilisation and re-distribution of deep-water *Halophila* seeds has been linked to physical disturbance of the sediment with major storm events such as cyclones and hurricanes (Bell et al. 2008, Fonseca et al. 2008). The impacts of Tropical Cyclone Ita in March 2014 led to complete loss of above-ground biomass at the Lizard Island site as well as 5 – 10 cm of sediment burial over the monitoring plots. The *H. decipiens* seed bank provided relatively quick recovery with new shoots germinating within three months post-cyclone, once the sediments added from the storm moved off the site and conditions for germination were present. *H. ovalis* recruitment was delayed compared to *H. decipiens*, supporting evidence for the importance of a seed bank for these deep-water seagrass populations in coping with episodic disturbances. We hypothesise that vegetative fragments or seeds from nearby shallow *H. ovalis* meadows eventually recruited into the site from either megaherbivore faecal deposits (Tol et al., 2017) or waterborne transport. A second cyclone, TC Nathan, in early 2015 had less severe effects on the local monitoring site. There was no major sediment movement and some remaining shoots were left on the site following the storm. The cyclone did not affect total above-ground biomass and shoot densities during the growing season in 2015, but there was a significant shift to *H. decipiens* dominance with very little *H. ovalis* remaining at the end of the study; a further indication of rapid colonisation from a locally germinated *H. decipiens* seed bank, rather than an undetected *H. ovalis* seed bank.

On smaller spatial and temporal scales, bioturbation by macroinvertebrate activity may be instrumental in seed burial and emergence (Vonk et al., 2008; Blackburn and Orth, 2013; Kneer et al., 2013). Fonseca et al. (2008) posited that bioturbation by callianassid

shrimp was responsible for re-distributing the buried seed bank of a west Florida shelf meadow based on associated dense stands of *H. decipiens* around the opening of excavation mounds. The presence of similar mounds also frequently covered with dense ramets of *H. decipiens* at our monitoring sites supports this hypothesis. In particular, this burrowing and mounding activity around the Lizard Island site by apheid shrimps (see Supp. Figure 2) may be responsible for the well mixed seed bank, rather than the clear stratified pattern at Green Island and Keswick Island. The positive feedback of such bioturbation on seagrass densities is in contrast to studies measuring negative correlations between Callianasa and seagrass cover and productivity (Suchanek, 1983; DeWitt, 2009). Nevertheless, shrimp burrowing may actively contribute to deep-water *Halophila* seed emergence to facilitate germination success. This activity may also lead to seed loss as well as moving seeds into the deeper sediment layers which we quantified in this study. While mounding activity was remarkably higher in the Lizard Island meadow, some burrowing activity and mounding was still present at Green Island and Keswick Island. Without this activity, the potential deeper seeds may effectively be lost from the seed bank under normal circumstances due to limited germination capacity from such depths as measured in other studies (Jarvis et al., 2014; Jarvis and Moore, 2015; Jørgensen et al., 2019). However, as the first cyclone impact at Lizard Island demonstrated, periodic large-scale disturbances have the capacity to expose these deeper seeds, and there may in fact be an advantage to have a deep seed reserve for population maintenance where such periodic large-scale impacts occur.

The net loss of seeds over the annual cycle can also result from wave action, bottom currents, and smaller storm activity re-distributing seeds both within the local site and

further afield (Bell et al. 2008). While not directly observed at the deep-water sites, local populations of dugong or sea turtles could act as a conduit to spread seeds; megafauna feeding and re-depositing of seeds through their excrement makes them important vectors for seagrass dispersal (McMahon et al., 2014; Tol et al., 2017).

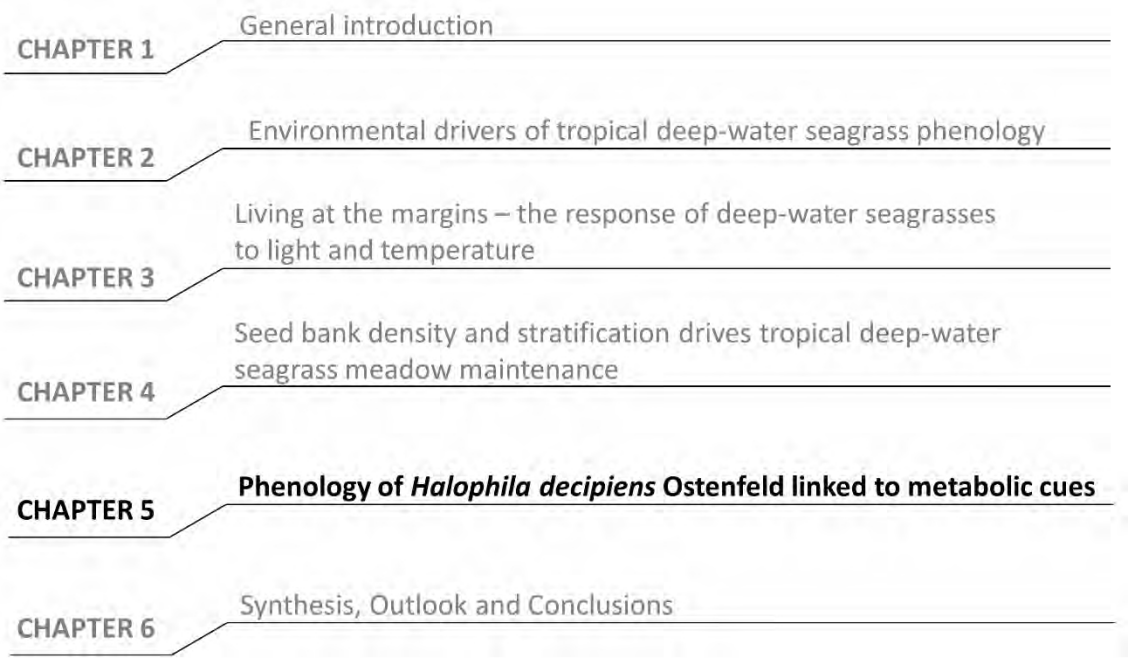
Seagrass seeds can remain dormant for a period of time until conditions are favourable for germination and re-establishment of the meadow (Kenworthy, 2000; Hammerstrom et al., 2006). For *Halophila* spp., it is thought that seeds can remain viable for up to 24 months (McMillan, 1991). The presence of seeds remaining in the sediments throughout the year despite low above-ground biomass in some years suggests germination of the seed bank was not high. The cues driving germination for seagrasses are poorly understood (Kendrick et al., 2017). In particular, the studies that have investigated *Halophila* spp. seed germination have had mixed results (Birch, 1981; McMillan, 1988a; Kuo and Kirkman, 1992; Statton et al., 2017; Strydom et al., 2017a). Light and temperature have been tested as germination cues for shallow populations of *H. ovalis* (Statton et al., 2017; Strydom et al., 2017a), but there has been little testing on *H. decipiens* or *H. tricostata*. The significantly lower above-ground biomass in the 2013/14 and 2015/16 growing season at Green Island may be a reflection of poor germination—whether due to a missing seasonal cue or poor seed viability is unclear—given the abundance of seeds found in the sediment equal to other years with much higher seasonal biomass. Interestingly, germination in the subsequent year did not appear to be affected suggesting the fewer number of seeds added to the seed bank in the low production year may have been supplemented by viable seeds left over from the previous cycle.

The phenology of deep-water *Halophila* spp. examined in Chapter 2 together with the present study of seed banks indicates larger inter-species variation than has previously been described (Kenworthy et al. 1989, Inglis 2000, Hammerstrom et al. 2006). In particular, *H. decipiens* appears to follow a more classic annual life history strategy, whereas *H. ovalis*, *H. tricostata* and *H. spinulosa* maintain some minimal standing crop year-round and may put less effort into flowering, fruiting and setting a substantial seed bank. Exploring the underlying metabolic patterns in deep-water seagrasses may help untangle some of the observed life history patterns and how a species responds at the cellular level to their environment (see Chapter 5).

This study provides an important insight into the seasonal and inter-annual changes in seed bank density, and the implications this may have for local seagrass resilience. The results have emphasised the critical importance in understanding seed-bank dynamics for these deep-water *Halophila* populations for assessments of resilience and meadow health. As considered in similar deep-water seagrass meadows (Hovey et al., 2015), the state of the seed bank may be a critical determinant of deep-water seagrass population dynamics. However, not all populations on the north-east coast of Australia have a one-size-fits-all pattern, even within this genus, with some species or meadows more reliant on asexual population maintenance, similar to many shallow seagrass meadows in the region (Rasheed, 1999; 2004). This distinction among species in deep-water communities is important when considering management strategies appropriate for local species resilience (Hovey et al., 2015). *H. ovalis* and *H. spinulosa* seeds were not detected over the four-year monitoring program at any of the sites. It may be that these

species rely more heavily on a balanced cycle of seed production and maintenance of above-ground shoots. In contrast, the most prolific deep-water seagrass species *H. decipiens* has a high reliance on a seed bank for long-term resilience, with its annual growth habit. If critical growing conditions required to generate sexual reproductive output is impaired, these seagrasses may be susceptible to losing their fundamental capacity for annual recovery. This study provides an insight of the interplay between the seasonality of the standing crop and the seeds responsible for annual meadow maintenance and highlights the importance of understanding seed bank dynamics for individual species and at local spatial scales.

CHAPTER 5 Phenology of *Halophila decipiens* Ostenfeld linked to metabolic cues



Chapter 5 will be submitted to the journal 'Frontiers in Marine Science' in April 2021 as: Chartrand K.M.[*1,2], Kuzhiumparambil U.[2], Pernice M.[2], Ralph P.J.[2] In prep. Phenology of *Halophila decipiens* Ostenfeld linked to metabolic cues. *Frontiers in Marine Science*.

[1] Centre for Tropical Water & Aquatic Ecosystem Research, James Cook University, Cairns, Queensland, Australia.
[2] Climate Change Cluster, University of Technology Sydney, Broadway, New South Wales, Australia.

Abstract

Seagrasses have adapted to thrive in a fully submerged marine environment; a unique physical challenge to the growth and development of a vascular flowering plant. The vast majority of seagrasses follow a perennial-like growth pattern that parallels environmental conditions while maintaining a standing crop year-round. *Halophila decipiens*, a pan-tropical species is a known exception, whereby it germinates from seed, grows rapidly, flowers and then fully senesces over a number of months each year. While seagrass growth patterns are well described, concomitant metabolic patterns known to drive the transition between key life stages and associated with the phenotypic response to the environment seen in terrestrial plants have not been studied in seagrasses. We explored the endogenous compounds, namely hormones and the broader metabolomic profile, within the observed life stages of the annual-like seagrass species *H. decipiens*. Above- and below-ground tissues were sampled over four key time points of plant development during an annual growth cycle. Key hormone classes as well as untargeted LC-MS and GC-MS metabolomic profiling found consistent and clear shifts in above- and below-ground tissues around plant maturity and flowering in September. The strong signal in both above- and below-ground tissues mimics profiles found in some annual land plants that progress through a defined life stage transition at flowering which ultimately leads to programmed senescence and the production of fruits. Jasmonic acid, polyamines, amino acids and fatty acids were the most prominent identified changes in leaves. Parallel patterns in these compounds are also known in the terrestrial literature that drive plant responses to an external stress or stimuli such as plant defense. We discuss how the annual growth pattern of *H. decipiens* and associated metabolic cues of this seagrass species is in line with an evolutionary adaptation to

living in a deep-water, low light and often disturbed environment where chances of survival year-round are unlikely.

5.2 Introduction

The sequence of events in a plant's life cycle is universal: germination and vegetative growth, followed by a reproductive phase, after which ensues final senescence. The transition through life cycle stages is in response to cues coming from the physical environment in which a plant has adapted over time. Annual versus perennial life histories demonstrate two broad selective strategies by which plants have evolved to cope with external stimuli in order to maximize survival and success (Friedman and Rubin, 2015). At the heart of both strategies, plant growth and development originates from meristems—a group of undifferentiated cells driving tissue and organ development. Environmental stimuli turn on and off key genes to regulate molecular pathways which drive the developmental switch between life cycle stages such as vegetative growth to flowering (Huijser and Schmid, 2011). Annual plants undergo these changes within a single cycle, whereas perennials have adapted to surviving unfavourable growing periods and preserving meristematic growth for when favourable conditions return (Tan and Swain, 2006).

Key transition points of growth and differentiation at each life stage are controlled by cross-talk between environmental cues and endogenous signals. Hormones are well described as driving flowering, fruiting, seed set and senescence in terrestrial plants (Gan and Amasino, 1995; Davies, 2010; Matsoukas, 2014). Sugars have also been highlighted as important regulators of life stage transitions. They interact with

hormones to drive key development processes such as juvenile to adult plant phase, flowering and final plant senescence by the re-allocation of sugars between sink and source tissues such that leaves senesce when fruits mature (Smeekens et al., 2010; Davies and Gan, 2012; Thomas, 2013; Yu et al., 2013).

The study of metabolomics offers a comprehensive means to analyse the suite of compounds, including hormones, produced by an organism's underlying gene expression. The metabolome is therefore a description of the final step in a long pathway of genetically driven signals activated or deactivated in response to the environment, which modulates the ultimate phenotypic response observed at the whole plant level (Patti et al., 2012; Guijas et al., 2018; Hu et al., 2018). The metabolome can be assessed against the complex hormone pathways and gene-networks responsible for plant growth (Mochida and Shinozaki, 2011; Johnson et al., 2016). Metabolomics has produced a substantial body of work on land plants driving advances from agriculture to plant-based medicines (Putri et al., 2013).

While a relatively less well studied and diverse lineage of angiosperms compared to their terrestrial counterparts, seagrasses are a unique subset of flowering monocotyledon plants (Order Alismatales) that have evolved unique adaptations to thrive in a fully submerged, saline environment (den Hartog, 1970; Larkum et al., 2018). They are recognised for their wide-ranging distribution and considerable value to ecosystem services (Costanza et al., 2014; Nordlund et al., 2016; Scott et al., 2018). Ecological and physiological attributes of seagrasses have been studied extensively and described in the context of how the environment structures seagrass growth condition and how they can

resist or tolerate change in order to better manage and conserve their presence in shallow coastal zones (McMahon et al., 2013; Coles et al., 2015; Kilminster et al., 2015). However, the fundamental principles of plant biology at the molecular level and how these pathways and signalling compounds have evolved to uniquely adapt to a submerged, marine environment is less well understood.

A handful of studies have examined metabolite profiles and their role in marine plants (Marián et al., 2000; Kumar et al., 2016; de Kock et al., 2020). These have expanded the potential range of indicators to assess the seagrass stress response, opening the door to a new suite of best-practice methodologies to manage marine ecosystem health (Griffiths et al., 2020).

Classification of seagrasses based on their life history attributes has also been an approach used to synthesise primary scientific knowledge into a form that can best be used for marine management practice (Kilminster et al., 2015). As with land plants, some seagrass species are considered fast growing with an annual-like life history (Kenworthy, 2000; Hammerstrom et al., 2006), whereas the vast majority of described seagrasses persist year round in a perennial form (Vermaat et al., 1995; Arnaud-Haond et al., 2012). With the uptake of metabolomics in seagrass research (Macreadie et al., 2014), we can began to explore species-specific phenology—the underlying endogenous or metabolic cues that drive seasonal or annual patterns in various seagrass species—to drive best management practice of these vulnerable coastal marine plants.

Halophila decipiens, prolific around the world in tropical and sub-tropical climates, has been shown to grow over an annual cycle; colonisation and rapid shoot proliferation is followed by immense fruit and seed production, before the complete dieback of plant material and local storage of seeds in the sediments until conditions are again favourable for germination and then shoot growth in the next annual cycle (see Chapter 1 and 3 and references therein). This species is often found growing in a low light environment at depth or in turbid inshore waters close to its physiological limits (see Chapters 1and 2). However, decline in annual plant biomass has been documented to precede the loss of light from seasonal changes or temperatures has been known to negatively affect the physiology of the species (Chartrand et al., 2018).

The goal of the present study was to explore the endogenous compounds, namely hormones and the broader metabolomic profile, within the observed life stages of the annual-like seagrass species, *H. decipiens*. We also evaluated whether these compounds parallel observed patterns in the terrestrial literature around key hormone pathways and metabolites known to drive plant growth phase transitions.

5.3 Methods

Samples of *H. decipiens* were collected from the mono-specific deep-water meadow off Green Island (Figure 2.1) in 2016. Collections were made at four key time points (T_1: 22 August, T_2: 22 September, T_3:1 November, and T_4: 24 November) to correspond with growth patterns and plant development stages observed during sampling and over the four year monitoring program (see Chapter 1)—vegetative growth, flowering, early fruiting and fruit maturation/leaf senescence, respectively. Rhizomes with intact roots,

shoots and reproductive structures when present were haphazardly collected from the meadow and kept in the dark to avoid physiological shock on bringing them up to high light surface conditions. Material was brought immediately to the surface where material was kept shaded and submerged in seawater while three replicates each of above-ground and below-ground samples as well as fruits (the two latter time points only) were isolated and immediately snap frozen in liquid nitrogen to halt metabolic activity. Samples were collected for hormone analysis and metabolome profiling for each tissue type (n=3) equating to 12 samples in the Time 1 and 2 sampling periods and 18 when fruits were present at Time 3 and 4. All material was transported via a dry shipper to University Technology Sydney and stored in -80ºC prior to extractions.

Hormones were extracted using protocols developed by Pan et al. (2008) as a rapid and sensitive method to quantify multiple classes of phytohormones in crude plant extracts. Hormone standards were sourced from DHI, Denmark. Briefly, samples were ground into a powder while in liquid nitrogen using a mortar and pestle before 1 mL of solvent, 1-propanol/H_2O/concentrated HCl (2:1:0.002, vol/vol/vol), with internal standards added. Samples were kept at 4ºC while sonicated for 5 min and then vortexed for 10 min, before being incubated overnight in a chilled state. Samples were then centrifuged for 5 min at 2,000 rpm and the supernatant collected. 1 mL of fresh solvent was then added followed by a 15 min incubation at 4ºC before samples were again centrifuged for 5 min at 2,000 rpm. The two supernatants were combined and 1 mL of CH_2Cl_2 added. Samples were vortexed for 30 min at 4ºC and centrifuged again for 5 min at 2,000 rpm in a chilled state. The lower layer (approx. 1 mL) was collected and 0.1 mL of the extract was transferred into a fresh vial for injection into the LC-MS/MS column

for analysis. The remaining extract was dried under nitrogen laminar flow at 4ºC before being re-dissolved in 0.2 MeOH and stored at -80ºC.

Hormone analysis was performed using Agilent6490 iFunnel QQQ LC-MS/MS(Agilent Technologies, Santa Clara, CA, USA) equipped with Dual AJS ESI, coupled with a 1260 infinity HPLC system (Agilent Technologies, Santa Clara, CA, USA). Separation was performed at 18°C on an Agilent Zorbax Eclipse XDB-C18 column (150 × 4.6 mm i.d., 2.7 μm). The HPLC program consisted of a linear gradient of A (milli-Q water) to B (methanol) over 15 min at a flow rate of 0.8 mL min^{-1} as follows: 0–10 min, 20–95% B; 10–11 min, 95-20% B; 11-15 min, 20 % B. Nitrogen was used as the nebulizing gas. A dual Automatic Jet Stream (AJS) Electrospray Ionisation (ESI) source was kept at a voltage of 3500 V in negative ion mode. Mass spectra were acquired with source conditions as follows: gas temperature 350°C, drying gas 4 L min^{-1} (N_2), nebulizer pressure 35 psi (N_2) and Vcap 3,500 V, fragmentor 160 V and skimmer 65 V. Samples were analysed in multiple reaction monitoring mode. The following transitions were monitored with fragmentor voltage of 380 V. IAA (m/z 174 → 130, collision energy (CE -18 eV), IBA (m/z 202 → 116, CE -15eV), and JA (m/z 209 → 59, CE -18eV). Quantification was performed using the five-point calibration curve plotted using analytical standards.

5.3.1 Untargeted metabolomics

Untargeted metabolomics were performed using gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) in

order to comprehensively assess both primary and secondary metabolites, thus get a complete picture of metabolomic adjustment.

5.3.2 GC-MS

Seagrass samples were homogenised using 750 µL of extraction solution (100% methanol spiked with three internal standards: 100 µmol l^{-1} DL-valine-d8, 60 µmol L^{-1} stearic acid-d3, 60 µmol L^{-1} 5-α-cholestane) per 20 mg of sample material. An additional extraction was performed using 750 µL of 50% methanol. Samples (20 mg) were incubated for 20 min with methanol and 50% methanol sequentially, and then spun down in a micro-centrifuge at 13,000 rpm for 10 min. Supernatants were collected, pooled, and stored at -20ºC until further analysis. 150 µL of pooled supernatant was transferred into a glass vial and evaporated to dryness under a gentle stream of nitrogen. Samples were derivatized by adding 20 µL of 20 mg mL^{-1} methoxylamine in pyridine then incubating samples at 37°C for 2 h at 750 rpm in the agitator. Then, 20 µL of N-methyl-N-(trimethylsilyl) trifluoroacetamide was added and samples were incubated at 37°C for 30 min at 750 rpm in the agitator. Samples were subsequently incubated at room temperature for 1 h, and finally 1 µL was injected for GC-MS analysis.

Samples were run on a GC-MS-QP2020 (Shimadzu Corporation, Kyoto, Japan) equipped with an AOC-20is autosampler (Shimadzu Corporation). The column used was an SH-Rxi-5Sil MS fused silica capillary column (30.0 m×0.25 mm×0.25 µm) operating in electron impact mode at 70 eV. Helium was used as the carrier gas at a constant flow of 1.0 mL min^{-1} and an injection volume of 1 µL, with an injector temperature of 280°C and an ion source temperature of 230°C. The temperature

gradient of the oven was 70°C for 1 min, then 7°C per minute to 325°C. The scan range was m/z 50–600. Samples were run in a randomized order to account for any potential experimental drift affecting experimental groups. A quality control (QC) sample composed of replicates from each sample group was also injected periodically.

