Impact of black swan grazing and anthropogenic contaminants on New Zealand seagrass meadows

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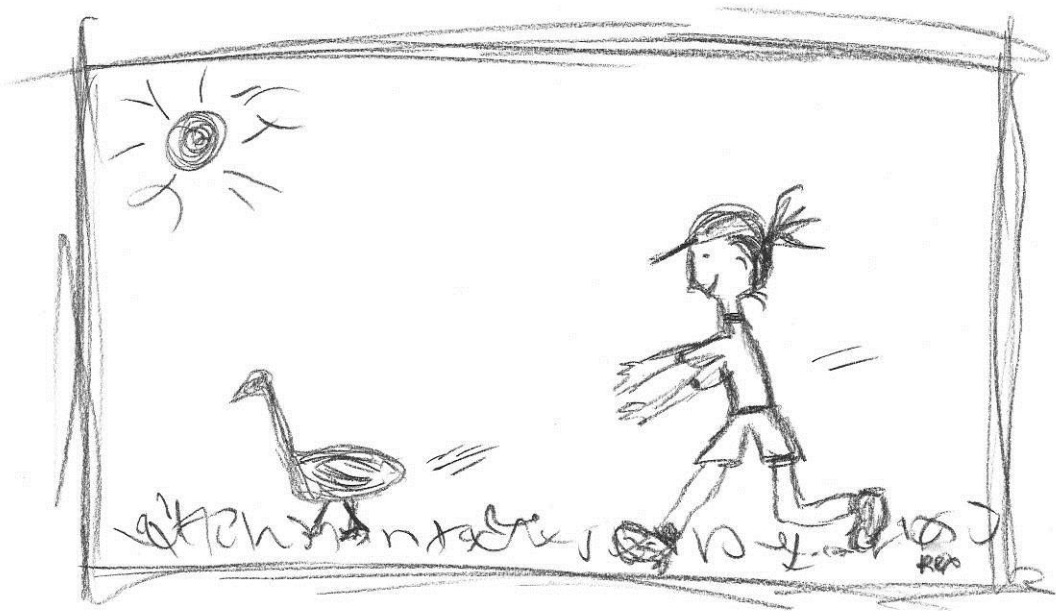
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Abstract

Seagrass is the only marine angiosperm (flowering plant) and decline of seagrass meadows has been reported worldwide. The important ecological and economic values of this marine habitat make it essential to preserve. Seagrass meadows of New Zealand are composed of only one species, *Zostera muelleri*. They are mainly present in sheltered, intertidal sand flats in harbours. Seagrass decline has been recorded in many New Zealand estuaries during the last century. Grazing by the black swan (*Cygnus atratus*) and anthropogenic contaminants resulting from catchment development are postulated to contribute to seagrass meadow loss in New Zealand and these factors have been assessed in this study. The aim of this study was to quantify black swan grazing pressure and examine effects of potential anthropogenic contaminants (specifically nutrient enrichment, sedimentation and herbicide residues) on seagrass meadows in New Zealand. The impact of current levels of these anthropogenic contaminants on seagrass resilience to swan grazing was also assessed *in situ*.

To quantify black swan grazing pressure on seagrass meadows and to evaluate the potential relationship between swan grazing pressure and seagrass standing stock, observations and measurements were conducted in Tauranga Harbour where more than a third of the seagrass meadows disappeared between 1959 to 1996. Observations have shown that black swans graze intensively on *Zostera muelleri* meadows during high tide and were most numerous in the harbour during autumn. Black swan grazing resulted in the formation of circular devegetated patches (0.28 m^2), where 92 % of

shoots, 25 % of roots and 99 % of rhizomes are removed. The consumption rate was estimated to be 394 g dry weight (DW) swan^{-1} day^{-1}. Significant seagrass loss on an annual basis was detected at the most swan populated site, which also had the highest swan grazing pressure (19-20 % of biomass consumed annually). This suggests a potential threshold of 19-20 % of annual seagrass biomass removal above which black swan grazing may contribute to substantial damage on seagrass meadows. Applied at the harbour scale, where there is a total of ~29 km^2 of seagrass meadows, this corresponds to a swan population (i.e. grazing pressure) > 4630 birds.

To identify the potential environmental stressors affecting seagrass condition, two contrasting harbours (Tauranga and Aotea) were surveyed where a range of environmental parameters, including parameters indicative of anthropogenic contamination, and seagrass condition metrics were measured. The survey showed that many of the seagrass condition metrics were positively correlated with sediment porewater ammonium (NH$_4$-N) concentrations suggesting that nitrogen availability regulates seagrass growth. The presence of higher porewater nitrate (NO$_3$-N) concentrations was linked to higher proportions of urban and crop land use in subcatchments, these latter parameters being negatively correlated with light availability during high tide. In spite of this, current levels of nutrient enrichment were apparently not detrimental to seagrass condition with evidence of N-limited plant growth and abundant light availability. Herbicide compounds were detected in seagrass sediment and at consistently higher concentrations in Tauranga harbour, which is the harbour more exposed to developed land use. The seagrass photosynthetic potential was lower in more herbicide contaminated sediments, although there was no evidence of any impact on seagrass biomass or morphometry.

In a final experiment, black swan grazing was simulated *in situ* at four sites in Tauranga Harbour differing in exposure to potential anthropogenic contaminants and environmental characteristics. Grazing was simulated by removing seagrass biomass from patches (0.25 m^2) in the meadow equivalent to natural swan removals (see above). Two levels of grazing intensity were applied to these patches; ~100 % of biomass removed (high) and ~ 40% of biomass removed (low). Results showed that the cover and the biomass of the low grazing intensity patches were not significantly different to controls (0 % of biomass removed) during the entire experiment. For the high grazing intensity patches, although the cover was restored after 9 months, biomass recovery was incomplete with only 30 % of total biomass regenerated, after one year. This suggests that swan grazing at high intensity can cause long-lasting damage to seagrass meadows, and that > 3 years is probably needed for a devegetated patch to fully recover from grazing (assuming a constant plant regeneration rate). The resilience response was similar across all sites examined in this harbour, suggesting that the current magnitude and range of environmental variability and potential contaminant levels within the seagrass meadows do not affect the ability of seagrass to regenerate from grazing.

