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Relative importance of different seagrass re-establishment strategies in tropical Queensland, Australia

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Abstract

Seagrass meadows are widespread coastal habitats that provide vitally important ecosystem services. Despite this importance they are reported to be declining at an alarming rate. Initiatives to monitor seagrass habitats and understand the mechanisms for recovery after loss are increasingly urgent. One aspect of these initiatives is to evaluate and predict the role of meadow connectivity and the importance of different dispersal mechanisms. Seagrasses disperse through both asexual and sexual processes. Clonal growth through rhizome extension enables rapid local recolonisation, while long distance dispersal occurs through abiotic movements of buoyant vegetative fragments. Seagrasses also produce flowers, fruits and seeds, however the seeds for most tropical species are negatively buoyant, limiting dispersal potential by abiotic vectors (such as currents). Recent research has found some aquatic herbivores (small fish, estuarine terrapin and some waterfowl) also disperse seagrass seeds. This biotic dispersal is a potentially important dispersal pathway that is relatively overlooked. There is little research available on the role of biotic dispersal in connectivity among seagrass meadows but particularly for tropical seagrasses. Increasing our understanding of how tropical seagrasses disperse will enhance seagrass management.

In north-east Australia, within the Great Barrier Reef World Heritage Area (GBRWHA), there is an estimated 35,000 km^2 of seagrass meadows, with around 12 tropical species present; some of the most common are *Zostera muelleri*, *Halodule uninervis* and *Halophila* species. Seagrasses provide many ecosystem services (provide nurseries, stabilise sediments, absorb excess nutrients and maintain coral health), with an estimated value of US$28,916/ha per year. This region also houses one of the world's largest populations of marine mega-herbivores; dugongs and green sea turtles, animals which are potentially important biotic dispersal vectors for seagrass. The goal of my thesis was to evaluate the processes associated with key abiotic and biotic dispersal pathways for tropical seagrass species. My objectives were to: **(1a)** determine whether marine mega-herbivores disperse viable tropical seagrass seeds; if so, **(1b)** determine whether seeds ingested and excreted by marine mega-herbivores had different germination outcomes compared to seeds dropped by the plant; and **(2)** quantify the number of viable seagrass fragments floating freely in the water column that could be available for abiotic dispersal and how this number varies over time.

1a) To determine biotic dispersal strategies, I collected marine mega-herbivore faeces that were floating in coastal bays in the central GBRWHA where dugongs and green sea turtles were foraging. I removed all seagrass seeds from the faeces by sieving in the laboratory. Viability of seeds found in faeces after gut passage was estimated by staining with a Tetrazolium dye solution (1.5%). I found that marine mega-herbivores were capable of dispersing 1 – 2 seeds per g DW of at least four tropical seagrass species (*Z. muelleri*, *H. uninervis*, *H. ovalis* and *Halophila spp*.), and that nearly 10% of these seeds were viable. *Z. muelleri* was the most common, representing 94% percent of all seeds. This equated to more than 500,000 viable seeds being dispersed in the GBRWHA per day by marine mega-herbivores, and they have the potential to disperse seagrass seeds for distances up to 650 km.

It is difficult to determine whether faeces come from turtle or dugong because of diet overlaps and morphological similarities in the faecal mass. I addressed this problem by creating short robust primers from the mitochondrial control region to distinguish between dugong and green sea turtle faeces. These primers were tested on 61 faecal samples from the central GBRWHA and Northern Territory, Australia and some from known dugong origin from Toba Aquarium in Japan. The primers successfully distinguished between dugong and green sea turtle DNA. They showed that all the samples collected from the GBRWHA were from *Chelonia mydas* (green sea turtle). Two samples from Bing Bong in the Northern Territory (with seagrass seeds present) were a match for *Dugong dugon* and 10 for *C. mydas*, confirming that both marine mega-herbivores do disperse seagrass seeds. *Cymodocea serrulata*, a new seagrass seed species was found in the Bing Bong samples in both marine mega-herbivores. It is likely that sea turtles play a larger role in biotic dispersal of tropical seagrass than dugongs, with the majority of faecal samples collected in the GBRWHA returning a positive match for *C. mydas*.

The largest number of seeds were found in green sea turtle faecal samples collected from Gladstone Harbour, (11 – 29 seeds per g DW), up to seven times more than found at other locations. Seed density in green sea turtle faeces, excluding Gladstone Harbour, was similar to the density found in the previous year (2 – 3 seeds per g DW). The density of seeds in marine mega-herbivore faeces varies substantially, possibly depending on fruiting success in individual foraging meadows. New calculations for maximum seed dispersal by green sea turtles, using updated population data for the southern GBR, estimates up to 120,000 viable seeds being dispersed per day during the fruiting season.

1b) To examine whether seeds ingested and excreted by marine mega-herbivores had different germination outcomes I performed a 60-day germination experiment with seeds from *Z. muelleri*. Germination times for seeds removed from faeces were compared to germination times for seeds collected from the plant (and allowed to naturally drop off of the plant in a mesocosm). All non-germinated seeds at the end of the experiment were tested for viability, using a Tetrazolium dye solution (1.5%). *Z. muelleri* seeds ingested and excreted by marine mega-herbivores germinated significantly faster (up to four times faster) and had a significantly greater maximum germination (up to two times more) than seeds allowed to drop from the plant. Un-germinated seeds which were ingested and excreted by marine mega-herbivores were significantly less likely to be viable after 60 days, unlike seeds dropped by the plant. Low viability of un-germinated seeds passed by marine mega-herbivores means that this mode of dispersal is best as a mechanism for immediate recovery/ re-establishment of meadows. The higher levels of viability of un-germinated seeds dropped by the plant means this pathway provides a longer-term recovery mechanism such as the ability to establish an *in-situ* viable seed bank.

2) To quantify the number of viable asexual propagules for abiotic dispersal I collected fragments that were floating freely within the top 70 cm of the water column at two intertidal seagrass meadows in the central GBRWHA. Fragments collected, pieces of seagrass with at least rhizome and one leaf present, were classified as viable or non-viable (based on the presence of an apical meristem or growing tip). Individual fragments were measured for characteristics including leaf shoot count, leaf count, leaf length, rhizome length, biomass, presence of roots, and presence of reproductive structures. I analysed whether wind speed, wind category, meadow location, seagrass density, growing or senescent season, and dugong foraging intensity influenced the density of total fragments and viable fragments. I found that adjacent seagrass meadow density is positively associated with total fragment density. There were nearly five times more viable fragments during the growing season (0.63 ± 0.08SE vs. 0.13 ± 0.03SE). Medium wind speeds (20-25 km/h) influenced the density of total fragments

and viable fragments, indicating moderate wave action (an effect of wind speed) is a factor in fragment creation.

My thesis provides significant new insights into the dispersal mechanisms of tropical seagrass species. I found biotic dispersal by marine mega-herbivores enables seagrass seeds to be dispersed to locations not accessible by abiotic means. These seeds will have a far greater germination success and will germinate faster. Previous dispersal and connectivity models for tropical seagrasses lacked data on biotic seed dispersal potential and on the availability of fragments for dispersal. My thesis has filled this knowledge gap, allowing for more accurate models, which can be used to assist in seagrass conservation and restoration actions.

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Publications produced during my PhD candidature

Publications from my PhD

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:**4458. https://www.nature.com/articles/s41598-017-04421-1.pdf

Tol. S.J., Jarvis. J.C., York. P.H., Congdon. B.C. and Coles. R.G. (2020). Mutualistic relationships in marine angiosperms: enhanced germination of seeds by mega-herbivores. Biotropica, Under review, Manuscript ID: BITR-20-268

Tol. S.J., Carter, A.C., York. P.H., Jarvis. J.C., Grech. A., Congdon. B.C. and Coles. R.G. (2020). Vegetative fragment production as a means of propagule dispersal for a tropical seagrass meadow. Annals of Botany, Under review, Manuscript #: 20635

Tol. S.J., Harrison. M., Groom. R., Gilbert. J., Blair. D., Coles. R.G. and Congdon. B.C. (2021) Using DNA to distinguish between faeces of *Dugong dugon* and *Chelonia mydas*: non-invasive sampling for IUCN-listed megafauna. Conservation Genetic Resources. 10.1007/s12686-020-01187-z

Other publications produced during my PhD

Tol. S.J., Coles. R.G. and Congdon. B.C. (2016) *Dugong dugon* feeding in tropical Australian seagrass meadows: implications for conservation planning. *Peer J* 4:e2194 https://doi.org/10.7717/peerj.2194

Grech. A., Hanert. E., McKenzie. L., Rasheed. M.A., Thomas C., **Tol. S.J.**, Wang. M, Waycott. M., Wolter. J. and Coles. R.G. (2018) Predicting the cumulative effect of multiple disturbances on seagrass connectivity. *Global Change Biology*: 1-12. https://onlinelibrary.wiley.com/doi/pdf/10.1111/gcb.14127

Conference Presentations

Tol. S.J., Coles. R.G., Jarvis. J.C., York. P.H. and Congdon. B.C. (2015) Seagrass seed dispersal by marine mega-herbivores: Dugongs (*Dugong dugon*) and green sea turtles (*Chelonia mydas*). The 52nd Australian Marine Science Association Annual Conference, Geelong, Australia

Tol. S.J., Jarvis. J.C., York. P.H., Bell. I., Coles. R.G. and Congdon. B.C. (2015) Seagrass dispersal by *Dugong dugon* and *Chelonia mydas* in the Great Barrier Reef lagoon, Australia. 24th Coastal and Estuarine Research Federation Biannual Conference, Portland, Oregon.

Tol. S.J., Coles. R.G., York. P.H., Jarvis. J.C., Bell. I. and Congdon. B.C. (2016) Quantifying the role of dugongs and green sea turtles in seagrass dispersal. The New Zealand Marine Sciences Society and Australian Marine Science Association Joint Conference, Wellington, New Zealand

Tol. S.J., Jarvis. J.C., York. P.H., Congdon. B.C. and Coles. R.G. (2019) Dugongs and green sea turtles increase seagrass germination through ingestion and excretion. 25th Coastal and Estuarine Research Federation Biannual Conference, Mobile, Alabama.

Poster Presentations

Tol. S.J., Carter. A., York. P.H., Grech. A., Congdon. B.C. and Coles. R.G. (2019) Waterborne seagrass fragments as dispersal propagules in the Great Barrier Reef. The 57th Australian Marine Science Association Annual Conference, Freemantle, Australia

Chapter 1

General introduction

Understanding the re-establishment potential for tropical seagrasses within the Great Barrier Reef World Heritage Area requires a quantitative knowledge of seagrass dispersal capabilities. In this chapter, I identify an approach to quantify the biotic dispersal capabilities of seagrass seeds by marine mega-herbivores and the abiotic dispersal of viable seagrass fragments. I also provide a rationale for the outline of my thesis, its objectives and structure.

Seagrass: an important marine habitat

How a species disperses directly impacts the population dynamics (Roughgarden et al. 1988, Hanski 1999), population genetics (McCauley et al. 1996, Cowen and Sponaugle 2009) and evolutionary path (Palumbi 1994), and influences connectivity and resilience (Kinlan and Gaines 2003). Angiosperms are sessile organisms and rely on dispersal not only to expand their range, but to escape areas where growth is no longer favourable (Kinlan and Gaines 2003). The dispersal pathways for terrestrial angiosperms have been well studied, with complex relationships and networks established for both biotic and abiotic dispersal (Herrera 1989, Eriksson and Bremer 1992). However, dispersal pathways and how they affect populations and community networks is an area of research that has only been recently emerging for marine angiosperms (Kendrick et al. 2012, McMahon et al. 2014, Kendrick et al. 2017). There is a sense of urgency to have a greater understanding of these dispersal processes to improve our understanding of connectivity and resilience now that we have entered into the Anthropocene era and are experiencing rapid habitat loss and fragmentation (Kinlan and Gaines 2003, Orth et al. 2006a, Kendrick et al. 2017).

Seagrasses are marine angiosperms which adapted to the marine environment approximately 100 million years ago (Larkum et al. 2006). Seagrasses are not true grasses, but are an ecological group from the families Zosteraceae, Cymodoceaceae, Posidoniaceae and Potamogetonaceae, comprising of approximately 72 different described species throughout the world; 54 of which are sub-tropical and tropical species (Short and Coles 2001, Short et al. 2007, Short et al. 2011) (Figure 1.1). Seagrass meadows provide many ecosystem services to the marine environment, not only for humans but for many iconic and culturally important species (Green and Short 2003, Orth et al. 2006a, Waycott et al. 2009). These ecological services benefit either directly to the habitat (e.g. stabilising sediment (Potouroglou et al. 2017), providing nurseries for juvenile fish and invertebrates (Coles et al. 1993), and providing food for a variety of organisms; such as many invertebrates, herbivorous fish, sea turtles and dugongs (Tol et al. 2016)), or indirectly by impacting connecting ecosystems (e.g. filtering excess nutrient (Mellors et al. 2005), sequestering carbon (York et al. 2018b), and increasing coral reefs ability to fight off disease (Lamb et al. 2017)).

Similar to terrestrial angiosperms, seagrasses are capable of reproducing both sexually and asexually. Seagrasses are the only marine plants which produce fruits and seeds underwater (Hemminga and Duarte 2000, Larkum et al. 2006, Orth et al. 2006b). They are also capable of clonal growth, through meadow expansion or growth from a fragment/propagule after settlement to a new location (Larkum et al. 2006, Orth et al. 2006b, Sherman et al. 2018). These two modes of reproduction have proven to be highly effective, as seagrasses inhabit the coastline of every continent except Antarctica (Hemminga and Duarte 2000, Green and Short 2003, Larkum et al. 2006).

Globally, seagrass meadows are declining due to increased nutrient loads from agricultural and urban run-off, coastal development, hydrological alterations, damaging fishing practices and increases in severe weather (Short and Wyllie-Echeverria 1996, Orth et al. 2006a, Waycott et al. 2009, Grech et al. 2011). Although the western Pacific has among the highest global seagrass biodiversity, this region has not been immune to declines, resulting from dense human populations close to the coast (Green and Short 2003, Short et al. 2014, Coles et al. 2015). Within Australia, north-east Queensland is a seagrass biodiversity hotspot, with up to 12 species present (Coles et al. 2002). During 2010-11, severe weather events (e.g. repeated flooding and a category 5 tropical cyclone - TC Yasi) led to a drastic reduction in coastal seagrass meadows along approximately 1,300 km of coastline in Queensland between Cairns and Hervey Bay (McKenzie et al. 2012). TC Yasi is believed to be responsible for the loss of 98% of intertidal seagrass meadows along its over 400 km coastal path (McKenzie et al. 2012, Rasheed et al. 2014, McKenna et al. 2015). These severe weather events added to an overall decline that has been occurring in north-eastern Queensland since 2006 (Devlin et al. 2012, Coles et al. 2015, McKenna et al. 2015). Recovery of many of these sites can take multiple years (Rasheed et al. 2019, McKenna et al. 2020). It is possible that climate change will further negatively affect seagrass meadows in this region, with predictions of increased intensity of weather events having the potential to reduce the ability of the seagrass plants to recover (Orth et al. 2006a, Bjork et al. 2008, Waycott et al. 2009, Short et al. 2011). With increasing pressures on seagrass meadows, it is becoming clear that further understanding of tropical seagrass resistance and recovery is required for effective conservation.

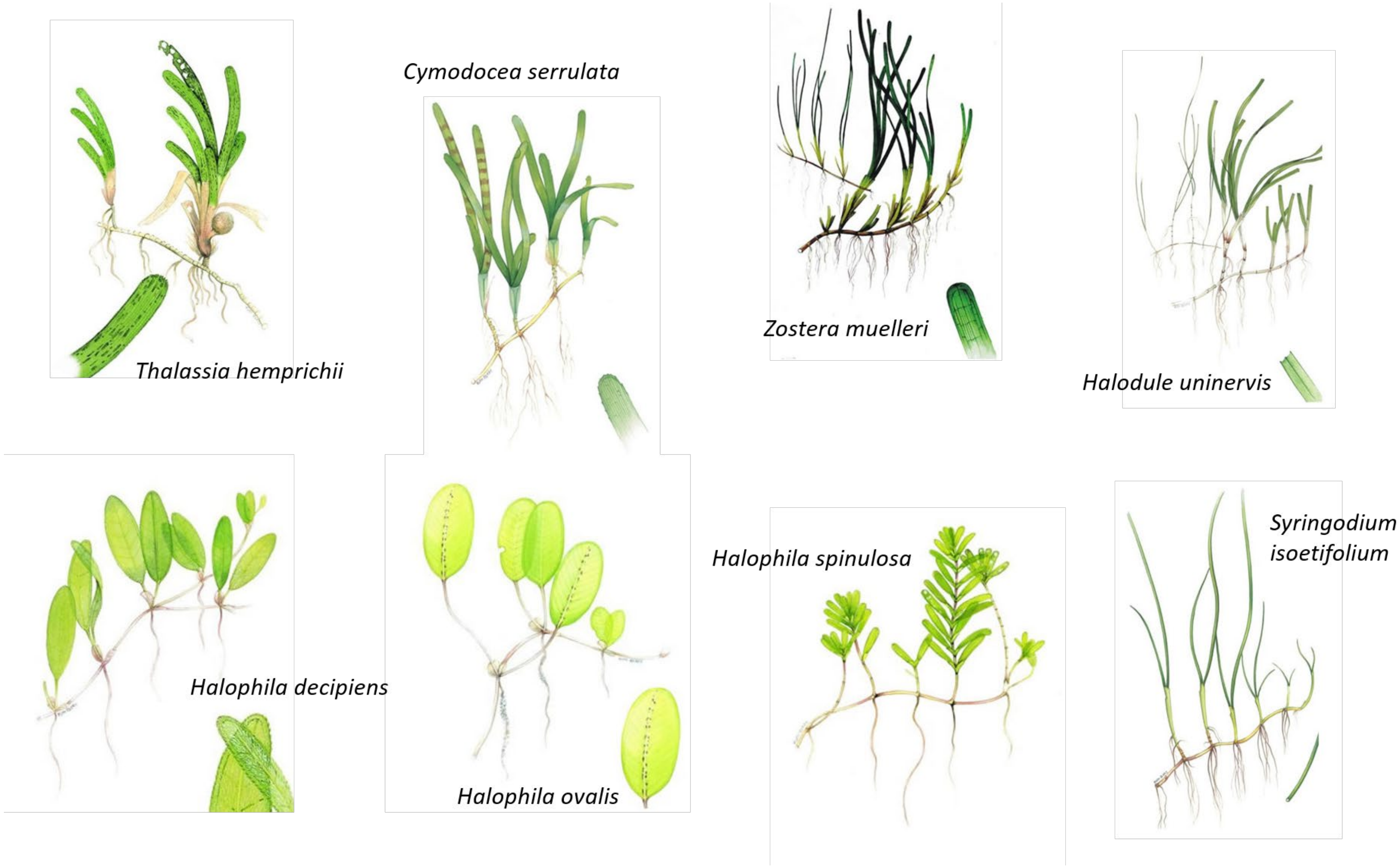


Figure 1.1: Some of the seagrass species commonly found in seagrass meadows in the Great Barrier Reef World Heritage Area, Queensland Australia; illustrations by Ruth Berry “Queensland Seagrasses – Art & Sciences”, Cairns Australia, 2000.

The role of sexual and asexual dispersal strategies

Understanding the dispersal of a species is critical if it has suffered a reduction in population size, has the potential for shifts in distribution and to assist in recovery after disturbance (Clobert et al. 2001). Therefore, to effectively manage a species or habitat you need to understand the modes of dispersal pathways (Clobert et al. 2001). Understanding and quantifying the potential dispersal modes of seagrasses will enable models to predict areas greatest at risk of reduced or inhibited recovery after a large-scale loss (Kendrick et al. 2012, McMahon et al. 2014).

Dispersal occurs through either biotic (dispersal by animals) or abiotic (wind, currents, tides etc.) pathways (Howe and Smallwood 1982, Cook 1985). The dominant mode of dispersal for aquatic (freshwater, euryhaline and marine) angiosperms is via the movement of viable plant propagules through abiotic processes. This can be through both sexual propagules (movement of reproductive shoots such as seeds, fruits and rhipidia (Grace 1993, Philbrick and Les 1996, Smith et al. 2018)) or asexual propagules (meadow expansion via rhizome growth or through successful recruitment from a viable vegetative fragment (Cook 1985, Hall et al. 2006)). For seagrasses, abiotic dispersal has proven to be highly effective, allowing for high levels of connectivity and even evidence of cross ocean dispersal (Erftemeijer et al. 2008, Källström et al. 2008, Smith et al. 2018).

For the majority of plant species, long-distance dispersal increases the success of seedling survival (Cook 1985). Parental competition can greatly reduce the survival of a seedling, especially in long lived species, therefore it is advantageous to disperse propagules over long-distances to increase the chance of decreasing competition (Cook 1985). Successful dispersal outside of a population enhances genetic diversity, which can enhance the resilience of a population or habitat (Kendrick et al. 2017, McMahon et al. 2018). Long-distance dispersal for seagrass is defined when a dispersal event affects connectivity and the metapopulation structure on a species and meadow level genetically; this is usually a dispersal distance greater than a few kilometres (Kendrick et al. 2017).

Sexual reproduction in angiosperms results in the production of fruits and seeds (Harper 1977). This mode of reproduction has a high energetic cost attached, however this is offset by the enhancement in genetic diversity (Cook 1985). Most aquatic angiosperms flower and produce seeds annually (Cook 1990, Larkum et al. 2006), with many seagrass species known to have a period of time each year in which they flower and produce fruits (Green and Short 2003, Waycott et al. 2004). However, sexual reproduction for aquatic angiosperms is found to be increased during times of stress (Inglis 2000b, Koch et al. 2010, Kendrick et al. 2012). Many of these seagrass species produce seeds with a hard seed coat and are capable of remaining in a transient (up to a few months) or persistent (up to a few years) dormant state in the seabed (Orth et al. 2006b, McMahon et al. 2014). This state of dormancy allows for the creation of a 'seed bank', which provides a source of recovery after loss (van Lent and Verschuure 1994, van Lent and Verschnure 1995, Orth et al. 2000).

The majority of seagrasses produce negatively buoyant seeds (11 out of 13 genera) (Orth et al. 2006b). A lack in seed buoyancy effects the abiotic dispersal potential, limiting the range to 10s to 100s of meters, and therefore within the meadow (Kendrick et al. 2012, McMahon et al. 2014). For a reproductive structure to undergo an abiotic long-distant dispersal movement, it would need to be attached to a floating fragment; known as 'rafting' (Kendrick et al. 2012, McMahon et al. 2014, Smith et al. 2018). Alternatively, if endozoochory (the dispersal of a seed by the ingestion and excretion by a vertebrate) occurs, there is the potential for biotic long-distance dispersal, especially if the animal vector is capable of large movements (Kendrick et al. 2012, McMahon et al. 2014, York et al. 2017).

Seed production in tropical seagrass species is lower than those recorded for temperate species; with 594 (±322) seeds/ m^2 and 1119 (± 313) seeds/ m^2 recorded for *Halophila ovalis* and *Zostera muelleri* respectively in Gladstone harbour (Bryant et al. 2016) compared to thousands per m^2 for *Zostera marina* (Olesen 1999). However, even seed numbers around 10 times lower appear to be sufficient to ensure a healthy seed bank (Harwell and Orth 2002b, Jarvis et al. 2015, Reason et al. 2020). These high seed productions are necessary, as germination success is relatively low for many seagrass seeds, especially those which undergo a time of dormancy (Orth et al. 2000). Additionally, seeds which undergo dormancy require a 'dormancy break' to initiate germination (such as a reduction in oxygen, salinity and/ or temperature, or scarification of the seed coat), if these conditions are not met it can reduce germination success (Orth et al. 2000, Jarvis 2009). Therefore, high seed output is necessary to ensure seedling production.

High seed production can also assist in a long-distance dispersal strategy, if endozoochory occurs. Many terrestrial angiosperms heavily rely on endozoochory for long-distance dispersal, and an added benefit from the ingestion and excretion of the seed is enhanced germination (Law 1985, Traveset 1998, Traveset and Verdú 2002). Some temperate seagrass species gain inter-meadow (100's meters) or long-distance (10s kilometres) connectivity through biotic dispersal of seeds by small marine fish, terrapin and water fowl (Charalambidou et al. 2003, Sumoski and Orth 2012, Tulipani and Lipcius 2014). Enhanced germination is known to occur for one tropical seagrass species (*Halophila ovalis*) after ingestion and excretion by water fowl and small marine fish (Wu et al. 2016). However, many seagrass species (both temperate and tropical) and potential biotic vectors are critically under studied.

Seagrasses are also capable of asexual growth by rhizomes, a buried horizontal root-like component similar to vegetative growth of some terrestrial plants (Hemminga and Duarte 2000, Larkum et al. 2006). This can be done by either the extension of the meadow through the growth of existing rhizomes or a viable vegetative fragment can come lose and disperse and establish in a new location (Den Hartog 1970). Dispersal by vegetative fragments for aquatic angiosperms is known to be a highly successful dispersal mode, with some invasive species traversing across vast bodies of water to colonise previously uninhabited continents (Barrett et al. 1993, Philbrick and Les 1996, Vallejo-Marín et al. 2010, Thomson et al. 2014). Dispersal over large distances is more common by vegetative fragments than seeds in aquatic angiosperms, which may in part be due to high rates of asexual growth (Barrett et al. 1993, Cox 1993, Conacher et al. 1994a, Philbrick and Les 1996, Vallejo-Marín et al. 2010, McMahon et al. 2014). However, the production of these fragments, how many are available for dispersal, and how many are viable, is still not currently known for most seagrass species.

How well populations are connected will determine their resilience to disturbance or loss (Baguette et al. 2013, Grech et al. 2018). Genetic data suggests that there is a high level of connectivity within seagrasses, however the direction of this connectivity is not clearly understood (Waycott et al. 2005, Kendrick et al. 2017). To effectively manage seagrass meadows, especially populations determined to have low resilience, this extent and direction of connectivity is necessary (Grech et al. 2016, Grech et al. 2018). To quantify and establish the direction of connectivity, we need to first gain a comprehensive understanding into the biotic and abiotic dispersal pathways of seagrass seeds and fragments.

Marine mega-herbivores interaction with seagrass

The evolutionary history of marine mega-herbivores, sirenians (dugong and manatee) and green sea turtles, covers almost the entire time span when seagrasses colonised the marine environment. The green sea turtle has existed for around 120 million years (Wyneken et al. 2013). This animal has a circumtropical distribution, allowing them access to at least 54 different sub-tropical and tropical seagrass species (Short and Coles 2001, Short et al. 2011, Marsh and Sobtzick 2019). Many extant species in the order Sirenia began feeding on seagrasses approximately 50-60 million years ago, with species in the family Dugongidae feeding predominately on either one or a select few species (Marsh et al. 2011). Today the dugong, the only species remaining in the family Dugongidae, feed predominately on at least 25 out of the 26 seagrass species within their Australian range. Dugongs can be found in warm waters ranging from the west Pacific Ocean to the east coast of Africa (Preen 1992, Aragones 1996, Marsh et al. 2011).

Marine mega-herbivores consume large volumes of seagrass. Dugongs have been estimated to consume up to 40 kg wet weight of seagrass daily (Aragones 1996, Marsh et al. 2011), while green sea turtles consume up to 2.5% of their body weight daily (Hadjichristophorou and Grove 1983). Both dugongs and green sea turtles have been observed to alter seagrass species composition and nutrient content due to these high levels of frequent foraging; commonly referred to as ‘cultivation grazing’ or ‘grazing plots’ (Preen 1995, Hearne et al. 2019, Scott et al. 2020). When foraging, these animals are capable of removing the entire plant (leaves and rhizomes), with dugongs doing so regularly (Marsh et al. 2011, Tol et al. 2016, Scott et al. 2020). This high volume of consumption has led to suggestions that these marine mega-herbivores could cause significant declines in habitat function, with some evidence immerging where populations of the marine mega-herbivores are increasing (Fourqurean et al. 2010).

However, there are equals calls that these marine mega-herbivores could play an important role in seagrass dispersal (Orth et al. 2006b, McMahon et al. 2014).

Seagrass meadows provide a key component of the marine food web (Heck Jr and Valentine 2006, Heck Jr et al. 2008). These seagrass plants have not evolved to become unpalatable (Heck Jr and Valentine 2006), suggesting it is possible that a co-evolutionary mutualistic relationship has formed. When large animals (such as large herbivorous fish, stingrays, sirenians and green sea-turtles) forage on or in a seagrass meadow, vegetative fragments may be created (Thayer et al. 1984, Green and Short 2003). These fragments can become free and float in the water column (Thayer et al. 1984) and may contribute to the number of fragments dispersing along coasts and in the ocean. Additionally, incidental consumption of fruits and seeds by these animals have been suggested to lead to long-distance dispersal (Orth et al. 2006b, McMahon et al. 2014, York et al. 2017).

Data on biotic movement of seagrass seeds and vegetative fragments is lacking, with only some work performed to date, and mainly on animals with small dispersal ranges or who are not marine species (Clausen et al. 2002, Les et al. 2003, Sumoski and Orth 2012, Tulipani and Lipcius 2014). Marine mega-herbivores, sirenians (particularly dugongs) and green sea-turtles, spend the majority of their lives within seagrass meadows and are known to travel long distances; 100’s to 1000’s of kilometres (Luschi et al. 1996, Cheng 2000, Sheppard et al. 2006, Hobbs et al. 2007). This makes these species the perfect candidate to determine if there is a relationship between animal movement and seagrass dispersal.

Figure 1.2: Marine mega-herbivores **a)** *Dugong dugon* (dugong) swimming off Orman Reef in the Torres Strait, Australia; and **b)** *Chelonia mydas* (green sea turtle) foraging on seagrass in an intertidal meadow at Lizard Island, Queensland Australia (photos courtesy by the Seagrass and Coastal Ecology Group, The Centre for Tropical Water and Aquatic Ecosystem Research Services).

Thesis objectives

Loss of seagrass cover globally is increasing (Orth et al. 2006a, Waycott et al. 2009, Short et al. 2014) and seagrass habitats in the Great Barrier Reef World Heritage Area (GBRWHA) are under threat from commercial, agricultural and residential development (Grech et al. 2011). Connectivity among seagrass meadows in the GBRWHA influences their capacity to replenish, recruit and recover from disturbances. A critical understanding of their abiotic and biotic dispersal mechanisms of seagrass is therefore necessary for effective management. For example, efforts to address seagrass loss by attempting to restore or rehabilitate meadows has had mixed results and a high financial cost due to poor ecological knowledge (Paling et al. 2009, van Katwijk et al. 2016, Kenworthy et al. 2018, Ambo-Rappe et al. 2019). Dispersal information is needed to support the cost-efficient allocation of restoration and rehabilitation resources, as dispersal determines the capacity for individual seagrass meadows to naturally replenish and recover with propagules from other meadows. Understanding of dispersal is necessary to provide confidence to Government that their management resources are invested strategically and in the right location by ruling out for example sites that should recover naturally (Grech et al. 2018).

The goal of my thesis was to determine the importance of abiotic and biotic dispersal of tropical seagrass in the GBRWHA. Biotic dispersal was concentrated on marine mega-herbivores (dugongs and green sea turtles) due to their potential for long distance (100s of kilometres) dispersal. To achieve my thesis goal, I identified the following objectives:

1. a) Determine whether marine mega-herbivores disperse viable seagrass seeds via endozoochory, and estimate the number of seeds and total potential dispersal distance;
1. b) Determine if seeds that pass through the gut of marine mega-herbivores have an increased germination rate for time and proportion; and
2. Quantify the number of viable floating seagrass fragments within the GBRWHA coastal seagrass meadows, and establish which environmental factors influence these numbers.

Objective 1a: Determine whether marine mega-herbivores disperse viable seagrass seeds via endozoochory, and estimate the number of seeds and total potential dispersal distance.

Research on the biotic role of seagrass dispersal has concentrated on waterfowl, freshwater turtles, and small fresh and marine fish (Clausen et al. 2002, Charalambidou et al. 2003, Sumoski and Orth 2012, Tulipani and Lipcius 2014, Wu et al. 2016). The potential for long distance dispersal revealed by these studies is limited due to the size of the organism, gut retention times and the animal's movement behaviour (home range), and/ or its constraint to fresh and brackish waters. Long distance biotic dispersal has been long suggested to occur by marine mega-herbivores; sirenians (dugongs and manatees) and sea turtles, through the incidental ingestion and passing of seagrass fruits and seeds (endozoochory) (Orth et al. 2006b, McMahon et al. 2014, York et al. 2017). Confirmation on whether marine mega-herbivores disperse viable seagrass seeds is necessary to understand seagrass dispersal potential. I have addressed this gap in knowledge in Chapters 2 and 3.

Objective 1b: Determine if seeds that pass through the gut of marine mega-herbivores have an increased germination rate for time and proportion.

If marine mega-herbivores disperse viable seagrass seeds, the next gap in knowledge is whether this dispersal mode changes the overall germination success or breaks the seeds dormancy to instigate quicker germination. Quantifying whether there is a net positive, negative or neutral effect of seed ingestion and excretion by a marine mega-herbivore on seed germination and viability will determine the importance of their dispersal potential. I have addressed this gap in knowledge in Chapter 4.

Objective 2: Quantify the number of viable floating seagrass fragments within the GBRWHA, and quantify what abiotic and biotic factors can predict fragment numbers, and establish which environmental factors influence these numbers.

Long distance dispersal can occur in seagrass via floating asexual propagules (fragments of rhizomes and shoot material). Present knowledge on the quantity of available floating fragments and the proportion which are viable is limited. There is limited data available for temperate species, and almost none for tropical species. Many abiotic factors are known to create fragments (such as wind, tides and currents), and grazing by mega-herbivores has also been suggested as a source. There are no models presently that can predict which environmental factors initiate fragment availability and to what quantity. Additionally, information on biotic fragment creation is lacking. I have addressed these knowledge gaps in Chapter 5.

Study Site

The GBRWHA spans approximately 2,300 kilometres along the north-eastern coast of Queensland, Australia; and extends across a latitudinal range of 10°41′ S to 24° 30′ S (Figure 1.3). Within the 348,000 km^2 of the world heritage site, there are around 3,000 coral reefs, 600 continental islands, 300 coral cays and 150 inshore mangrove islands, and the system is considered to be the world's largest living organism. The GBRWHA boasts one of the greatest levels of biodiversity globally, and is considered to be of international importance due to its "outstanding universal values". However, many factors are currently impacting the existence of this national treasure as we currently know it. Impacts from commercial and recreational fishing, agricultural, residential and industrial run-off, as well as climate change (increased sea surface temperatures, increased intensity of storms, cyclones and floods, and rising sea levels) have led to the heritage site listed as being of "significant concern" (IUCN 2017). To ensure the survival of the GBRWHA, further research into the ecosystems and habitats within the heritage area is needed.

Seagrass habitats are vital to the health of the GBRWHA. The GBRWHA contains nearly 35,000 km^2 of seagrass meadows; just over 3,000 km^2 in waters less than 15 meters and greater than 31,000 km^2 over waters greater than 15 meters (Coles et al. 2015, Carter et al. 2016). Many of these meadows have been extensively mapped and monitored for nearly two decades, with annual trends available on meadow size, species presence and biomass (e.g., Carter et al. 2016, McKenna et al. 2020, Smith et al. 2020). The seagrass meadows within the GBRWHA are also home to one of the largest remaining dugong (Sobtzick et al. 2014, Sobtzick et al. 2015, Sobtzick et al. 2017) and green sea turtle populations globally (Chaloupka and Limpus 2001, Sobtzick et al. 2017, Limpus et al. 2018). This makes these meadows an ideal location to study the potential dispersal relationship between these marine mega-herbivores and seagrass.

