

The resilience of tropical seagrasses to chronic stress and physical disturbances

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DECLARATION

I hereby declare that this thesis is my original work and it has been written by me in its entirety. I have duly acknowledged all the sources of information, which have been used in the thesis.

This thesis has also not been submitted for any degree in any university previously.

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SUMMARY

Seagrasses are known to have one of the highest light requirements of any plant group, and are therefore sensitive to changes in water quality — especially turbidity. While seagrasses possess a variety of mechanisms for coping with light reduction, little is known about how acclimation to turbid environments affects their overall resilience, and in what way recovery is dampened when stressors are combined. This thesis tests the effects of chronic light reduction and physical disturbance on seagrass resilience. It also examines how frequent disturbances can impact resilience in the long term and whether an improvement in abiotic conditions necessarily results in recovery of the system to its original state.

Despite their sensitivity to changes in water quality, seagrasses can and do exist in busy port waters, as seen in the case of Singapore. Twelve species of seagrass have been recorded in this highly urbanised city-state, with the three largest meadows covering a 33.7 ha of intertidal shore. A reconstruction of the historical extent of seagrass in Singapore suggests that close to 45% of Singapore's original seagrass beds have been lost, largely through land reclamation. A Vulnerability Analysis suggests that the top threat to seagrass loss was, and continues to be, land reclamation; hence a comprehensive management and conservation plan is needed to ensure the continued presence of seagrasses in Singapore.

Acclimation to sub-optimal (low) light conditions for extended periods of time affects the resilience of seagrasses. Temporal light reduction, by adding two different levels of shading to *Halophila ovalis* plants in two meadows with distinct light histories: one characterised by a low light (turbid) environment and the other by a relatively high light (clear) environment, resulted in complete mortality for both shading treatments

in the meadow with a turbid light environment. These contrasting results for the same species in two different locations indicate that acclimation to chronic low light regimes can affect seagrass resilience and highlights the importance of light history in determining the outcome of exposure to further (short-term) stress.

While climax seagrasses such as *Thalassodendron ciliatum* are thought to be able to cope better with disturbances because of their larger carbohydrate reserves, prolonged exposure to low light coupled with physical disturbances increases the sensitivity of this species to seasonal light reduction. When exposed to long-term light reduction, physically disturbed *T. ciliatum* was unable to recover to its original starting state. This further emphasises that exposure to chronic stress dampens ecosystem resilience, resulting in reduced recovery.

Seagrasses have a diminished capacity for recovery when physical disturbance is coupled with a sustained period of low light stress. The recovery capacity of seagrasses is further reduced if they experience repeated physical disturbances. Plants exposed to light reduction and repeated disturbances were unable to recover fully, even when they were returned to their ambient conditions, highlighting that the resilience of seagrasses is reduced when stress is prolonged.

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*“It is not despair, for despair is only for those who see the end beyond all doubt.
We do not.”*

– Gandalf the Grey

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CHAPTER 1. INTRODUCTION

1.1. Seagrasses: An overview

Seagrasses are marine angiosperms that grow in the sheltered coastal waters of every continent except Antarctica (den Hartog, 1970; Green & Short, 2003). As a taxonomic group, seagrasses are paraphyletic and with origins in diverse lineages that have converged on similar morphologies and physiologies well adapted to life underwater (Larkum & den Hartog, 1989). There are approximately 65 species worldwide belonging to 10 genera and four families (den Hartog, 1970; Green & Short, 2003). As with other marine organisms, seagrass diversity is highest in the tropical Indo-Pacific region, which harbours 23 species. Seagrasses can be found in a variety of estuarine, intertidal, shallow-coastal (Carruthers et al., 2002) and deep-water habitats (Erftemeijer & Stapel, 1999) where they can occur as meadows or in discontinuous patches.

Seagrasses are perhaps better defined as an ecological rather than taxonomic group (den Hartog & Kuo, 2006) as they fulfill several ecological roles within the marine environment. Seagrass meadows provide a range of ecosystem services such as nutrient cycling (Hemminga et al., 1999; McGlathery et al., 2007), food provision to humans (Cullen-Unsworth et al., 2013) and endangered large grazers such as dugongs and turtles (Bell & Pollard, 1989; Heck & Valentine, 2006), coastal protection by stabilizing sediments and sediment accretion (Madsen et al., 2001; Gacia et al., 2003) and mitigating climate change through carbon sequestration (Duarte et al., 2005; Fourqurean et al., 2012). Seagrasses are considered one of the most valuable ecosystems on earth, ranking third after wetlands and mangrove habitats, and ahead of all terrestrial habitats including tropical rainforests (Costanza et al., 1997). Despite the recognition of their significant economic and ecological

value, the past five decades has seen substantial loss and degradation of seagrass meadows worldwide, largely driven by declining water quality from human activities (Orth et al., 2006; Waycott et al., 2009).

Like their terrestrial counterparts, seagrasses require light for photosynthesis and growth. However, due to the inherent properties of water, light is attenuated as it enters the water column due to absorption and scattering, and the degree to which this happens depends on factors such as dissolved organic matter, sediment load, nutrient load and depth (chapter on optical properties, Ralph et al., 2007). As such, light availability is a primary limiting factor for seagrass depth distribution (Dennison, 1987). Seagrasses are known to have one of the highest light requirements of any plant group, with some species needed as much as 25% of incident radiation, compared to as low as 1% for other angiosperms (Dennison et al., 1993, Erftemeijer & Lewis, 2006). Light requirements vary, which together with water clarity affects their depth distribution, but the majority of species are found in depths of 20 m or less (Hemminga & Duarte, 2000). Seagrasses have often been termed coastal canaries due to their efficacy as indicators of change in water quality (Dennison et al., 1993; Orth et al., 2006).

On the one hand, the relatively high light requirements of seagrasses is due to the need to maintain a positive carbon balance for growth while at the same time meet the oxygen demand of non-photosynthetic tissues such as the roots and rhizomes, which exist in an anoxic sediment environment (Hemminga, 1998) This non-photosynthetic biomass, can be a burden under prolonged low light conditions, especially if they constitute a relatively large proportion of the plant's total biomass (Hemminga, 1998). On the other hand, the ability of seagrasses to store

carbohydrates in their rhizomes offers a source of energy reserves that enables seagrass to support metabolic demands during periods of low light (Hemminga, 1998; Vermaat, 2009). In this respect, seagrasses with a larger below ground biomass of rhizomes and roots are considered to be better able to survive periods of light deprivation (Vermaat, 1997; Alcoverro et al., 1999). The effects of light deprivation on seagrass have been researched widely (see reviews in Erftemeijer & Lewis, 2006; Lee et al., 2007; Ralph et al., 2007;) and it is known that they possess a variety of mechanisms that allow them to cope with periods of light reduction. These mechanisms can manifest at different scales, ranging from physiological responses at the level of individual cells or plant tissues, to meadow scale processes, depending on the duration and intensity of light deprivation (Fig. 1.1).

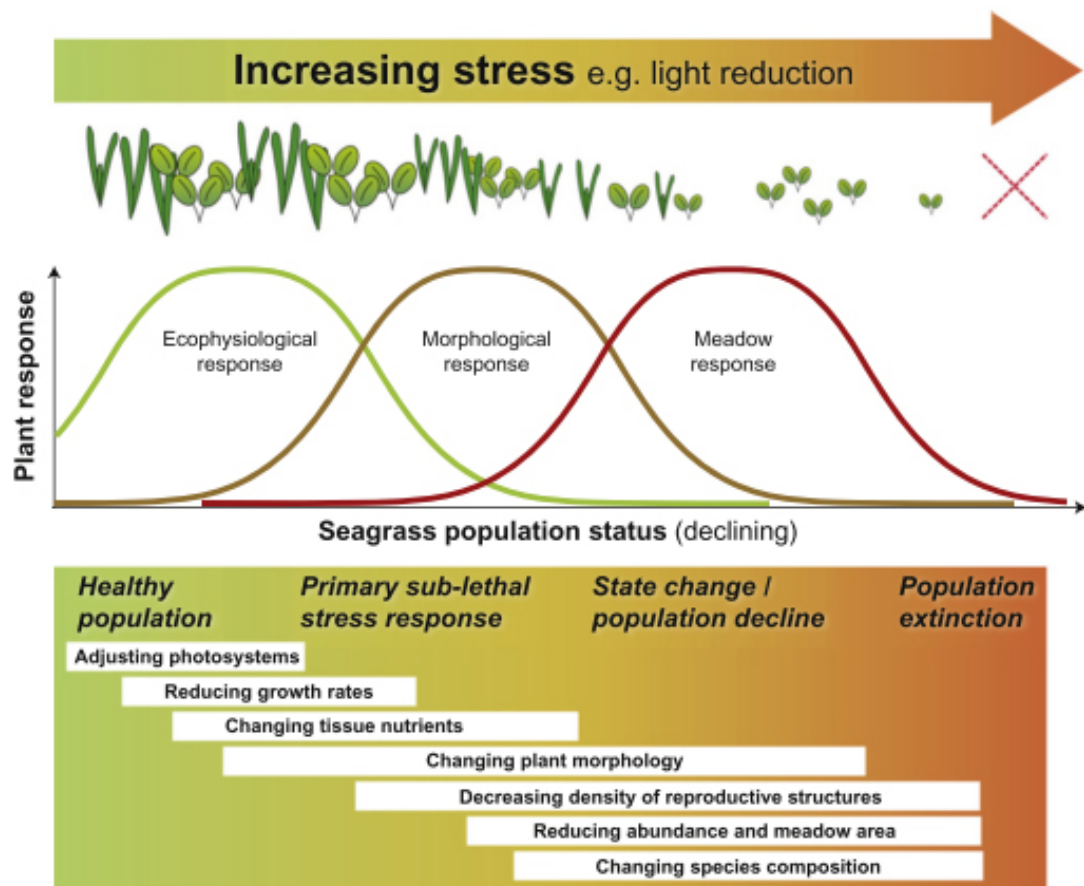


Figure 1.1. The relationship between plant response at the ecophysiological, morphological and meadow levels to increasing stress. (Figure from Collier et al., 2012, with permission).

Much of the early research on seagrass and light focused on responses to light reduction (Dennison, 1987). More recently, there has been a shift in direction, with more studies examining mixed effects and interactions between light and other environmental parameters such as nutrients (Moore & Wetzel, 2000; Ibarra-Obando, 2004), temperature (York et al., 2013) and seasonality (Lavery et al., 2009). There has also been a push towards understanding the different response variables and identifying appropriate indicators for assessing seagrass state and health (McMahon et al., 2013). This is especially pertinent given that seagrasses are common in sheltered and shallow coastal waters and are therefore often subjected to the rapid rate of coastal modification and disturbance from anthropogenic activities. There have been several documented cases of seagrass loss following water quality deterioration as a result of eutrophication (Cardoso et al., 2004; Burkholder et al., 2007), turbidity and sedimentation from dredging (Erftemeijer & Lewis, 2006), flooding (Campbell & McKenzie, 2004) and contamination or pollution (Cambridge & McComb, 1984).

Anthropogenic impacts have been identified as the leading cause for seagrass loss and decline worldwide (Orth et al., 2006; Waycott et al., 2009), perhaps unsurprising given the rapid rate of urbanization of coastal cities and modification of coastlines in the past few decades. In East Asia alone, there are 12 coastal cities with a population of more than 15 million (Gill & Kharas, 2007) that are still expanding, placing an unprecedented strain on natural near shore resources and habitats (Yeung, 2001). There have been several documented cases of large-scale losses associated with development, such as increased pollution, eutrophication (Burkholder et al., 2007) and turbidity and sedimentation caused by construction and dredging (Erftemeijer & Lewis, 2006), and these continue to be major threats to the survival of

seagrass meadows worldwide (Orth et al., 2006; Waycott et al., 2009; Grech et al., 2012).

The resilience of an ecosystem refers to its capacity to withstand disturbance while retaining its functions, structure and ability to recover from such disturbances (Beisner et al., 2003; Walker et al., 2004). Maintaining ecosystem resilience is of utmost importance in order for an ecosystem to continue providing vital ecosystem services. Seagrass resilience is a concept that has been discussed in the literature (Björk et al., 2008, Carr et al., 2012b) but, as yet, there is no unified concept of resilience in seagrass meadows. However, several factors are known to contribute to seagrass recovery from disturbances such as species diversity (Peterson et al., 2002; Cardoso et al., 2004) genetic diversity (Hughes & Stachowicz, 2004; Elhers et al., 2008) and genetic and habitat connectivity (Poraccini et al., 2007; Kendrick et al., 2012), whereas changes in abiotic conditions such as water organic nutrient content, turbidity, water flow and sediment anoxia contribute to meadow decline and hampers recovery and restoration efforts (Björk et al., 2008).

It is generally accepted that water quality declines lead to loss of seagrass, the role of chronic stressors in eroding resilience prior to system collapse is poorly studied. In coral reefs, empirical data suggests that chronic eutrophication and overfishing erode resilience to disease and coral bleaching (Nyström et al., 2000; Folke et al., 2004), but that these chronic stressors are not in themselves lethal and the ecosystem can persist and show little sign of stress until collapse is triggered by a separate event initiating the transition to algae-dominated reefs (Bellwood et al., 2004). Thus, it is possible that a similar situation exists for seagrasses, where seagrasses that are frequently or continuously exposed to chronic non-lethal stress or disturbances,

continue to exist without showing obvious signs of stress. To date however, there is no experimental evidence to support this hypothesis. Seagrass meadows that exist in waters adjacent to urban coastal settlements are potentially useful settings for exploring the themes of non-lethal chronic stress, light requirements, the response and recovery from frequent disturbances and how these relate to resilience of seagrass ecosystems.

Singapore, an island city-state, is an excellent example of a massive conurbation situated adjacent to critical marine tropical ecosystems. However, unlike their mangrove and coral reef counterparts, there are very few studies on seagrasses in Singapore and a comprehensive examination of the extent, distribution and species diversity has never been carried out, save for a few preliminary surveys (Loo et al., 1990, 1996). Nevertheless, seagrasses are thought to be common on Singapore shores (Chuang, 1961) and, given the long history of coastal modification around the island (Hilton & Manning, 1995), and the associated increase in sedimentation and turbidity that results from such activities (Dikou & van Woesik, 2006), it provides an excellent opportunity for exploring some of the abovementioned themes.

1.2. Research objectives

The impetus for this PhD project was to understand the impacts of dredging and coastal reclamation on sensitive marine habitats such as seagrasses. Carried out in the setting of Singapore's waters, where such activities are common, there was some urgency to understand the distribution, extent and diversity of seagrasses and explore the reasons and mechanisms behind their continued survival. This is a large and ongoing task, to which this thesis makes a contribution by addressing the following key objectives:

1. Review the diversity, distribution and extent of remaining seagrass habitats in Singapore;
2. Examine the past distribution of seagrass in Singapore, the changes in distribution and the proximate reasons for changes;
3. Understand how a non-lethal but chronic light environment affects seagrass response to disturbances and how these responses may vary with species and location;
4. Understand the responses of seagrasses to frequent physical disturbance and how this affects resilience in the long term.

The results from this thesis should will inform the decision making and management of seagrasses with the aim of ensuring their continued existence in Singapore waters and the region.

1.3. Thesis structure and overview of data chapters

I would like to first clarify the use of style and the personal (I/we) and possessive (my/our) pronouns in this thesis. The use of “I/my” in the first and last chapters refers to my own work and ideas as the sole author. The use of “we/our” in Chapters 2 to 6 refers to the first author (myself) and co-authors. In all the chapters, I am the lead author and data collector, but receiving comments, suggestions, language edits and technical inputs from the co-authors. The exception to this was Chapter 3, where co-author Eugene Chen carried out most of the fieldwork and data collection, while I assisted with project formulation, data analysis and wrote the paper. Three of the five data chapters have been published and are presented here verbatim. A brief description of each data chapter is presented below:

Chapter 2. The diversity and distribution of seagrass in Singapore.

Compared to their mangrove and coral reef counterparts, seagrass habitats in Singapore have been largely overlooked. In this chapter, I present the first comprehensive study compiling the species composition, distribution and current status of seagrasses in Singapore waters. I also mapped (using satellite imagery) the extent of the three largest seagrass meadows known in Singapore. This chapter has been fully published as Yaakub et al. (2013a) in the journal *Nature in Singapore*.

Chapter 3. Courage under fire: Seagrass persistence adjacent to a highly urbanised city-state.

This chapter examines the changes in the distribution and extent of seagrass in Singapore waters. I reconstructed the historic extent and diversity of local seagrass meadows through herbarium records and backwards extrapolation from contemporary seagrass locations. Seagrass-Watch habitat monitoring data were used to determine the status and trends of present day seagrass meadows and a Vulnerability Analysis was carried out to identify the current threats to seagrasses in Singapore. The chapter concludes with suggested reasons for the continued persistence of seagrass in Singapore waters, despite the extensive coastal modification that has taken place, and provides recommendations for the conservation and management of seagrasses in Singapore. This chapter is “in press” as Yaakub et al. (2014) in the journal *Marine Pollution Bulletin*.

Chapter 4. Chronic light reduction reduced overall resilience to additional shading stress in the seagrass *Halophila ovalis*.

The ability to tolerate and/or acclimatize to an altered light regime provides seagrasses with essential mechanisms to survive sub-optimal (low) light levels.

However, relatively little is known regarding how acclimation to a sub-optimal light environment for extended periods affects the resilience of seagrasses. In this chapter I examined how chronic low-light stress on seagrasses impacts the plants' response to additional stress. I compared the morphological, photosynthetic and shoot density responses of two different monospecific seagrass meadows in Singapore that have two very different light histories, one characterized by a low light (turbid) environment and the other by a relatively high light (clear) environment. This chapter has been fully published as Yaakub et al., (2013b) in the journal *Marine Pollution Bulletin*.

Chapter 5. Chronic light reduction impairs recovery from seasonal and physical disturbances in the climax seagrass species *Thalassodendron ciliatum* (Forsskål) den Hartog.

Climax species are generally thought to cope better with disturbances for longer periods due to their larger carbohydrate stores in their roots and rhizomes. Similarly, their recovery is thought to be slower due to a slower growth strategy. Here, I tested the resilience and recovery responses of disturbed and undisturbed *Thalassodendron ciliatum* plots, which were subjected to experimentally induced shading. This research also serves to contrast the response of a climax species to that of the pioneer species, *Halophila ovalis*, studied in the previous chapter. This chapter is currently being prepared for journal submission.

Chapter 6. Resilience of seagrass to repeated disturbances and low-light stress: Experimental evidence for critical slowing down.

Restoration of seagrass habitats is typically costly, labour intensive, and is rarely successful. One explanation for the limited success in restoring seagrass habitats is the existence of alternative stable states. It has been proposed that, once a critical

threshold is passed, positive environmental conditions that surpass previous ambient conditions are needed before the habitat can return to its previous state. In this paper, I explored the mechanism of resilience and recovery in meadows of the pioneer species *Halophila ovalis* by inducing a physical disturbance coupled with the added stress of low light. This chapter is currently being prepared for journal submission.

CHAPTER 2. THE DIVERSITY AND DISTRIBUTION OF SEAGRASS IN SINGAPORE¹

2.1. Introduction

Seagrasses are flowering plants that are commonly found in both tropical and temperate coastal waters (Green & Short, 2003). They have a widespread distribution that encompasses every continent except Antarctica, but the highest diversity of seagrass species is centered in the tropical Indo-Pacific (Waycott et al., 2004). Seagrass habitats are an important component of the marine environment, providing vital services such as nutrient cycling, food provision, and climate change mitigation (Orth et al., 2006; Waycott et al., 2009). Similar to other marine ecosystems, seagrass habitats are under threat from human activities such as coastal development and increased nutrient input. These impacts have brought about accelerated decline in seagrass habitats globally (Waycott et al., 2009).

Seagrasses were common in Singapore up to the late 1950s and early 1960s, with large meadows found off the eastern coast of Singapore Island and around some offshore islands (Chuang, 1961; Johnson, 1973). The World Atlas of Seagrasses list 11 species present in Singapore waters (Green & Short, 2003), but there is a general paucity of published information on local seagrass diversity and ecology, especially in the scientific literature (Ooi et al., 2011). In fact, they are so poorly studied that in an initial assessment of the national conservation status of plants and animals, five of the 11 species of seagrass were listed as locally extinct (Turner et al., 1994), while a later survey only found seven species spread across 12 locations (Loo et al., 1996). Even more elusive than species diversity is information on the distribution and extent

¹This paper has been fully published as Yaakub, S.M., R.L.F. Lim, W.L. Lim, P.A. Todd, 2013. The diversity and distribution of seagrass in Singapore. *Nature in Singapore*, 6: 105–111.

of seagrass habitats in Singapore's coastal waters. This is possibly owing to the fact that seagrass habitats, unlike their coral reef and mangrove counterparts, have never been a feature in marine navigational maps, making even an estimate of their true extent difficult to determine.

It is generally acknowledged that in order for sound management of resources to exist, there needs to be an inventory and a baseline of those resources (Chua, 2006). While such inventories and baselines exist for other marine habitats in Singapore such as coral reefs (Burke et al., 2002) and mangroves (Yee et al., 2010), there are currently no reliable baseline available for seagrass habitats. Hence, the aim of this paper is to provide a comprehensive checklist for the diversity of seagrass species and a baseline for their distribution. We also map the extent of the three largest remaining seagrass meadows in Singapore.

2.2. Materials and Methods

2.2.1. Seagrass diversity and distribution in Singapore.

To determine their present distribution, herbarium records of all seagrass species (families Hydrocharitaceae and Cymodoceaceae [formerly Potamogetonaceae]) lodged with the Singapore Botanic Gardens Herbarium (SING) since 2000 were compiled and sorted by area (Eastern and Western Johore Straits, Southern Mainland, Southern Offshore Islands, and Southern Patch Reefs). Data from surveys carried out between 2007 and 2010 were used to corroborate herbarium specimens.

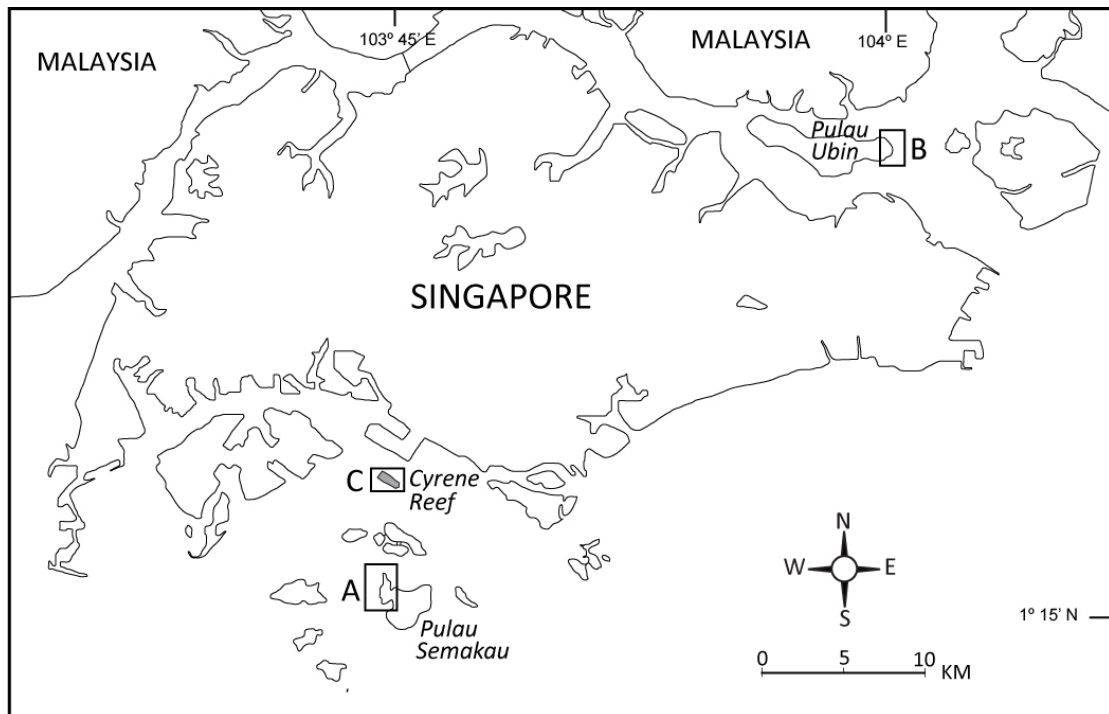


Figure 2.1. The locations of Singapore's three largest seagrass meadows: A, Pulau Semakau; B, Chek Jawa, Pulau Ubin; C, Cyrene Reef.