5.3.3 LC-MS

For LC-MS metabolite extraction, frozen plant tissue was placed in vials containing 2 mL of 100% methanol (LC-MS Grade, BandJ Brand, Honeywell, Shanghai, China) spiked with 0.005 mmol L^{-1} aminoanthracene (Sigma-Aldrich, Castle Hill, Australia) as an internal standard. Samples were homogenized and then vortexed for 30 s per sample in centrifuge tubes. Resulting extracts were collected into clean scintillation vials, and 1 mL of 70% methanol was added to vials to ensure extraction of hydrophilic metabolites. Samples in 70% methanol were vortexed for another 30 s and the resulting extract was combined with the 100% methanol extract. The combined extract was stored at −20°C until further analysis. Prior to LC-MS analysis, the extracts were filtered using a 0.22 µm Hydraflavon syringe filter (MicroAnalytix Pty Ltd, Taren Point, Australia) to remove any particulate matter and into clean 2 mL HPLC vials.

Untargeted LC-MS metabolite profiling was conducted using a reverse phase (C18) technique targeting semi-polar to hydrophobic secondary metabolites (De Vos et al., 2007). Samples were analyzed on 6550 iFunnel Q-TOF LC-MS (Agilent Technologies, Santa Clara, CA, USA) equipped with dual automatic jet stream electrospray ionization (AJS ESI), coupled with a 1260 infinity HPLC system (Agilent Technologies). Separation was performed at 25°C on an Agilent Zorbax Eclipse XDB-C18 column

(100×4.6 mm i.d., 1.8 μm). The HPLC program consisted of a linear gradient of milli-Q water (with 1% formic acid) to 100% acetonitrile (with 1% formic acid) over 12 min, followed by isocratic elution at 100% acetonitrile (with 1% formic acid) at a flow rate of 1 mL min^{-1}. Nitrogen was used as the nebulizing gas. The dual AJS ESI source was kept at a voltage of 3500 V in positive ion mode. Mass spectra were acquired with source conditions as follows: gas temperature 350°C, drying gas 4 L min^{-1} (N_2), nebulizer pressure 35 psi (N_2) and Vcap 3500 V, fragmentor 160 V and skimmer 65 V. The mass range scanned was 70– 1700 m/z, at a scan rate of 2 spectra s^{-1}. Because analysis was untargeted, generic settings were applied to obtain as many compounds as possible. All samples were injected in a single batch and a randomized injection order was used to account for any experimental drift. A QC sample composed of replicates from each sample group was analyzed at the beginning, middle and end of the batch. External mass calibration was performed using a calibrating solution monitoring signals at m/z in positive polarity. Data were processed using Mass Hunter Qualitative analysis software (v.B.06.00 Agilent Technologies). All solvents used were of high purity grade from Honeywell Burdick and Jackson (Chem-Supply, Gillman, Australia). LC-MS grade formic acid was obtained from Sigma-Aldrich.

5.3.4 Data Analysis

Hormones were analysed using R v.3.4.4 (R Core Team, 2018) with each hormone class analysed as the linear response to the predictors tissue type (*Tissue*: above-ground, below-ground, and fruits) and sampling time point (*Tpt*: T_1, T_2, T_3, T_4). Data was also grouped and analysed by whether plants were showing signs of reproductive output (ie. flowering/fruiting), *Phase I* (T_1+ T_2) and *Phase II* (T_3+ T_4).

To determine the optimal model, a global model was created for each response variable where all explanatory variables and interactions were considered. Sub-model sets were then generated using the dredge function in the MuMIn package (Bartoń, 2013). The best-fit models were considered to be those with the lowest Akaike's information criterion (AICc) and highest Akaike weight (w), which by definition contain the best set of explanatory factors for adequately predicting each response variable (Burnham and Anderson, 2002; Wagenmakers and Farrell, 2004). Models with AIC_c values within 2 of each other were considered strong models and are presented with the chosen model being the simplest of this sub-set which was further used for multiple comparison analysis (Burnham and Anderson, 2002). All models were validated by assessing Pearson residuals against fitted model values and standard model diagnostics including residuals were inspected for heteroscedasticity and non-normality. Post-hoc comparisons were made on estimated marginal means using the Bonferonni adjustment method in the emmeans package (Lenth, 2016).

Raw GC–MS data converted to CDF format using GC-MS solution software (v. 4.0, Shimadzu, Kyoto, Japan), were subsequently imported into XCMS (v. 3.2, Galaxy Project Metabolomics, Roscoff, France) to perform non-linear retention time alignment, matched filtration, peak detection and peak matching. Grouping was performed with a bandwidth value set to 10 and the resulting peak list comprising of features (ions, retention time, intensity) was area-normalized using the total peak area of internal standards and used for further statistical analysis. Statistical analysis of the ~ normalized m/zRT features were performed using MetaboAnalyst 4.0 software.

Normalized peak areas were log-transformed and auto-scaled prior to statistical analysis. Features showing statistically significant (ANOVA; $p < 0.001$) differences among the groups were used to annotate peaks. Metabolite profiling and tentative metabolite identification was performed using GC-MS solution software by combining mass spectra and database consultation (NIST17, match with library > 70 %).

Raw LC–MS data were uploaded to Agilent Mass Hunter Profinder software (version B.06.00) and processed using the Batch Recursive Feature Extraction to obtain a group of ions characterized by retention time, peak area and accurate mass as molecular features. This was performed by using a minimum absolute abundance threshold of 10,000 counts with an m/z range of 100-1,700. The charge state was set to 2 and minimum number of ions in the isotopic distribution was set to 2, following the isotope model of "common organic molecules". The molecular features were binned and aligned as a function of retention time, fragmentation pattern and m/z value across the data matrix, using a tolerance window of ± 0.1 % + 0.2 min retention time and ± 10 ppm + 2 mDa mass window. Allowed ion species were H^+, Na^+, K^+, NH_4^+ and neutral losses of H_2O, and CO_2. Data files were transformed into .CEF files containing extracted compounds, neutral mass, retention time, and abundance, and exported to Mass Profiler Professional (MPP) software package (version B.14.9.1, Agilent Technologies, Santa Clara, CA, USA).

Data were normalized with the internal standard, evaluated and filtered to remove low quality and inconsistent mass spectral features (only those appearing in 75 % of samples in at least one condition were considered). Thereafter, compound abundance values in

each sample were baselined to the median of each compounds in all samples. Statistical analysis and data visualization of resultant molecular features were performed using mass profiler professional (MPP) software (v8.2, Agilent, USA).

5.4 Results

5.4.1 Hormones

Hormones detected within each tissue type came from four major classes: jasmonates, auxins, abscisic acid and cytokinins (Figure 5.1). The most abundant across all tissue types was jasmonic acid (JA) which was found at each time point. There was a significant effect of both *Tissue* and *Tpt* on JA ($R^2 = 0.64$, $F(4,19) = 11.4$, $p < 0.001$), but no significant interaction of these factors for above-ground and below-ground sampled at a given time point (Table 5.1). above-ground had overall lower JA than below-ground ($F = 13.9$, $p = 0.001$), while T_2 (flowering phase) had significantly higher JA across both tissue types combined ($F = 10.5$, $p = 0.0003$). In the second half of the growing period when fruits were present, both below-ground and fruits had significantly greater JA than above-ground ($F = 7.8$, $p < 0.001$; Figure 5.1a).

Indole-3-acetic acid, the main auxin in higher plants, was present in both T_1 and T_2 in above-ground tissues (Figure 5.1b); however, high variability led to no measurable difference by time point or tissue type between above-ground and below-ground in the first half of sampling. In T_3 and T_4, above-ground samples had no detectable IAA, but it was still present at a significant level in below-ground tissues along with fruits ($R^2 = 0.55$, $F = 10.6$, $p = 0.001$).

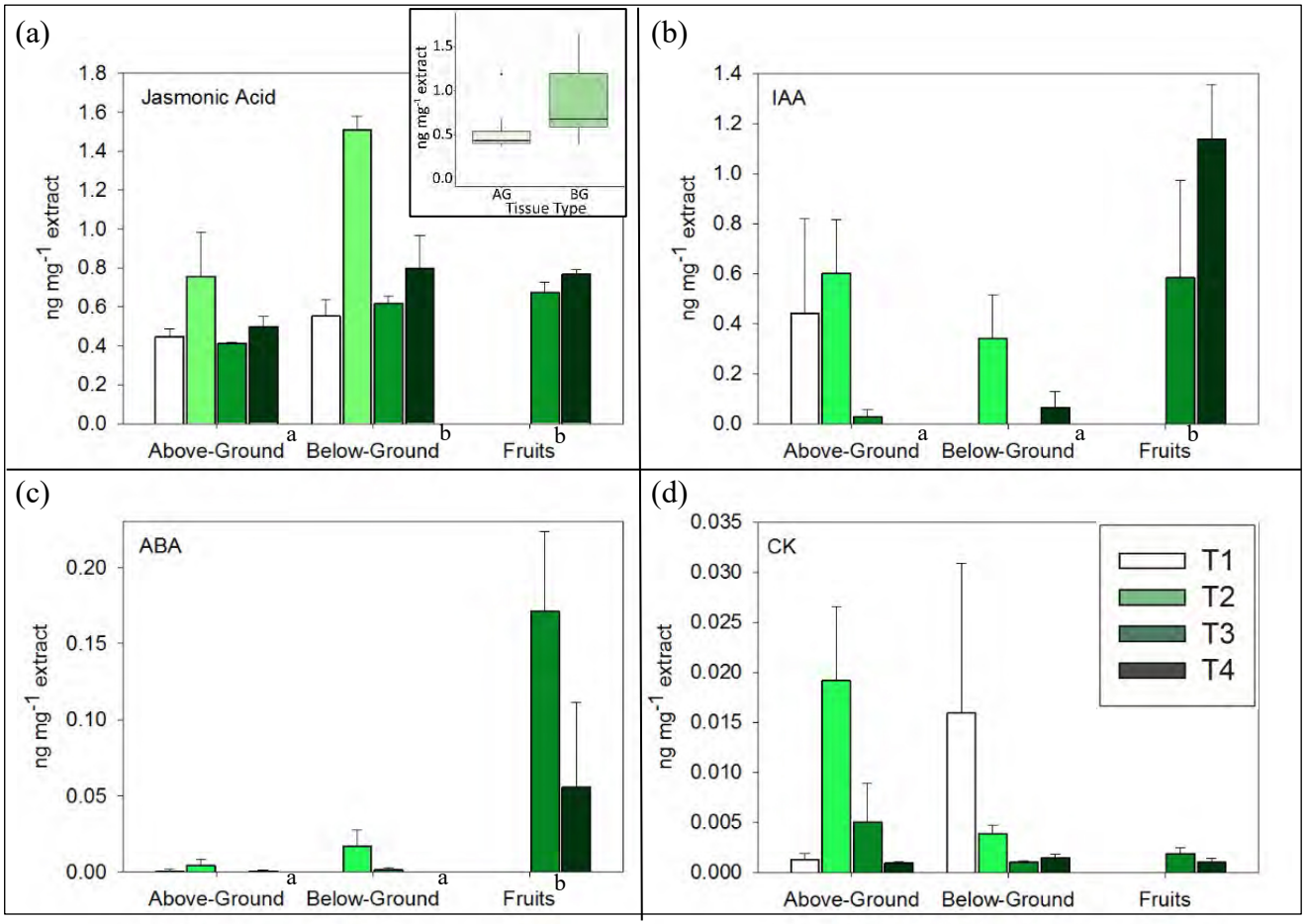


Figure 5.1 Mean hormone extracts by life stage and tissue type (a) jasmonic acid (inset: total pool for tissue types sampled at all time points, above-ground and below-ground), (b) indole-3-acetic acid (IAA), (c) abscisic acid (ABA), and (d) cytokinins (CK). Note y-axis scales vary due to the extremely low concentration in some hormone classes. Error bars indicate ± SEM (n = 3). Differing letters indicate significant differences among time points.

Table 5.1 Overall fit of selected best models predicting hormone content by class in *H. decipiens* from Green Island. Predictors tested were sampling time point (*Tpt*) and tissue type (*Tissue*); *F* is the F-statistic.

Hormone Class	Models	df	Log Lik	AIC_c	ΔAIC_c	*w*
Jasmonic Acid	***[Horm] = Tpt + Tissue + ε***	6	4.58	7.8	0.00	0.749
	[Horm] = Tpt x *Tissue* + *Tpt* + T*issue* + *ε*	9	10.42	10.0	2.24	0.244
IAA	*[Horm] = Tpt + ε*	5	-2.6	18.6	0.00	0.345
	[Horm] = 1 + ε	**2**	**-7.20**	**19.0**	**0.34**	**0.291**
Abscisic Acid	*[Horm] = Tpt + ε*	5	86.45	-159.6	0.00	0.360
	[Horm] = 1 + ε	**2**	**82.05**	**-159.5**	**0.04**	**0.354**
Cytokinin	***[Horm] = 1 + ε***	**2**	**74.29**	**-144.0**	**0.00**	**0.730**
	[Horm] = T*issue + ε*	3	74.32	-141.4	2.57	0.201

Abscisic acid (ABA), while identified in all tissue types, was at extremely low levels compared to JA and IAA (Figure 5.1.c). Both above-ground and below-ground tissues had very little or no quantifiable ABA in any samples compared to fruits which had a significant overall concentration when present ($R^2 = 0.58$, $F = 20.0$, $p < 0.0001$; Table 5.1).

Cytokinins were present at all sampling time points and in all tissues (Figure 5.1d); however, the very low quantity and high variability meant no significant trends were detected (Table 5.1).

5.4.2 GC-MS

A total of 27 unique and differentially expressed ($p < 0.001$) metabolites were resolved by GC-MS in above-ground and below-ground tissues after cross-referencing with the NIST17 library. PCA of all annotated compounds suggested a clear metabolic shift in both above- and below-ground tissues with all replicate samples at T_3, the 22 September sampling when flowering was observed (Figure 5.2). In above-ground tissues, all samples from a given time point clustered closely (Figure 5.2a). In addition to T_3 separating out distinctly from other time points, T_1 (juvenile phase) samples were discrete from both November time points (T_3 and T_4). Below-ground samples were still tightly clustered by sampling time but with greater overlap in expression across T_1, T_3 and T_4 (Figure 5.2b). Metabolites ranged from amino acids, fatty acids, polyamines, saccharides and sterols (Figure 5.3 and 5.4).

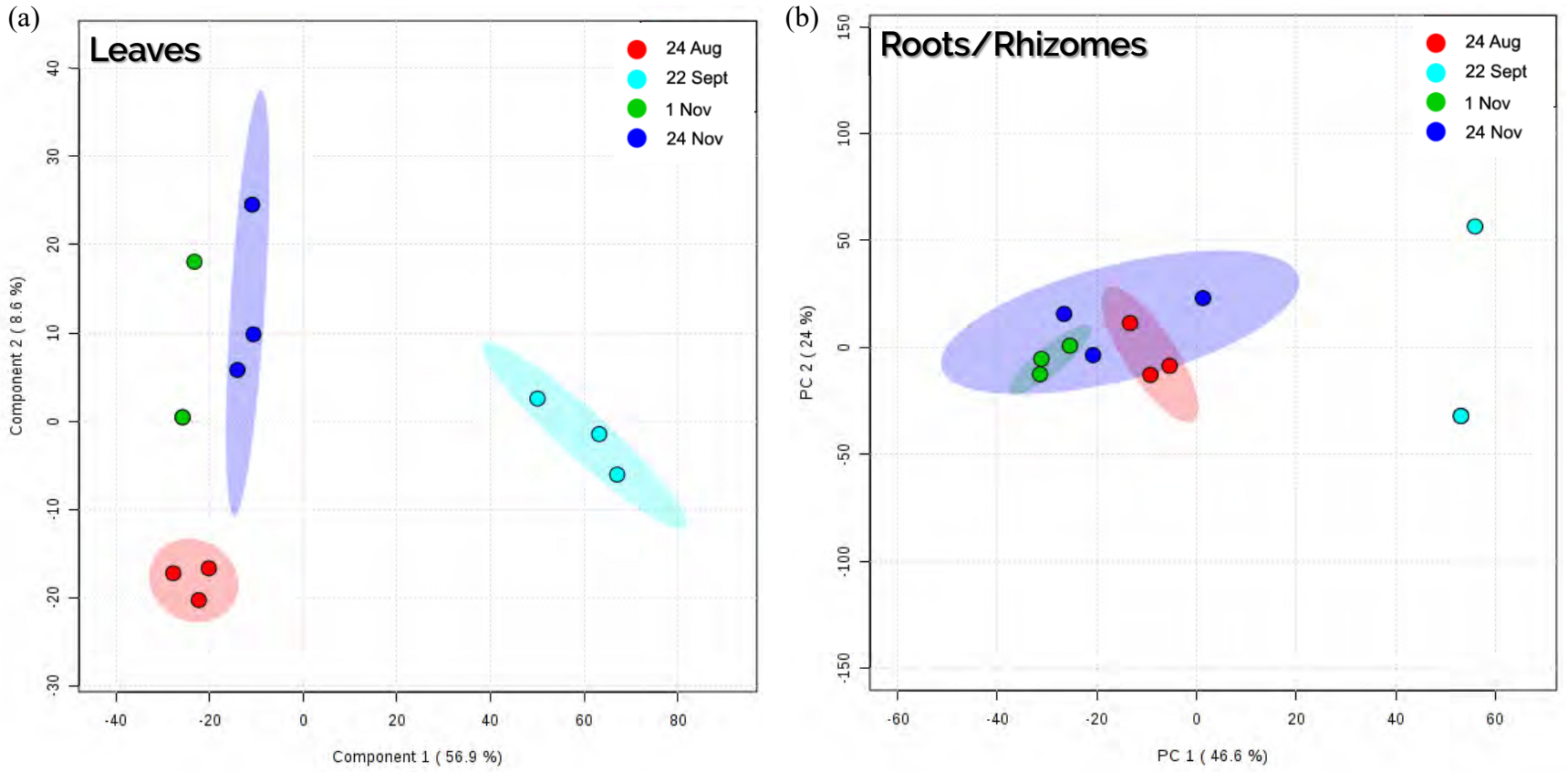


Figure 5.2 Principal components analysis models based on targeted GC-MS data showing a clear shift in metabolome profiles in both (a) above- and (b) below-ground tissues on 22 September, when local flowering was observed in the meadow.

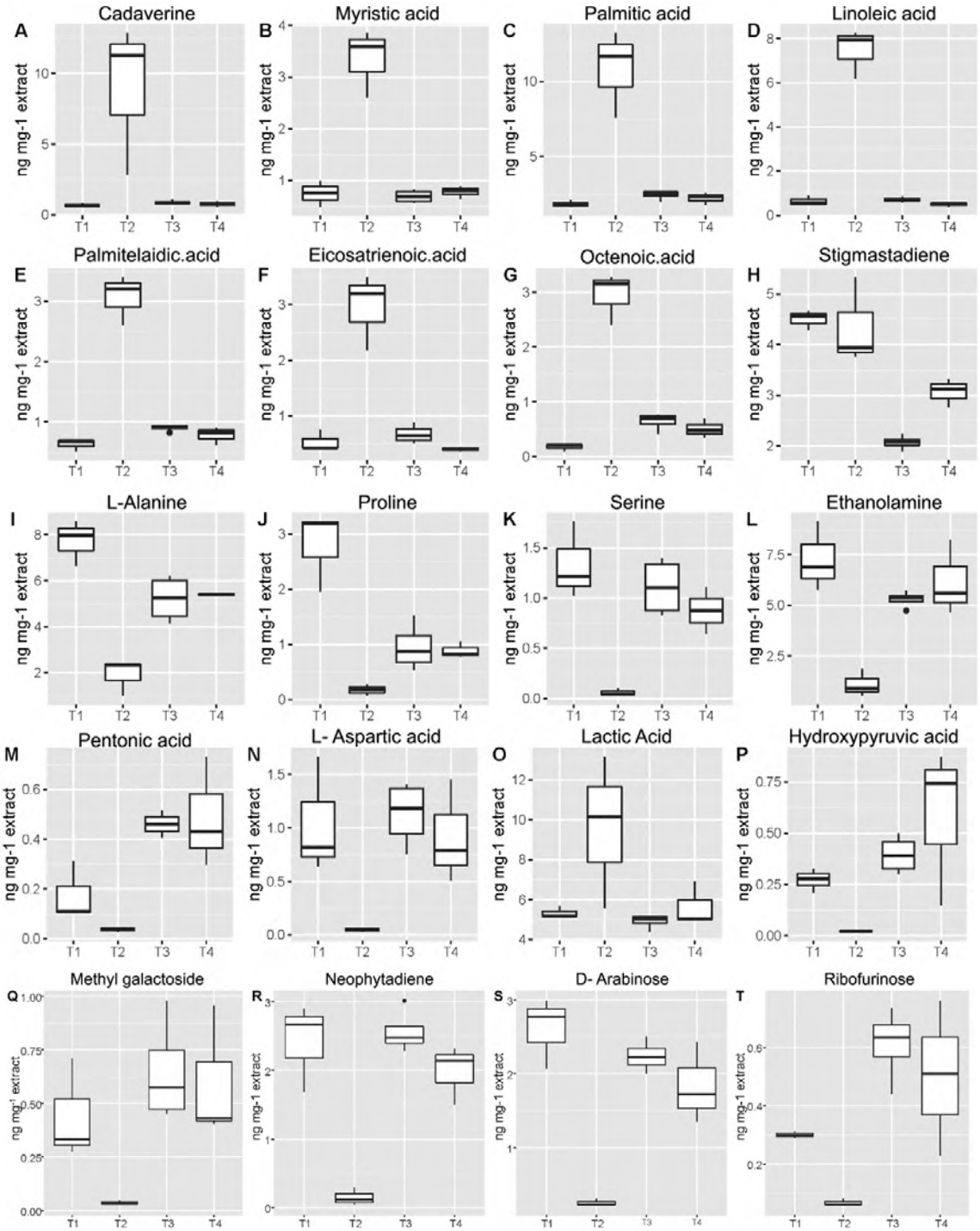


Figure 5.3 Compound changes among time points from above-ground tissues extracted by GC-MS. (a - h) are polyamines and fatty acids exhibiting significant spikes at T_2, when flowering was observed. (i – t) are amino acids and polysaccharides, which increase in the last two time points as membranes and proteins degrade with plant senescence. Boxes represent the interquartile range of values, with the lower boundary the 25th percentile, a line within the box marks the median, and the upper boundary the 75th percentile. Whiskers (error bars) above and below the box represent the 90^{th} and 10^{th} percentiles. (n = 3)

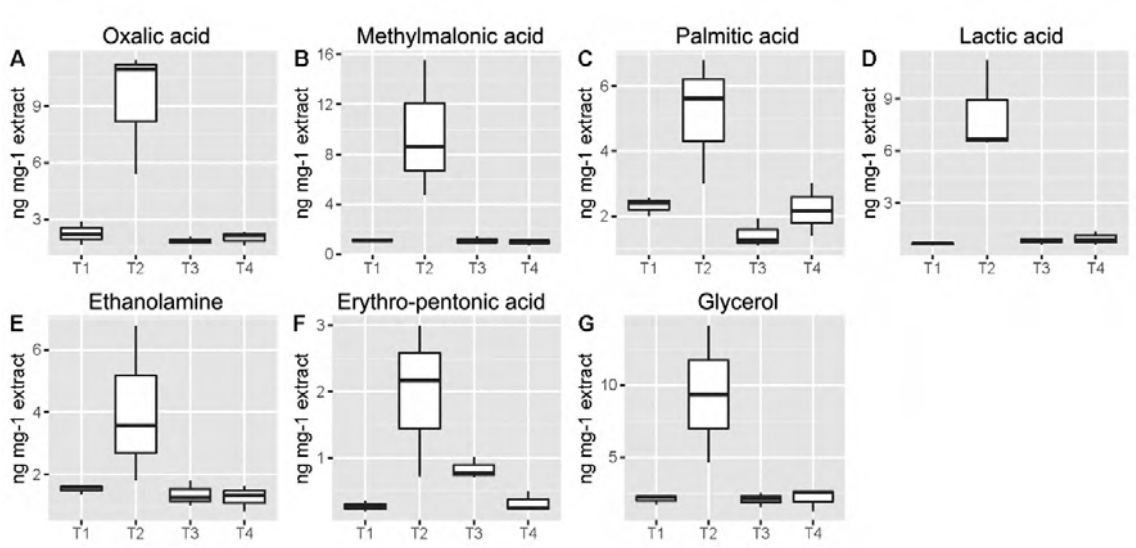


Figure 5.4 Compound changes among time points from below-ground tissues extracted by GC-MS. Boxes represent the interquartile range of values, with the lower boundary the 25th percentile, a line within the box marks the median, and the upper boundary the 75th percentile. Whiskers (error bars) above and below the box represent the 90th and 10th percentiles. (n = 3)

In above-ground tissues, 20 annotated metabolites showed strong differential expression of profile patterns at T_2 and were grouped somewhat by metabolite class (Figure 5.3). Polyamines and fatty acids were extremely low except for the relative spikes at T_2. In contrast, amino acids dropped to extremely low or nil levels from T_1 to T_2 followed by a return to relatively high levels at T_3 and T_4 (Figure 5.3a-n).