A total of five seagrass meadows were chosen for my thesis research; Cleveland Bay in Townsville, Upstart Bay just north of Bowen, Pioneer Bay and Midge Point in the Whitsundays, and Gladstone Harbour in Gladstone (Figure 1.3). These seagrass meadows were chosen as they have extensive intertidal and subtidal meadows, as well as the knowledge that these meadows are routinely foraged in large numbers by dugong and green sea turtles (Sobtzick et al. 2017, Limpus et al. 2018). Additionally, these meadows were ideal locations for my research, as the meadows are located within bays and harbours providing protection from the wind, as well as having a low level of boat interference (excluding Gladstone Harbour for boat traffic); factors which increased our chances of successfully collecting faecal samples and floating seagrass fragments.

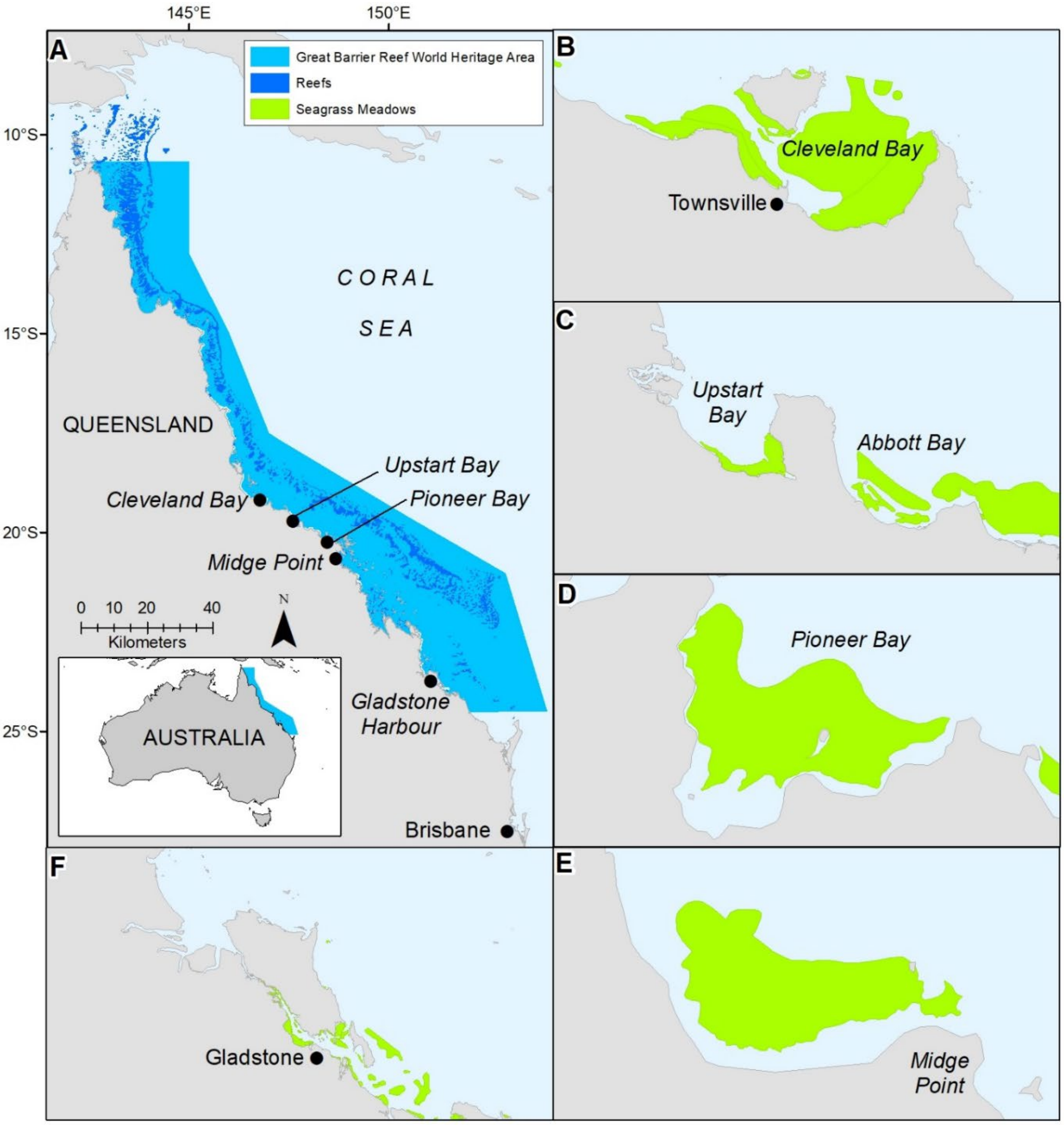


Figure 1.3: Map of seagrass meadows in the Great Barrier Reef World Heritage Area, Queensland, Australia; map created by Alana Grech.

Thesis Outline

This thesis is composed as a series of data chapters that have been written in a format and style for publication in peer review journals. The overall structure of this thesis is illustrated in Figure 1.4. Authorship of the publications are shared with my thesis committee, Brad Congdon and Rob Coles (Chapters 2-5), Jessie Jarvis and Paul York (Chapters 2, 4 & 5), and assistance from Alana Grech (Chapter 2, and 5), David Blair and Mellissa Harrison (Chapter 3) and Alex Carter (Chapter 5).

Tables and figures are shown throughout the chapter's text, with additional supporting material provided in the appendices. I created all tables, figures and photos within this thesis unless stated otherwise.

Chapter 1 provides an introduction, and outlines the different dispersal mechanisms of tropical seagrasses and highlights the knowledge gaps.

Chapter 2 describes the existence of endozoochory of tropical seagrass seeds by marine mega-herbivores; dugong and green sea turtles. This chapter also estimates the potential maximum dispersal distance and the number of viable seeds dispersed per day during peak reproductive season in the GBRWHA.

Chapter 3 develops PCR primer pairs to determine species origin (of either dugong or green sea turtle) of faecal samples collected *in-situ*. This chapter also provides a re-evaluation after DNA identification of the seagrass seed dispersal via endozoochory by marine mega-herbivores outlined in Chapter 2.

Chapter 4 quantifies the increase in germination success of seagrass seeds (*Zostera muelleri*) in their tropical range after ingestion and excretion by marine mega-herbivores; dugong and green sea turtles.

Chapter 5 quantifies the number of viable floating seagrass fragments available along the coastline of the GBRWHA, and investigates which biotic and abiotic factors predict the number of fragments.

Chapter 6 provides a summary of the previous data chapters and discusses the implications for seagrass management of these modes of dispersal in the GBRWHA. This chapter summarises the dispersal of seagrass and discusses how this can affect connectivity among meadows, and describes how this knew knowledge can assist in seagrass management.

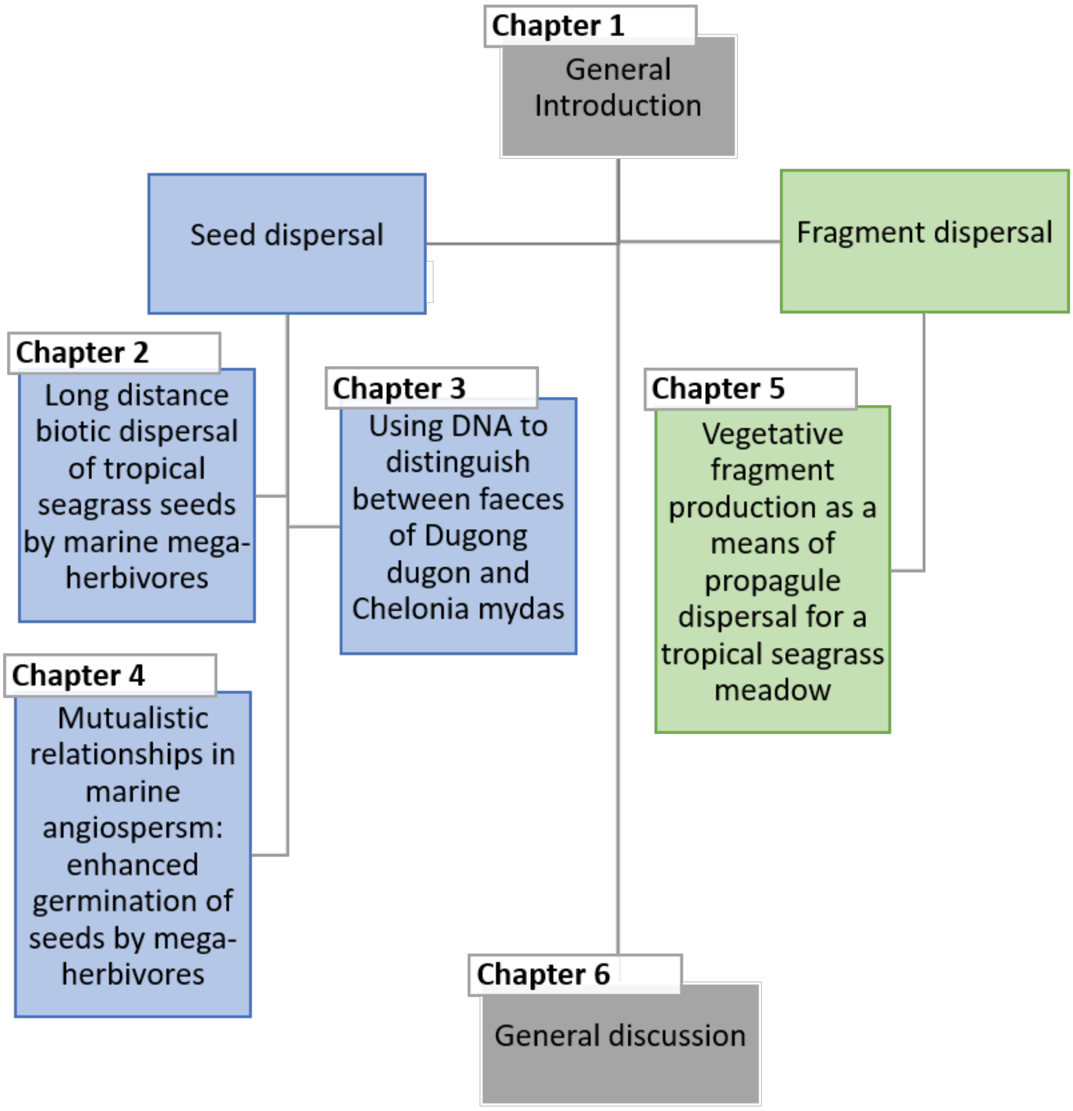


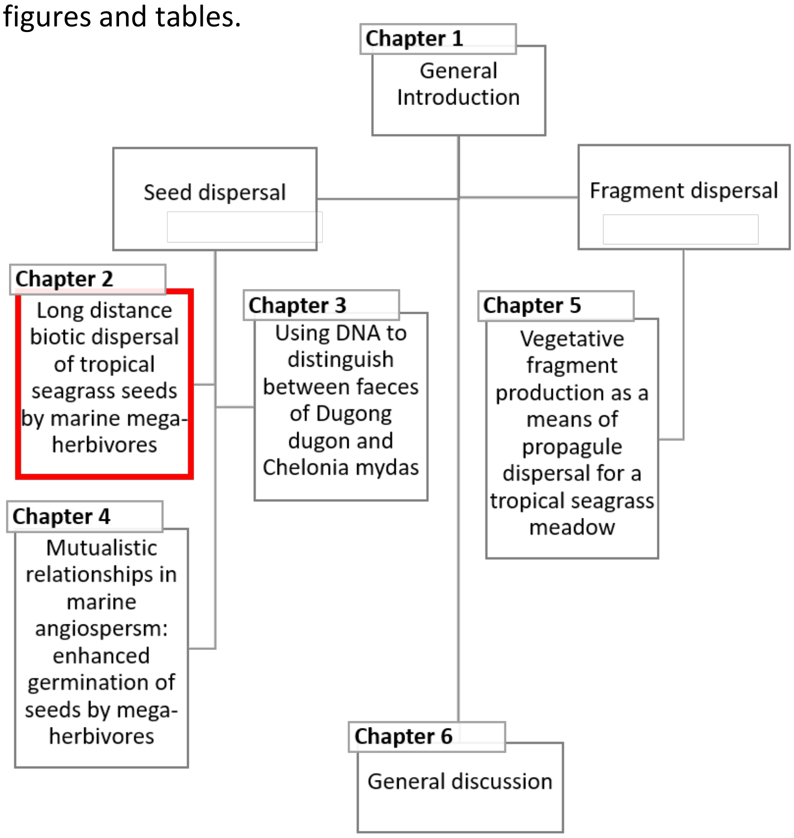
Figure 1.4: Thesis chapter structure.

Chapter 2

Long distance biotic dispersal of tropical seagrass seeds by marine mega-herbivores [1]

In this chapter, I investigate whether marine mega-herbivores (dugongs and green sea turtles) disperse tropical seagrass seeds, and investigate if those seeds are viable. Using published data on dugong and green sea turtle population estimates, travel speed and movements, as well as data on gut retention time, I calculate the maximum dispersal distance and quantify the maximum number of viable seeds that could be dispersed within the GBRWHA by this mechanism.

Tol and Coles co-developed the research question. Tol collected the data and performed the data analysis with assistance from Jarvis. Tol wrote the first draft of the paper which was revised with editorial input from Jarvis, York, Grech, Congdon and Coles. Tol, Jarvis and Grech developed the figures and tables.



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[1] **Tol. SJ**, Jarvis. JC, York. PH, Grech. A, Congdon. BC and Coles. RG. 2017. Long distance biotic dispersal of tropical seagrass seeds by marine mega-herbivores. *Scientific Reports* **7**, 1-8, DOI: 10.1038/s41598-017-04421-1

Introduction

Dispersal is a critical stage in the life history of nearly all plant species, and limitations on this process may reduce connectivity between populations, lower resilience to natural and anthropogenic disturbances and inhibit recovery from large-scale declines due to propagule limitation (Kendrick et al. 2012, McMahon et al. 2014). Although individual plant species may rely on one species-specific dispersal mechanisms (e.g. wind, rain) (Brodie 1955), the majority of plant species use multiple dispersal mechanisms via both abiotic and biotic vectors (Harper 1977, Schupp and Fuentes 1995).

Biotic dispersal of seeds by fauna occurs via bioturbation and the creation of drifting fragments that contain reproductive structures (Figuerola et al. 2003, Blackburn and Orth 2013), the attachment of propagules to grazers (e.g. seeds stuck in waterfowl plumage) and subsequent deposition in a new location (Delefosse and Kristensen 2012, Sumoski and Orth 2012), or via direct consumption and excretion during grazing (Inglis 2000a, Jarvis and Moore 2010). Depending upon the size and mobility of the dispersal organism, seeds or plant propagules may be dispersed centimetres to kilometres (Brodie 1955, Kendrick et al. 2012, McMahon et al. 2014). For those seeds ingested during the dispersal process, the physical damage to seed coats during digestion and excretion may increase germination success through the process of scarification, or the splitting of the seed coat (Harper 1977, Schupp and Fuentes 1995). This process alleviates physical dormancy imposed by the seed coat and initiates the germination process, serving as a primary germination cue for many terrestrial species (Baskin and Baskin 2014). Terrestrial plant-herbivore interactions are well studied, but little is known of these relationships in marine environments (Campbell and McKenzie 2004, Jarvis and Moore 2010, Baskin and Baskin 2014).

Seagrasses are marine angiosperms which produce flowers, fruits and seeds almost entirely underwater (Den Hartog 1970). Between 117,000 km^2 and 500,000 km^2 (Green and Short 2003, Waycott et al. 2009) of seagrass meadows are found in shallow coastal waters around the world's continents with the exception of Antarctica (Hemminga and Duarte 2000, Green and Short 2003, Larkum et al. 2006). Within these habitats, seagrasses provide essential ecosystem functions including filtering nutrient run-off from terrestrial sources and stabilising marine sediment (Costanza et al. 1997, Duarte 2002), providing nursery grounds for economically important fisheries species (Coles et al. 1993, Heck Jr et al. 2003), and sequestering carbon (Fourqurean et al. 2012, Macreadie et al. 2014). However, many seagrass meadows are under pressure from anthropogenic impacts (e.g. coastal development, fishing practices, agricultural and urban runoff) (Waycott et al. 2009) due to their proximity to areas of high human population (Grech et al. 2011, Coles et al. 2015). As a result of these stressors, approximately 7% of the known global area of seagrass is thought to be lost annually (Waycott et al. 2009). Many factors that influence seagrass meadow maintenance and recovery have been examined in both temperate and tropical habitats, but little is known concerning the modes of dispersal for these populations (Kendrick et al. 2012).

The resilience of seagrass meadows is dependent on interactions between physical (e.g., location, climate, water currents and tidal flow) and biological factors (e.g. species and genetic diversity, life history strategy, population connectivity) (Orth et al. 2006a, Unsworth et al. 2015, Grech et al. 2016). Following large-scale losses of seagrass, seed germination from the sediment seed bank and subsequent seedling growth, is thought to be one of the main pathways for natural revegetation of disturbed habitats (Campbell and McKenzie 2004, Jarvis and Moore 2010). Seed banks are replenished via seed production within the meadow and from the input of seeds, or propagules, from other more distant meadows. Deposition of seeds from outside sources results in more abundant seed banks and an increase in genetic diversity, culminating in an increased resilience to disturbance (Unsworth et al. 2015, Grech et al. 2016). However, most seagrass seeds are negatively buoyant. This inhibits dispersal by abiotic means unless seeds are attached to floating plant fragments (Den Hartog 1970, McMahon et al. 2014). Significant knowledge gaps remain on mechanisms of seed dispersal in seagrass, levels of connectivity between meadows from seed dispersal (Kendrick et al. 2012, McMahon et al. 2014), and particularly on the importance of biological dispersal vectors for maintaining the resilience and recovery capacity of seagrass meadows.

Research has primarily focused on abiotic dispersal of seagrass seeds and propagules (Kendrick et al. 2012, Grech et al. 2016). However, herbivores and omnivores in the marine environment, including crustaceans, echinoderms, fish, reptiles, birds, sea turtles and Sirenians (dugongs and manatees), consume seagrass either directly or indirectly while feeding (Inglis 2000a, Fenner and Thompson 2005, Jarvis and Moore 2010, Blackburn and Orth 2013, Baskin and Baskin 2014). As a result, fruiting bodies and seeds can be consumed, providing a potential for biotic dispersal (Kendrick et al. 2012). Recent laboratory studies have shown that seeds of *Zostera marina* and *Halophila ovalis* can survive consumption by herbivorous fish and maintain or enhance their ability to germinate (Tulipani and Lipcius 2014, Wu et al. 2016). However, the ability of seeds to survive digestion by herbivores is species specific, as *Thalassia hemprichii* seeds did not survive consumption by waterfowl and fish under controlled laboratory conditions (Wu et al. 2016). While these results indicate biotic dispersal of some seagrass by specific vectors is possible, there still remains a dearth of information on the role of animals, particularly mega-herbivores, in seagrass dispersal.

In tropical habitats marine mega-herbivores, dugongs (*Dugong dugon*) and green sea turtles (*Chelonia mydas*), consume seagrasses in shallow coastal waters (Heck Jr and Valentine 2006, Marsh et al. 2011, Wyneken et al. 2013). Dugongs conusme almost exclusively seagrass and include in their diet 24 out of the 26 seagrass species that co-occur in Australian waters (Marsh et al. 2011). Green sea turtles consume either a seagrass or algae dominated diet depending on their home range, however they will consume both food types when present (Garnett et al. 1985). Dugongs can consume up to 40 kg wet weight of seagrass daily (Aragones 1996, Marsh et al. 2011) and green sea turtles consume up to 2.5% of their body weight in algae and/or seagrass daily (Hadjichristophorou and Grove 1983). Both marine mega-herbivores have digestion times ranging from several days to weeks; 6-8 days for dugongs and 7-14 days for green sea turtles (time variations are dependent on the digesta/food consumed, with an herbivorous diet taking longer to digest) (Hadjichristophorou and Grove 1983, Lanyon and Marsh 1995, Brand et al. 1999). Dugongs and green sea turtles actively move across their home ranges (green sea turtle median home range of 75.7 km^2; dugong median home range of 453.2 km^2) (Cleguer et al. 2016) and can travel large distances when actively migrating (tagged dugongs have been observed to move at 25.9 ±2.23 (SE) km per day when undertaking macro-scale movements, while green sea turtles can travel up to 40 km per day) (Luschi et al. 1996, Cheng 2000, Sheppard et al. 2006). The traits of high consumption rates, slow digestion and long-distance movement combine to make dugongs and green sea turtles suitable vectors for long distance dispersal of seagrasses (Fig. 2.1).

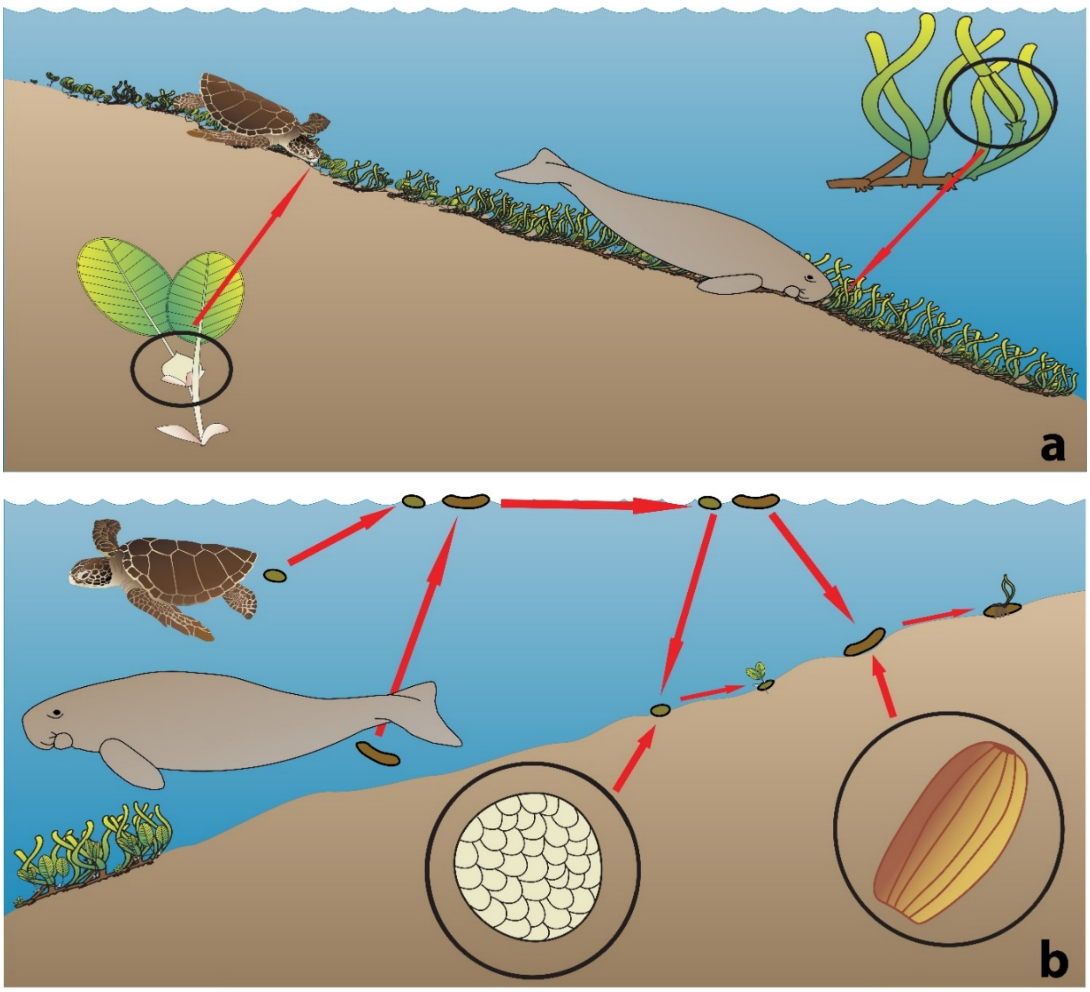


Figure 2.1: Illustration of biotic dispersal of seagrass seeds by marine mega- herbivores. a) Ingestion of seagrass seeds or propagules by a dugong or green sea turtle allows long distance dispersal of the seed; b) before settlement and growth into a seedling. Figure created by Samantha Tol, using images provided by Tracy Saxby, Catherine Collier, Diana Kleine, and the Integration and Application Network, University of Maryland Centre for Environmental Science (ian.umces.edu/imagelibrary/).

For mega-herbivores in marine systems to disperse seagrasses, seeds must remain viable after passage through the gut; a factor not explored in previous studies. My objectives in the present study were to (1) determine whether seagrass seeds pass through the digestive systems of marine mega-herbivores and remain viable and (2) determine the species, number, and potential dispersal distances for those seagrass species consumed by dugongs and green sea turtles in the Great Barrier Reef (GBR) Region. My results quantify the potential for marine mega-herbivores to disperse seagrass seeds at different spatial scales and provide direct evidence of biotic dispersal by marine mega-herbivores.

Methods

Faecal Collection

Dugong and green sea turtle faecal samples were collected monthly between September and December 2014 from three coastal seagrass meadows in central GBR, Queensland; Pioneer Bay near the Whitsunday Island Group, Upstart Bay near the city of Bowen and Cleveland Bay near the city of Townsville (Fig. 2.2). Seagrass meadows at all sites consisted of mixed species assemblages and extended from the intertidal zone to the subtidal zone (Table 2.1). Samples were collected during the period of seagrass maximum sexual reproduction, when 9 out of the 14 species present in the region flower (Waycott et al. 2004). During sample collections, green sea turtles were observed foraging at all sites, while dugongs were seen foraging at Pioneer Bay and Cleveland Bay. As it is not possible under field conditions to confidently determine from which of the two species the faeces had originated, and genetic differentiation was at the time outside the scope of this research, faecal samples from dugongs and green sea turtles were combined under a single ‘marine mega-herbivore’ category.

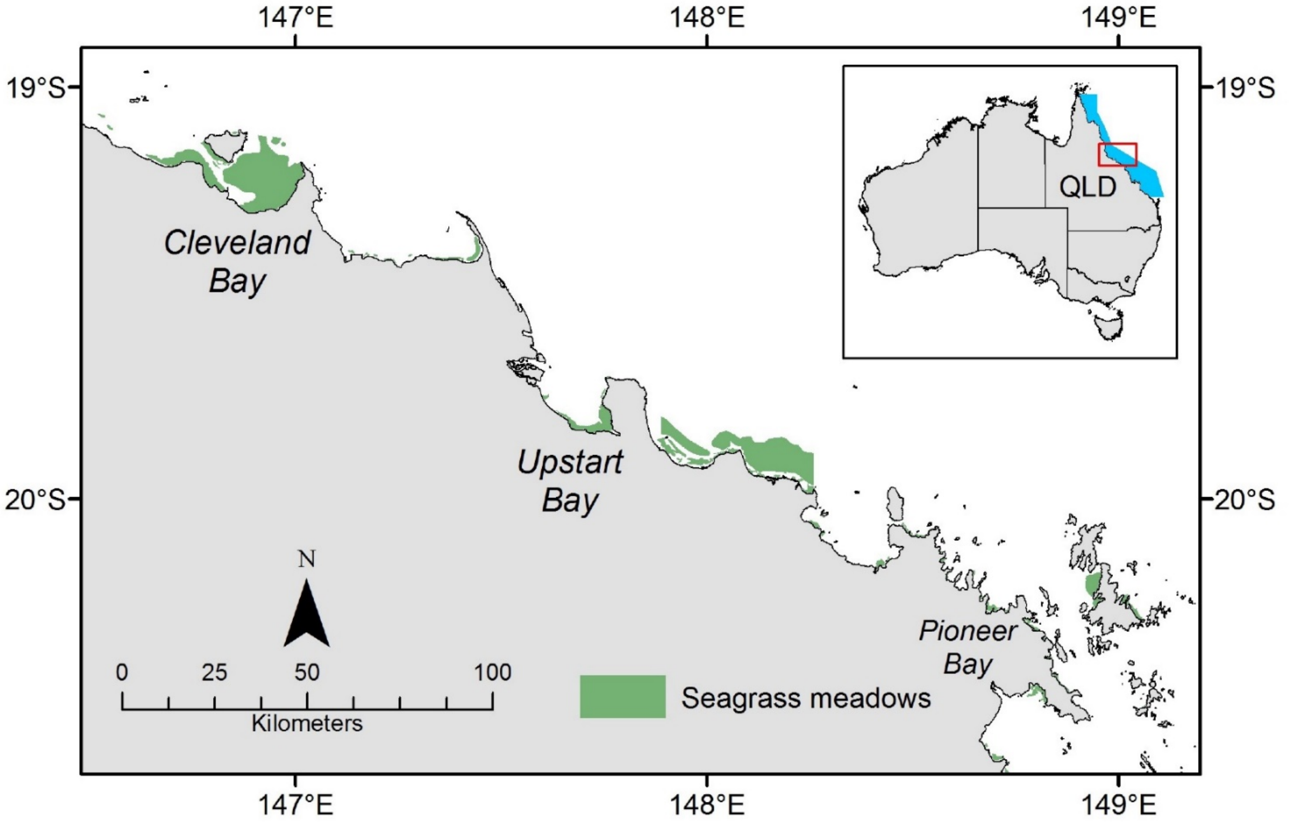


Figure 2.2: Map of intertidal seagrass meadows (green) in the central Great Barrier Reef, Queensland (QLD) Australia where mega-herbivore faecal matter samples were collected between September and December 2014. The inset map shows the location of the central region (red box) of the Great Barrier Reef World Heritage Area (blue). The map is overlayed with a subset of current known seagrass meadows (available from http://eatlas.org.au/data/uuid/77998615-bbab-4270-bcb1-96c46f56f85a).

Table 2.1: Description of intertidal seagrass meadows in northern Queensland, Australia; Pioneer Bay in the Whitsunday Island Group, Upstart Bay near the city of Bowen and Cleveland Bay near the city of Townsville; biomass measurement for above ground only.

Location	Site Description	Species Present	Dominant Species	Total Mean Biomass (gdw m^2)	Total area (ha)
Pioneer Bay	Sand/mud sediment	*Halophila spp.* *Halophila ovalis* *Halodule uninervis* *Zostera muelleri*	*Zostera muelleri /* *Halodule uninervis*	0.59 ±0.1	141.1 ±40
Upstart Bay	Mud/sand sediment	*Cymodocea serrulata* *Halophila decipiens* *Halophila ovalis* *Halophila spinulosa* *Halophila tricostata* *Halodule uninervis* *Zostera muelleri*	*Zostera muelleri*	21.0 ±5.3	2987 ±532
Cleveland Bay	Sand/mud sediment	*Cymodocea serrulata* *Halophila decipiens* *Halophila ovalis* *Halophila spinulosa* *Halodule uninervis* *Zostera muelleri*	*Zostera muelleri* (Intertidal) *Halodule uninervis* (Subtidal)	10.98 ±1.06 (Intertidal) 6.34 ±0.46 (Subtidal)	1479.81 ±101.3 (Intertidal) 4016.85 ±438.9 (Subtidal)

Seed Collection

A total of 60 faecal samples were collected over four months; 4 samples in September, 15 in October, 19 in November and 22 in December. Faecal samples were transported on ice to the laboratory where they were stored at 3-5°C until processing. All samples were processed within 30 days of collection. Samples were sieved across three size fractions to aid in seed species identification (1.4mm, 750 µm and 250 µm) and then placed in 34 ± 1 (SE) PSU seawater to prevent osmotic stress (Conacher et al. 1994a). Seagrass seeds found in faecal samples were identified to species and counted prior to the removal of the seed coat. Once the seed coat was removed all non-germinated seeds were stained with a 1.5% Tetrazolium solution for a total of 48 hours to determine viability (Conacher et al. 1994a). A positive stain (tissue turns brown to red in colour; Fig. 2.3a) indicated normal cellular metabolism within cells, signifying that the seed was still active and capable of germination (Conacher et al. 1994a, The Tetrazolium Subcommittee of the Association of Offical Seed Analysts 2005). Any seeds removed from the faeces that had already germinated were counted as viable (Fig. 2.3b). Seeds with a split seed coat were also counted per sample. Faecal matter was dried at 220°C in an oven until a consistent dry weight was reached. Samples were then weighed and reported as g DW.

Statistical Analysis

Due to the low number of seeds collected per sample, seed data was combined across seagrass species and reported as the total number of seagrass seeds per g DW of faecal matter, the proportion of seeds with a split seed coat per g DW of faecal matter and the proportion of viable seagrass seeds per g DW of faecal matter. Statistical analyses were conducted in the statistical software environment R (R version 3.2.5) (R Core Team 2014). Differences in the abundance of seagrass seeds per g DW of facecal matter over time (month) and site were analysed using generalized linear mixed effects models with a quasi-Poisson distribution (GLM) (R Core Team 2014); a Tukey post hoc test was applied. Quasi-Poisson regression is a generalized form of Poisson regression which corrects for overdispersion in count data (O'Hara and Kotze 2010). Changes over time (month) and site in the proportion of seeds with a split seed coat and the proportions of seeds that stained as viable (positive stain + germinated seeds) per g DW of faecal matter were analyzed using logistic regression mixed effects models with a binomial distribution (GLM) (R Core Team 2014); a Tukey post hoc test was applied. Logistic regression was selected due to the binary response variable and the small number of seeds found per sample (Warton and Hui 2011).

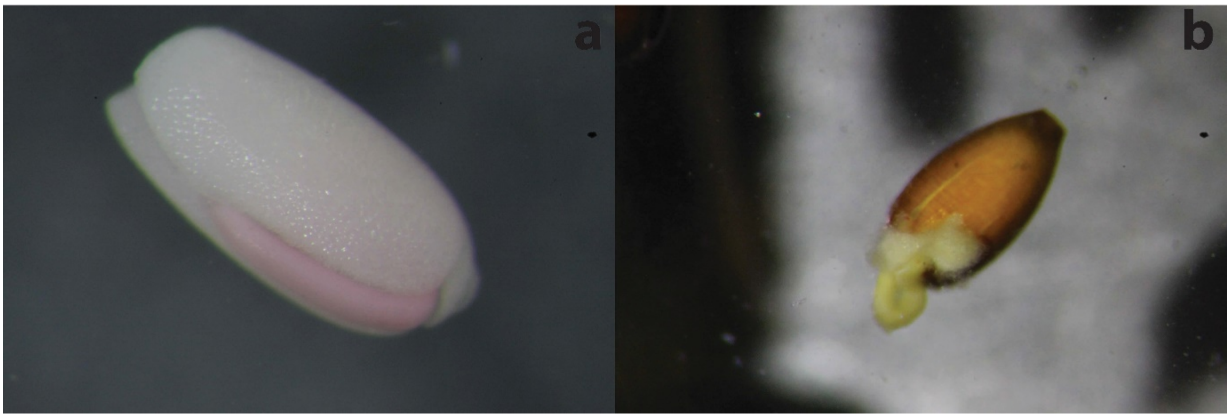


Figure 2.3: **a)** A *Zostera muelleri* seed (with seed coat removed) taken from marine mega-herbivore faeces returned a positive stain, turning the cotyledon pink; **b)** A germinated *Zostera muelleri* seed removed from marine mega-herbivore faeces.

Results

Marine mega-herbivore faecal samples collected floating on the water's surface varied in size and mass; ranging from 0.002 g DW to 7.696 g DW, with an average of 1.37 ± 0.18 (SE) g DW (Fig. 2.4).

Figure 2.4: **a)** Marine mega-herbivore faeces collected floating on the water's surface in Pioneer Bay at Airlie Beach in the Whitsundays, northern Queensland Australia; **b)** Floating marine mega-herbivore faeces floating on the water's surface. Picture taken at Pioneer Bay in the Whitsunday Island Group, northern Queensland Australia.