In conclusion, from these results, I can not conclude that anthropogenic contaminant or black swan grazing pressure are by themselves responsible for seagrass decline in Tauranga Harbour. However, the study suggests that herbicide residues have the potential to detrimentally affect the seagrass photosynthetic activity as do high grazing pressure by the black swan on the seagrass meadow condition. Overall, this study contributes to the understanding of impacts of black swan grazing pressure combined with potential contaminant effects on seagrass ecosystems. It emphasizes the importance of herbivory in temperate seagrass systems, which it is

often underestimated and highlights the seagrass habitat contamination due to coastal development. It provides crucial information that will assist resource management agencies to preserve this valuable natural ecosystem.

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Acknowledgments

Nō reira tēnā koutou, tēnā koutou, tēnā koutou katoa!

Preface

The main body of this thesis comprises three chapters (Chapters 2-4). Chapters 2-4 have been prepared in a paper format to assist submission for publication in peer reviewed scientific journals. I assumed the responsibility of the field work programme, laboratory and data analysis, and for writing this thesis. Except where referenced, the material in this thesis was produced from my own ideas and work undertaken under the supervision of Conrad Pilditch, Fleur Matheson and Arnaud Elger.

Chapter 2 has been submitted to *Aquatic biology*, under the title "Black swan (*Cygnus atratus*) grazing pressure and impacts on temperate intertidal seagrass (*Zostera muelleri*) meadows" by V.M. Dos Santos, F.E. Matheson, C.A. Pilditch and A. Elger.

Chapter 3 has been submitted to *Marine Pollution Bulletin*, under the title "Influence of nutrient enrichment, sedimentation and herbicide residues on seagrass condition in two contrasting estuaries" by V.M. Dos Santos, F.E. Matheson, C.A. Pilditch, G. Merlina and A. Elger.

Chapter 4 has been prepared to be submitted in a peer reviewed scientific journal, under the title "Seagrass resilience to waterfowl grazing at sites differing in anthropogenic contaminants and other environmental characteristics" by V.M. Dos Santos, F.E. Matheson, C.A. Pilditch, and A. Elger.

Chapter 1

General introduction

Zostera muelleri meadows in New Zealand

1.   General introduction

1.1. *Seagrass origin, species and occurrence*

The origin of angiosperms (flowering plants) is accepted to be about 400 million years ago (Raven 1977), and it is just about 100 million years ago that angiosperms colonised the marine environment (Den Hartog 1970). These marine angiosperms are commonly called seagrasses and belong to the Magnoliophyta division, order Alismatale. Five families of seagrass are present worldwide, which are Posidoniaceae, Zosteraceae, Hydrocharitaceae, Cymodoceaceae and Ruppiaceae. Seagrasses are present in coastal marine waters in all continents except Antarctica (Short et al 2007). Seagrass biodiversity is not equally spread around the world and varies with latitude, with the highest diversity around the equator.

1.2. *Ecological and economical importance of seagrass meadows*

Seagrass meadows play a key role in coastal ecosystems and are one of the most productive marine ecosystems, influencing environmental abiotic parameters and supporting a great abundance and diversity of species belonging to many phyla. Seagrasses can purify estuarine water by sequestering toxic compounds from the water column (e.g., copper, zinc, lead, manganese, nickel, cadmium; Ward 1987, Hoven et al 1999) and by absorbing nutrients which potentially reduces eutrophication and phytoplankton blooms (Short and Short 1984). Seagrass photosynthetic activity releases oxygen that is vital for surrounding living organisms and the root-rhizome system plays an important role in oxygenating the superficial sediment layers. Moreover, the strong root-rhizome anchoring system binds and stabilises sediments

which prevents coastal erosion and provides habitat complexity in soft sediment environments (Orth et al 1984, Short and Short 1984).

Seagrass meadows provide a nursery area for offshore fish. They offer shelter and habitat for endangered species such as dugong, manatee, green turtle and sea horses (Hemminga and Duarte 2000). Moreover, many commercial fisheries, such as fish (snapper, flounder and rabbitfish), molluscs (oysters, mussels, scallops and clams), and crustaceans (shrimp, lobster and crabs) use the seagrass habitat. Seagrass meadows also provide food for a range of organisms. The micro-organisms process seagrass detrital matter, detritivores (e.g., crabs and worms) feed on decaying seagrass, and herbivores (waterfowl, dugong, manatee, turtle, fish, sea urchin and other invertebrates) directly consume fresh seagrass leaves and/or roots. Threatened herbivores such as the dugong and the green turtle are highly dependant on the presence of this unique food resource, which greatly increases the ecological value of this marine ecosystem. Commercial fisheries also derive an economic benefit from this ecosystem, which is estimated to be in the order of US$19,004 per ha per year (Costanza et al 1997).

1.3. Potential stressors affecting seagrass

Within the last century, seagrass decline has been recorded worldwide (Short and Willie-Echeverria 1996, Waycott et al 2009). The ecological and economic value of this ecosystem makes it essential to investigate the potential causes of this decline (see Fig. 1.1). Overgrazing by herbivores has been postulated as a potential threat to seagrass meadows since they disturb the structure of the seagrass habitat by removing a large amount of plant biomass (River and Short 2007). The foraging technique varies amongst herbivores, with fish, turtle and invertebrates cropping only the

seagrass leaves (Kuiper-Linley et al 2007, Unsworth et al 2007, Vergés et al 2008) and dugongs, manatee and birds creating pits and/or trails in the seagrass meadow removing and/or damaging roots and rhizomes as well as leaves (Masini et al 2001, Hugues et al 2004, Tinkler et al 2009). Seagrass usually recovers from grazing through seed germination and vegetative regrowth from remnant or adjacent plants (Preen 1995). It is even known that the abrasive action of grazing by large herbivores such as dugongs stimulates seed germination thus facilitating seagrass recovery after grazing (Peterken and Conacher 1997). The persistence of seagrass ecosystems therefore suggests that there is a balance between the regeneration of seagrass meadows and the natural densities of herbivores. However, the introduction of new herbivore species or increases in natural grazer populations, that often result in overgrazing (River and Short 2007, Eklöf et al 2008), may detrimentally affect this equilibrium. Under intensive grazing, a high proportion of biomass is removed, especially rhizomes, which may delay or prevent recovery in the longer-term (Rivers and Short 2007). Herbivores that graze on both above and below ground biomass usually remove the meristem (the part of the plant where growth is initiated) of seagrass, which highly impedes recovery. Moreover, consumption or a poor recruitment of seeds will reduce the recovery capacity after grazing. A better understanding of how different types of herbivory can disturb seagrass meadows and the factors influencing seagrass regeneration from grazing by various herbivores is crucial to preserve these ecosystems.