2.2.2. *Extent of Singapore's three largest seagrass meadows.*

The three largest seagrass meadows in Singapore were chosen for mapping (Fig. 2.1) as these are regularly monitored sites under the Seagrass-Watch program (McKenzie et al., 2009). The first meadow is found near Pulau [=island] Semakau, an offshore island southwest of Singapore Island ($1^{\circ}12.54'N$, $103^{\circ}45.40'E$). The island is a conglomerate of two islands, Pulau Semakau and Pulau Sakeng, combined to create an offshore landfill. The eastern shoreline was largely undisturbed during the construction of a 7km rock bund that joins the two islands, but substantial tracts of mangroves on the northern and western side of the island were destroyed (Chou & Tun, 2007). The second is at Chek Jawa Wetlands, a mudflat situated on the eastern tip of Pulau Ubin, off the northeastern coast of Singapore ($1^{\circ}24.56'N$ $103^{\circ}59.52'E$). This site is influenced by the Johor River, which discharges a high sediment load into the area. The seagrass meadow at Chek Jawa is situated within an intertidal lagoon, with seagrass growing on the periphery of the lagoon on the seaward side. The third

meadow is located at Cyrene Reef and refers to a group of three patch reefs, the largest being Terumbu [=reef] Pandan (1°15.52'N 103°45.27'E), which has a substantial seagrass meadow growing on the reef top. The reefs are situated just off Pasir Panjang Container Terminal and are bounded by the oil and petrochemical refinery islands of Pulau Bukom to the south and Jurong Island to the west.

WorldView-2 satellite images (1-m resolution) from 2010 and 2011 were used to map the extent of the three seagrass meadows. These were supplemented by Geoeye-1 satellite images when cloud-free Worldview-2 images could not be obtained. The images used were acquired at low tide when possible in order to increase the accuracy of the classification study. Hierarchical unsupervised classification, coupled with filtering and contextual editing, was applied to each image. In addition, groundtruthing (with a handheld GARMIN GPS 76Cx, at an accuracy of ± 10 m) was also conducted at randomly chosen sampling points. GPS-tagged field photographs and videos were also taken during low tide conditions in order to relate image data to real features on the ground and therefore minimise errors in the classification.

2.3. Results

2.3.1. Seagrass diversity and distribution in Singapore.

A total of 12 species of seagrass were recorded at 32 sites in Singapore (Table 2.1). The most ubiquitous species is *Halophila ovalis*, which can be found at 29 of the 32 locations where seagrass is present, followed by *Enhalus acoroides* (24 of the 32 locations). The species that occur least frequently are *Halodule pinifolia*, *Halophila decipiens*, and *Halophila minor*.

Of these, *Halodule pinifolia* and *Halophila minor*/*Halophila ovata* exist only in herbarium records, as there has been no positive identification of either species during field surveys. A new addition to Singapore's seagrass diversity was made when a specimen of *Halophila decipiens* was discovered off Pulau Semakau in 2007 (SING 2008-273).

Table 2.1. The distribution of seagrass species by location. Data are compiled from records from the Singapore Botanic Gardens Herbarium (SING) and supplemented by field surveys conducted between 2006–2009. '**' refers to specimen/locations that only exist in Herbarium records; '+' denotes species observed during field observations (2007–2010); and '#' indicates field observations that have been corroborated with a lodged herbarium specimen. Species codes: CR = *Cymodocea rotundata*; CS = *Cymodocea serrulata*; HP = *Halodule pinifolia*; HU = *Halodule uninervis*; SI = *Syringodium isoetifolium*; EA = *Enhalus acoroides*; HB = *Halophila beccarii*; HD = *Halophila decipiens*; HM/HV = *Halophila minor* / *Halophila ovata* (*H. minor* and *H. ovata* are considered synonyms of each other and thus combined in this study [Short & Waycott, 2010, Short et al., 2010]); HO = *Halophila ovalis*; HS = *Halophila spinulosa*; TH = *Thalassia hemprichii*; P. = pulau (island); T. = terumbu (reef).

LOCATIONS	FAMILY/SPECIES											
	Cymodoceaceae					Hydrocharitaceae						
	CR	CS	HP	HU	SI	EA	HB	HD	HM/HV	HO	HS	TH
Western Johore Straits												
Tuas						+				+		
Lim Chu Kang Mangroves							#					
Sungei Buloh + Kranji						+	#					
Mandai							+					
P. Pergam						*		*	*	*	*	
Eastern Johore Straits												
Pasir Ris Park										#		
Changi				+		*		#		+	+	
Tanah Merah	+					+				+		
P. Ubin	#		*	#		#	#			#	#	#
P. Sekudu						+				#	+	
P. Tekong						+				+		
Beting Bronok										+		
Southern Mainland												
Labrador Beach						#				+		+
Belayer Creek										+		
East Coast Park				+		+				+		
Southern Offshore Islands												
P. Belakang Mati (Sentosa Is.)				+		+				#		
P. Tekukor										#		#
P. Sikajang Pelepah (Lazarus Is.)										+		+
P. Sikajang Bendera (St. John's Is.)										+		
P. Tembakul (Kusu Is.)						+				+		
P. Hantu						+				+		
P. Semakau		#		#	#	#		#		#		#
P. Jong										+		+
P. Sudong						+				+		
P. Pawai	#	#		#	+	#				#		#
P. Biola				#		#				#		#
P. Satumu (Raffles Lighthouse)						+				#		
Southern Patch Reefs												
T. Pandan (Cyrene Reef)	#	#		#	#	#		+		#		#
T. Pempang Laut					+	+				+		+
Beting Bemban Besar		+			+	+				+		+
T. Raya						+				+		
T. Semakau		+				+				+		

There appears to be a north–south dichotomy in the distribution of some species, with *Halophila beccarii* and *Halophila spinulosa* restricted only to locations in the north, and *Cymodocea serrulata* being restricted only to offshore southern islands and reefs. The largest seagrass meadows in Singapore are those found at Pulau Semakau, Cyrene Reef, and Chek Jawa, although sizeable beds are also present at Changi, Pasir Ris, Pulau Sekudu, Labrador Beach, and Pulau Pawai (Fig. 2.1). The three locations with the highest number of species of seagrass are also Chek Jawa, Pulau Semakau, and Cyrene Reef (Table 2.1).

2.3.2. Extent of Singapore's three largest seagrass meadows.

The total extent of the three mapped seagrass meadows (Fig. 2.2) was approximately 33.7 ha, with the largest meadow found at Cyrene Reef (14 ha), followed by Pulau Semakau (13.7 ha) and Chek Jawa (6.5 ha).

Pulau Semakau has the largest continuous seagrass meadow in Singapore, measuring approximately 2 km in length along the intertidal on the western shore of the island (Fig. 2.2A). This meadow is predominantly *Enhalus acoroides*, interspersed with a mix of *Cymodocea serrulata*, *Syringodium isoetifolium*, and *Thalassia hemprichii*. Smaller patches of seagrass (mainly pioneering *Halophila ovalis*) are found elsewhere in the intertidal zone. No seagrass meadows on the reefs adjacent to Pulau Semakau were detected from the satellite images, but ground surveys showed there are some seagrass on these reefs (Table 2.1).

In comparison, the results indicate that seagrass cover at Chek Jawa is patchy with cover more dense in some areas (such as in the southern end) than others (Fig. 2.2B). However, groundtruthing indicated that the aboveground seagrass cover at

Chek Jawa is actually quite homogeneous, but species composition varies, with the southern end dominated by *Cymodocea rotundata* and the rest of the meadow composed of a mix of *Halophila ovalis* and *Halodule uninervis*.

At 14 ha, Cyrene Reef is the largest seagrass meadow in Singapore (Fig. 2.2C). Seagrass cover at Cyrene Reef is mostly concentrated on the reef top in the centre of the reef, although seagrass is also found growing close to the reef edge in a coral rubble area. Similar to Chek Jawa, results indicate that the seagrass cover at Cyrene Reef is not continuous, but instead is comprised of a number of large patches. The seagrass meadow at Cyrene Reef is multi-specific, predominantly made up of *Enhalus acoroides*, *Cymodocea serrulata*, *Syringodium isoetifolium*, and *Thalassia hemprichii*. Other species found at Cyrene Reef are *Cymodocea rotundata*, *Halodule uninervis*, and *Halophila ovalis*.

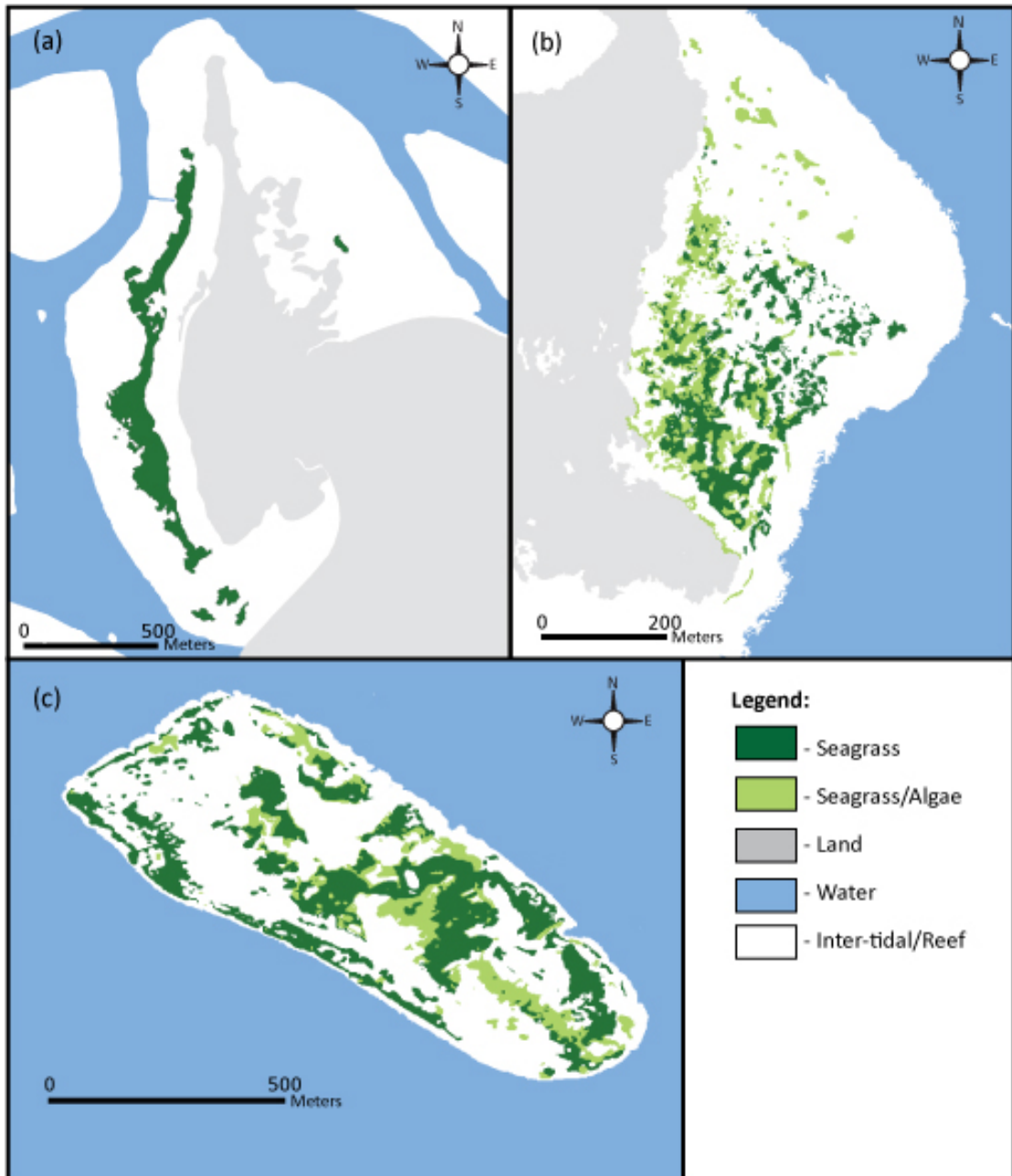


Figure 2.2. Maps generated from satellite images of Singapore's three largest seagrass meadows. A, Pulau Semakau; B, Chek Jawa, Pulau Ubin; and C, Cyrene Reef. Please see Fig. 1.2 for their locations with respect to Singapore Island.

2.4. Discussion

Despite its limited coastal waters, Singapore's total seagrass species diversity is comparable to that of neighbouring countries such as Malaysia, Indonesia, and Thailand. However, while seagrasses occur on many sandy shores and mudflats in Singapore, the species richness at these sites tends to be low, usually comprising of

Enhalus acoroides and *Halophila ovalis* (two of the most common species). There are only a few areas in Singapore where seagrasses form substantial and multi-specific meadows, the largest of which are Chek Jawa, Pulau Semakau, and Cyrene Reef.

It is interesting to note that two species, *Halodule pinifolia* and *Halophila minor*/*Halophila ovata*, only exist as herbarium specimens. Both these species were recorded as part of Singapore's seagrass flora in previous literature (Loo et al., 1996) but are conspicuously missing in recent field surveys. This absence may stem from the fact that there are some contentions regarding the legitimacy of both species. *Halophila minor* and *Halophila ovata* are both thought to be small-leaved variants belonging to the *Halophila ovalis* species complex, whereas *Halodule pinifolia* is thought to be the narrow-leaved version of *Halodule uninervis* (Waycott et al., 2004). Studies on the genetics of both these taxa have failed to lend support to *Halodule pinifolia* and *Halophila minor*/*Halophila ovata* being distinct species (Waycott et al., 2006; Lucas et al., 2012). In light of this evidence, it is possible that the herbarium specimens are actually wrongly identified. Unfortunately, an attempt to re-determine all of the specimens failed as some of the key identifying features of these species do not preserve well when dried.

The present study represents the first attempt at quantifying the extent of seagrass habitats in Singapore waters through remote sensing. As no previous areal estimates exist for seagrass habitats, this represents a baseline. Naturally, the total extent of the larger seagrass meadows in Singapore is small when compared to those of neighbouring countries (Green & Short, 2003), as there are only a few sites where seagrasses form substantial meadows. Yet, despite the lack of large meadows, there

exists a diversity of seagrass habitat types. In tropical regions such as North-Eastern Australia, seagrass habitats are typically classified as estuarine, coastal, reef-associated or deep-water meadows (Carruthers et al., 2002), and three of these meadow types can be found in Singapore.

The seagrass meadows found in the waters to the north of Singapore Island tend to be estuarine and mangrove associated, whereas meadows found among the Southern Islands are reef associated and found on reef tops, such as Cyrene Reef, or intertidal/coastal meadows like Pulau Semakau. This is expected as there are large river catchments on the Malay Peninsula that drain into the northern straits between Singapore and Malaysia, such as the Johor River that discharges into the area between the eastern tip of Pulau Ubin and Pulau Tekong in the northeastern part of Singapore. On the other hand, the islands and reefs off the southern coast of Singapore are geologically distinct from the northern coast (Lu et al., 2005), with considerably less freshwater influence. This variety of seagrass habitat types should help support high biodiversity because while ecosystem functions of seagrass meadows generally remain the same, the different combinations of species composition, sediment type, and proximity to other marine habitats generally results in different meadow types supporting different suites of flora and fauna (Heck & Wetstone, 1977; Livingston, 1984; Lee et al., 2012a).

Each of the three seagrass meadows mapped presented a unique challenge for the remote sensing exercise. We encountered a sub-pixel effect (Doerffer et al., 1989) occurring for sites with smaller-leaved species such as *Halophila ovalis* and *Halodule uninervis*, which may be the cause for patchiness observed in the processed imagery. In such locations, the total leaf area of these small species within a 2 × 2

m2 WorldView-2 pixel may be too sparse for detection. Instead, the net signal from a pixel will be dominated by the underlying sand/substrate. In the case of Chek Jawa, the issue of sub-pixel patchiness may lead to an underestimation of the actual extent of the meadow. Sub-pixel patchiness remains a challenge in remote sensing at varying spatial scales, particularly when oceanographic features are concerned (Lee et al., 2012b).

While Cyrene Reef and Pulau Semakau share a similar suite of species, seagrass cover at the two sites is different. Cyrene is a reef-associated meadow and it is not unusual for these meadow types to have micro-habitats such as sand mounds, sand bars, and coral rubble interspersed within the seagrass cover (Carruthers et al., 2002). Its location on a reef also influences the sediment composition present, with a large percentage of sand and coral rubble that the seagrass grows on. In contrast, Pulau Semakau is an intertidal seagrass meadow with a landward edge and is likely to have terrigenous and mangrove influences in its sediment mix, which appears conducive to the growth of both *Enhalus acoroides* and *Thalassia hemprichii* (Ertemeijer, 1994; Tanaka & Kayanne, 2007).

2.5. Conclusions

Twelve species of seagrass have been recorded in Singapore, half the number found in the Indo-Pacific (Ooi et al., 2011). However, two species, *Halodule pinifolia* and *Halophila minor*/*Halophila ovata*, have not been encountered during field surveys for many decades, casting doubt on their present status. We compiled a list of locations supporting seagrasses and the species found there (Table 2.1) which, together with the maps of the three largest seagrass meadows, provides a baseline for detecting change over time.

CHAPTER 3. COURAGE UNDER FIRE: SEAGRASS PERSISTENCE ADJACENT TO A HIGHLY URBANISED COASTAL CITY²

3.1. Introduction

Coastlines worldwide are undergoing rapid urbanization and development. In East Asia alone, there are 12 coastal cities with a population of more than 15 million (Gill & Kharas, 2007) that are still expanding, placing an unprecedented strain on natural nearshore resources and habitats (Yeung, 2001). The impacts of development, such as increased pollution, eutrophication and sedimentation caused by construction, reclamation and dredging, are major threats to coastal marine ecosystems such as coral reefs and seagrass meadows (Hughes et al., 2003; Waycott et al., 2009; Grech et al., 2012). Seagrasses are habitat forming marine angiosperms that are common in shallow coastal waters. They provide a range of valuable ecosystem services (Costanza et al., 1997) but are being degraded at an alarming rate with associated reductions in their diversity, resilience and ecosystem functions (Orth et al., 2006; Grech et al., 2012). Without appropriate management, the reported widespread loss of seagrass habitats is predicted to continue (Waycott et al., 2009).

Singapore is a highly urbanised island city state located at the southern tip of the Malay Peninsula and comprises of one main island and more than 60 smaller islands. Despite her small size and limited natural resources, Singapore is an economic powerhouse in the region with a per capita GDP that rivals most of the developed world (Department of Statistics Singapore, 2013). With a total land area of 714.3 km² and a population of 5.3 million, Singapore is representative of the types of changes and challenges that are currently facing many other coastal cities

² This chapter has been published as Yaakub, S.M., McKenzie, L.J., Erftemeijer, P.L.A., Bouma, T., Todd, P.A., 2013. Courage under fire: Seagrass persistence adjacent to a highly urbanized coast. *Marine Pollution Bulletin* (in press).

experiencing rapid expansion and industrialization. Due to Singapore's equatorial setting and its vicinity to the Coral Triangle, it supports a wide variety of nearshore habitats including mangroves, coral reefs and seagrass meadows, all of which sustain high biodiversity (Huang et al., 2006, 2009; Chou, 2008; Lee et al., 2012).

During the late 1960s to 1970s, Singapore initiated a number of large-scale land reclamation projects to ease the burden of land scarcity coupled with rapid population growth. This systematically obliterated a large proportion of coastal habitats (Todd & Chou, 2005), mostly through seaward expansion from the southeastern mainland and also the amalgamation of a group of 11 islands just off the southwestern coast. In total, land reclamation has resulted in the loss of an estimated 60% of coral reef area and 95% of mangroves (Chou, 2008) but, to date, the loss of seagrass meadows has not been documented. Mangroves are represented as forest on Singapore's maps and corals reefs are clearly delineated due to their potential as a shipping hazard. Seagrass meadows have traditionally not been treated as a navigational hazard; hence, historical records of their distribution are scarce, although they have been described as "common" on Singapore shores (Chuang, 1961). In this paper, we examine the factors that contribute to the survival of seagrasses in Singapore's busy port waters. We explore the history of local seagrass meadows from pre-reclamation to present day through a historical reconstruction of their past distribution and diversity using a multi-method approach. We then determine the current state of seagrasses in Singapore through analysis of a long-term monitoring dataset for the three largest seagrass meadows. Lastly, we identify present and future threats to seagrass habitats and make recommendations for management of seagrass resources in highly urbanised coastal areas.

3.2. Materials and Methods

3.2.1. Historical reconstruction.

We reconstructed the distribution and diversity of seagrass meadows in Singapore by triangulating (LeCompte & Preissle, 1993) three main lines of evidence: herbarium records, topographic maps and research interviews. We identified all seagrass specimens collected in Singapore prior to 1970 from three herbaria that were determined to have seagrass collections from Singapore: the Singapore Herbarium at the Singapore Botanic Gardens, the Herbarium Pacificum of the Bernice Pauahi Bishop Museum in Honolulu, Hawaii and the National Herbarium of the Naturalis Biodiversity Centre in Leiden, The Netherlands. From these specimens, we identified gazetteer location and species, and secondary site-specific information (e.g. sediment type, meadow size) where available. For each gazetteer location, we listed the species that occurred in that location to reconstruct meadow type and species composition, supplemented with site descriptions from den Hartog (1970). The gazetteer locations of herbarium specimens were then overlaid on maps of the Survey Department of the Federation of Malaya from 1946 and 1969. In the vicinity of gazetteer locations, we identified intertidal geographical features such as reef flats, mudflats, sand bars and sand shoals that had the potential to support seagrass growth. To explore another avenue for historical information, we sought out people that utilized seagrass meadows, including recreational and subsistence fishers, naval officers and former residents of villages that existed at locations that were near reclaimed areas, and conducted purposive interviews (Guest, 2006). We interviewed six people between the ages of 50 and 73 who fitted the above profile with the purpose of extracting information such as the approximate locations where seagrass meadows were found, their extent, how they were utilised, the time period in which they frequented these meadows and other nearby or co-occurring habitats.

Respondents were given visual aids (photos of seagrass species and seagrass meadows) and presented with an old map of Singapore to help them identify areas where they utilised seagrass meadows.

3.2.2. Predicting seagrass extent.

By overlaying the herbarium gazetteer locations and information from interviews on intertidal geographical features on maps, we were able to determine the approximate intertidal areas that supported seagrass meadows in the past (circa 1964–1970). To ensure that the historical reconstruction was representative of the full extent of seagrass in the past, we also took into account present day distribution of seagrass in Singapore (from Yaakub et al., 2013a) and included locations that have not undergone coastline modification or reclamation, as these were likely to have also supported seagrass in the 1960s (even if they were not represented by herbarium specimens). Each location was assigned into three base categories of seagrass meadows based on species composition, habitat type and geomorphologic association: i.e. sand/mud flat, fringing reef, and reef platform. These categories were based on an earlier assessment of seagrass meadows in Singapore (Yaakub et al., 2013a) and in accordance with previous studies (Carruthers et al., 2002; Waycott et al., 2004). The intertidal area for each site was estimated using ArcGIS® (Environmental Systems Research Institute) from a 2011, 1:20,000 map. The boundary for each intertidal sand/mudflat and reef platform followed that which was demarcated on the map. For areas that have been reclaimed, we calculated the lost area from a 1946 1:63,360 map of Singapore, using the squares method after Hilton and Manning (1995). As it is unlikely that seagrass would occupy the entire intertidal area, we calibrated the extent of seagrass by applying an occupation estimate. This was obtained by dividing the actual area of the seagrass meadow by the actual size

of the intertidal area in order to obtain an occupation proportion. We calculated the occupation proportion from existing literature for Singapore (Yaakub et al., 2013a) supplemented with additional field mapping of eight seagrass meadows and obtained an average that is typical of each of the three meadow classifications established in the previous paragraph. This occupation proportion was then applied to the intertidal areas identified earlier to obtain a retrospective prediction of seagrass extent in Singapore.

3.2.3. Seagrass-Watch monitoring data.

Status and trends in present-day Singapore seagrass abundance were determined over five years of monitoring (2007–2012) using the Seagrass-Watch protocols (McKenzie et al., 2009). Seagrass-Watch is a participatory monitoring program, a component of which encourages citizen science by empowering people with the knowledge and skills needed to collect data using scientifically rigorous methods. In Singapore, the data are collected by TeamSeaGrass, a group of trained volunteers collaborating with the National Parks Board. In 2007, three monitoring locations: Chek Jawa, Pulau Semakau and Cyrene Reef (Fig. 3.1) were established within intertidal seagrass communities at locations representative of Singapore's varying habitat characteristics e.g. island fringing reef, coastal/estuarine lagoon and patch reefs. These are also three of the largest seagrass meadows. Within locations, 2–3 replicate sites (50 m × 50 m), 200–250 m apart, were permanently marked with stakes and GPS coordinates and surveyed three to four times per year (every three to four months).