In below-ground tissues, only 7 metabolites including the fatty acid palmitic acid and other carboxylic acids were differentially expressed among time points ($p < 0.001$), with all relatively high at T_2 (Figure 5.4).

5.4.3 LC-MS

Biochemical trends obtained from LC-MS analysis were consistent with results the GC-MS results, where the metabolite profile showed similar trends with T_2 significantly up- or down-regulated relative to other time points in both above-ground and below-ground tissues (Figure 5.5a-b). A clear clustering pattern was observed between fruit samples as well. (Figure 5.5c).

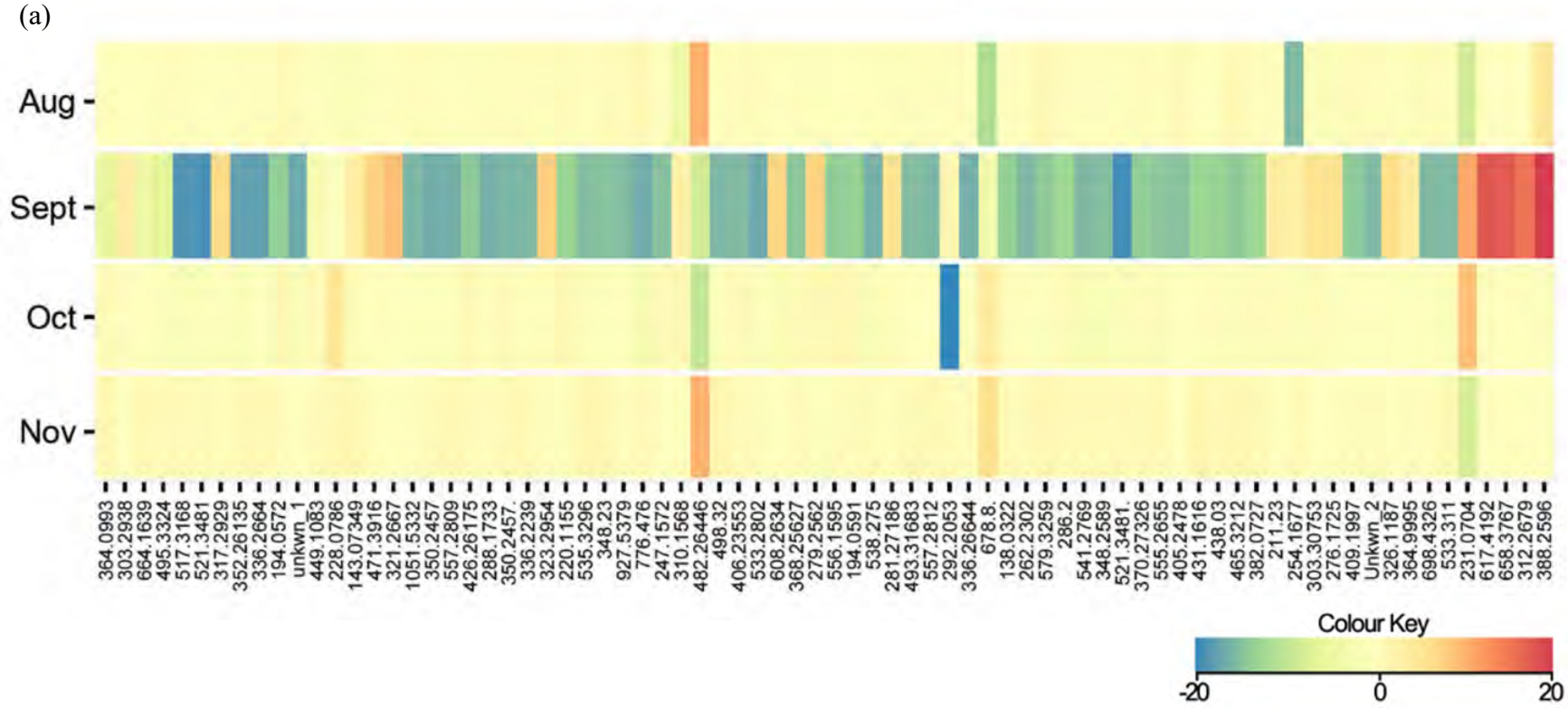


Figure 5.5 Heat map of normalized intensity values of metabolites extracted during untargeted LC-MS by time point in (a) above-ground, (b) below-ground and (c) fruits. Identities are defined by molecular mass as a conservative approach rather than by Metlin library findings.

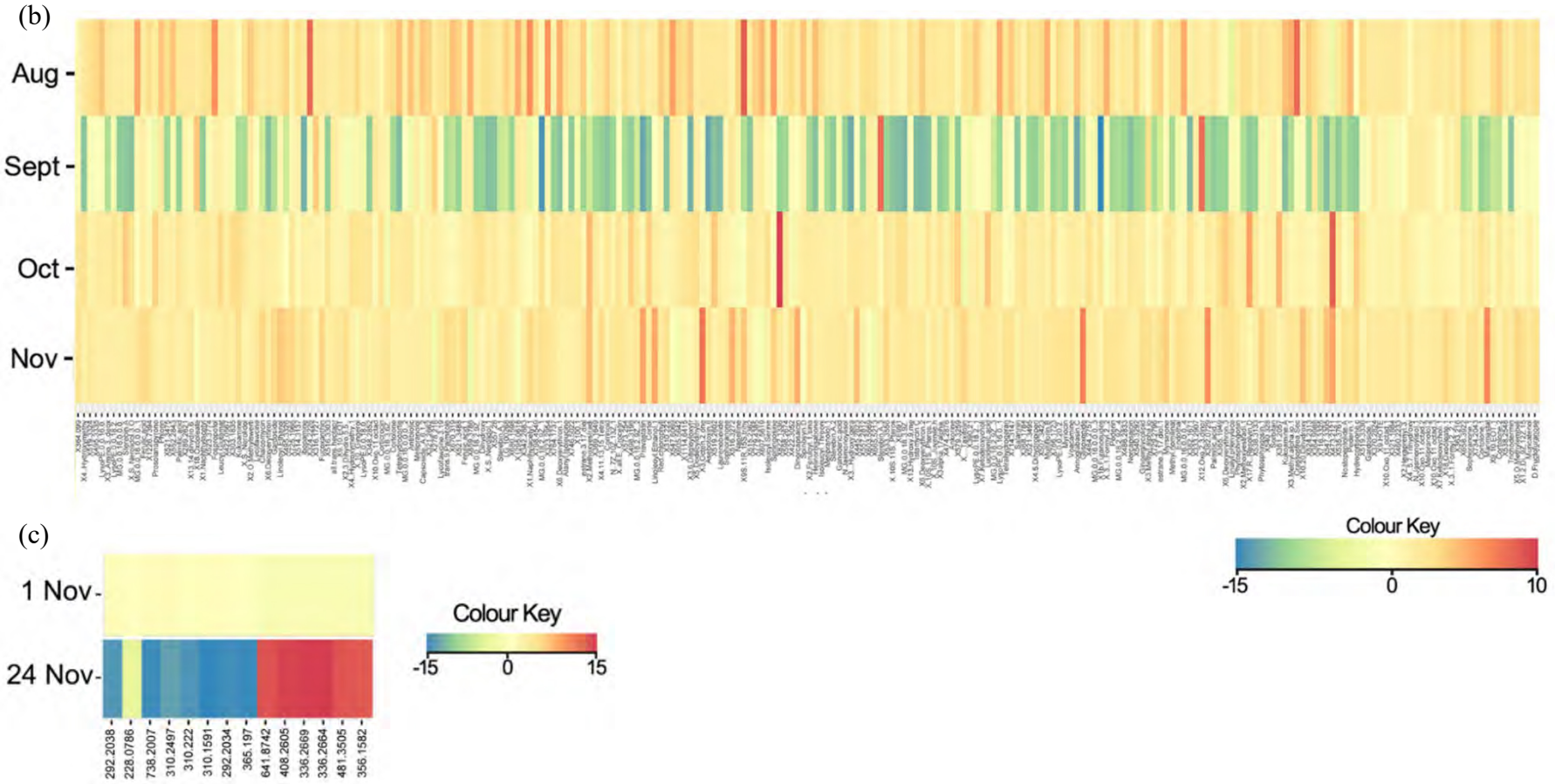


Figure 5.5. (continued) Heat map of normalized intensity values of metabolites extracted during untargeted LC-MS by time point in (a) above-ground, (b) below-ground and (c) fruits. Identities are defined by molecular mass as a conservative approach rather than by Metlin library findings. (n=3, except for below-ground 22 Sept n=2)

5.5 Discussion

Seagrasses have been studied extensively as a sentinel marine plant, researched for how changes in the marine environment drive seagrass condition to better manage and mitigate impacts in the coastal zone (Coles et al., 2015; Kilminster et al., 2015; McMahon et al., 2013). However, studies are only beginning to emerge examining the underlying molecular pathways and compounds that have evolved to support a uniquely adapted lifestyle in a submerged, marine environment (Gu et al., 2012; Dattolo et al., 2014; Marín-Guirao et al., 2017).

Our work is the first study, to the best of our knowledge, to document spatio-temporal trends of endogenous hormones resident in seagrass tissues. No previous studies have described endogenous levels of hormones in seagrasses and the lack of such studies and the implications on understanding seagrass biology was recognised some time ago (Orth et al., 2000). Instead, previous studies have looked at the benefits of exogenous hormone application using known treatment benefits based on terrestrial examples (Koch and Durako, 1991; Glasby et al., 2015). Another study examined the microbial community associated with seagrasses and its' production of compounds including phytohormones around roots and rhizomes (Kurtz et al., 2003).

This work builds on results from a long-term monitoring study documenting a clear annual cycle of the deep-water seagrass *Halophila decipiens* (see Chapter 2). It establishes significant trends in hormones and broader metabolite profiles of this pan-tropical marine plant at varying life cycle stages and across different tissue types. Samples from both above- and below-ground tissues collected in late September when

plants appeared mature and began to flower had a metabolome signature that was unique from the other three time periods. The overall expression of the metabolome at this time point was strongly correlated with high levels of jasmonic acid, polyamines and fatty acids as well as an equivalent lack of amino acids which rose significantly over the subsequent sampling periods when fruit set and leaf senescence progressed.

In annual terrestrial plants, senescence is thought to be invoked at the onset of the reproductive phase (Tan and Swain et al. 2006; Davies and Gan 2012). The initiation of reproduction involves the process of converting purely vegetative shoots into floral organs or inflorescences, a process known to be driven by a wide number of phytohormones, photoreceptors, and other cues such as vernalization (periods of exposure to low temperature) which can act independently or in concert (Srikanth and Schmid, 2011). Once these pathways have been initiated, the process cannot be reversed and with that, comes the onset of plant senescence.

The presence of jasmonic acid at high levels in both above- and below-ground *H. decipiens* tissues during the flowering phase aligns with terrestrial studies that have found this hormone can play a critical function in flowering plants (Creelman and Mullet, 1995; Tan and Swain, 2006; Yuan and Zhang, 2015; Huang et al., 2017). *Arabidopsis*, tomato, rice and maize all have conserved jasmonic acid signaling pathways which biologically function to affect varying elements of reproduction from controlling inflorescence and stamen development to sex determination (Yuan and Zhang, 2015). In a duckweed species—like seagrasses, also from the order

Alismatales—abiotic stress was found to induce the production of jasmonic acid and other related oxylipin compounds which promoted flowering (Yokoyama et al., 2000).

Below ground, jasmonates are known to stunt root growth while promoting lateral root elongation and rhizome formation through crosstalk with other key hormones such as auxins (Rayirath et al., 2011; Wasternack, 2014). The peak in jasmonates in *H. decipiens* below-ground tissues during the flowering phase may be a sign of high productivity and rhizome extension; not an unexpected signal during the September and October sampling period when the deep-water meadow was rapidly expanding (see Chapter 1).

Other well-studied hormones known to drive plant development and senescence were not found in our study. For instance, gibberellins are more commonly known to play a key role in promoting floral induction (Srikanth and Schmid, 2011). While not able to be detected in *H. decipiens* tissues in this study, the transcriptome of another seagrass species, *Posidonia australis*, showed strong activation of genes linked to gibberellin coordinating flowering (Entrambasaguas et al., 2017). Ethylene, a hormone well known for its role in plant development and senescence also was not detected in this study. Research on *Zostera marina*, a widely studied temperate seagrass, found the lack of ethylene signaling pathways in its transcriptome and suggested this hormone may be absent more broadly in submerged marine macrophytes given ethylene is a volatile gaseous hormone (Golicz et al. 2017).

The metabolome, the closest molecular level quantification of an organism's phenotype, represents the current expression of underlying genes and the cellular processes that are driven both by external stimuli and built-in life history strategies (Guijas et al., 2018). The untargeted expression of the metabolome of *H. decipiens* over the sampled life cycle found a strong and unique signature expressed during the second sampling event, which corresponded with significantly higher jasmonic acid in hormone samples. The differentially expressed metabolome was both in above-ground and below-ground tissues and consistent between GC-MS and LC-MS approaches suggesting a distinct, whole-plant physiological state at a time of rapid meadow expansion and flowering.

Wounding (i.e., mechanical damage) and various abiotic stresses are known to affect plant metabolite composition (Creelman and Mullet, 1995; Upchurch, 2008; Vu et al., 2015; Hou et al., 2016). A wounding response typically occurs first at the site of damage before a systemic response via an activated network of pathways that coordinate a broader response in other parts of the plant (Savatin et al., 2014). The physical damage to leaves from herbivory can affect lipids in plant membranes (Vu et al., 2015; Genva et al., 2019). Jasmonates are also well described for their role in triggering metabolic pathways of defense and wound response to insects and pathogens in land plants (Wasternack and Hause, 2013). In seagrass communities, fish grazing can restructure the meadow by direct shoot removal (Bessey et al., 2016; Scott et al., 2021) and even small invertebrates can directly consume leaf matter or indirectly wound seagrasses when feeding on epiphyte assemblages (Orth and Van Montfrans, 1984; Brearley et al., 2008). In this study, no obvious damage to shoots was observed during sampling or over the previous four years of monitoring during the September/October

period, although it is possible grazing was missed due to behavioral changes in the fish and invertebrate communities when divers were on site. The high levels of jasmonates and fatty acids in late September in this study may be a response to some external stimuli, mirroring a land plant response with the production of deterrent secondary metabolites (Wasternack, 2014). In *Arabidopsis thaliana*, fatty acid levels have been found to increase from wounding after only 45 minutes and last for at least six hours (Vu et al., 2015). The physical collection of seagrasses in essence is similar to a grazing behavior and therefore could induce a plant defense response; however, samples were snap frozen in liquid nitrogen on site to immobilize further metabolic plant activity. In addition, a similar peak in the signal would be expected at all time points if the spike was related to mechanical damage from collecting samples.

Studies of metabolites in seagrasses have been limited prior to the development of the field of metabolomics. Studies to date have focused on phenolics, namely flavonoids for their antimicrobial or antifouling bioactivity (Zidorn, 2016). Outbreaks of the tissue 'wasting disease' linked to a slime mold (*Labyrinthula zosterae*) in temperate eelgrass populations, concentrated on the protective role phenolics may play in disease, fouling and deterring herbivory (Vergeer and Develi, 1997). One study by Enerstvedt et al. (2017) considered the ecological significance of seasonal flux in flavonoids from *Zostera* spp. tissues off Norway. More recently, metabolomics profiling has been applied in ecological studies looking for early indicators of stress related to environmental perturbations (Griffiths et al., 2020) and is an emerging field of study for marine macrophytes (Macreadie et al., 2014; Kumar et al., 2016).

Metabolomic profiling across different growth stages has been widely used in the study of crop plants to facilitate higher biomass yields and/or quality of harvests (Lombardo et al., 2011; Zhang et al., 2018; Qin et al., 2019; Yang et al., 2020). Qin et al. (2019) found key metabolome changes occurring around flowering compared to post-flowering stages. Zhang et al. (2018) also found significant swings in amino acids, proteins, carbohydrates and fatty acids in leaves of tobacco over the plant's life cycle. These terrestrial studies provide a constructive framework from which marine plant metabolite signatures can be evaluated.

In the current study, saturated and unsaturated fatty acids—namely palmitic acid, palmitelaidic acid, myristic acid, linoleic acid, eicosatrienoic acid, and octenoic acid—spiked during the flowering phase before declining to negligible levels in the subsequent fruiting and leaf senescence phase samples (Figure 5.3). One of the most dominant forms of saturated fatty acids in plants, animals, and microorganisms is palmitic acid. It was strongly upregulated in above- and below-ground tissues at the time of flowering in this study. Fatty acids are known for a variety of roles in mediating plant responses. Fatty acids can increase with plant defense and regulate salt, drought, and heavy metal tolerance as well as wound-induced responses and defense against insect and herbivore feeding in plants (Upchurch 2008, Hou et al 2016). The upregulation in fatty acids—known precursors of jasmonic acid (Creelman and Mulpuri, 2002) which also was elevated at flowering in our study—indicates a strong response or preemptive change in seagrass leaves that coincides with the time of flowering and maturation of shoots in the meadow. The driver of this change is uncertain and again

could be a protective response to a negative external stimuli (herbivory or poor environmental conditions) or a deliberate signal, following plant stress.

One of the more remarkable metabolites elevated at flowering in this study is the polyamine cadaverine. Polyamines are a group of compounds also recognised for modulating plant growth and development as well as response to environmental stresses (Chen et al., 2019). The most common polyamines in plant tissues are putrescine, spermidine and spermine. One study of the seagrass *Cymodocea nodosa* sought to quantify the presence of these more commonly found polyamines known as important plant growth regulators (Marián et al., 2000). Cadaverine, however, is less well studied but has been found in a variety of plants such as rice, wheat, corn and legumes (Tomar et al., 2013; Jancewicz et al., 2016). This compound is known to modulate an environmental stress response (Jancewicz et al., 2016) or assist in plant development, namely reproductive function such as flower initiation and flower development (Huang et al., 2004; Kiełkowska and Dziurka, 2020). It is possible that cadaverine in *H. decipiens* above-ground tissues is part of a modulated shift during flowering or response to an external stimuli that elicits fruiting and fated plant senescence.

Amino acids levels identified from GC-MS in above-ground tissues had the opposite trend to polyamine and fatty acids with little to none during early flowering versus early vegetative growth, fruiting and senescent stages in above ground tissues. Amino acids serve a number of roles from signaling to the biosynthesis of proteins (Häusler et al., 2014; Hildebrandt et al., 2015) making it hard to know from this study alone how the changes in amino acid concentration are directly tied to changes across life cycle stages.

However, the unique overall signature across multiple metabolites and the metabolome as a whole at flowering is in line with a clear event occurring within seagrass tissues at the flowering stage. Perhaps, the increase in simple amino acids during the latter two stages could be due to the breakdown of proteins as tissues senesce and resources are re-allocated into alternative sinks such as fruits rather than in leaves. A similar increase was seen with the upregulated levels of polysaccharides in both above- and below-ground tissues, may be a sign of the breakdown of stored sugars and the metabolite remobilization from senescing leaves to alternative sinks such as fruit and seed development (Moschen et al., 2016).