Other factors may also contribute to seagrass decline or play a role in decreasing seagrass resilience to grazing pressure. Due to the increase in human population around the coast, seagrass meadows are often exposed to anthropogenic contaminants. Urbanisation of coastal areas, along with lack of efficient waste water management,

has been shown to result in large losses of seagrass (Kemp et al 1983, Larkum and West 1990, Short and Willie-Echeverria 1996, Peters et al 1997). Coastal runoff leaches nutrients, fine sediment particles and other contaminants into estuarine water, which can detrimentally affect seagrass meadows. Nutrient over-enrichment of the water column often results in growth of competitive algae that considerably reduces light penetration to seagrass meadows by reducing water clarity (Duarte 1991, Williams and Rucklshaus 1993, Kendrick et al 2002, Greve and Krausen-Jensen 2005, Burkholder et al 2007, Sugimoto et al 2007). The suspension of fine mineral or organic particles in the water column may also limit light penetration and availability to seagrasses, and increased fluxes of fine sediment into estuaries can result in smothering of plants (Burkholder et al 2007).
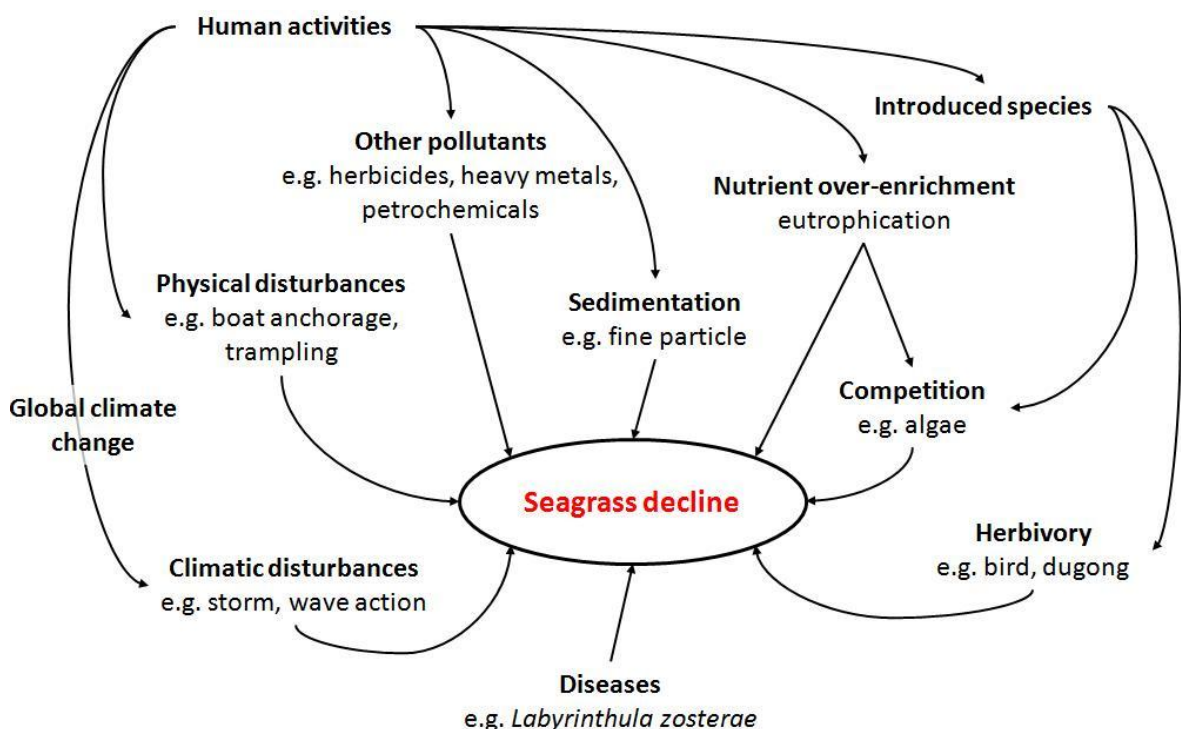
Figure 1.1: Stressors that can potentially affect seagrass standing stock or growth abilities, and contribute to its decline. Arrows indicate the proposed direction of impact.

Globally, along with herbivory, siltation and eutrophication of coastal areas are considered to be the major threats to the persistence of seagrass meadows (Burkholder et al 2007), but other anthropogenic pollutants (e.g., herbicides used in agriculture or in antifouling paints) may also be detrimental. Various herbicide components (e.g., *s*-triazines and substituted ureas) have been detected in estuarine water and sediment (Scarlett et al 1999, Haynes et al 2000, Macinnis-Ng et Ralph 2004, Shahidul Islam and Tanaka 2004, Lam et al 2005, McMahon et al 2005, Zhang et al 2008). These herbicides can detrimentally affect the mitosis process and/or the photosystem of aquatic plants (Coutris et al 2011), and thus may impede seagrass growth or regeneration after a grazing event.

Beyond the need to study how individual stressors affect seagrass meadows, studies that emphasise the combined effects of multiple stressors are required. Indeed, although seagrass herbivory (as well as biomass losses resulting from other causes such as wave action) is often part of the natural dynamics of seagrass meadows, the introduction of new grazer species, or an increase in grazer population size, combined with the relatively recent contamination of coastal areas due to human activities, may disturb the seagrass ecosystem. Relatively few studies have investigated the combined effect of multiple stressors on seagrass meadows and examined which of these may be more important (Ibarra-Obando et al 2004, Eklöf et al 2009). For instance, Eklöf et al (2009) showed that reduced water clarity (simulated by shading) negatively affected the seagrass resilience after black swan grazing damaged above- and below-ground biomass. In contrast Ibarra-Obando et al (2004) showed no interactive effect of nutrient loading, light reduction and leaf removal mimicking sea-urchin grazing. Research on the cumulative (and possibly interactive) impacts of multiple stressors

affecting seagrass beds is key for understanding and disentangling how the ecosystem responds to such pressures.