At each site, during each monitoring event, observers recorded the percent seagrass cover within a 50 cm × 50 cm quadrat every 5 m along three 50 m transects, placed

25 m apart, running perpendicular to the shoreline. A total of 33 quadrats were sampled per site. Details of the Seagrass-Watch monitoring protocols are described in McKenzie et al. (2000, 2003). Data collected from each survey was compiled and submitted to Seagrass-Watch HQ where program scientists checked the data for compliance and provided an independent assessment of their accuracy and quality. For this study, we averaged the above ground percent cover data for each of the three locations to obtain a general trend of seagrass cover between 2007 and 2012.

3.2.4. Vulnerability analysis.

A vulnerability analysis (VA) was conducted in July 2012 during a workshop with 39 participants from Singaporean academic and research institutions, government agencies and non-government organisations. Workshop participants had backgrounds in biology, ecology and management of coastal areas and worked or intended to work on seagrass habitats around Singapore. Workshop participants were divided randomly into groups and asked to first identify anthropogenic activities that affect or threaten seagrass in Singapore waters. To collect and quantitatively synthesise opinion on the ecological effects of anthropogenic threats to seagrasses, we used a systematic and standardised method (from Halpern et al., 2007; Selkoe et al., 2008), scoring each threat with respect to five vulnerability factors. In contrast to most other threat ranking exercises, this method provides detail on the reasons behind a particular ranking of threats to an area and why the ranking may vary across the ecological landscape (Grech et al., 2011).

To assess the relative vulnerability of seagrass to each threat, we used vulnerability factors with attributes of how a threat affects a seagrass ecosystem, including: spatial scale, frequency, functional impact, resistance of the ecological community to

the threat, and recovery time needed for the affected aspects of the community to return to their 'natural' or previous state following removal or remediation of the threat (Halpern et al., 2007). Using a ranking system, Spatial scale was scored from 1 (<1 km²) to 6 (>10,000 km²) based on a logarithmic system; Frequency from 1 (rare) to 4 (persistent); Functional Impact from 1 (single species) to 4 (entire community); Resistance from 1 (high resistance) to 3 (low resistance); and Recovery time from 1 (<1 year) to 4 (>100 years). A score of 0 represented No impact or Not applicable (Table 3.1). Scores for each threat's vulnerability factor were then rescaled so that all factors had the same range of values (as per Selkoe et al., 2008) and a single mean vulnerability score calculated (Grech et al., 2012).

Table 3.1. Scoring system for the five vulnerability factors (based on Halpern et al., 2007 and Grech et al., 2011).

Score	Scale	Frequency	Components of vulnerability			Certainty
			Functional Impact	Resistance	Recovery time	
0	No impact	Never occurs	No impact	Not applicable	No impact	Not at all certain
1	<1km ²	Rare	Single species	High resistance	<1 year	Low certainty
2	1 – 10 km ²	Occasional	Single tropic level	Moderate resistance	1 – 10 years	Moderate certainty
3	10 – 100 km ²	Annual or regular	Multiple tropic level	Low resistance	10 – 100 years	High certainty
4	100 – 1,000 km ²	Persistent	Entire community		>100 years	Very certain

3.3. Results

3.3.1. Historical reconstruction.

There were 78 records of seagrasses belonging to 11 species lodged in the three herbaria collected prior to 1970. These species were: *Cymodocea rotundata*, *C. serrulata*, *Halodule pinifolia*, *Halodule uninervis* and *Syringodium isoetifolium* from the Family Cymodoceaceae; and *Enhalus acoroides*, *Halophila beccarii*, *Halophila minor*, *Halophila ovalis*, *Halophila spinulosa* and *Thalassia hemprichii* from the Family Hydrocharitaceae. From the herbarium records, 14 gazetter locations were

identified that had geographical features that historically supported seagrass meadows (Fig. 3.1a), and an additional seven secondary locations were identified as having had seagrass meadows through interviews (Fig. 3.1a). Forty of the 78 herbarium seagrass specimens were collected from the east coast of Singapore, which was identified on the 1946 map as a large sandy shoal (Fig. 3.1a) and all six interview respondents confirmed that there had been an extensive meadow in this area. Herbarium specimens from this location included seven species, suggesting that this was a multi-species meadow (Table 3.2, Fig. 3.1a). Seagrasses could also be found on intertidal reef flats of almost every offshore island up to the late 1960s (Table 3.2, Fig. 3.1a). Although not represented through herbarium records, we identified a further 14 locations that were likely to have supported seagrass in the past (Table 3.2) due to the fact that they have not undergone extensive coastal modification and currently support seagrass meadows (Fig. 3.1b). These locations will be included in the calculations of predicted past extent in the following section.

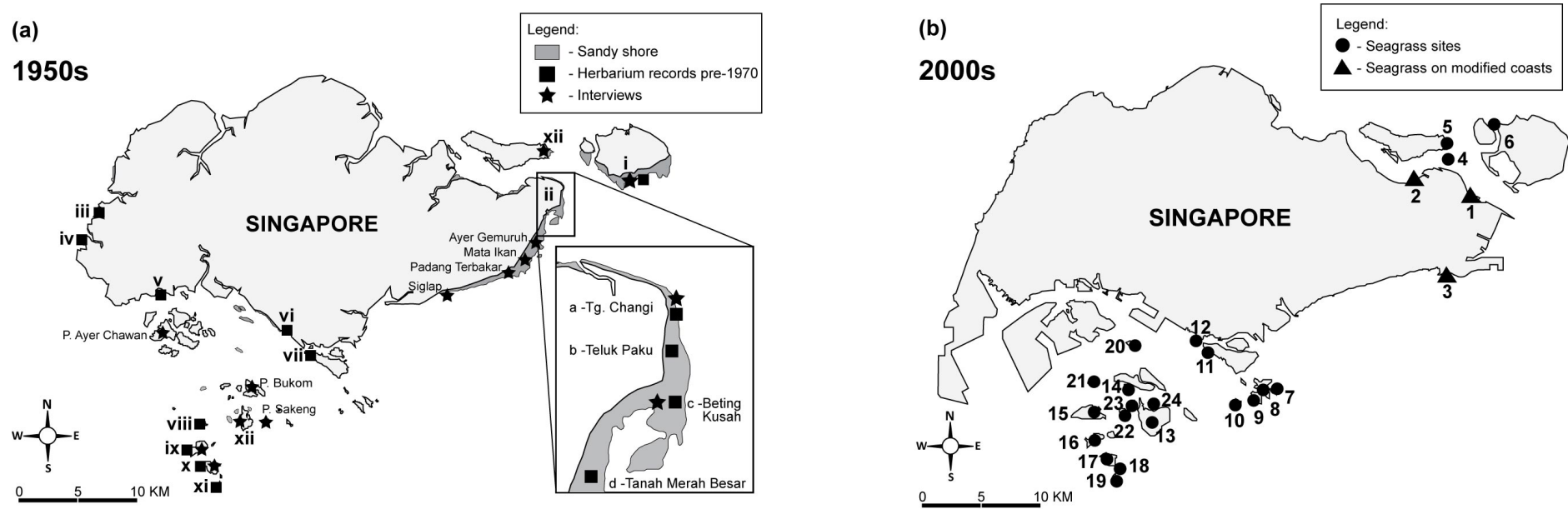


Figure 3.1. Map of Singapore (a) circa-1950, pre-major coastal modifications with historical distribution of seagrasses. Squares refer to herbarium gazetteer locations prior to 1970 and stars refer to sites identified as seagrass meadows through interviews. Locations are numbered and corresponding names can be found in Table 3.2. Note that location marked (ii) on the map comprises four gazetteer locations that have been combined in Table 3.2 and (b) from 2011 with present day seagrass distribution as per Table 3.2. Seagrass sites are as per Yaakub et al. (2013a), with circles indicating natural or unmodified coasts, and triangles indicating areas that have newly formed intertidal areas from reclamation.

Table 3.2. The historical distribution and extent of sites with seagrass meadows pre-1970s, showing meadow species composition and habitat descriptions. Locations are listed first according to sites that appear in herbarium records pre-1970 and correspond to Fig. 3.1. The remaining sites listed correspond to locations in Fig. 3.2, as listed in Yaakub et al. (2013a) and present day (post 1970 – present) herbarium records. P = Pulau (Malay for island), Tg. = Tanjung (Malay for headland/cape), T = Terumbu (Malay for reef), B = Beting (Malay for shoal/sandbank). Species composition is based on herbarium records and records in den Hartog (1970). Habitat descriptions with 1 derived from descriptions in den Hartog (1970); 2 from geographical features of 1946 1:63, 360 map of Singapore; and 3 from oral interviews.

Herbarium gazetteer location (pre-1970)	# on map (Fig.1)	Species Composition	Habitat description	Original intertidal area		Area lost from reclamation		
				Total inter-tidal (ha)	Predicted seagrass (ha)	Inter-tidal lost (ha)	Predicted seagrass loss since 1970 (ha)	Predicted remaining seagrass area (ha)
Sand/Mud flats								
P. Tekong	i	HO, HS	Sand/mud with sub-tidal meadows to 5m ¹	431	129.3	265.4	79.6	49.7
^a Tg. Changi, ^b Teluk Paku, ^c Beting Kusah, ^d Tanah Merah Besar	ii	CR, CS, EA, HP, HU, HB, HM, HO, HS, SI, TH	Sand/mud flats ^{1, 2} , extensive and dense ^{1, 3}	88.3	26.5	88.3	26.5	0.0
Bajau	iii	EA	Mud flat ²	14	4.2	14.0	4.2	0.0
Pasir Laba	iv	HM, HO, TH	Mud flat ¹	23.7	7.1	23.7	7.1	0.0
P. Samulun	v	CS, EA, HO	Mud flat ¹	8.9	2.7	8.9	2.7	0.0
Fringing reef flat								
Labrador (incl Pasir Panjang Beach)	vi	EA, HM, HO, HP	Rocky shore with fringing reef flat ²	26.3	1.8	24.7	1.7	0.1
P. Belakang Mati	vii	HM	Sandy shore ¹ with fringing reef ²	138	9.7	135.4	9.5	0.2
P. Sudong	viii	SI	Fringing reef ²	230	16.1	167.7	11.7	4.4
P. Pawai	ix	HU, HM	Sandy shore ¹ with fringing reef ^{2, 3}	138.4	9.7	0	0	9.7
P. Senang	x	EA, HU, HO, HP, TH	Sandy shore ¹ with fringing reef ^{2, 3}	97.8	6.8	0	0	6.8
P. Satumu (Raffles Lighthouse)	xi	HO	Fringing reef ²	0.8	0.1	0	0	0.1
Sub-total					214.0		143.0	71.0
Present day sites in Singapore likely to have existed pre-1970	# on map (Fig. 2)	Species composition	Habitat description	Total inter-tidal (ha)	Predicted seagrass (ha)	Inter-tidal lost (ha)	Predicted seagrass loss since 1970 (ha)	Predicted remaining seagrass area (ha)
Sand/Mud flats								
P. Ubin	2	CR, HU, EA, HB, HO, HS, TH	Sand/Mud flat	93.1	27.9	57.1	17.1	10.8

P. Sekudu	3	EA, HO, HS	Sand flat/shoal	12.8	3.8	0.0	0.0	3.8
Fringing reef flat								
P. Biola	14	HU, EA, HO, TH	Fringing reef	7.3	0.5	0.0	0.0	0.5
P. Semakau	15	CS, HU, SI, EA, HD, HO, TH	Sandy shore with fringing reef	139.4	9.8	19.9	1.4	8.4
P. Hantu	18	EA, HO	Fringing reef	25.6	1.8	0.0	0.0	1.8
Sisters Island	22	EA, HO	Fringing reef	7.2	0.5	0.0	0.0	0.2
St. Johns Island	23	EA, HO	Fringing reef	7.1	0.5	0.0	0.0	0.5
Lazarus Island	24	HO, TH	Fringing reef	10	0.7	0.0	0.0	0.7
P. Kusu	25	EA, HO	Fringing reef	5	0.4	0.0	0.0	0.4
Patch Reefs								
B. Bemban Besar	16	CS, SI, EA, HO, TH	Sandy reef-top	60.6	26.1	0.0	0.0	26.0
T. Raya	17	EA, HO	Sandy reef-top	39.3	16.9	0.0	0.0	16.9
T. Pempang Laut	19	SI, EA, HO, TH	Sandy reef-top	39.9	17.2	0.0	0.0	17.2
T. Semakau	20	CS, EA, HO	Sandy reef-top	26.2	11.3	0.0	0.0	11.3
T. Pandan (Cyrene Reef)	21	CR, CS, HU, SI, EA, HD, HO, TH	Sandy reef-top	51.5	22.1	0.0	0.0	22.2
Sub-total					139.6		18.5	120.7
Total original predicted seagrass area (ha)					353.5	Predicted seagrass loss	161.5	
Newly formed intertidal areas with seagrass	# on map (Fig. 2)	Species composition	Habitat description			Created intertidal area (ha)	Predicted seagrass area (ha)	
Changi Beach	1	HU, EA, HD, HO, HS	Sandy shore			3.9	1.2	
Pasir Ris Beach	2	HO	Sandy shore with mudflat			3.4	1.0	
Tanah Merah Beach	3	CR, EA, HO	Sandy shore			26.0	7.8	
Sub-total						33.3	10.0	
Total remaining seagrass area (ha)							201.7	

3.3.2. Predicted extent of seagrass in Singapore: past and present.

Based on our field surveys, the average occupation percentage of seagrass on intertidal habitats is 30% for sand/mud flats, 7% for fringing reef flats, and 43% for patch reef platforms (Table 3). These occupation percentages were applied to the locations identified to have supported seagrass meadows historically (Fig. 3.1a; Table 3.2), plus three locations that were created during the process of land reclamation (Fig. 3.1b, Table 3.2). The hindcast for the predicted extent of seagrass meadows prior to 1970 was approximately 353.5 ha (Table 3.2), of which 45.7% (161.5 ha) has since been lost to either coastal modification or land reclamation (Table 3.3, Fig. 3.1a and b). The present day extent of seagrass in Singapore is estimated to be approximately 201 ha, encompassing the three new locations that were not included in the historical hindcast as these were newly formed intertidal areas (a result of reclamation) that did not exist before 1970 (Fig. 3.1b, Table 3.1).

Table 3.3. Calculation of occupation proportion based on the mapped extent of seagrass meadows divided by the total intertidal extent of the site. Sites denoted with # are mapped manually and sites marked with / were mapped using GIS, taken from Yaakub et al. (2013a).

Site	Total inter-tidal area (ha)	Seagrass extent (ha)	Occupation proportion
Sand/Mud flat			
Changi Beach #	3.9	1.05	0.25
P. Sekudu #	12.8	4.6	0.36
Chek Jawa* (P. Ubin)	23.2	6.5	0.28
		Average for sand/mud flats	0.3
Fringing reef flats			
Lazarus Island#	10	0.2	0.02
P. Semakau*	120	13.7	0.11
P. Semakau #	120	11.9	0.10
Sisters Island #	7.2	0.2	0.03
St. Johns #	7	0.8	0.11
		Average for fringing reef flats	0.07
Fringing Reefs			
Cyrene Reefs*	45	14	0.38
Cyrene Reefs #	45	17.5	0.39
T. Raya #	39.3	20.1	0.51
		Average for patch reefs	0.43

3.3.3. Current state of seagrass meadows in Singapore.

Long-term monitoring of selected sites (Fig. 3.1b) over the past five years to determine the status and trends of seagrass in Singapore waters reveals some notable differences among habitats, both in species composition and abundance. At the Chek Jawa long-term monitoring site, seagrass abundance ranged from 25% to 65% between 2007 and 2012 (Fig. 3.2a). There has been little change between years with abundances remaining relatively stable after an initial moderate increase during the first 18 months of monitoring (Fig. 3.2a). Within calendar years, however, the abundance fluctuated seasonally, typically reaching maxima during the second quarter. Seagrass abundance at the Cyrene Reef long-term monitoring sites similarly fluctuated seasonally within years but showed a stable annual trend over the 2007–2012 monitoring period (Fig. 3.2b). The Pulau Semakau long-term monitoring sites showed the largest variation in seagrass abundance from 4% to 49% on average, with one site having substantially lower seagrass abundance. This was also the only location where seagrass abundance showed a marked decline at all three monitoring sites between 2007 and 2012 (Fig. 3.2c).

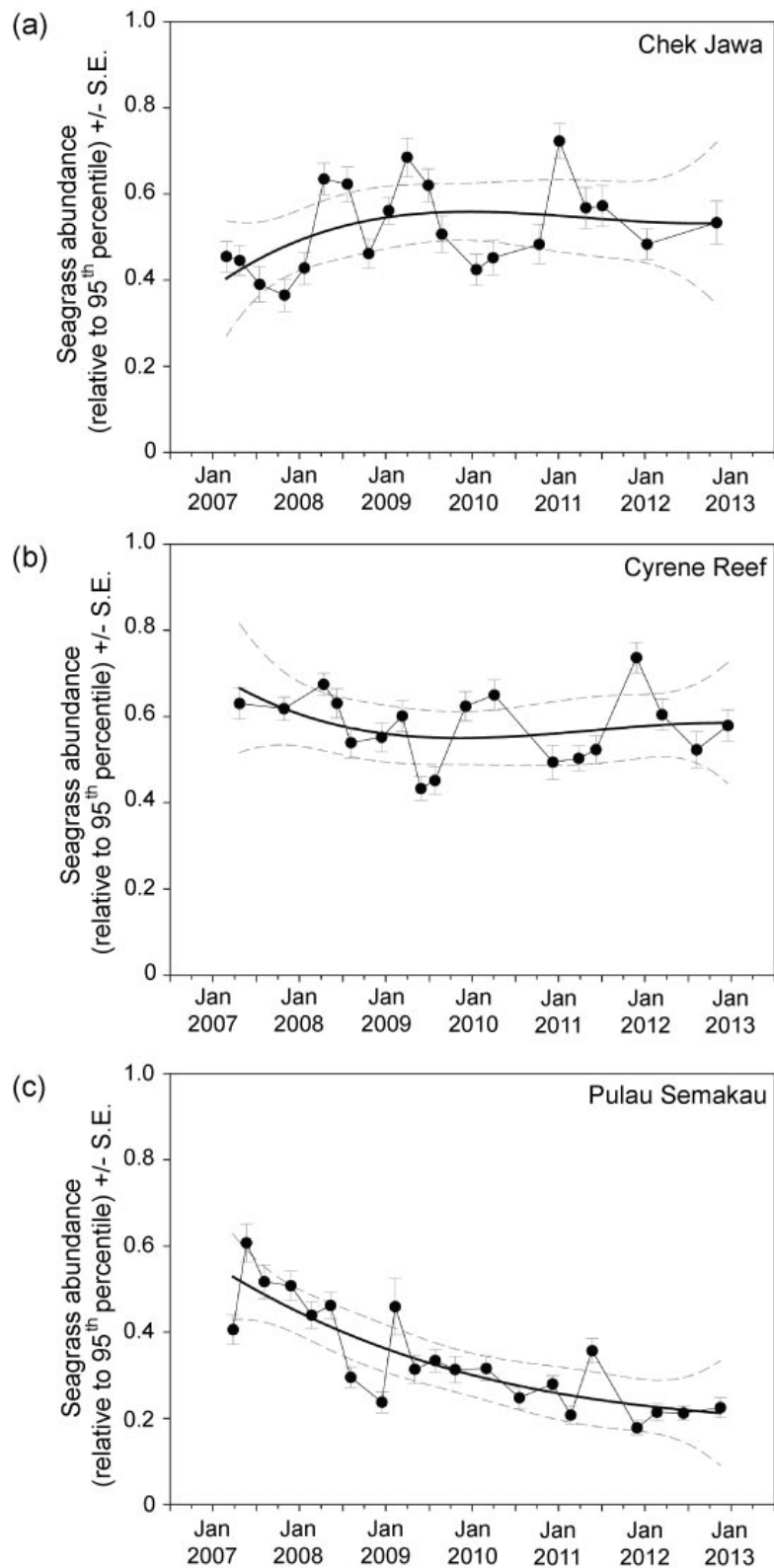


Figure 3.2. Generalised trends in seagrass abundance for each long-term monitoring location (sites pooled) relative to the 95th percentile (equally scaled). The 95th percentile was calculated for each site across all data. Trendline is 3rd order polynomial, 95% confidence intervals displayed, (a) Chek Jawa (2 sites), (b) Cyrene Reef (2 sites) and (c) Pulau Semakau (3 sites).

3.3.4. Vulnerability analysis.

The vulnerability analysis identified land reclamation as the greatest threat to seagrasses in Singapore (Table 3.4). Land reclamation had the highest relative impact score (3.5) ahead of nine other anthropogenic activities, with recreational netting/fishing having the lowest relative impact score (1.4). Port-related operations (e.g. dredging, shipping movements, recreational boating activities) constituted 40% of the total vulnerability score. Overall, certainty scores ranged from 2.0 to 3.3 suggesting a moderate level of certainty across the groups of participants in relation to their estimates of vulnerability scores for each of the threats. Workshop participants had a high certainty in their estimates of vulnerability scores for impacts from land reclamation, dredging, shipping accidents, and recreational netting (Table 3.4), but a low certainty for impacts from aquaculture/fisheries.

Table 3.4. Vulnerability and Certainty scores for 10 anthropogenic activities in Singapore derived from a vulnerability assessment conducted at a workshop. Activities are organised from the highest to lowest vulnerability scores.

Threat	Vulnerability (relative impact) score	Certainty
Land reclamation	3.5	3.3
Dredging	2.8	3
Urban runoff (incl. from Malaysia)	2.5	2.7
Shipping movements (ship wake/resuspension)	2.5	2.3
Boating activities (recreation/commercial) e.g. groundings	2.4	2.7
Shipping accident - oil spills	2.3	3
Industrial runoff	2.3	2.5
Tourism (trampling)	2	2.7
Aquaculture/Fisheries	2	2
Recreational netting/fishing	1.4	3

3.4. Discussion

This study is the first to reconstruct the historical distribution of seagrass in Singapore up to the late 1960s, which was previously only described as common (Chuang, 1961). An examination of the loss of seagrass meadows in Singapore was

previously hampered by a lack of information on the past and present distribution and, more importantly, extent of seagrass meadows in local waters. Our method of hindcasting seagrass extent by triangulating various information sources, which encompasses geography, biology and local and/or traditional knowledge, provides a simple, inexpensive and pragmatic approach for estimating original baselines in locations which are data deficient and where satellite imagery and aerial photographs are not available. While the disappearance of coral reefs and mangroves in Singapore has been well documented (Hilton & Manning, 1995; Chou, 2008), the loss of seagrass meadows has gone largely unnoticed, a trend which is common throughout Southeast Asia, reflecting the general paucity of knowledge with respect to seagrass meadows in one of the most speciose marine bioregions (Waycott et al., 2009; Ooi et al., 2011). Our estimated 45% loss of seagrass extent in Singapore provides a useful figure for comparing against losses of other marine habitats such as mangroves and coral reefs, which have suffered higher losses at 95% and 60% respectively (Chou, 2008). It is worth noting, however, that 45% seagrass loss in Singapore is likely to be an underestimation, as we did not include in the analysis, some sites that were identified as seagrass meadows in interviews, but which could not be triangulated with either geographical features, herbarium specimens or known in present day surveys as having seagrass meadows. Our analysis also did not include subtidal meadows although there is documented evidence of seagrasses up to 5 m depths, both historically (den Hartog, 1970) and in the present day (Yaakub et al., 2013a).

The main driver for loss of seagrass habitats in Singapore has been land reclamation, as indicated by their past and present distribution and also by the results of the Vulnerability analysis. Land reclamation is likely to remain a top threat

to seagrass meadows in Singapore, as the pressures of space constraints arising from an increasing population and urban expansion continue. While the impacts of land reclamation are immediate, tangible and generally irreversible (Duarte, 2002), impacts from dredging, urban runoff and shipping activities, are not immediately apparent and could manifest over a longer time scale. Dredging, for example, is a common practice in Singapore's shipping lanes (Chou, 1996), and has been shown to have a detrimental impact on seagrasses by increasing light attenuation and reducing overall water quality (Erftemeijer & Lewis, 2006). The proximity, intensity and frequency of dredging activities in Singapore waters is likely to be a major contributing factor that could compromise the continued persistence of seagrass meadows, with more frequent and intense dredging activities affecting the ability of seagrass to cope with additional stress (Yaakub et al., 2013b).