The changes in light and temperature or other seasonal shift in the environment over the growing cycle of *H. decipiens* may in itself be driving the shift in the metabolomic signature rather than the actual event of flowering modulating further endogenous change. Additional work in a controlled laboratory environment would help untangle the specific cause and effect of external stimuli, flowering and the fate of this annual-like species.

Measures of endogenous hormones together with metabolomic profiling provide a better understanding of seagrass phenology. This study provides the first evidence of life stages being modulated not only by environmental conditions such as light and temperature, but also by underlying metabolic cues that may drive the ultimate fate of flowering, fruiting and plant senescence in an annual-like seagrass. This study found a strong and consistent shift in the metabolic makeup of *H. decipiens* at the time of flowering and elevated plant growth in late September. The strong signal in both above-

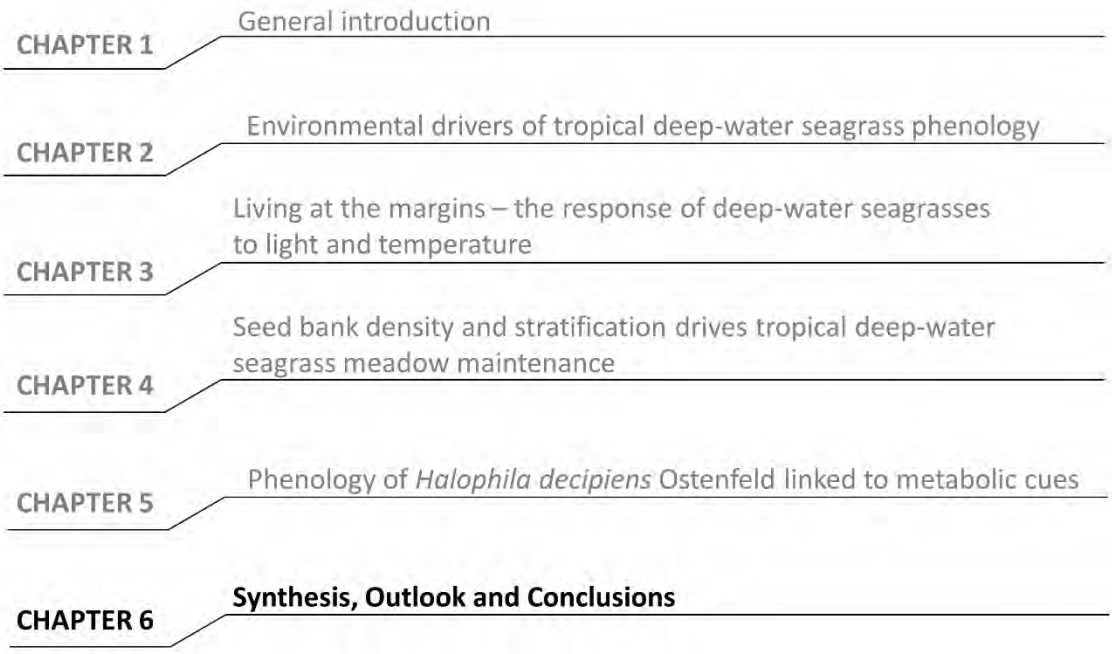
and below-ground tissues mimics profiles found in some annual land plants that go through a notable life stage transition at flowering which ultimately leads to programmed senescence and the production of fruits. These metabolic changes are often detected well before the onset of senescence (Moschen et al., 2016). With a growing need to mitigate anthropogenic stressors on marine plants, it is vital to explore how such metabolomic signatures linked to plant phenology could play a role in managing and mitigating the duration and timing of stress conditions (Wu et al., 2017). For example, results from this study suggest monitoring hormone and metabolome signatures to protect *H. decipiens* up to and during flowering would be a valuable management strategy to ensure the process of fruiting and seed set progresses to ensure the ongoing maintenance of annual meadows. Metabolomic signatures can be developed as bioindicators of aquatic health or condition (Pomfret et al., 2020) and may be an appropriate application to better manage and mitigate impacts around key life stage development in seagrasses.

5.6 Conclusion

The annual growth pattern and associated metabolic cues of this seagrass species suggests an evolutionary adaptation to living at the margins (i.e. deep-water, low light) and in disturbed environments where chances of survival year-round are risky. *Halophila decipiens* produces and dispenses a large number of seeds each year into a local seed bank used to generate successive annual populations (see Chapter 3). This strategy of annual resource allocation into seed production is an effort to maximize chances of survival for this small-bodied species (Friedman and Rubin, 2015). This is the first study to show a submerged marine macrophyte goes through a developmental

switch linked to the onset of flowering and appears to be modulated in part by the underlying plant metabolome, similar to annual land plants (Guijas et al., 2018). Further research is needed into the relationship between the phenotype (i.e. metabolome) and the underlying plant genome in order to map the metabolic pathways of this annual-like seagrass and whether other seagrasses have retained the blueprint of metabolic cues and reproductive strategies well described in terrestrial plants.

CHAPTER 6 Synthesis, Outlook and Conclusions



6.1 Summary

Seagrasses provide irreplaceable ecosystem services (Costanza et al., 1997; Cullen-Unsworth et al., 2014; Nordlund et al., 2016; Ruiz-Frau et al., 2017) and yet threats from coastal development (Waycott et al., 2009) and climate related impacts (Zimmerman, 2020) jeopardise their global footprint and function in our oceans. Efforts to better manage and mitigate threats to seagrass meadows have led to enhanced investment and research into their distribution, ecological drivers and bioindicators of their health (McMahon et al., 2013; Kilminster et al., 2015; McKenzie et al., 2020). In the Great Barrier Reef Marine Park (GBRMP), much effort has been made to translate our mechanistic understanding of marine plants into impactful management of both acute disturbances (e.g. dredging, Chartrand et al., 2018) and chronic stressors (e.g. eutrophication, Udy et al., 2019). However, these applied research outcomes have primarily focused on shallow seagrass communities, synthesising results and deriving relationships that could be used by managers and regulators.

The goal of this thesis was to deepen our understanding of the ecological dynamics and underlying biological drivers of deep-water seagrasses from the Great Barrier Reef in order to fill a knowledge gap that can be applied for their better management and the communities they support. To achieve this goal, I (i) studied the seasonal growth dynamics of deep-water seagrasses, characterising key environmental parameters linked with growth and senescence; (ii) evaluated light and temperature as drivers of seagrass abundance and determined light thresholds for the dominant *Halophila* species; (iii) quantified *Halophila* seed banks over time and space, evaluating the role of seed

stratification on germination patterns; and (iv) investigated what role endogenous cues play in the phenology of *H. decipiens*, the only known pan-tropical seagrass species.

6.2 Thesis outcomes

Objective 1: Establish the seasonal growth dynamics of Great Barrier Reef deep-water seagrasses, characterising key environmental parameters linked with growth and senescence

In Chapter 2, I demonstrated that deep-water GBR seagrasses from the *Halophila* genus do not all follow the same cyclic annual growth pattern. *Halophila decipiens* follows a true annual cycle of germination, rapid growth and meadow expansion, before flowering, fruiting, and full plant senescence when fruits release mature seeds for subsequent years' regeneration. This pattern was found in three distinct deep-water populations and the cycle proceeded prior to declines in local light availability or any detectable change in the environment that could drive a stress response leading to die-off. *Halophila ovalis*, conversely, persisted year-round aside from its absence in the months following severe cyclone disturbance. The findings emphasise the importance in understanding differences among species as well as locations in shaping the phenology of GBR species. They are not all the same and a nuanced approach to management rather than a one size fits all strategy is required

Objective 2: Evaluate light and temperature as drivers of seagrass abundance and determine light thresholds for the dominant *Halophila* species

In Chapter 3, I investigated the specific physiological limits of the two most common deep-water seagrasses, *H. decipiens* and *H. spinulosa*. Limiting light led to decreased shoot density for both species after two and four weeks, respectively, yet neither species was affected by increases in temperature with or without compounded light stress. Photoacclimation and physiological adjustments by either species were not adequate to compensate for reduced irradiance suggesting these plants reside at the margins of their functional limits. The disparity in time until a negative morphological response from low light underscored the importance in understanding species-specific response to environmental perturbations. Findings also reinforced, together with Chapter 2, that deep-water seagrasses are growing near their physiological limit where small reductions in light from ambient conditions can curtail growth and lead to meadow loss.

Objective 3: Quantify *Halophila* seed banks over time and space and evaluate the role of seed stratification on germination

Results presented in Chapter 2 established growth contrasts among the GBR deep-water seagrass species. *Halophila decipiens* and *H. tricostata* died off as part of their annual cycle, while *H. ovalis* reduced in biomass but persisted year-round like a more classic perennial plant. Chapter 4 explored the specific role reproductive output played in structuring the meadow and these contrasts in growth dynamics among deep-water species established in Chapter 2 and 3. I quantified the intra- and inter-annual changes

in species-specific seed bank densities and stratification from the three Great Barrier Reef deep-water seagrass meadows monitored in Chapter 2, how these seed banks relate to localized seed production (i.e. fruiting), and the extent of seed retention following annual plant senescence. I also examined how seed bank capacity drives recovery from severe and localized natural disturbance events (ie. cyclones) in an opportunistic case study.

Objective 4: Investigate what role the metabolome and specific hormones may play in the phenology of the only known pan-tropical seagrass species

In Chapter 2 and 3 there was strong evidence that *H. decipiens* condition declined prior to light falling below positive growth thresholds, indicating there may be an element of programmed senescence for these plants and a true annual life cycle. In Chapter 5, I looked at the underlying metabolic function associated with the annual life history traits established in Chapters 2, 3 and 4. Endogenous cues known to initiate the transition between key life stages in terrestrial plants have not been studied in seagrasses. I explored key hormone classes and the broader metabolomic profile within the observed life stages of the annual-like seagrass species *H. decipiens*. I measured consistent and clear shifts in both hormones and the untargeted LC-MS and GC-MS metabolomic profiles of above- and below-ground tissues around plant maturity and flowering in September; aligning with patterns observed in terrestrial plants (Huijser and Schmid, 2011; Qin et al., 2019). This is the first known study to link metabolomic regulation with plant growth and development in seagrasses and provided further evidence that *H. decipiens* may be a true annual species.

6.3 Application & Management Implications

6.3.1 Seagrass Insurance Policy Drives Management Approach

In recent times, there has been an increased focus on using the maintenance of ecologically relevant light thresholds for seagrass to mitigate against the impacts from coastal development and runoff. This has included active management of the light environment and the interval of allowable attenuated light in lieu of more traditional and indirect measures of turbidity (Chartrand et al., 2016; Collier et al., 2016b). This approach has been broadly successful for coastal seagrass communities that often flourish in protected embayments and waterways where they are in direct competition for space with commercial use areas such as port facilities and growing city centres (Grech et al., 2011). With deep-water seagrasses growing near their physiological limit, small reductions in ambient light could drive deep-water seagrass losses relatively quickly and well before management can enact steps to relieve pressure such as relocating a dredge or halting works entirely. Other management strategies therefore need to be considered that would pre-empt loss of light in place of reactive action plans once aberrant light attenuation has occurred.

One such approach is the use of ecological windows (Fraser et al., 2017; Wu et al., 2018). This approach allows for risk mitigation by managers using windows when the impact of dredging stress does not impair seagrass resilience. Deep-water seagrasses such as *H. decipiens* which follow a clear annual pattern supports such a strategy. Light reducing activities such as dredging would be best timed when seagrass exists as a seed bank and therefore not actively photosynthesising which necessitates a good light environment.

This thesis also describes how some deep-water GBR seagrass populations have synchronised their life stage to natural disturbance cycles. *H. decipiens* and likely *H. tricostata* rely entirely on a seed bank to regenerate each growing cycle or risk prolonged meadow loss. The process of seed generation is therefore a critical insurance policy put in place with the onset of flowering. Parallels between *H. decipiens* and annual land plants in the endogenous cues and growth patterns affirms that the initiation of flowering is a trigger that once pulled, cannot be undone in an annual cycle. Fruit and seed development is largely preordained as energy is re-directed and plant senescence is all but inevitable to ensure seeds mature and are released back into the environment. These findings together underscore the need to maintain light specifically around this window of early growth and above all, flower initiation. The need to produce a vast supply of seeds to store below ground during adverse growing conditions means it is critical the reproductive phase of its life cycle is not circumvented. Ensuring that poor light does not impede flowering and fruit production should be a key management goal. This paradigm shift in managing more ephemeral type seagrasses has been suggested before (Hovey et al., 2015); however this study provides explicit knowledge of a turning point in plant development, which has direct implications for managing critical phenological events rather than meadow maintenance as a whole. This thesis has therefore provided an important enhancement to the application of light thresholds as a management approach and particularly for species like *H. decipiens* to ensure they are not pushed beyond a point of no return.

6.3.2 One Size Does Not Fit All

Species-specific management strategies are warranted based on disparities in morphological traits, growth patterns and the phenology of *Halophila* spp found in this thesis. As described above, *H. decipiens* can significantly die-back in less than two weeks under low light conditions. *H. spinulosa* can persist through short periods (< 4 weeks) of light attenuation based on findings in Chapter 3, which to some extent may be due to the larger anatomical structures which can support primary metabolism and augment losses of photosynthetic carbon fixation (Collier et al., 2009; Griffiths et al., 2020). Uncoupled from observed cyclone impacts, *H. ovalis* persisted year-round in the Lizard Island meadow with similar diminutive morphology to *H. decipiens*; an attribute unlikely to support metabolic demands to withstand light loss. *Halophila* spp. from the three meadows also varied in producing and relying on a local seed bank. Observations from where *H. spinulosa* was collected in Chapter 3 have found variable production of fruits in the local population indicating this species may produce fruits intermittently in response to growing conditions rather than follow an obligate pattern of seed production each year. Without this evidence for a wide range of strategies and growing patterns in *Halophila* spp., deep-water seagrass management strategies would likely rely on a more broad-brush approach. This thesis has enriched the understanding of a specialised subset of seagrasses, their ecology and an understudied community of seagrasses with important ecosystem services (Fonseca, 1989; Esteban et al., 2018; York et al., 2018; Hayes et al., 2020) and points to a requirement for a more nuanced approach to deep-water seagrass management.

Importantly, unvegetated areas can be important seagrass habitat that can mistakenly be deemed otherwise due to the time of year or sporadic nature of a survey. If surveyed from January to May, many *Halophila* spp. may be absent in the form of a living above-ground meadow but thriving in the form of a living seed bank. The direct removal of seeds from dredging the seafloor for land reclamation or burial of seeds beyond depths germination is possible, could alter the presence of the meadow in future years (Birch, 1981; Erftemeijer and Robin Lewis, 2006). However, the threat is likely low given 1) the large, patchy scale at which deep-water seagrasses are found in the GBR lagoon (Coles et al., 2009) and 2) the re-distribution of sediments by natural wave and bottom stress means seeds are likely relocated over large areas during storms and large tidal action as a means to disperse seeds and support meadow generation (Fonseca et al., 2008). It is important to account for varying seagrass life history strategies and distinguish between a truly absent seagrass meadow and one that may be naturally present but locked away in the sediments until growing conditions return.

6.4 Future Directions

The research outcomes from this thesis have opened a window to explore many avenues of deep-water seagrass communities and the broader seagrass research field. Specifically, this thesis opens up a number of emerging lines of research including:

1. What are the growth dynamics and drivers of lesser studied *Halophila* species? Little is still known about other more elusive species that did not grow in the deep-water meadows studied here, but are relatively common and make up a substantial part of the deep-water seagrass habitat of the GBR (Coles et al 2009). The one-year

dataset at Keswick Island was not sufficiently long enough to explore the *H. tricostata* growth dynamics and *H. spinulosa* was only encountered off Bowen where it could be collected for laboratory studies. This limited timeframe studying these genetically and morphologically similar species (Waycott et al., 2002) is part of a handful of studies on either *H. spinulosa* or *H. tricostata* over the last 40 years (Birch, 1981; Kuo et al., 1993; Burkholder et al., 2013; Weatherall et al., 2016). Specific focus on these frequently observed but little studied Indo-Pacific species would be a natural progression after demonstrating the diversity of phenology, resilience and reproductive outcomes for deep-water *Halophila* spp.

2. What extrinsic factors drive annual seed germination in *Halophila* spp.? The strong annual cyclic pattern of germination and rapid growth in *H. decipiens* was dependent on the magnitude of the seed bank generated during the previous year (Chapter 4). It is not clear, however, what drives germination itself following the wet season. The most common factor studied to drive germination for *Halophila* spp. has been light availability (McMillan, 1987; 1988b; Strydom et al., 2017a). One study did explore other common seed germination cues for *H. spinulosa* but could not identify any clear extrinsic factor responsible for germination patterns (Birch, 1981). Some exploratory experiments into the role of light, specifically spectrally-adjusted light to mimic deep-water conditions, was not able to detect a significant effect on germination success (pers. obs.).

3. What role do burrowing crustaceans (and other burrowing organisms) play in cycling seed, seed bank viability and germination? The burrowing and mounding

activity described in Chapter 4 around the Lizard Island site may be an example of an important positive feedback mechanism as suggested by Fonseca et al. (2008) for deep-water seagrass seed banks. A study exploring the mechanical action and re-distribution of seeds by infauna and its effect on the seed bank would provide useful insights into this relationship.

4. What proportion of the deep-water seed bank remains in the sediment and viable over time? Many studies assess seed viability in shallow seagrasses using well established stains. These have been ineffective when trialled on *Halophila* seeds. New research by Waite et al. (2020) is exploring viability tests applying alternative methods used for terrestrial plants that x-ray seeds to observe the seed coat as an indicator of viability. Further work is needed to see if it works on other deep-water species besides *H. ovalis*.

5. How do these deep-water *Halophila* species function as pioneer species in shallower turbid environments? *Halophila* species as a whole are considered important early colonising seagrasses; establishing in a new area of sediment quicker as so-called "r" strategists than other slower and more enduring seagrasses species (Kilminster et al., 2015; Connolly et al., 2018). Plants can change the biotic and abiotic characteristics of soil and in turn affecting successional processes (Piercey et al., 2020). Little is known still as to how this small-bodied genera influences sediment chemistry or sediment stabilisation to facilitate more enduring seagrasses to establish. Whether early colonising species are a necessity for more enduring seagrasses to re-establish or simply present first as a consequence of their

life history strategy is unclear, but studies have shown tropical meadow recovery time can be accelerated by such faster-growing species (Kenworthy et al., 2018). Further research in GBR-specific meadows would be insightful.

6. Are the endogenous signals manifested in *H. decipiens* regulated in a similar pattern for other annual-like seagrasses and how else can metabolomics integrate into ecological seagrass studies? It was encouraging to see endogenous signals, well known in terrestrial literature, adhere to an expected pattern over the life cycle of a seagrass. There is a wealth of research directions to explore seagrass growth and development with metabolomics and even further by incorporating other 'omics' into seagrass research (Davey et al., 2016; Kumar et al., 2016). We have limited understanding of adaptation of plants to a submerged lifestyle at the molecular level but clues are starting to emerge (Franssen et al., 2011; Golicz et al., 2015; Griffiths et al., 2020; Nguyen et al., 2020; Ruocco et al., 2020). Further exploration around how external stimuli can trigger internal cues at key life stage events (e.g. flowering) will play an important step in understanding the interplay of the plant's environment, evolutionary adaptation, and its phenotypic expression through metabolic pathways. Better understanding of these relationships will promote better management of acute anthropogenic disturbances like dredging and a more comprehensive and integrative approach to implement restoration programs (Orth et al., 2002; Tan et al., 2020).

7. Can metabolomics or targeted metabolite profiling be applied as a realistic management tool? Aquatic biomonitoring uses various metrics or biomarkers to

assess environmental condition. Metabolomics is an emerging field of applied research that has the potential to assess overall organism fitness and can detect stress-driven changes as a new holistic type of biomarker (Pomfret et al., 2020). This thesis provides early evidence that the seagrass metabolome may act as a bioindicator of marine health and provide a new management tool to mitigate impacts, such as around key life stage development in deep-water seagrasses. Exploring how a rapid test or measure of metabolic state in seagrasses could act as a bioindicator is a valuable area of future research emerging from this thesis.

8. Is there a case of misidentification in *Halophila* species? For a biological or ecological study, the correct identification of the subject matter underpins the outcomes. The anatomy of *H. decipiens* includes the clear presence of minute hairs on both the adaxial and abaxial surfaces of each leaf. Larkum (1995) described a new tropical reef species of *Halophila* endemic to the Coral Sea, *Halophila capricorni*, with near identical anatomical features except for the lack of hairs on the adaxial leaf surface. *H. capricorni* was described as growing in more classic reef environments offshore in clearer water than where *H. decipiens* is typically found. Observations during this thesis at both Green Island and Lizard Island found shoots that fit the physical description of *H. capricorni* when observed with the naked eye. On closer inspection under the microscope, the lack of hairs was actually reduced hair length on the adaxial surface, but they were always present confirming what would be deemed *H. decipiens*. Recent sequencing has also confirmed that shoots would be deemed *H. capricorni* based on Larkum's description was in fact the identical to *H. decipiens* samples (van Dijk pers.

comm.). Further sampling and herbarium specimens need to be collected and sequencing run across a variety of GBR reef and inshore environments to confirm the disparities in growth form are purely adaptation to the local growing environment rather than a divergent lineage in the genera. A study into the role of these micro-hairs on the physiology of *H decipiens* would be a fascinating look into the adaptation of this species within the niche of marginal habitats it has embraced and one I hope to advance.

6.5 Conclusion

In summary, this thesis has led to major advances in our understanding of deep-water seagrass habitats in the tropics. It has provided new insights on the growth strategies that drive spatial and seasonal dynamics of tropical deep-water *Halophila* communities in the Great Barrier Reef lagoon including:

1. *H decipiens* is a true annual plant with programmed senescence irrespective of variations in light and temperature levels during normal growing conditions and is corroborated by underlying metabolic profiles that mimic terrestrial plant patterns in life stage transition.