1.4. Thesis overview and objectives

To assess how seagrass is affected by multiple stressors, this study examines separately the impacts of herbivory and anthropogenic contaminants (nutrient enrichment, sedimentation and herbicide contamination) on seagrass meadow structure and condition, and then investigates the possibility of an interactive effect of these stressors on the resilience of seagrass meadows. To implement this, I used a model system that incorporates a major herbivore, the black swan (*Cygnus atratus* Latham 1790) that grazes on mono-specific meadows of *Zostera muelleri* Irmisch ex Asch. (Zosteraceae) (Jacobs et al 2006, Jones et al 2008).

The black swan population in New Zealand has long been considered as introduced by man from Australia in 1863. However, natural migration also occurred (Howard-Williams et al 1987, Heather and Robertson 1996). Fossil records also indicate that swans were already established in New Zealand prior to European settlement with *C. sumnerensis*, a species which is now referred to *C. atratus* (Worthy and Holdaway 2002). However, little is known about the historical abundance of swans in New Zealand and whether swan grazing has been a constant or sporadic selective pressure on New Zealand seagrass through evolutionary time. Nowadays, the black swan is considered as a native species of New Zealand and Australia. The New Zealand population is estimated to be ~ 35,000 birds (Kear 2005). The breeding populations congregate mostly in lakes whereas non-breeders mainly occupy estuaries, particularly during the summer moulting season, where they feed on seagrass (Williams 1981).

Zostera muelleri meadows occur predominantly on sheltered, intertidal sand flats in harbours. Low water clarity enables seagrass meadows to extend into the subtital zone in some locations (Schwarz 2004). Seagrass decline has been recorded in many New Zealand estuaries during the last century (Inglis 2003). Wasting disease detected during the 1960s, overgrazing by black swans and anthropogenic sedimentation and eutrophication have been postulated to contribute to this decline (Armiger 1965, Inglis 2003, Reed et al 2004, Park 1999). To our knowledge no studies have quantified black swan grazing impacts on New Zealand seagrass meadows and investigated the impact of anthropogenic contaminants on seagrass.

The present study is a combination of experiments, observations and measurements conducted *in situ* in mainly one estuary (Tauranga harbour), where urban land use is developing, and where the black swan population is increasing. The aim of the thesis is to quantify and identify the different pressures that detrimentally affect seagrass condition and examine a possible synergistic impact of these stressors on seagrass meadows.

1.5. *Organisation of the thesis*

The main body of the thesis comprises three chapters (2 to 4) each assessing the impact of stressors on seagrass meadows. Chapter 2 quantifies black swan grazing pressure and impact on seagrass meadows using an original alternative approach to traditional bird enclosure or exclosure experiments. Chapter 3 investigates, *in situ*, the relationships between current environmental parameters and seagrass condition. Chapter 4 examines seagrass meadow resilience to grazing pressure at sites differing in their environmental characteristics, notably the exposure to contaminants. The specific objectives of each chapter are:

▪ (Chapter 2) To document black swan grazing behaviour in estuaries and quantify consumption rates and disturbance impacts on seagrass beds. This chapter includes (1) observations on the timing and feeding patterns of swan grazing, (2) experiments to quantify *in situ* black swan consumption rates on seagrass and determine the nature of plant material eaten by the swan, and (3) analyses to examine possible relationships between swan grazing pressure and seagrass standing stock biomass at seasonal and annual time scales.

▪ (Chapter 3) To select and quantify a range of potential stressors and identify those environmental variables most strongly correlated with seagrass condition. This chapter consists of a single large survey of seagrass condition and potential environmental and contaminant stressors at ten sites in two harbours.

▪ (Chapter 4) To evaluate how seagrass meadow structure and recovery are affected by different levels of grazing intensity across sites that varied in exposure to anthropogenic contaminants and other environmental characteristics. This chapter consists of a grazing simulation experiment based on the black swan foraging behaviour quantified in chapter two, at sites with contrasting environmental stressors as defined in chapter three.

Chapter 2

Black swan (*Cygnus atratus*) grazing pressure and impacts on temperate intertidal seagrass (*Zostera muelleri*) meadows

Swan count at Katikati site, Tauranga Harbour.

2. Black swan (*Cygnus atratus*) grazing pressure and impacts on temperate intertidal seagrass (*Zostera muelleri*) meadows

2.1. Introduction

Seagrasses are found in the coastal zones of all continents except Antarctica (Short et al. 2007) and contribute significantly to coastal primary production (Hemminga and Duarte 2000, Beck et al. 2001). Globally, seagrasses are an important food source for a variety of marine herbivores including dugongs, manatees, turtles, fish, sea-urchins and waterfowl (Thayer et al 1984, Heck and Valentine 2006). Smaller grazers (e.g., sea-urchins, fish, and turtles) tend to remove only the leaves and shoots of plants while larger grazers (e.g., manatees, dugongs and waterfowl) will also uproot and consume roots and rhizomes (Jacob et al 1981, Vermaat and Verhagen 1996, Mathers et al. 1998, Hugues et al 2004, Tinkler et al 2009). In addition to the loss of biomass, grazing activities can disturb the structure of seagrass meadows by creating a mosaic of defoliated or devegetated patches (Jacob et al 1981, Thayer et al 1984). This can also lead to a change in environmental conditions within the meadow (e.g., current velocities, sediment quality) that is less conducive to seagrass re-growth (Kendrick et al 2002, Eklöf et al 2008), and meadows may become more vulnerable to erosive forces (Fonseca and Bell 1998). However, grazing effects might not always be detrimental. Removal of epiphytised leaves and senescent tissue by grazing may

reduce the susceptibility to slime mold (*Labyrinthula* sp.) infection (Jackson 2001). Periodic biomass removal may facilitate erosion of elevated intertidalbeds, counteracting excessive sedimentation and plant exposure to desiccation stress, thus assisting these meadows to regenerate on an annual basis (Jacob et al 1981, Nacked and Reise 2000). In some settings, seagrasses have been shown to compensate for low to moderate grazing losses (removal of 5 to 40 % of leaf biomass) by increasing their above-ground growth rate (Vergés et al 2008).