Despite these threats, our data shows that the seagrass cover at two of the three monitoring sites is stable or increasing, and that there has been no decrease in species diversity (Yaakub et al., 2013b). Given the relatively long history of coastal modification in Singapore's waters (Hilton & Manning, 1995), and the impact it has had on other sensitive marine habitats such as coral reefs (Dikou & van Woesik, 2006), it seems rather incongruous that seagrass meadows should still exist in Singapore waters. There are a number of factors that might explain their persistence, the first being that Singapore possesses substantial areas of suitable substrates in waters shallow enough to allow sufficient light penetration for seagrass growth (Dennison et al., 1993). Secondly, there are strict laws governing emissions and the treatment and disposal of waste and sewage, which prevents pollution on land and of water bodies that drain into the sea (Environmental Protection and Management Act of 1999, Chapter 94A). Port waters are also protected from shipping pollution by the

Prevention of Pollution of the Sea Act of 1990 (Chapter 243) administered by the Maritime Port Authority of Singapore (MPA), which has also implemented a series of programmes and initiatives to encourage responsible and environmentally friendly maritime practices (Maritime Port Authority of Singapore, 2013). Lastly, Singapore has very limited farming activity, thus reducing the potential for eutrophication of coastal waters through agricultural runoff. These factors appear to have acted in concert to successfully minimise industrial pollution and eutrophication of the marine environment in Singapore, which has been shown to be less susceptible to algal blooms compared to the nearby Johore Straits (Gin et al., 2006). The combination of eutrophic waters and high turbidity has been linked to the widespread loss of seagrasses worldwide (Cambridge & McComb, 1984; McGlathery, 2001; Burkholder et al., 2007; Freeman et al., 2008).

With rapid urbanisation of coastal areas, there is an urgent need to assess strategies for sustainable coastal development to ensure the continued existence and functioning of coastal habitats. A review of the impacts of dredging on seagrasses (Ertemeijer & Lewis, 2006) yielded several documented cases of decline, although not all occurred in urban waters. Hong Kong has had a long history of land reclamation (Glaser et al., 1991) and the construction of the airport at Chek Lap Kok was implicated for the decline and loss of seagrass habitats in nearby adjacent areas (Fong, 1999). Coastal development was also identified as the primary cause for the loss of *Zostera marina* and *Posidonia oceanica* meadows in Gibraltar (Bull et al., 2010) whereas, in Bahrain, it was the cumulative effects of coastal development and unregulated sewage outfall that caused widespread seagrass losses (Zainal et al., 2012). In comparison the persistence of seagrass in Singapore waters presents an anomaly and while it might seem like a good case study for urban conservation, in

reality there is minimal active management of seagrasses and other local marine habitats (as evidenced by the lack of marine protected areas, MPAs), and their continued existence is primarily an artifact of strict controls on anthropogenic discharge into Singapore waters (Gin et al., 2006).

Singapore lacks a basic legal framework that ensures that the demands of industry and infrastructure can be met in the most sustainable manner. Even though it is a signatory to various international conventions and agreements on biodiversity conservation and environment (Chua, 2006), Singapore currently has no legislation to protect its remaining marine ecological resources, and in this it falls behind neighboring ASEAN states such as Malaysia, Indonesia, the Philippines and Thailand, all of whom have adopted Environmental Impact Assessment (EIA) legislation (Briffett, 1999). In recent years, there appears to be a concerted effort on the part of governing bodies to conduct EIAs prior to major reclamation and dredging works, which may reflect a shift in government attitude (Chou, 2008) but these rarely incorporate a widely publicised stakeholder consultation component. Effective management of marine resources calls for a multi-disciplinary approach that engages all stakeholders due to the interdependent and interlinked nature of the marine environment (Chua, 2006). Singapore's National Biodiversity Strategy and Action Plan presented in 2009 outlines several key strategies for conserving biodiversity and integrated coastal management (National Parks Board, 2009) but it remains to be seen how these actions translate into real plans for marine conservation, when there is no formal documentation or framework for the implementation of such broad goals.

There is a general deficiency of information on the health, resilience, and long-term trends in seagrass abundance within the Southeast Asian and tropical Indo-Pacific

regions (Waycott et al., 2009). Seagrass meadows in this region are likely to be undergoing rapid changes (UNEP, 2004), given the expansion of major coastal cities such as Jakarta, Manila and Ho Chi Minh (Yeung, 2001) and the associated impacts to nearby environments from coastal modification, pollution and urban runoff (Turner et al., 1996; Todd et al., 2010). For example, there are documented examples of seagrass decline from eutrophication and pollution in Manila Bay in the Philippines (Lopez, 2000). Urbanisation is not just a threat to marine habitats in waters adjacent to large conurbations, but also to those in smaller towns and satellite cities that are being developed for industry or tourism (Chua et al., 1989; Wong, 1998). There is thus an urgent need to quantitatively assess seagrass resources in the region and determine how they are being managed with respect to land use change, infrastructure development, and subsequent water quality issues.

The most compelling reason for seagrass persistence in Singapore waters is that, despite the high turbidity and sedimentation from large-scale reclamations, these are not compounded with highly eutrophic waters from open sewage discharge or runoff. If this tight control of sewage and pollution discharge was complemented with a framework for managing future coastal development in Singapore, such as mandatory and transparent environmental impact assessments, seagrasses may continue to persist in Singapore's waters. The Singapore experience is important to coastal resource managers and planners elsewhere in the Asian tropics as it shows that diverse marine habitats can exist in busy port waters in the absence of multiple and cumulative anthropogenic impacts. However, the remaining seagrass meadows in Singapore are still under threat from future development plans as there are neither MPAs nor any environmental protection laws that pertain to conserving Singapore's marine biodiversity. There is a clear need for firm policies that translate Singapore's

existing “paper” plans for integrated coastal management and marine conservation into action.

CHAPTER 4. CHRONIC LIGHT REDUCTION REDUCES OVERALL RESILIENCE TO ADDITIONAL SHADING STRESS IN THE SEAGRASS *HALOPHILA OVALIS*³

4.1. Introduction

Seagrasses are important habitat-building marine angiosperms that grow in sheltered coastal waters in both temperate and tropical regions (Green & Short, 2003). They provide a range of ecosystem services such as nutrient cycling (Hemminga et al., 1999; McGlathery et al., 2007), nursery grounds for many fish and crustacean species (Bell & Pollard, 1989), food for endangered large grazers such as dugongs and turtles (Heck & Valentine, 2006), coastal protection by sediment accretion and stabilisation (Madsen et al., 2001; Gacia et al., 2003) and mitigating climate change through carbon sequestration (Duarte et al., 2005; Fourqurean et al., 2012) among others. The past five decades has seen a substantial loss and degradation of seagrass meadows worldwide, largely driven by declining water clarity from human activities (Orth et al., 2006; Waycott et al., 2009).

Light is an important limiting factor for seagrass growth, making seagrass meadows vulnerable to the effects of turbidity (Ralph et al., 2007). Most seagrass species are able to respond to reductions in ambient light levels by adjusting their physiology and/or morphology at the leaf level, before stress effects manifest at the meadow scale through changes in shoot density (Ralph et al., 2007; Collier et al., 2012). This indicates that seagrasses have the capacity to acclimate to a changing light environment.

³ This paper has been published as Yaakub, S.M., Chen, E., Bouma, T.J., Erftemeijer, P.L.A., Todd, P.A. (2013) Chronic light reduction reduces overall resilience to additional shading stress in the seagrass *Halophila ovalis*. Marine Pollution Bulletin (*in press*).

The capacity of seagrasses to acclimate to reduced light is largely related to the individual species' energy reserves, and their ability to mobilize these reserves to maintain a positive carbon-budget (Dennison & Alberte, 1985). Carbohydrate reserves allow seagrass plants to survive periods when there is no or too little photosynthetic activity to meet respiratory demands (Alcoverro et al., 2001). It has been suggested that species with larger rhizome stores are able to cope with periods of low light better than species with small rhizome stores (Alcoverro et al., 1999; Walker et al., 1999). More holistic and complex models incorporate the ability of seagrass plants to acclimate to changes in light availability through photo-physiological and morphological changes in order to improve the ratio between photosynthetic carbon gain and respiratory carbon losses (Ralph et al., 2007, McMahon et al., 2013). Understanding the ability of seagrasses to acclimate to light reduction is critical given that coastal environments are frequently subject to disturbances ranging from natural processes such as flood plumes and storms (Preen et al., 1995, Campbell & McKenzie, 2004), to anthropogenic coastal modification activities such as land reclamation and dredging (Erftemeijer & Lewis, 2006), all of which typically increase turbidity levels and sedimentation rates in proximate seagrass habitats.

The ability to acclimate to an altered light regime provides seagrasses with essential mechanisms to survive sub-optimal (i.e. low) light conditions. However, relatively little is known about how exposure to chronic sub-optimal light affects the plants' ability to cope with further temporal reductions in light. On the one hand, using carbohydrate reserves to maintain respiration and growth during periods of low light (Alcoverro et al., 2001) is expected to deplete storage reserves and therefore reduce resilience, leaving the plant more susceptible to further temporal light reduction. On the other

hand, seagrasses have been shown to alter their carbon budget through photo-physiological acclimation to align with metabolic demands under reduced light (Lee et al., 2007) and, in the process, create an adjusted light requirement threshold, which may increase overall resilience to further light reduction (Dennison & Alberte, 1986; Zimmerman et al., 1995). In order to better understand how chronic sub-optimal light affects seagrass ability to cope with additional temporary decreases in light availability, we examined the effects of shading on two monospecific *Halophila ovalis* meadows characterized by different ambient light regimes. We hypothesized that plants growing in chronic sub-optimal light environments are less able to cope with additional reductions in light availability.

4.2. Materials and Methods

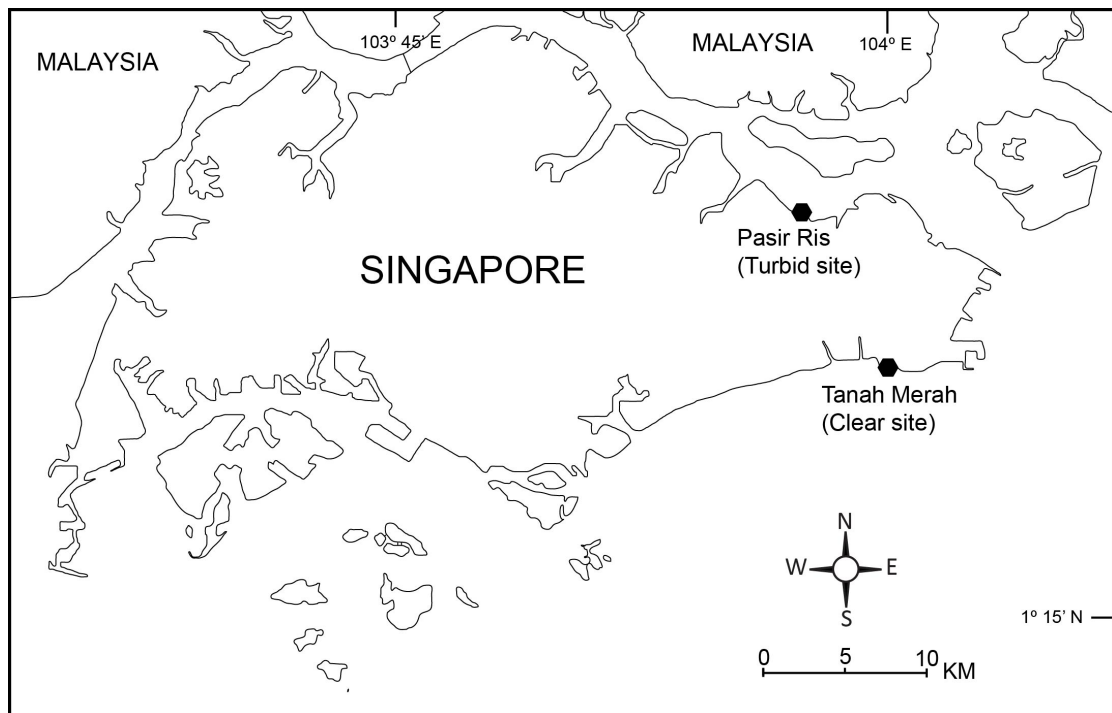


Figure 4.1. Map of Singapore showing the two study sites.

4.2.1. Study sites.

This study was carried out between December 2009 and February 2010. Two study sites, Pasir Ris Park Beach (1°22'56.40"N, 103°57'51.66"E) on the northeastern coast and Tanah Merah (1°18'41.08"N, 103°59'44.97"E) on the southeastern coast of mainland Singapore (Fig. 4.1) were chosen on the basis of having dense monospecific stands of the seagrass *Halophila ovalis* R. Br. (Hook) f. *Halophila ovalis* is widely distributed in the Indo-West Pacific (den Hartog, 1970) and is the most common seagrass species in Singapore waters (Yaakub et al., 2013a). It is distinguished by a pair of oval shaped leaves that arise directly from a rhizome and exhibits a wide tolerance to environmental perturbations and high morphological plasticity (Waycott et al., 2004). Compared to larger and late successional stage seagrass species, *H. ovalis* has a high turnover rate and responds to environmental stressors relatively quickly (Erftemeijer & Stapel, 1999).

Both study sites experience semi-diurnal tides with a tidal range between 0.1 and 3.2 m (MPA, 2008, 2009). We measured the area of the monospecific *H. ovalis* beds both at Pasir Ris, which was approximately 3000 m², and at Tanah Merah which was approximately 1300 m². Within each of these two meadows, a representative area with >75% percent cover measuring 375 m² (25 m × 15 m) was chosen as the experimental site. Based on regular observations of water clarity since 2007 (pers. obs. and this study), Pasir Ris Park Beach was characterised as a site with high turbidity, and is henceforth referred to as the “turbid site”. In contrast, Tanah Merah was generally much less turbid, and is henceforth referred to as the “clear site”. To check that the sites maintained these differences in turbidity, light loggers were deployed for the duration of the experiment.

To assess the extent to which seagrasses in both meadows had acclimated to ambient light conditions, the following parameters were measured before commencing the experiment: shoot density, leaf length, -width and -surface area, and maximum quantum yield (F_v/F_m). The methodology for quantifying the above-mentioned parameters is described below.

4.2.2. Experimental design.

Shading frames, measuring 1.0 m long × 1.0 m wide × 0.6 m high, were constructed from polyvinyl chloride (PVC) pipes and deployed at both sites. Pilot studies showed that dense shade nets typically used to create high shading levels are easily clogged by sediment in areas with high turbidity. To prevent such fouling, we designed a more open shading apparatus that consisted of multiple layers of plastic netting with a coarse (17 mm) mesh suspended on top of each other (Fig. 4.2). These sheets were cable-tied to the frames with 150 mm in between three layers to simulate moderate shading (~43% light transmission), and with 75 mm in between five layers to simulate high shading (~20% light transmission). For each layer, the plastic mesh was rotated 45° from the position of the adjacent layer to maximize the shading effect. The bottom mesh layer for the shaded treatments was always fixed at 0.3 m above the substrate to minimize hydrodynamic interference with the seagrass. Using multiple layers of plastic mesh allowed sediment to move more freely with the water, with no enhanced sediment accretion on plants or nets observed between shaded and control treatments. Control frames did not have any plastic mesh attached.

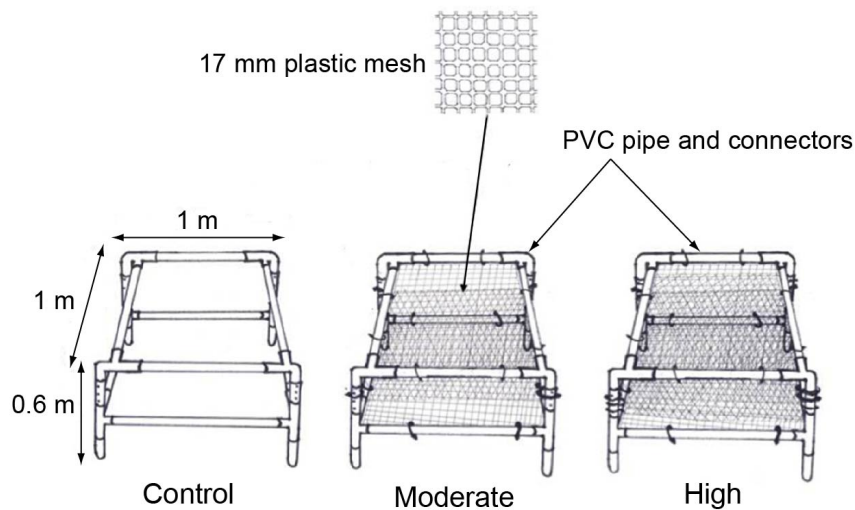


Figure 4.2. Shading frames deployed at the two study sites. The three level were: no plastic mesh (control), three layers of mesh (moderate shading) and five layers of mesh (high shading).

Six replicates of each of the three treatments were deployed 1 m apart in a six by three Latin square arrangement at each of the study sites. Seagrasses within the shading plot were not disconnected from those outside the shading plot in compliance to the research permit granted by the National Parks Board, Singapore. Sampling and measurements were confined to the central 0.3 m × 0.3 m plot in order to minimize edge effects although potential carbon transfer from shoots outside the shading area to shaded plants could not be excluded. Maintenance and cleaning of shading frames was conducted during the low spring tides of every month.

4.2.3. *Light and temperature measurements.*

Three temperature/light data loggers (model UA-002-08, HOBO, Onset Computer Corp, Bourne, MA) were deployed at each of the study sites to monitor light availability and temperature. Loggers were secured to steel poles under randomly-selected frames of each treatment in the middle of the plot. All loggers were launched simultaneously at a predetermined time and set to log every 30 min. After data were downloaded at the end of each month during low tide, loggers were cleaned and

redeployed randomly. Due to biofouling and sediment deposition on the loggers over the course of the month, only light readings from the first week after cleaning and replacement were used. As temperature recording was not affected by biofouling, monthly mean temperature was obtained from 30 min interval measurements for the duration of the experiment.

An UA-002-08 Hobo logger and a Li-Cor LI-250A light meter (Li-Cor, Lincoln, NE), attached to a Li-Cor 2p LI-192 quantum sensor, were mounted on the rooftop of the National University of Singapore (NUS), to measure luminosity and photosynthetically active radiation (PAR) from 9 February – 11 February 2010 (3 days). Data were collected between 10:00-15:00 hrs daily at 30 min intervals simultaneously. Readings were then used to establish a linear relationship between the data from the two loggers that yielded a correlation of $r^2 = 0.85$, thus allowing conversion of luminosity measurements by the Hobo logger (in Lux) at the study site into PAR equivalents (in $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$).

4.2.4. *Plant measurements.*

Shoot density at both study sites was measured by counting all shoots within a 0.3 m \times 0.3 m sampling quadrat placed in the middle of the 1 m \times 1 m shading plots after the frames were removed at the end of the experiment. In addition, approximately 10 cm \times 10 cm of *H. ovalis* (whole plant) were haphazardly collected from the 0.3 m \times 0.3 m center of each plot. Samples collected were kept in a cooler for transportation to the laboratory, where they were rinsed with seawater before processing.

4.2.5. *Leaf morphometry.*

From the collected leaf material, 10 leaves from each replicate plot were randomly

chosen for sampling. From each leaf, three morphological parameters were measured: leaf length, leaf width and total leaf area; using a LI-3000C portable area meter (Li-Cor, Lincoln, NE) in combination with the LI-3050C transparent belt conveyer accessory (Li-Cor, Lincoln, NE). This equipment was able to measure all three parameters at the same time with 1 mm² resolution and a precision of 2%. The mean of each parameter (per replicate plot) was used in the final analysis.

4.2.6. *Chlorophyll fluorescence.*

Five haphazardly-selected *H. ovalis* leaves were chosen from each plot and dark-adapted with Walz Diving PAM dark-acclimation leaf clips before being exposed to a short period of saturating light (>2000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) using a Diving-PAM[®] (pulse amplitude modulated) fluorometer (PAM, Walz, Germany) to obtain measurements of maximum quantum yield (F_v/F_m). Fluorescence measurements were performed on the leaves within 30 to 60 min of collection as measurements could not be carried out *in situ* due to time constraints and tidal limitations. Leaves were placed in a dark cooler box on ice until measurements were taken. Mean F_v/F_m values (per replicate plot) were used in the final analysis.

4.2.7. *Data analysis.*

Due to continuous logging, only descriptive statistics were calculated for the light and temperature data. For biological data parametric analyses were performed using SPSS Statistics 17.0 (SPSS Inc, Chicago, IL) after checking for normality and homogeneity of variance. In the event when either of these assumptions was violated, data were log₁₀-transformed. If assumptions were still not met, non-parametric tests were applied.

One-way MANOVA was performed on shoot density, leaf morphometry and photosynthetic efficiency within each site at the start of the experiment to check that these variables were similar across all treatments, thus obviating the need to conduct “before and after” tests. Independent samples *t*-tests were then conducted to test whether the same suite of parameters were significantly different between sites (Bonferroni corrections were made for the three leaf morphology measurements).

A one-way MANOVA could not be performed at the end of the experiment to test for differences among treatments as assumptions were not met. Hence, univariate analyses, with Bonferroni corrections, were performed using one-way ANOVAs ($\alpha = 0.05$) and post-hoc Tukey tests for pairwise comparisons of shoot density and F_v/F_m . Kruskal-Wallis tests and post-hoc Mann-Whitney/Wilcoxon tests were performed on leaf length, width and area as data failed to meet parametric assumptions even after transformation. No statistical comparisons between the two sites were made as, by the end of the study, there was no seagrass in the plots of the moderate and high shade treatment at the turbid site.

4.3. Results

4.3.1. Before shading.

MANOVA indicated no significant differences for all parameters measured among treatments within the clear site ($F_{14,18}=0.537$, $p=0.879$) and within the turbid site ($F_{14,18}=0.564$, $p=0.860$), indicating homogeneity of the meadows at the beginning of the experiment. The two sites differed significantly from each other in all parameters tested except one (maximum quantum yield, F_v/F_m), with the turbid site having lower mean shoot density ($t_{26}=-4.8$, $p<0.001$) and plants with leaves that were longer

($t_{20}=120.53$, $p<0.001$), wider ($t_{23.5}=95.33$, $p<0.001$) and greater in area ($t_{34}=292.79$) than those at the clear site (Figs. 4.3 & 4.4).

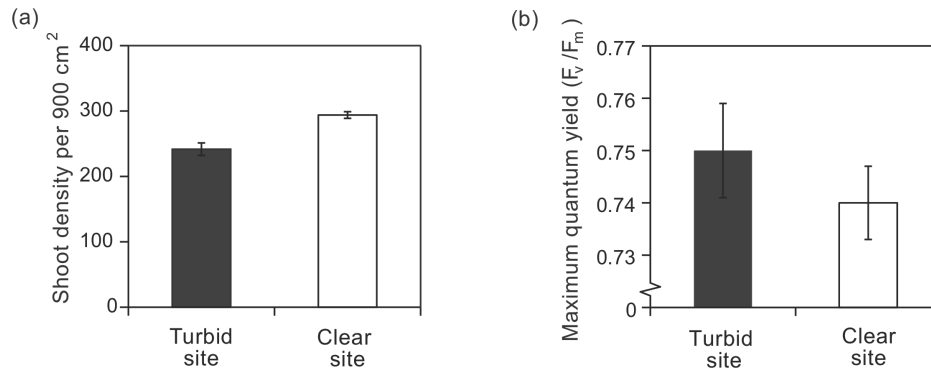


Figure 4.3. The means (\pm S.E.) of (a) shoot density and (b) maximum quantum yield (F_v/F_m) between the turbid and clear sites at the start of the experiment.

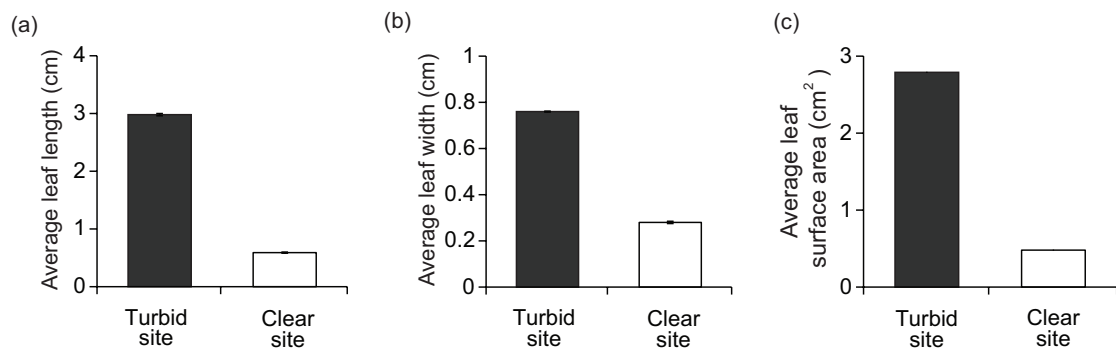


Figure 4.4. The means (\pm S.E.) of (a) leaf length, (b) leaf width and (c) leaf surface area between the clear and turbid sites at the start of the experiment.