2. A local, substantial seed bank is important for longevity of *Halophila* meadows. The presence of a persistent seed bank likely ensures deep-water GBR seagrasses can re-populate areas when suitable germination conditions return.

3. Deep-water *Halophila* communities reside at the margins of their functional limits. As such, relatively short periods of light attenuating events, like dredging or flood plumes, may be detrimental to deep-water seagrass populations. Even relatively short periods of increased light attenuation can affect key life history events used to ensure long-term meadow maintenance such as flowering and seed bank generation.

4. Light thresholds have been derived that can be used to manage acute stress events such as dredging campaigns. Their application is most critical during key growing windows, i.e. leading up and including when plants are flowering/fruiting.

5. This thesis highlights hitherto undetected responses of closely related species to light and temperature conditions. This has implications for making broad-based management decisions using information from a single studied species to forecast broader genera outcomes.

REFERENCES

Abal, E.G., Loneragan, N., Bowen, P., Perry, C.J., Udy, J.W., and Dennison, W.C. (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., Collier, C.J., Uthicke, S., Ow, Y.X., Langlois, L., and O'Brien, K.R. (2017). Model fit versus biological relevance: Evaluating photosynthesis-temperature models for three tropical seagrass species. *Scientific reports* 7.

Alcoverro, T., Manzanera, M., and Romero, J. (2001). Annual metabolic carbon balance of the seagrass *Posidonia oceanica*: the importance of carbohydrate reserves. *Marine Ecology Progress Series* 211**,** 105-116.

Alcoverro, T., Zimmerman, R.C., Kohrs, D.G., and Alberte, R.S. (1999). Resource allocation and sucrose mobilization in light-limited eelgrass *Zostera marina*. *Marine Ecology Progress Series* 187**,** 121-131. doi: 10.3354/meps187121.

Arnaud-Haond, S., Duarte, C.M., Diaz-Almela, E., Marbà, N., Sintes, T., and Serrão, E.A. (2012). Implications of extreme life span in clonal organisms: Millenary clones in meadows of the threatened seagrass *Posidonia oceanica*. *PLOS ONE* 7(2)**,** e30454. doi: 10.1371/journal.pone.0030454.

Atkinson, M., and Smith, S. (1983). C: N: P ratios of benthic marine plants. *Limnology and Oceanography* 28(3)**,** 568-574.

Barbier, E.B., Hacker, S.D., Kennedy, C., Koch, E.W., Stier, A.C., and Silliman, B.R. (2011). The value of estuarine and coastal ecosystem services. *Ecological Monographs* 81(2)**,** 169-193.

Bartoń, K. (2013). MuMIn: Multi-model inference. R package version 1.9. 13. *The Comprehensive R Archive Network (CRAN), Vienna, Austria*.

Bates, D., Maechler, M., and Bolker, B. (2012). lme4: Linear mixed-effects models using S4 classes.

Beca-Carretero, P., Guihéneuf, F., Winters, G., and Stengel, D.B. (2019). Depth-induced adjustment of fatty acid and pigment composition suggests high biochemical plasticity in the tropical seagrass *Halophila stipulacea*. *Marine Ecology Progress Series* 608**,** 105-117.

Beer, S., Mtolera, M., Lyimo, T., and Björk, M. (2006). The photosynthetic performance of the tropical seagrass *Halophila ovalis* in the upper intertidal. *Aquatic botany* 84(4)**,** 367-371.

Beer, S., and Waisel, Y. (1982). Effects of light and pressure on photosynthesis in two seagrasses. *Aquatic Botany* 13(0)**,** 331-337. doi: 10.1016/0304-3770(82)90068-7.

Bell, S., Fonseca, M., and Kenworthy, W. (2008). Dynamics of a subtropical seagrass landscape: links between disturbance and mobile seed banks. *Landscape Ecology* 23(0)**,** 67-74. doi: 10.1007/s10980-007-9137-z.

Bessey, C., Heithaus, M., Fourqurean, J., Gastrich, K., and Burkholder, D. (2016). Importance of teleost macrograzers to seagrass composition in a subtropical ecosystem with abundant populations of megagrazers and predators. *Marine Ecology Progress Series* 553**,** 81-92.

Biber, P.D., Paerl, H.W., Gallegos, C.L., Kenworthy, W.J., and Fonseca, M.S. (2005). Evaluating indicators of seagrass stress to light. *Estuarine indicators. CRC Press, Boca Raton, Florida***,** 193-209.

Birch, W. (1981). Morphology of germinating seeds of the seagrass *Halophila spinulosa* (R. Br.) Aschers.(Hydrocharitaceae). *Aquatic Botany* 11**,** 79-90.

Bité, J.S., Campbell, S.J., McKenzie, L.J., and Coles, R.G. (2007). Chlorophyll fluorescence measures of seagrasses *Halophila ovalis* and *Zostera capricorni* reveal differences in response to experimental shading. *Marine Biology* 152(2)**,** 405.

Blackburn, N.J., and Orth, R.J. (2013). Seed burial in eelgrass *Zostera marina*: the role of infauna. *Marine Ecology Progress Series* 474**,** 135-145.

Borum, J., Larkum, A.W.D., Orth, R.J., Duarte, C.M., Sand-Jensen, K., Binzer, T., et al. (2006). "Oxygen Movement in Seagrasses," in *Seagrasses: Biology, Ecology and Conservation*. (Springer Netherlands), 255-270.

Brearley, A., Kendrick, G.A., and Walker, D.I. (2008). How does burrowing by the isopod *Limnoria agrostisa* (Crustacea: Limnoriidae) affect the leaf canopy of the southern Australian seagrass *Amphibolis griffithii*? *Marine Biology* 156(1)**,** 65-77. doi: 10.1007/s00227-008-1065-1.

BREE (2012). "Australian bulk commodity export and infrastructure: Outlook to 2025". (Canberra, Australia).

Bryant, C.V., Davies, J.D., Jarvis, J.C., Tol, S., and Rasheed, M.A. (2013). "Seagrasses in Port Curtis and Rodds Bay 2013: Annual long term monitoring, biannual Western Basin surveys & updated baseline survey". (Cairns: Centre for Tropical Water & Aquatic Ecosystem Research (TropWATER), James Cook University).

Buesa, R. (1975). Population biomass and metabolic rates of marine angiosperms on the northwestern Cuban shelf. *Aquatic Botany* 1**,** 11-23.

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

Burke, M., Dennison, W., and Moore, K. (1996). Non-structural carbohydrate reserves of eelgrass *Zostera marina*. *Marine Ecology Progress Series* 137**,** 195-201. doi: 10.3354/meps137195.

Burkholder, D.A., Fourqurean, J.W., and Heithaus, M.R. (2013). Spatial pattern in seagrass stoichiometry indicates both N-limited and P-limited regions of an iconic P-limited subtropical bay. *Marine Ecology Progress Series* 472**,** 101-115.

Burnham, K., and Anderson, D. (2002). Model Selection and Multimodal Inference: A Practical Information-Theoretic Approach. 2nd edn Springer-Verlag. *New York, NY*.

Cabaço, S., and Santos, R. (2010). Reproduction of the eelgrass *Zostera marina* at the species southern distributional limit in the Eastern Atlantic. *Marine Ecology* 31(2)**,** 300-308.

Cabaço, S., and Santos, R. (2012). Seagrass reproductive effort as an ecological indicator of disturbance. *Ecological Indicators* 23(0)**,** 116-122. doi: 10.1016/j.ecolind.2012.03.022.

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

Cambridge, M.L., and Lambers, H. (1998). Specific leaf area and functional leaf anatomy in Western Australian seagrasses. *Inherent variation in plant growth. Physiological mechanisms and ecological consequences. Leiden, the Netherlands: Backhuys Publishers***,** 89-99.

Campbell, S.J., McKenzie, L.J., Kerville, S.P., and Bité, J.S. (2007). Patterns in tropical seagrass photosynthesis in relation to light, depth and habitat. *Estuarine, Coastal and Shelf Science* 73(3-4)**,** 551-562.

Campbell, S.J., and Miller, C.J. (2002). Shoot and abundance characteristics of the seagrass *Heterozostera tasmanica* in Westernport estuary (south-eastern Australia). *Aquatic Botany* 73(1)**,** 33-46.

Carruthers, T.J.B., Dennison, W.C., Longstaff, B.J., Waycott, M., Abal, E.G., McKenzie, L.J., et al. (2002). Seagrass habitats of Northeast Australia: Models of key processes and controls. *Bulletin of Marine Science* 71(3)**,** 1153-1169.

Chartrand, K.M., Bryant, C.V., Carter, A.B., Ralph, P.J., and 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. doi: doi.org/10.3389/fmars.2016.00106.

Chartrand, K.M., Szabó, M., Sinutok, S., Rasheed, M.A., and Ralph, P.J. (2018). Living at the margins–The response of deep-water seagrasses to light and temperature renders them susceptible to acute impacts. *Marine environmental research* 136**,** 126-138.

Chen, D., Shao, Q., Yin, L., Younis, A., and Zheng, B. (2019). Polyamine function in plants: Metabolism, regulation on development, and roles in abiotic stress responses. *Frontiers in Plant Science* 9(1945). doi: 10.3389/fpls.2018.01945.

Coles, R., Long, W.L., Squire, B., Squire, L., and 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.

Coles, R., McKenzie, L., De'ath, G., Roelofs, A.J., and Lee Long, W. (2009). Spatial distribution of deepwater seagrass in the inter-reef lagoon of the Great Barrier Reef World Heritage Area. *Marine Ecology Progress Series* 392**,** 57-68. doi: 10.3354/meps08197.

Coles, R.G., Rasheed, M.A., McKenzie, L.J., Grech, A., York, P.H., Sheaves, M., et al. (2015). The Great Barrier Reef World Heritage Area seagrasses: managing this iconic Australian ecosystem resource for the future. *Estuarine, Coastal and Shelf Science* 153**,** A1-A12.

Collier, C., Adams, M., Langlois, L., Waycott, M., O'Brien, K., Maxwell, P., et al. (2016a). Thresholds for morphological response to light reduction for four tropical seagrass species. *Ecological Indicators* 67**,** 358-366. doi: doi:10.1016/j.ecolind.2016.02.050.

Collier, C., and Waycott, M. (2014). Temperature extremes reduce seagrass growth and induce mortality. *Marine pollution bulletin* 83(2)**,** 483-490.

Collier, C., Waycott, M., and McKenzie, L. (2012a). Light thresholds derived from seagrass loss in the coastal zone of the northern Great Barrier Reef, Australia. *Ecological Indicators* 23**,** 211-219.

Collier, C.J., Chartrand K.M., Honchin C., Fletcher A., and M.A., R. (2016b). "Light thresholds for seagrasses of the GBR: a synthesis and guiding document. Including knowledge gaps and future priorities", in: *Report to the National Environmental Science Programme.* (Cairns).

Collier, C.J., Lavery, P.S., Ralph, P.J., and Masini, R.J. (2009). Shade-induced response and recovery of the seagrass *Posidonia sinuosa*. *Journal of Experimental Marine Biology and Ecology* 370(1)**,** 89-103.

Collier, C.J., Uthicke, S., and 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. doi: 10.4319/lo.2011.56.6.2200.

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

Connolly, R.M., Jackson, E.L., Macreadie, P.I., Maxwell, P.S., and O'Brien, K.R. (2018). "Seagrass dynamics and resilience," in *Seagrasses of Australia*. (Springer), 197-212.

Cosgrove, J., and Borowitzka, M.A. (2010). "Chlorophyll fluorescence terminology: an introduction," in *Chlorophyll a fluorescence in aquatic sciences: methods and applications*. (Springer), 1-17.

Costa, M.M., Barrote, I., Silva, J., and Santos, R. (2014). *Photosynthetic pigment content in seagrasses. In: Effect of high CO_2 and ocean acidification on photosynthesis and response to oxidative stress in seagrasses.* . Universidade do Algarve.

Costanza, R., d'Arge, R., De Groot, R., Farber, S., Grasso, M., Hannon, B., et al. (1997). The value of the world's ecosystem services and natural capital. *Nature* 387(6630)**,** 253-260.

Costanza, R., de Groot, R., Sutton, P., van der Ploeg, S., Anderson, S.J., Kubiszewski, I., et al. (2014). Changes in the global value of ecosystem services. *Global Environmental Change* 26**,** 152-158.

Creelman, R.A., and Mullet, J.E. (1995). Jasmonic acid distribution and action in plants: regulation during development and response to biotic and abiotic stress. *Proceedings of the National Academy of Sciences* 92(10)**,** 4114-4119.

Creelman, R.A., and Mulpuri, R. (2002). The oxylipin pathway in Arabidopsis. *The arabidopsis book* 1**,** e0012-e0012. doi: 10.1199/tab.0012.

Cullen-Unsworth, L., and Unsworth, R. (2013). Seagrass meadows, ecosystem services, and sustainability. *Environment: Science and Policy for Sustainable Development* 55(3)**,** 14-28.

Cullen-Unsworth, L.C., Nordlund, L.M., Paddock, J., Baker, S., McKenzie, L.J., and Unsworth, R.K. (2014). Seagrass meadows globally as a coupled social–ecological system: Implications for human wellbeing. *Marine Pollution Bulletin* 83(2)**,** 387-397.

Cummings, M.E., and Zimmerman, R.C. (2003). Light harvesting and the package effect in the seagrasses *Thalassia testudinum* Banks ex König and *Zostera marina* L.: optical constraints on photoacclimation. *Aquatic Botany* 75(3)**,** 261-274. doi: 10.1016/S0304-3770(02)00180-8.

Czerny, A.B., and Dunton, K.H. (1995). The effects of in situ light reduction on the growth of two subtropical seagrasses, *Thalassia testudinum* and *Halodule wrightii*. *Estuaries and Coasts* 18(2)**,** 418-427.

Dattolo, E., Ruocco, M., Brunet, C., Lorenti, M., Lauritano, C., D'esposito, D., et al. (2014). Response of the seagrass Posidonia oceanica to different light environments: Insights from a combined molecular and photo-physiological study. *Marine environmental research* 101**,** 225-236.

Davey, P.A., Pernice, M., Sablok, G., Larkum, A., Lee, H.T., Golicz, A., et al. (2016). The emergence of molecular profiling and omics techniques in seagrass biology; furthering our understanding of seagrasses. *Functional & Integrative Genomics***,** 1-16.

Davies, P., and Gan, S. (2012). Towards an integrated view of monocarpic plant senescence. *Russian Journal of Plant Physiology* 59(4), 467-478.
Davies, P.J. (2010). "The plant hormones: their nature, occurrence, and functions," in *Plant hormones*. (Springer), 1-15.
Dawes, C.J., Lobban, C.S., and Tomasko, D.A. (1989). A comparison of the physiological ecology of the seagrasses *Halophila decipiens* Ostenfeld and *H. Johnsonii* Eiseman from Florida. *Aquatic Botany* 33(1–2), 149-154. doi: 10.1016/0304-3770(89)90028-4.
de Kock, W., Hasler-Sheetal, H., Holmer, M., Tsapakis, M., and Apostolaki, E.T. (2020). Metabolomics and traditional indicators unveil stress of a seagrass (*Cymodocea nodosa*) meadow at intermediate distance from a fish farm. *Ecological Indicators* 109, 105765. doi: 10.1016/j.ecolind.2019.105765.
de los Santos, C., Brun, F., Bouma, T., Vergara, J., and Perez-Llorens, J. (2010). Acclimation of seagrass *Zostera noltii* to co-occurring hydrodynamic and light stresses. *Marine Ecology Progress Series* 398, 127-135. doi: 10.3354/meps08343.
den Hartog, C. (1970). *The sea-grasses of the world.* Amsterdam: North-Holland Publishing Company.
den Hartog, C., and Kuo, J. (2006). "Taxonomy and biogeography of seagrasses," in *Seagrasses: Biology, Ecology and Conservation*. (Springer), 1-23.
Dennison, W.C. (1987). Effects of light on seagrass photosynthesis, growth and depth distribution. *Aquatic Botany* 27(1), 15-26.
Dennison, W.C., and Alberte, R.S. (1986). Photoadaptation and growth of *Zostera marina* L.(eelgrass) transplants along a depth gradient. *Journal of Experimental Marine Biology and Ecology* 98(3), 265-282.
Dennison, W.C., Orth, R.J., Moore, K.A., Stevenson, J.C., Carter, V., Kollar, S., et al. (1993). Assessing water quality with submersed aquatic vegetation. *BioScience* 43(2), 86-94. doi: 10.2307/1311969.
Department of Agriculture and Fisheries (2015). Available: http://www.daf.qld.gov.au [Accessed].
Devlin, M., McKinna, L., Alvarez-Romero, J., Petus, C., Abott, B., Harkness, P., et al. (2012). Mapping the pollutants in surface riverine flood plume waters in the Great Barrier Reef, Australia. *Marine Pollution Bulletin* 65(4-9), 224-235.
DeWitt, T.H. (2009). 10.0 The Effects of Bioturbation and Bioirrigation on Seagrasses. *Seagrasses and Protective Criteria: A Review and Assessment of Research Status*.
Duarte, C.M. (1990). Seagrass nutrient content. *Marine Ecology Progress Series* 6(2), 201-207.
Duarte, C.M. (1991). Seagrass depth limits. *Aquatic Botany* 40(4), 363-377.
Duarte, C.M., and Chiscano, C.L. (1999). Seagrass biomass and production: a reassessment. *Aquatic Botany* 65(1), 159-174.
Duarte, C.M., Marbà, N., Gacia, E., Fourqurean, J.W., Beggins, J., Barrón, C., et al. (2010). Seagrass community metabolism: Assessing the carbon sink capacity of seagrass meadows. *Global Biogeochemical Cycles* 24(4), GB4032. doi: 10.1029/2010GB003793.
Duarte, C.M., Terrados, J., Agawin, N.S., Fortes, M.D., Bach, S., and Kenworthy, W.J. (1997). Response of a mixed Philippine seagrass meadow to experimental burial. *Marine Ecology Progress Series* 147, 285-294.

Durako, M.J. (2007). Leaf optical properties and photosynthetic leaf absorptances in several Australian seagrasses. *Aquatic Botany* 87(1), 83-89. doi: 10.1016/j.aquabot.2007.03.005.

Durako, M.J., Kunzelman, J.I., Kenworthy, W.J., and Hammerstrom, K.K. (2003). Depth-related variability in the photobiology of two populations of *Halophila johnsonii* and *Halophila decipiens*. *Marine Biology* 142(6), 1219-1228. doi: 10.1007/s00227-003-1038-3.

Enerstvedt, K.H., Lundberg, A., Sj, I.K., Fadnes, P., and Jordheim, M. (2017). Characterization and seasonal variation of individual flavonoids in Zostera marina and Zostera noltii from Norwegian coastal waters. *Biochemical Systematics and Ecology* 74, 42-50.

Enriquez, S. (2005). Light absorption efficiency and the package effect in the leaves of the seagrass *Thalassia testudinum*. *Marine Ecology Progress Series* 289, 141-150. doi: 10.3354/meps289141.

Enríquez, S., and Rodríguez-Román, A. (2006). Effect of water flow on the photosynthesis of three marine macrophytes from a fringing-reef lagoon. *Marine Ecology Progress Series* 323, 119-132.

Entrambasaguas, L., Jahnke, M., Biffali, E., Borra, M., Sanges, R., Marín-Guirao, L., et al. (2017). Tissue-specific transcriptomic profiling provides new insights into the reproductive ecology and biology of the iconic seagrass species *Posidonia oceanica*. *Marine Genomics* 35, 51-61. doi: 10.1016/j.margen.2017.05.006.

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

Erftemeijer, P.L.A., and Stapel, J. (1999). Primary production of deep-water *Halophila ovalis* meadows. *Aquatic Botany* 65(1-4), 71-82.

Esteban, N., Unsworth, R., Gourlay, J., and Hays, G. (2018). The discovery of deep-water seagrass meadows in a pristine Indian Ocean wilderness revealed by tracking green turtles. *Marine Pollution Bulletin* 134, 99-105.

Evans, J.R. (2009). Potential errors in electron transport rates calculated from chlorophyll fluorescence as revealed by a multilayer leaf model. *Plant and Cell Physiology* 50(4), 698-706.

Fabricius, K., Logan, M., Weeks, S., Lewis, S., and Brodie, J. (2016). Changes in water clarity in response to river discharges on the Great Barrier Reef continental shelf: 2002–2013. *Estuarine, Coastal and Shelf Science* 173, A1-A15.

Falkowski, P.G., and Raven, J.A. (2007). *Aquatic Photosynthesis.* Princeton University Press.

Ferreira, C., Horta, P.A., Almeida, G.M., Zitta, C.S., de M. Oliveira, E., Gueye, M.B.Y.B., et al. (2015). Anatomical and ultrastructural adaptations of seagrass leaves: an evaluation of the southern Atlantic groups. *Protoplasma* 252(1), 3-20. doi: 10.1007/s00709-014-0661-9.

Fong, Y., Huang, Y., Gilbert, P.B., and Permar, S.R. (2017). chngpt: threshold regression model estimation and inference. *BMC bioinformatics* 18(1), 454.

Fonseca, M.S. (1989). Sediment stabilization by *Halophila decipiens* in comparison to other seagrasses. *Estuarine, Coastal and Shelf Science* 29(5), 501-507.

Fonseca, M.S., Kenworthy, W.J., Griffith, E., Hall, M.O., Finkbeiner, M., and Bell, S.S. (2008). Factors influencing landscape pattern of the seagrass *Halophila decipiens* in an oceanic setting. *Estuarine, Coastal and Shelf Science* 76(1), 163-174.

Fournier, D.A., Skaug, H.J., Ancheta, J., Ianelli, J., Magnusson, A., Maunder, M.N., et al. (2012). AD Model Builder: using automatic differentiation for statistical inference of highly parameterized complex nonlinear models. *Optimization Methods and Software* 27(2)**,** 233-249.

Fourqurean, J., and Zieman, J. (1991). Photosynthesis, respiration and whole plant carbon budget of the seagrass *Thalassia testudinum*. *Marine Ecology Progress Series* 69(1)**,** 161-170.