Seeds

I found seagrass seeds in 56% of the marine mega-herbivore faecal samples. Seagrass seeds included *Zostera muelleri* (n = 219), *Halodule uninervis* (n = 3) *Halophila decipiens* (n = 7) and unidentified *Halophila spp.* (n = 4). The mean number of seagrass seeds was between 2-3 seeds per g DW faecal matter (Fig. 2.5a). The maximum density of seagrass seeds found within faecal samples were from Cleveland Bay (n = 153), followed by Pioneer Bay (n = 72), and Upstart Bay (n = 9). The month of December has significantly less seeds than the months of October (p = 0.005) and November (p = 0.0006); seed abundance per sample (n = 8) is lowest in December, as is the percentage of faecal samples that contained seeds (September = 75.0%; October = 73.3%; November = 57.9%; December = 19.2%). Upstart Bay is significantly different to Pioneer Bay (p = 0.0005) and Cleveland Bay (p = 0.0054). Upstart Bay produced the least number of seeds (UB = 8; PB = 70; CB = 150) as well as the lowest percentage of faecal samples that contained seeds (UB = 30.0%; PB = 45.0%; CB = 60.0%) (Supplementary Table 2.1).

Except for *H. decipiens*, all species of seagrass seeds found in faecal samples included at least one seed with a split coat (*Z. muelleri* split seeds = 39.73%; *Halodule uninervis* split seeds = 100%; *Halophila spp.* split seeds = 50%). Time and/or site of collection had no significant effect on the proportion of seeds which had their seed coat split (site p = 0.661; time p = 0.291) (Fig. 2.5b and Fig. 2.6; Supplementary Table 2.2).

Viability

Germination of seeds collected from mega-herbivore faeces occurred prior to viability testing for *Z. muelleri*, *Halophila decipiens* and *Halophila spp.*, but not for *H. uninervis*. As germination is an indication of viability, these seeds were considered viable. Of the remaining non-germinated seeds, only *Z. muelleri* seeds tested as viable. The mean percentage of viable *Z. muelleri* seeds collected was 9.13% ± 4.61% (SE). Although the percentage of viable seeds was lower in samples collected in the earlier months (September and October) and peaked towards the end of the year (November and December), time of collection and/or site did not have a significant effect on the proportion of viable seeds (site p = 0.753; time p = 0.9525) (Fig. 2.5c; Supplementary Table 2.3).

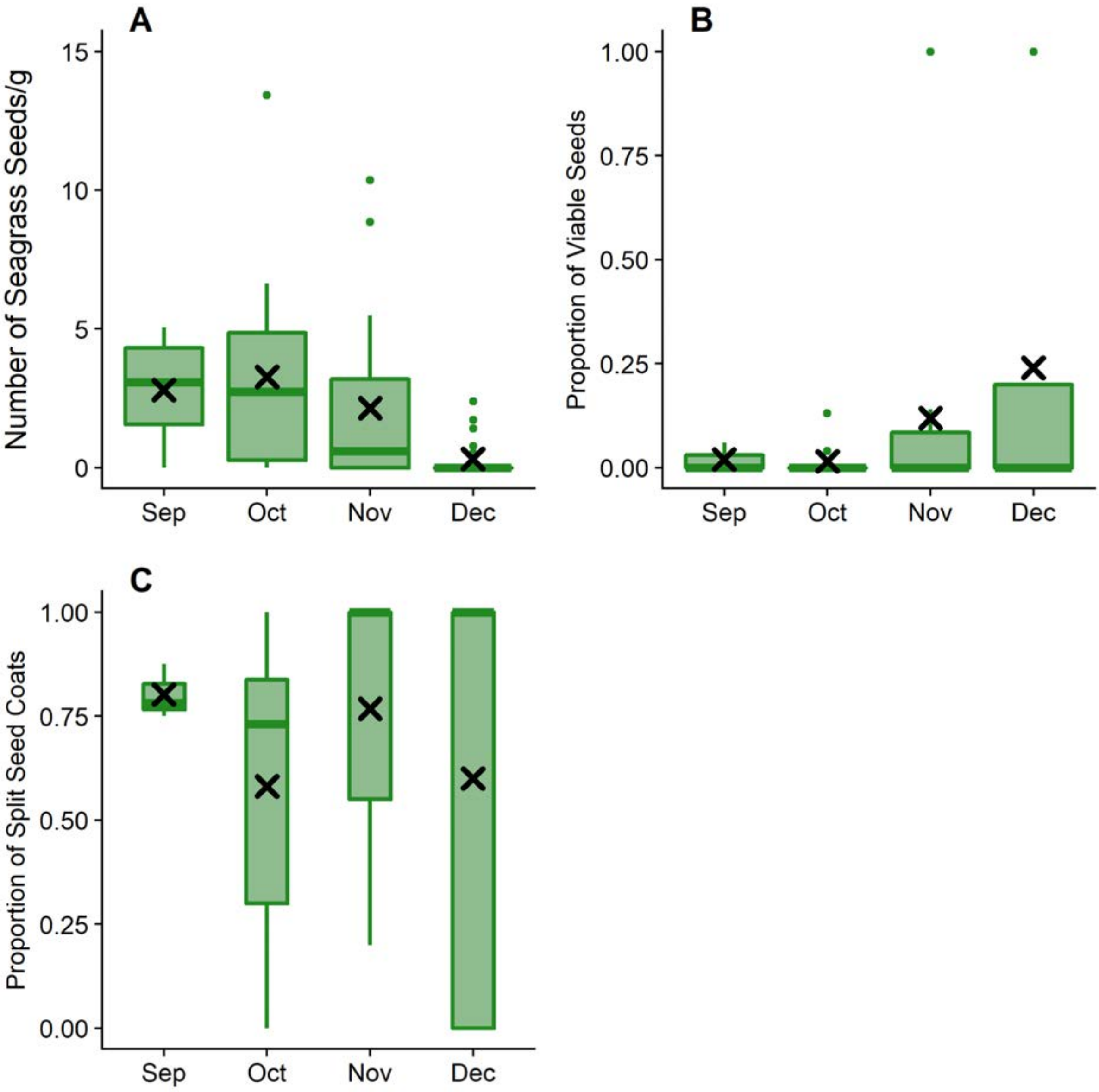


Figure 2.5: Mean monthly **a)** number of seagrass seeds (*Zostera muelleri*, *Halodule uninervis*, *Halophila ovalis*, and *Halophila spp*.) per g DW mega-herbivore faecal matter; **b)** proportion of seeds with a split seed coat per g DW faecal matter; **c)** proportion of viable seagrass seeds found per g DW faecal matter collected across all sampling sites between September and December 2014. Crosses indicate data outliers for the sampled months and the box plots indicating the median, quartiles and 95 percentiles.

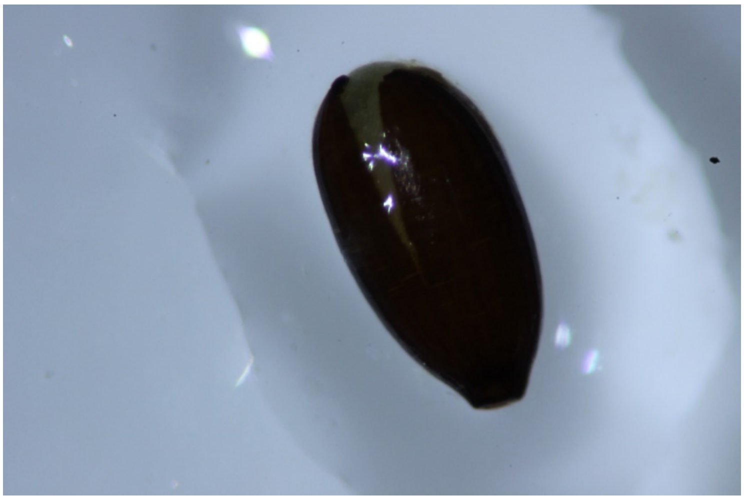


Figure 2.6: *Zostera muelleri* seed with a split coat; the seed was removed from a marine mega-herbivore faecal sample collected in Cleveland Bay, north Queensland Australia, in 2014.

Discussion

This is the first study to confirm the role of marine mega-herbivores, *Dugong dugon* and *Chelonia mydas*, in the biotic dispersal of seagrass seeds. I found 2-3 seeds per g DW in marine mega-herbivore faecal matter, with 9.13% ± 4.61% (SE) of these seeds maintaining viability after digestion. I found over half of the seeds excreted had a split seed coat, potentially increasing the chance of germination (Conacher et al. 1994a). Time and site had only a minor effect on the number of seeds found per gram of marine mega-herbivore faeces. This is most likely due to different seagrass locations peaking in reproduction in the flowering season for these species.

For most seagrass species, long distance movement of reproductive structures (flowers, fruits and seeds) is possible only when these structures are attached to floating fragments (Orth et al. 2006b, Källström et al. 2008, McMahon et al. 2014, Grech et al. 2016). The potential movement of viable seeds by biotic dispersal, confirmed in my study, is an alternative and likely important pathway by which seagrass can colonise new or recovering locations. By identifying a potential long-distance dispersal vector for seagrasses, these results have direct implications for an increased understanding of the connectivity between seagrass meadows and existing natural resilience and recovery processes in seagrass meadows.

The dispersal capability of mega-herbivores depends on the availability of plant reproductive material, the number of mega-herbivores in the region, the amount of time the seagrass material stays within the digestive systems of herbivores and the potential distance travelled between consumption and excretion of the plant material (Kendrick et al. 2012, McMahon et al. 2014). In the GBR the availability of viable seagrass reproductive material varies temporally, spatially and between species (Waycott et al. 2004, McKenzie et al. 2014). In this study, maximum seed abundances in mega-herbivore faecal samples occurred in September and October while the greatest proportion of viable seeds were found in November and December. This reflects the dominance of *Z. muelleri* seeds in the samples and follows the observed periods of maximum flowering/reproductive density for *Z. muelleri* in this region (Conacher et al. 1994b). In addition to its greater availably to mega-herbivores compared to other seagrass species across the sites sampled (Table 2.1), the abundance of *Z. muelleri* seeds in the collected mega-herbivore faeces can be attributed to its flowering strategy. *Zostera muelleri* seeds are produced on branching flowering shoots that occur at the top of the meadow canopy (Conacher et al. 1994b). The location and concentration of seeds above the sediment surface may make it easier for herbivores, green sea turtles in particular, to consume the seeds compared to species which flower at the base of the plant (e.g. *H. uninervis*) (Inglis 2000b). Therefore, the impacts of the timing and flowering shoot morphology on the biotic dispersal capability of seagrasses by mega-herbivores is likely to be species specific.

The GBR region supports a dugong population of at least 4,000 – 6,000 individuals (Marsh et al. 2007, Sobtzick et al. 2015) and an estimated population of 855,000 (95% CI: 55,000 – 1,200,000) green sea turtles (green sea turtle estimates based on best available data collected in the southern GBR) (Chaloupka and Limpus 2001). If these marine mega-herbivores conservatively pass one average size faeces per day, they have the potential to disperse as many as 500,000 viable seeds daily during the peak seagrass reproductive season (September to November). Based on current mega-herbivore population estimates, that would be an average of up to 2,500 viable seeds per day for dugongs and up to 500,000 viable seeds per day for green sea turtles. However, caution should be given when using these estimated seed dispersal numbers due to limitations in mega-herbivore abundance and migration data. Refined estimates of the number of individuals in the resident and migratory dugong and green sea turtle populations in the GBR, and the variability in the proportion of the turtle population that migrate into and out of the region, will be necessary to improve the accuracy of these dispersal estimates. However, the numbers presented here are likely to be conservative.

Due to the importance of connectivity in maintaining resilient seagrass meadows, it is important to quantify how many and how far seeds are dispersed (Waycott et al. 2007, McMahon et al. 2014). For biotic dispersal of seeds by mega-herbivores, dispersal distance is dependent on how far individual herbivores move while the seagrass material moves through their digestive system. Green sea turtles have a digesta retention time of 156 – 325 hours (Brand et al. 1999), with an average travelling speed of 1.89 ± 0.12 (SE) km h (Luschi et al. 1996, Cheng 2000), creating a potential dispersal distance of 277 – 652 km. However, most green sea turtles have a median home range of 75.7 km^2 which may result in local rather than long-distance dispersal when turtles are not migrating (Cleguer et al. 2016). Dugong gut passage times range from 146 - 166 hours (Lanyon and Marsh 1995). They can travel on average at a speed of 1.3 ± 0.11 (SE) km h when swimming long distances (Sheppard et al. 2006), potentially leading to a dispersal distance of 173 - 234 km; a distance less than the median home range of dugongs (453.2 km^2) (Cleguer et al. 2016). However, pedigree analysis in south-east Queensland found that only 1-3% of dugongs undertake largescale movements (Cope et al. 2015), while recapture and telemetric data found long distance movement across foraging grounds is possible, with travel distances ranging up to 560 km (Sheppard et al. 2006, Cope et al. 2015). This suggests that dugongs have the potential to be long distance dispersers, however are more likely to disperse locally, similar to green sea turtles.

The actual distance travelled by seeds collected in this study cannot be measured. However, we found seeds in marine mega-herbivore faeces at one of my sites (Pioneer Bay) 100s of kilometres from where seagrass flowering is common (McKenzie et al. 2014), supporting the conclusion that these herbivores are an effective disperser of seagrass seeds. Distances shown in this study for seed dispersal, and previous studies on abiotic dispersal, indicate that there is a strong potential for the dispersal of seagrass among isolated meadows on reefs and islands and for the dispersal among countries by these mechanisms (Seminoff 2004, Marsh et al. 2011, Grech et al. 2016).

Increases in the severity and occurrence of storms that have the ability to decimate seagrass meadows (Short and Neckles 1999, Duarte 2002, Orth et al. 2006a, McKenna et al. 2015) have led to questions about the ability of seagrasses to re-establish or re-colonise after large losses (Orth et al. 2006a, Grech et al. 2016, York et al. 2017). The present understanding of seagrass recovery mechanisms highlights the importance of the scale of the disturbance. Seagrass biomass loss on the scale of meters to tens of meters can recover primarily through rhizome extension (Orth et al. 2006b, Kendrick et al. 2012, McMahon et al. 2014, Grech et al. 2016). Seagrass expansion by clonal growth can occur quickly, allowing for rapid recovery from small scale impacts (Orth et al. 2006b, McMahon et al. 2014). However, clonal growth alone would be slow to re-colonise a large area if most seagrass biomass was lost (McMahon et al. 2014). To re-colonise a large area devoid of seagrass, input from either viable seagrass fragments or seeds would be required (Orth et al. 2006b, McMahon et al. 2014). Some coastal seagrass meadows have low connectivity to other meadows based on abiotic factors alone (such as wind and currents) (Grech et al. 2016). Marine mega-herbivores may be vital for connectivity and gene flow among these meadows, as these animals are more likely to deposit seeds in habitats viable for seagrass growth compared to abiotic processes. The process of digestion aids in scarification, which through splitting the seed coat, removes physical dormancy and provides a cue for germination for some plant species (Baskin and Baskin 2014). Ultimately this may enhance germination rates and contribute to the recovery of impacted meadows. Deposition of genetically distinct seeds via mega-herbivore dispersal would increase the genetic diversity of meadows, thereby increasing their resiliency to disturbance events (Hughes and Stachowicz 2011).

My study has confirmed biotic dispersal of viable seagrass seeds by marine mega-herbivores. As a result, tropical seagrass seeds have the potential to be dispersed far greater distances (in the hundreds of kilometres) than most previous reports suggest. The potential importance of mega herbivores in biotic dispersal is further enhanced by the large geographic distribution of these animals (green sea turtles from the tropical and sub-tropical ocean basins (Seminoff 2004) and dugongs from the east coast of Africa to the Indo-Pacific between latitudes 27 degrees north and south (Marsh et al. 2011)), which includes a large proportion of the world's tropical and sub-tropical seagrass meadows. Biotic dispersal is also more likely to carry seeds to areas that are viable habitats for seagrass to grow, due to mega-herbivores actively searching for seagrass as a primary food source. This dispersal mode has the ability to contribute to the resilience of seagrass meadows and aid in recovery after loss. My findings suggest that the conservation of green sea turtles and dugongs is likely to be far more important for maintaining the delicate balance between seagrass meadow recovery and loss than previously realised. A detailed understanding of the interconnection between marine mega-herbivores and their seagrass food is necessary to ensure a sustainable future for both these animals and their seagrass habitats.

Chapter Summary

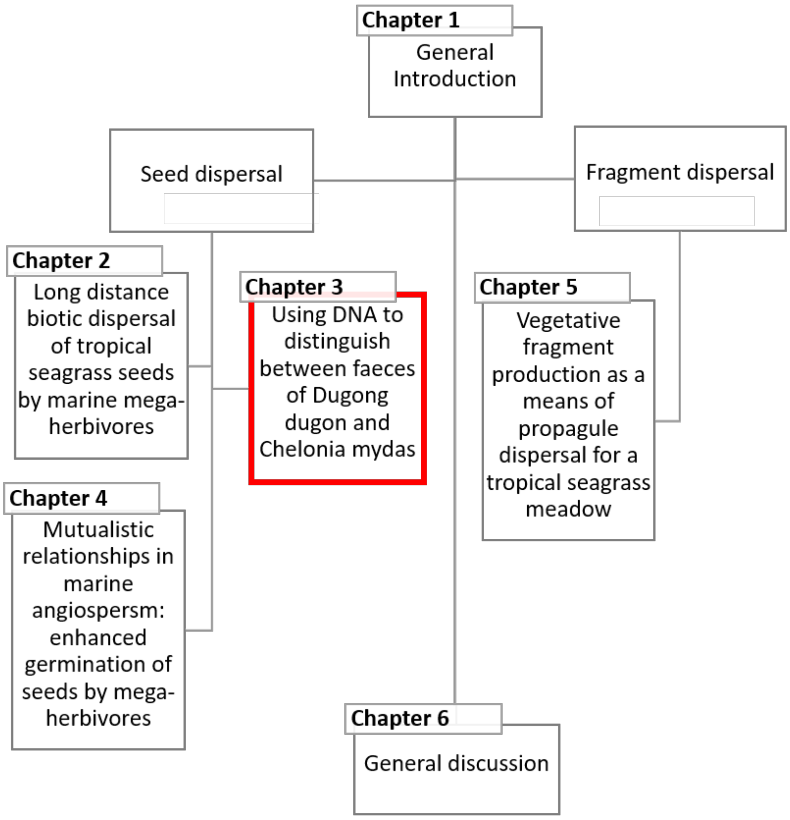
- Terrestrial plants use an array of animals as vectors for dispersal, however little is known of biotic dispersal of marine angiosperms such as seagrasses. My research confirms for the first time that dugongs (*Dugong dugon*) and green sea turtles (*Chelonia mydas*) disperse seagrass seeds in the GBRWHA.
- Marine mega-herbivores consume and pass in faecal matter viable tropical seagrass seeds for at least four different species (*Z. muelleri*, *H. uninervis*, *H. decipiens* and an unidentified *Halophila spp*.).
- 2 – 3 seagrass seeds per g DW of faecal matter were found during the peak of the seagrass reproductive season (September to December), with viability on excretion of 9.13% (± 4.61% SE).
- Using population estimates for these mega-herbivores, and data on digestion time (hrs), average daily movement (km/ h) and numbers of viable seagrass seeds excreted (per g DW), I calculated potential seagrass seed dispersal distances. Dugongs and green sea turtle populations within the GBRWHA can disperse >500,000 viable seagrass seeds daily, with a maximum dispersal distance of approximately 650 km.
- Biotic dispersal of tropical seagrass seeds by dugongs and green sea turtles provides a large-scale mechanism that enhances connectivity among seagrass meadows, and aids in resilience and recovery of these coastal habitats.

Chapter 3

Using DNA to distinguish between faeces of *Dugong dugon* and *Chelonia mydas*: non-invasive sampling for IUCN-listed marine megafauna [2]

In this chapter, I develop PCR primer pairs and methodology capable of determining the species origin of mega-herbivore (dugong and green sea turtle) faeces. Using this information, I re-evaluate the biotic tropical seagrass seed dispersal potential by these marine mega-herbivores described in Chapter 2.

Tol, Coles, Congdon and Blair co-developed the research question. Tol, Marsh, Blair and Harrison collected the data, with assistance from Groom and Gilbert. Tol performed the data analysis, with assistance from Blair. Tol wrote the first draft of the chapter, which was revised with editorial input from Coles, York and Congdon. Tol and York developed the figures and tables.



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[2] modified from: **Tol. SJ**, Harrison. M, Groom. R, Gilbert. J, Blair. D, Coles. RG, and Congdon. BC. 2021. Using DNA to distinguish between faeces of *Dugong dugon* and *Chelonia mydas*: non-invasive sampling for IUCN-listed marine megafauna. *Conservation Genetic Resources*. 10.1007/s12686-020-01187-z

Introduction

Marine mega-herbivores, *Dugong dugon* (dugong) and *Chelonia mydas* (green sea turtle), are important for marine tourism (Gerrard 1999, Campbell 2003, Conservation & Biodiversity Operations Branch 2018) and have cultural significance to Indigenous communities (Hudson 1983, Campbell 2003, McNiven 2004). Both species have experienced large population declines over the past century from habitat loss, hunting pressures, invasive species interactions, capture in fisheries nets, and boat strikes (Campbell 2003, Marsh et al. 2011). The International Union for the Conservation of Nature (IUCN) lists the dugong as vulnerable to extinction, and the green sea turtle as endangered (Seminoff 2004, Marsh and Sobtzick 2019).

The Great Barrier Reef World Heritage Area (GBRWHA) is home to one of the last large populations of dugongs (Marsh et al. 2011, Sobtzick et al. 2015, Sobtzick et al. 2017), and houses one of the largest populations and rookeries of green sea turtles (Chaloupka and Limpus 2001, Fuentes et al. 2010). Within the GBRWHA, both turtles and dugongs rely on the estimated 35,000 km^2 of seagrass meadows (Coles et al. 2015, Carter et al. 2016) as a major food source (Marsh et al. 2011, Wyneken et al. 2013). Declines in seagrass cover and biomass have been followed by large losses of dugongs and green sea turtles (Preen and Marsh 1995, Meager and Limpus 2012a, b), highlighting that the conservation of these foraging grounds is vital for the survival of mega-herbivores.

Studying how these animals use and impact seagrasses can enhance not only the conservation efforts for these meadows, but also aid in the management of the marine mega-herbivores. Previous research has found that foraging by both dugongs and green sea turtles enhances seagrass species diversity (Preen 1995, Aragones and Marsh 2000), maintains meadow health (Aragones and Marsh 2000, Christianen et al. 2012) and they act as biotic dispersal vectors for seagrass seeds (Chapter 2, Tol et al. 2017). All of these ecological interactions assist in the survival, connectivity and therefore resilience of seagrass meadows, which in turn is vital for the survival of these marine mega-herbivores. However, we do not have a full understanding of the relative contribution of seed dispersal provided by dugongs and green sea turtles.

In Chapter two, I calculated the extent of viable seagrass seed transmission in green sea turtle and dugong faeces, and how this dispersal process plays a valuable ecological role in seagrass dispersal and connectivity. However, this understanding was compromised by my inability to separate the faeces of green sea turtle and dugong. This was due to the similarity in composition and appearance of the faecal masses owing to their overlap in diet and digesta retention time (Lanyon and Marsh 1995, Brand et al. 1999). In this Chapter, I address this problem by using DNA extracted from faeces as a means of species-specific identification. I then use these identifications to re-evaluate the biotic seagrass seed dispersal potential of each mega-herbivore.

Methods

Tissue Collection

Dugong skin was provided by the University of Queensland; collected from Moreton Bay in June 2014 (ethics permit: SBS/290/11). Green sea turtle skin was collected in March 2019 from the James Cook University Cairns Aquarium (ethics permit: A2416). Both tissue samples were stored in 98% ethanol until DNA extraction.

Faecal Collection

A total of 57 faecal samples, from either dugong or green sea turtle, were collected *in-situ* from known seagrass foraging meadows between October and November 2018. Nine faeces were collected from Gladstone Harbour and 36 from Cleveland Bay, Townsville within the central GBRWHA. The remaining 12 faecal samples were gifted from Dr. Rachel Groom, and were collected from Bing Bong in the Gulf of Carpentaria, Northern Territory in November 2018 (Figure 3.1). Four known dugong faecal samples were provided from Toba Aquarium, Japan in October 2019, where two dugongs are housed.

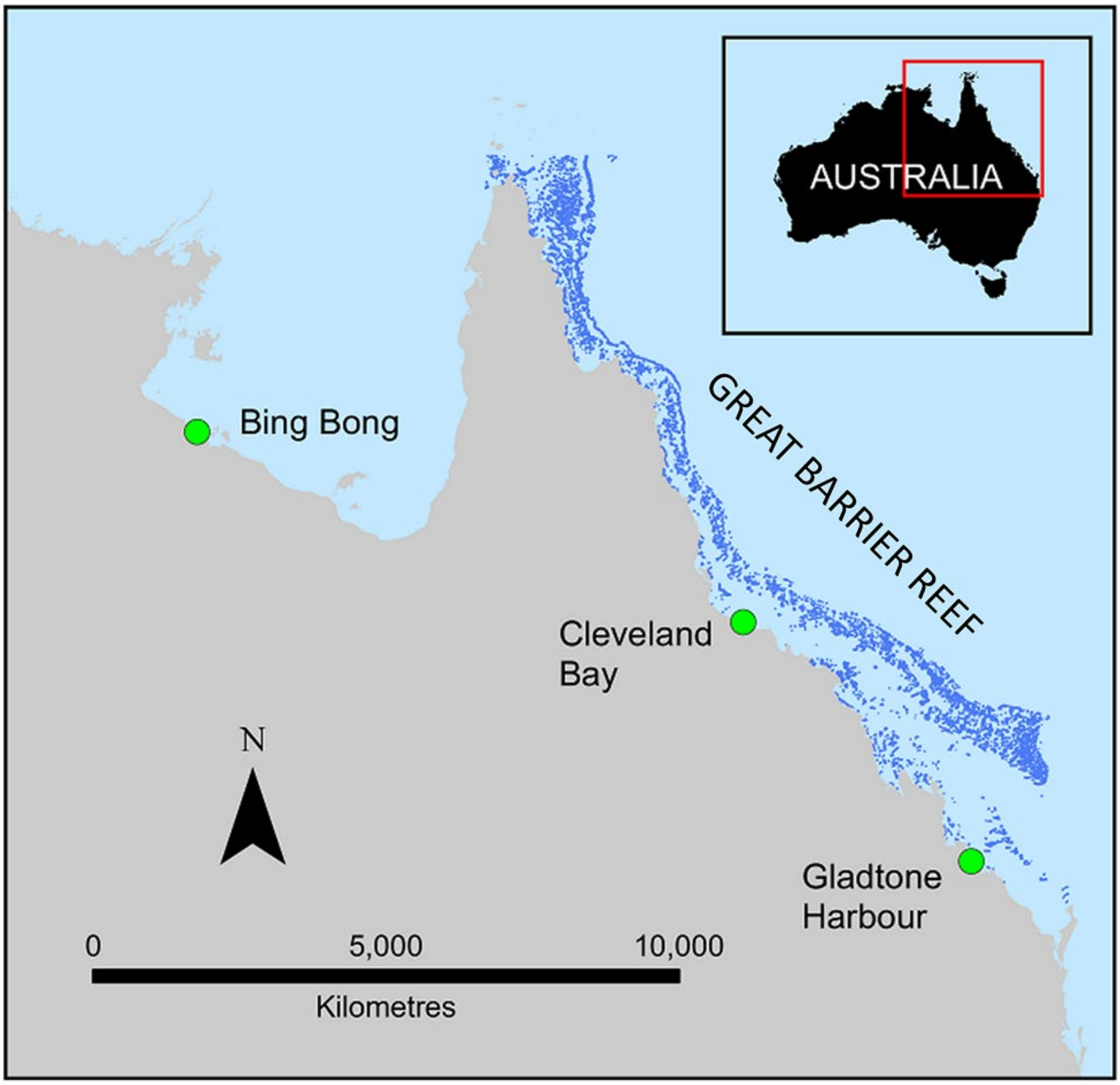


Figure 3.1: Site map for *in-situ* marine mega-herbivore faecal collection at Bing Bong, Northern Territory, and Gladstone Harbour in Gladstone and Cleveland Bay in Townsville, Queensland, Australia.

The exposed surface of the faecal mass was sampled to increase the chance of intestinal epithelial cells being present. Gladstone and Townsville faeces were placed in liquid nitrogen immediately after collection and stored at -90 °C, while samples from Japan were stored in 98% ethanol and samples from Bing Bong were stored at -20 °C.

Seed Collection

Faecal samples were sieved in laboratory grade sieves (1.4 mm, 750 µm and 250 µm) to retrieve any seagrass seeds present. After sieving, all remaining faecal matter larger than 125 µm per sample was dried at 60 °C until a consistent dry weight was obtained. All seeds retrieved from the central GBRWHA faecal samples were placed in a germination study, and were not available to be stained for viability. Seeds retrieved from the Bing Bong faecal samples were not able to be tested for viability, as the faeces had been frozen before sieving.

Primer Creation

Primers were designed using BioEdit software (Hall 1999). Faecal DNA is often of poor quality. We therefore designed primers to amplify a short region of the mitochondrial control region for dugongs (based on GenBank accession KJ944385) and green sea turtles (based on GenBank accession AB012104) (Table 3.1); as mtDNA is known to be less prone to degradation during environmental exposure. The portion of the control region we targeted is highly conserved within a species, but distinct between species; based on BLAST results.

Table 3.1: Primer sequences for species identification of *Dugong dugon* (dugong) and *Chelonia mydas* (green sea turtle).

Species	**Primer sequences (5' – 3')**	**Annealing Temp (°C)**	**Product size (bp)**
Dugong dugon	F: CGCGCGCTATGTACTTCGT R: GGGGTAAGTAGTGTAATGCACG	65 °C	110
Chelonia mydas	F: TAAATTCTCTGCCGTGCCCA R: TGTTTAGGGCCAAATCAACTGT	68 °C	122

NB: Product size after removal of the primer sequences for *Dugong dugon* is 69 bp, and for *Chelonia mydas* is 80 bp.

DNA extraction

Primers were tested using DNA extracted from tissue samples of both species. Genomic DNA was extracted using a DNeasy tissue kit (Qiagen Inc.), following the manufacturer's protocol. DNA was also extracted from the 61 faecal samples using the QIAamp Fast DNA Stool Mini Kit (Qiagen Inc.), following the manufacturer's protocol.

All samples were amplified for each primer pair using a standardised PCR protocol, with a negative control, and dugong and green sea turtle tissue samples as positive controls. We used iProof High-Fidelity DNA Polymerase (Bio-Rad Laboratories Inc.), following the manufacturer's guidelines for 'Typical Reaction Setup' with a 20µL reaction volume and a final concentration of 0.4 µM for each primer (1.5 µM $MgCl^2$ and 200 µM each dNTP). Cycling was carried out using a T100 Thermal Cycler (Bio-Rad Laboratories Inc.) under the following conditions: 98 °C for 45 s, followed by 35 cycles of 98 °C for 10 s, 65 °C (for dugong primers) or 68 °C (for turtle primers) for 30 s, 72 °C for 15 s, and a final elongation at 72 °C for 5 min. Amplified fragments were separated and visualised using a 1% agarose gel; a standard DNA ladder was included as a size marker.

Sequencing

Bidirectional sequencing of PCR products using the Sanger method was performed at the Australian Genome Research Facility (Brisbane, Queensland, Australia). We used BioEdit for sequence alignment (Hall 1999).

Statistical Analysis

We re-analysed the density of seagrass seeds in known green sea turtle faeces. Prior to analysis, all seed abundance data obtained from the faecal samples was examined for outliers and normality, and predictor variables assessed for collinearity using variance inflation factors (Zuur et al. 2010). All statistical analyses were conducted in the statistical software environment R (R version 4.0.2; R Core Team 2020).

All seagrass seed species were combined to ensure a robust statistical analysis, as there were insufficient numbers of seeds across all species from all locations. Only seeds removed from green sea turtle faeces were analysed, due to insufficient numbers of dugong faeces. A generalized linear model (GLM) with a negative binomial distribution to correct for overdispersion ('MASS' package, Venables and Ripley 2002) was used to quantify the effects of faecal collection site; data on time of collection was insufficient to include in the analysis. Residuals were inspected visually for patterns by plotting the fitted versus response variables. Post hoc 'tukey' adjustment analysis for pairwise comparisons were computed using the 'emmeans' package (Lenth 2020).

Results

DNA extraction

The dugong primers successfully amplified from the dugong tissue sample and six of the 61 faecal samples (two from Bing Bong and four known dugong samples from Toba Aquarium, Japan). The green sea turtle primers did not amplify products from these same samples. The green sea turtle primer pair successfully amplified from the green sea turtle tissue sample and 55 of the faecal samples (nine from Gladstone Harbour, 36 from Cleveland Bay and ten from Bing Bong). The dugong primer pair did not amplify from these samples (Figure 3.2).

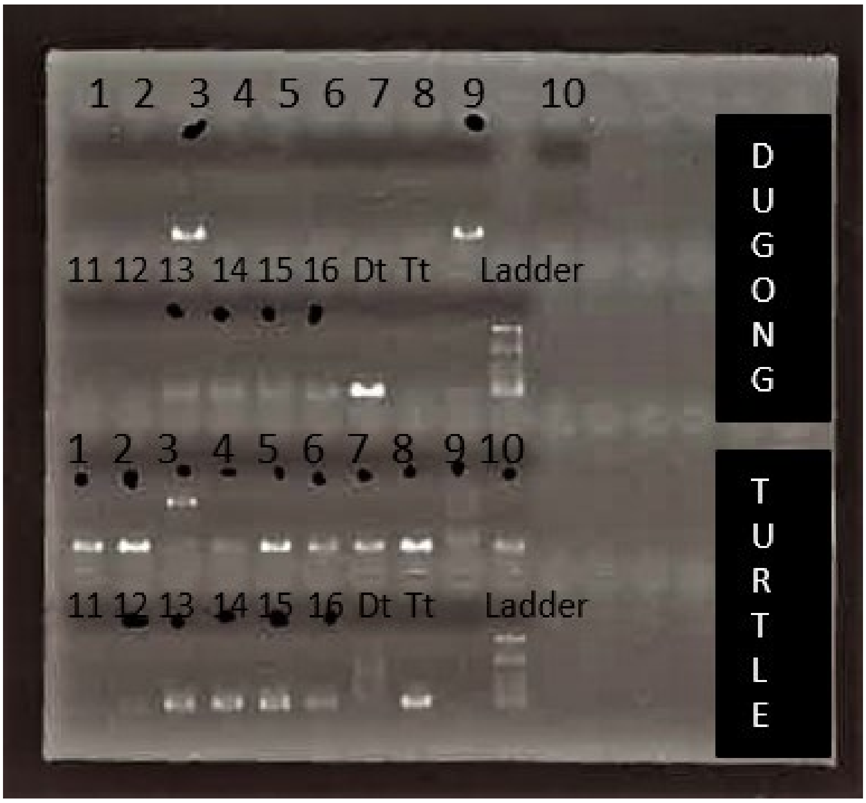


Figure 3.2: Agarose gel showing PCR products amplified from 16 faecal samples using primer pairs for *Chelonia mydas* (green sea turtle) and *Dugong dugon* (dugong). Samples 3 and 9 yielded a band for the dugong primers but not for the green sea turtle primers. Samples 1, 2, 4 – 8, 10 and 13 – 16 produced a band for the green sea turtle primers but not for the dugong primer pair. Samples 11 and 12 yielded turtle sequences despite a clear PCR product not being observed in the gel. A band could only be amplified from sea turtle tissue (Tt) and dugong tissue (Dt) samples using their corresponding primers. All faecal masses were collected from within Australian waters, from Gladstone Harbour and Cleveland Bay, Townsville in Queensland and Bing Bong in the Northern Territory.