4.3.2. Shading experiment.

The turbid site consistently received less light on average across all treatments compared to the clear site for the duration of the experiment (Figs. 4.5a & 4.5b), with the controls at the clear site receiving, on average, twice the amount of light compared to the controls at the turbid site (Figs. 4.5c & 4.5d). The average light level received by each of the control, moderate and high shade treatments at the turbid site was consistently lower than the amount of light received at its corresponding

treatment at the clear site, but higher than the next treatment level, thus forming a gradient of light (i.e. average PAR received at the clear site moderate treatment > turbid site moderate treatment > clear site high treatment) with the control treatments at the clear site receiving the most light and the high shade treatment at the turbid site receiving the least (Figs. 5c & 5d). Temperature was relatively constant between the two sites, with a 3-month average of $29.5^{\circ}\text{C} \pm 0.9$ at the turbid site and $28.7^{\circ}\text{C} \pm 1.06$ at the clear site.

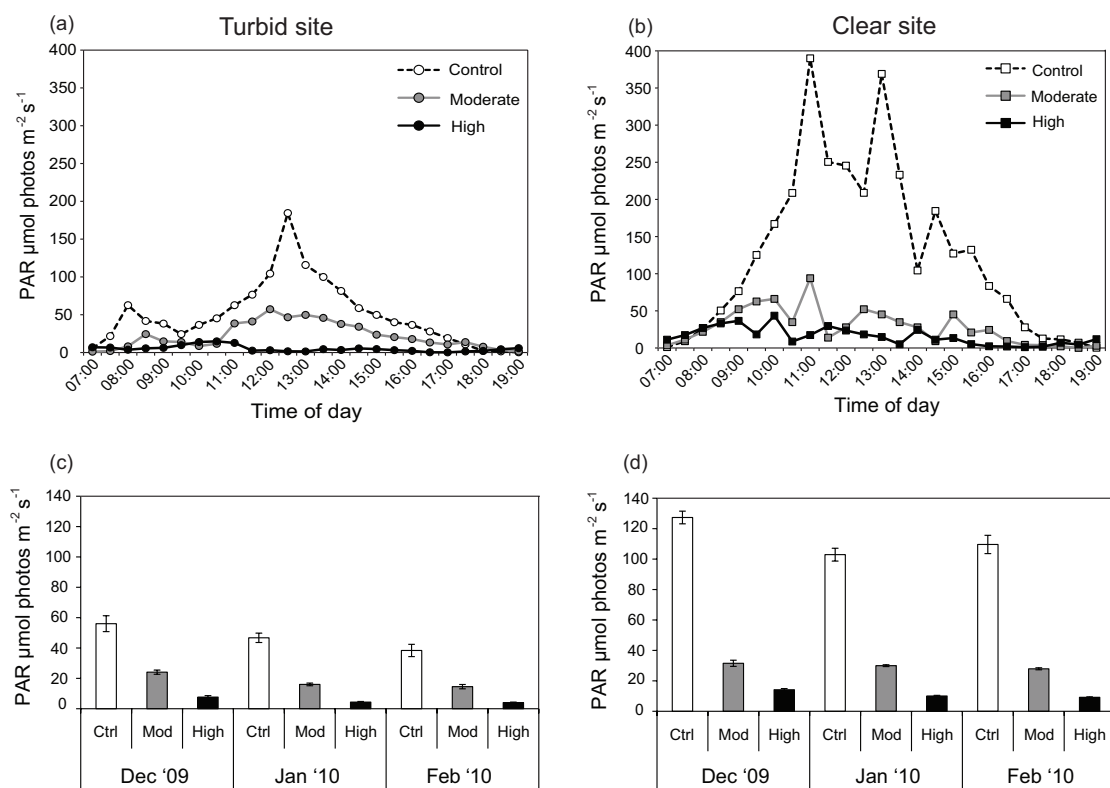


Figure 4.5. A typical daily light curve of photosynthetically active radiation (PAR) taken the third day after deployment in December 2009 at the turbid site (a) and clear site (b) over a 12 hour daylight period between 07:00h and 19:00h. Monthly means (\pm S.E.) of daily means for each treatment within each site for the turbid site (c) and the clear site (d).

There was a decrease in shoot density with increased shading at the clear site, with significant differences ($F_{2,15}=129.14$, $p<0.001$) detected among the control, moderate and high shade treatments (Fig. 4.6). At the turbid site, there were no shoots remaining in the moderate and high shade plots by the end of the experiment (Fig.

4.6). It is important to note that, while on average the high shade treatment at the clear site received less light than the moderate shade treatment at the turbid site, plants survived in the former while no plants survived in the latter.

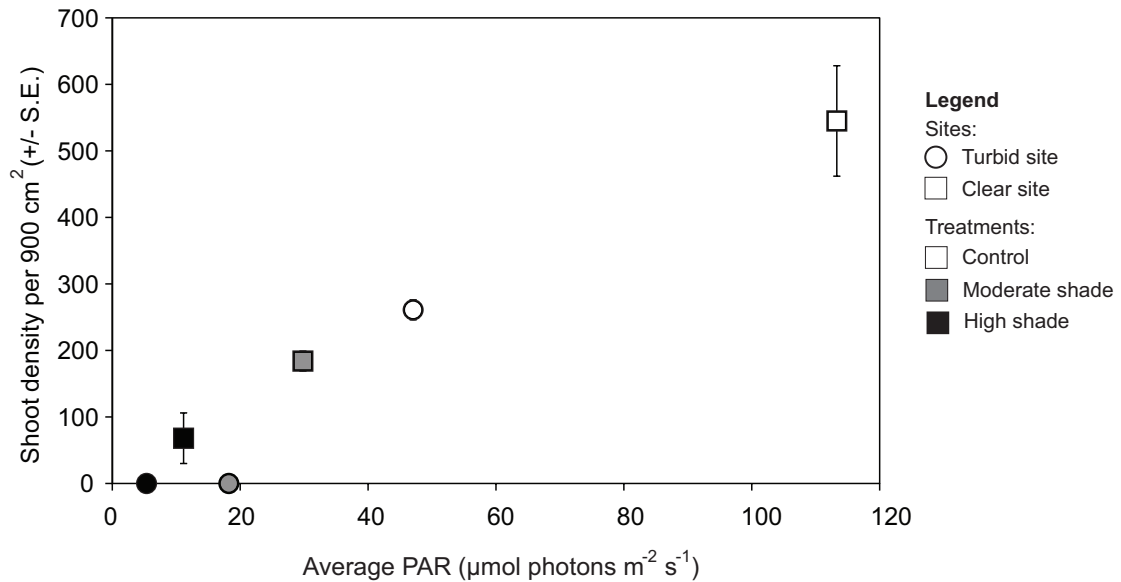


Figure 4.6. *Halophila ovalis* mean (\pm S.E.) shoot densities at the end of the experiment plotted against the average PAR received in each treatment at each site.

There were significant differences detected in the photochemical efficiency of PSII (F_v/F_m) among treatments at the clear site ($F_{2,14}=261.79$, $p<0.001$), with *H. ovalis* in the moderate treatment having the highest F_v/F_m value, followed by those in the high shade treatment. Plants in the control treatment registered a mean value that was lower than both the shaded treatments (Fig. 4.7), indicating increased photochemical efficiency in the two shaded treatments. The photochemical efficiency of *H. ovalis* in the control treatment at the turbid site did not differ significantly from the values recorded at the start of the experiment ($F_v/F_m=0.74 \pm 0.004$). No values are reported for the plants in the shading treatments at the turbid site as none survived.

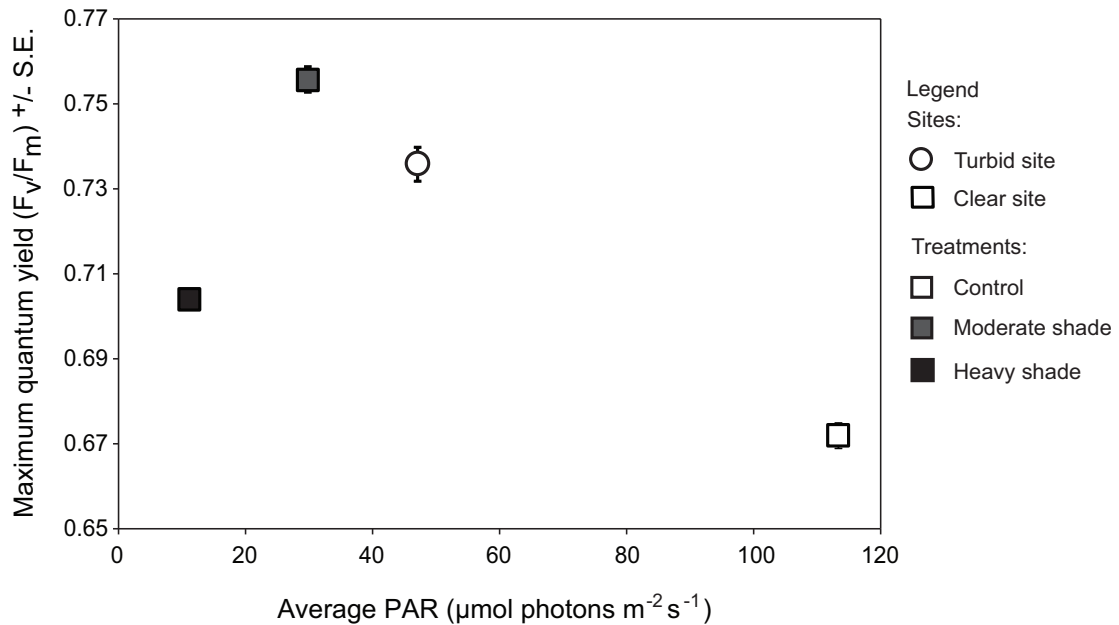


Figure 4.7. *Halophila ovalis* mean (\pm S.E.) maximum quantum yield (F_v/F_m) at each of the three treatments at the clear site and the control treatment at the turbid site at the end of the experiment. Moderate and high shade treatments at turbid site are not shown as there were no surviving leaves in the plots.

There were significant differences in leaf length ($\chi^2=15.27$, $df=2$, $p<0.001$), width ($\chi^2=12.21$, $df=2$, $p<0.001$) and area ($\chi^2=15.17$, $df=2$, $p<0.001$) between treatments at the clear site, with a trend of reduced leaf size with decreasing light. Leaf length and area showed significant differences between treatments, with controls having the longest and largest leaves, followed by moderate and heavy shade treatments (Figs. 4.8a & 4.8c). Leaf widths differed significantly between the control and two shading treatments, but no significant differences were detected between the moderate and the high shade treatments (Fig. 4.8b).

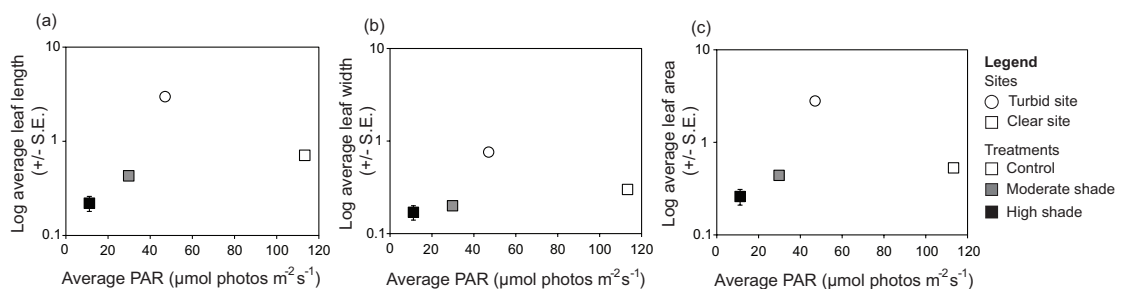


Figure 4.8. *Halophila ovalis* mean (\pm S.E.) leaf length (a), leaf width (b) and surface area (c) measurements for each of the treatments at the clear site and for the control treatment at the turbid site at the end of the experiment. Moderate and high shade treatments at turbid site are not shown as there were no surviving leaves in the plots.

4.4. Discussion

Both short- and long-term responses of seagrasses to light reduction are well documented (Lee et al., 2007; Ralph et. al., 2007) and several papers have emphasised the importance of obtaining a minimum light requirement (MLR) threshold for species in order to determine how much light is needed for survival (Dennison et al., 1993; Erftemeijer & Lewis, 2006), especially when setting criteria for environmental mitigation and monitoring plans (McMahon et al., 2013). This study demonstrates how local light history alters species light requirement thresholds by showing that *H. ovalis* plants subject to chronic low light environments have a diminished capacity for coping with additional reductions in light. This concept of “light history” has been alluded to in the literature (Longstaff et al., 1999; Lee et al., 2007) but this is, to our knowledge, the first study to demonstrate that acclimation to chronic low light regimes can affect seagrass resilience to further stress when compared to plants from a more favourable light environment.

4.4.1. Predicting the additive effects of temporary acute stress

It is possible for seagrasses to alter their carbon budget in order to maintain a positive balance even in sub-optimal light conditions by adjusting photosynthetic carbon fixing to meet with respiratory and carbohydrate storage demands of the plant (Dennison & Alberte, 1985; Hemminga, 1998; Alcoverro et al., 2001). The *H. ovalis* plants at both sites in this study did not display obvious signs of stress (sub-lethal or otherwise) at the start of the experiment, but they did show signs of acclimation to local light conditions, as both meadows had been in existence for several years prior

to our experiment (Yaakub et al., 2013a). Yet, the application of additional short-term shading to plants at the turbid site resulted in complete mortality, which can be attributed to additional stress brought about by acclimation to maintain a positive carbon balance in a low light environment (McMahon et al., 2013). In our study, *H. ovalis* plants were found to survive at average light levels as low as $11 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ at the clear site, but experienced complete mortality at light levels averaging $18 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ at the turbid site. It is generally accepted that critical light thresholds are species-specific (Erftemeijer & Lewis, 2006), but our results indicate that different thresholds may exist for the same species at different locations—a result of acclimation to past light conditions. When this location-specific threshold is exceeded, plants are no longer able to compensate for additional temporal stress and mortality ensues. This demonstrates the difficulty of setting minimum light requirements (MLR) for such acute temporal disturbances as the plant's MLR is conditional on its acclimation to a prior light climate.

This study shows that acclimation to chronic low light conditions drives the response of seagrass to further temporal reductions in light, which manifests at physiological, morphological and meadow scales. In Fig. 4.9 (adapted from Erftemeijer et al. 2012) we present a conceptual diagram that shows how plants with different light history respond to further temporal reductions in light. Plants that exist in chronically low light and high stress environments move through the stage of manifesting physiological and morphological change faster than their counterparts growing in low stress and high light environments. The application of a MLR concept is useful as long as it is coupled with the realization that it is not a constant level, but varies depending on the light levels experienced during the plant's life history.

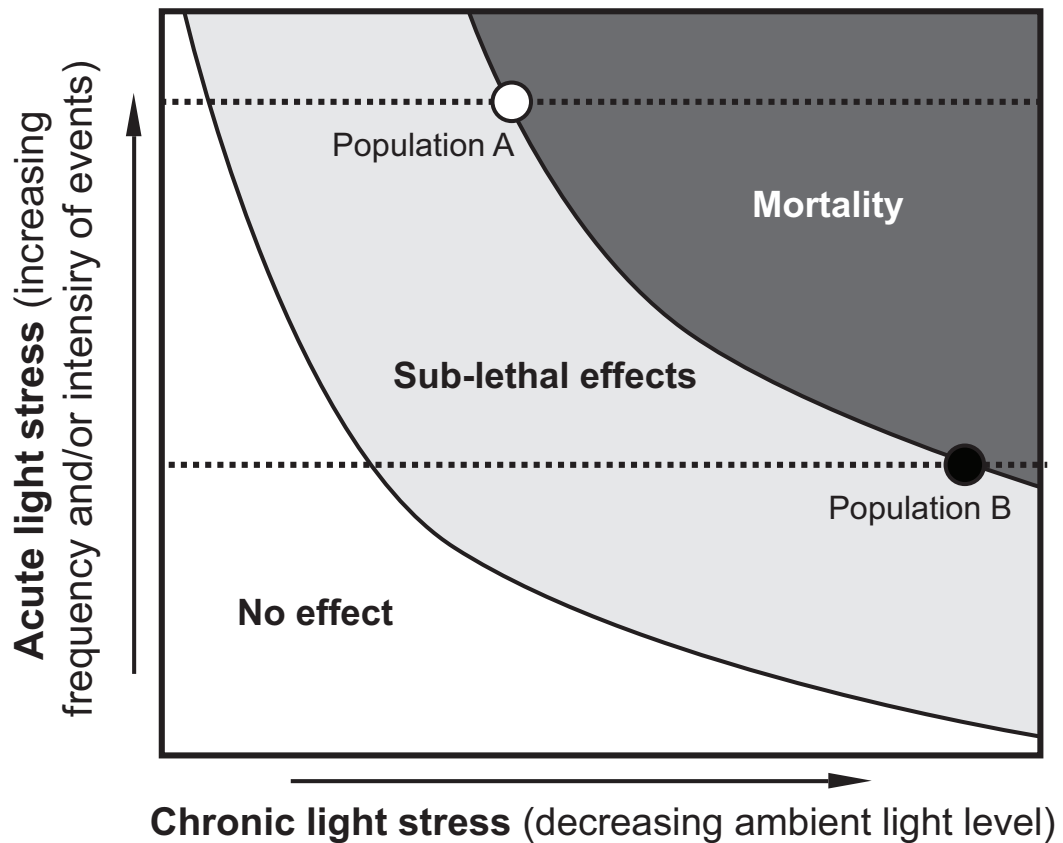


Figure 4.9. A conceptual diagram showing the combined effect of chronic light stress experienced during growth (X-axis) and acute temporal light stress (Y-axis). Two hypothetical populations, A and B, of the same species exist at different levels of chronic stress. Population B, which grows in a low light environment, reaches its minimum light requirement (MLR) threshold when subjected to moderate additional shading. However, a much higher intensity stress event is required before Population A reaches its MLR.

4.4.2. Physiological and morphological responses of *H. ovalis* to light reduction

Shoot density responses of *H. ovalis* to shading at the clear and turbid sites were very different. Seagrasses at the clear site showed a pattern of decreasing shoot density with increased shading, similar to that reported in previous studies (Lee et al., 1997; Ralph et al., 2007), while seagrasses from the turbid site showed complete mortality at light levels that were just half that of natural ambient light conditions. As a genus, *Halophila* typically has low light requirements (Dennison et al., 1993) but studies on *H. ovalis* have reported minimum light requirements of ~16% of surface irradiance (SI) for plants growing at depths of approximately 10 m (Schwarz et al., 2000; Campbell et al., 2007) and a light compensation point of approximately 33 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$, measured at depths of ~15m (Erftemeijer & Stapel, 1999).

Seagrasses possess the ability to acclimate to changes in light availability at physiological, morphological and meadow-wide scales, in both the short and longer term. In response to short-term changes and fluctuations in photon flux for example, photo-physiological acclimation can take place within hours to adjust to a new equilibrium for optimal light capture (Ralph et al., 2007). We detected significant differences among treatments in the photochemical efficiency at the clear site, with the shading treatments having higher F_v/F_m values, which concur with previous research on the effects of light reduction of laboratory-cultured *H. ovalis* (Ralph, 1999). This confirms that *H. ovalis* has the ability to photo-acclimate rapidly in response to reduced light. In fact, there are indications of photo-inhibition in the control treatments at the clear site as F_v/F_m values were similar to those reported in a previous experiment where the same species was subjected to high irradiances (Ralph, 1999).

At the end of the three-month experimental period, surviving *H. ovalis* plants in shaded treatments at the clear site showed significantly altered morphologies with reduced leaf lengths, widths and surface areas compared to those growing in control treatments. This represents a longer-term response to light deprivation and is agreement with what has been documented in other studies on the effects of light deprivation on seagrasses (Gordon et al., 1994; Collier et al., 2012), as a decrease in leaf size reduces the overall respiratory demands of the plant (Campbell & Miller, 2002). However, the opposite response has also been observed, where a decrease in light availability resulted in an increase in leaf width (Eklöf et. al., 2009) and leaf length (Longstaff & Dennison, 1999). Such morphological adjustments are related to a seagrass' size and growth strategy, with smaller species (capable of rapid tissue

turnover rates) likely to exhibit altered morphologies more quickly when subjected to a new light environment compared to structurally large species (Walker et al., 1999). *Halophila ovalis* has previously been observed to change its shape (McMillan, 1983) in response to a new light environment and shows a range of morphological variation along environmental gradients (Waycott et al., 2004; Hedge et al., 2009).

4.4.3. Practical implications for management and monitoring

While it is important to establish MLR for seagrass species, to best characterize the optimal light environment for seagrass growth it would be myopic to apply this standard across the board. It is evident from our results that MLR differ within species and is dependent on existing light conditions. From the perspective of best practices for management and environmental monitoring this is relevant as light requirements are often emphasised, for example, when setting thresholds for turbidity during dredging operations. In this scenario, applying a minimum light requirement obtained from a relatively healthy meadow to one that is already experiencing sub-lethal stress would be a mistake, as the latter is likely to have a lower resilience to any further reductions in light. In light of results from the present study, it is prudent to propose a lower turbidity threshold for meadows that have a history of chronic low light stress. As chronic light reduction is a common problem in coastal environments, this concept of light history warrants further examination to determine how it applies to different species as well as to understand the mechanism behind this variable resilience to shading.

CHAPTER 5. CHRONIC LIGHT REDUCTION IMPAIRS RECOVERY FROM SEASONAL AND PHYSICAL DISTURBANCES IN THE CLIMAX SEAGRASS SPECIES, *THALASSODENDRON CILIATUM* (FORSSKÅL) DEN HARTOG

5.1. Introduction

Disturbances play an intrinsic role in the maintenance of diversity in ecosystems (Connell, 1978). The ability to recover from cyclical or seasonal disturbances, such as woodlands from bushfires, coral reefs from hurricanes and deserts from flood is a component of the ecosystem's resilience. Ecosystem resilience refers to the ability of ecosystems to absorb impact and recover from disturbance without switching to an alternative state (Beisner, 2003). There is growing empirical evidence for the erosion of the resilience of ecosystems to such disturbances (van Nes & Scheffer, 2007) which, in some cases, has led to these ecosystems shifting beyond their tipping points into an alternative state (Scheffer et al., 2001). The catalyst behind the erosion of ecosystem resilience is thought to be exposure to sustained or chronic stressors (usually anthropogenic in origin) that dampen the recovery from disturbances as the ecosystem approaches a tipping point (Dakos et al., 2008). The effects of these anthropogenic stressors are often insidious and not immediately apparent, as ecosystems can continue to exist in stressful environments for long periods without showing obvious symptoms of stress (Yaakub et al., 2013b) or drastic change (Dikou & van Woesik, 2006). Ergo, subsequent changes into an alternate state are often described as abrupt, sudden and disproportional shifts (Beisner et al., 2003).

Long term or chronically stressed environments are common in densely populated areas such as coastlines. Today, more than half the world's population dwell in coastal areas (Hinrichson, 1998), often in rapidly developing conurbations or newly emerging mega-cities (Redclift et al., 2011). Critical marine ecosystems, such as

mangroves, coral reefs, and seagrass habitats, that inhabit coastal waters adjacent to such cities are often subject to disturbances and stressors from coastal modification, urban and industrial run off and land use change. Such long-term chronic stress has been shown to erode ecosystem resilience (Nyström et al., 2000) and may help explain the global loss of these critical coastal habitats (Pandolfi et al., 2003; Waycott et al., 2009; Polidoro et al., 2010).

Seagrasses are habitat-building marine angiosperms that grow in shallow, sheltered coastal waters. The decline of seagrass meadows worldwide has been largely attributed to a reduction in water quality, including eutrophication and turbidity, as a result of human activity (Waycott et al., 2009). Light is a primary limiting factor for seagrass growth and distribution (Hemminga & Duarte, 2000; Ralph et al., 2007). When coping with light reduction, seagrasses can adopt a spectrum of survival strategies, ranging from short term ecophysiological responses such as altering photosystems, followed by medium term modifications in morphology, and finally undergoing meadow scale changes such as a reduction in shoot density (Waycott et al., 2005; Ralph et al., 2007). Short lived pioneer species with a rapid turnover rate such as *Halophila ovalis* and *Halodule uninervis* are generally thought to be less able to cope for prolonged durations of light reduction as they are structurally small and do not have the carbohydrate reserves that climax species such as *Enhalus acoroides* and *Thalassia hemprichii* possess (Duarte, 1991; Vermaat, 2009). Climax species are generally expected to be able to cope better with long-term stressors, but nothing is known about how exposure to these affects their resilience to subsequent disturbance. In this study, we aim to examine the effect that a non-lethal chronic stress (in this case light reduction) has on the resilience of climax species to

additional physical and seasonal disturbances, using *Thalassodendron ciliatum* as a model species.