Franssen, S.U., Gu, J., Bergmann, N., Winters, G., Klostermeier, U.C., Rosenstiel, P., et al. (2011). Transcriptomic resilience to global warming in the seagrass Zostera marina, a marine foundation species. *Proceedings of the National Academy of Sciences* 108(48)**,** 19276-19281.

Fraser, M.W., Short, J., Kendrick, G., McLean, D., Keesing, J., Byrne, M., et al. (2017). Effects of dredging on critical ecological processes for marine invertebrates, seagrasses and macroalgae, and the potential for management with environmental windows using Western Australia as a case study. *Ecological Indicators* 78**,** 229-242.

Friedman, J., and Rubin, M.J. (2015). All in good time: understanding annual and perennial strategies in plants. *American journal of botany* 102(4)**,** 497-499.

Gan, S., and Amasino, R.M. (1995). Inhibition of leaf senescence by autoregulated production of cytokinin. *Science* 270(5244)**,** 1986-1988.

Gattuso, J.-P., Gentili, B., Duarte, C., Kleypas, J., Middelburg, J., and Antoine, D. (2006). Light availability in the coastal ocean: impact on the distribution of benthic photosynthetic organisms and contribution to primary production. *Biogeosciences Discussions* 3(4)**,** 895-959.

Genva, M., Akong, F.O., Andersson, M.X., Deleu, M., Lins, L., and Fauconnier, M.-L. (2019). New insights into the biosynthesis of esterified oxylipins and their involvement in plant defense and developmental mechanisms. *Phytochemistry Reviews* 18(1)**,** 343-358.

Glasby, T.M., Taylor, S.L., and Housefield, G.P. (2015). Factors influencing the growth of seagrass seedlings: A case study of *Posidonia australis*. *Aquatic Botany* 120**,** 251-259.

Golicz, A.A., Schliep, M., Lee, H.T., Larkum, A.W., Dolferus, R., Batley, J., et al. (2015). Genome-wide survey of the seagrass *Zostera muelleri* suggests modification of the ethylene signalling network. *Journal of Experimental Botany***,** eru510.

Goodman, J.L., Moore, K.A., and Dennison, W.C. (1995). Photosynthetic responses of eelgrass (*Zostera marina* L.) to light and sediment sulfide in a shallow barrier island lagoon. *Aquatic Botany* 50(1)**,** 37-47.

Gordon, D., Grey, K., Chase, S., and Simpson, C. (1994). Changes to the structure and productivity of a *Posidonia sinuosa* meadow during and after imposed shading. *Aquatic Botany* 47(3)**,** 265-275.

Great Barrier Reef Marine Park Authority (2015). "Great Barrier Reef Outlook Report 2014". (Townsville: GBRMPA).

Grech, A., Bos, M., Brodie, J., Coles, R., Dale, A., Gilbert, R., et al. (2013). Guiding principles for the improved governance of port and shipping impacts in the Great Barrier Reef. *Marine Pollution Bulletin* 75(1)**,** 8-20.

Grech, A., Chartrand-Miller, K., Erftemeijer, P., Fonseca, M., McKenzie, L., Rasheed, M., et al. (2012). A comparison of threats, vulnerabilities and management

approaches in global seagrass bioregions. *Environmental Research Letters* 7(2), 024006. doi: 10.1088/1748-9326/7/2/024006.
Grech, A., Coles, R., and Marsh, H. (2011). A broad-scale assessment of the risk to coastal seagrasses from cumulative threats. *Marine Policy* 35(5), 560-567. doi: 10.1016/j.marpol.2011.03.003.
Grech, A., Wolter, J., Coles, R., McKenzie, L., Rasheed, M., Thomas, C., et al. (2016). Spatial patterns of seagrass dispersal and settlement. *Diversity and Distributions* 22(11), 1150-1162.
Green, E., and Short, F. (2003). World Atlas of Seagrasses. Prepared by the UNEP World Conservation Monitoring Centre. *University of California, Press Berkeley, USA*.
Grice, A.M., Loneragan, N.R., and Dennison, W.C. (1996). Light intensity and the interactions between physiology, morphology and stable isotope ratios in five species of seagrass. *Journal of Experimental Marine Biology and Ecology* 195(1), 91-110.
Griffiths, L., Melvin, S., Connolly, R., Pearson, R., and Brown, C. (2020). Metabolomic indicators for low-light stress in seagrass. *Ecological Indicators* 114, 106316.
Gu, J., Weber, K., Klemp, E., Winters, G., Franssen, S.U., Wienpahl, I., et al. (2012). Identifying core features of adaptive metabolic mechanisms for chronic heat stress attenuation contributing to systems robustness. *Integrative Biology* 4(5), 480-493.
Guijas, C., Montenegro-Burke, J.R., Warth, B., Spilker, M.E., and Siuzdak, G. (2018). Metabolomics activity screening for identifying metabolites that modulate phenotype. *Nature Biotechnology* 36(4), 316-320. doi: 10.1038/nbt.4101.
Hammerstrom, K.K., Kenworthy, W.J., Fonseca, M.S., and Whitfield, P.E. (2006). Seed bank, biomass, and productivity of *Halophila decipiens*, a deep water seagrass on the west Florida continental shelf. *Aquatic Botany* 84(2), 110-120.
Häusler, R.E., Ludewig, F., and Krueger, S. (2014). Amino acids – a life between metabolism and signaling. *Plant Science* 229, 225-237.
Hayes, M.A., McClure, E.C., York, P.H., Jinks, K.I., Rasheed, M.A., Sheaves, M., et al. (2020). The differential importance of deep and shallow seagrass to Nekton assemblages of The Great Barrier Reef. *Diversity* 12(8), 292.
Hays, G.C., Alcoverro, T., Christianen, M.J., Duarte, C.M., Hamann, M., Macreadie, P.I., et al. (2018). New tools to identify the location of seagrass meadows: marine grazers as habitat indicators. *Frontiers in Marine Science* 5, 9.
Heck, K.L.J., Carruthers, T.J., Duarte, C.M., Hughes, A.R., Kendrick, G., Orth, R.J., et al. (2008). Trophic transfers from seagrass meadows subsidize diverse marine and terrestrial consumers. *Ecosystems* 11(7), 1198-1210. doi: 10.1007/s10021-008-9155-y.
Hemminga, M.A., and Duarte, C.M. (2000). *Seagrass ecology.* Cambridge University Press.
Hildebrandt, T.M., Nesi, A.N., Araújo, W.L., and Braun, H.-P. (2015). Amino acid catabolism in plants. *Molecular Plant* 8(11), 1563-1579.
Hou, Q., Ufer, G., and Bartels, D. (2016). Lipid signalling in plant responses to abiotic stress. *Plant, Cell & Environment* 39(5), 1029-1048.
Hovey, R.K., Statton, J., Fraser, M.W., Ruiz-Montoya, L., Zavala-Perez, A., Rees, M., et al. (2015). Strategy for assessing impacts in ephemeral tropical seagrasses. *Marine pollution bulletin* 101(2), 594-599.

Hu, L., Robert, C.A., Cadot, S., Zhang, X., Ye, M., Li, B., et al. (2018). Root exudate metabolites drive plant-soil feedbacks on growth and defense by shaping the rhizosphere microbiota. *Nature Communications* 9(1)**,** 1-13.

Huang, C.-K., Chang, B.-S., Wang, K.-C., Her, S.-J., Chen, T.-W., Chen, Y.-A., et al. (2004). Changes in polyamine pattern are involved in floral initiation and development in *Polianthes tuberosa*. *Journal of plant physiology* 161(6)**,** 709-713.

Huang, H., Liu, B., Liu, L., and Song, S. (2017). Jasmonate action in plant growth and development. *Journal of Experimental Botany* 68(6)**,** 1349-1359.

Hughes, A.R., Williams, S.L., Duarte, C.M., Heck Jr, K.L., and Waycott, M. (2008). Associations of concern: declining seagrasses and threatened dependent species. *Frontiers in Ecology and the Environment* 7(5)**,** 242-246. doi: 10.1890/080041.

Huijser, P., and Schmid, M. (2011). The control of developmental phase transitions in plants. *Development* 138(19)**,** 4117-4129.

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

Jacobs, R., and Dicks, B. (1985). Seagrasses in the Zeit Bay area and at Ras Gharib (Egyptian Red Sea coast). *Aquatic Botany* 23(2)**,** 137-147.

Jancewicz, A.L., Gibbs, N.M., and Masson, P.H. (2016). Cadaverine's functional role in plant development and environmental response. *Frontiers in plant science* 7**,** 870-870. doi: 10.3389/fpls.2016.00870.

Jarvis, J.C., and Moore, K.A. (2015). Effects of seed source, sediment type, and burial depth on mixed-annual and perennial *Zostera marina* L. seed germination and seedling establishment. *Estuaries and Coasts* 38(3)**,** 964-978.

Jarvis, J.C., Moore, K.A., and Kenworthy, W.J. (2014). Persistence of *Zostera marina* L.(eelgrass) seeds in the sediment seed bank. *Journal of Experimental Marine Biology and Ecology* 459**,** 126-136.

Johnson, C.H., Ivanisevic, J., and Siuzdak, G. (2016). Metabolomics: beyond biomarkers and towards mechanisms. *Nature Reviews Molecular Cell Biology* 17(7)**,** 451-459. doi: 10.1038/nrm.2016.25.

Jørgensen, M.S., Labouriau, R., and Olesen, B. (2019). Seed size and burial depth influence *Zostera marina* L.(eelgrass) seed survival, seedling emergence and initial seedling biomass development. *PLOS ONE* 14(4)**,** e0215157.

Josselyn, M., Fonseca, M., Niesen, T., and Larson, R. (1986). Biomass, production and decomposition of a deep water seagrass, *Halophila decipiens* Ostenfeld. *Aquatic Botany* 25**,** 47-61.

Kahn, A.E., and Durako, M.J. (2009). Wavelength-specific photosynthetic responses of *Halophila johnsonii* from marine-influenced versus river-influenced habitats. *Aquatic Botany* 91(3)**,** 245-249.

Karkalas, J.J. (1985). An improved enzymatic method for the determination of native and modified starch. *Journal of the Science of Food and Agriculture* 36**,** 1019-1027.

Kendrick, G.A., Orth, R.J., Statton, J., Hovey, R., Ruiz Montoya, L., Lowe, R.J., et al. (2017). Demographic and genetic connectivity: the role and consequences of reproduction, dispersal and recruitment in seagrasses. *Biological Reviews* 92(2)**,** 921-938. doi: 10.1111/brv.12261.

Kendrick, G.A., Waycott, M., Carruthers, T.J., Cambridge, M.L., Hovey, R., Krauss, S.L., et al. (2012). The central role of dispersal in the maintenance and persistence of seagrass populations. *Bioscience* 62(1)**,** 56-65.

Kenworthy, W. (2000). The role of sexual reproduction in maintaining populations of *Halophila decipiens*: Implications for the biodiversity and conservation of tropical seagrass ecosystems. *Pacific Conservation Biology* 5(4)**,** 260.

Kenworthy, W.J., Currin, C.A., Fonseca, M.S., and Smith, G. (1989). Production, decomposition, and heterotrophic utilization of the seagrass *Halophila decipiens* in a submarine canyon. *Marine Ecology Progress Series* 51(3)**,** 277-290.

Kenworthy, W.J., Hall, M.O., Hammerstrom, K.K., Merello, M., and Schwartzschild, A. (2018). Restoration of tropical seagrass beds using wild bird fertilization and sediment regrading. *Ecological Engineering* 112**,** 72-81. doi: 10.1016/j.ecoleng.2017.12.008.

Kenworthy, W.J., Wyllie-Echeverria, S., Coles, R.G., Pergent, G., and Pergent-Martini, C. (2006). "Seagrass conservation biology: an interdisciplinary science for protection of the seagrass biome," in *Seagrasses: Biology, ecology and conservation*. (Springer), 595-623.

Kiełkowska, A., and Dziurka, M. (2020). Changes in polyamine pattern mediates sex differentiation and unisexual flower development in monoecious cucumber (*Cucumis sativus* L.). *Physiologia Plantarum*.

Kilminster, K., McMahon, K., Waycott, M., Kendrick, G.A., Scanes, P., McKenzie, L., et al. (2015). Unravelling complexity in seagrass systems for management: Australia as a microcosm. *Science of the Total Environment*.

Kirk, J.T.O. (1994). *Light and photosynthesis in aquatic ecosystems.* Cambridge University Press.

Kirkman, H. (1978). Decline of seagrass in northern areas of Moreton Bay, Queensland. *Aquatic Botany* 5**,** 63-76.

Kitajima, K., and Hogan, K. (2003). Increases of chlorophyll a/b ratios during acclimation of tropical woody seedlings to nitrogen limitation and high light. *Plant, Cell & Environment* 26(6)**,** 857-865.

Kneer, D., Asmus, H., and Jompa, J. (2013). Do burrowing callianassid shrimp control the lower boundary of tropical seagrass beds? *Journal of Experimental Marine Biology and Ecology* 446**,** 262-272.

Koch, E., and Durako, M.J. (1991). In vitro studies of the submerged angiosperm *Ruppia maritima*: Auxin and cytokinin effects on plant growth and development. *Marine Biology* 110(1)**,** 1-6.

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

Krause-Jensen, D., Middelboe, A.L., Sand-Jensen, K., and Christensen, P.B. (2000). Eelgrass, *Zostera marina*, growth along depth gradients: upper boundaries of the variation as a powerful predictive tool. *Oikos* 91(2)**,** 233-244. doi: 10.1034/j.1600-0706.2001.910204.x.

Kroon, F.J., Kuhnert, P.M., Henderson, B.L., Wilkinson, S.N., Kinsey-Henderson, A., Abbott, B., et al. (2012). River loads of suspended solids, nitrogen, phosphorus and herbicides delivered to the Great Barrier Reef lagoon. *Marine Pollution Bulletin* 65(4-9)**,** 167-181.

Kumar, M., Kuzhiumparambil, U., Pernice, M., Jiang, Z., and Ralph, P.J. (2016). Metabolomics: an emerging frontier of systems biology in marine macrophytes. *Algal Research* 16**,** 76-92.

Kuo, J., and den Hartog, C. (2006). "Seagrass morphology, anatomy, and ultrastructure," in *Seagrasses: Biology, Ecology and Conservation*. (Springer), 51-87.

Kuo, J., and Kirkman, H. (1992). Fruits, seeds and germination in the seagrass *Halophila ovalis* (Hydrocharitaceae). *Botanica marina* 35(3)**,** 197-204.

Kuo, J., and Kirkman, H. (1995). *Halophila decipiens* Ostenfeld in estuaries of southwestern Australia. *Aquatic Botany* 51(3)**,** 335-340.

Kuo, J., Long, W.L., and Coles, R. (1993). Occurrence and fruit and seed biology of *Halophila tricostata* Greenway (Hydrocharitaceae). *Marine and Freshwater Research* 44(1)**,** 43-57.

Kurtz, J.C., Yates, D.F., Macauley, J.M., Quarles, R.L., Genthner, F.J., Chancy, C.A., et al. (2003). Effects of light reduction on growth of the submerged macrophyte Vallisneria americana and the community of root-associated heterotrophic bacteria. *Journal of Experimental Marine Biology and Ecology* 291(2)**,** 199-218.

Kuznetsova, A., Brockhoff, P.B., and Christensen, R.H.B. (2015). Package 'lmerTest'. *R package version***,** 2.0-29.

Larkum, A. (1995). *Halophila capricorni* (Hydrocharitaceae): a new species of seagrass from the Coral Sea. *Aquatic Botany* 51(3)**,** 319-328.

Larkum, A.W., Drew, E.A., and Ralph, P.J. (2006). "Photosynthesis and metabolism in seagrasses at the cellular level," in *Seagrasses: Biology, Ecology and Conservation*. (Springer).

Larkum, A.W.D., Waycott, M., and Conran, J.G. (2018). "Evolution and Biogeography of Seagrasses," in *Seagrasses of Australia: Structure, Ecology and Conservation,* eds. A.W.D. Larkum, G.A. Kendrick & P.J. Ralph. (Springer International Publishing), 3-29.

Lee, K.-S., and Dunton, K.H. (1997). Effect of in situ light reduction on the maintenance, growth and partitioning of carbon resources in *Thalassia testudinum* banks ex König. *Journal of Experimental Marine Biology and Ecology* 210(1)**,** 53-73. doi: 10.1016/S0022-0981(96)02720-7.

Lee, K.-S., Park, S.R., and 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.

Lenth, R.V. (2016). Least-squares means: the R Package lsmeans. *Journal of Statistical Software* 69**,** 1-33.

Leong, T.-Y., and Anderson, J.M. (1984). Adaptation of the thylakoid membranes of pea chloroplasts to light intensities. I. Study on the distribution of chlorophyll-protein complexes. *Photosynthesis Research* 5(2)**,** 105-115.

Lewis, S.E., Olley, J., Furuichi, T., Sharma, A., and Burton, J. (2014). Complex sediment deposition history on a wide continental shelf: Implications for the calculation of accumulation rates on the Great Barrier Reef. *Earth and Planetary Science Letters* 393**,** 146-158.

Lichtenthaler, H., Buschmann, C., Döll, M., Fietz, H.-J., Bach, T., Kozel, U., et al. (1981). Photosynthetic activity, chloroplast ultrastructure, and leaf characteristics of high-light and low-light plants and of sun and shade leaves. *Photosynthesis Research* 2(2)**,** 115-141.

Lombardo, V.A., Osorio, S., Borsani, J., Lauxmann, M.A., Bustamante, C.A., Budde, C.O., et al. (2011). Metabolic profiling during peach fruit development and ripening reveals the metabolic networks that underpin each developmental stage. *Plant Physiology* 157(4)**,** 1696-1710.

Longstaff, B.J., and Dennison, W.C. (1999). Seagrass survival during pulsed turbidity events: the effects of light deprivation on the seagrasses *Halodule pinifolia* and *Halophila ovalis*. *Aquatic Botany* 65(1)**,** 105-121.

Longstaff, B.J., Loneragan, N.R., O'Donohue, M.J., and Dennison, W.C. (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.

Loya, Y., Puglise, K.A., and Bridge, T.C. (2019). *Mesophotic coral ecosystems.* Springer.

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

Marba, N., and Duarte, C.M. (1998). Rhizome elongation and seagrass clonal growth. *Marine Ecology Progress Series* 174.

Margvelashvili, N., Andrewartha, J., Baird, M., Herzfeld, M., Jones, E., Mongin, M., et al. (2018). Simulated fate of catchment-derived sediment on the Great Barrier Reef shelf. *Marine Pollution Bulletin* 135**,** 954-962.

Marián, F.D., Garcia-Jimenez, P., and Robaina, R.R. (2000). Polyamine levels in the seagrass *Cymodocea nodosa*. *Aquatic Botany* 68(2)**,** 179-184. doi: 10.1016/S0304-3770(00)00111-X.

Marín-Guirao, L., Entrambasaguas, L., Dattolo, E., Ruiz, J.M., and Procaccini, G. (2017). Molecular mechanisms behind the physiological resistance to intense transient warming in an iconic marine plant. *Frontiers in plant science* 8**,** 1142.

Marsh Jr, J.A., Dennison, W.C., and Alberte, R.S. (1986). Effects of temperature on photosynthesis and respiration in eelgrass (*Zostera marina* L.). *Journal of Experimental Marine Biology and Ecology* 101(3)**,** 257-267.

Masini, R., Cary, J., Simpson, C., and McComb, A. (1995). Effects of light and temperature on the photosynthesis of temperate meadow-forming seagrasses in Western Australia. *Aquatic Botany* 49(4)**,** 239-254.

Matsoukas, I.G. (2014). Interplay between sugar and hormone signaling pathways modulate floral signal transduction. *Frontiers in Genetics* 5.

Mazzuca, S., Bjork, M., Beer, S., Felisberto, P., Gobert, S., Procaccini, G., et al. (2013). Establishing research strategies, methodologies and technologies to link genomics and proteomics to seagrass productivity, community metabolism, and ecosystem carbon fluxes. *Frontiers in Plant Science* 4(38). doi: 10.3389/fpls.2013.00038.

McKenna, S., Jarvis, J., Sankey, T., Reason, C., Coles, R., and Rasheed, M. (2015). Declines of seagrasses in a tropical harbour, North Queensland, Australia, are not the result of a single event. *Journal of Biosciences* 40(2)**,** 389-398.

McKenzie, L. (1994). Seasonal changes in biomass and shoot characteristics of a *Zostera capricorni* Aschers. Dominant meadow in Cairns Harbour, northern Queensland. *Marine and Freshwater Research* 45(7)**,** 1337-1352. doi: 10.1071/MF9941337.

McKenzie, L., Nordlund, L.M., Jones, B.L., Cullen-Unsworth, L.C., Roelfsema, C.M., and Unsworth, R. (2020). The global distribution of seagrass meadows. *Environmental Research Letters*.

McKenzie, L.J., Collier, C.J., and Waycott, M. (2012). "In: Reef Rescue Marine Monitoring Program: Nearshore Seagrass, Annual Report for the sampling period 1st July 2010–31st May 2011.". (Cairns: Fisheries Queensland).

McMahon, K., Collier, C., and Lavery, P.S. (2013). Identifying robust bioindicators of light stress in seagrasses: A meta-analysis. *Ecological Indicators* 30(0)**,** 7-15. doi: 10.1016/j.ecolind.2013.01.030.

McMahon, K., van Dijk, K.-j., Ruiz-Montoya, L., Kendrick, G.A., Krauss, S.L., Waycott, M., et al. (2014). The movement ecology of seagrasses. *Proceedings of the Royal Society B: Biological Sciences* 281(1795)**,** 20140878.

McMillan, C. (1987). Seed germination and seedling morphology of the seagrass, *Halophila engelmannii* (Hydrocharitaceae). *Aquatic Botany* 28(2)**,** 179-188.

McMillan, C. (1988a). Seed germination and seedling development of *Halophila decipiens* Ostenfeld (Hydrocharitaceae) from Panama. *Aquatic Botany* 31(1)**,** 169-176.

McMillan, C. (1988b). The seed reserve of *Halophila decipiens* Ostenfeld (Hydrocharitaceae) in Panama. *Aquatic Botany* 31(1-2)**,** 177-182.