All samples returned positive sequence results, although a few chromatograms were not of high quality, even when sequencing was repeated. Nevertheless, the readable sequences were unambiguous for either sea turtle or dugong. Samples amplified using the dugong primer pair were identical to *D. dugon* sequences in GenBank (MK986817 or MH704430), while readable sequences obtained using the green sea turtle primer pair were all identical and matched those of *C. mydas* (e.g. LC522564) (Supplementary Material 3.1 and 3.2).

Seagrass Seeds

A total of 346 seagrass seeds from at least five different seagrass species were collected from 55 green sea turtle faecal samples. Nearly 60% of all turtle faeces had at least one seagrass seed present; 47% at Cleveland Bay, 100% at Gladstone Harbour and 60% at Bing Bong. The average green sea turtle faeces from the GBRWHA was 1.280 g DW (± 0.150 SE; n = 45), while the average green sea turtle faeces from Bing Bong was 4.650 g DW (± 0.874 SE; n = 10).

A total of 14 *Cymodocea serrulata* seeds were collected from two dugong faecal samples from Bing Bong; both faeces had seeds present (Table 3.2). The average *in-situ* collected dugong faeces weighed 2.223 g DW (± 0.212 SE; n = 2).

Table 3.2: Number of seagrass seeds, by species, found in *Chelonia mydas* (green sea turtle) and *Dugong dugon* (dugong) faeces at three different intertidal/ subtidal seagrass meadows in Australia; Gladstone Harbour and Cleveland Bay in Central GBRWHA eastern Queensland, and Bing Bong in the Gulf of Carpentaria Northern Territory.

	Chelonia mydas			***Dugong dugon***
	Gladstone (n = 9)	**Cleveland Bay (n = 36)**	**Bing Bong (n = 10)**	**Bing Bong (n = 2)**
Cymodocea serrulata	0	0	28	14
Halodule uninervis	1	16	0	0
Halophila decipiens	0	103	64	0
Halophila spp.	0	2	0	0
Zostera muelleri	117	3	0	0
Total	**118**	**124**	**92**	**14**

There were significantly more seagrass seeds in faecal masses collected in Gladstone harbour (20 seeds per g DW ± 9 SE) than Cleveland Bay and Bing Bong (3 seeds per g DW ± 1 SE) (Figure 3.3 and Supplementary Table 3.3).

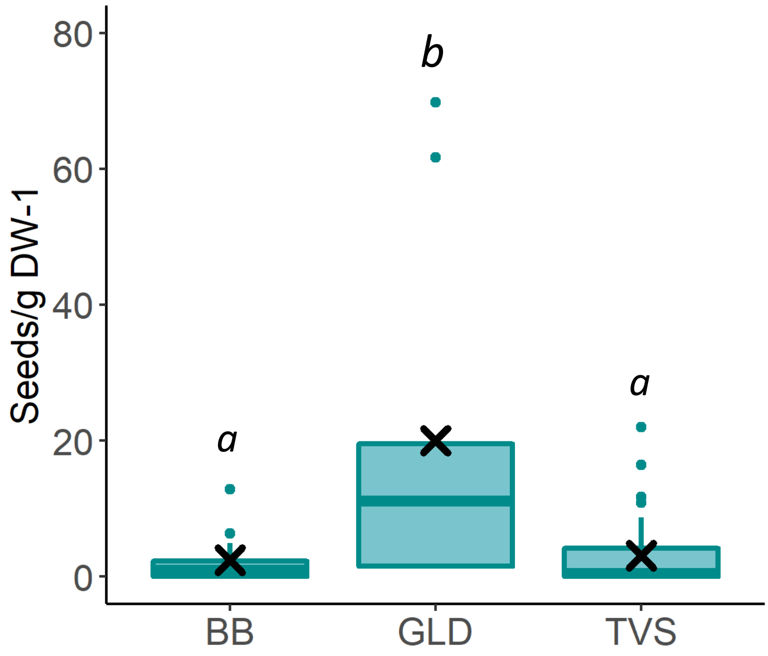


Figure 3.3: Boxplot of the number of seagrass seeds/g dry weight of green sea turtle (*Chelonia mydas*) faeces, collected from Gladstone Harbour (GLD) and Cleveland Bay (TVS) in Townsville, Queensland east coast, and Bing Bong (BB) in the Gulf of Carpentaria Northern Territory, Australia; X depicts the mean, and the letters show sites which were significantly different analysed with a Tukey post-hoc.

Discussion

My primer pairs were successful in identifying species of origin for dugong and green sea turtle faeces. To my knowledge, this is only the second study to successfully extract DNA from dugong faeces after Tikel et al. (1996) and the first for green sea turtles faeces. These primers will enable researchers to confirm the presence of dugongs and green sea turtles in foraging grounds, without directly impacting the animals. This new research also discovered another tropical seagrass species (*Cymodocea serrulata*) not previously found in faeces and being dispersed by marine mega-herbivores, bringing the total number of species found in faeces to at least five. This research also conclusively demonstrated that both dugongs and green sea turtles disperse tropical seagrass seeds.

Faecal samples confirmed to have been collected from green sea turtles in this analysis show a similar seed density as found in Chapter two (1 – 2 seeds per g DW), if faecal samples collected from Gladstone Harbour are excluded. The fruiting season in Gladstone Harbour during 2018 was one of the largest fruiting events witnessed in previous years when surveys have been carried out (Emma Jackson 2018, personal communication 11th October). This suggests that greater seed density in faecal matter at this location reflects the increased reproductive output in the meadows during collection. These numbers are likely to be at the upper end of the range for *Z. muelleri* on the Great Barrier Reef. Seed density is likely to be related to individual seagrass meadow fruiting, with large fluctuations of seed density in marine mega-herbivore faecal masses depending on seagrass reproductive conditions. Fluctuations in fruiting are likely to influence the number of seeds effectively dispersed by marine mega-herbivores at any given time.

Now that we have calculated the number of seagrass seeds being dispersed by green sea turtles (compared to dugongs), we are able to revise their seagrass seed dispersal estimate. This can be calculated using the updated figure for the average weighted GBRWHA green sea turtle faeces (one 1.280 g ± 0.150 SE faeces per day conservatively), combined with the seed viability value determined in Chapter two (9.13% ± 4.61 SE) and the latest green sea turtle population numbers for the southern GBRWHA (135,471 ± 19,802 SE) (Sobtzick et al. 2017); the most accurate available data. Using the smaller seeds per g DW figure for a conservative estimate, there is the potential for more than 120,000 viable seagrass seeds being dispersed per day within the southern GBRWHA. This figure, when corrected for area (only approximately 1,100km of coastline in the southern GBRWHA compared to 2,300 km for the entire GBRWHA), is approximately half that estimated in the previous chapter. This estimate reduction is predominately due to the green sea turtle population estimate being significantly smaller than that used in the previous chapter. However, this figure is still large enough to be adding significantly to seagrass dispersal. There is also the potential for this estimate to be far greater if seagrass meadows have a high yielding fruiting season.

Green sea turtles have a long gut retention time due to their herbivorous diet; 156 – 325 hours (Brand et al. 1999). Tagging studies in Gladstone (during the same year as my faeces collection) found turtle migrations can be as long as 65 – 220 kilometres (when measured in a straight line) for an unspecified time frame (Limpus et al. 2018). This can allow for seed dispersal in the 100s kilometres range. However, this tagging study also shows that the majority of sea turtle movements were within the harbour (Limpus et al. 2018) and likely only shifting seeds among harbour meadows. These findings have implications on the role of biotic seagrass seed dispersal by green sea turtles, further suggesting that the majority of dispersal provided would be between meadows within a bay or harbour (10s kilometres). It is likely the seed dispersal relationship among meadows will be related to the scale of the meadows and the foraging and migration behaviour of the herbivores, and understanding those relationships may vary among locations.

We were surprised with the low number of dugong faeces collected during my study as dugong were observed in large numbers during collection. The larger number of confirmed green sea turtle faeces compared with confirmed dugong faeces could be due to multiple reasons; such as increased differences in buoyancy of faecal matter between herbivores biasing sample collection (i.e., more of the dugong faeces may sink than previously thought), or differences in population sizes for dugongs to green sea turtles. The most conservative green sea turtle population is more than 60 times greater than the population estimate for dugongs in the southern GBRWHA (Sobtzick et al. 2017), so green sea turtles likely play a larger role in seagrass seed dispersal. If dugong populations were to increase, their role/ importance would also increase, especially considering that their home ranges are larger than sea turtles (Cleguer et al. 2016).

The confirmation of both dugong and green sea turtles providing biotic dispersal of at least five different seagrass species, emphasises their importance to the ecology of seagrass meadows. Their role in seagrass seed dispersal (Tol et al. 2017), along with their importance in shaping meadow health and species persistence (Preen 1995, Aragones and Marsh 2000, Marsh et al. 2018) makes these marine mega-herbivores vital to the enhanced resilience of seagrass ecosystems. Dugongs and green sea turtles have been affected by many anthropogenic factors leading to their populations declining globally (Campbell 2003, Marsh and Sobtzick 2019). To ensure seagrass ecosystems continue to function effectively, it is important we ensure the conservation of these two marine mega-herbivores.

Ethics Declaration

Ethics were approved by The University of Queensland Animal Ethics Committee and James Cook University Ethics Committee. Dugong skin collection ethics number: SBS/290/11. Green sea turtle skin collection ethics number: A2416.

Chapter Summary

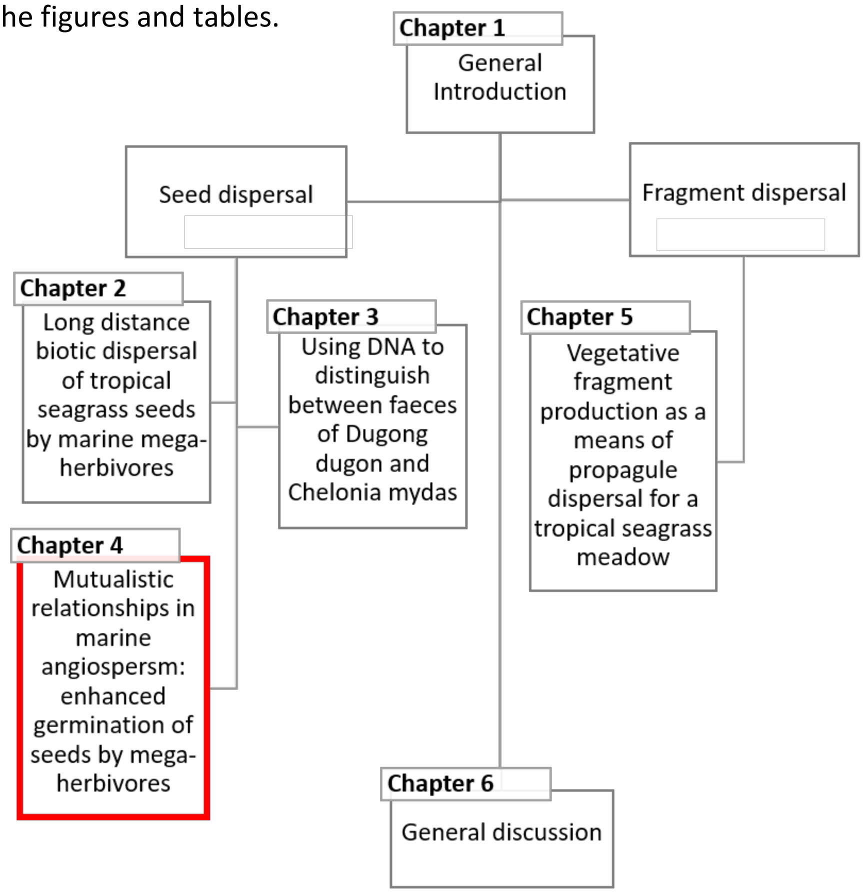
- Dugongs and green sea turtles provide important ecological services to seagrasses, however, the populations of these marine mega-herbivores are declining locally and globally. One such ecological service these marine mega-herbivores provide is the biotic dispersal of seagrass seeds.
- In this chapter, we created two PCR primer pairs (one for green sea turtles and one for dugongs) to enable us to successfully distinguish species origin of the faecal masses.
- I confirmed that both marine mega-herbivores do disperse seagrass seeds, and found another species of seagrass seed (*Cymodocea serrulata*) to be dispersed by both marine mega-herbivores.
- I found there to be a similar seed density (2-3 seeds per g DW of faeces) in green sea turtle faeces to that found in found Chapter two, except at Gladstone Harbour. In Gladstone Harbour, I found a high amount of seed density in green sea turtle faeces (11-29 seeds per g DW of faeces), and that faecal seed density is most likely associated with the volume of fruiting.
- Chapter 3 re-evaluates previous data and found green sea turtles to be dispersing approximately more than 120,000 seeds per day within the southern GBRWA. This new figure is approximately half that calculated in Chapter two, however, even at this reduced estimate green sea turtles would still be providing a vital service in seagrass seed dispersal.
- The survival of seagrasses and recovery/ re-establishment after loss is linked to the survival and behaviour of marine mega herbivores and GBRWHA conservation strategies can be improved by integrating this knowledge.

Chapter 4

Mutualistic relationships in marine angiosperms: enhanced germination of seeds by mega-herbivores [3]

In this chapter I determine whether tropical seagrass seeds of *Zostera muelleri* ingested and excreted by marine mega-herbivores (dugongs and green sea turtles) have a different germination outcome compared to seeds dropped by the plant. I also quantify whether un-germinated ingested seeds have a different viability outcome compared to seeds dropped by the plant, and what impacts this has on the connectivity of seagrass meadows.

Tol, Coles and Jarvis co-developed the research question. Tol and Coles collected the data. Tol performed the data analysis, with assistance from Jarvis. Tol wrote the first draft of the paper which was revised with editorial input from Jarvis, York, Congdon and Coles. Tol developed the figures and tables.



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[3] **Tol. SJ**, Jarvis. JC, York. PH, Congdon. BC and Coles. RG. Mutualistic relationships in marine angiosperms: enhanced germination of seeds by mega-herbivores. *BioTropica,* Under Review, Manuscript ID: BITR-20-268.

Introduction

Mutually beneficial interactions between plants and animals are ubiquitous in nature and lead to the co-evolution of species that drive the production and maintenance of biodiversity (Law 1985, Thompson and Cunningham 2002, Bascompte and Jordano 2007). Plant-animal mutualisms are fundamental processes that have contributed to angiosperms becoming the dominant plant form on earth (Howe and Smallwood 1982, Law 1985, Tiffney 2004). Flowers and fruits attract insects, birds, reptiles and mammals by providing a food source, while the plant benefits through enhanced pollination, seed dispersal and or germination (Ridley 1930, Faegri and Van Der Pijl 1980, Herrera 1989, Klein et al. 2008). These mutualistic relationships are so successful, it is estimated that nearly all angiosperms benefit from one or more symbiotic interactions (Faegri and Van Der Pijl 1980, Kiester et al. 1984, Law 1985, Jordano 1987).

Seeds carried by animals, either on their feathers, fur or skin (epizoochory) or through ingestion and excretion (endozoochory), can be dispersed equal or greater distances than by abiotic factors (such as wind and currents), decreasing competition with the parent plant (Ridley 1930, Howe and Smallwood 1982, Herrera 1989). Seed ingestion and excretion can create dormancy breaks (such as scarification of the seed coat) that disrupt dormancy and increase the rate and overall success of germination (Law 1985, Traveset 1998). This effect is found in approximately half the instances of gut passage through an animal (Traveset 1998). Dormancy-breaking during gut passage is influenced by a combination of seed and herbivore characteristics, such as seed size, seed coat, and fruit flesh, as well as gut retention time and gut chemical composition (Traveset 1998, Traveset and Verdú 2002, Jaganathan et al. 2016).

An ecosystem's robustness relies on a network of symbiotic interactions, where multiple animals and plant species interact in a combination of mutualism, commensalism and competition (Bascompte and Jordano 2007, Bascompte 2019). However, this balance can be disrupted through a loss or decrease in one or more species within a community network (Bascompte and Jordano 2007). For an ecosystem to remain stable, it is important to understand these community interactions on a species by species basis. A plethora of these mutualistic relationships among terrestrial plants and animals are well studied (Chapman

1989, Eriksson 1992, Bascompte and Jordano 2007, Costa et al. 2014), however there is a lack in knowledge of these interactions among aquatic plants and animals in aquatic environments (York et al. 2017, Costa-Pereira et al. 2018).

Seagrasses are angiosperms which adapted to the marine environment around 100 million years ago, and produce flowers, fruits and seeds almost exclusively underwater (Hemminga and Duarte 2000, Waycott et al. 2006). They are found globally along the coast of all continents except Antarctica, with over 70 different seagrass species described; 54 of them in tropical waters (Hemminga and Duarte 2000, Green and Short 2003, Short et al. 2007). Seagrass ecosystems provide many important ecosystem services (Mellors et al. 2005, Macreadie et al. 2014, Lamb et al. 2017, James et al. 2019, Lefcheck et al. 2019), estimated at a value of US$28,916/ha per year (Costanza et al. 2014). Seagrass meadows are a vital source of food for many herbivorous animals, such as crustaceans, echinoderms, fish, seabirds, sea turtles and sirenians (dugongs and manatees) (Hemminga and Duarte 2000, Valentine and Duffy 2006, York et al. 2018a). There is likely to be a symbiotic community network within seagrass ecosystems, where some of these herbivores act as pollinators (for example isopods cross pollinate flowers of *Thalassia testudinum* in the Caribbean (van Tussenbroek et al. 2012, van Tussenbroek et al. 2016)), dispersal vectors (Sumoski and Orth 2012, Tulipani and Lipcius 2014, Tol et al. 2017) and germination enhancers (Wu et al. 2016) with our knowledge of these processes increasing dramatically in recent years. Sea turtles and sirenians have fed on seagrass plants for at least 60 million years, leading to expectations of a co-evolved mutualistic relationship (Thayer et al. 1984, Tol et al. 2017).

Seagrass plants are capable of asexual and sexual reproduction (Orth et al. 2006b, Kendrick et al. 2012, Sherman et al. 2018). Asexual reproduction through rhizomal growth provides expansion within a meadow (Kendrick et al. 2012, Sherman et al. 2018), while vegetative fragments/propagules can disperse vast distances (Kendrick et al. 2012, McMahon et al. 2014, Smith et al. 2018). Seagrasses are also capable of producing fruits and seeds, of which the majority are negatively buoyant (Kendrick et al. 2012, McMahon et al. 2014). They have a dispersal potential from tens to hundreds of meters once separated from the flowering shoot, but can disperse greater distances, tens to hundreds of kilometres, if attached to a floating plant fragment (Erftemeijer et al. 2008, Källström et al. 2008).

Biotic dispersal via endozoochory has been documented in as many as six seagrass species, with multiple animals acting as dispersal vectors; seabirds, estuarine turtles, marine fish and marine mega-herbivores (dugongs and green sea turtles) (Charalambidou et al. 2003, Sumoski and Orth 2012, Wu et al. 2016, Tol et al. 2017). Seabirds, some fish, terrapin, sirenians and green sea turtles can travel long distances during gut retention times, making them important biotic dispersal vectors (Charalambidou et al. 2003, Tulipani and Lipcius 2014, Wu et al. 2016, Tol et al. 2017). All of the known seagrass species involved in endozoochory produce seeds with a hard protective coat and can remain dormant from two months to two years (Green and Short 2003, Waycott et al. 2004, Orth et al. 2006b, Kilminster et al. 2015). Reduced temperatures, low oxygen, fresh water pulses, burial depth, light, and scarification are known germination cues for some seagrass species (Loques et al. 1990, Harrison 1991, Kawasaki 1993, Moore et al. 1993, Conacher et al. 1994a, Brenchley and Probert 1998, Jarvis and Moore 2015, Stafford-Bell et al. 2016, Wu et al. 2016, Cumming et al. 2017). Scarification of seagrass seed coatings has been observed in over 60% of the seeds found in marine mega-herbivore faeces (Tol et al. 2017). Scarification is known to reduce physical dormancy and enhance germination for terrestrial plants (Law 1985, Baskin and Baskin 2003), however, the effect on germination of gut passage for seagrass seeds via marine mega-herbivores is presently unknown.

The aim of our study is to determine whether ingestion and excretion of seagrass seeds by marine mega-herbivores (green sea turtles (*Chelonia mydas*) and dugongs (*Dugong dugon*)) has an effect on time to germination and germination success, compared with seeds dropped naturally from the plant. We focus on *Zostera muelleri*, a seagrass species found along the entire eastern and southern Australian coast (Waycott et al. 2004). This seagrass species was the most common seagrass seed found in marine mega-herbivore faeces in north eastern-Australia, and nearly 10% the those seeds remained viable after excretion (Tol et al. 2017). Germination studies undertaken in temperate regions found *Z. muelleri* had increased germination outcomes in low temperatures, low salinity, low light and anerobic conditions (Conacher et al. 1994a, Brenchley and Probert 1998, Stafford-Bell et al. 2016). However, the salinity levels needed to produce optimal germination in the above studies is rarely achieved in coastal seagrass meadows in the Great Barrier Reef (GBR) (Schaffelke et al. 2009), so we chose to look at only temperature as a treatment. Our findings will improve the understanding of the ecological relationship between seagrass plants and the megafauna which rely on them as a food source.

Methods

Zostera muelleri sexual reproduction

Peak flowering and seed production for *Zostera muelleri* occurs during the spring to summer months in the southern hemisphere; September to January (Conacher et al. 1994b, Waycott et al. 2004). For this species, multiple seeds are enclosed in a reproductive shoot known as a spathe (Conacher et al. 1994b, Waycott et al. 2004). These seeds have a hard seed coat and a dormancy of less than one year, enabling the creation of a seed bank that can provide seeds to replenish a meadow (Conacher et al. 1994a, McMahon et al. 2014, Stafford-Bell et al. 2016, Reason et al. 2020). Known germination cues for *Z. muelleri* include reduced temperature, reduced salinity, low light, low oxygen and scarification of the seed coat (Conacher et al. 1994a, Brenchley and Probert 1998, Stafford-Bell et al. 2016).

Seed collection: Mega-herbivores

Marine mega-herbivore faecal samples were collected during *Z. muelleri* peak flowering from two north-east Queensland coastal locations; Gladstone Harbour in October 2018 and Cleveland Bay in Townsville in November 2015 and November 2018 (Figure 1). Viability of seeds removed from marine mega-herbivore faeces does not change between September to December (Tol et al. 2017). The seagrass meadows at the collection sites extended from the intertidal zone in to the subtidal zone, and all were mixed species assemblages (Table S1) (McKenna et al. 2020, Smith et al. 2020). Both locations are known feeding grounds for dugongs and green sea turtles.

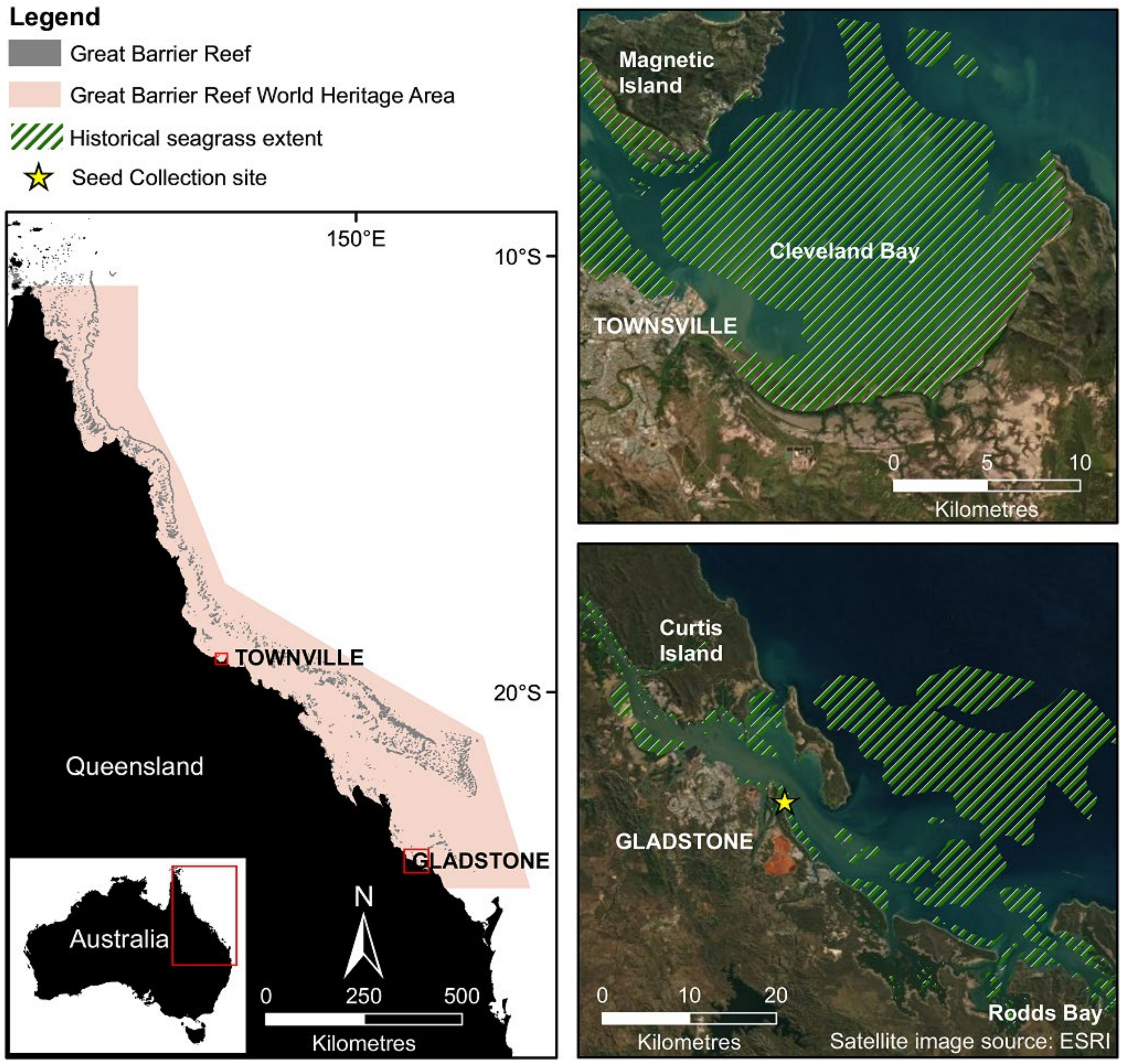


Figure 4.1: Site map showing where seed samples (Lillie Beach, Gladstone Harbour) and marine mega-herbivore faeces were collected (Gladstone Harbour and Cleveland Bay), in central Great Barrier Reef World Heritage Area, Australia.

For this paper, dugong and green sea turtle faeces are referred to as marine mega-herbivore faeces as they are indistinguishable due to similar diets (Tol et al. 2017). This results in the experimental design including seeds that originate from either of the two marine mega-herbivores. Seeds retrieved from the faecal samples were randomly assigned to temperature treatments to address this issue. Faecal samples were transported chilled and stored between 3-5 °C to prevent seed decay. Before retrieved seeds were placed in the germination experiment, faecal samples were stored chilled for less than 60 days (all medium and high temperature treatment seeds and one block in the low temperature treatment were stored for less than 30 days, while three blocks from the low temperature treatment were stored for less than 60 days). Previous research on *Z. muelleri* and *Z. japonica* has found that cold storage of seeds for less than 60 days does not impact seed viability (Grice 1994, Yue et al. 2019). Samples were sieved through laboratory grade sieves (1.4 mm, 750 µm and 250 µm) to ensure seeds would be retained, and all seeds found were placed in sterile artificial seawater at a salinity of 34 ppt to prevent osmotic stress until use in germination experiments.

Seed collection: Fresh seeds

Z. muelleri spathes were harvested from Lillies Beach in Gladstone Harbour (Figure 1) in August 2018, and placed in a mesocosm to mature. The mesocosm replicated the seagrass meadow conditions (tides, light and temperature), with filtered seawater sourced from Gladstone harbour. The seeds were allowed to mature and drop naturally from the spathe, before collection in early October 2018. All harvested seeds were stored in sterile artificial seawater (salinity of 34 ppt) at 3-5 °C to prevent decay (Grice 1994) until placement in the germination experiment (no greater than seven days).

Germination

To reduce confounding issues, the germination experiment concentrated on one environmental factor (Jarvis and Moore 2008, Fernández-Torquemada and Sánchez-Lizaso 2013, Stafford-Bell et al. 2016, Xu et al. 2016, Cumming et al. 2017), temperature (three temperature treatments, low (19 °C ± 1), medium (26 °C ± 1) and high (32 °C ± 1)), and seed deposition treatment (passed through an herbivores gut or dropped from the plants). The temperature treatment levels were derived from long term temperature datasets within the central Great Barrier Reef World Heritage Area (GBRWHA) region (McKenzie et al. 2014, Australian Institute of Marine Science 2019).

Individual seeds were treated as replicates and were set up in blocks of 10 – 20 seeds per petri dish. Seeds removed from the plant were sufficient in number for four blocks, with 20 seeds per block; n= 80 seeds per temperature treatment. Seeds removed from marine mega-herbivore faeces were sufficient in number for four blocks in the low temperature treatment and three blocks in the medium and high temperature treatment, with 10 seeds per block; n

= 40 seeds for the low temperature treatment and 30 seeds each for the medium and high temperature treatments. All seeds selected for the experiment were mature, having a dark brown and hard seed coat (Conacher et al. 1994b). Seeds were held in a petri dish on two 125 mm diameter Whatman Wet Strengthened filter papers and a sterile sponge. Seeds were distributed evenly and secured to ensure they did not touch each other. All replicates were submerged in artificial seawater and kept at an average salinity of 34 ppt (± 1), reflecting the natural salinity conditions of intertidal and subtidal seagrass meadows within the central GBRWHA region (Schaffelke et al. 2009). All seeds were monitored daily for 60 days for signs of germination. Germination was defined as the rupture and growth of the cotyledon (Churchill 1983, Brenchley and Probert 1998, Orth et al. 2000). The high temperature treatment for seeds from marine mega-herbivore faeces was conducted from December 2015 to February 2016. All other treatments for seeds removed from the plant and from faeces were conducted between November 2018 and March 2019 due to the availability of faecal material and seeds.

Viability

On day 60 of the germination experiment, all non-germinated seeds were tested for viability. Seed coats were removed and the 'naked' seeds were left submerged in 1.5% Tetrazolium dye solution for 48 hours without light. If a seeds' cotyledon stained pink from the Tetrazolium dye, this was used to indicate respiration and to classify the seed as viable (Conacher et al. 1994a, The Tetrazolium Subcommittee of the Association of Offical Seed Analysts 2005).

Statistical Analysis

Data was examined for outliers, collinearity and variance inflation prior to analysis (Zuur et al. 2010). Survival analysis, using a Kaplan-Meier survivorship function with a Cox model, was used to investigate the effect of seed deposition type and the effect of temperature on time to germination (McNair et al. 2012). Each seed was analysed independently as time-to-event analysis is based on the distribution of germination times of individual seeds (McNair et al. 2012, Jarvis and Moore 2015). A zero/one inflated beta binomial (ZOIB) regression analysis was performed using a 'gamlss' package to quantify the effects of seed deposition type and temperature on maximum percent germination (Rigby and Stasinopoulos 2005). Residuals were inspected visually for patterns by plotting the fitted versus response variables. The best-fit model was considered to be the simplest model with the lowest Akaike information criterion (AIC) score (Zuur et al. 2010) which was calculated using the 'MuMIn' package (Barton 2019). Post hoc analysis for pairwise comparisons were computed from the contrasts between factors with a 'tukey' adjustment using the 'emmeans' and 'lsmeans' packages (Lenth 2016, Lenth 2020). To analyse the proportion of seeds which did not germinate but remained viable after the 60-day germination study, I used a Mann-Whitney U Test, as the data did not conform to a normal distribution. There was only sufficient data to analyse seed deposition for the viability analysis and not sufficient data for separate temperature treatments. All statistical analyses was performed using the statistical software environment R (R version 3.6.3; R Core Team 2020).

Results

Mean time to germinate

Seeds which passed through a marine mega-herbivore germinated 10 – 35 days earlier than seeds released from the plant directly (18% - 61% faster) (Table 4.1 & Figure 4.2). For all temperature treatments, seeds which passed through a marine mega-herbivore germinated significantly quicker than seeds released directly from the plant ($p < 0.005$; Tables 4.2 & 4.3). Seeds which were ingested and excreted by a marine mega-herbivore in the medium temperature treatment, germinated significantly faster than the low temperature treatment ($p < 0.001$; Tables 4.1 & 4.2). Mean time to germination was not different across temperature treatments for seeds taken from the plant ($p > 0.05$; Tables 4.1 & 4.2).

Table 4.1: Mean time to germinate (MTG) and maximum percentage germination results (± SE) for *Zostera muelleri* seeds which were ingested and excreted by a marine mega-herbivore (dugong or green sea turtle) compared with seeds dropped from the plant across three temperate treatments. Results based on Time to Survival analysis, using a Cox model. Seeds were collected from central Great Barrier Reef World Heritage Area, Australia.

Seed Deposition	**Temp. (°C)**	**MTG (days)**	**Max. % Germ**
Herbivore	18	44 ± 2	73 ± 1
	26	22 ± 3	87 ± 2
	32	35 ± 3	83 ± 2
Plant	18	53 ± 1	29 ± 2
	26	56 ± 1	20 ± 1
	32	53 ± 1	36 ± 1

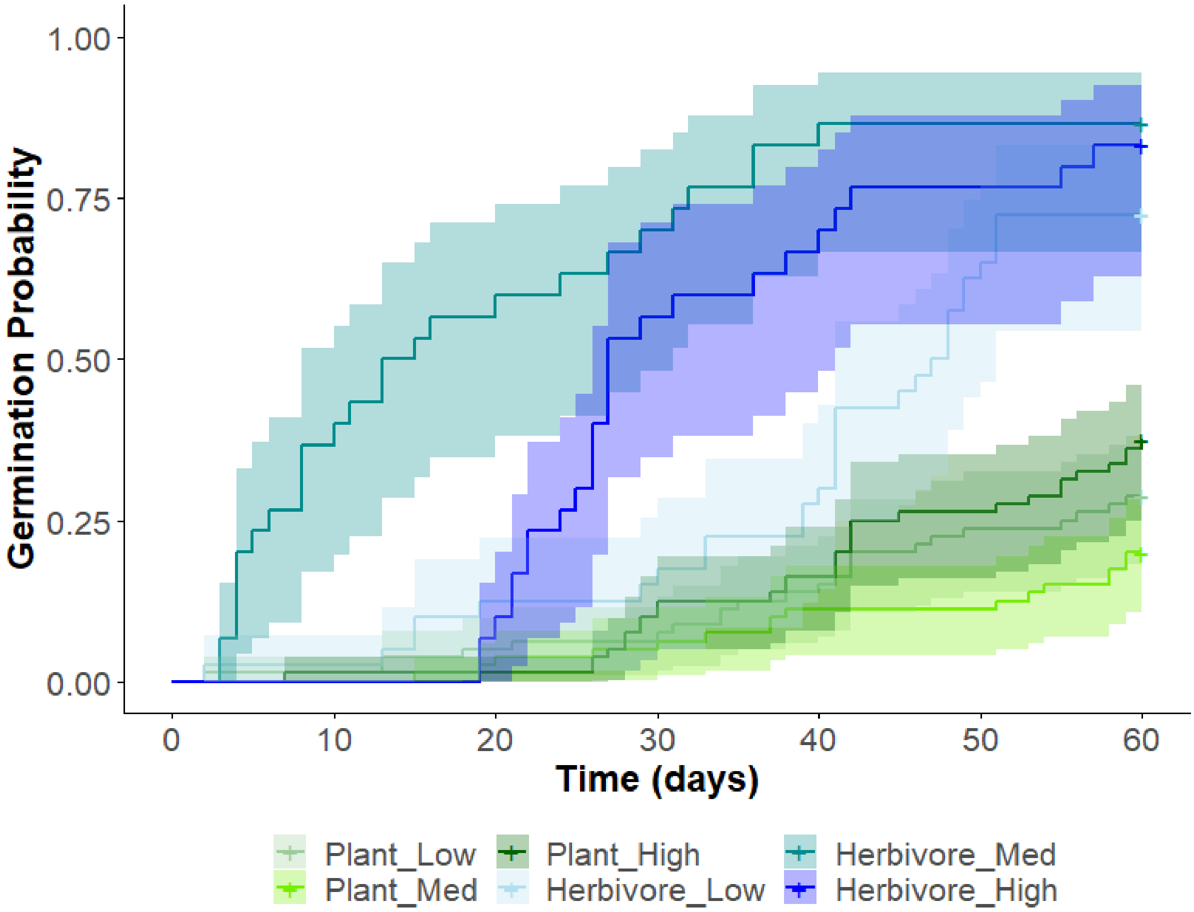


Figure 4.2: Mean germination time (95% CI) for *Zostera muelleri* seeds which were ingested and excreted by a marine mega-herbivore (dugong or green sea turtle) compared with seeds dropped from the plant across three temperate treatments (low = 19 °C, medium = 26 °C, and high = 32 °C). Seeds were collected from central Great Barrier Reef World Heritage Area, Australia.