Avian grazing on intertidal seagrasses is often a seasonal occurrence with grazing events linked to migration and lasting for a few weeks to a few months (Jacob et al 1981, Thayer et al 1984, Portig et al 1994, Baldwin and Lovvorn 1994, Ganter 2000, Nacked and Reise 2000, River and Short 2007). Studies on migratory geese and wigeon in the Northern Hemisphere have documented intensive grazing events during the autumn and/or winter period. During these events, grazing can remove moderate to very high proportions of the seagrass biomass (20 - 98 %) (Portig et al 1994, Baldwin and Lovvorn 1994, Nacked and Reise 2000, Rivers and Short 2007). Recovery usually proceeds in the following growing season by vegetative expansion of remnant rhizomes and leaves and/or through germination of seeds (Jacob et al 1981). However, recovery can be slowed, or prevented, following high intensity grazing because of significant damage to, or consumption of, plant rhizomes or meristems (e.g., Rivers and Short 2007, Alcoverro and Mariani 2002). The recovery process is also dependent on the frequency of disturbance, the availability of resources to enable re-growth (e.g., sufficient light and nutrients) and the colonisation strategy of the plant species (Di Carlo and Kenworthy 2008) (i.e., recovery may be hindered by a lack of dissemination structures (e.g., seeds) (Kendrick et al 2002, Altstatt 2003, Orth et al 2006, Eklöf et al 2008)).

In New Zealand, the black swan (*Cygnus atratus* Latham 1790) is the only large grazer of intertidal seagrass meadows. The black swan is a native species of New Zealand and Australia (Worthy and Holdaway 2002) and the New Zealand population is estimated to be ~ 35,000 birds (Kear 2005). Black swans are present in New Zealand lakes and estuaries all year. However, the non-breeding birds tend to congregate in large estuaries during the moulting season (summer-autumn period) where they graze as flocks on the intertidal seagrass meadows (Williams 1981). Evaluating the swans grazing pressure is an important first step toward understanding its role in the dynamics of seagrass meadows.

Zostera muelleri Irmisch ex Asch. (Zosteraceae) is the only seagrass species in New Zealand (Jacobs et al. 2006, Jones et al 2008) and it forms meadows occurring predominantly on sheltered, intertidal sand flats in estuaries. Seagrass meadows have declined in many New Zealand estuaries during the last century (Park 1999, Inglis 2003, Reed et al 2004). Seagrass wasting disease, which was detected during the 1960s (Armiger 1965), may have contributed to the decline together with other potential causes including anthropogenic sedimentation and eutrophication (Park 1999, Inglis 2003). Overgrazing by black swans is also postulated as a factor contributing to seagrass decline in some locations (Park 1999).

In temperate climates, seagrass communities are often subject to strong seasonal variations in biomass (Duarte and Chiscano 1999, Turner and Schwarz 2006a). Moreover swans not only migrate to estuaries seasonally but also can move within an estuary when resources within a site have been exhausted (Percival and Evans 1997). Thus, observations and measurements that account for such variations in grazer and seagrass abundance are necessary to accurately assess the effects of herbivory. I used such an approach to examine whether grazing by black swans might contribute to

seagrass decline in a large New Zealand estuary, Tauranga Harbour. In 1959, 22 % of Tauranga Harbour surface area was covered by both intertidal (89 %) and subtidal (11 %) *Z. muelleri*. By 1996, more than one-third of the seagrass meadows had disappeared, reducing the seagrass coverage to 15 % from which 27 % of the intertidal and 90 % of the subtidal seagrass was lost (Park 1999), while black swan numbers in this harbour have steadily increased (from 1900 to 5100 birds in mid-summer between 1979 and 2010; Eastern Region Fish and Game Council, unpublished data). The increased grazing pressure on seagrass in this harbour, which may be due to decline of macrophytes in inland lakes, may have contributed to the observed decline in meadow area, particularly for intertidal plants and those shallow subtidal plants within the reach of grazing swans (i.e. <1m depth). Our aims in this study were to (1) document the grazing behaviour of black swans feeding on seagrass meadows; (2) quantify *in situ* seagrass consumption rates by black swans and grazed patch characteristics; and finally, (3) to examine possible relationships between swan grazing pressure and seagrass standing stock biomass at seasonal and annual time scales. To quantify seagrass consumption rates I used an original, alternative approach to traditional bird enclosure and exclosure experiments. Enclosure and exclosure experiments were considered for this study but were highly problematic for the following reasons; (1) enclosure facilities to pen large, mobile birds like swans for controlled feeding experiments are rare (i.e. only in zoos) and the birds natural behaviour may be altered in such situations and not representative of that *in situ*; (2) cages or other structures to exclude birds from parts of the seagrass meadow are prone to fouling with floating seaweed and other flotsam, and if adjacent to populated areas are highly vulnerable to tampering or removal.

2.2. Materials and methods

2.2.1. Study sites

The study was conducted in Tauranga Harbour on the North Island of New Zealand (Latitude: 37° 41' 7", Longitude: 176° 9' 58") (Fig. 1). This harbour has a surface area of 201 km^2, of which ~14 % (29 km²) is covered by seagrass meadows (Park 1999). Five sites considered representative of Tauranga Harbour were chosen (Fig. 2.1), varying in seagrass meadow extent and swan occupation. Field observations and measurements were made between April 2007 (autumn) and February 2010 (summer).
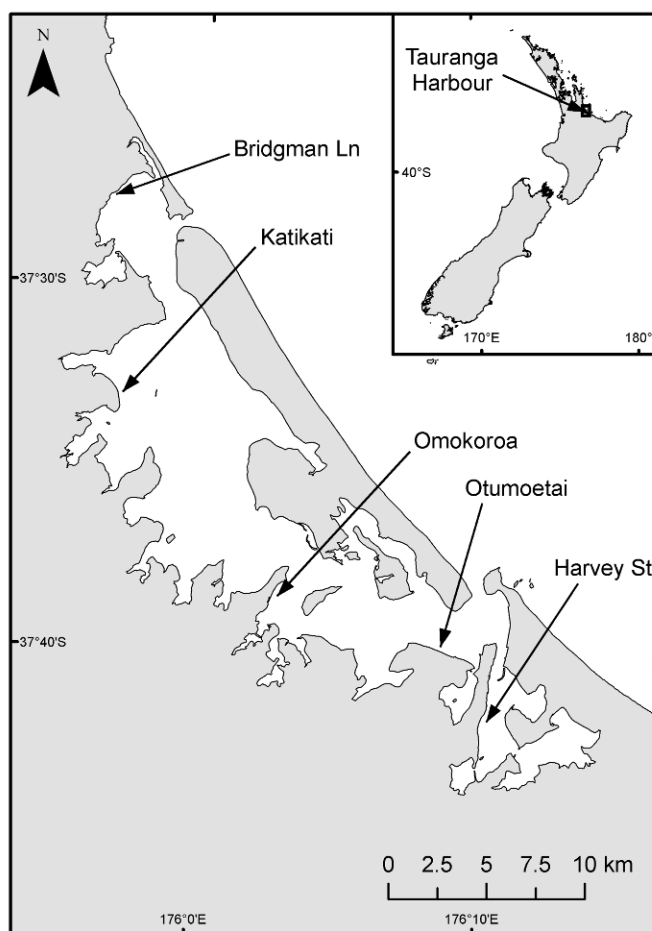
Figure 2.1: Location of the five study sites in Tauranga Harbour, North Island, New Zealand.