McMillan, C. (1988c). The seed reserve of *Halophila engelmannii* (Hydrocharitaceae) in Redfish bay, Texas. *Aquatic Botany* 30(3)**,** 253-259.

McMillan, C. (1991). The longevity of seagrass seeds. *Aquatic Botany* 40(2)**,** 195-198. doi: 10.1016/0304-3770(91)90097-O.

McMillan, C., and Soong, K. (1989). An annual cycle of flowering, fruiting and seed reserve for *Halophila decipiens* Ostenfeld (Hydrocharitaceae) in Panama. *Aquatic botany* 34(4)**,** 375-379.

Mellors, J.E. (1991). An evaluation of a rapid visual technique for estimating seagrass biomass. *Aquatic Botany* 42(1)**,** 67-73. doi: 10.1016/0304-3770(91)90106-F.

Mercado, J.M., del Pilar Sánchez-Saavedra, M., Correa-Reyes, G., Lubián, L., Montero, O., and Figueroa, F.L. (2004). Blue light effect on growth, light absorption characteristics and photosynthesis of five benthic diatom strains. *Aquatic Botany* 78(3)**,** 265-277.

Mochida, K., and Shinozaki, K. (2011). Advances in omics and bioinformatics tools for systems analyses of plant functions. *Plant and Cell Physiology* 52(12)**,** 2017-2038.

Moore, K.A., Shields, E.C., Parrish, D.B., and Orth, R.J. (2012). Eelgrass survival in two contrasting systems: role of turbidity and summer water temperatures. *Marine Ecology Progress Series* 448**,** 247-258.

Moschen, S., Bengoa Luoni, S., Di Rienzo, J.A., Caro, M.d.P., Tohge, T., Watanabe, M., et al. (2016). Integrating transcriptomic and metabolomic analysis to understand natural leaf senescence in sunflower. *Plant Biotechnology Journal* 14(2)**,** 719-734.

Nguyen, H.M., Kim, M., Ralph, P.J., Marín-Guirao, L., Pernice, M., and Procaccini, G. (2020). Stress memory in seagrasses: First insight into the effects of thermal priming and the role of epigenetic modifications. *Frontiers in Plant Science* 11(494). doi: 10.3389/fpls.2020.00494.

Nieuwenhuis, R., te Grotenhuis, H., and Pelzer, B. (2012). Influence. ME: tools for detecting influential data in mixed effects models.

Nordlund, L.M., Koch, E.W., Barbier, E.B., and Creed, J.C. (2016). Seagrass ecosystem services and their variability across genera and geographical regions. *PLoS One* 11(10)**,** e0163091.

O'Brien, K.R., Adams, M.P., Ferguson, A.J., Samper-Villarreal, J., Maxwell, P.S., Baird, M.E., et al. (2018). "Seagrass resistance to light deprivation: Implications for resilience," in *Seagrasses of Australia*. (Springer), 287-311.

Olesen, B., Enríquez, S., Duarte, C.M., and Sand-Jensen, K. (2002). Depth-acclimation of photosynthesis, morphology and demography of *Posidonia oceanica* and *Cymodocea nodosa* in the Spanish Mediterranean Sea. *Marine Ecology Progress Series* 236**,** 89-97.

Orth, R., Batiuk, R., Bergstrom, P., and Moore, K. (2002). A perspective on two decades of policies and regulations influencing the protection and restoration of submerged aquatic vegetation in Chesapeake Bay, USA. *Bulletin of Marine Science* 71(3)**,** 1391-1403.

Orth, R.J., Harwell, M.C., Bailey, E.M., Bartholomew, A., Jawad, J.T., Lombana, A.V., et al. (2000). A review of issues in seagrass seed dormancy and germination: implications for conservation and restoration. *Marine Ecology Progress Series* 200**,** 277-288.

Orth, R.J., Harwell, M.C., and Inglis, G.J. (2006). "Ecology of seagrass seeds and seagrass dispersal processes," in *Seagrasses: Biology, Ecology and Conservation*. (Springer).

Orth, R.J., and Van Montfrans, J. (1984). Epiphyte-seagrass relationships with an emphasis on the role of micrograzing: a review. *Aquatic Botany* 18(1-2)**,** 43-69.

Osmond, B., Chow, W.S., Wyber, R., Zavafer, A., Keller, B., Pogson, B.J., et al. (2017). Relative functional and optical absorption cross-sections of PSII and other photosynthetic parameters monitored *in situ*, at a distance with a time resolution of a few seconds, using a prototype light induced fluorescence transient (LIFT) device. *Functional Plant Biology*. doi: 10.1071/FP17024.

Pan, X., Welti, R., and Wang, X. (2008). Simultaneous quantification of major phytohormones and related compounds in crude plant extracts by liquid chromatography–electrospray tandem mass spectrometry. *Phytochemistry* 69(8)**,** 1773-1781. doi: 10.1016/j.phytochem.2008.02.008.

Patti, G.J., Yanes, O., and Siuzdak, G. (2012). Metabolomics: the apogee of the omics trilogy. *Nature Reviews Molecular Cell Biology* 13(4)**,** 263-269. doi: 10.1038/nrm3314.

Peralta, P., Brun, F.G., Pérez-Lloréns, J., and Bouma, T.J. (2006). Direct effects of current velocity on the growth, morphometry and architecture of seagrasses: a case study on *Zostera noltii*. *Marine Ecology Progress Series* 327.

Petrou, K., Jimenez-Denness, I., Chartrand, K., McCormack, C., Rasheed, M., and Ralph, P. (2013). Seasonal heterogeneity in the photophysiological response to air exposure in two tropical intertidal seagrass species. *Marine Ecology Progress Series* 482**,** 93-106. doi: 10.3354/meps10229.

Piercey, R.S., Gribben, P.E., Hanley, T.C., Moles, A.T., and Hughes, A.R. (2020). Incorporating marine macrophytes in plant–soil feedbacks: Emerging evidence and opportunities to advance the field. *Journal of Ecology*.

Poethig, R.S. (2003). Phase change and the regulation of developmental timing in plants. *Science* 301(5631)**,** 334-336.

Pomfret, S.M., Brua, R.B., Izral, N.M., and Yates, A.G. (2020). Metabolomics for biomonitoring: an evaluation of the metabolome as an indicator of aquatic ecosystem health. *Environmental Reviews* 28(1)**,** 89-98.

Preen, A., and Marsh, H. (1995). Response of dugongs to large-scale loss of seagrass from Hervey Bay, Queensland Australia. *Wildlife Research* 22(4)**,** 507-519.

Productivity Commission (2003). "Industries, land use and water quality in the Great Barrier Reef catchment". (Canberra).

Putri, S.P., Nakayama, Y., Matsuda, F., Uchikata, T., Kobayashi, S., Matsubara, A., et al. (2013). Current metabolomics: Practical applications. *Journal of Bioscience and Bioengineering* 115(6)**,** 579-589. doi: 10.1016/j.jbiosc.2012.12.007.

Qin, Z., Liao, D., Chen, Y., Zhang, C., An, R., Zeng, Q., et al. (2019). A widely metabolomic analysis revealed metabolic alterations of *Epimedium Pubescens* leaves at different growth stages. *Molecules (Basel, Switzerland)* 25(1)**,** 137. doi: 10.3390/molecules25010137.

R Core Team (2018). R version 3.4.4 "Someone to Lean On".

Ralph, P., and Burchett, M. (1998). Photosynthetic response of *Halophila ovalis* to heavy metal stress. *Environmental Pollution* 103(1)**,** 91-101.

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

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

Rasheed, M.A. (1999). Recovery of experimentally created gaps within a tropical *Zostera capricorni* (Aschers.) seagrass meadow, Queensland Australia. *Journal of Experimental Marine Biology and Ecology* 235(2)**,** 183-200. doi: 10.1016/S0022-0981(98)00158-0.

Rasheed, M.A. (2004). Recovery and succession in a multi-species tropical seagrass meadow following experimental disturbance: the role of sexual and asexual reproduction. *Journal of Experimental Marine Biology and Ecology* 310(1)**,** 13-45.

Rasheed, M.A., Dew, K.R., McKenzie, L.J., Coles, R.G., Kerville, S.P., and Campbell, S.J. (2008). Productivity, carbon assimilation and intra-annual change in tropical reef platform seagrass communities of the Torres Strait, north-eastern Australia. *Continental Shelf Research* 28(16)**,** 2292-2303.

Rasheed, M.A., McKenna, S.A., Carter, A.B., and Coles, R.G. (2014). Contrasting recovery of shallow and deep water seagrass communities following climate associated losses in tropical north Queensland, Australia. *Marine Pollution Bulletin* 83(2)**,** 491-499.

Rayirath, U.P., Lada, R.R., Caldwell, C.D., Asiedu, S.K., and Sibley, K.J. (2011). Role of ethylene and jasmonic acid on rhizome induction and growth in rhubarb (Rheum rhabarbarum L.). *Plant Cell, Tissue and Organ Culture (PCTOC)* 105(2)**,** 253-263.

Ritchie, R.J. (2006). Consistent sets of spectrophotometric chlorophyll equations for acetone, methanol and ethanol solvents. *Photosynthesis Research* 89(1)**,** 27-41.

Roberts, D.G., McComb, A.J., and Kuo, J. (1984). The structure and continuity of the lacunar system of the seagrass *Halophila ovalis* (R. Br.) Hook f. (Hydrocharitaceae). *Aquatic Botany* 18(4)**,** 377-388. doi: 10.1016/0304-3770(84)90058-5.

Rodriguez, M.C.G., and Simó, T.C. (1981). *Halophila decipiens* Ostenfeld (Hidrocharitaceae) una fanerógama marina nueva para el Atlántico Oriental. *Vieraea: Folia scientarum biologicarum canariensium* (11)**,** 207-216.

Ruban, A.V. (2014). Evolution under the sun: optimizing light harvesting in photosynthesis. *Journal of Experimental Botany* 66(1)**,** 7-23.

Ruiz-Frau, A., Gelcich, S., Hendriks, I., Duarte, C.M., and Marbà, N. (2017). Current state of seagrass ecosystem services: research and policy integration. *Ocean & Coastal Management* 149**,** 107-115.

Ruocco, M., Ambrosino, L., Jahnke, M., Chiusano, M.L., Barrote, I., Procaccini, G., et al. (2020). m6A RNA methylation in marine plants: First insights and relevance for biological rhythms. *International Journal of Molecular Sciences* 21(20)**,** 7508.

Saunders, M.I., Bayraktarov, E., Roelfsema, C.M., Leon, J.X., Samper-Villarreal, J., Phinn, S.R., et al. (2015). Spatial and temporal variability of seagrass at Lizard Island, Great Barrier Reef. *Botanica Marina* 58(1)**,** 35-49.

Savatin, D.V., Gramegna, G., Modesti, V., and Cervone, F. (2014). Wounding in the plant tissue: the defense of a dangerous passage. *Frontiers in Plant Science* 5(470). doi: 10.3389/fpls.2014.00470.

Schreiber, U. (2004). "Pulse-amplitude-modulation (PAM) fluorometry and saturation pulse method: an overview," in *Chlorophyll a Fluorescence*. (Springer), 279-319.

Schreiber, U., and Klughammer, C. (2013). Wavelength-dependent photodamage to *Chlorella* investigated with a new type of multi-color PAM chlorophyll fluorometer. *Photosynthesis Research* 114(3)**,** 165-177.

Schreiber, U., Klughammer, C., and Kolbowski, J. (2012). Assessment of wavelength-dependent parameters of photosynthetic electron transport with a new type of multi-color PAM chlorophyll fluorometer. *Photosynthesis Research* 113(1-3)**,** 127-144.

Scott, A.L., York, P.H., Duncan, C., Macreadie, P.I., Connolly, R.M., Ellis, M.T., et al. (2018). The role of herbivory in structuring tropical seagrass ecosystem service delivery. *Frontiers in Plant Science* 9**,** 127.

Scott, A.L., York, P.H., and Rasheed, M.A. (2021). Spatial and temporal patterns in macroherbivore grazing in a multi-species tropical seagrass meadow of the Great Barrier Reef. *Diversity* 13(1)**,** 12.

Sharon, Y., Levitan, O., Spungin, D., Berman-Frank, B., and Beer, S. (2011). Photoacclimation of the seagrass *Halophila stipulacea* to the dim irradiance at its 48-meter depth limit. *Limnology and Oceanography* 56(1)**,** 357.

Sharon, Y., Silva J, Santos R, Runcie JW, Chernihovsky M, Beer S (2009). Photosynthetic responses of *Halophila stipulacea* to a light gradient. II. Acclimations following transplantation. *Aquatic Biology* 7**,** 153-157. doi: 10.3354/ab00148.

Short, F.T., and Duarte, C.M. (2001). Methods for the measurement of seagrass growth and production. *Global seagrass research methods***,** 155-182.

Short, F.T., and Neckles, H.A. (1999). The effects of global climate change on seagrasses. *Aquatic Botany* 63(3)**,** 169-196.

Smeekens, S. (2000). Sugar-induced signal transduction in plants. *Annual Review of Plant Biology* 51(1)**,** 49-81.

Smeekens, S., Ma, J., Hanson, J., and Rolland, F. (2010). Sugar signals and molecular networks controlling plant growth. *Current Opinion in Plant Biology* 13(3)**,** 273-278.

Smith, H. (1982). Light quality, photoperception, and plant strategy. *Annual review of plant physiology* 33(1)**,** 481-518.

Srikanth, A., and Schmid, M. (2011). Regulation of flowering time: all roads lead to Rome. *Cellular and Molecular Life Sciences* 68(12)**,** 2013-2037.

Statton, J., Sellers, R., Dixon, K.W., Kilminster, K., Merritt, D.J., and Kendrick, G.A. (2017). Seed dormancy and germination of *Halophila ovalis* mediated by simulated seasonal temperature changes. *Estuarine, Coastal and Shelf Science* 198**,** 156-162.

Stoeckl, N., Hicks, C.C., Mills, M., Fabricius, K., Esparon, M., Kroon, F., et al. (2011). The economic value of ecosystem services in the Great Barrier Reef: our state of knowledge. *Annals of the New York Academy of Sciences* 1219(1)**,** 113-133.

Strasser, R., and Govindjee (Year). "On the OJIP fluorescence transient in leaves and D1 mutants of *Chlamydomonas-reinhardtii*", in: *Photosynthesis Research*: Kluwer Academic Publ), 135-135.

Strydom, S., McMahon, K., Kendrick, G.A., Statton, J., and Lavery, P.S. (2017a). Seagrass *Halophila ovalis* is affected by light quality across different life history stages. *Marine Ecology Progress Series* 572**,** 103-116.

Strydom, S., McMahon, K., and Lavery, P.S. (2017b). Response of the seagrass *Halophila ovalis* to altered light quality in a simulated dredge plume. *Marine Pollution Bulletin* 121(1-2)**,** 323-330.

Suchanek, T.H. (1983). Control of seagrass communities and sediment distribution by Callianassa (Crustacea, Thalassinidea) bioturbation. *Journal of Marine Research* 41(2)**,** 281-298.

Szabó, M., Wangpraseurt, D., Tamburic, B., Larkum, A.W., Schreiber, U., Suggett, D.J., et al. (2014). Effective light absorption and absolute electron transport rates in the coral *Pocillopora damicornis*. *Plant Physiology and Biochemistry* 83**,** 159-167.

Tan, F.-C., and Swain, S.M. (2006). Genetics of flower initiation and development in annual and perennial plants. *Physiologia Plantarum* 128(1)**,** 8-17. doi: 10.1111/j.1399-3054.2006.00724.x.

Tan, Y.M., Dalby, O., Kendrick, G.A., Statton, J., Sinclair, E.A., Fraser, M.W., et al. (2020). Seagrass restoration is possible: Insights and lessons from Australia and New Zealand. *Frontiers in Marine Science* 7(617). doi: 10.3389/fmars.2020.00617.

Taylor, H.A., and Rasheed, M. (2010). *Torres Strait Dugong Sanctuary seagrass baseline survey.* Department of employment, Economic Development and Innovation.

Terrados, J., Duarte, C.M., Fortes, M.D., Borum, J., Agawin, N.S., Bach, S., et al. (1998). Changes in community structure and biomass of seagrass communities along gradients of siltation in SE Asia. *Estuarine, Coastal and Shelf Science* 46(5)**,** 757-768.

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

Thomas, H. (2013). Senescence, ageing and death of the whole plant. *New Phytologist* 197(3)**,** 696-711.

Tol, S.J., Jarvis, J.C., York, P.H., Grech, A., Congdon, B.C., and Coles, R.G. (2017). Long distance biotic dispersal of tropical seagrass seeds by marine mega-herbivores. *Scientific Reports* 7(1)**,** 4458.

Tomar, P.C., Lakra, N., and Mishra, S. (2013). Cadaverine: a lysine catabolite involved in plant growth and development. *Plant signaling & behavior* 8(10)**,** e25850.

Touchette, B.W., and 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. doi: 10.1016/S0022-0981(00)00196-9.

Udy, J., Waycott, M., Carter, A., Collier, C., Kilminster, K., Rasheed, M., et al. (2019). Monitoring seagrass within the Reef 2050 Integrated Monitoring and Reporting Program: Final report of the seagrass expert group.

Upchurch, R.G. (2008). Fatty acid unsaturation, mobilization, and regulation in the response of plants to stress. *Biotechnology Letters* 30(6)**,** 967-977.

Vergeer, L.H., and Develi, A. (1997). Phenolic acids in healthy and infected leaves of *Zostera marina* and their growth-limiting properties towards *Labyrinthula zosterae*. *Aquatic Botany* 58(1)**,** 65-72.

Vermaat, J., Agawin, N., Duarte, C., Fortes, M., Marba, N., and Uri, J. (1995). Meadow maintenance, growth and productivity of a mixed Philippine seagrass bed. *Marine Ecology Progress Series* 124(1)**,** 215-225.

Vogelmann, T.C., and Evans, J. (2002). Profiles of light absorption and chlorophyll within spinach leaves from chlorophyll fluorescence. *Plant, Cell & Environment* 25(10)**,** 1313-1323.

Voitsekhovskaja, O., and Tyutereva, E. (2015). Chlorophyll b in angiosperms: functions in photosynthesis, signaling and ontogenetic regulation. *Journal of Plant Physiology* 189**,** 51-64.

Vonk, J.A., Kneer, D., Stapel, J., and Asmus, H. (2008). Shrimp burrow in tropical seagrass meadows: An important sink for litter. *Estuarine, Coastal and Shelf Science* 79(1)**,** 79-85. doi: 10.1016/j.ecss.2008.03.003.

Vu, H.S., Roston, R., Shiva, S., Hur, M., Wurtele, E.S., Wang, X., et al. (2015). Modifications of membrane lipids in response to wounding of Arabidopsis thaliana leaves. *Plant Signaling & Behavior* 10(9)**,** e1056422-e1056422. doi: 10.1080/15592324.2015.1056422.

Wagenmakers, E.-J., and Farrell, S. (2004). AIC model selection using Akaike weights. *Psychonomic Bulletin & Review* 11(1)**,** 192-196. doi: 10.3758/bf03206482.

Waite, B., Statton, J., and Kendrick, G.A. (2020). Temperature Stratification and Monochromatic Light Break Dormancy and Facilitate On-Demand In Situ Germination in the Seagrass *Halophila ovalis*, with Seed Viability Determined by a Novel X-Ray Analysis. *Estuaries and Coasts***,** 1-10.

Walker, D., Dennison, W., and Edgar, G. (1999). Status of Australian seagrass research and knowledge. *Seagrass in Australia. Strategic review and development of an R&D plan. CSIRO, Canberra Australia***,** 1-24.

Walker, D., Kendrick, G., and McComb, A. (1988). The distribution of seagrass species in Shark Bay, Western Australia, with notes on their ecology. *Aquatic Botany* 30(4)**,** 305-317.

Walters, R.G. (2005). Towards an understanding of photosynthetic acclimation. *Journal of Experimental Botany* 56(411)**,** 435-447.

Waltham, N., Buelow, C., Iles, J.A., Whinney, J., Ramsby, B., and Macdonald, R. (2019). "Port of Mackay and Hay Point ambient marine water quality monitoring program (July 2018 – July 2019)". Centre for Tropical Water & Aquatic Ecosystem Research (TropWATER), James Cook University).

Ware, M.A., Belgio, E., and Ruban, A.V. (2015). Photoprotective capacity of non-photochemical quenching in plants acclimated to different light intensities. *Photosynthesis Research* 126(2)**,** 261-274. doi: 10.1007/s11120-015-0102-4.

Wasternack, C. (2014). "Jasmonates in plant growth and stress responses," in *Phytohormones: A window to metabolism, signaling and biotechnological applications*. (Springer), 221-263.

Wasternack, C., and Hause, B. (2013). Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 review in Annals of Botany. *Annals of Botany* 111(6)**,** 1021-1058.

Waycott, M., Duarte, C.M., Carruthers, T.J., Orth, R.J., Dennison, W.C., Olyarnik, S., et al. (2009). Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Proceedings of the National Academy of Sciences, USA* 106(30)**,** 12377-12381.

Waycott, M., Freshwater, D.W., York, R.A., Calladine, A., and Kenworthy, W.J. (2002). Evolutionary trends in the seagrass genus *Halophila* (Thouars): insights from molecular phylogeny. *Bulletin of Marine Science* 71(3)**,** 1299-1308.

Waycott, M., McMahon, K., Mellors, J., Calladine, A., and Kleine, D. (2004). *A guide to tropical seagrasses of the Indo-West Pacific.* Townsville, Australia: James Cook University.

Weatherall, E.J., Jackson, E.L., Hendry, R., and Campbell, M.L. (2016). Quantifying the dispersal potential of seagrass vegetative fragments: a comparison of multiple subtropical species. *Estuarine, Coastal and Shelf Science* 169**,** 207-215.

Weir, K.L., Wilson, J.R., and White, R.J. (1977). CSIRO Australian Division Tropical Paper No. 20 CSIRO).