Table 4.2: Mean time to germinate results for *Zostera muelleri* seeds which were ingested and excreted by a marine mega-herbivore (dugong or green sea turtle) compared with seeds dropped from the plant across three temperate treatments (low = 19 °C, medium = 26 °C, and high = 32 °C). Results based on Time to Survival analysis, using a Cox model, with an 'emmeans' post hoc. Seeds were collected from central Great Barrier Reef World Heritage Area, Australia.

Parameter	**SE**	***Z* ratio**	***p*-value**
Herbivore vs. Plant	0.171	-10.631	<0.0001*
Low Temp	0.212	-1.609	0.2418
Med Temp	0.195	-2.163	0.0776
High Temp	0.209	-0.385	0.9215
Plant Low - Herbivore Low	0.280	-4.354	0.0002*
Plant Low - Herbivore Med	0.290	-8.040	<.0001*
Plant Low - Herbivore High	0.292	-6.069	<.0001*
Plant Med – Herbivore Low	0.313	5.270	<.0001*
Plant Med - Herbivore Med	0.322	-8.581	<.0001*
Plant Med - Herbivore High	0.323	-6.803	<.0001*
Plant High – Herbivore Low	0.261	3.550	0.0052*
Plant High – Herbivore Med	0.272	7.506	<.0001*
Plant High - Herbivore High	0.274	-5.405	<.0001*
Plant Low - Plant Med	0.326	1.314	0.7777
Plant Low - Plant High	0.277	-1.057	0.8982
Plant Med - Plant High	0.310	-2.327	0.1829
Herbivore Low - Herbivore Med	0.273	-4.075	0.0007*
Herbivore Low - Herbivore High	0.275	-2.007	0.3379
Herbivore Med - Herbivore High	0.281	1.993	0.3463

***significant**

Table 4.3: Maximum germination results for *Zostera muelleri* seeds which were ingested and excreted by a marine mega-herbivore (dugong or green sea turtle) compared with seeds dropped from the plant across three temperate treatments (low = 19 °C, medium = 26 °C, and high = 32 °C). Results based on a zero/one inflation beta (ZOIB) regression, with a 'lsmeans' post hoc with an alpha of 0.05. Seeds were collected from central Great Barrier Reef World Heritage Area, Australia.

Parameter	**lsmean**	**SE**	**Group**
Plant Low	-0.908	0.247	a
Plant Med	-1.386	0.280	a
Plant High	-0.565	0.233	a
Herbivore Low	0.969	0.354	b
Herbivore Med	1.872	0.537	b
Herbivore High	1.609	0.490	b

Maximum germination

All seeds that passed through an herbivore had significantly greater maximum germination compared to seeds dropped by the plant, with no effect of temperature ($p < 0.05$; Table 4.1 & 4.3). Seeds which passed through a marine mega-herbivore had two to four times greater maximum percentage germination than the best performing temperature treatment for seeds dropped from the plant (Table 4.1 & Figure 4.2). The highest germination percentage for seeds passed by an herbivore was from the medium temperature treatment, and the highest germination percentage for seeds dropped by the plant was from the high temperature treatment (Table 4.1 & Figure 4.2).

Viability of non-germinated seeds

There were significantly more viable seeds remaining in the plant dropped seed treatments after the 60-day germination period compared to the mega-herbivore treatments ($W = 18$, $p = 0.004$; Figure 4.3). Nearly all non-germinated seeds which were dropped by the plant were viable (95% ± 2% SE); 98% viable at 19 °C (n = 56), 97% viable at 26 °C (n = 62) and 90% viable at 32 °C (n = 45). Temperature did not affect viability of non-germinated seeds dropped by the plant. Nearly all of the non-germinated seeds which were ingested and excreted by marine mega-herbivores were not viable at the end of the germination experiment (25% ± 16% SE). All non-germinated ingested seeds in the low (19 °C n = 8) and medium (26 °C n = 4) temperature treatments were not viable, however all ingested seeds in high temperature treatments were viable after 60 days (100% viable at 32 °C; n = 5).

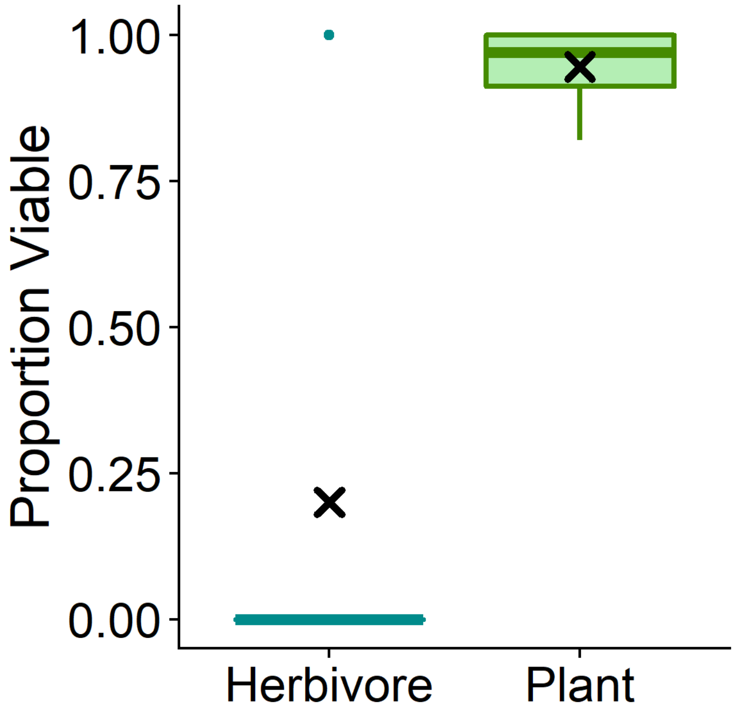


Figure 4.3: Proportion of non-germinated seeds viable after 60 days in a germination experiment, for *Zostera muelleri* seeds which were ingested and excreted by a marine mega-herbivore (dugong or green sea turtle) compared with seeds dropped from the plant; X represents mean viability. Seeds were collected from central Great Barrier Reef World Heritage Area, Australia.

Discussion

This is the first study on the effects of ingestion and excretion by marine mega-herbivores on germination of seagrass seeds that I am aware of. Marine mega-herbivores have a positive impact on successful *Z. muelleri* seed germination as seeds ingested and excreted by marine mega-herbivores germinate quicker and have a higher germination percentage than seeds dropped into the environment from a parent plant. However, viability of seeds excreted by marine mega-herbivores that did not germinate after 60 days was low or non-existent, while nearly all seeds dropped from the plant that did not germinate remained viable.

Seeds which pass through the gut of a marine mega-herbivore undergo scarification. Previous research found greater than 60% of seeds passed by a marine mega-herbivore had a split seed coat (Tol et al. 2017). A split seed coat has been found to break seed dormancy and increase germination success for *Zostera* species (Conacher et al. 1994a, Jarvis and Moore 2015). During ingestion and digestion by an herbivore, seed coats can be scarified by mechanical or chemical (stomach acid) forces and or by osmotic stress (Traveset 1998, Samuels and Levey 2005). Greater maximum germination and a quicker time to germinate could also be explained by the 'sterilisation' of the seed through contact with stomach acids, which may remove potentially lethal fungal infections (Govers et al. 2016). *Zostera* seeds are known to have a high percentage of seeds contaminated with bacteria and fungi which reduce or prevent germination (Govers et al. 2016). These pathogens can be eliminated with the use of an acidic salt (copper sulphate) (Govers et al. 2017). Stomach acids could provide a similar 'sterilisation' of the seed, reducing the presence of diseases and consequently increase germination.

Seeds which passed through a marine mega-herbivore were also significantly different for time to germinate in the medium temperature compared to the low temperature treatment; there was no effect of temperature for maximum percentage germination. This suggests that seeds germinate quicker if they do not go through a temperature change after they have been ingested and excreted by a marine mega-herbivore. Seeds which are ingested by a marine mega-herbivore are likely to be exposed to higher temperatures than external sea surface temperatures while passing through the gut. The body temperature of green sea turtles are consistently 1 – 2 °C above ambient temperature when inactive, and can reach high temperatures of 31.3 °C when basking and 37.1 °C when undertaking vigorous activities (Heath and McGinnis 1980, Standora et al. 1982, Whittow and Balazs 1982). Dugongs, as mammals, also maintain a constant temperature with the best estimate reaching a maximum of 32.5 °C (Lanyon et al. 2010). A sudden decrease in temperature after passing through the gut of a marine mega-herbivore which can take anywhere between 6 – 13.5 days (Hadjichristophorou and Grove 1983, Lanyon and Marsh 1995, Brand et al. 1999), may cause a delay in germination, even after the seeds have had their dormancy broken.

Over the three different temperature treatments seeds dropped by the plant did not show any difference in time to germinate or in maximum percentage germination. This suggest that *Z. muelleri* seeds which have been dropped by the plant may need to undergo some type of stress to break dormancy (Orth et al. 2000, Orth et al. 2006b), and that temperature does not act as a germination cue for *Z. muelleri* in the tropics. This is unlike temperate regions where *Z. muelleri* is also found, and for other *Zostera* species (Brenchley and Probert 1998, Abe et al. 2008, Cumming et al. 2017). Seeds which have passed through a marine mega-herbivore have already undergone a form of dormancy break, and temperature stress had no additional germination benefits. Marine mega-herbivores are providing a dormancy break for *Z. muelleri* seeds, while providing long distance dispersal, rapid seedling germination and potentially increasing seagrass genetic diversity at dispersal sites.

Non-germinated seeds directly from the plant retained viability after 60 days. High seed viability over time is an effective method in establishing a seed bank for the recovery of a seagrass meadow after loss (Jarvis and Moore 2010, Jarvis et al. 2015). Nearly all the non-germinated seeds which passed through a marine mega-herbivore were not viable after 60 days. Seeds passed by marine mega-herbivores are unlikely to add to a viable seed bank, and will only assist in the recovery or expansion of a meadow by rapid germination and transmission of seedlings. Green sea turtles and dugongs, when searching for foraging locations, are likely to transport viable seeds to locations suitable for meadow growth, and therefore dispersing seeds outside of a source meadow further than is capable by abiotic means (McMahon et al. 2014, Tol et al. 2017). Dispersal of viable seeds capable of rapid germination outside of a source meadow enhances meadow connectivity and genetic diversity and may assist to recolonise suitable habitat after loss or decline (McMahon et al. 2014, Grech et al. 2018, Sherman et al. 2018).

Asexual reproduction is a successful mode of reproduction for seagrasses, due to meadow expansion and the long-distance dispersal potential of fragments (Kendrick et al. 2012, Sherman et al. 2018, Smith et al. 2018). Sexual reproduction is important for meadow continuity and recovery after loss in the form of seed banks and promoting and maintaining genetic diversity (Jarvis and Moore 2010, Kendrick et al. 2012, McMahon et al. 2014). Sexual reproduction does not generally assist long-distance dispersal for most seagrasses, as most species fruits and seeds are negatively buoyant and can only travel 100s of meters (Kendrick et al. 2012, McMahon et al. 2014, Sherman et al. 2018). My findings show that sexual reproduction in *Z. muelleri* has at least two successful reproductive pathways; one that builds resilience through direct deposition and formation of a local seed bank that allows for recovery after disturbance; and another that relies on colonization and connectivity through long distance dispersal and rapid germination via endozoochory by marine mega-herbivores (Tol et al. 2017).

Quantifying the germination success of seagrass seeds ingested and excreted by marine mega-herbivores, compared to seeds dropped naturally from the plant, brings forward more questions for future research. The consumption of seagrass fruits and seeds is widely considered incidental, due to the small size of the fruits and seeds (Marsh et al. 2011, Kendrick et al. 2012, McMahon et al. 2014). However, due to the impact that these biotic vectors have on the dispersal and germination of at least two known seagrass seed species (*Z. muelleri* in this study and *Halophila ovalis* in Wu et al. (2016)), it would be ideal to determine if incidental ingestion or targeted ingestion is occurring. The addition of sediment in a germination trial would provide a more ecologically meaningful outcome, as sediment burial is a known germination cue for many seagrass species (Moore et al. 1993, Jarvis and Moore 2015). Finally, it would be meaningful to determine if nutrient input from the herbivores faeces is a factor in the improved germination outcomes.

Turtles and dugongs are integral mutualistic partners within seagrass ecosystem community networks. These animals are listed as endangered and vulnerable respectively by the IUCN, due to large population reductions, hunting pressures, fishing by-catch and habitat destruction (Seminoff 2004, Marsh and Sobtzick 2019). If one or both of these animals were to be lost the seagrass ecosystem community network would become less robust, leading to a flow on effect for other animal species within the network. Protection and conservation of these two marine mega-herbivores is important to maintain a strong and resilient seagrass ecosystem community. The research I describe here, will contribute to this important process.

Chapter Summary

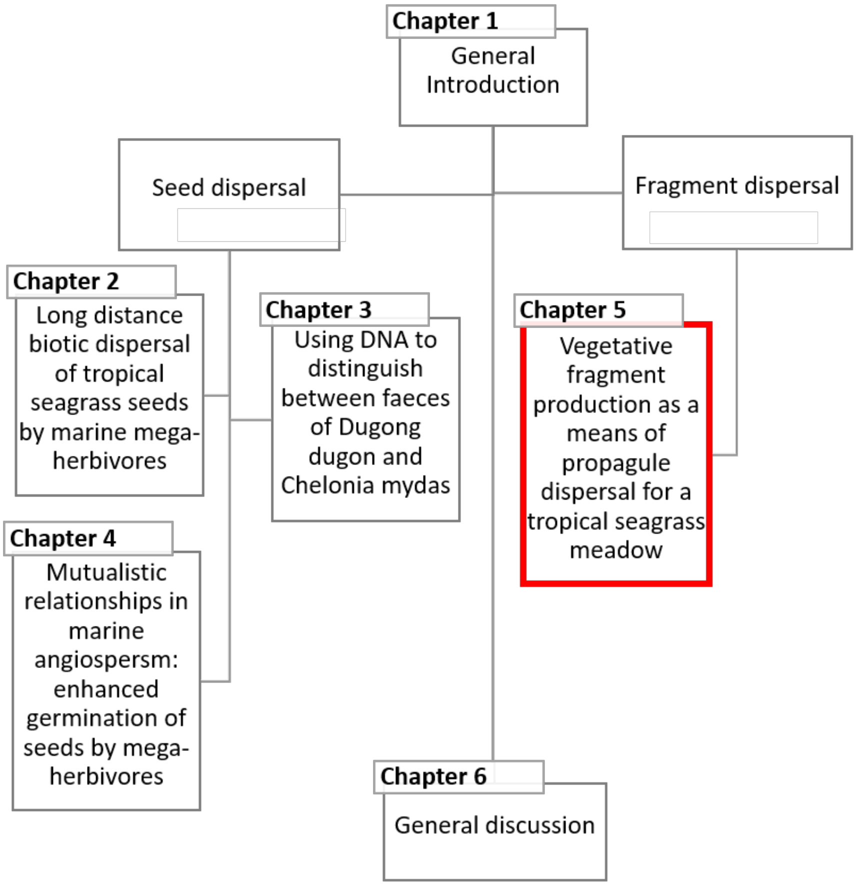
- Angiosperms have co-evolved with animals over thousands of years leading to an array of mutualistic relationships. Passage of plant seeds through animal intestines leads to an important mutualism providing the animal with food and the plant with seed dispersal and enhanced germination. This phenomenon is well studied in terrestrial angiosperms but there is less research in aquatic environments. This chapter studied the effect of gut-passage in marine mega-herbivores (green sea turtles and dugongs) on seed germination for a common Australian seagrass, *Zostera muelleri*.
- Ingested and excreted seeds had a significantly greater germination rate (two to four times greater) and germinated significantly faster (18 – 61% faster) than seeds from the plant. Temperature did not add any benefit to germination success for either seed deposition treatment.
- Seeds released directly from the plant have a slow, low rate of germination.
- Excreted seeds which had not germinated at the end of the experiment were significantly less likely to be viable compared with seeds taken directly from the plant.
- Seeds from the plant that did not germinate, maintained viability and could provide seeds to a seed bank that would be available for meadow recovery.
- This chapter is the first record of marine mega-herbivores enhancing germination of *Z. muelleri* seeds. By transporting seeds to new locations and enhancing germination these animals are important in seagrass meadow resilience and connectivity.

Chapter 5

Vegetative fragment production as a means of propagule dispersal for a tropical seagrass meadow [4]

In this chapter I quantify the number of viable seagrass fragments within two tropical coastal intertidal and subtidal seagrass meadows in the Central GBRWHA. I also determine which abiotic and biotic factors influence the density of fragments available for dispersal, and describe the characteristics of these fragments.

Tol, York and Coles co-developed the research question. Tol and Coles collected the data. Tol and Carter performed the data analysis. Tol wrote the first draft of the paper which was revised with editorial input from York, Jarvis, Carter, Grech, Congdon and Coles. Tol developed the figures and tables, with assistance from Carter and York.



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[4] **Tol. SJ**, Carter. AB, York. PH, Jarvis. JC, Grech. A, Congdon. BC, and Coles. RG. Vegetative fragment production as a means of propagule dispersal for a tropical seagrass meadow. *Annals of Botany,* Under Review, Manuscript #: 20635.

Introduction

Information on a species' dispersal mechanisms is important for the design of effective management and conservation actions (Clobert et al. 2001, Van der Stocken et al. 2019). Dispersal success underpins the resilience of a species, including the ability to colonize new habitats or to recover from loss (Cowen et al. 2006, Van der Stocken et al. 2019). Comprehensive information on dispersal attributes is necessary to inform biophysical models of dispersal and to estimate potential connectivity (Kendrick et al. 2012, Grech et al. 2018). Connectivity models provide critical information for management and restoration programs, as they can be used to predict recovery potential based on whether subpopulations within a metapopulation are highly connected or isolated (Källström et al. 2008, Grech et al. 2016, Grech et al. 2018, Jahnke et al. 2020). For seagrass, an ecological grouping of marine angiosperms, high levels of connectivity among meadows support species replenishment and recovery after disturbance events such as cyclones and large floods (Cowen et al. 2006, Grech et al. 2018). Quantifying the factors that enable propagule creation and dispersal is key to understanding how populations of seagrass within discrete meadows are connected in an ecological network (Kendrick et al. 2012).

Evaluating dispersal potential is an important step in estimating an organism's ability to colonise new locations. For dispersal to be successful, many factors must align before and after settlement. The capacity for aquatic propagules/ fragments to disperse is influenced by wind, tide and current flows, and survival time (Kendrick et al. 2012, Baguette et al. 2013). In order to understand these factors, it is imperative to quantify the density of these propagules available for dispersal. The settlement location also needs to meet the requirements for growth and survival if an organism is to successfully establish and grow (Bonte et al. 2012, Baguette et al. 2013). Marine plants, which primarily disperse abiotically, are limited to locations of optimal environmental conditions (e.g. sediment type, exposure and depth) (McMahon et al. 2014, Sherman et al. 2018). Successful settlement therefore requires production of large numbers of propagules or viable plant fragments to mitigate the low probability of settling at an appropriate location (Bonte et al. 2012, Baguette et al. 2013). Therefore, quantifying dispersal potential and connectivity for marine plants requires a diverse range of knowledge on physical and biological factors (Erftemeijer et al. 2008, Källström et al. 2008, Grech et al. 2016).

Seagrasses grow along all continental coastlines except Antarctica (Hemminga and Duarte 2000, Green and Short 2003, Larkum et al. 2006). Seagrass habitats provide important ecosystem services, such as commercial fisheries habitat (Watson et al. 1993, Unsworth et al. 2010), stabilisation of sediments that prevent coastal erosion (Hemminga and Duarte 2000, Potouroglou et al. 2017), absorption of excess nutrients, and keeping coral reefs healthy by filtering pathogens and particulates from the water column (Mellors et al. 2005, Lamb et al. 2017). In some locations they can be a valuable mechanism for carbon sequestration (Kennedy et al. 2010, Macreadie et al. 2014). Despite their importance, seagrass meadows are not well protected in most of the world and have been declining globally due to multiple impacts (Waycott et al. 2009). A large proportion (approximately 40%) of the human population resides in tropical coastal regions (Creel 2003, Lai et al. 2015), exposing tropical seagrass meadows to multiple anthropogenic stressors (Waycott et al. 2009, Grech et al. 2011). These stressors include, but are not limited to: increased nutrient loads from agricultural and urban run-off, coastal development, hydrological alterations, damaging fishing practices, and extreme weather events (Orth et al. 2006a, Waycott et al. 2009, Grech et al. 2011).

Seagrasses are the only marine angiosperms that can flower and fruit submerged in seawater (Hemminga and Duarte 2000, Larkum et al. 2006), but are also capable of growing clonally through asexual growth by rhizomes (Hemminga and Duarte 2000, Larkum et al. 2006). Seagrass seeds are generally negatively buoyant and settle close to the parent plant if no other dispersal vectors are involved (Orth et al. 2006b, Kendrick et al. 2012, McMahon et al. 2014). Long distance (greater than 100s of metres) abiotic dispersal of seagrass can occur through the movement of viable propagules such as buoyant vegetative fragments (e.g. Thomson et al. 2014, Smith et al. 2018) or through seeds attached to rafting seagrass (e.g. Erftemeijer et al. 2008, Källström et al. 2008) via wind and tidal currents. Biotic processes such as ingestion and excretion of seagrass seeds by marine herbivores can also assist long distance dispersal (Charalambidou et al. 2003, Sumoski and Orth 2012, Wu et al. 2016, Tol et al. 2017).

In the Great Barrier Reef World Heritage Area (GBRWHA) in north-eastern Australia, seagrass meadows are foraging grounds for endangered green sea turtles (*Chelonia mydas*), and for one of the last remaining large populations of dugong (*Dugong dugon*) (Marsh et al. 2011, Jensen et al. 2016). Green sea turtles and dugongs are theorised to create seagrass fragments when foraging (Kendrick et al. 2012, McMahon et al. 2014). However, green sea turtles predominately crop leaves when foraging (Bjorndal 1980), making it difficult to identify foraging within a meadow. Dugongs commonly forage in an 'excavation' style, where they remove the entire plant (leaves, rhizomes and roots) and leave behind visible bare serpentine indentations in the meadow (Tol et al. 2016). This foraging style provides an easy measure of herbivore feeding within a meadow over a short time frame (Tol et al. 2016). Dugongs are known to interact with seagrass meadows and can stimulate new growth through grazing (Preen 1995) or move seeds through their faeces (Tol et al. 2017), however their role in propagule (fragment) creation and dispersal has not been quantified.

Tropical seagrasses are known to differ in their morphology (for example, smaller and shorter blades) and biology (for example, fast growing) compared to temperate species, which can impact dispersal (Short et al. 2007). Models have been used to estimate spatial scales of seagrass dispersal and the levels of connectivity, however, most variables used are specific to temperate species or based on a range of assumptions which could render them inaccurate for tropical species (Erftemeijer et al. 2008, Källström et al. 2008, Ruiz-Montoya et al. 2012, Ruiz-Montoya et al. 2015, Grech et al. 2016, Grech et al. 2018). The limited information available on seagrass propagule dispersal is mostly concentrated on buoyancy and not on availability and potential viability of fragments (Hall et al. 2006, Källström et al. 2008, Thomson et al. 2014, York et al. 2017), and quantitative information on these biological variables is incomplete (Kendrick et al. 2012, McMahon et al. 2014).

The goal of my study was to assess the attributes of seagrass fragments and quantify the fragments available for dispersal for two of the most common tropical seagrass species in the GBRWHA; *Z. muelleri* and *H. uninervis*. At two seagrass meadows in the central GBRWHA, I measured the number of total and viable fragments, describe the average fragment dimensions and characteristics, and quantified which factors (growing/senescent season, wind speed, wind direction, seagrass meadow density, and dugong foraging intensity) influence the formation and density of floating fragments. My analysis will provide critical inputs to dispersal and connectivity models that inform seagrass ecosystem management and conservation.

Methods

Data collection

Positively buoyant seagrass fragments were collected from sheltered seagrass meadows at Pioneer Bay and at Midge Point in the central GBRWHA, Queensland Australia (Figure 5.1). Both meadows have minimal boat/ anchor traffic and a similar species composition (Supplementary Table 5.1).

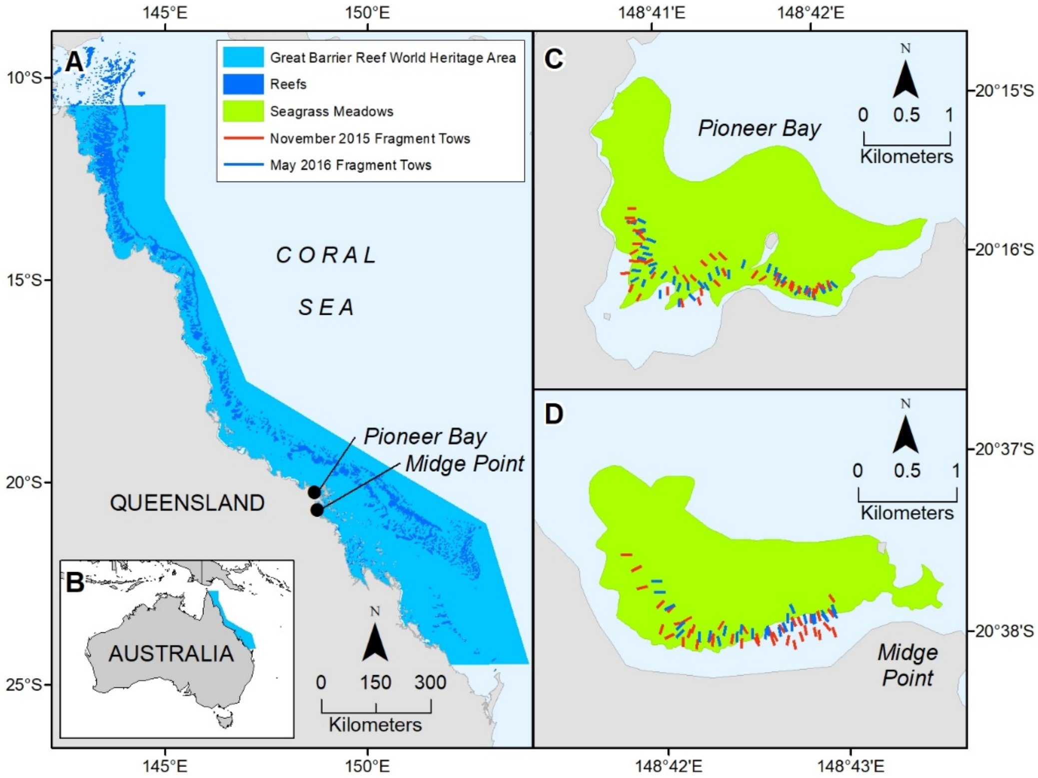


Figure 5.1: Study site map, showing the location of the Great Barrier Reef World Heritage Area relative to Queensland (A) and Australia (B), and fragment tow locations in Pioneer Bay (C) and Midge Point (D) seagrass meadows.

Seagrass fragments were collected during the growing (September to February) and senescent/ dormant (March to August) seasons for tropical seagrass species in the southern hemisphere (Sherman et al. 2018). A total of 156 tows were performed during the growing season (21-26th November 2015; Pioneer Bay n = 78 and Midge Point n = 78), and 130 tows were performed during the senescent season (4-8th May 2016; Pioneer Bay n = 78 and Midge Point n = 65). Seagrass fragments were collected from the water's surface by towing two partially buoyant nets (mesh size 0.5 cm), one on either side of the vessel, along a transect 100 m perpendicular to the coast, commencing within 30 minutes after high tide. Each net was attached to a 1x1 m frame; the top edge of the opening was positioned approximately 30 cm above and the bottom edge 70 cm below the water surface, to ensure all floating fragments in its path were captured.

I defined a fragment as any detached piece of seagrass where a rhizome and a leaf or leaves were present (Ewanchuk and Williams 1996, Hall et al. 2006). Fragment characteristics were recorded for each species, including presence of reproductive organelles and roots, number of shoots and leaves, length of leaves and rhizome (mm), and biomass (g dry weight). Viability of each fragment was assessed by the presence of a growing tip or apical meristem (Ewanchuk and Williams 1996). All fragments were dried in an oven at 60 °C up to 72 hours to obtain a constant dry weight for biomass.

Seagrass density and dugong foraging intensity were estimated from quadrats placed haphazardly within meadows exposed during low tides. Seagrass density was estimated at each sampling location and seasonal event from ten 0.25 m^2 quadrats using shoot counts for each species in four 0.01 m^2 quadrats placed within each 0.25 m^2 quadrat. Dugong foraging intensity was estimated for every sampling day, by counting the presence of dugong feeding trails in 100 x 1 m^2 quadrats. Dugong foraging intensity was calculated for each high tide by subtracting the total number of quadrats with feeding trails at low tide for Time 0 (the low tide preceding high tide fragment tows) from the number of quadrats with feeding trails at Time 1 (the low tide following fragment tows). Wind data (wind speed km/h and wind direction) was obtained from the Australian Bureau of Meteorology Hamilton Island airport weather station (Station number 033106); located approximately 45 km from both meadows. Wind speed was averaged for the 12 hours before towing began each sample day and assigned to three categories; low (< 20 km/h), medium (20 – 25 km/h) and high (> 25 km/h). Wind direction was grouped into three categories; east, southeast and south. There was no north, northeast and northwest winds during my sample times, which is consistent with the climatology of the sampling period.

Statistical analysis

All data prior to analysis was examined for outliers and normality, and predictor variables assessed for collinearity using variance inflation factors (Zuur et al. 2010). All statistical analyses were conducted in the statistical software environment R (R version 4.0.2; R Core Team 2020).

Differences in shoot count, leaf count, leaf length, rhizome length and biomass between viable and non-viable fragments for each species (*Z. muelleri* and *H. uninervis*), were analysed using a Mann-Whitney U Test, as the data did not conform to a normal distribution. To ensure a robust statistical analysis, fragments were pooled across sites.

Statistical analyses for seagrass fragment density was conducted for *Z. muelleri* and *H. uninervis* combined due to their morphological similarities (Coles et al. 2002, Waycott et al. 2004). A set of analytical models were developled to quantify the effects of season (T), location (S), meadow density (SC), dugong foraging intensity (DFI), and wind speed (WS) and direction (WD) on fragment density for total seagrass fragments (tsf = T + S + SC + DFI + WS + WD) and viable seagrass fragments (vsf = T + S + SC + DFI + WS + WD). The best-fit model was considered to be the simplest model with the lowest Akaike information criterion (AIC) score calculated from log likelihood ratios derived from all regression analyses (Zuur et al. 2010). Differences between all AIC models were analyzed and ranked using the 'MuMIn' package (Barton 2019). A generalized linear model (GLM) with a negative binomial distribution ('MASS' package (Venables and Ripley 2002)) was used to the density of total and viable seagrass fragments. A negative binomal distribution was selected to correct for overdispersion (Lindén and Mäntyniemi 2011). Residuals were inspected visually for patterns by plotting the fitted versus response variables. Post hoc 'tukey' adjustment analysis for pairwise comparisons were computed using the 'emmeans' package (Lenth 2020).

A small number of *Halophila ovalis* fragments were collected during this experiment, however the sample size was too small for statistical analysis.

Results

A total of 264 fragments were collected in the growing season (*Z. muelleri* = 91%; *H. uninervis* = 8%; *H. ovalis* = 1%) and 46 fragments in the senescent season (*Z. muelleri* = 18.5%; *H. uninervis* = 80%; *H. ovalis* = 1.5%). Just over one third of all fragments collected were classified as viable (38%; Table 5.1). Nearly all fragments collected across both locations and season had roots present (96%). Five fragments had fruits/ seeds attached, and all were collected in the growing season; one *H. uninervis* fragment with a seed attached (from Pioneer Bay), one *H. ovalis* fragment with a fruit attached (from Pioneer Bay) and three *Z. muelleri* fragments with spathes attached (two from Pioneer Bay and one from Midge Point) (Figure 5.2).

Table 5.1: Number of seagrass fragments collected from Pioneer Bay and Midge Point in November 2015 (growing season) and May 2016 (senescent season), in the Central GBRWHA, Queensland Australia. Viable fragments were determined by the presence of a growing tip/apical meristem.

		Total Fragments			**Viable Fragments**		
		Z. muelleri	***H. uninervis***	***H. ovalis***	***Z. muelleri***	***H. uninervis***	***H. ovalis***
Pioneer Bay							
	November 2015	115	6	3	33	3	1
	May 2016	12	24	2	6	5	1
Midge Point							
	November 2015	101	12	0	56	6	0
	May 2016	1	33	1	0	6	0

Figure 5.2: Seagrass fragments collected floating on the water's surface in the central GBRWHA, Queensland Australia during November 2015; **a)** *Zostera muelleri* fragment with spathes attached; **b)** *Halophila ovalis* fragments with an immature fruit attached; and **c)** a *Halodule uninervis* fragment with a seed attached.

Viable *Z. muelleri* fragments had more shoots and leaves, and longer rhizomes, but shorter leaves than non-viable fragments (Figure 5.3; Supplementary Table 5.2). An average viable *Z. muelleri* fragment had 2.5 (± 0.1 SE) shoots, 7.1 (± 0.4 SE) leaves, an average leaf length of 28 mm (± 1 SE), a rhizome length of 26 mm (± 2 SE), and weighed 0.026 g (± 0.001 SE). Viable *H. uninervis* fragments had more shoots and leaves than non-viable fragments, but no difference in leaf and rhizome lengths (Figure 5.3; Supplementary Table 5.3). An average *H. uninervis* viable fragment had 2.1 (± 0.2 SE) shoots, 4.5 (± 0.6 SE) leaves, an average leaf length of 20 mm (± 1 SE), a rhizome length of 19 mm (± 2 SE), and weighed 0.005 g (± 0.001 SE). There was no difference in biomass for both *Z. muelleri* and *H. uninervis* viable and non-viable fragments (Figure 5.3; Supplementary Table 5.3). Only 18% of all fragments had greater than two shoots, with the maximum number being six shoots for *Z. muelleri*, five shoots for *H. uninervis* and four shoots for *H. ovalis*.