Thalassodendron ciliatum (Forsskål) den Hartog is a climax stage seagrass species that grows on rocky substrates, thus occurring most commonly in reef associated habitats (Waycott et al., 2004). It is distinguished by having terminal leaf clusters growing on vertical stems with thick rhizomes and roots, which allows this species to attach itself to hard substrates (den Hartog, 1970; Waycott et al., 2007). In a comparison of the structural properties of seagrasses, *T. ciliatum* had the sixth largest rhizome diameter (Duarte, 1991). Rhizome diameter is relatively good indicator of carbohydrate storage capability (Vermaat, 2009), which serves as buffer during stressful or adverse periods (Alcoverro et al., 1999; Walker et al., 1999). Perhaps due to its perceived potential for carbohydrate storage, there are no studies examining the response of *T. ciliatum* to light reduction (McMahon et al., 2013) and, more importantly, how light reduction affects the resilience of this climax species to subsequent disturbances. Hence, we examined the effects of chronic light reduction stress and its impact on the recovery response of *T. ciliatum* to subsequent disturbances. In particular, we tested whether prolonged exposure to low light affects this species' ability to recover from physical and seasonal disturbances.

5.2. Materials and Methods

5.2.1. Study site

The study was carried out between May 2011 and May 2012 at Teluk Bakau (1° 4' 25.71" N 104° 38' 40.92" E) on the eastern coast of Pulau Bintan in the Riau Archipelago, Indonesia (Figure 5.1). Teluk Bakau is a shallow and relatively undisturbed bay with an extensive reef flat, mostly dominated by dense stands of *T.*

ciliatum, with a rim of corals growing on the reef crest. The bay is characterised by diurnal tides, and seagrasses in the inner bay may be exposed at the lowest astronomical tides, but the mid- to outer bay is a fully subtidal seagrass meadow. Water depth in the outer bay ranges from approximately 0.5 m to 6 m, and *T. ciliatum* can be found in these areas forming large sub-tidal monospecific meadows on the outer reef crest, but this species can also be found inter-mixed with other seagrass species in the inter-tidal zone (Kuriandewa & Supriyadi, 2006).

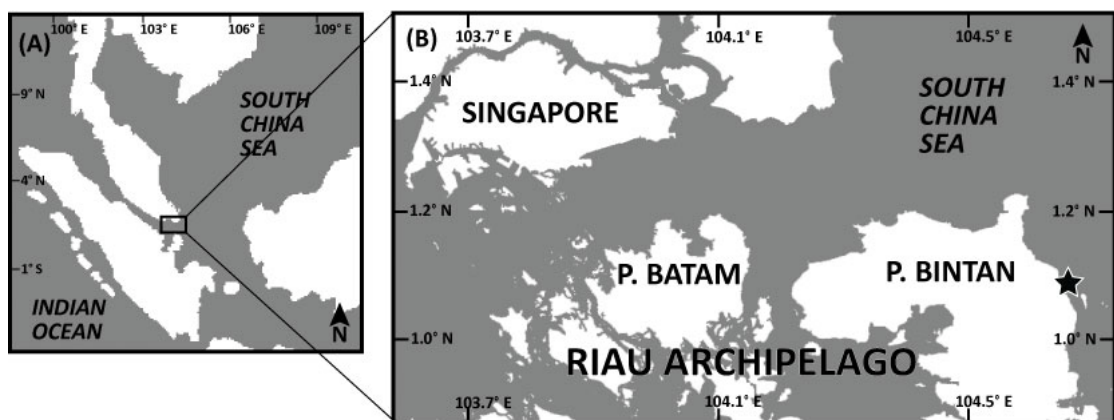


Figure 5.1. (a) Map of Southeast Asia showing relative position of P. Bintan, Indonesia; (b) showing location of field site on P. Bintan.

5.2.2. Experimental design

Twenty-four experimental plots (4 treatments × 6 replicates) were established in the mid to outer section of the bay, following randomly generated compass bearings and kick-swims using a combination of snorkeling and SCUBA diving. Each experimental plot was given a minimum 10 m radius from the next closest plot and randomly assigned a treatment. To simulate light deprivation, a 2 × 2 m² square shade cloth was suspended from four 1.5 m angle iron rods that were manually embedded ~30 cm into the substrate (Fig. 5.2a). The black polypropylene knitted mesh shade cloth (that reduced light by 70%) was suspended approximately 30 cm above the seagrass canopy and attached to the angle irons with cable ties (Fig. 5.2a). Each shade cloth

had its corners reinforced with black fabric tape and a metal grommet to prevent tearing and was replaced every four weeks from May 2011 to November 2011 to minimise the (interactive) effects of fouling. Unshaded plots were established by marking out an equivalent $2 \times 2 \text{ m}^2$ area with one angle iron rod at each corner. Within each plot, all required sampling, as described below, was carried out within a $0.5 \times 0.5 \text{ m}^2$ area established in the centre of the $2 \times 2 \text{ m}^2$ plot, in order to minimise edge effects.

The four treatments were a combination of: No shade – disturbed (TR1); No shade – undisturbed (TR2 – control plots); 70% shade – disturbed (TR3) and 70% shade – undisturbed (TR4). The experimental duration was then broken up into four phases (Fig. 5.2). Light reduction using shade cloths was introduced in Phase 1 for three months. Shading continued into Phase 2, at the start of which, a physical disturbance was introduced. The physical disturbance was simulated by severing all leaf clusters within the middle $0.5 \times 0.5 \text{ m}^2$ area in TR1 and TR3 experimental plots while leaving the shoots and rhizomes intact (Fig. 5.2b). Shading was applied for a total duration of six months between May 2011 and November 2011. Shade cloth was removed in November 2011 at the start of Phase 3, which coincided with the beginning of the Northeast monsoon season. During the monsoon season, the study site experienced increased rainfall, higher wave energy due to stronger winds, and greater turbidity (likely as a result of the combination of rainfall and winds; Yaakub, S.M., pers. obs.). Phase 4 marked the end of the monsoon season during which treatments were left to recover. At the end of each phase, shoot density measurements were taken by haphazardly placing four replicate $0.15 \times 0.15 \text{ m}^2$ quadrats within the fixed $0.5 \times 0.5 \text{ m}^2$ sampling area in the middle of the plot. Within each of the $0.15 \times 0.15 \text{ m}^2$

quadrats, four stems were haphazardly chosen and the number of leaves in each leaf cluster was recorded.

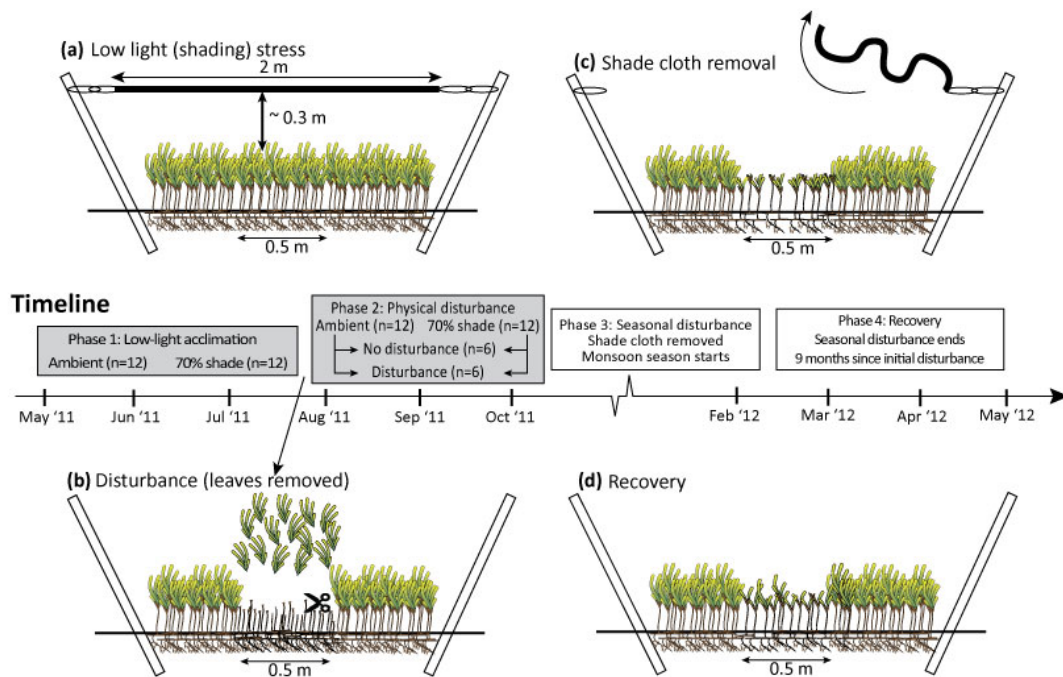


Figure 5.2. The experimental design and timeline showing (a) shading set up; (b) disturbance that was applied to the disturbed treatments; (c) removal of shade cloth and (d) set up during recovery period (post-shading).

5.2.3. Light measurements

The amount of Photosynthetically Active Radiation (PAR) received in the shaded and unshaded treatments was monitored for the duration of the experiment. PAR was recorded using the Odyssey submersible photosynthetic irradiance recording system loggers (Dataflow Systems Pty. Ltd.), which were attached to steel angle bars positioned in the middle of three randomly chosen plots from each of the shaded and unshaded treatments of the experimental plots. The loggers were collected and replaced monthly and, although there was little observed fouling or sedimentation on the sensors, only the first two week's worth of light data were used to ensure accuracy. Within that fortnight, measurements between 0730 h and 1930 h for each day were averaged to obtain a monthly PAR average. Some loggers were lost, but

we were able to collect data from at least two replicate loggers a month, with the exception of the monsoon period and for Phase 4 when only readings were available for April 2012.

5.2.4. Data analysis

Due to continuous logging, only descriptive statistics were calculated for the light (PAR) data. For each plot, average seagrass shoot count was obtained from the four replicate 0.15 × 0.15 m² quadrats. Average number of leaves per cluster was obtained from the sixteen replicate counts within each plot. Leaf biomass was estimated by multiplying the average number of shoots by the average number of leaves per cluster for each experimental plot. For the statistical analyses, the end of each phase was taken as a measurement time point. A one-way MANOVA was used to test for overall differences between response variables, followed by separate two-way ANOVA and one-way ANOVAs with treatments (TR1-4) and time (Phase 1-4) as factors. Post-hoc pairwise t tests were applied to the two-way and one-way ANOVA results. All data were analysed in R version 3.0.1 (The R Foundation for Statistical Computing, 2013).

5.3. Results

There was an overall significant effect of treatment on shoot density, leaf cluster count and leaf biomass against time (Pillais' Trace=0.52, $F_{(1,6)}=3.26$, $p<0.05$). Subsequent two-way ANOVAs for individual response variables indicated significant main effects for treatment and time, with no significant interaction between the two (Table 5.1). There were significant differences in shoot density post-monsoon (Phase 3) and at the end of the experiment (Phase 4), whereas leaf counts per cluster and leaf biomass showed significant differences between post-disturbance (Phase 2) and

post-monsoon recovery (Phase 4) (Table 5.2). Post-hoc pairwise comparisons for shoot density and leaf biomass identified significant differences across all treatments except TR1 (unshaded-disturbed) and TR2 (controls) (Table 5.2) because there was full recovery, whereas shading was an overriding factor driving differences in leaf cluster counts (*post-hoc* tests indicated significant differences detected between shaded, unshaded and disturbed treatments) (Table 5.2).

Table 5.1. Results of two-way ANOVA testing for effects of time and treatment for shoot density, leaves per cluster counts and leaf biomass. Significant p-values are highlighted in bold. Ti = time (i.e. Phase), Tr = treatment.

	<i>d.f.</i>	F	<i>p</i>
Shoot density			
Time	3	3.636	< 0.05
Treatment	3	30.899	< 0.001
Ti × Tr	9	0.483	n.s.
Leaves per cluster			
Time	3	2.823	< 0.05
Treatment	3	11.409	< 0.001
Ti × Tr	9	0.663	n.s.
Leaf biomass			
Time	3	4.162	< 0.01
Treatment	3	25.579	< 0.001
Ti × Tr	9	0.793	n.s.

Table 5.2. Result matrix of Pairwise comparisons t tests with pooled SD for the main effects of Time (i.e. Phase) and Treatment. * indicates significance, - indicates not significant

		Time			Treatment		
		T1	T2	T3	TR1	TR2	TR3
Shoot Density	T2	-			TR2	-	
	T3	-	-		TR3	*	*
	T4	-	-	*	TR4	*	*
		T1	T2	T3	TR1	TR2	TR3
Leaves per cluster	T2	-			TR2	-	
	T3	-	-		TR3	*	*
	T4	-	*	-	TR4	*	*
		T1	T2	T3	TR1	TR2	TR3
Leaf biomass	T2	-			TR2	-	
	T3	-	-		TR3	*	*
	T4	-	*	-	TR4	*	*
		T1	T2	T3	TR1	TR2	TR3

5.3.1. Shoot density responses

The treatments had significant effects on shoot density within all four time phases (Table 5.3). Shading reduced average densities to less than 10 shoots per 225 cm²,

while treatments receiving ambient light maintained shoot densities of more than 20 shoots per 225 cm² (Fig. 5.3a). Shading also had an effect on the recovery from disturbance, with disturbed shaded plots having lower shoot densities compared to unshaded disturbed plots, which recovered to similar levels of the unshaded and undisturbed control plots within 2.5 mo (Fig. 5.3b). The monsoon period exacerbated shoot density differences between shaded and unshaded plots, but all treatments showed a reduction in average shoot density (Fig. 5.3c). Post-hoc tests indicate that the combination of shading and disturbance had a significant effect on shoot density (Table 5.2) where significant differences were detected between the TR1 (No shade – disturbed) and TR3 (70% shade – disturbed) even after light levels were restored to ambient, and shoot densities remained below 10 shoots per 225 cm² (Fig. 5.3d).

Table 5.3. Results of one-way ANOVA testing for effects of treatment for shoot density, leaves per cluster counts and leaf biomass within each of the four experimental phases. Significant p-values are highlighted in bold.

	<i>d.f.</i>	Phase 1		Phase 2		Phase 3		Phase 4	
		F	<i>p</i>	F	<i>p</i>	F	<i>p</i>	F	<i>p</i>
Shoot density	3	9.965	< 0.001	9.404	< 0.001	6.686	< 0.01	7.016	< 0.01
Leaves per cluster	3	1.014	n.s.	3.568	< 0.05	7.105	< 0.01	3.228	< 0.05
Leaf biomass	3	7.009	< 0.01	6.694	< 0.01	8.279	< 0.001	6.433	< 0.01

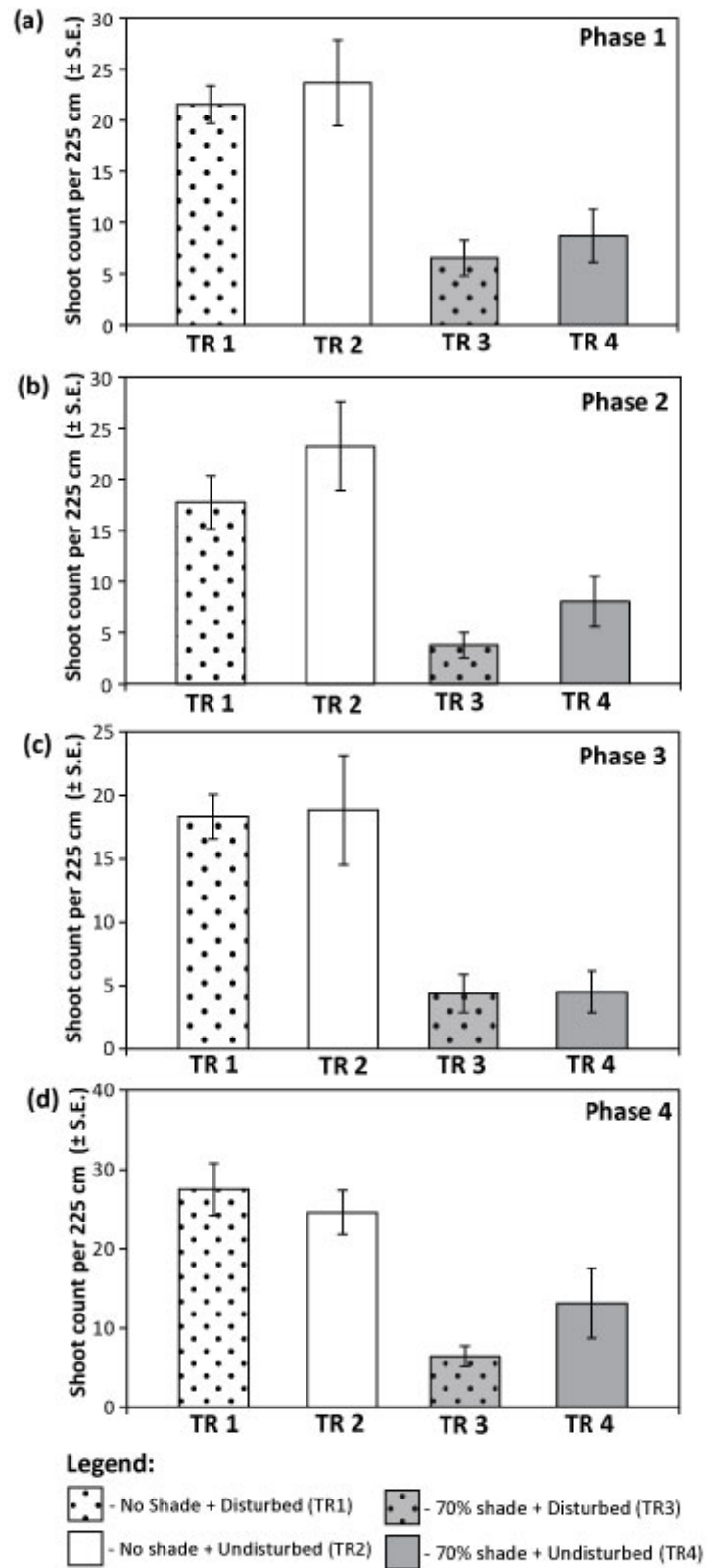


Figure 5.3. The results of shoot density from the four experimental phases and four corresponding treatments.

5.3.2. Leaf cluster count responses

The treatments had significant effects on leaf count within all time phases except Phase 2 (Table 5.3). Although, after three months, there were marginally fewer leaves per cluster in the shaded treatments, this was not significant (Fig. 5.4a). Post-disturbance, the number of leaves per cluster in the disturbed – shaded treatments (TR3) had significantly fewer leaves per cluster compared to the unshaded-undisturbed and control treatments (TR1 & TR2), but there were no differences detected between these and the shaded and undisturbed plots (TR4) (Fig. 4b). The monsoon season heightened differences between shaded and unshaded treatments, with unshaded treatments having a significantly higher number of leaves per cluster than shaded treatments at the end of Phase 3 (Fig. 5.4c). At the end of Phase 4, TR3 still had significantly lower leaf counts compared to TR1 and TR2 (Fig. 5.4d).

5.3.3. Leaf biomass responses

The treatments had significant effects on biomass within all four time phases (Table 5.3). Shading was again an overriding factor affecting leaf biomass as there were highly significant differences in leaf biomass between shaded and unshaded treatments in Phases 1 and 3 (Figs. 5.5a & 5.5c). Shading affected recovery from disturbance, evidenced by the differences detected in leaf biomass between TR3 and TR1 in Phase 2 (Fig. 5.5b). A return to ambient light levels post monsoon (Phase 4) showed that leaf biomass in TR3 had still not fully recovered compared to both unshaded treatments (TR1 and TR2), as opposed to leaf biomass in the shaded-undisturbed (TR4) treatment, which was not significantly different from the control (TR2) treatment (Fig. 5.5d).

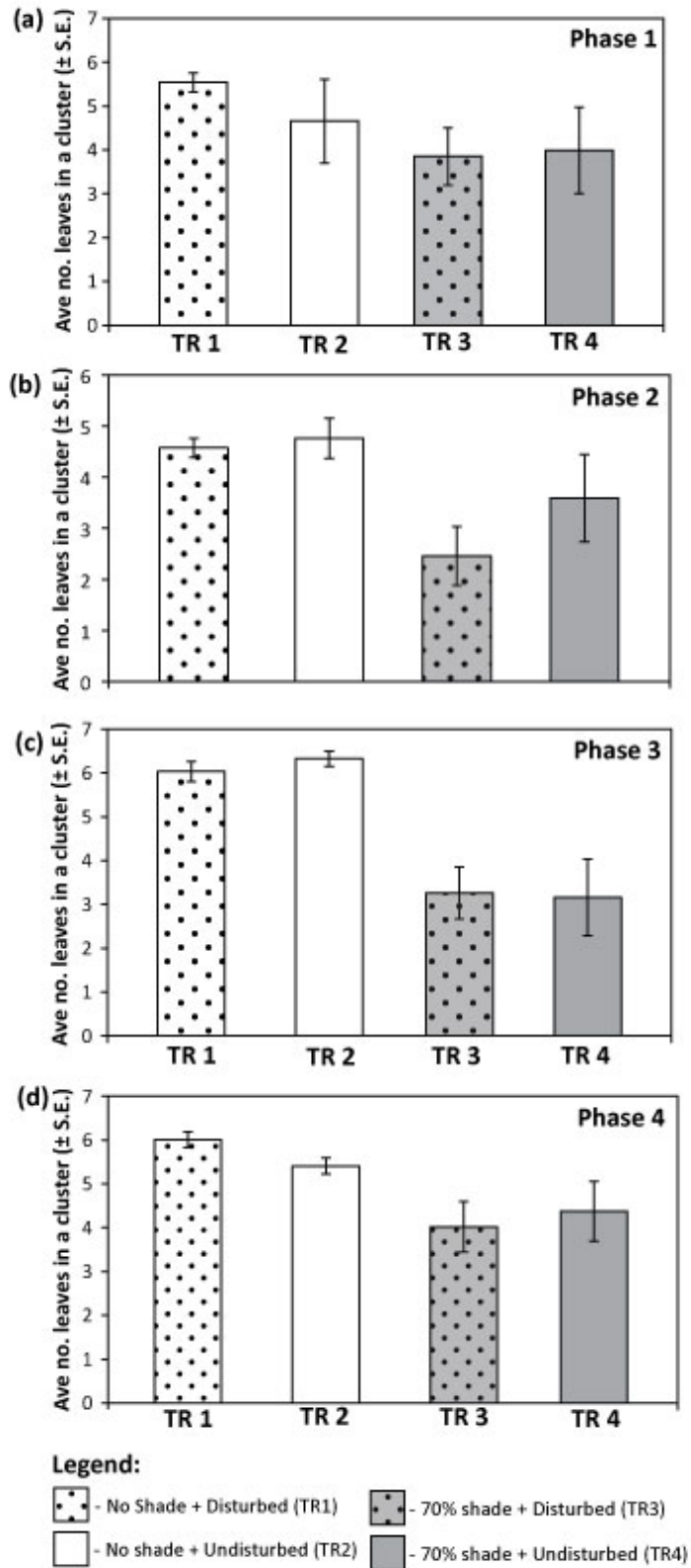


Figure 5.4. The results of leaf number from the four experimental phases and four corresponding treatments.