Williams, S.L., and Dennison, W.C. (1990). Light availability and diurnal growth of a green macroalga (*Caulerpa cupressoides*) and a seagrass (*Halophila decipiens*). *Marine Biology* 106(3)**,** 437-443. doi: 10.1007/BF01344324.

Wood, S. (2006). *Generalized additive models: an introduction with R.* CRC press.

Wu, P.P.-Y., Mengersen, K., McMahon, K., Kendrick, G.A., Chartrand, K., York, P.H., et al. (2017). Timing anthropogenic stressors to mitigate their impact on marine ecosystem resilience. *Nature Communications* 8(1)**,** 1263. doi: 10.1038/s41467-017-01306-9.

Wu, P.P.Y., McMahon, K., Rasheed, M.A., Kendrick, G.A., York, P.H., Chartrand, K., et al. (2018). Managing seagrass resilience under cumulative dredging affecting light: Predicting risk using dynamic Bayesian networks. *Journal of Applied Ecology* 55(3)**,** 1339-1350.

Yang, J., Su, L., Li, D., Luo, L., Sun, K., Yang, M., et al. (2020). Dynamic transcriptome and metabolome analyses of two types of rice during the seed germination and young seedling growth stages. *BMC Genomics* 21(1)**,** 603. doi: 10.1186/s12864-020-07024-9.

Yokoyama, M., Yamaguchi, S., Inomata, S., Komatsu, K., Yoshida, S., Iida, T., et al. (2000). Stress-induced factor involved in flower formation of *Lemna* is an α-ketol derivative of linolenic acid. *Plant and Cell Physiology* 41(1)**,** 110-113. doi: 10.1093/pcp/41.1.110.

York, P., Carter, A., Chartrand, K.M., Sankey, T., Wells, L., and Rasheed, M.A. (2015). Dynamics of a deep-water seagrass population on the Great Barrier Reef: annual occurrence and response to a major dredging program. *Scientific Reports*. doi: 10.1038/srep13167.

York, P., and Rasheed, M. (2018). "Annual Seagrass Monitoring in the Mackay-Hay Point Region – 2017". JCU Centre for Tropical Water & Aquatic Ecosystem Research Publication).

York, P.H., Gruber, R.K., Hill, R., Ralph, P.J., Booth, D.J., and Macreadie, P.I. (2013). Physiological and morphological responses of the temperate seagrass *Zostera muelleri* to multiple stressors: investigating the interactive effects of light and temperature. *PloS One* 8(10)**,** e76377.

York, P.H., Macreadie, P.I., and Rasheed, M.A. (2018). Blue Carbon stocks of Great Barrier Reef deep-water seagrasses. *Biology Letters* 14(12)**,** 20180529.

Yu, S., Cao, L., Zhou, C.-M., Zhang, T.-Q., Lian, H., Sun, Y., et al. (2013). Sugar is an endogenous cue for juvenile-to-adult phase transition in plants. *Elife* 2**,** e00269.

Yuan, Z., and Zhang, D. (2015). Roles of jasmonate signalling in plant inflorescence and flower development. *Current Opinion in Plant Biology* 27**,** 44-51.

Zarranz, M.E., Gonzalez-Henriquez, N., García-Jiménez, P., and Robaina, R.R. (2010). Restoration of *Cymodocea nodosa* (Uchria) Ascherson seagrass meadows through seed propagation: seed storage and influences of plant hormones and mineral nutrients on seedling growth in vitro. *Botanica Marina* 53(5)**,** 439-448.

Zhang, L., Zhang, X., Ji, H., Wang, W., Liu, J., Wang, F., et al. (2018). Metabolic profiling of tobacco leaves at different growth stages or different stalk positions by gas chromatography–mass spectrometry. *Industrial Crops and Products* 116**,** 46-55.

Zidorn, C. (2016). Secondary metabolites of seagrasses (Alismatales and Potamogetonales; Alismatidae): Chemical diversity, bioactivity, and ecological function. *Phytochemistry* 124**,** 5-28.

Zimmerman, R.C. (2007). "Light and photosynthesis in seagrass meadows," in *Seagrasses: Biology, Ecology and Conservation*. (Springer), 303-321.

Zimmerman, R.C. (2020). Scaling up: Predicting the Impacts of Climate Change on Seagrass Ecosystems. *Estuaries and Coasts***,** 1-19.

Zivcak, M., Brestic, M., Kalaji, H.M., and Govindjee (2014). Photosynthetic responses of sun- and shade-grown barley leaves to high light: is the lower PSII connectivity in shade leaves associated with protection against excess of light? *Photosynthesis Research* 119(3)**,** 339-354. doi: 10.1007/s11120-014-9969-8.

Zuur, A., Ieno, E., Walker, N., Saveliev, A., and Smith, G. (2009). "Mixed effects models and extensions in ecology with R. New York: Springer".).

APPENDICES

Appendix A

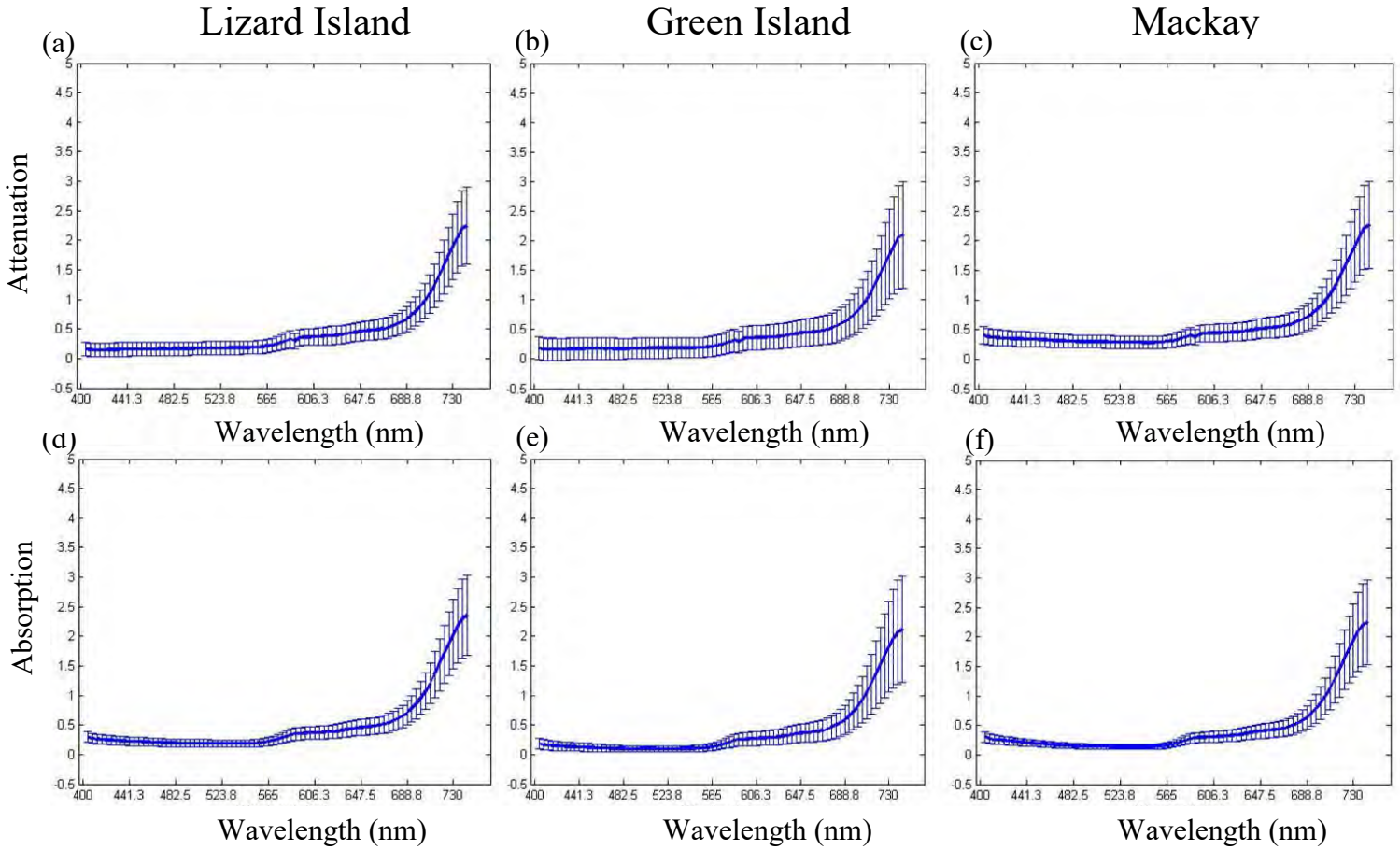


Figure A.1 November 2012, spectrally-resolved, depth-averaged attenuation (a-c) and absorption (d-f) at Green Island, Lizard Island and a previously monitored Mackay seagrass meadow inshore from the Keswick Island site.

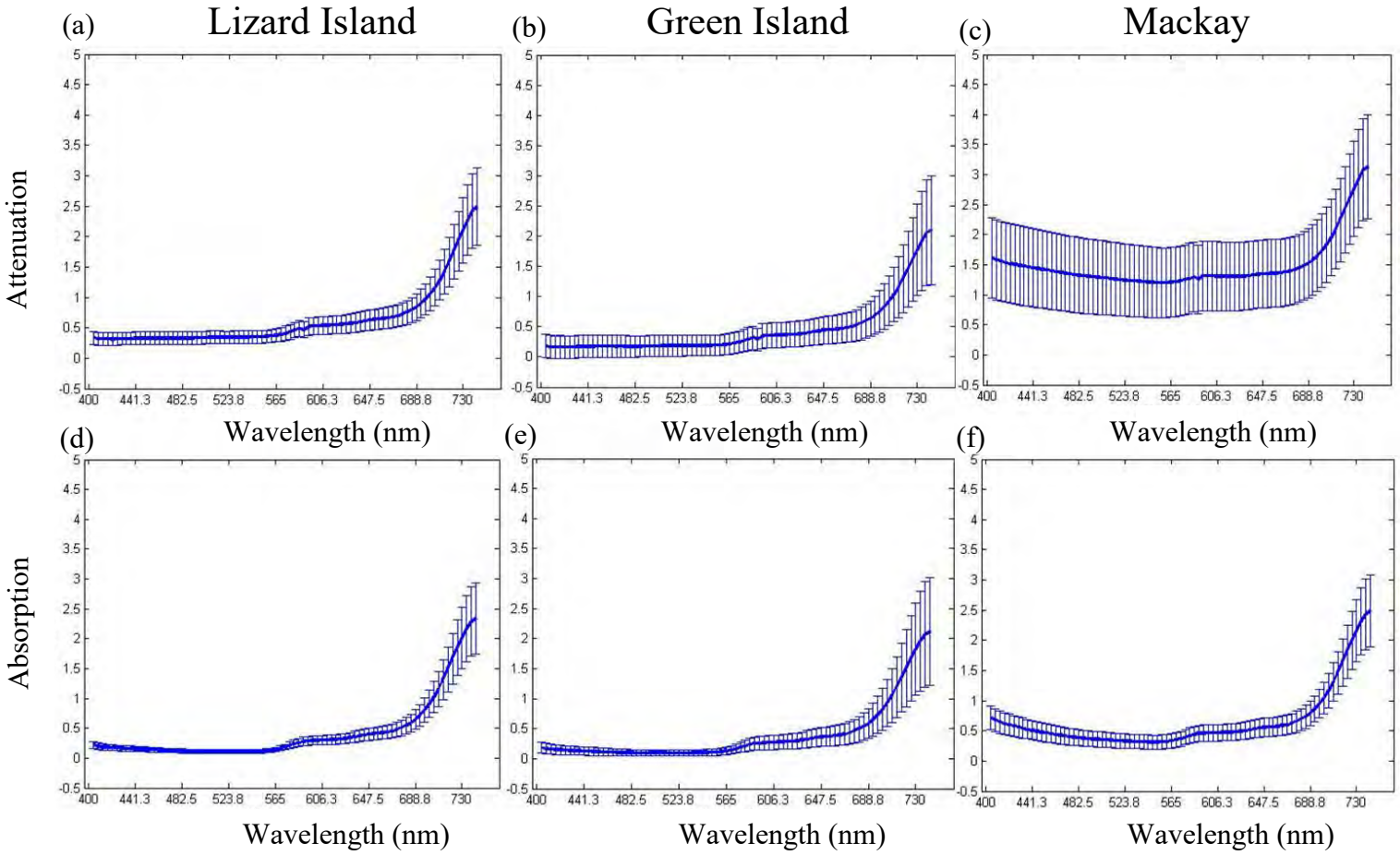


Figure A.2 February 2013, spectrally-resolved, depth-averaged attenuation (a-c) and absorption (d-f) at Green Island, Lizard Island and a previously monitored Mackay seagrass meadow inshore from the Keswick Island site.

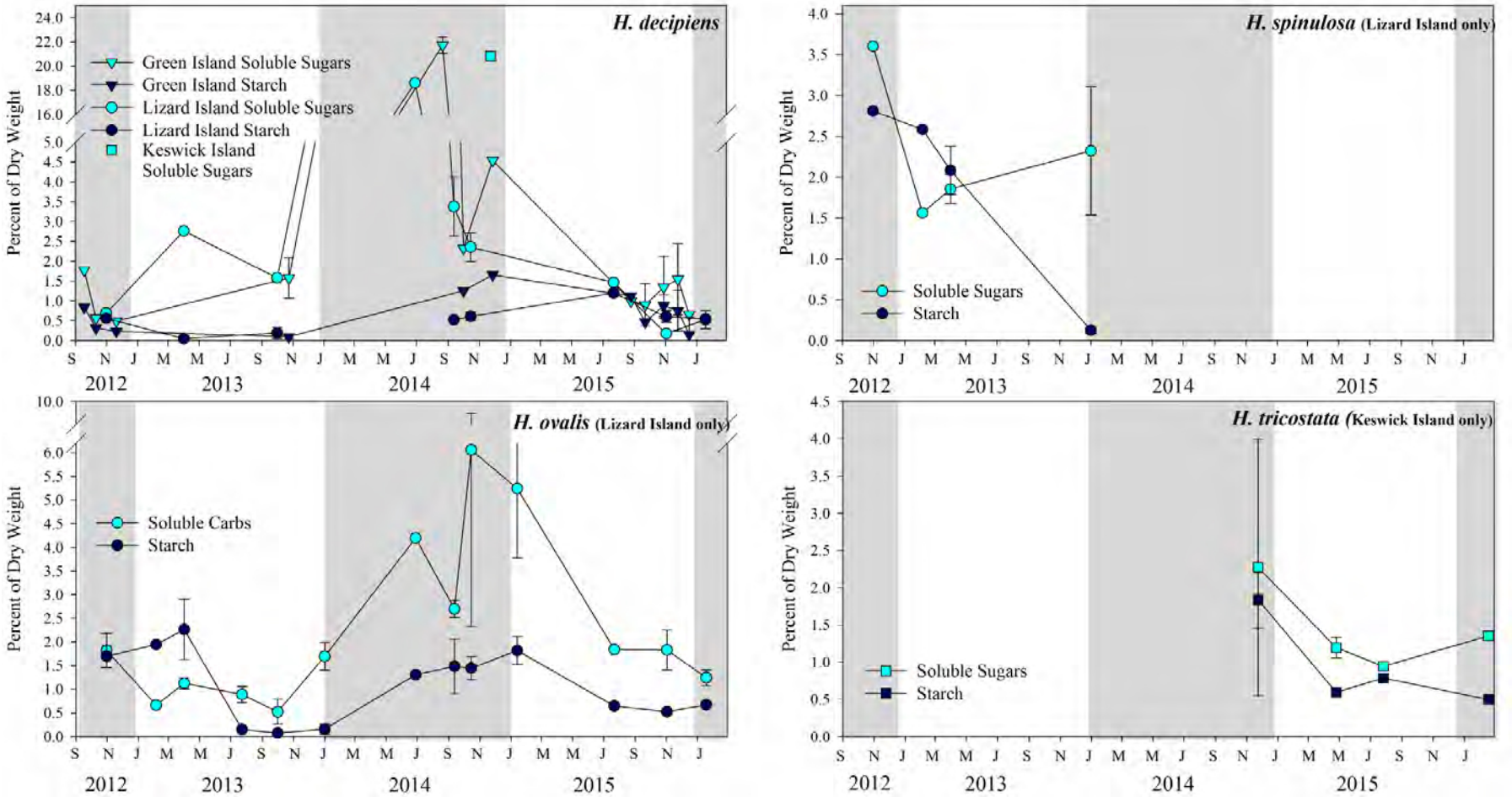


Figure A.3 Below-ground carbohydrates measured in (a) *H. decipiens*, (b) *H. ovalis*, (c) *H. spinulosa*, and (d) *H. tricostata* at Green Island, Lizard Island, and Keswick Island measured as a percentage of root/rhizome dry weight. Due to diminutive size of plant material, samples were often combined to achieve minimum analytical weights. Where replicates were available, means are presented with error bars representing ± S.E. Note change of scale in y-axis for each species. *negligible biomass at site in April 2015; could not collect

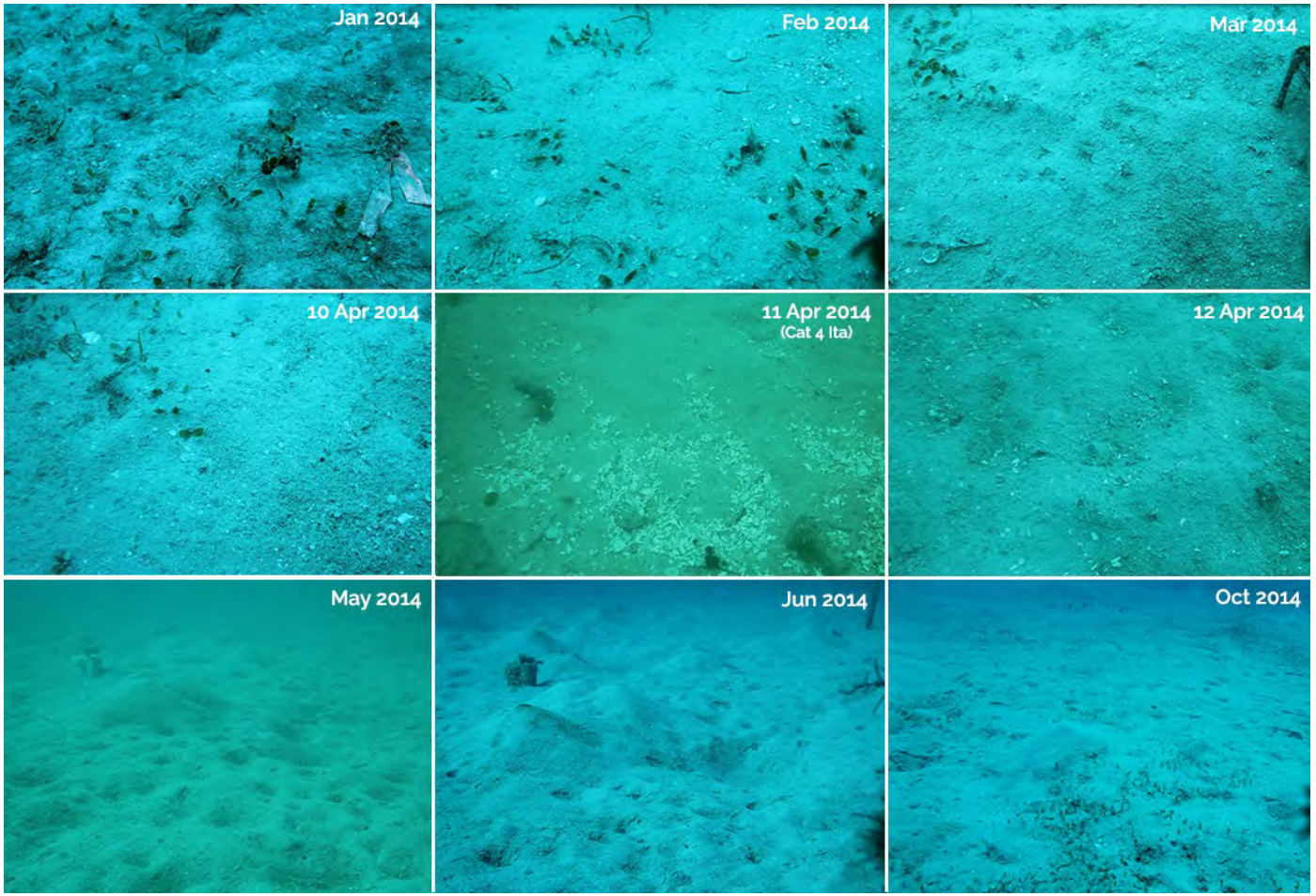


Figure A.4 Cyclone Ita time-series of still frames taken prior, during, and following the passing of the category 4 system directly over the Lizard Island long-term monitoring site on 11 April 2014. Still frames were taken pointing downward over a 0.25 m^2 surface until May 2014 when the camera angle was re-directed to capture the broader meadow-scale changes.

Appendix B

Figure B.1 Global models run in R for total seed core and seed stratification data for each monitoring location. All models included a random error term ε. edf is the estimated degrees of freedom. *Y* is *Year; DY* is the day in growing year (June – July); *CIta* is a fixed factor for the 12 months post-Cyclone Ita data at Lizard Island; *M* is a fixed factor for sampling month at Keswick Island; and *Depth* is a fixed factor for the three sediment layers seeds were counted in the stratification models. All stratified seed models had a random effect (β) of core.

Total Seed Bank	**Global Model**
Green Island	$Seed_{bank} = s(DY*Y) + Y + s(DY) + \varepsilon$
Lizard Island	$Seed_{bank} = s(DY*Y) + Y * CIta + s(DY) + \varepsilon$
Keswick Island	$Seed_{bank} = s(DY*Y) + s(DY) + Y + \varepsilon$
Seed Stratification	
Green Island	$Seed_{depth} = s(DY*Depth) + s(DY) + Depth * Y + \beta_{core} + \varepsilon$
Lizard Island	$Seed_{depth} = s(DY*Depth) + Depth * Y * CIta + \beta_{core} + \varepsilon$
Keswick Island	$Seed_{depth} = Depth * M + \beta_{core} + \varepsilon$