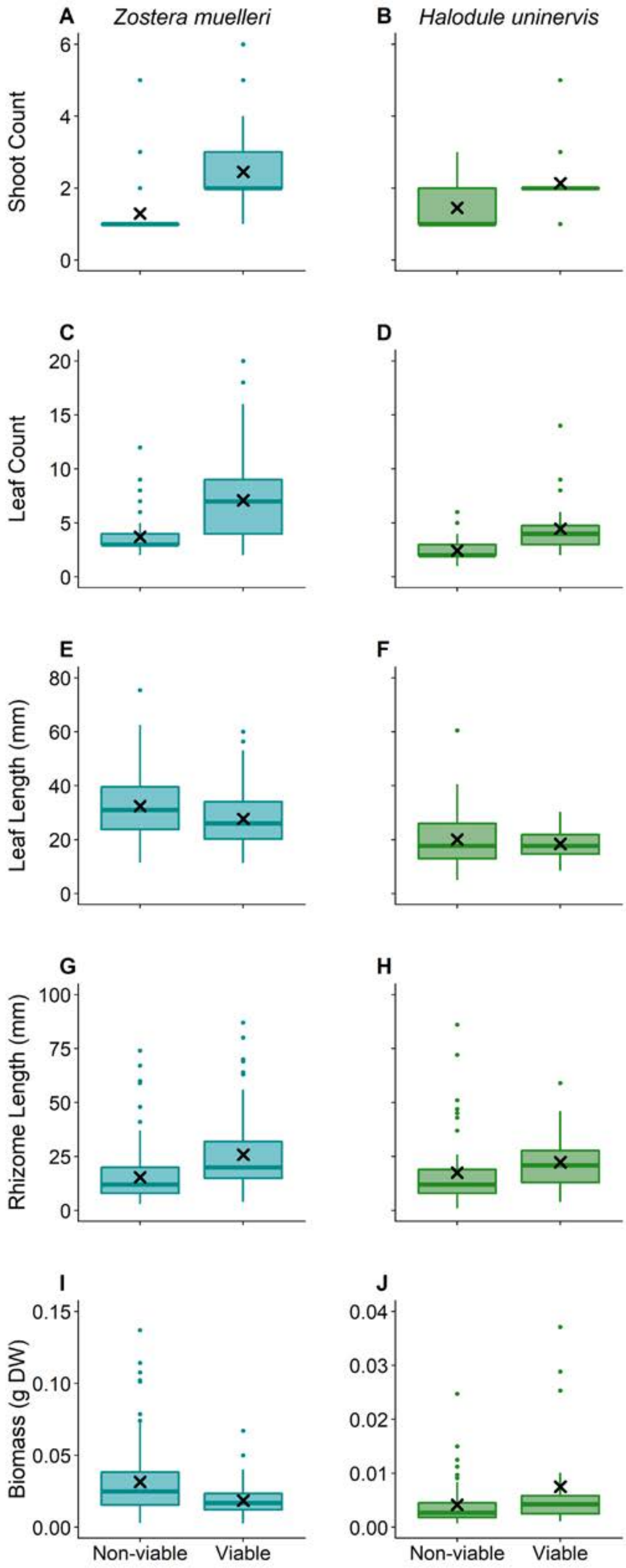


Figure 5.3: Boxplots of floating seagrass fragment characteristics for *Zostera muelleri* (n = 93 viable and 136 non-viable) and *Halodule uninervis* (n = 22 viable and 53 non-viable); **X** depicts the mean value. Fragments were collected from Pioneer Bay and Midge Point in the Central GBRWHA, Queensland Australia.

There was a significant and positive relationship between number of total fragments and seagrass meadow density (Figure 5.4a; Supplementary Table 5.4). There was no difference in total number of fragments between the growing and senescent season. There were significantly more viable fragments during the growing season (0.63 ± 0.08 SE fragments 100 m^{-2}) than the senescent season (0.13 ± 0.03 SE fragments 100 m^{-2}) (Figure 5.5a & Supplementary Table 5.5). Medium winds produced significantly more of both total and viable fragments than low or high winds (Figure 5.4b and 5.5b; Supplementary Table 5.4 and 5.5). Wind direction, meadow location and dugong foraging intensity did not impact fragment density.

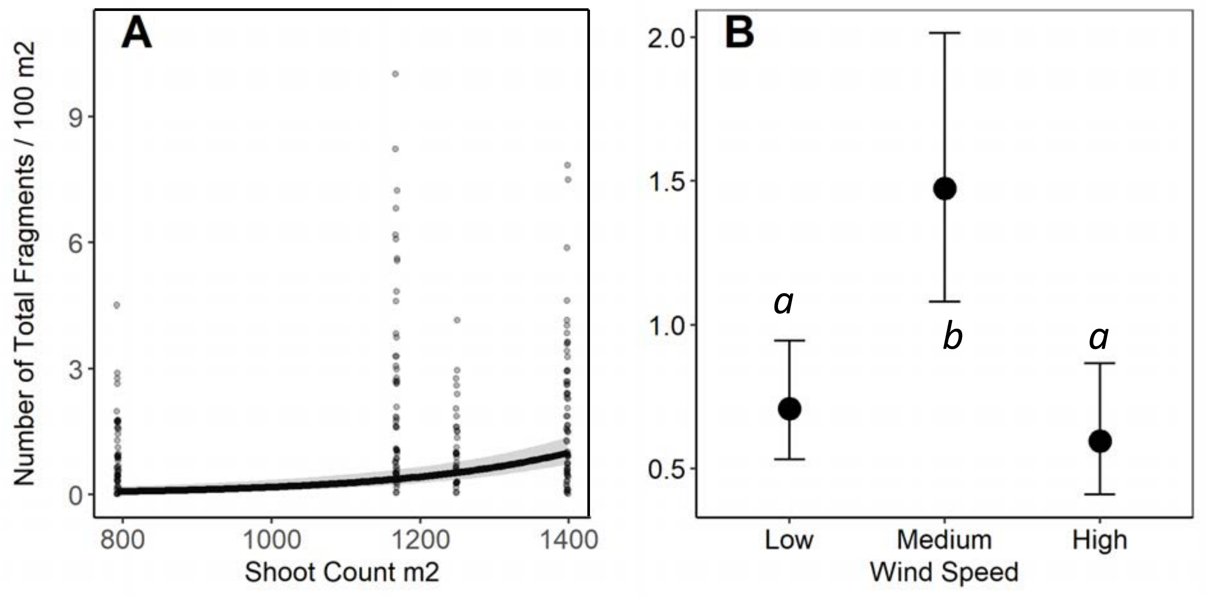


Figure 5.4: Number of total seagrass fragments by **a)** shoot count 100 m^{-2} (95% CI); and **b)** wind speed (low = < 20 km/h, medium = 20-25 km/h, and high = > 25 km/h; letters indicate which wind speeds were significantly different, analysed with a Tukey post-hoc), for coastal seagrass meadows in the Central GBRWHA, Queensland Australia.

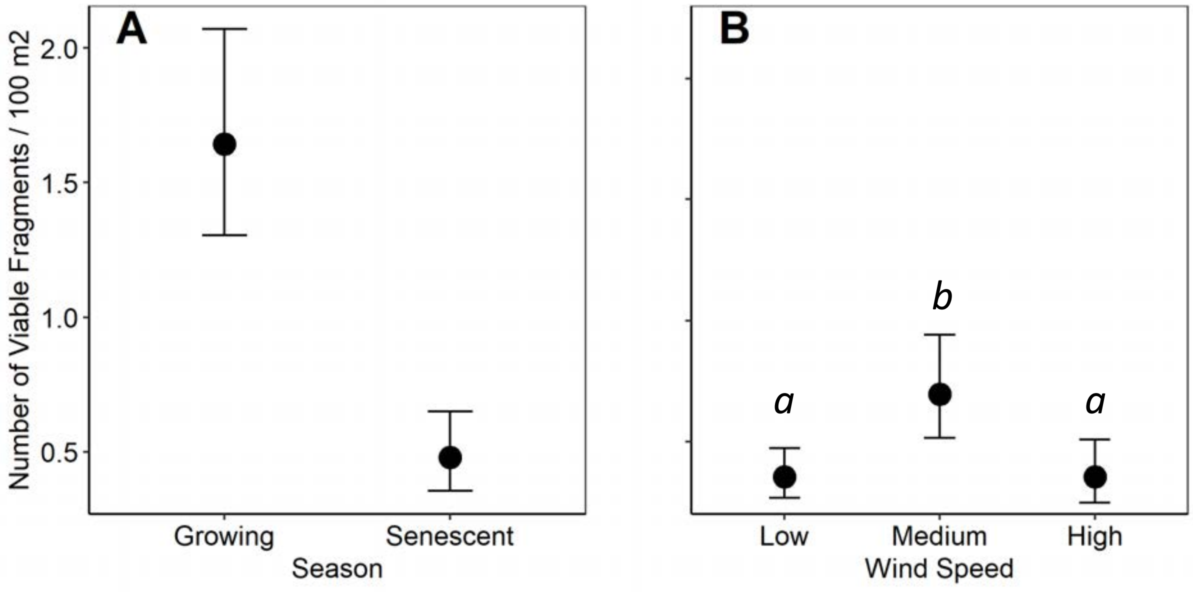


Figure 5.5: Average number of viable seagrass fragments by **a)** growing and senescent season; and **b)** wind speed (low = < 20 km/h, medium = 20-25 km/h, and high = > 25 km/h; letters indicate which wind speeds were significantly different, analysed with a Tukey post-hoc), for coastal seagrass meadows in the Central GBRWHA, Queensland Australia.

Discussion

My study quantified seagrass fragment density in two tropical coastal seagrass meadows, and found over one third of these fragments floating freely in the water were viable. If these fragments settle in a location with suitable environmental attributes, they have the potential to establish as a new plant and contribute to seagrass meadow growth and connectivity. At my study locations, total fragment density was influenced by seagrass meadow density and wind speed, while viable fragment density was influenced by season and wind speed. Surprisingly, foraging by dugongs did not affect seagrass fragment density.

Less than 2% of all fragments collected in my study had a fruit/ spathe attached, indicating that abiotic dispersal plays a role in the dispersal of non-reproductive shoots. Viable fragments for *Z. muelleri* and *H. uninervis* had more shoots and consequently more leaves than non-viable fragments. A greater number of shoots on viable fragments would be expected, as the fragment is capable of continuing to grow when a growing tip or apical meristem is present (Hemminga and Duarte 2000, Larkum et al. 2006, Thomson et al. 2014). Conversely, when a fragment is incapable of rhizome extension from a growing tip, they could re-direct growth into leaves (Hemminga and Duarte 2000, Larkum et al. 2006). This might explain why my *Z. muelleri* viable fragments had longer rhizomes, and why non-viable fragments had longer leaves. It is likely that viable floating fragments have the ability to continue to grow in the water column, increasing their ability to establish into a new plant if they settle in a suitable environment.

I found that the density of floating seagrass fragments was dependent on seagrass meadow density and wind speed, while the density of floating viable seagrass fragments was dependent on season and wind speed. As seagrass meadow density increases, there are more plants available for the creation of fragments. Additionally, there were significantly more viable fragments (nearly five times more) floating on the water's surface during the seagrass growing season, compared to the senescent season. Many seagrass species (including *H. uninervis* and *Z. muelleri*) grow during the warmer months and remain dormant during the cooler months (Marba et al. 1996, Waycott et al. 2004, Chartrand et al. 2016, Sherman et al. 2018). Increased growth during the "growing" season would produce greater volumes of growing tips compared to the "senescent" season, increasing the chance of growing tips being present on a fragment.

My study found significantly more fragments in the water during times of medium wind speed (20-25 km/h). Physical disturbance by waves, an effect of wind speed, may create seagrass fragments either due to sediment erosion or through rhizome breakage (Kendrick et al. 2012, McMahon et al. 2014). Low winds produce low energy waves with little impact on the seabed (Donelan et al. 2012) and fragment drift speeds will be similar to tidal flows (Ryan et al. 2018). At the other end of the scale, high winds can cause wave crests to begin to break, which can wash fragments out of the water column and up on to the adjacent shore as wrack (Simeone et al. 2013). Wave exposure was one of the most important factors in predicting the presence of seagrass meadows in the GBRWHA (Carruthers et al. 2002, Grech and Coles 2010). If wave action is consistently too high, seagrass species are incapable of maintaining anchorage (Carruthers et al. 2002). This is also evident in extreme weather events, such as storms, cyclones and hurricanes, where high sediment turnover leads to the erosion of intertidal and subtidal seagrass meadows (Carruthers et al. 2002, Orth et al. 2006a, Pollard and Greenway 2013, McKenna et al. 2015). My study supports this, as high wind and waves would also make it less likely for propagules in the form of fragments to be available for transportation to new sites and assist in replenishment, as they are removed from the system and washed ashore as wrack.

I expected that active foraging by dugongs would increase the density of seagrass fragments (Kendrick et al. 2012, McMahon et al. 2014, York et al. 2017), as direct grazing and bioturbation by herbivores is theorised to create fragments/ propagules (Figuerola and Green 2002, Bakker et al. 2016). Dugongs consume up to 40 kg wet weight of seagrass daily (Aragones 1996, Marsh et al. 2011), and consume the whole plant when undertaking excavation foraging (Marsh et al. 2011, Tol et al. 2016). These two factors combined suggest a high level of bioturbation within active foraging meadows. My results do not support this theory at my study sites. However, it should be noted that dugongs, including at my study sites, produce large volumes of seagrass wrack which did not meet my requirements to be classified as a fragment. Current population estimates for dugongs within the central GBRWHA (Whitsundays to Hinchinbrook Island, ~350 kilometres of coastline) is approximately 2,000 (Sobtzick et al. 2017). The central GBRWHA dugong population density is relatively smaller than other local populations in northern (Torres Strait approximately 15,700 individuals (Sobtzick et al. 2014)) and southern (Hervey Bay approximately 2055 (Sobtzick et al. 2017)) Queensland. At my study sites, the amount of dugong foraging could be considered low and might explain why I found a negligible effect on fragment frequency. Areas with denser dugong populations may still provide a source of fragment creation, and increase seagrass fragment frequency and dispersal potential.

Although fragment density initially appears low in my study, with less than one fragment 100 m^{-2}, when scaled up to a meadow size, the number of available fragments for dispersal is considerable. The seagrass meadow at Pioneer Bay is 4.63 km^2 in size, and the meadow at Midge Point is 3.19 km^2 (Carter et al. 2016). Therefore, when scaled up to the area of the meadow, there should be between 25,465 – 32,873 viable fragments in Pioneer Bay, and 17,545 – 22,649 viable fragments in Midge Point per day during the growing season, and 4,630 – 7,408 viable fragments in Pioneer Bay and 3,190 – 5,104 viable fragments in Midge Point per day during the senescent season. A large number of fragments would be lost due to a multitude of reasons, such as being eaten by various herbivores (Valentine and Duffy 2006), deterioration over time (Weatherall et al. 2016), and settlement in a location incompatible with growth (Grech et al. 2016). However, the large number of fragments we have quantified in my study from relatively small tropical seagrass meadows make successful dispersal by vegetative fragments highly probable.

Dispersal and connectivity are fundamental processes that shape the distribution, structure and resilience of marine ecosystems. The forces of ocean waves and currents are efficient vectors for long distance dispersal, and facilitate the replenishment and recovery of seagrass populations after disturbance (Nathan 2008). Biophysical models predict dispersal and connectivity by combining oceanographic simulations with biological information on species life histories. Previous studies using data from biophysical models have been limited in predicting the potential connectivity of seagrass meadows because information on the number of fragments in tropical meadows has not been available (e.g. Grech et al. 2018). The new information on available floating seagrass propagules generated in this study is germane to informing estimations of connectivity and advances the predictive capacity and understanding of replenishment, recruitment and recovery in seagrass ecosystems.

Chapter Summary

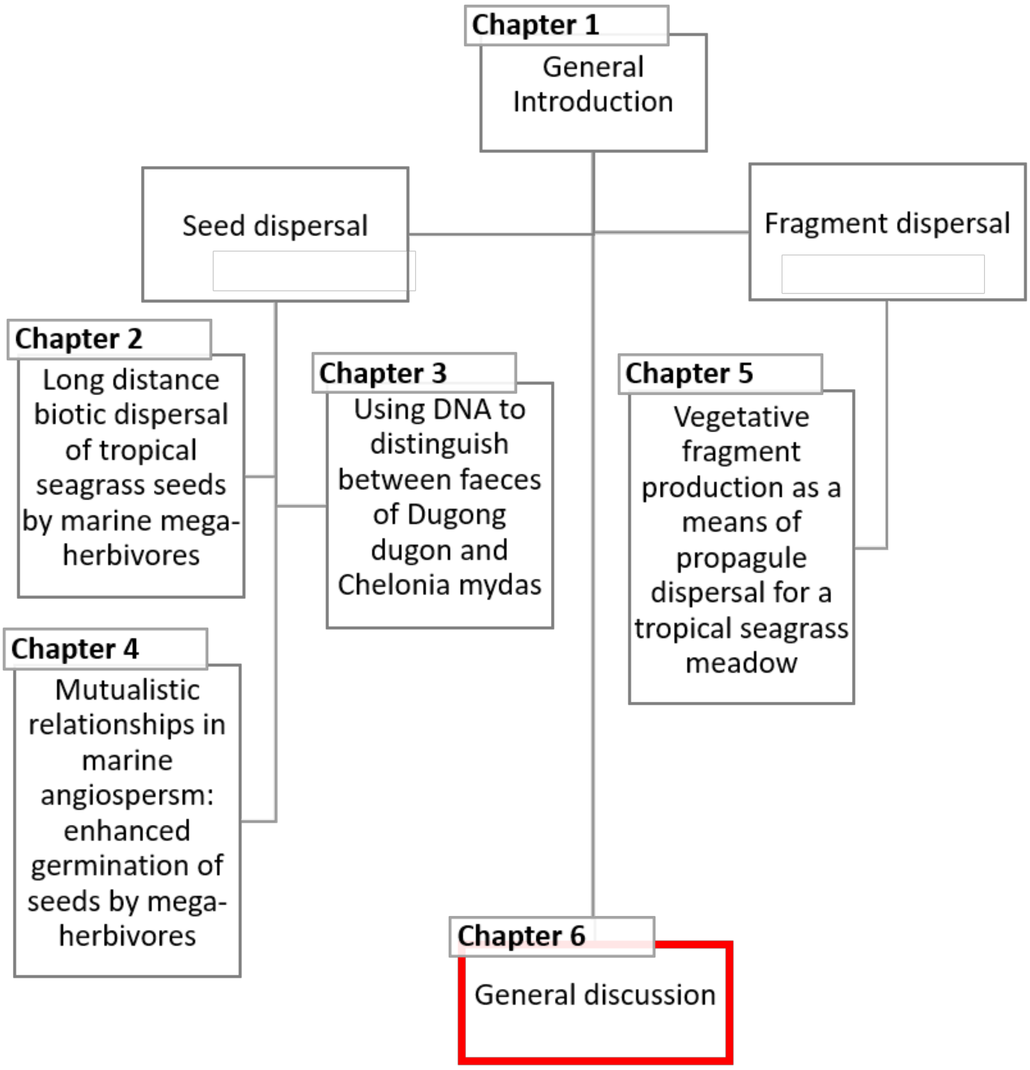
- Long distance dispersal of seagrasses contributes to the replenishment and recovery of tropical coastal habitats exposed to disturbance events, such as cyclones and infrastructure development. However, the present understanding of the attributes of seagrass fragments is primarily from temperate species and regions.
- I found that seagrass meadow density and wind speed influenced the total fragment density, while season and wind speed influenced the density of viable fragments. Dugong foraging intensity did not impact fragment density.
- My results indicate that wave action from winds and high seagrass meadow density increases seagrass fragment creation, and that more viable fragments are produced during the growing than the senescence season.
- Seagrass fragments classified as viable for *Z. muelleri* and *H. uninervis* had significantly more shoots and leaves than non-viable fragments.
- Over a third (38%) of all fragments collected were viable.
- I collected 0.63 (± 0.08 SE) floating viable fragments 100 m^{-2} in the growing season, and 0.13 (± 0.03 SE) viable fragments 100 m^{-2} in the senescent season.
- There is likely to be a large number of viable seagrass fragments available for long distance dispersal. This study's outputs will enhance dispersal and connectivity models that inform seagrass ecosystem management and conservation.

Chapter 6

General discussion

In this chapter, I summarise my thesis findings and describe their implications for the future management and conservation of tropical seagrass meadows. Finally, I outline potential future research endeavours to complete the picture of the dispersal mechanisms for at least two tropical seagrass species.

Tol wrote the first draft of the paper, which was revised with editorial input from York, Grech, Jarvis, Congdon and Coles.



Relative importance of different seagrass re-establishment strategies in tropical Queensland, Australia

Key Findings

The key findings of the work undertaken in this thesis are summarised as follows:

Overall

- Marine mega-herbivores provide a means for seed dispersal and rapid germination, and assist in the connectivity, resilience, and replenishment/ recovery of tropical seagrass meadows.
- There are numerous *Z. muelleri* and *H. uninervis* viable fragments floating in the water column which are being dispersed abiotically in the central GBRWHA; suggesting there is enough fragments to survive for long-distance dispersal over time.

Biotic dispersal

- Marine mega-herbivores, dugong and green sea turtles, disperse seagrass seeds of at least five tropical species (*Cymodocea serrulata*, *Halophila decipiens*, *Halophila spp.*, *Halodule uninervis*, and *Zostera muelleri*).
- Nearly 10% of these seeds are viable after ingestion and excretion, and more than 60% of the seeds passed have a split seed coat (a known dormancy break).
- On a daily basis and during seagrass fruiting times, my analysis indicates up to 120,000 seeds are being dispersed in the southern GBRWHA, and as many as 500,000 seeds dispersed over the entire GBRWHA.
- Seagrass seed dispersal by marine mega-herbivores has the potential to move seeds up to ~ 650 km. Distances in the 10s kilometres are more likely to be common.

Genetic Analysis

- I found that it was impossible to visually distinguish between the faeces of dugong and green sea turtles collected *in-situ*. I was able to determine species of origin by developing PCR primer pairs that create robust and distinct PCR products that can be analysed using Sanger sequencing.

- I am now able to confirm, using these primers, that both dugong and green sea turtles are biotic dispersers for tropical seagrass seeds.

Germination

- Seeds which have been ingested and excreted by a marine mega-herbivores germinated 18% - 61% faster and had two to four times greater maximum germination success, compared to seeds that had not been consumed, over a two-month period.
- Un-germinated seeds passed by marine mega-herbivores had a significantly lower viability (25% ±16% SE) compared to un-germinated seeds dropped by the plant (95% ± 2% SE).
- Enhanced germination of seeds passed by a marine mega-herbivore means that these seeds are unlikely to form part of a persistent seed bank but could lead to the immediate replenishment of a meadow.

Fragment dispersal

- I have demonstrated that over one third (38%) of the seagrass fragments being dispersed were classified as viable.
- Meadow density and moderate wind speeds (20 – 25 km/hr) were a factor in total fragment creation. There were more viable fragments dispersed during the growing season (0.63 ± 0.08 SE fragments per 100 m^2 per day) compared to the senescent season (0.03 ± 0.03 SE fragments per 100 m^2 per day).
- Foraging by dugongs was not a significant factor in the creation of seagrass fragments.
- I estimate that my two study sites during the growing season release 43,000 – 55,000 viable seagrass fragments for dispersal each day.

Implications for Tropical Seagrass Conservation and Management

Introduction

Seagrass habitats play a vital role in the health and resilience of multiple species which either reside within the meadow (e.g. fish and mega fauna), or benefit from their services (e.g. coral reef ecosystems) (Nordlund et al. 2018). To ensure that these ecosystems continue to provide ecosystem services, they must be managed effectively. For this to be possible, it is vital to understand how seagrass meadows are connected spatially. The rate of exchange or connectivity among populations affects population and metapopulation dynamics, genetics, responses to species invasions and disease transmission, species expansion, and the replenishment of populations after disturbance events (Kinlan and Gaines 2003, Cowen and Sponaugle 2009). In the case of marine plants, long-distance seed dispersal is critical to the distribution, structure and resilience of populations (Kendrick et al. 2012, Kendrick et al. 2017). Seed dispersal results in the movement of genetic material between populations or into new previously uncolonized areas (McMahon et al. 2014). Increasing the dispersal distance of a reproductive organelle, allows for greater genetic connectivity and the potential to enhance seagrass resilience (Kendrick et al. 2012, McMahon et al. 2014, O'Brien et al. 2018). Clonal meadows have a lower resilience than meadows with genetic diversity, as higher generic diversity increase the chance of having different genes with different stress limitations (Hughes and Stachowicz 2004, Hughes and Stachowicz 2011). Increasing genetic diversity through the input of seeds from different meadows may improve sustainability in the face of increasing threats.

The arrival of the Anthropocene has brought with it rapid changes to the condition and level of connectivity among seagrass coastal habitats as they become increasingly fragmented by human activities; such as urban, industrial and agricultural development, and commercial and subsistence fishing (Unsworth et al. 2018). Coastal habitats also face a changing global climate, resulting in warming sea temperatures and increased intensity of tropical storms (Orth et al. 2006a, Waycott et al. 2009). Identifying, protecting and restoring connectivity is therefore critical to effective conservation outcomes as it provides support for the replenishment, recruitment and recovery of coastal habitats after disturbance events. To accurately understand connectivity, we need to understand how seagrass species disperse

(Kendrick et al. 2017, O'Brien et al. 2018). There is a dearth of research on the dispersal pathways for seagrass species, especially tropical species (Kendrick et al. 2012, McMahon et al. 2014). Enhancing our understanding of seagrass dispersal enables a greater ability to predict and assist their capacity to recover after loss or disturbance (O'Brien et al. 2018).

Terrestrial plant dispersal processes are well studied compared to aquatic plants (Cook 1990), and dispersal pathways for most terrestrial angiosperms are well established. Even complex symbiotic relationships are well understood to the point where information is available to assist in the appropriate conservation and management to preserve these connections and individual species (Fleming and Holland 1998, Webber and Woodrow 2004, Youngsteadt et al. 2009). In the realm of marine plants, especially seagrass, this information is lacking in comparison. There is more research on dispersal processes and establishment success for temperate seagrasses than tropical species (McMahon et al. 2014). However, overall a huge gap remains world-wide for biotic dispersal of seagrass (Kendrick et al. 2017). Theories on the possibility for long-distance biotic dispersal have been discussed for some time (Orth et al. 2006b, Sumoski and Orth 2012, McMahon et al. 2014), but the research to establish biotic pathways has been difficult because of the reduced population of some of the suspected dispersers (marine mega-herbivores: dugong, manatee and green sea turtle) (Seminoff 2004, Bjork et al. 2008, Deutsch et al. 2008, Marmontel et al. 2016, Marsh and Sobtzick 2019, Diagne 2015). Field work is time consuming and requires knowledge of both seagrass plant physiology and the behaviours and movements of the animal dispersers. With both seagrass meadows and the dispersers under threat in recent years these studies are a priority.

Biotic Dispersal

Seed Dispersal

The majority of seagrass seeds (11 of the 13 genera) have limited abiotic long-distance dispersal potential as they are negatively buoyant (Kendrick et al. 2012, McMahon et al. 2014). Abiotic seed dispersal is within the 10s of meters range, and even with partial assistance from small biotic dispersers is only within a meadow (Orth et al. 2006b, Sumoski and Orth 2012, McMahon et al. 2014). Previously known occurrences of long-distance dispersal of seeds have been through abiotic dispersal of rafting seagrass shoots with attached fruiting bodies

(Harwell and Orth 2002a, Källström et al. 2008, Ruiz-Montoya et al. 2012). Confirmation of viable seagrass seeds found in marine mega-herbivore faeces is evidence that seagrass seeds dispersed by herbivores play a significant role in long-distance connectivity and re-establishment (dispersal among meadows not just within); information that was not previously known.

Dispersal of seagrass seeds by marine mega-herbivores is likely to be targeted. Dugongs and green sea turtles will more likely search for food in an area where seagrass is capable of growing. As a result, targeted dispersal has a higher probability of delivering seagrass seeds to a location compatible with growth compared with passive abiotic dispersal. Increasing the number of seeds dispersed to a suitable settlement location increases the potential for successful germination and seedling establishment. Abiotic dispersal is by tidal flows, wind speeds, currents and weather, therefore delivery to a suitable location is not guaranteed (Bonte et al. 2012, Baguette et al. 2013). Non-targeted (abiotic) dispersal would have a reduced probability of successful settlement compared with targeted biotic dispersal.

Biotic dispersal of seagrass seeds by marine mega-herbivores increases connectivity among seagrass meadows. This connectivity would most likely be within meadows of relatively close proximity (<100 km), due to the movement behaviour of marine mega-herbivores. In my thesis, we estimate a potential for a maximum dispersal of viable seagrass seeds of up to 650 kilometres for green sea turtles and up to 230 kilometres for dugongs. However, many sea turtles do not swim these distances regularly and stay within a defined home range. The distance travelled is likely to be far less; an average of 75 km^2 (Cleguer et al. 2016, Limpus et al. 2018). Increased connectivity among seagrass meadows by the feeding behaviour of marine mega-herbivores increases seagrass resilience, as targeted dispersal of viable seagrass seeds will increase the chance of meadow recovery after disturbance.

Germination

My research found viable seeds of *Zostera muelleri* dispersed by marine mega-herbivores have a far greater maximum germination percentage (over the short to medium term; 2 months) and germinate more rapidly, compared to non-consumed seeds dropped by a plant. Rapid germination and larger numbers allow for the possibility of a quick establishment of multiple seedlings. Seagrass seedling survival is increased by the presence of other seagrass plants, due to the reduction in sediment disturbance and decreased wave impact, and a reduction in direct herbivory impact by meso-grazers (Balestri et al. 1998, Rivers et al. 2011). Therefore, the probability of seedling survival from marine mega-herbivore delivered seeds is greater due to the greater likelihood of recruitment to a suitable habitat and enhancement of the number of seeds germinating.

Seeds excreted by marine mega-herbivores play less of a role in the establishment of seed banks compared to seeds dropped by the plant. Seeds which are capable of remaining dormant in the sediment for a prolonged period of time (e.g. *Zostera spp.*, *Halophila spp.*, *Cymodocea spp*. etc) can provide a 'back-up' supply of readily available seeds to re-establish the meadow if a disturbance causes a wide scale loss (Orth et al. 2000). My research emphasised this capability for *Z. muelleri* seeds taken from the plant, with greater than 90% viability of un-germinated seeds after 60 days. However, seeds excreted by marine mega-herbivores only had a quarter of un-germinated seeds viable after 60 days. Therefore, seeds dispersed by marine mega-herbivores will be contributing less to the seed bank, than those that fall directly from seagrass plants. Seed banks are considered to be important for the resilience for some seagrass meadows (Orth et al. 2000, Johnson et al. 2020). Endozoochory provides an additional and more immediate pathway for a meadow to be reseeded and recover and/ or to add to a meadow's genetic diversity. Endozoochory itself and the influence on the timing and success of germination are major steps forward in our understanding of meadow connectivity and tropical seagrass ecology.

Abiotic Dispersal

Fragment Frequency

For abiotic dispersal to be successful, a large number of propagules needs to be produced. This ensures the chance of at least one propagule surviving the dispersal journey and ending in an appropriate location for growth (Bonte et al. 2012, Baguette et al. 2013). Quantifying that there are large numbers of viable fragments being produced daily, year-round, confirms this mode of abiotic dispersal by vegetative fragments is valid for at least two tropical seagrass species in the GBRWHA. The large numbers suggest that the chance of long-distance dispersal via abiotic modes is possible as even loss by herbivory, decay and removal from the system (loss of buoyancy) should still enable a small probability of at least one propagule surviving and settling at an appropriate location (Smith et al. 2018).

The movement of at least one viable vegetative propagule over a large distance, once every few decades or centuries, is enough to maintain genetic connectivity (Bonte et al. 2012, Baguette et al. 2013). The close genetic relationships between seagrass meadows along the GBRWHA coastline for most species would indicate that connectivity is high and the exchange of genetic material between populations is common, however the direction of this connectivity has not been clear (Waycott et al. 2005, McMahon et al. 2014, Kendrick et al. 2017). By measuring the number and variability of fragments being dispersed, it is possible to make direction specific dispersal models more accurate. If we are able to produce more accurate dispersal and connectivity models, it is possible to include the direction of dispersal, and allow a better understanding of the patterns of genetic connectivity and source-sink dynamics among seagrass meadows (Kendrick et al. 2017).

Implications for Management and Conservation

Intensive seagrass research in the GBRWHA started approximately 35 years ago. The research evolved from mapping the presence of meadows and the species within them (Lee Long et al. 1993, Lee Long et al. 1996), to long-term monitoring programs (e.g. McKenna et al. 2020) and to research directed towards informing the understanding of the GBRWHA ecosystems, how they interact, and the implications for management. Recent research has predicted where meadows are likely to be established (Grech and Coles 2010), determined which meadows are at risk of decline (Grech et al. 2011), quantified impacts of foraging from meso-grazers to mega-grazers (Scott et al. 2018, Hoffmann et al. 2020, Scott et al. 2020), assessed carbon sequestration potential (York et al. 2018b), and identified different seagrass communities and determining their connectivity (Carter et al. 2016, Grech et al. 2016, Grech et al. 2018).

On-going research on meadow connectivity is vital for conservation of seagrasses in the GBRWHA. Connectivity influences (1) the recovery potential of individual meadows and patches; (2) the relative importance of meadows and patches to the replenishment (and therefore recruitment and recovery) of other areas; and (3) the location of meadows that are important stepping stones, connecting various different parts of the habitat networks. It is likely that within certain spatial scale some meadows will have a greater importance to connectivity than others due to physical constraints (Grech et al. 2018), as well as feeding and movement parameters of herbivores. Current seagrass models for dispersal and connectivity within the GBRWHA are based only on abiotic dispersal pathways (Grech et al. 2016, Grech et al. 2018). These models indicate that most seagrass meadows lost at spatial scales of up to 100 – 200 kilometres of coastline could recover without intervention due to the level of connectivity via abiotic dispersal of seagrass propagules (Grech et al. 2018). However, these models also indicate that large-scale loss greater than 400 kilometres may be beyond the scope of short-term recovery based on abiotic dispersal alone. Such losses have been experienced previously due to tropical cyclones, such as TC Yasi and TC Hamish (Grech et al. 2018). Large-scale losses will most likely only recover via 'stepping stones', as meadows expand in from the edges of the loss via abiotic processes. This can delay the time it takes for full recovery (Grech et al. 2018). Losses that extend over prolonged periods place stress on many species which rely on seagrass habitats. My thesis provides information to enhance seagrass connectivity models within the GBRWHA and other tropical regions. It emphasises that the seagrass meadows in the GBRWHA are likely to be well connected by movement of propagules at scales of up to 650 kilometres both by the biotic and abiotic processes outlined.

There are recent management initiatives in the GBRWHA that propose restoration as a primary means of maintaining ecosystems particularly for coral but also for seagrass meadows (Burrows et al. 2019, Tan et al. 2020). My research suggests that the addition of biotic dispersal should not change the model outcome for large-scale loss events, as the effects from biotic dispersal is limited at greater than 400 kilometres. However, the addition of biotic dispersal should assist in recovery, provided the scale of the loss is less than 400 kilometres. Propagules are available in large numbers at this scale for recovery from both biotic and abiotic processes. Time to recover is site dependant, and is most likely to follow the recovery of shoreline topography, light availability and the return of suitable bottom sediments (van Katwijk et al. 2009, McKenna et al. 2015). Without the recovery of this 'landing zone', restoration intervention and the availability of viable propagules from any process will not result in a viable meadow.

My research shows that the dispersal and connectivity of tropical seagrass meadows in the GBRWHA is more complicated than previously known, and that there are potential overlaps in the mechanisms of connectivity and connectivity pathways. Some meadows found previously to have low levels of connectivity (e.g. Upstart Bay (Grech et al. 2018)) may have connectivity via the process of endozoochory. The addition of long-distance dispersal through biotic means suggests that seagrass meadows may be more resilient to loss than previously thought. However, the dispersal potential from marine mega-herbivores is within the same scale as abiotic pathways, and large-scale losses greater than circa 400 kilometres will still have potentially disastrous outcomes for recovery times.