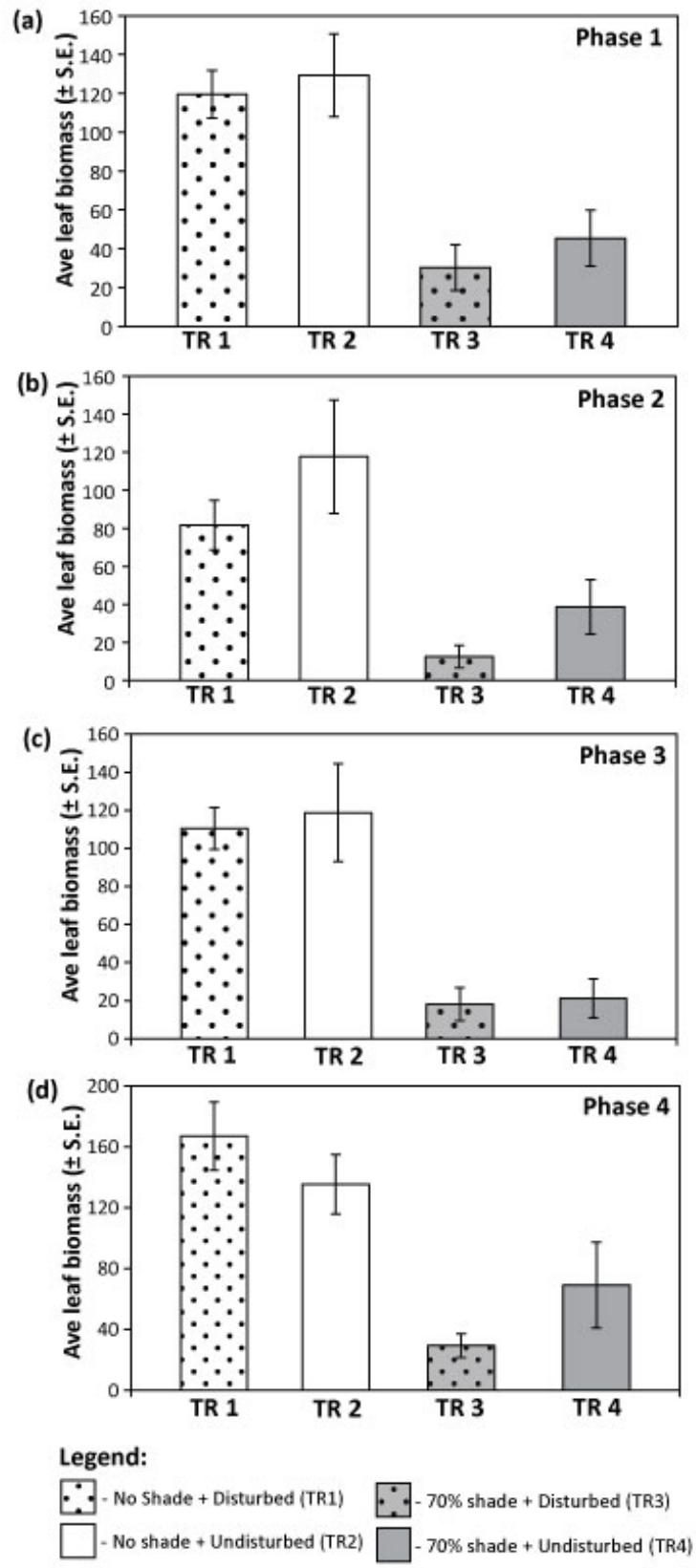


Figure 5.5. The results of leaf biomass from the four experimental phases and four corresponding treatments.

5.3.4. Light measurements

On average the ambient PAR received in May 2011 and July 2011 was more than $1309 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ compared to the average ambient PAR in October ($942 \mu\text{mol photons m}^{-2} \text{s}^{-1}$), highlighting seasonal differences (Fig. 5.6a). Shaded treatments also received, on average, higher PAR levels in May/July 2011 compared to October 2011 (more than 400 vs $92 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) (Fig. 5.6). A typical daily light curve for PAR in the unshaded treatments peaked between 12:00 and 13:00 h at $3000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ and at around $2000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ in shaded treatments (Fig. 5.6b). Point light measurements during the monsoon season in December 2011 indicated that the ambient PAR received was $92 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ which is lower than the average levels received in the high shade treatments throughout the duration of shading treatments between May and November 2011.

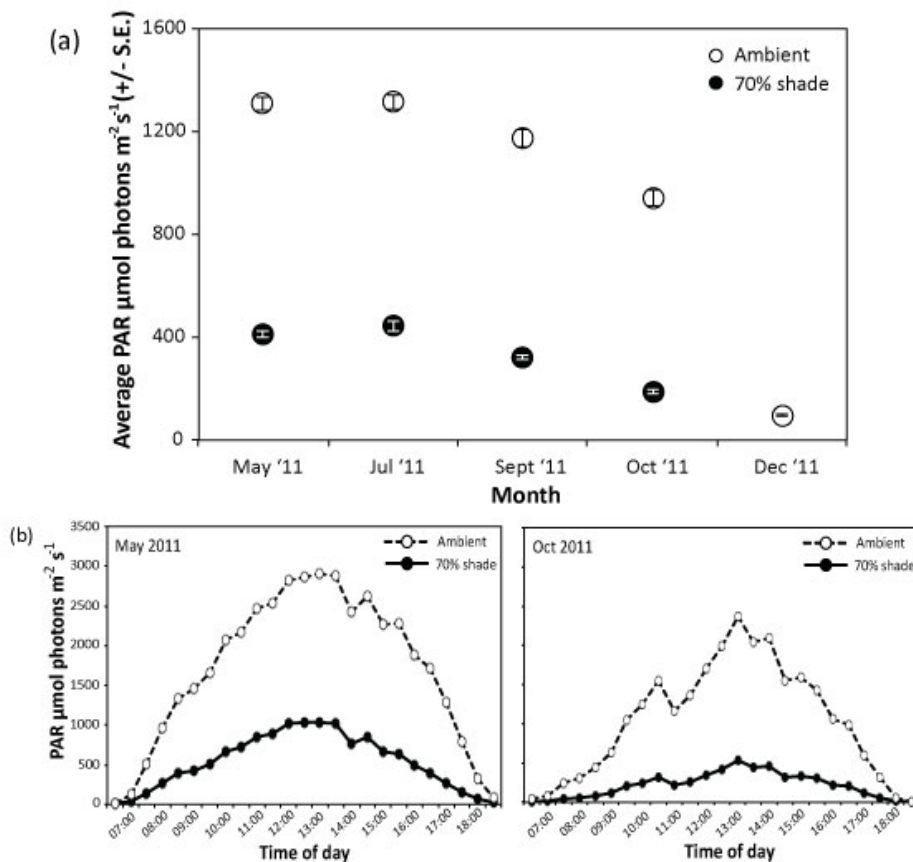


Figure 5.6. Light measurements obtained from Bintan showing (a) the average light (PAR) received for the ambient and shaded treatments for the duration of the shading period; (b) a typical daily light curve for May and October 2011, showing a reduction in the amount of light received.

5.4. Discussion

Sustained periods of light reduction have a detrimental effect on seagrasses (see review in Ralph et al., 2007) but the effect of prolonged light reduction on seagrass resilience to subsequent disturbances has not previously been tested. Yaakub et al. (2013b) demonstrated that seagrasses with a history of chronic low light are more susceptible to further reductions in light, but the present study also shows that light history is an important factor that determines the response of seagrass to physical and seasonal disturbance. The results indicate that low light conditions hampered the recovery from small disturbances in the short-term and, in the longer term, impeded the ability of plants to recover from natural seasonal disturbances, even after ambient light levels were restored.

Short-term responses to light reduction showed that *T. ciliatum* is not immune to light reduction, with an average ~70% decrease in ambient light over three months resulting in a ~50% reduction in shoot density and ~70% reduction in leaf biomass. This is comparable to changes observed in another seagrass species of similar morphology, *Amphibolis griffithii* (Lavery et al., 2009), which underwent a comparable decrease in leaf density and biomass after three months of 70% photosynthetic photon flux density (PPFD) reduction. *T. ciliatum* and *A. griffithii* are at the higher end of the form-function model for seagrasses (Walker et al., 1999), placing them closer to climax species that are hypothesized to be more tolerant to light reduction due to larger carbohydrate storage reserves (Vermaat, 1997). In contrast, in a pioneering seagrass species with thin rhizomes, *Halophila ovalis*, shoot density decreased by 80% after three months of 70% light reduction (Yaakub et al., 2013b).

We found that shading did not, however, significantly affect the average number of *T. ciliatum* leaves per cluster—contrary to the response of *A. griffithii* in Lavery et al.'s (2009) study. Another study on four tropical seagrass species also showed that number of leaves per shoot responded quickly and strongly to light reduction (Collier et al., 2011). Our unexpected results could be due to species-specific growth controls on form and function, for instance, it has been shown that *T. ciliatum* devotes a large percentage of its resources to leaf production and stem (vertical) extension (Duarte et al., 1996), and it is possible that resources were not reallocated in the first three months of light reduction, thus maintaining leaf numbers at the expense of shoot density.

Seasonal variability and disturbances are natural processes within seagrass meadows that affect biomass and production (Erftemeijer & Herman, 1994; Elkalay et al., 2003; Pergent-Martini et al., 2005). The present study identified differences in shoot density before and after the monsoon season, which concurs with a previous study that showed seasonal variation in growth rates (Duarte et al., 1996). Under ambient light conditions, *T. ciliatum* appears to be able to recover relatively quickly from physical disturbances, most likely because this species is capable of rapid vertical growth and of regeneration through branching from a vertical stem (Brouns, 1985). However, it is clear from our results that recovery from seasonal disturbance can be adversely affected by chronic stress from low light. The effects of chronic low light are compounded when coupled with physical disturbance, as seen from the slower recovery of the treatments subjected to shading and disturbance. The observation that shoot density in the shaded-disturbed treatments did not recover to the same density as those in control plots suggests increased sensitivity to further disturbance in seagrasses that have experienced significant light reduction and could

reflect a reduced resilience of the system (Yaakub et al., 2013b). This study underlines the role of light history and how a system under chronic stress may be less able to recover from seasonal disturbances, even after the chronic stress has been removed.

Changes in water quality brought about by eutrophication and coastal modification have produced a general trend of decline and loss of seagrass meadows worldwide (Waycott et al., 2009). The exposure to long term or chronic stress has been shown to erode ecosystem resilience to disturbances (Nyström et al., 2000), which has important implications for how marine habitats are managed. The effects of multiple stressors to a system are generally cumulative or magnified (Crain et al., 2008) and there is evidence that, under such circumstances, the recovery response of seagrasses is dampened (Yaakub et al., 2013b). The minimum light requirements for a disturbed or stressed seagrass meadow are therefore unlikely to be the same as those for a relatively pristine meadow. This highlights a critical drawback of establishing static minimum light requirement thresholds for aquatic plants (Dennison et al., 1993), which has been the focus of many environmental monitoring and management plans for seagrasses (Erftemeijer & Lewis, 2006) and other marine organisms (PIANC, 2010). We suggest a more dynamic concept of minimum light requirements is necessary; one that takes into consideration stressors (e.g. reduced light) and disturbances in the meadow's recent past.

CHAPTER 6. RESILIENCE OF SEAGRASS TO REPEATED DISTURBANCES AND LOW-LIGHT STRESS: EXPERIMENTAL EVIDENCE FOR CRITICAL SLOWING DOWN

6.1. Introduction

Regime shifts are defined as abrupt transitions between contrasting and persistent states in a system; the end results are frequently referred to as alternative stable states. Empirical evidence for regime shifts has been detected in various systems, including freshwater lakes (Carpenter et al., 2011) and coral reefs (deYoung et al., 2008). Understanding the theory behind such shifts can provide fundamental insights into understanding ecosystem dynamics. For example, it explains how ecosystems are relatively resilient to environmental degradation due to positive feedback loops within the system (Scheffer & Carpenter, 2003; Carr et al., 2012a), but that an abrupt transition to a contrasting stable state is possible once a critical threshold is surpassed (Scheffer et al., 2009; Carr et al., 2012a; McGlathery et al., 2012). Restoring a collapsed ecosystem to its previous state is often hampered by hysteresis effects, which require abiotic conditions to far surpass levels that allowed the ecosystem to persist originally (Beisner et al., 2003). This explains some of the difficulties encountered in habitat restoration, reinforces the notion that preventing ecosystem collapse far preferable to restoring a collapsed ecosystem, and emphasises the need for suitable indicators of nearness to collapse (Carpenter & Brock, 2006; Andersen et al., 2009).

Recently, theoretical studies indicated that “critical slowing down” may be a suitable indicator for predicting impending ecosystem collapse (van Nes & Scheffer, 2007; Scheffer et al., 2009; Drake & Griffen, 2010). This concept predicts that recovery from disturbances slows down as the ecosystem approaches a critical threshold or

tipping point (Drake, 2013) and that ecosystems become increasingly sensitive to additional disturbances that occur independent of the underlying stress. This has important consequences for ecosystem management, as it implies that threshold values derived under undisturbed conditions may not be representative of many real world situations as it is likely that these undisturbed systems have a higher stress tolerance than systems with frequent disturbances and/or various other types and levels of stress. There are some studies with experimental evidence for critical slowing down (see Dai et al., 2012) but in ecology such evidence is generally limited to *a posteriori* observations (see Schröder et al., 2005) and, to our knowledge, there are no experimental studies examining critical slowing down from repeated disturbances. Here we examine the effect of multiple disturbances and subsequent recovery responses in a seagrass meadow. Seagrass meadows represent an ecosystem with a demonstrated alternative stable state (van der Heide et al., 2007; de Boer, 2007; Carr et al., 2012a, 2012b) that are often subjected to the combined effects of various environmental stressors and repeated physical disturbance.

Seagrasses are habitat forming marine angiosperms that play a vital role in the health and productivity of both tropical and temperate coastal waters worldwide (Green & Short, 2003; Orth et al., 2006). Despite being a valuable coastal resource (Costanza et al., 1997), there has been an unprecedented loss of seagrass area worldwide largely due to anthropogenic activities such as urban and agricultural runoff, coastal development and dredging that led directly or indirectly to light reduction (Waycott et al., 2009; Grech et al., 2012). Light is a primary factor limiting seagrass growth and the effect of light deprivation stress on seagrass has been researched widely (see reviews in Dennison et al., 1993; Ralph et al., 2007), revealing some general strategies that enable seagrasses to cope with periods of low

light, such as increasing their photochemical efficiency (Ralph, 1999; Durako et al., 2003; Beer et al., 2006), and altering their morphology (Collier et al., 2009; Ochieng et al., 2010) and meadow density (Ruiz & Romero, 2001; Collier et al., 2007). In nature, seagrass meadows are also often subjected to frequent and/or cyclical physical disturbances, which result in the removal or loss of seagrass from the substrate. Such disturbances can be attributed to natural causes such as animal grazing (Aragones et al., 2006; Skilleter et al., 2007) and burrowing (Burkenbusch et al., 2007) or from various human activities including boating damage (Zieman, 1976; Engeman et al., 2008), dredging (Erftemeijer & Lewis, 2006), as well as digging, gleaning and harvesting (Japar et al., 2006; Unsworth & Cullen, 2010). The process of physical disturbance and recovery is an important dynamic in the persistence of seagrass meadows (Bell et al., 1999) but the impacts of increasing and repeated human-induced disturbances are not yet understood.

Studies that focus solely on the effects of a single stressor or disturbance fail to accommodate the fact that, in reality, stressors rarely occur in isolation and as such have limited applicability to the management of ecosystems where multiple and combined disturbances occur frequently (Crain et al., 2008). In the present study, we aimed to determine *in situ* the response of the seagrass *Halophila ovalis* to repeated disturbances (gap creation) while simultaneously exposed to three contrasting light treatments: ambient light (no stress), moderate light (moderate stress) and high shade (high stress). We questioned whether *i)* seagrass recovery response decreases with repetition of this disturbance? *ii)* Does such a response vary with the additional stress level imposed by chronic shading? *iii)* Does the meadow retain its capacity to fully recover following removal of the (light) stress after several disturbances? Answering these questions will provide insights regarding the extent to

which repeated physical disturbances in combination with low light stress lead to critical slowing down of ecosystem recovery.

6.2. Materials and Methods

6.2.1. Study site

This study was conducted between March 2011 and June 2012 for a total duration of 15 months. The study site was located in the Chek Jawa Wetlands (1° 24' 29.06" N, 103° 59' 32.28" E) on Pulau Ubin, an island located off the northeastern coast of Singapore Island (Figure 6.1a). The study site is characterized by semi-diurnal tides with a tidal range between 0 and 3.0 m (MPA, 2011, 2012). The inter-tidal seagrass meadow at Chek Jawa measures approximately 3.2 ha and consists predominantly of *Halophila ovalis* (Yaakub et al., 2013), especially in the northern end of the meadow where we conducted our experiment. *Halophila ovalis* (Hook Br.) is a fast growing seagrass species and was chosen for this study as it has been shown to react quickly to change and disturbance (Longstaff et al., 1999).

6.2.2. Experimental design—imposing three stress levels

To simulate light reduction, 1 × 1 m² shading frames were made from Polyvinyl chloride (PVC) pipes with layers of coarse mesh plastic netting suspended on top of each other (following the methods and specifications in Yaakub et al., in press). These shading frames have been shown to be effective in simulating shading in highly turbid environments as they allow sediments to fall through the netting compared to traditional shade cloths which clog rapidly (Fig. 6.1b). Three light treatments were imposed: no shade (ambient light), moderate shade (~40% light transmission), and high shade (~20% light transmission). To ensure minimal movement, shading frames were secured to angle iron bars that were driven into the

substrate. The plastic mesh was cleaned and serviced at fortnightly or monthly intervals during suitably safe low tide periods and were changed every four months throughout the duration of the experiment. Six replicates of each treatment (no shade, moderate shade and high shade) were randomly placed 2 m apart in an arrangement of six columns and three rows (Fig. 6.1c). An additional frame at the end of each row was maintained as a light-monitoring treatment (Fig. 6.1c).

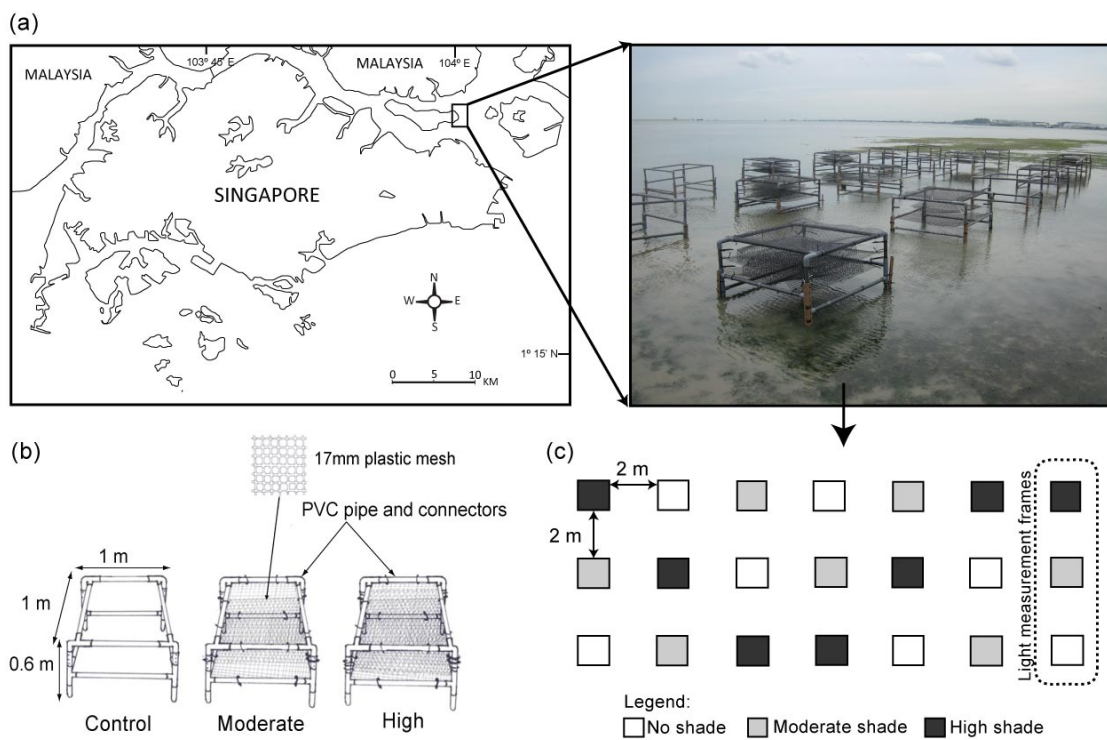


Figure 6.1. Study site (a) and experimental layout of shading frames (c). Shading frame design (b) as described in Yaakub et al., (2013b).

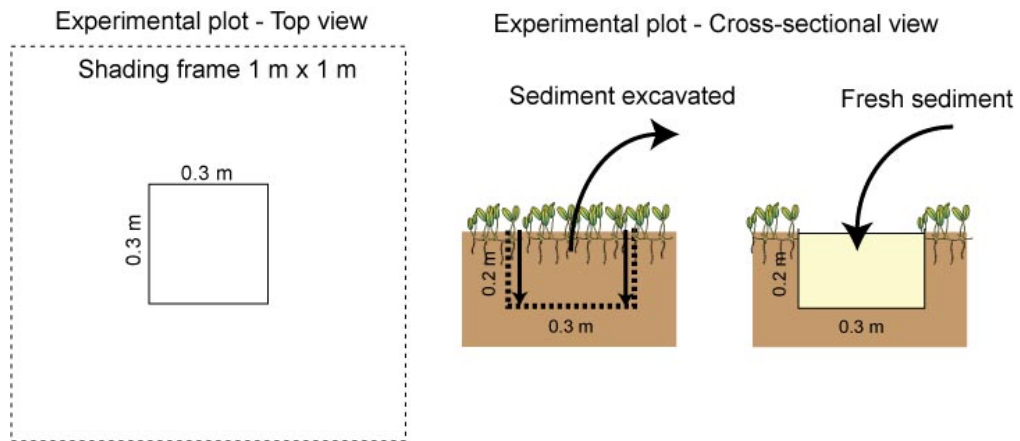


Figure 6.2. Diagram of the disturbance experiment. Showing the layout of each plot within the $1 \times 1 \text{ m}^2$ shading frame.

6.2.3. Experimental design — imposing repeated disturbances & undisturbed controls

A $0.3 \times 0.3 \text{ m}^2$ square in the middle of each experimental plot (no shade, moderate shade and high shade) was marked with plastic pegs and excavated to a depth of 0.2 m to simulate a physical disturbance. Excavated plots were re-filled to ambient level with new sediment from adjacent areas of bare substrate (Figure 6.2). A shaded buffer zone of 0.35 m was maintained around the circumference of the excavated plot to minimize edge effects, although carbon transfer from shoots outside the shading area to shaded plants could not be fully excluded as the rhizomes at the boundary of the shading frame were not severed.

The excavated plots were monitored for recovery at fortnightly or monthly intervals. The number of shoots of *H. ovalis* in each of the excavated plots was counted and recorded. Where the number of shoots in the plots exceeded 50, three subsamples of $0.1 \times 0.1 \text{ m}^2$ quadrats were haphazardly placed within the plot and the number of shoots counted and recorded. In addition to the disturbed treatment plots, the number of shoots in six randomly chosen $0.3 \times 0.3 \text{ m}^2$ control plots from the meadow surrounding the experimental plot was also counted. These control plots received

ambient light and were left intact throughout the experiment. The shoot density in these control plots was used to calculate the relative recovery from the disturbed treatment plots. The plots were considered to have full recovery when the shoot density in the no shade treatments was no longer significantly different from the shoot density of control plots. A repeat disturbance event (by excavation using the same methodology) was re-introduced across all treatment plots when the no-shade treatment plots had recovered to the same shoot density as the control plots.

There were four disturbance events simulated during the course of the experiment: the initial disturbance on 25 March 2011, and repeat disturbances on 20 June 2011, 15 October 2011 and 13 March 2012. At the fourth and final disturbance in March 2012, shade netting on all moderate and high shade treatments were removed and treatments plots were left to recover for a period of 10 weeks.

6.2.4. Light measurements

The amount of Photosynthetically Active Radiation (PAR) received in each of the three treatments was monitored for the duration of the experiment. PAR was recorded using the Odyssey submersible photosynthetic irradiance recording system loggers (Dataflow Systems Pty. Ltd.), which were attached to steel angle bars positioned in the middle of the light monitoring frames. The loggers were collected and replaced every fortnight when tides allowed or every month when tides were unsuitable.

6.2.5. Data analysis

Due to continuous logging, only descriptive statistics were calculated for the light (PAR) data. An unpaired t-test was used to determine when shoot density in the no

shade treatments reached ambient levels, i.e. similar to those in the control plots in the surrounding meadow. After the first disturbance, shoot densities in the no shade treatments reached ambient meadow levels at the 10-week mark, at which point they were analysed using a one-way ANOVA. For subsequent disturbances, we analysed the “recovered proportion”, which was determined by dividing shoot density in treatment plots by average ambient shoot density of control plots. This was done to account for any fluctuations in ambient meadow shoot density that could occur over the period of the experiment. To determine whether there were differences in recovery, the “recovered proportion” of shoots at 10 weeks after each physical disturbance was compared with a two-way ANOVA with shading treatment as a fixed factor and disturbance (1st, 2nd and 3rd) as a random factor. Tukey’s HSD was used for post-hoc comparisons. All data were analysed using R version 3.0.1 (The R Foundation for Statistical Computing, 2013).