2.2.2. Swan numbers and grazing patterns

Once a month for a two-year period (March 2008 to February 2010), swan numbers were counted at each site during the day at low tide using binoculars (Asahi Pentax Binoculars, 10 × 50 Field 55º) to assess seasonal patterns. Diel grazing behaviour was assessed periodically at 2 sites (Omokoroa and Katikati) where swans were relatively numerous (> 50) and they could be observed with minimal disturbance. Swan observations were made for four contrasting tide/diel scenarios: low tide (1) and high tide (2) around midday, and low tide (3) and high tide (4) around midnight. Observations were made for either 12 or 24 h on 3 or 4 different occasions for each scenario during the period when swans were most abundant in the harbour (December to May). Swans were counted and their behaviours ((1) sleeping, (2) grazing, or (3) other activity (e.g., loafing, flying, swimming and walking)) observed and recorded every 15 min using binoculars. Infra-red binoculars (ATN Voyager Night Vision) were used during night-time observations.

2.2.3. Quantifying patch size and biomass removal

Circular defoliated patches within the seagrass meadows were clearly visible and attributable to swan grazing. The most recently-made patches were identified by the presence of adjacent fresh swan faeces and/or uprooted seagrass fragments. I measured the dimensions of 10 freshly grazed patches at 2 sites (Omokoroa and Bridgman Ln.) on 3 separate occasions (November 2007, February and March 2008) to determine the average size of the defoliated patches.

I also determined the average amount of above-ground and below-ground biomass removed from these defoliated patches. On three occasions at the Omokoroa site (March, April and May 2008), I collected single cores (8 cm diameter × 10 cm depth)

from the centre of 10 grazed patches and another set of 10 cores randomly selected from adjacent ungrazed areas of the meadow. For the latter, I carefully selected areas that had no obvious signs of recent grazing (e.g., leaf breakage, exposed roots, bare patches). Plant material was extracted from the cores and separated into shoot, root and rhizome fractions. All samples were dried (70°C for 48 h) to determine dry weight (DW).

2.2.4.   *Seagrass consumption rates, calorific value and biomass*

Seagrass consumption rates were estimated *in situ* 5 times across 2 sites (Omokoroa and Katikati), in summer-autumn (January 2008, March to May 2008 and February 2009). Within the seagrass meadow, an observation plot (100 × 100 m or 75 × 75 m) was marked in each corner with buoys at low tide and all swan faeces were removed. The number of swans within the plot was counted every 15 min for either 12 or 24 h to determine mean swan density during the experiment. The duration of the experiment was based on the following assumptions: (1) that the rate of throughput does not exceed 6 hours (the black swan has a mean digestive tract length of 3.83 m (n = 6, V. Dos Santos, unpublished data) which lies between that of the whooper swan (3.17 m) and the mute swan (4.05 m) which have food retention times of 3 and 6 hours, respectively (Clausen et al 2002)) and (2) that swan numbers entering versus exiting the plot remained relatively constant for the duration of the observation period. At the end of the observation period all faeces within the plot were collected and the DW (70°C for 48 h) determined. Seagrass samples were also collected and dried on one occasion. I used lignin as an indigestible marker (herbivorous birds are unable to digest lignin; Newsholme and Start 1973), to estimate the quantity of seagrass consumed from the quantity of faeces collected. The lignin content (mean ± SE) as a % DW of faeces (L_F) (7.6 ± 0.6 %; n = 3) and seagrass (L_S) (5.8 ± 0.4 %; n = 3) was

determined using a Tecator Fibretec System (Robertson and Van Soest 1981). I calculated the digestibility (%) of seagrass as:

$$\text{(Eq. 1)} \quad d = \left(1 - \left(L_S / L_F\right)\right) * 100$$

24 % of the seagrass was digested and thus lost to the environment. I therefore applied a correction factor (S_d) of 1.31 (L_F/L_S) to our calculations of seagrass consumption rates (see Eq. 2).

I also noted that a loss of swan faeces could occur from plots due to faeces degradation and/or transport by tidal currents. I assessed this potential loss term on 3 occasions (Omokoroa site, May 2008, January and February 2010) under conditions (tidal range, wind speed) similar to those encountered during our seagrass consumption measurements. Faecal samples (n = 20, for each occasion) were collected at low tide, marked using insoluble spray-paint, weighed (wet weight, WW) and distributed at randomly chosen locations within a 100 × 100 m plot area. On the following low tide intact marked faeces remaining in the plot were collected and reweighed; 53 ± 17 % (WW) of the faeces were lost.

Seagrass consumption rates (CR; g DW swan^{-1} d^{-1}) were estimated using:

$$\text{(Eq. 2)} \quad CR = \frac{\left(Q_f * F_w * S_d\right)}{n * t}$$

where Q_f is the quantity of faeces collected (g DW), F_w is the correction factor for the amount of faeces washed away by one tide cycle (2.11 for a 12 h experiment (100/(100-53)) or 4.46 when 2 tide cycles occurred during a 24 h experiment (100/(100-53))^2), S_d is the correction factor for the amount of seagrass lost via digestion (1.31), and n is the average number of swans present in the plot area during the observation/feeding period t (either 0.5 or 1 day).