My thesis enhances the understanding of how marine mega-herbivore (dugongs and green sea turtles) and seagrass meadows are ecologically linked in a dispersal, loss, recovery, and resiliency cycle. To ensure the successful conservation and management of one, it is beneficial to conserve and protect the other. Without marine mega-herbivores there would be less connectivity among meadows, greatly reducing the resilience of seagrass meadows to external impacts. Focusing on protecting the herbivores and increasing their numbers where they have been depleted may cascade down to improving seagrass meadow connectivity and resilience.

Research Opportunities

My thesis research is part of an initiative to investigate how seagrass meadows interact through abiotic and biotic means (Coles et al. 2015, Grech et al. 2016, Grech et al. 2018). This initiative was triggered by major losses of seagrass during previous tropical cyclones and floods, and the need to understand the process by which natural recovery occurs and at what scale (Coles et al. 2015). These research initiatives also provided guidance as to when and at what scale a loss may require an intervention such as physical restoration by replanting (Grech et al. 2018). The data chapters within my thesis have improved our knowledge of tropical seagrass species dispersal and connectivity. The research undertaken for my thesis fills some of the gaps in knowledge that form the basis for seagrass dispersal and connectivity models (Grech et al. 2016, Grech et al. 2018). I outline below areas where more research would enhance these models and improve our advice to management.

Seagrass Seed Dispersal

Species not included in this study

There are at least 12 tropical seagrass species in the GBRWHA (Coles et al. 2002, Coles et al. 2015). Seven seagrass species not included in the present research for logistic reasons remain to be studied. For each of these species, it is necessary to quantify if green sea turtles and dugongs provide biotic dispersal of viable seeds. The common traits of the five species known to be dispersed by marine mega-herbivores is a hard seed coat and seed dormancy (Waycott et al. 2004, Tol et al. 2017). Based on this knowledge, even though they were not found during my study, *Cymodocea rotundata* and *Syringodium isoetifolium* are also likely candidates for viable biotic dispersal. It is less likely that the seeds of *Thalassia hemprichii*, *Enhalus acoroides* and *Thalassodendron ciliatum* are biotically dispersed, due to the plants reproductive strategy, or the soft fleshy seed coat and lack of dormancy (Waycott et al. 2004) making surviving the passage through a mega-herbivore gut unlikely.

Seed Taxonomy

Three species in the GBRWHA were included in the *Halophila spp*. group found in Chapter two *(H. ovalis*, *H. spinulosa* and *H. tricostata*). Because of the visual similarity of the seeds, and the need to stain each seed found for viability, I was not able to identify these seeds to species level. To complete our understanding of the role marine mega-herbivores play in biotic dispersal, either genetic analysis or scanning electron micrography (SEM) would be required to enable correct identification for these *Halophila* species (Kuo et al. 1993, Bujang et al. 2006). All three species are likely candidates for dispersal by marine mega-herbivores. Time constraints of a thesis did not allow this to be pursued.

Seagrass Seed Germination

Species not included in this study

Germination experiments for other seagrass species that are dispersed by marine mega-herbivores would be needed to fully understand the role these animals play in seed dispersal. There is little information on germination for many of the tropical seagrass species found in the GBRWHA, so further research would be advantageous to our general knowledge of tropical seagrasses. In Chapter two, I noted that some germination of *H. decipiens* and the unknown *Halophila spp*. occurred. I predict a similar germination outcome for these two species as was seen for *Z. muelleri*.

Seedling Survival

Germination is only the beginning of the life cycle of a seagrass plant, and is not an indication of seedling survival (Kendrick et al. 2017). For dispersal by marine mega-herbivores to be important for seagrass connectivity, it is essential to verify seedling survivorship and if it is different to germinated seeds dispersed by dugongs and green sea turtles compared to germinated seeds dropped by the plant.

Nutrient Input

Seeds ingested and excreted by marine mega-herbivores are in a pocket of nutrient rich faeces, in comparison to the bare sediment. The majority of marine mega-herbivore faeces is partially digested seagrass leaves and rhizomes. It is likely that the faecal mass provides a readily available supply of nitrogen and phosphorous for the germinated seed, potentially increasing seedling survival. This is an area of research worth following.

Other marine mega-herbivores

Manatees are similar to dugongs and have the potential to disperse seagrass seeds. Manatees occupy marine, brackish and freshwater habitats and may aid in biotic dispersal for a suite of marine, brackish and aquatic vegetation. There are three species of manatee (four including sub-species) found in the northern hemisphere (Domning 2001, Marsh et al. 2011), and just like dugongs they are listed as Vulnerable by the IUCN with decreasing populations (Deutsch et al. 2008, Marmontel et al. 2016, Diagne 2015). These animals may provide a vital role in aquatic biotic dispersal. A better understanding of how these animals interact with their food will assist with conservation and management of the manatees and the seagrasses and other aquatic vegetation they rely on.

Fragment Dispersal

Viable Fragment Numbers

My thesis research was able to quantify the number of fragments being dispersed for two of the tropical seagrass species present in coastal seagrass meadows in the central GBRWHA. It would be useful to research fragment availability for the remaining seagrass species and expand the study to multiple meadows throughout the GBRWHA.

Other seagrass foragers

There are other bioturbators who could create fragments when foraging; such as waterfowl, sea turtles, stingrays and fish. Studying the role these animals play in the creation of viable fragments for dispersal would assist in understanding/ quantifying biotic dispersal of seagrass by fragments.

Fragment Float Time and Survival

Calculating the quantity of viable fragments for seagrass species is only the beginning in providing the input for modelling dispersal and connectivity for seagrass. The next step is to determine the time each species is capable of floating while still viable. Float time will determine maximum dispersal capability (Grech et al. 2016, Kendrick et al. 2017). Loss by externalities such as herbivory, epiphyte growth and loss of buoyancy during float time also needs to be quantified for models to be accurate (Thiel and Gutow 2004).

Wind Drag Effect

Current seagrass dispersal models use a variety of wind drag coefficients (Grech et al. 2016). Calculating the effect wind speed has on the movement of seagrass fragments in the water will increase the accuracy of dispersal and connectivity models. This data would be desirable for all tropical seagrass species.

Fragment Settlement

To fully understand seagrass fragment dispersal, we need to quantify the proportion of fragments which settle in an appropriate location for successful growth, and to estimate the proportion of those fragments which successfully establish into a plant (Kendrick et al. 2012, McMahon et al. 2014). Settlement success may vary depending on the growth trait of the species; i.e. colonising (fast growing would most likely have a quick settlement time and the potential for high proportions of survival) compared to opportunistic and persistent (slower growing and more likely have a longer settlement time and therefore have a lower proportion of survival).

Conclusion

My research has advanced our knowledge of seagrass ecology and provided vital information to guide management agencies in managing seagrass meadows by:

- Confirming the existence of biotic seed dispersal by marine mega-herbivores of at least five different tropical seagrass species, and that these seeds have the ability to be dispersed in large numbers and among meadows;
- Creating effective PCR primer pairs to identify species origin of marine mega-herbivore faeces (dugong and green sea turtle);
- Confirming that *Zostera muelleri* seeds ingested and excreted by marine mega-herbivores germinate faster and have a high germination percentage than seeds released by the plant;
- Confirming that there are large numbers of viable seagrass fragments being dispersed from tropical coastal seagrass meadows for at least two tropical seagrass species; and
- Highlighting that biotic dispersal is an important dispersal pathway for tropical seagrasses, and this measure needs to be included in future dispersal and connectivity models to ensure accuracy and to enable better management of these coastal habitats.

References

Abe, M., A. Kurashima, and M. Maegawa. 2008. Temperature requirements for seed germination and seedling growth of *Zostera marina* from central Japan. Fisheries Science **74**:589-593.

Ambo-Rappe, R., Y. La Nafie, S. Limbong, N. Asriani, N. Handayani, and E. Lisdayanti. 2019. Restoration of seagrass Enhalus acoroides using a combination of generative and vegetative techniques. Biodiversitas Journal of Biological Diversity **20**:3358-3363.

Aragones, L. 1996. Dugongs and green turtles: grazers in the tropical seagrass ecosystem. James Cook University, Townsville.

Aragones, L., and H. Marsh. 2000. Impact of dugong grazing and turtle cropping on tropical seagrass communities. Pacific Conservation Biology **5**:277-288.

Australian Institute of Marine Science. 2019. Temperature Loggers, Data Centre <http://maps.aims.gov.au/index.html?intro=false&z=4&ll=142.91883,-17.51872&l0=aims_aims:AIMS%20-%20Temperature%20Loggers,ea_World_NE2-coast-cities-reefs_Baselayer>.

Baguette, M., S. Blanchet, D. Legrand, V. Stevens, and C. Turlure. 2013. Individual dispersal, landscape connectivity and ecological networks. Biological Reviews **88**:310-326.

Bakker, E., K. Wood, J. Pagès, G. Veen, M. Christianen, L. Santamaría, B. Nolet, and S. Hilt. 2016. Herbivory on freshwater and marine macrophytes: a review and perspective. Aquatic Botany **135**:18-36.

Balestri, E., L. Piazzib, and F. Cinelli. 1998. Survival and growth of transplanted and natural seedlings of *Posidonia oceanica* (L.) Delile in a damaged coastal area. Journal of Experimental Marine Biology and Ecology **228**:209-225.

Barrett, S., C. Eckert, and B. Husband. 1993. Evolutionary processes in aquatic plant populations. Aquatic Botany **44**:105-145.

Barton, K. 2019. MuMIn: Multi-model inference. R Package Version 0.12.2/r18.

Bascompte, J. 2019. Mutualism and biodiversity. Current Biology **29**:R467-R470.

Bascompte, J., and P. Jordano. 2007. Plant-animal mutualistic networks: the architecture of biodiversity. Annual Review of Ecology, Evolution, and Systematics **38**:567-593.

Baskin, C., and J. Baskin. 2014. Seeds: ecology, biogeography, and, evolution of dormancy and germination. Vol 2. Academic Press, University of Kentucky.

Baskin, J., and C. Baskin. 2003. Classification, biogeography, and phylogenetic relationships of seed dormancy. Pages 518-544 *in* RD Smith, JB Dickie, SH Linington, HW Pritchard, and R. Probert, editors. Seed conservation: turning science into practice. Royal Botanic Gardens, Kew.

Bjork, M., F. Short, E. Mcleod, and S. Beer. 2008. Managing seagrasses for resilience to climate change. IUCN, Gland, Switzerland.

Bjorndal, K. A. 1980. Nutrition and grazing behavior of the green turtle *Chelonia mydas*. Marine Biology **56**:147-154.

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

Bonte, D., H. Van Dyck, J. Bullock, A. Coulon, M. Delgado, M. Gibbs, V. Lehouck, E. Matthysen, K. Mustin, M. Saastamoinen, N. Schtickzelle, V. Stevens, S. Vandewoestijne, M. Baguette, K. Barton, T. Benton, A. Chaput-Bardy, J. Clobert, C. Dytham, T. Hovestadt, C. Meier, S. Palmer, C. Turlure, and J. Travis. 2012. Costs of dispersal. Biological Reviews **87**:290-312.

Brand, S., J. Lanyon, and C. Limpus. 1999. Digesta composition and retention times in wild immature green turtles, *Chelonia mydas*: a preliminary investigation. Marine and Freshwater Research **50**:145-147.

Brenchley, J., and R. Probert. 1998. Seed germination responses to some environmental factors in the seagrass *Zostera capricorni* from eastern Australia. Aquatic Botany **62**:177-188.

Brodie, H. 1955. Springboard plant dispersal mechanisms operated by rain. Canadian Journal of Botany **33**:156-167.

Bryant, C., J. Davies, T. Sankey, and M. Rasheed. 2016. Long Term Seagrass Monitoring in Port Curtis: Quarterly Permanent Transect Monitoring Progress Report 2009 to 2015. Centre for Tropical Water & Aquatic Ecosystem Research (TropWATER), James Cook University, Cairns.

Bujang, J., M. Zakaria, S. Awing, S. Nojima, and H. Ogawa. 2006. Seed coat sculpturing in Halophila. Coastal Marine Science **30**:240-242.

Burrows, D., J. Purandare, L. Bay, N. Cook, D. Koopman, S. Long, P. Lundgren, D. Mead, S. Morris, M. Newlands, C. Roth, D. Wachenfeld, A. Smith, and I. McLeod. 2019. Symposium report: Great Barrier Reef restoration symposium, 2018. Ecological Management and Restoration **20**:175-178.

Campbell, L. 2003. 12 Contemporary Culture, Use, and Conservation of Sea Turtles. Pages 307-338 *in* P. L. Lutz and J. A. Musick, editors. The Biology of Sea Turtles. CRC Press, Washington, D.C. United States of America.

Campbell, S., and L. McKenzie. 2004. Flood related loss and recovery of intertidal seagrass meadows in southern Queensland, Australia. Estuarine, Coastal and Shelf Science **60**:477-490.

Carruthers, T., W. Dennison, B. Longstaff, M. Waycott, E. Abal, L. McKenzie, and W. Lee Long. 2002. Seagrass habitats of northeast Australia: models of key processes and controls. Bulletin of Marine Science **71**:1153-1169.

Carter, A., S. McKenna, M. Rasheed, L. McKenzie, and R. Coles. 2016. Seagrass mapping synthesis: A resource for coastal management in the Great Barrier Reef World Heritage Area. Report to the National Environmental Science Programme. Reef and Rainforest Research Centre Limited, Cairns Australia.

Chaloupka, M., and C. Limpus. 2001. Trends in the abundance of sea turtles resident in southern Great Barrier Reef waters. Biological Conservation **102**:235-249.

Chapman, C. 1989. Primate seed dispersal: the fate of dispersed seeds. Biotropica **21**:148-154.

Charalambidou, I., L. Santamaria, and O. Langevoord. 2003. Effect of ingestion by five avian dispersers on the retention time, retrieval and germination of *Ruppia maritima* seeds. Functional Ecology **17**:747-753.

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

Cheng, I.-J. 2000. Post-nesting migrations of green turtles (*Chelonia mydas*) at Wan-an Island, Penghu Archipelago, Taiwan. Marine Biology **137**:747-754.

Christianen, M., L. Govers, T. Bouma, W. Kiswara, J. Roelofs, L. Lamers, and M. van Katwijk. 2012. Marine megaherbivore grazing may increase seagrass tolerance to high nutrient loads. Journal of Ecology **100**:546-560.

Churchill, A. 1983. Field studies on seed germination and seedling development in *Zostera marina* L. Aquatic Botany **16**:21-29.

Clausen, P., B. Nolet, A. Fox, and M. Klaassen. 2002. Long-distance endozoochorous dispersal of submerged macrophyte seeds by migratory waterbirds in northern Europe—a critical review of possibilities and limitations. Acta oecologica **23**:191-203.

Cleguer, C., S. Preston, R. Hagihara, T. Shimada, M. Hamann, S. Simpson, F. Loban, G. Bowie, R. Fujii, and H. Marsh. 2016. Working with the community to understand use of space by dugongs and green turtles in Torres Strait: A project in collaboration with the Mura Badulgal RegisteredNative Title Bodies Corporate. Report to the National Environment Science Program, Reef and Rainforest Research Centre Limited, Cairns.

Clobert, J., E. Danchin, A. Dhondt, and J. Nichols. 2001. Dispersal. Oxford University Press, Oxford.

Coles, R., W. Lee Long, R. Watson, and K. Derbyshire. 1993. Distribution of seagrasses, and their fish and penaeid prawn communities, in Cairns Harbour, a tropical estuary, Northern Queensland, Australia. Australian Journal of Marine and Freshwater Research **44**:193-210.

Coles, R., L. McKenzie, and S. Campbell. 2002. The seagrasses of eastern Australia. Pages 131-147 *in* E. Green and F. Short, editors. The World Atlas of Seagrasses: present status and future conservation. University of California Press.

Coles, R., M. Rasheed, L. McKenzie, A. Grech, P. York, M. Sheaves, S. McKenna, and C. Bryant. 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.

Conacher, C., I. Poiner, J. Butler, S. Pun, and D. Tree. 1994a. Germination, storage and viability testing of seeds of *Zostera capricorni* Aschers. from a tropical bay in Australia. Aquatic Botany **49**:47-58.

Conacher, C., I. Poiner, and M. O'donohue. 1994b. Morphology, flowering and seed production of *Zostera capricorni* Aschers. in subtropical Australia. Aquatic Botany **49**:33-46.

Conservation & Biodiversity Operations Branch, D. o. E. a. S. 2018. Marine Turtle Conservation Strategy-Queensland, Brisbane. Department of Environment and Science, Queensland Government.

Cook, C. 1990. Aquatic plant book. SPB Academic Publishing, The Netherlands.

Cook, R. 1985. Growth and development in clonal plant populations. Pages 259-296 *in* J. Jackson, L. Buss, and R. Cook, editors. Population biology and evolution of clonal organisms. Yale University Press, New Haven, CT.

Cope, R., P. Pollett, J. Lanyon, and J. Seddon. 2015. Indirect detection of genetic dispersal (movement and breeding events) through pedigree analysis of dugong populations in southern Queensland, Australia. Biological Conservation **181**:91-101.

Costa-Pereira, R., S. Correa, and M. Galetti. 2018. Fishing-down within populations harms seed dispersal mutualism. Biotropica **50**:319-325.

Costa, J., J. Ramos, L. da Silva, S. Timoteo, P. Araújo, M. Felgueiras, A. Rosa, C. Matos, P. Encarnação, and P. Tenreiro. 2014. Endozoochory largely outweighs epizoochory in migrating passerines. Journal of Avian Biology **45**:59-64.

Costanza, R., R. d'Arge, R. De Groot, S. Farber, M. Grasso, B. Hannon, K. Limburg, S. Naeem, R. O'neill, and J. Paruelo. 1997. The value of the world's ecosystem services and natural capital. Nature **387**:253-260.

Costanza, R., R. De Groot, P. Sutton, S. Van der Ploeg, S. Anderson, I. Kubiszewski, S. Farber, and R. Turner. 2014. Changes in the global value of ecosystem services. Global environmental change **26**:152-158.

Cowen, R., C. Paris, and A. Srinivasan. 2006. Scaling of connectivity in marine populations. Science **311**:522-527.

Cowen, R., and S. Sponaugle. 2009. Larval dispersal and marine population connectivity. Annual Review of Marine Science **1**:443-466.

Cox, P. 1993. Water-pollinated plants. Scientific American **269**:50-74.

Creel, L. 2003. Ripple effects: population and coastal regions. Population Reference Bureau, Washington, DC.

Cumming, E., J. Jarvis, C. Sherman, P. York, and T. Smith. 2017. Seed germination in a southern Australian temperate seagrass. PeerJ **5**:e3114.

Delefosse, M., and E. Kristensen. 2012. Burial of *Zostera marina* seeds in sediment inhabited by three polychaetes: laboratory and field studies. Journal of Sea Research **71**:41-49.

Den Hartog, C. 1970. The sea-grasses of the world. North-Holland Pub. Co.

Deutsch, C. J., C. Self-Sullivan, and A. Mignucci-Giannoni. 2008. *Trichechus manatus*. The IUCN Red List of Threatened Species 2008: e.T22103A9356917. https://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T22103A9356917.en. .

Devlin, M., J. Brodie, A. Wenger, E. da Silva, J. Alvarez- Romero, J. Waterhouse, and L. McKenzie. 2012. Extreme weather conditions in the Great Barrier Reef: Drivers of change? Page 5 *in* Proceedings of the 12th International Coral Reef Symposium, Cairns, Australia.

Diagne, L. 2015. *Trichechus senegalensis* (errata version published in 2016). The IUCN Red List of Threatened Species 2015: e.T22104A97168578. https://dx.doi.org/10.2305/IUCN.UK.2015-4.RLTS.T22104A81904980.en. .

Domning, D. 2001. Sirenians, seagrasses, and Cenozoic ecological change in the Caribbean. Palaeogeography, Palaeoclimatology, Palaeoecology **166**:27-50.

Donelan, M., M. Curcic, S. S. Chen, and A. Magnusson. 2012. Modeling waves and wind stress. Journal of Geophysical Research: Oceans **117**.

Duarte, C. 2002. The future of seagrass meadows. Environmental conservation **29**:192-206.

Erftemeijer, P., J. van Beek, C. Ochieng, Z. Jager, and H. Los. 2008. Eelgrass seed dispersal via floating generative shoots in the Dutch Wadden Sea: a model approach. Marine Ecology Progress Series **358**:115-124.

Eriksson, O. 1992. Evolution of seed dispersal and recruitment in clonal plants. Oikos **63**:439-448.

Eriksson, O., and B. Bremer. 1992. Pollination systems, dispersal modes, life forms, and diversification rates in angiosperm families. Evolution **46**:258-266.

Ewanchuk, P., and S. Williams. 1996. Survival and re-establishment of vegetative fragments of eelgrass (*Zostera marina*). Canadian Journal of Botany **74**:1584-1590.

Faegri, K., and L. Van Der Pijl. 1980. The Principles of Pollination Ecology. Pergamon Press, Oxford, England.

Fenner, M., and K. Thompson. 2005. The ecology of seeds. Cambridge University Press, Cambridge, UK.

Fernández-Torquemada, Y., and J. Sánchez-Lizaso. 2013. Effects of salinity on seed germination and early seedling growth of the Mediterranean seagrass *Posidonia oceanica* (L.) Delile. Estuarine, Coastal and Shelf Science **119**:64-70.

Figuerola, J., and A. Green. 2002. Dispersal of aquatic organisms by waterbirds: a review of past research and priorities for future studies. Freshwater biology **47**:483-494.

Figuerola, J., A. Green, and L. Santamaría. 2003. Passive internal transport of aquatic organisms by waterfowl in Doñana, south-west Spain. Global Ecology and Biogeography **12**:427-436.

Fleming, T., and J. Holland. 1998. The evolution of obligate pollination mutualisms: senita cactus and senita moth. Oecologia **114**:368-375.

Fourqurean, J., C. Duarte, H. Kennedy, N. Marbà, M. Holmer, M. Mateo, E. Apostolaki, G. Kendrick, D. Krause-Jensen, and K. McGlathery. 2012. Seagrass ecosystems as a globally significant carbon stock. Nature geoscience **5**:505-509.

Fourqurean, J., S. Manuel, K. Coates, W. Kenworthy, and S. Smith. 2010. Effects of excluding sea turtle herbivores from a seagrass bed: overgrazing may have led to loss of seagrass meadows in Bermuda. Marine Ecology Progress Series **419**:223-232.

Fuentes, M., C. Limpus, M. Hamann, and J. Dawson. 2010. Potential impacts of projected sea-level rise on sea turtle rookeries. Aquatic Conservation: marine and freshwater ecosystems **20**:132-139.

Garnett, S., I. Price, and F. Scott. 1985. The diet of the green turtle, *Chelonia mydas* (L.), in Torres Strait. Wildlife Research **12**:103-112.

Gerrard, C. 1999. Dugong-watching tourism and encounter response of the dugong (*Dugong dugon*) in Shark Bay, Western Australia. University of Calgary.

Govers, L., W. Man, J. Meffert, T. Bouma, P. van Rijswick, J. Heusinkveld, R. Orth, M. van Katwijk, and T. van der Heide. 2016. Marine *Phytophthora* species can hamper conservation and restoration of vegetated coastal ecosystems. Proc. R. Soc. B **283**.

Govers, L., E. Van Der Zee, J. Meffert, P. Van Rijswick, W. Man, J. Heusinkveld, and T. Van Der Heide. 2017. Copper treatment during storage reduces *Phytophthora* and *Halophytophthora* infection of *Zostera marina* seeds used for restoration. Scientific Reports **7**.

Grace, J. 1993. The adaptive significance of clonal reproduction in angiosperms: an aquatic perspective. Aquatic Botany **44**:159-180.

Grech, A., and R. Coles. 2010. An ecosystem-scale predictive model of coastal seagrass distribution. Aquatic Conservation: marine and freshwater ecosystems **20**:437-444.

Grech, A., R. Coles, and H. Marsh. 2011. A broad-scale assessment of the risk to coastal seagrasses from cumulative threats. Marine Policy **35**:560-567.

Grech, A., E. Hanert, L. McKenzie, M. Rasheed, C. Thomas, S. Tol, M. Wang, M. Waycott, J. Wolter, and R. Coles. 2018. Predicting the cumulative effect of multiple disturbances on seagrass connectivity. Global change biology:1-12.

Grech, A., J. Wolter, R. Coles, L. McKenzie, M. Rasheed, C. Thomas, M. Waycott, and E. Hanert. 2016. Spatial patterns of seagrass dispersal and settlement. Diversity and Distributions **22**:1150-1162.

Green, E., and F. Short. 2003. World atlas of seagrasses. University of California Press.

Grice, A. 1994. Seed germination and seedling development of *Zostera capricorni* Ashers. CSIRO, Division of Fisheries Vaction Scholarship in Marine Botany.

Hadjichristophorou, M., and D. Grove. 1983. A study of appetite, digestion and growth in juvenile green turtle (*Chelonia mydas* L.) fed on artificial diets. Aquaculture **30**:191-201.

Hall, L., M. Hanisak, and R. Virnstein. 2006. Fragments of the seagrasses *Halodule wrightii* and *Halophila johnsonii* as potential recruits in Indian River Lagoon, Florida. Marine Ecology Progress Series **310**:109-117.

Hall, T. 1999. BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. Nucleic Acids Symposium Series **41**:95-98.

Hanski, I. 1999. Metapopulation ecology. Oxford University Press, New York.

Harper, J. 1977. Population Biology of Plants. Academic Press, The University of California.

Harrison, P. 1991. Mechanisms of seed dormancy in an annual population of *Zostera marina* (eelgrass) from the Netherlands. Canadian Journal of Botany **69**:1972-1976.

Harwell, M., and R. Orth. 2002a. Long-distance dispersal potential in a marine macrophyte. Ecology **83**:3319-3330.

Harwell, M., and R. Orth. 2002b. Seed bank patterns in Chesapeake Bay eelgrass (*Zostera marina* L.): a bay-wide perspective. Estuaries **25**:1196-1204.

Hearne, E., R. Johnson, A. Gulick, A. Candelmo, A. Bolten, and K. Bjorndal. 2019. Effects of green turtle grazing on seagrass and macroalgae diversity vary spatially among seagrass meadows. Aquatic Botany **152**:10-15.

Heath, M., and S. McGinnis. 1980. Body temperature and heat transfer in the green sea turtle, *Chelonia mydas*. Copeia:767-773.

Heck Jr, K., T. Carruthers, C. Duarte, A. Hughes, G. Kendrick, R. Orth, and S. Williams. 2008. Trophic transfers from seagrass meadows subsidize diverse marine and terrestrial consumers. Ecosystems **11**:1198-1210.

Heck Jr, K., G. Hays, and R. Orth. 2003. Critical evaluation of the nursery role hypothesis for seagrass meadows. Marine Ecology Progress Series **253**:123-136.

Heck Jr, K., and J. Valentine. 2006. Plant–herbivore interactions in seagrass meadows. Journal of Experimental Marine Biology and Ecology **330**:420-436.

Hemminga, M., and C. Duarte. 2000. Seagrass ecology. Cambridge University Press, Cambridge, United Kingdom.

Herrera, C. 1989. Seed dispersal by animals: a role in angiosperm diversification? American Naturalist **133**:309-322.

Hobbs, J.-P., A. Frisch, J. Hender, and J. Gilligan. 2007. Long-distance oceanic movement of a solitary dugong (*Dugong dugon*) to the Cocos (Keeling) Islands. Aquatic Mammals **33**:175-178.

Hoffmann, L., W. Edwards, P. York, and M. Rasheed. 2020. Richness of Primary Producers and Consumer Abundance Mediate Epiphyte Loads in a Tropical Seagrass System. Diversity **12**:384.

Howe, H., and J. Smallwood. 1982. Ecology of seed dispersal. Annual review of ecology and systematics **13**:201-228.

Hudson, B. 1983. Dugongs: Can the Kiwai survive without them. Pages 20-25 *in* 11th International Congress of Anthropological and Ethno-logical Sciences, Vancouver, Canada, August.

Hughes, A., and J. Stachowicz. 2004. Genetic diversity enhances the resistance of a seagrass ecosystem to disturbance. Proceedings of the National Academy of Sciences **101**:8998-9002.

Hughes, R., and J. Stachowicz. 2011. Seagrass genotypic diversity increases disturbance response via complementarity and dominance. Journal of Ecology **99**:445-453.

Inglis, G. 2000a. Disturbance-related heterogeneity in the seed banks of a marine angiosperm. Journal of Ecology **88**:88-99.

Inglis, G. 2000b. Variation in the recruitment behaviour of seagrass seeds: implications for population dynamics and resource management. Pacific Conservation Biology **5**:251.

IUCN. 2017. IUCN World Heritage Outlook: Great Barrier Reef - 2017 Conservation Outlook Assessment. International Union for Conservation of Nature and Natural Resources Gland, Switzerland.

Jaganathan, G., K. Yule, and B. Liu. 2016. On the evolutionary and ecological value of breaking physical dormancy by endozoochory. Perspectives in Plant Ecology, Evolution and Systematics **22**:11-22.

Jahnke, M., P. Moksnes, J. Olsen, N. Serra Serra, M. Nilsson Jacobi, K. Kuusemäe, H. Corell, and P. Jonsson. 2020. Integrating genetics, biophysical, and demographic insights identifies critical sites for seagrass conservation. Ecological Applications:e02121.

James, R., R. Silva, B. van Tussenbroek, M. Escudero-Castillo, I. Mariño-Tapia, H. Dijkstra, R. Van Westen, J. Pietrzak, A. Candy, and C. Katsman. 2019. Maintaining tropical beaches with seagrass and algae: a promising alternative to engineering solutions. Bioscience **69**:136-142.

Jarvis, J. 2009. Function of seed-bank ecology in mid-Atlantic semi-annual and perennial *Zostera marina* beds. PhD. The College of William & Mary in Virginia, Viginia, United States of America.

Jarvis, J., and K. Moore. 2008. Influence of environmental factors on *Vallisneria americana* seed germination. Aquatic Botany **88**:283-294.

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

Jarvis, J., and K. Moore. 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**:964-978.

Jarvis, J., E. Scott, C. Bryant, and M. Rasheed. 2015. Port Curtis Seagrass Seed Bank Density and Viability Studies - Year 1. James Cook University, Cairns, Report produced for the Ecosystem Research and Monitoring Program Advisory Panel as part of Gladstone Ports Corporation's Ecosystem Research and Monitoring Program. Centre for Tropical Water & Aquatic Ecosystem Research (TropWATER) Publication 15/35.

Jensen, M., I. Bell, C. Limpus, M. Hamann, S. Ambar, T. Whap, C. David, and N. FitzSimmons. 2016. Spatial and temporal genetic variation among size classes of green turtles

(Chelonia mydas) provides information on oceanic dispersal and population dynamics. Marine Ecology Progress Series **543**:241-256.

Johnson, A., E. Shields, G. Kendrick, and R. Orth. 2020. Recovery Dynamics of the Seagrass *Zostera marina* Following Mass Mortalities from Two Extreme Climatic Events. Estuaries and Coasts:1-10.

Jordano, P. 1987. Patterns of Mutualistic Interactions in Pollination and Seed Dispersal: Connectance, Dependence Asymmetries, and Coevolution. The American Naturalist **129**:657-677.

Källström, B., A. Nyqvist, P. Åberg, M. Bodin, and C. André. 2008. Seed rafting as a dispersal strategy for eelgrass (*Zostera marina*). Aquatic Botany **88**:148-153.

Kawasaki, Y. 1993. Effects of some environmental factors on seed germination and seedling growth of Zostera marina L. Pages 28-35 *in* International Workshop on Seagrass Biology; Itominato. 1993 Proceedings, Ocean Research Institute, University of Tokyo.

Kendrick, G., R. Orth, J. Statton, R. Hovey, L. Ruiz Montoya, R. Lowe, S. Krauss, and E. Sinclair. 2017. Demographic and genetic connectivity: the role and consequences of reproduction, dispersal and recruitment in seagrasses. Biological Reviews **92**:921-938.

Kendrick, G., M. Waycott, T. Carruthers, M. Cambridge, R. Hovey, S. Krauss, P. Lavery, D. Les, R. Lowe, and O. i Vidal. 2012. The central role of dispersal in the maintenance and persistence of seagrass populations. Bioscience **62**:56-65.

Kennedy, H., J. Beggins, C. Duarte, J. Fourqurean, M. Holmer, N. Marbà, and J. Middelburg. 2010. Seagrass sediments as a global carbon sink: Isotopic constraints. Global Biogeochemical Cycles **24**.

Kenworthy, W., M. Hall, K. Hammerstrom, M. Merello, and A. Schwartzschild. 2018. Restoration of tropical seagrass beds using wild bird fertilization and sediment regrading. Ecological Engineering **112**:72-81.

Kiester, A., R. Lande, and D. Schemske. 1984. Models of Coevolution and Speciation in Plants and their Pollinators. The American Naturalist **124**:220-243.

Kilminster, K., K. McMahon, M. Waycott, G. Kendrick, P. Scanes, L. McKenzie, K. O'Brien, M. Lyons, A. Ferguson, and P. Maxwell. 2015. Unravelling complexity in seagrass systems for management: Australia as a microcosm. Science of The Total Environment **534**:97-109.

Kinlan, B., and S. Gaines. 2003. Propagule dispersal in marine and terrestrial environments: a community perspective. Ecology **84**:2007-2020.

Klein, A., S. Cunningham, M. Bos, and I. Steffan-Dewenter. 2008. Advances in pollination ecology from tropical plantation crops. Ecology **89**:935-943.

Koch, E., M. Ailstock, D. Booth, D. Shafer, and A. Magoun. 2010. The role of currents and waves in the dispersal of submersed angiosperm seeds and seedlings. Restoration ecology **18**:584-595.

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

Lai, S., L. Loke, M. Hilton, T. Bouma, and P. Todd. 2015. The effects of urbanisation on coastal habitats and the potential for ecological engineering: A Singapore case study. Ocean & Coastal Management **103**:78-85.

Lamb, J., J. Van De Water, D. Bourne, C. Altier, M. Hein, E. Fiorenza, N. Abu, J. Jompa, and C. Harvell. 2017. Seagrass ecosystems reduce exposure to bacterial pathogens of humans, fishes, and invertebrates. Science **355**:731-733.

Lanyon, J., and H. Marsh. 1995. Digesta passage times in the dugong. Australian Journal of Zoology **43**:119-127.

Lanyon, J., H. Sneath, T. Long, and R. Bonde. 2010. Physiological Response of Wild Dugongs (*Dugong dugon*) to Out-of-Water Sampling for Health Assessment. Aquatic Mammals **36**:46-58.

Larkum, A., R. Orth, and C. Duarte. 2006. Seagrasses: Biology, Ecology and Conservation. Springer, Dordrecht, The Netherlands.

Law, R. 1985. Evolution in a mutualistic environment. Pages 145-170 *in* D. Boucher, editor. The biology of mutualism: Ecology and evolution. Oxford University Press, New York.

Lee Long, W., R. Coles, and L. McKenzie. 1996. Deepwater seagrasses in Northeastern Australia: how deep, how meaningful? Pages 25-29 *in* Seagrass Biology: Proceedings of an International Workshop, Rottnest Island, Western Australia.

Lee Long, W., J. Mellors, and R. Coles. 1993. Seagrasses between Cape York and Hervey Bay, Queensland, Australia. Marine and Freshwater Research **44**:19-31.

Lefcheck, J., B. Hughes, A. Johnson, B. Pfirrmann, D. Rasher, A. Smyth, B. Williams, M. Beck, and R. Orth. 2019. Are coastal habitats important nurseries? A meta-analysis. Conservation Letters **12**:e12645.

Lenth, R. 2016. Least-Squares Means: The R Package lsmeans. Journal of Statistical Software **69**:1-33.