6.3. Results

6.3.1. First disturbance and shading treatments

Shoot densities in the no shade treatment were found to reach comparable densities to those found in the control treatments at the 10-week mark ($t_6=0.25$, $p>0.05$). The degree of shading had a significant effect on the recovery of *H. ovalis* 10 weeks after the initial disturbance, ($F_{2,15}=91.79$, $p<0.001$), with lower shoot densities in the high shade (2.8 ± 0.8) and moderate shade (10.9 ± 2.4) treatments, compared to the no shade treatments (50.8 ± 3.9) (Fig. 6.3). However, Tukey’s HSD post-hoc comparisons showed that there were no significant differences in shoot density between moderate and high shade treatments.

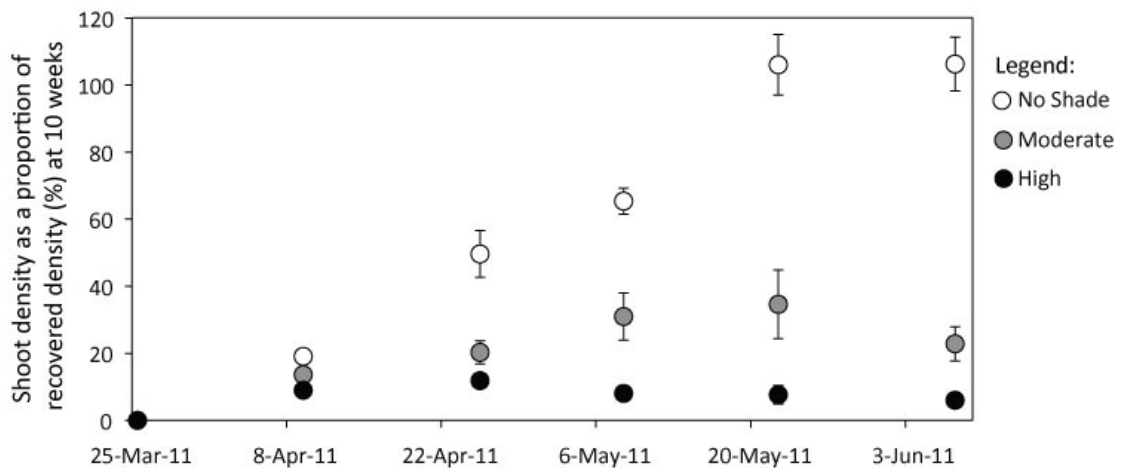


Figure 6.3. A plot of shoot density as a proportion of recovered density at 10 weeks, plot against time. Significant differences were maintained between treatments just a month after the disturbance.

6.3.2. Comparing recovery between repeated physical disturbances

There was a significant effect of shading treatment across all three physical disturbances ($F_{2,45}=548.9$, $p<0.001$), with a pattern of decreased recovery with increased shading (Figure 6.4a). There was also a significant effect of each repeated physical disturbance ($F_{2,15}=7.3$, $p<0.001$), as recovery with respect to control plots was different at each of the time points analysed. There was a significant effect of the interaction between treatment and repeated physical disturbances ($F_{4,45}=3.2$, $p<0.05$), and Tukey's HSD post-hoc comparisons showed significant differences between the moderate shade treatment in the initial disturbance and high shade treatment in the subsequent second and third disturbances (Table 6.1). This shows a reduction in recovery potential in the second and third disturbances compared to the initial disturbance, which corresponds to approximately 55% reduction in recovery proportion in moderate shade and 78% reduction in high shade treatments between the first and second disturbance and a 75% and 96% reduction in recovery in the moderate and high shade treatments between the third and initial disturbances (Fig. 6.4b).

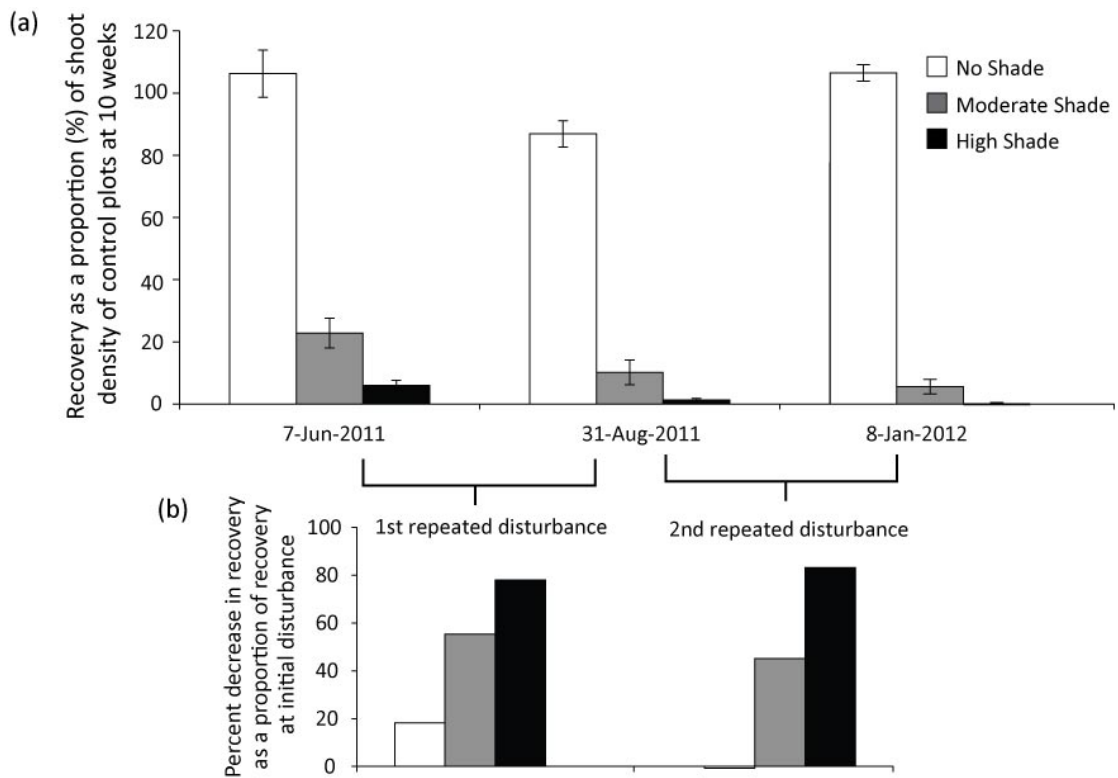


Figure 6.4. (a) Showing recovery of treatment plots as a proportion of control plots at 10-weeks after disturbance; (b) the percent decrease in shoot densities as a proportion of recovery after initial disturbance.

Table 6.1. Result matrix of Pairwise comparisons *t* tests with pooled SD for the main effects of each disturbance (by date) and treatment. * indicates $p < 0.05$; ** indicates $p < 0.01$; *** indicates $p < 0.001$; - indicates not significant.

		7 June 2011			31 Aug 2011			8 Jan 2012		
		No Shade	Moderate	High	No Shade	Moderate	High	No Shade	Moderate	High
7 June 2011	No Shade	-								
	Moderate	***	-							
	High	***	0	-						
31 Aug 2011	No Shade	*	***	***	-					
	Moderate	***	0	0	***	-				
	High	***	*	0	***	0	-			
8 Jan 2012	No Shade	0	***	***	*	***	***	-		
	Moderate	***	0	0	***	0	0	***	-	
	High	***	**	0	***	0	0	***	0	-

6.3.3. Recovery after the removal of shading stress

The combination of chronic low-light stress and repeated physical disturbances had a lasting effect on the recovery proportion even after ambient light levels were restored (shading stress was removed), and there were significantly lower relative shoot densities ($F_{2,15}=6.38$, $p<0.01$) in the moderate and high shade treatments compared to the no shade treatments after 10 weeks (Fig. 6.5). However, there was no significant difference between relative shoot density in the moderate and high shade treatments.

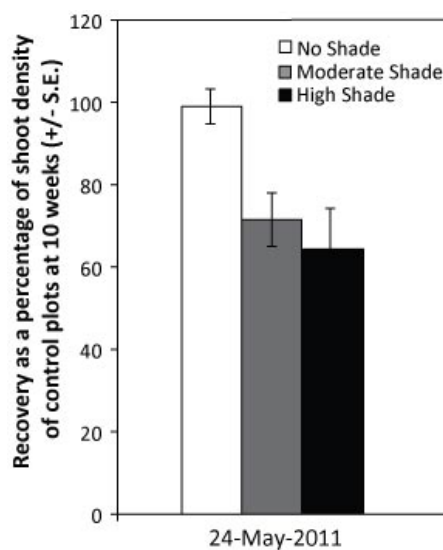


Figure 6.5. Shoot recovery at 10-weeks with each subsequent disturbance, with the final recovery of all treatment plots.

6.3.4. Light attenuation

The amount of PAR received was found to vary throughout the experiment, but the relative differences in light reaching the seagrass in each treatment was maintained (Fig. 6.6). The June 2011 growing period received higher light than the August 2011 and January 2012 periods. On average, no shade treatments received more than $800 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ per day, with the exception of September and December of 2011, when light levels dipped below $800 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ per day. Moderate shade treatments received on average $200 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ per day between

March 2001 and July 2011, but on average less than 200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ per day from August 2011 to Dec 2011. High shade treatments received more than 100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ per day on average between March 2011 and July 2011, but PAR dropped to less than that level between the months of August 2011 and January 2012, with December 2011 having the lowest light daily average PAR at just 7 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ per day.

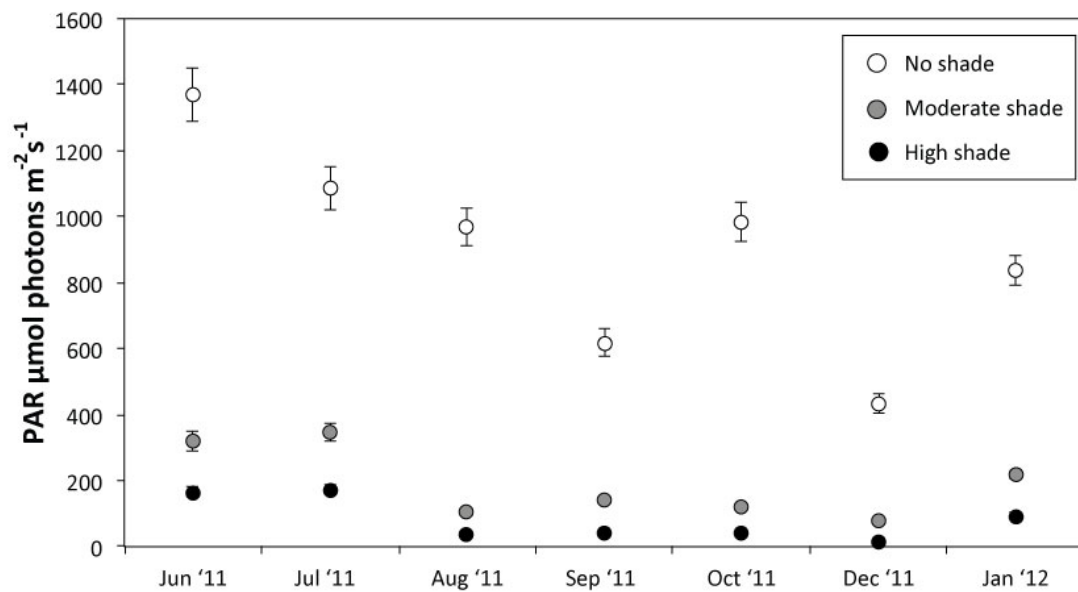


Figure 6.6. The daily average PAR $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (\pm S.E.) received for each treatment throughout the duration of the experiment. The averages are derived from two one-week periods within the month with the exception of October and December 2011 when tides were not suitable for retrieval and re-deployment of light loggers.

6.4. Discussion

The resilience of an ecosystem is the magnitude of disturbance it can withstand before shifting to an alternative state (Scheffer et al., 2009). Empirical evidence suggests that frequent disturbances or chronic stress can result in slower and diminished capacity for recovery, a phenomenon known as critical slowing down (van Nes & Scheffer, 2007), but evidence for this phenomenon is limited to controlled laboratory experiments (see Dai et al., 2012). To our knowledge, this study is the first experimental evidence for critical slowing down set in an ecological context. The

results show that seagrass habitats, an ecosystem with a known alternative stable state, shows a diminished capacity for recovery when physical disturbance is coupled with a sustained period of low light stress, and that the recovery capacity is diminished with subsequent disturbances in a *H. ovalis* meadow.

6.4.1. Recovery responses to combined shading and disturbance

There have been no prior studies that examined seagrass recovery from disturbance under manipulated low light conditions. Given that the stressors we simulated in this study are quite common and often co-occur (Erftemeijer & Lewis, 2007), this is surprising. Our study showed that recovery was hampered by light reduction, with lower shoot densities in the low light treatments, which concurs with other literature on light reduction (sans physical disturbance) on seagrass (Ralph et al., 2007; McMahon et al., 2013), but more importantly, it demonstrates that light reduction could severely impact recovery of seagrass meadows from repeated (or frequent) physical disturbances. This is probably because the processes that control growth during light deprivation are the same processes that govern recovery after disturbance under similar conditions (Longstaff et al., 1999; Longstaff & Dennison, 1999). Even though *H. ovalis* is considered a fast-growing pioneer species that is tolerant of low light conditions (Erftemeijer & Stapel, 1999), a 40% decrease (moderate shade in this study) in PAR from ambient resulted in a reduction in recovery when compared to the first recovery response. Literature on recovery of seagrass indicates that fast-growing species can recover from small-scale disturbances within weeks to months and recovery rates are species dependent (Preen, 1995; Nakaoka & Aioi, 1999; Rasheed, 1999). In our study, the unshaded treatments were able to recover to ambient meadow densities within 10 weeks of the

initial disturbance, but recovery in shaded treatments had plateaued at densities significantly lower than ambient.

Importantly, our study showed that there was a reduction in recovery with each subsequent disturbance as there was a difference between the recovery proportions in the recovery period after the first disturbance compared those after the second and third disturbances. Disturbances can be frequent or seasonal in natural systems and rarely occur in isolation, and the interactive and cumulative effects of multiple stressors have been shown to intensify negative impacts on marine systems (Crain et al., 2008). The effects of combined and interacting stressors and disturbance have been shown in a handful of other seagrass studies (e.g. Eklof et al., 2009) but none have mimicked repeated physical disturbances which are a ubiquitous aspect of seagrass meadows, through both natural and anthropogenic factors (Bell et al., 1999).

In our study, shoot densities in the shaded treatments did not recover to the same densities as the unshaded treatments, even after light conditions were reverted to ambient levels. This implies that above and beyond a diminished capacity to recover in response to deteriorating light conditions, the dampened recovery response persists even after the stressor is removed, indicating a shift in the threshold of *H. ovalis* plants that have been growing under a lower light environment. Yaakub et al. (2013b) determined that long-term acclimation by *H. ovalis* to chronically turbid conditions resulted in reduced resilience to additional stress. The results of the present study corroborate those findings and offers additional insights by showing a downward shift in the resilience threshold of these seagrasses which cannot be rectified simply by returning light levels to what they were before.

6.4.2. Implications for managing resilience

Disturbances are common in seagrass meadows, but the frequency and intensity of anthropogenic disturbances are often intensified near rapidly expanding coastal cities (Grech et al., 2011). Furthermore, these disturbances have been found to be cumulative (Crain et al., 2008), stressing the need for studies that examine these effects in order to inform management of key marine ecosystems with respect to resilience to such stressors. The results from this study have ramifications not just for seagrass management and restoration, but can be applied to other sensitive coastal ecosystems. Perhaps the most salient finding of the current study is that there is a lagged recovery of plots that were subjected to a long term or chronic stress, suggesting a hysteresis effect (Beisner et al., 2003; Schröder et al., 2005), as the treatments with a history of chronic low light stress did not have the same recovery from perturbations as the treatments that were not subjected to chronic low light, even when ambient light levels were restored. There is empirical evidence for this in coral reefs, where chronic stressors (in this case, nutrient and sediment loading) is eroding resilience of the system to further acute stressors such as bleaching events (Bellwood et al., 2004). However, this study is, to our knowledge, the first field-based experiment to show a hampered return to an original equilibrium. This again emphasises the need to manage chronic and long-term stressors that modify a system state over time, and warrants more careful management of ecosystems that are currently under chronic stress as they are more susceptible to additional disturbances (Yaakub et al., 2013b).

Combined stressors in marine systems calls for a more integrative and predictive rather than reactive style of management and the results from this study emphasises the fact that stressors and disturbances have to be managed together and not in

isolation. Simply removing the stressors may not ensure full recovery, suggesting that a preventative approach to ensure that the habitat does not pass a critical threshold (van Nes & Scheffer, 2007) is clearly preferable.

CHAPTER 7. CONCLUSIONS

7.1. State of seagrasses in Singapore

The idea that there are seagrass meadows (or perhaps any kind of significant marine life at all) in Singapore waters seems to be a paradoxical one to many researchers I have spoken with over the years. The results from Chapters 2 and 3 should quell any residual doubt that seagrass meadows do exist in Singapore. Chapter 3 in particular shows that it is possible for seagrass meadows to exist in waters adjacent to highly urbanised areas – if there is appropriate management of runoff and sewage outfall. Despite this positive finding, the future of Singapore’s seagrass meadows remain uncertain in the face of continuing stress and disturbance from anthropogenic activities such as land reclamation and dredging, which were identified as major threats in the vulnerability analysis (Chapter 3). There is a clear need to develop a framework for conserving these important habitats, and that an ad-hoc style of managing such resources is not sustainable. One aspect of this framework could be the implementation of marine protected areas, which should incorporate other marine habitats such as coral reefs and mangroves.

Existing literature on seagrass meadows in “urban” waters suggests that the situation in Singapore is an exception rather than the rule. In Hong Kong for example, land reclamation has resulted in the direct and indirect loss of seagrass meadows (Fong, 1999), as is the case in Gibraltar (Bull et al., 2010). Seagrass meadow health has also been shown to improve with increasing distance from urban city centres (Montefalcone et al., 2007). A comprehensive framework for managing and understanding seagrasses in “urban” settings, especially in newly developing satellite cities and rapidly urbanising centres, is critical for seagrass conservation. This framework should ideally incorporate inland and coastal land use change

assessments, urban and agricultural runoff, sewage discharge, port and shipping activities and an active monitoring and pre-emptive approach to the management of seagrass meadows near or around new developments.

7.2. Seagrass responses to light reduction and implications for resilience

Chapters 4 to 6 highlight some of the factors that affect the ability of seagrasses to recover from disturbances and the processes that might hinder a return to the original state. There is a substantial body of literature documenting how seagrasses adapt to light reduction (see Introduction). One of the first responses of seagrass to low light is to adjust their photosynthetic efficiency (Ralph et al., 2007), and the results from Chapter 4, comparing the responses of two seagrass meadows – one that has been subjected to a low light regime and another that is subjected to a relatively higher light regime – suggests that acclimation to a low light regime might be deleterious as it erodes resilience to additional reductions in light. This concept of light history is one that warrants more study, as it is important to understand how the duration and intensity of light reduction affects subsequent response to further disturbances. There may also be interactions with abiotic environmental parameters such as nutrients and hydrodynamics, which was not examined in this thesis, but have been shown to influence the maintenance and recovery of seagrass meadows (Koch, 2001). Nevertheless, the results from Chapter 4 challenge a critical assumption made about the minimum light requirements needed for seagrass survival, i.e. that the threshold for a particular species is fixed. My results demonstrate that the same species growing under differing light regimes has different minimum light requirements: a finding that has important implications for seagrass management.

In Chapter 5, I moved away from Singapore's impacted environment to a more unspoiled setting on the eastern coast of Bintan Island in Indonesia. This relatively undisturbed area allowed for the manipulation of a meadow growing in pristine conditions and a high light environment. It also allowed for a comparison of the responses of a more climax seagrass species (*T. ciliatum*), compared to the pioneer species (*H. ovalis*) used in the previous chapter. The results from Chapter 5 show that climax species are not immune to severe and long-term light reduction, despite being perceived to be better able to withstand such conditions due to having larger carbohydrate reserves. When unimpaired by low light availability, this species is able to recover relatively quickly from small physical disturbances (in this study: the loss of their leaves) and withstand a period of low light that comes with a seasonal monsoon. However, when subjected to chronic light reduction for the period preceding the monsoon, subsequent recovery is hampered and does not return to its previous undisturbed state; again showing that recovery from chronic light reduction is slow.

Chapter 6 continues with the theme of chronic light reduction but explores the added dimension of repeated physical disturbance and how this might affect recovery; firstly with each repeated disturbance, and secondly after the stress factors have been removed. The results show that there is a reduction in recovery of seagrass in the low light treatments with each subsequent disturbance. More interestingly, it demonstrates that after light levels have been restored to ambient, the dampened recovery response is still apparent in the low light treatments. This shows that physical disturbances when coupled with chronic light reduction results in a slower recovery and, by extension, an erosion of resilience. It is interesting to note that in both climax and pioneer species, long-term chronic light reduction affects resilience

to the extent that there is no return to a “normal” or previous state even after ambient light levels are restored. This implies an overall reduction of resilience that transcends the plant’s ability to tolerate light reduction and persists even after abiotic conditions have improved.

7.3. Future research directions

The work presented here is by no means exhaustive, and there are several avenues to explore based on the ideas brought forth from this research. Firstly, the GIS mapping of three seagrass meadows in Singapore does not reflect the full extent of seagrass in Singapore and there is still much to be done, especially in relation to mapping seagrass extent on reef platforms where ground truthing exercises indicate a high occurrence. Regular GIS mapping can also complement current seagrass monitoring programmes to determine changes at larger scales. The method of predicting seagrass extent employed in Chapter 3 is simple and inexpensive to conduct, but it could be developed into a more robust model by incorporating parameters such as bathymetry, water depth and hydrodynamic information.

One of the factors influencing the rate of recovery of gaps in seagrass beds is the perimeter to area ratio (Uhrin et al., 2011), which provides one explanation why larger gaps take a longer time to recover. It is well known that seagrasses exert a stabilizing effect on the sediments they grow in and there is an increased risk of erosion in a bare patch of substrate (Bos et al., 2007). A better understanding of recovery and resilience would be achieved if the studies conducted in Chapters 4 to 6 were scaled up to in order to determine the interaction between the size and extent of the disturbance and how subsequent recovery of gaps is affected by light reduction, as the relationship is unlikely to be linear.

The primary response measure in this thesis was shoot density, so an examination of other indicators of sub-lethal stress such as rhizome sugars, shoot carbon:nitrogen ratios and leaf growth and production (McMahon et al., 2013), would provide additional insights into what affect seagrass resilience. Another emerging area of research that could improve our understanding of seagrass resilience to stress and disturbance is that of ecogenomics (Procaccini et al., 2012). The inter-play of genes and environment may help determine how such stressors and disturbances affect selection outcomes in seagrass, especially in pioneer species with short generation times.

7.4. Management implications

“An idea that is not dangerous is unworthy of being called an idea at all.”

- Oscar Wilde

At the crux of this thesis is the concept that the history of stress and disturbance a seagrass meadow has been exposed to cannot be discounted when trying to predict its response to future disturbances or additional stress. While the idea itself is not as dangerous as the late Mr. Wilde might suggest, there are serious implications for management of seagrass habitats, especially those that already exist in stressful environments.

The most urgent aspect in need of review is that of environmental management plans (EMPs) used in environmental impact assessments (EIAs) for coastal modification or dredging activities. In most EMP baselines, impact criteria are set based on environmental conditions such as water quality, background sedimentation and turbidity rates and current condition of the stress receptor (e.g. seagrass or coral reef

habitats) (PIANC, 2010). These are the measurements against which future impacts from dredging (e.g. sediment overspill, turbidity levels) are predicted. One of the key impact criteria for seagrass is turbidity level (as a proxy for the amount of light received during a dredging exercise) and minimum light requirements for the different species present (Erftemeijer & Lewis, 2006). Used together, they set the acceptable threshold for allowable turbidity or light loss during the course of an EIA monitoring project. My research shows that individual species light thresholds, while informative, are too rigid and do not fully reflect actual light requirements, which vary depending on the historical and contemporary light climate at a particular site. It is possible that these minimum light requirements should be set very conservatively—especially for seagrass meadows that already inhabit chronically turbid or low light environments.

A second major finding of this thesis is that habitats in chronic low light environments that experience repeated or frequent disturbances have reduced resilience and are unable to recover to previous levels even after the stress has been removed. Applied in the context of a dredging operation, these results suggest that regular disturbance may be detrimental to seagrass resilience in the longer term and hamper recovery. Based on these outcomes, it is prudent to set additional criteria that address the light climate and disturbance history of the site. Much like a patient's medical record, it should be considered due diligence to assess the previous and current light climate, and frequency and intensity of disturbance, before exposing the impact receptor to additional stress.

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