To estimate the daily energy intake by the black swan, calorific values of both above and below-ground biomass of *Z. muelleri* were obtained by combustion of dried seagrass samples (from Omokoroa site, n = 6) in an Oxygen Bomb Calorimeter (PARR Oxygen Bomb Calorimeter, 1341 Plain Jacket Calorimeter). The black swan daily energy intake (E_I) was estimated in kilo-joule per day (kJ day^{-1}) by:

$$(\text{Eq. 3}) \quad E_I = CR * S_C$$

where S_C is the mean seagrass calorific value (kJ g^{-1}).

Seagrass meadow size (m^2) at each site was estimated during summer (2007/2008) using a tape measure as the distance occupied alongshore multiplied by distance occupied downshore. Seagrass biomass (S_b; g DW m^{-2}) was determined on a monthly basis for one year (February 2009 to February 2010) to compare to that removed by swan grazing. To avoid repeated destructive sampling of meadows, seagrass biomass was estimated from percentage (%) cover values in 6 plots (0.25 m^2) at all sites except Otumoetai. I established a relationship between % seagrass cover and biomass on one occasion by sampling 15 plots (0.25 m^2) at each of the 5 study sites. Percentage seagrass cover was determined and biomass measured from a core (8 cm diameter × 10 cm depth) collected in the middle of each plot, from which seagrass (above and below ground biomass) was extracted, dried (70°C for 48 h) and weighed. I converted monthly percentage cover measurements into biomass (g DW m^{-2}) using the following equation ($r^2 = 0.71$, p < 0.01):

$$(\text{Eq. 4}) \quad S_b = (\% \text{ cover - } 5.90)/\, 0.18.$$

Black swan grazing pressure (Sg) was expressed as a proportion (%) of the standing seagrass biomass and estimated using the following formula:

$$(\text{Eq. 5}) \quad Sg = \frac{CR * mn * t_m}{S_b * a} * 100$$

where *mn* is the monthly swan number in the plot area and t_m is the number of days in a month, *a* the size of the plot area (10,000 m^2). Monthly swan number in the plot area was estimated as 5.7 % of the swan number counted monthly at each site from March 2009 to February 2010. This percentage corresponds to the average proportion of the swan population present in the plot area during the consumption rate experiments. Seagrass biomass and grazing pressure data were pooled and presented as seasonally and annually averaged values for each site.

2.2.5. *Data analyses*

Bivariate linear regression analysis was used to examine relationships between swan numbers/density and seagrass meadow size. The amount of time swans spent grazing during the day versus the night, seagrass biomass in February 2009 versus February 2010 and seagrass calorific value of above versus below-ground biomass, were compared with a Student's *t*-test. Above, below-ground, root, rhizome and total biomass of grazed versus ungrazed areas, were compared with a paired Student's *t*-test. Significant temporal and spatial differences ($p < 0.05$) in swan numbers were explored with a three-way analysis of variance (ANOVA) where site, year, season and combined effects were tested. Seagrass biomass and grazing pressure among sites and seasons were examined using two-way ANOVA. Post-hoc Tukey HSD multiple comparison tests were used for pair-wise comparisons when applicable. Statistica Version 8 (StatSoft, Inc. Oklahoma, USA) was used for all statistical analyses.

2.3. Results

2.3.1. Seasonal swan numbers and daily grazing patterns

Swans generally congregated in the harbour from late spring to early winter (November to June) (Fig. 2.2). The swan population typically increased during the summer months to reach a maximum during autumn then decreased through winter and spring. The most populated sites were Bridgman Ln, Omokoroa and Katikati, with annual average swan numbers of 154, 88 and 53 respectively. Harvey St. and Otumoetai sites were much less populated by swans (annual average swan number 16 and 0 respectively) and were close to urban areas. Seasonal trends in swan numbers differed significantly among sites (three-way ANOVA, site × season effect $F_{9,64} =$ 9.49, p < 0.01). Swan numbers were significantly higher during autumn at Bridgman Ln. and Omokoroa sites compared to other sites (Tukey HSD test, p < 0.05).