Lenth, R. 2020. emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.4.8. https://CRAN.R-project.org/package=emmeans.

Les, D., D. Crawford, R. Kimball, M. Moody, and E. Landolt. 2003. Biogeography of discontinuously distributed hydrophytes: a molecular appraisal of intercontinental disjunctions. International Journal of Plant Sciences **164**:917-932.

Limpus, C., N. FitzSimmons, K. Finlayson, C. Hannon, D. Limpus, D. Pursell, J. Sergeev, and T. Shimada. 2018. Increase the Understanding of the Green Turtle Population in Port Curtis. Brisbane: Department of Environment and Science, Queensland Government. Report produced for the Ecosystem Research and Monitoring Program Advisory Panel as part of Gladstone Ports Corporation Ecosystem Research and Monitoring Program.

Lindén, A., and S. Mäntyniemi. 2011. Using the negative binomial distribution to model overdispersion in ecological count data. Ecology **92**:1414-1421.

Loques, F., G. Caye, and A. Meinesz. 1990. Germination in the marine phanerogam *Zostera noltii* Hornemann at Golfe Juan, French Mediterranean. Aquatic Botany **38**:249-260.

Luschi, P., F. Papi, H. Liew, E. Chan, and F. Bonadonna. 1996. Long-distance migration and homing after displacement in the green turtle (*Chelonia mydas*): a satellite tracking study. Journal of Comparative Physiology A **178**:447-452.

Macreadie, P., M. Baird, S. Trevathan-Tackett, A. Larkum, and P. Ralph. 2014. Quantifying and modelling the carbon sequestration capacity of seagrass meadows–a critical assessment. Marine Pollution Bulletin **83**:430-439.

Marba, N., J. Cebrian, S. Enriquez, and C. Duarte. 1996. Growth patterns of Western Mediterranean seagrasses: species-specific responses to seasonal forcing. Marine Ecology Progress Series **133**:203-215.

Marmontel, M., D. de Souza, and S. Kendall. 2016. *Trichechus inunguis*. The IUCN Red List of Threatened Species 2016: e.T22102A43793736. https://dx.doi.org/10.2305/IUCN.UK.2016-2.RLTS.T22102A43793736.en.

Marsh, H., A. Grech, and K. McMahon. 2018. Dugongs: Seagrass Community Specialists. Pages 629-661 *in* A. W. D. Larkum, G. A. Kendrick, and P. J. Ralph, editors. Seagrasses of Australia: Structure, Ecology and Conservation. Springer International Publishing, Cham.

Marsh, H., A. Hodgson, I. Lawler, A. Grech, and S. Delean. 2007. Condition, status and trends and projected futures of the dugong in the Northern Great Barrier Reef and Torres Strait; including identification and evaluation of the key threats and evaluation of available management options to improve its status. Marine and Tropical Sciences Research Facility Report Series. 1921359056, Reef and Rainforest Research Centre Limited, Cairns, Australia.

Marsh, H., T. O'Shea, and J. Reynolds III. 2011. Ecology and conservation of the sirenia: dugongs and manatees. Cambridge University Press.

Marsh, H., and M. Sobtzick. 2019. *Dugong dugon* (amended version of 2015 assessment). The IUCN Red List of Threatened Species 2019: e.T6909A160756767. https://dx.doi.org/10.2305/IUCN.UK.2015-4.RLTS.T6909A160756767.en. .

McCauley, E., W. Wilson, and A. de Roos. 1996. Dynamics of age-structured predator-prey populations in space: asymmetrical effects of mobility in juvenile and adult predators. Oikos **76**:485-497.

McKenna, S., K. Chartrand, C. Van De Wetering, J. Wells, A. Carter, and M. Rasheed. 2020. Port of Townsville Seagrass Monitoring Program: 2019, Publication 20/16. Centre for Tropical Water & Aquatic Ecosystem Research (TropWATER), James Cook University, Cairns.

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

McKenzie, L., C. Collier, and M. Waycott. 2012. Reef Rescue Marine Monitoring Program-Inshore Seagrass, Annual Report for the sampling period 1st July 2010–31st May 2011. Department of Employment, Economic Development and Innovation (Fisheries Queensland), Cairns, Australia.

McKenzie, L., C. Collier, and M. Waycott. 2014. Reef Rescue Marine Monitoring Program - Inshore seagrass, Annual Report for the sampling period 1st July 2011–31st May 2012. TropWATER, James Cook University, Cairns.

McMahon, K., E. Sinclair, C. Sherman, K. van Dijk, U. Hernawan, J. Verduin, and M. Waycott. 2018. Genetic connectivity in tropical and temperate Australian seagrass species.

Pages 155-194 *in* A. Larkum, G. Kendrick, and P. Ralph, editors. Seagrasses of Australia. Springer, Cham.

McMahon, K., K. van Dijk, L. Ruiz-Montoya, G. Kendrick, S. Krauss, M. Waycott, J. Verduin, R. Lowe, J. Statton, E. Brown, and C. Duarte. 2014. The movement ecology of seagrasses. Proceedings of the Royal Society B **281**:1-9.

McNair, J., A. Sunkara, and D. Frobish. 2012. How to analyse seed germination data using statistical time-to-event analysis: non-parametric and semi-parametric methods. Seed Science Research **22**:77-95.

McNiven, I. 2004. Saltwater people: spiritscapes, maritime rituals and the archaeology of Australian indigenous seascapes. World Archaeology **35**:329-349.

Meager, J., and C. Limpus. 2012a. Marine wildlife stranding and mortality database annual report 2011. I Dugong. *Conservation Technical Paper and Data Report 2011*. Threatened Species Unit, Department of Environmental and Heritage Protection, Brisbane, Queensland.

Meager, J., and C. Limpus. 2012b. Marine wildlife stranding and mortality database annual report 2011. III. Marine Turtle. Conservation Technical and Data Report. Threatened Species Unit, Department of Environment and Heritage Protection, Brisbane, Queensland.

Mellors, J., M. Waycott, and H. Marsh. 2005. Variation in biogeochemical parameters across intertidal seagrass meadows in the central Great Barrier Reef region. Marine Pollution Bulletin **51**:335-342.

Moore, K., R. Orth, and J. Nowak. 1993. Environmental regulation of seed germination in *Zostera marina* L.(eelgrass) in Chesapeake Bay: effects of light, oxygen and sediment burial. Aquatic Botany **45**:79-91.

Nathan, R. 2008. An emerging movement ecology paradigm. Proceedings of the National Academy of Sciences **105**:19050-19051.

Nordlund, L., E. Jackson, M. Nakaoka, J. Samper-Villarreal, P. Beca-Carretero, and J. Creed. 2018. Seagrass ecosystem services–What's next? Marine Pollution Bulletin **134**:145-151.

O'Brien, K., M. Waycott, P. Maxwell, G. Kendrick, J. Udy, A. Ferguson, K. Kilminster, P. Scanes, L. McKenzie, K. McMahon, M. Adams, J. Samper-Villarreal, C. Collier, M. Lyons, P. Mumby, L. Radke, M. Christianen, and W. Dennison. 2018. Seagrass ecosystem trajectory depends on the relative timescales of resistance, recovery and disturbance. Marine Pollution Bulletin **134**:166-176.

O'Hara, R., and J. Kotze. 2010. Do not log-transform count data. Nature Precedings:1-1.

Olesen, B. 1999. Reproduction in Danish eelgrass (*Zostera marina* L.) stands: size-dependence and biomass partitioning. Aquatic Botany **65**:209-219.

Orth, R., T. Carruthers, W. Dennison, C. Duarte, J. Fourqurean, K. Heck, R. Hughes, G. Kendrick, J. Kenworthy, and S. Olyarnik. 2006a. A global crisis for seagrass ecosystems. Bioscience **56**:987-996.

Orth, R., M. Harwell, E. Bailey, A. Bartholomew, J. Jawad, A. Lombana, K. Moore, J. Rhode, and H. Woods. 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., M. Harwell, and G. Inglis. 2006b. Ecology of seagrass seeds and seagrass dispersal processes. Pages 111-133 Seagrasses: Biology, ecology and conservation. Springer.

Paling, E., M. Fonseca, M. van Katwijk, and M. van Keulen. 2009. Seagrass restoration. Pages 687-713 *in* G. Perillo, E. Wolanski, D. Cahoon, and M. Brinson, editors. Coastal wetlands: An integrated ecosystems approach. Elsevier, Amsterdam, the Netherlands.

Palumbi, S. 1994. Genetic divergence, reproductive isolation, and marine speciation. Annual review of ecology and systematics **25**:547-572.

Philbrick, C., and D. Les. 1996. Evolution of Aquatic Angiosperm Reproductive Systems: What is the balance between sexual and asexual reproduction in aquatic angiosperms? Bioscience **46**:813-826.

Pollard, P., and M. Greenway. 2013. Seagrasses in tropical Australia, productive and abundant for decades decimated overnight. Journal of Biosciences **38**:157-166.

Potouroglou, M., J. Bull, K. Krauss, H. Kennedy, M. Fusi, D. Daffonchio, M. Mangora, M. Githaiga, K. Diele, and M. Huxham. 2017. Measuring the role of seagrasses in regulating sediment surface elevation. Scientific Reports **7**:11.

Preen, A. 1992. Interactions between dugongs and seagrasses in a subtropical environment. PhD Thesis. James Cook University, Townsville, Australia.

Preen, A. 1995. Impacts of dugong foraging on seagrass habitats: observational and experimental evidence for cultivation grazing. Marine ecology progress series. Oldendorf **124**:201-213.

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

R Core Team. 2014. R: A Language and Environment for Statistical Computing. Available at: https://www.R-project.org/, Vienna, Austria.

R Core Team. 2020. R: A Language and Environment for Statistical Computing. Available at: https://www.R-project.org/, Vienna, Austria.

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

Rasheed, M., C. Reason, and J. Wells. 2019. Seagrass habitat of Cairns Harbour and Trinity Inlet: Annual Monitoring Report 2018. Centre for Tropical Water and Aquatic Ecosystem Research Publication 19/13, Cairns.

Reason, C., S. McKenna, and M. Rasheed. 2020. Seagrass habitat of Cairns Harbour and Trinity Inlet: Cairns Shipping Development Program and Annual Monitoring Report 2019. JCU Publication. Centre for Tropical Water & Aquatic Ecosystem Research Publication 20/06, Cairns.

Ridley, H. 1930. Dispersal of plants throughout the world. Reeve & Co., Ashford.

Rigby, R., and D. Stasinopoulos. 2005. Generalized additive models for location, scale and shape. Journal of the Royal Statistical Society: Series C (Applied Statistics) **54**:507-554.

Rivers, D., G. Kendrick, and D. Walker. 2011. Microsites play an important role for seedling survival in the seagrass *Amphibolis antarctica*. Journal of Experimental Marine Biology and Ecology **401**:29-35.

Roughgarden, J., S. Gaines, and H. Possingham. 1988. Recruitment dynamics in complex life cycles. Science **241**:1460-1466.

Ruiz-Montoya, L., R. Lowe, and G. Kendrick. 2015. Contemporary connectivity is sustained by wind-and current-driven seed dispersal among seagrass meadows. Movement ecology **3**:1-9.

Ruiz-Montoya, L., R. Lowe, K. Van Niel, and G. Kendrick. 2012. The role of hydrodynamics on seed dispersal in seagrasses. Limnology and oceanography **57**:1257-1265.

Ryan, J., G. Peter, and E. Rebecca. 2018. Nearshore drift dynamics of natural versus artificial seagrass wrack. Estuarine, Coastal and Shelf Science **202**:164-171.

Samuels, I., and D. Levey. 2005. Effects of gut passage on seed germination: do experiments answer the questions they ask? Functional Ecology **19**:365-368.

Schaffelke, B., A. Thompson, J. Carleton, J. Davidson, J. Doyle, M. Furnas, K. Gunn, M. Skuza, M. Wright, and I. Zagorskis. 2009. Reef Rescue Marine Monitoring Program. Final Report of AIMS Activities 2008/09. Australian Institute of Marine Science, Townsville.

Schupp, E., and M. Fuentes. 1995. Spatial patterns of seed dispersal and the unification of plant population ecology. Ecoscience **2**:267-275.

Scott, A., P. York, C. Duncan, P. Macreadie, R. Connolly, M. Ellis, J. Jarvis, K. Jinks, H. Marsh, and M. Rasheed. 2018. The role of herbivory in structuring tropical seagrass ecosystem service delivery. Frontiers in Plant Science **9**:127.

Scott, A., P. York, and M. Rasheed. 2020. Green turtle (*Chelonia mydas*) grazing plot formation creates structural changes in a multi-species Great Barrier Reef seagrass meadow. Marine environmental research **162**:105183.

Seminoff, J., (Southwest Fisheries Science Center, U.S.) 2004. *Chelonia mydas.* The IUCN Red List of Threatened Species 2004: e.T4615A11037468. http://dx.doi.org/10.2305/IUCN.UK.2004.RLTS.T4615A11037468.en. Downloaded on 03 December 2019.

Sheppard, J., A. Preen, H. Marsh, I. Lawler, S. Whiting, and R. Jones. 2006. Movement heterogeneity of dugongs, *Dugong dugon* (Müller), over large spatial scales. Journal of Experimental Marine Biology and Ecology **334**:64-83.

Sherman, C., T. Smith, P. York, J. Jarvis, L. Ruiz-Montoya, and G. Kendrick. 2018. Reproductive, dispersal and recruitment strategies in Australian seagrasses. Pages 213-256 *in* A. Larkum, G. Kendrick, and P. Ralph, editors. Seagrasses of Australia. Springer, Cham.

Short, F., T. Carruthers, W. Dennison, and M. Waycott. 2007. Global seagrass distribution and diversity: a bioregional model. Journal of Experimental Marine Biology and Ecology **350**:3-20.

Short, F., and R. Coles. 2001. Global seagrass research methods. Elsevier, University of New Hampshire Durham, USA.

Short, F., R. Coles, M. Fortes, S. Victor, M. Salik, I. Isnain, J. Andrew, and A. Seno. 2014. Monitoring in the Western Pacific region shows evidence of seagrass decline in line with global trends. Marine Pollution Bulletin **83**:408-416.

Short, F., and H. Neckles. 1999. The effects of global climate change on seagrasses. Aquatic Botany **63**:169-196.

Short, F., B. Polidoro, S. Livingstone, K. Carpenter, S. Bandeira, J. Bujang, H. Calumpong, T. Carruthers, R. Coles, and W. Dennison. 2011. Extinction risk assessment of the world's seagrass species. Biological Conservation **144**:1961-1971.

Short, F., and S. Wyllie-Echeverria. 1996. Natural and human-induced disturbance of seagrasses. Environmental conservation **23**:17-27.

Simeone, S., S. De Muro, and G. De Falco. 2013. Seagrass berm deposition on a Mediterranean embayed beach. Estuarine, Coastal and Shelf Science **135**:171-181.

Smith, T., K. Chartrand, J. Wells, A. Carter, and M. Rasheed. 2020. Seagrasses in Port Curtis and Rodds Bay 2019 Annual long-term monitoring and whole of port survey. Centre for Tropical Water & Aquatic Ecosystem Research (TropWATER) Publication 20/02, James Cook University, Cairns.

Smith, T., P. York, B. Broitman, M. Thiel, G. Hays, E. van Sebille, N. Putman, P. Macreadie, and C. Sherman. 2018. Rare long-distance dispersal of a marine angiosperm across the Pacific Ocean. Global Ecology and Biogeography **27**:487-496.

Sobtzick, M., R. Hagihara, H. Penrose, A. Grech, C. Cleguer, and H. Marsh. 2014. An assessment of the distribution and abundance of dugongs in the Northern Great Barrier Reef and Torres Strait. Report to the National Environmental Research Program. Reef and Rainforest Research Centre Limited, Cairns.

Sobtzick, S., C. Cleguer, R. Hagihara, and H. Marsh. 2017. Distribution and abundance of dugong and large marine turtles in Moreton Bay, Hervey Bay and the Southern Great Barrier Reef. Report 17/21. TropWater, James Cook University, Townsville, Australia.

Sobtzick, S., R. Hagihara, A. Grech, R. Jones, and H. Marsh. 2015. Improving the time series of estimates of dugong abundance and distribution by incorporating revised availability bias corrections. Centre for Tropical Water and Aquatic Ecosystem Research (TropWATER) Publication, James Cook University, Townsville.

Stafford-Bell, R., A. Chariton, and R. Robinson. 2016. Germination and early-stage development in the seagrass, *Zostera muelleri* Irmisch ex Asch. in response to multiple stressors. Aquatic Botany **128**:18-25.

Standora, E., J. Spotila, and R. Foley. 1982. Regional endothermy in the sea turtle, *Chelonia mydas*. Journal of Thermal Biology **7**:159-165.

Sumoski, S., and R. Orth. 2012. Biotic dispersal in eelgrass Zostera marina. Marine Ecology Progress Series **471**:1-10.

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

Thayer, G., K. Bjorndal, J. Ogden, S. Williams, and J. Zieman. 1984. Role of larger herbvores in seagrass communities. Estuaries **7**:351-376.

The Tetrazolium Subcommittee of the Association of Offical Seed Analysts. 2005. Tetrazolium Testing Handbook. Association of Offical Seed Analysts, Las Cruces, NM USA.

Thiel, M., and L. Gutow. 2004. The ecology of rafting in the marine environment. I. The floating substrata. Oceanography and Marine Biology: an annual review **42**:181-264.

Thompson, J., and B. Cunningham. 2002. Geographic structure and dynamics of coevolutionary selection. Nature **417**:735-738.

Thomson, A., P. York, T. Smith, C. Sherman, D. Booth, M. Keough, J. Ross, and P. Macreadie. 2014. Seagrass viviparous propagules as a potential long-distance dispersal mechanism. Estuaries and Coasts.

Tiffney, B. 2004. Vertebrate dispersal of seed plants through time. Annual Review of Ecology, Evolution, and Systematics **35**:1-29.

Tikel, D., D. Blair, and H. Marsh. 1996. Marine mammal faeces as a source of DNA. Molecular Ecology **5**:456-457.

Tol, S., R. Coles, and B. Congdon. 2016. *Dugong dugon* feeding in tropical Australian seagrass meadows: implications for conservation planning. PeerJ **4**:1-17.

Tol, S., J. Jarvis, P. York, A. Grech, B. Congdon, and R. Coles. 2017. Long distance biotic dispersal of tropical seagrass seeds by marine mega-herbivores. Scientific Reports **7**:1-8.

Traveset, A. 1998. Effect of seed passage through vertebrate frugivores' guts on germination: a review. Perspectives in Plant Ecology, Evolution and Systematics **1**:151-190.

Traveset, A., and M. Verdú. 2002. 22 A Meta-analysis of the effect of gut treatment on seed germination. Pages 339-350 *in* D. J. Levey, W. R. Silva, and M. Galetti, editors. Seed dispersal and frugivory: Ecology, evolution, and conservation. CABI Publishing, Wallingford, UK.

Tulipani, D., and R. Lipcius. 2014. Evidence of Eelgrass (*Zostera marina*) Seed Dispersal by Northern Diamondback Terrapin (*Malaclemys terrapin terrapin*) in Lower Chesapeake Bay. PloS one **9**:e103346.

Unsworth, R., C. Collier, M. Waycott, L. Mckenzie, and L. Cullen-Unsworth. 2015. A framework for the resilience of seagrass ecosystems. Marine Pollution Bulletin **100**:34-46.

Unsworth, R., L. Cullen, J. Pretty, D. Smith, and J. Bell. 2010. Economic and subsistence values of the standing stocks of seagrass fisheries: Potential benefits of no-fishing marine protected area management. Ocean & Coastal Management **53**:218-224.

Unsworth, R., L. McKenzie, C. Collier, L. Cullen-Unsworth, C. Duarte, J. Eklöf, J. Jarvis, B. Jones, and L. Nordlund. 2018. Global challenges for seagrass conservation. Ambio:1-15.

Valentine, J., and J. Duffy. 2006. The central role of grazing in seagrass ecology. Pages 463-501 *in* Anthony W.D. Larkum, Robert J. Orth, and C. M. Duarte, editors. Seagrasses: Biology, Ecologyand Conservation. Springer, The Netherlands.

Vallejo-Marín, M., M. Dorken, and S. Barrett. 2010. The ecological and evolutionary consequences of clonality for plant mating. Annual Review of Ecology, Evolution, and Systematics **41**:193-213.

Van der Stocken, T., A. Wee, D. De Ryck, B. Vanschoenwinkel, D. Friess, F. Dahdouh-Guebas, M. Simard, N. Koedam, and E. Webb. 2019. A general framework for propagule dispersal in mangroves. Biological Reviews **94**:1547-1575.

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

van Katwijk, M., A. Thorhaug, N. Marbà, R. Orth, C. Duarte, G. Kendrick, I. Althuizen, E. Balestri, G. Bernard, and M. Cambridge. 2016. Global analysis of seagrass restoration: the importance of large-scale planting. Journal of Applied Ecology **53**:567-578.

van Lent, F., and J. Verschnure. 1995. Comparative study on populations of *Zostera marina* L.(eelgrass): experimental germination and growth. Journal of Experimental Marine Biology and Ecology **185**:77-91.

van Lent, F., and J. Verschuure. 1994. Intraspecific variability of *Zostera marina* L.(eelgrass) in the estuaries and lagoons of the southwestern Netherlands. I. Population dynamics. Aquatic Botany **48**:31-58.

van Tussenbroek, B., L. Monroy-Velazquez, and V. Solis-Weiss. 2012. Meso-fauna foraging on seagrass pollen may serve in marine zoophilous pollination. Marine Ecology Progress Series **469**:1-6.

van Tussenbroek, B., N. Villamil, J. Márquez-Guzmán, R. Wong, L. Monroy-Velázquez, and V. Solis-Weiss. 2016. Experimental evidence of pollination in marine flowers by invertebrate fauna. Nature communications **7**:1-6.

Venables, W., and B. Ripley. 2002. Modern Applied Statistics with S. Fourth Edition. Springer, New York.

Warton, D., and F. Hui. 2011. The arcsine is asinine: the analysis of proportions in ecology. Ecology **92**:3-10.

Watson, R., R. Coles, and W. Lee Long. 1993. Simulation estimates of annual yield and landed value for commercial penaeid prawns from a tropical seagrass habitat, northern Queensland, Australia. Marine and Freshwater Research **44**:211-219.

Waycott, M., C. Collier, K. McMahon, P. Ralph, L. McKenzie, J. Udy, and A. Grech. 2007. Vulnerability of seagrasses in the Great Barrier Reef to climate change. Pages 193-235 *in* J. E. Johnson and P. A. Marshall, editors. Climate Change and the Great Barrier Reef: A Vulnerability Assessment. Great Barrier Reef Marine Park Authority and Australia and the Australian Greenhouse Office, Australia.

Waycott, M., C. Duarte, T. Carruthers, R. Orth, W. Dennison, S. Olyarnik, A. Calladine, J. Fourqurean, K. Heck, and R. Hughes. 2009. Accelerating loss of seagrasses across the globe threatens coastal ecosystems. Proceedings of the National Academy of Sciences **106**:12377-12381.

Waycott, M., B. Longstaff, and J. Mellors. 2005. Seagrass population dynamics and water quality in the Great Barrier Reef region: A review and future research directions. Marine Pollution Bulletin **51**:343-350.

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

Waycott, M., G. Procaccini, D. Les, and T. Reusch. 2006. Seagrass Evolution, Ecology and Conservation: A genetic perspective. Pages 25-50 *in* Anthony W.D. Larkum, Robert J. Orth, and C. M. Duarte, editors. Seagrasses: Biology, Ecology and Conservation. Springer, The Netherlands.

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

Webber, B., and I. Woodrow. 2004. Cassowary frugivory, seed defleshing and fruit fly infestation influence the transition from seed to seedling in the rare Australian rainforest tree, *Ryparosa* sp. nov. 1 (Achariaceae). Functional Plant Biology **31**:505–516.

Whittow, G., and G. Balazs. 1982. Basking behavior of the Hawaiian green turtle (*Chelonia mydas*).

Wu, K., C. Chen, and K. Soong. 2016. Long distance dispersal potential of two seagrasses *Thalassia hemprichii* and *Halophila ovalis*. PloS one **11**:e0156585.

Wyneken, J., K. Lohmann, and J. Musick. 2013. The biology of sea turtles. CRC press, United States.

Xu, S., Y. Zhou, P. Wang, F. Wang, X. Zhang, and R. Gu. 2016. Salinity and temperature significantly influence seed germination, seedling establishment, and seedling growth of eelgrass *Zostera marina* L. PeerJ **4**:e2697.

York, P., G. Hyndes, M. Bishop, and R. Barnes. 2018a. Faunal assemblages of seagrass ecosystems. Pages 541-588 *in* A. Larkum, G. Kendrick, and P. Ralph, editors. Seagrasses of Australia. Springer, Cham.

York, P., P. Macreadie, and M. Rasheed. 2018b. Blue Carbon stocks of Great Barrier Reef deep-water seagrasses. Biology letters **14**:20180529.

York, P., T. Smith, R. Coles, S. McKenna, R. Connolly, A. Irving, E. Jackson, K. McMahon, J. Runcie, and C. Sherman. 2017. Identifying knowledge gaps in seagrass research and management: an Australian perspective. Marine environmental research **127**:163-172.

Youngsteadt, E., J. Baca, J. Osborne, and C. Schal. 2009. Species-specific seed dispersal in an obligate ant-plant mutualism. PloS one **4**:e4335.

Yue, S., Y. Zhang, Y. Zhou, S. Xu, S. Xu, X. Zhang, and R. Gu. 2019. Optimal long-term seed storage conditions for the endangered seagrass *Zostera japonica*: implications for habitat conservation and restoration. Plant Methods **15**:1-10.

Zuur, A., E. Ieno, and C. Elphick. 2010. A protocol for data exploration to avoid common statistical problems. Methods in Ecology and Evolution **1**:3-14.

Appendix A

Chapter 2 supporting material

Supplementary Table 2.1: Statistical results for a generalized linear model (GLM) using a Quassi-Poisson distribution (with a 'emmeans' Tukey post-hoc analysis) for the number of seagrass seeds per gram dry weight (g DW) of marine mega-herbivore faecal matter, collected from the Central GBRWHA, Queensland Australia 2014; CB = Cleveland Bay, UB = Upstart Bay, and PB = Pioneer Bay.

	Estimate	**Standard Error**	**df**	**Z. Ratio**	***p*. value**
Time					
Sep – Oct	-0.13	0.590	Inf	-0.221	0.9962
Sep – Nov	-1.40	1.104	Inf	-1.264	0.5858
Sep – Dec	1.80	0.738	Inf	2.439	0.0699
Oct – Nov	-1.27	1.007	Inf	-1.257	0.5903
Oct – Dec	1.93	0.582	Inf	3.318	0.0050*
Nov - Dec	3.20	0.822	Inf	3.890	0.0006*
Site					
CB – PB	0.916	0.914	Inf	1.003	0.5752
CB – UB	3.612	1.162	Inf	3.108	0.0054*
PB - UB	2.696	0.718	Inf	3.755	0.0005*

*** significant**

Supplementary Table 2.2: Statistical results for a logistic regression mixed effect model with a binomial distribution (with a 'emmeans' Tukey post-hoc analysis) for the proportion of seagrass seeds with a split seed coat found in marine mega-herbivore faecal matter, collected from the Central GBRWHA, Queensland Australia 2014; CB = Cleveland Bay, UB = Upstart Bay, and PB = Pioneer Bay.

	Estimate	**Standard Error**	**df**	**Z. Ratio**	***p*. value**
Time					
Sep – Oct	0.349	4.69	Inf	0.074	0.9999
Sep – Nov	0.161	77875.32	Inf	0.000	1.0000
Sep – Dec	19.439	34826.90	Inf	0.001	1.0000
Oct – Nov	-0.188	77875.32	Inf	0.000	1.0000
Oct – Dec	19.091	34826.90	Inf	0.001	1.0000
Nov - Dec	19.278	69653.80	Inf	0.000	1.0000
Site					
CB – PB	0.0	77875	Inf	0.000	1.0000
CB – UB	19.3	87646	Inf	0.000	1.0000
PB - UB	19.3	40215	Inf	0.000	1.0000

*** significant**

Supplementary Table 2.3: Statistical results for a logistic regression mixed effect model with a binomial distribution (with a 'emmeans' Tukey post-hoc analysis) for the proportion of viable seagrass seeds found in marine mega-herbivore faecal matter, collected from the Central GBRWHA, Queensland Australia 2014; CB = Cleveland Bay, UB = Upstart Bay, and PB = Pioneer Bay.

	Estimate	**Standard Error**	**df**	**Z. Ratio**	***p*. value**
Time					
Sep – Oct	0.291	0.753	Inf	0.386	0.9804
Sep – Nov	0.858	1.449	Inf	0.592	0.9346
Sep – Dec	0.473	0.957	Inf	0.494	0.9605
Oct – Nov	0.567	1.355	Inf	0.418	0.9754
Oct – Dec	0.181	0.807	Inf	0.225	0.9960
Nov - Dec	-0.385	1.088	Inf	-0.354	0.9848
Site					
CB – PB	-0.693	1.225	Inf	-0.566	0.8382
CB – UB	-1.078	1.420	Inf	-0.759	0.7280
PB - UB	-0.385	0.719	Inf	-0.536	0.8538

*** significant**

Appendix B

Chapter 3 supporting material

Supplementary Material 3.1: *Dugong dugon* (dugong) sequences of tissue sample (collected in Moreton Bay, Queensland Australia) and faecal samples (collected *in-situ* from Bing Bong Gulf of Carpentaria Northern Territory, Australia, and from Toba Aquarium, Japan).

```
Dugong_tissue_sample
GCATTATGTGCTCTTCCCCATAACAGTACTATATATGTTTTATCTTACATACACCATCCTATGTATAAT

DP1_F&R
GCATTATGTGCTCTTCCCCATAACAGTACTATATATGTTTTATCTTACATACACCATCCTATGTATAAT

DP4_F&R
GCATTATGTGCTCTTCCCCATAACAGTACTATATATGTTTTATCTTACATACACCATCCTATGTATAAT

DP2_R_only
GCATTATGTGCTCTTCCCCATAACAGTACTATATATGTTTTATCTtACATACACcatcctatgtataat

DP3_R_only
GCATTATGTGCTCTTCCCCATAACAGTACTATATATGTTTtATCTtACATACaccatcctatgtataat

RG3_F&R
GCATTATGTGCTCCTCCCCATAATAGTACTATATATGTTTTATCTTACATACACCATCCTATGTATAAT

RG9_F&R
GCATTATGTGCTCCTCCCCATAATAGTACTATATATGTTTTATCTTACATACACCATCCTATGTATAAT
```

Supplementary Material 3.2: *Chelonia mydas* (green sea turtle) sequences of tissue sample (collected in Cairns, Queensland Australia) and faecal samples (collected from Gladstone Harbour and Cleveland Bay Townsville in Queensland, and Bing Bong Gulf of Carpentaria Northern Territory, Australia).

Turtle_Tissue
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

P1
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

P2
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

P3
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

P4
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

P5
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

P6
aCAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

P7
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

P8
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

P9
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

P10
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

P11
ACAGAACAATACCcACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

P12

ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

P13
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

P14
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

P15
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

P16
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

P17
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

P18
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

P19
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

P20
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

P21
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

P22
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

P23
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

P24
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

P25
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

P26
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

P27
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

P28
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

P29
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

P30
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

P31
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

P32
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

P33
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

P34
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

P35
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

P36
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

P37
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

P38
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

P39
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

P40
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

P41

ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

P42
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

P43
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

P44
ACAGAACAATACCCACAATACCTATCTATGTATTAtTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

P45
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

RG1
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

RG2
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

RG4
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

RG5
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

RG6
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

RG7
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

RG8
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

RG10r
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTtACCactagcatangacnn
nnnnnnnnn

RG11
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAnTGTTA

RG12
ACAGAACAATACCCACAATACCTATCTATGTATTATTGTACATCTACTTATTTACCACTAGCATATGACCA
GTAATGTTA

Appendix C

Chapter 4 supporting material

Supplementary Table 4.1: Absence and presence of seagrass species at two intertidal and subtidal seagrass meadows in the central Great Barrier Reef World Heritage Area, Australia. Information from Smith et al. 2020 and McKenna et al. 2020.

Species	**Cleveland Bay, Townsville**	**Gladstone Harbour**
Cymodocea rotundata	Present	Absent
Cymodocea serrulata	Present	Absent
Enhalus acoroides	Present	Absent
Halodule uninervis	Present	Present
Halophila decipiens	Present	Present
Halophila ovalis	Present	Present
Halophila spinulosa	Present	Present
Thalassia hemprichii	Present	Absent
Zostera muelleri	Present	Present

Appendix D

Chapter 5 supporting material

Supplementary Table 5.1: Seagrass species presence at Pioneer Bay and Midge Point intertidal seagrass meadows in the Central GBRWHA, Queensland Australia; November 2015 (growing season) and May 2016 (senescent season).

		Total Area km^2	Average Shoot Count/m^2	Mean HU Percent Cover	Mean ZM Percent Cover	Mean HO Percent Cover
Pioneer Bay	November 2015	4.63 *	1168 ± 107.42	39.30 ± 6.34	55.68 ± 6.81	2.53 ± 0.72
	May 2016		793 ± 67.43	16.13 ± 5.09	77.88 ± 5.02	6.00 ± 2.05
Midge Point	November 2015	3.19 *	1398 ± 157.08	51.50 ± 5.73	45.25 ± 5.72	3.25 ± 1.58
	May 2016		1249 ± 108.00	63.65 ± 5.55	31.88 ± 5.46	4.60 ± 1.46

* Data taken from Carter et al. 2016

HU = *Halodule uninervis*; ZM = *Zostera muelleri*; HO = *Halophila ovalis*

Supplementary Table 5.2: Statistical results for a Mann-Whitney U Test for viable verse non-viable *Zostera muelleri* seagrass fragment characteristics at two coastal seagrass meadows in the Central GBRWHA, Queensland Australia.

	***W*. value**	***p*. value**
Shoot Count	4679.5	<0.0001*
Leaf Count	4555.5	<0.0001*
Leaf Length	12462	0.0257*
Rhizome Length	6023	<0.0001*
Biomass	12065	0.0901

*** significant**

Supplementary Table 5.3: Statistical results for a Mann-Whitney U Test for viable verse non-viable *Halodule uninervis* seagrass fragments characteristics at two coastal seagrass meadows in the Central GBRWHA, Queensland Australia; collected in November 2015 (growing season) and May 2016 (senescent season).

	***W*. value**	***p*. value**
Shoot Count	255	0.0006*
Leaf Count	190.5	<0.0001*
Leaf Length	466.5	0.8169
Rhizome Length	355	0.0801
Biomass	385	0.1802

*** significant**

Supplementary Table 5.4: Statistical results for a generalized linear model (GLM) using a negative binomial distribution for the number of seagrass fragments at two coastal seagrass meadows in the Central GBRWHA, Queensland Australia.

	Df	**Deviance**	**Residual Df**	**Residual Deviance**	***p*. value**
Null			285	320.55	
Site	1	0.517	284	320.03	0.47
Shoot Count m^2	2	7.890	282	312.14	<0.0001*
Wind speed	1	41.352	281	270.79	<0.001*

*** significant**

Supplementary Table 5.5: Statistical results for a generalized linear model (GLM) using a negative binomial distribution with an 'emmeans' post-hoc for the number of viable seagrass fragments at two coastal seagrass meadows in the Central GBRWHA, Queensland Australia.

	Estimate	**SE**	***Z*. ratio**	***p*. value**
Date	1.23	0.203	6.064	<0.0001*
Wind speed				
Low – Medium	-6.74e-01	0.215	-3.126	0.0050*
Low – High	-8.76e-05	0.234	0.000	1.0000
Medium – High	6.74e-01	0.240	2.803	0.0140*

*** significant**