Seagrass meadow sizes varied from 0.005 (Harvey St. and Otumoetai sites) to 0.72 km^2 (Bridgman Ln. site). Omokoroa and Katikati sites had intermediate meadow sizes of 0.24 and 0.09 km^2 respectively. I found a significant positive correlation between meadow size and annual average swan number ($r^2 = 0.92$, p < 0.01, n = 5) but no significant correlation between swan density and meadow size ($r^2 = 0.64$, p = 0.52, n = 5). I found no evidence of a critical meadow size for swan occupation in this study. The smallest meadow size measured in this study at two sites (both adjacent to urban areas) was 0.005 km^2. While one site (Harvey St.) was periodically occupied by a small number of the swans, swans were entirely absent from the other site (Otumoetai).
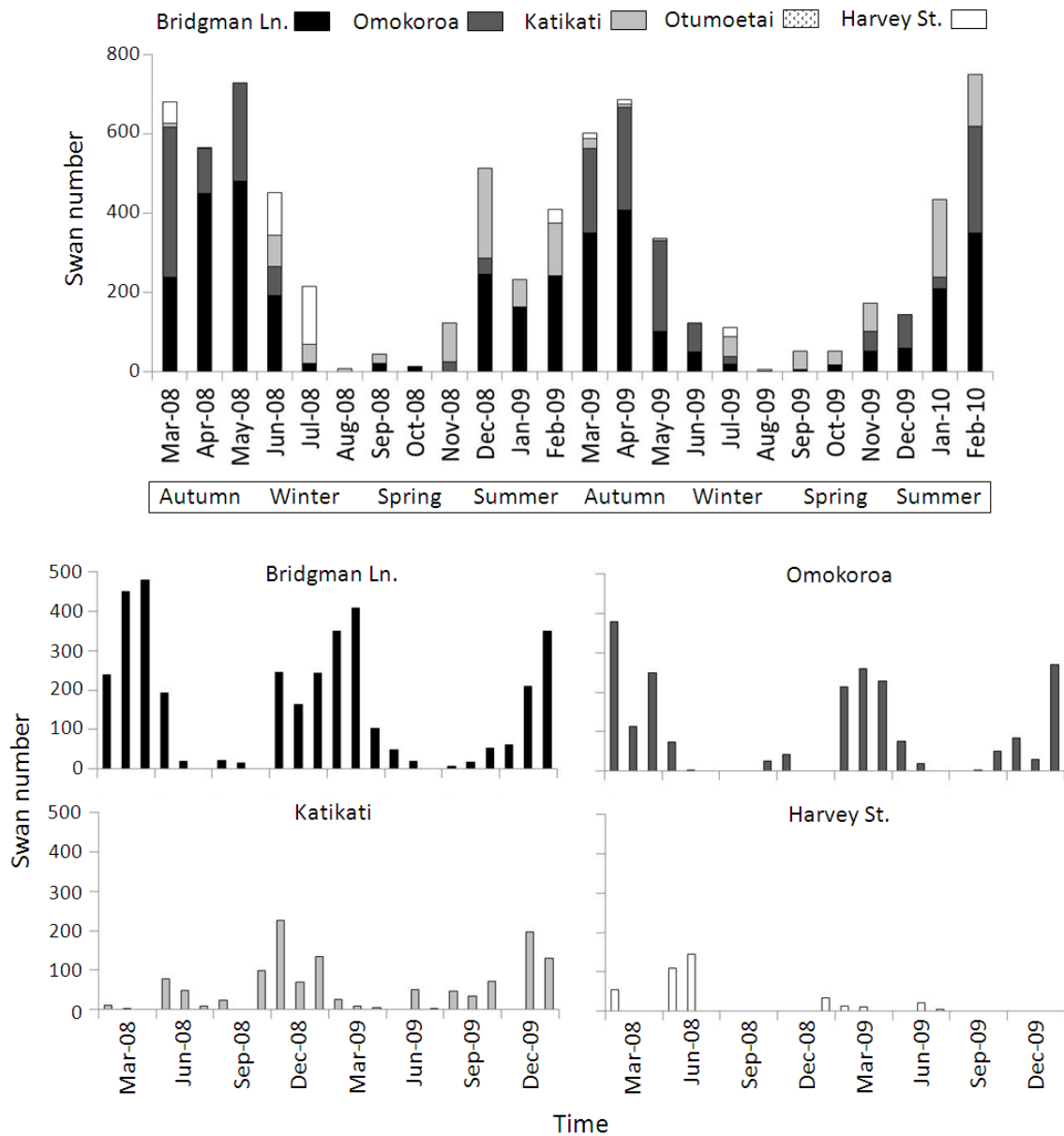
Figure 2.2: Numbers of black swans recorded monthly at each site in Tauranga Harbour from March 2008 to February 2010.The trend in the total count summed for all sites is illustrated in the top panel while counts for individual sites are shown in the lower panels. Black swan number is not separately illustrated for the Otumoetai site since no swans were present at this site during the two-year count period.

Our observations showed that black swans generally only grazed on the seagrass meadows when plants were covered by shallow water (i.e., during mid-high tide when water depth was less than ~1 m, the length of their necks), and that this grazing pattern was not affected by the day-night cycle (Fig. 2.3).

There was no significant difference (*t*-test, n = 6, p = 0.79) between the average amount of time spent grazing on meadows during the night (58 %) and the day (59 %) (Table 2.1). Swans were generally sleeping or grooming when the seagrass meadows were exposed at low tide.
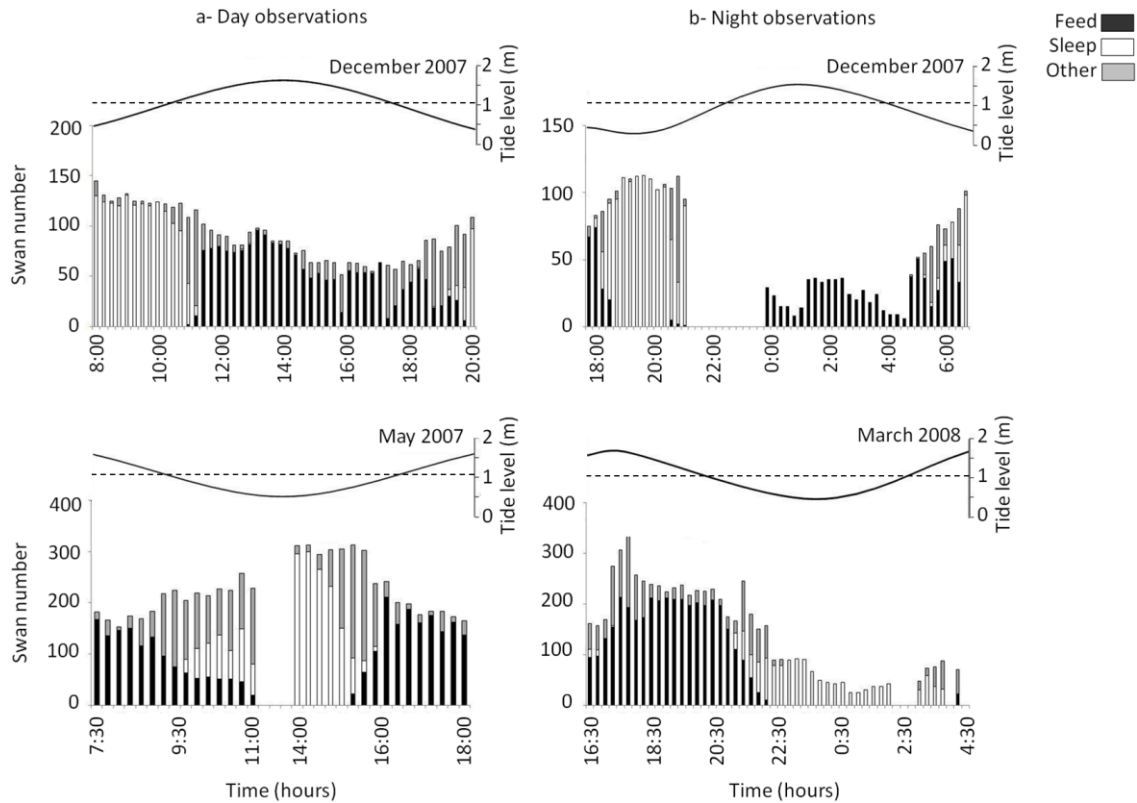
Figure 2.3: Examples of day and night swan behaviour results at the Omokoroa site. Counts were made every 15 min during the day (a; left graphs) and the night (b; right graphs) when high tide occurs at the middle of the observation period (upper graphs) and when low tide occurs at the middle of the observation period (lower graphs). The above solid curve represents the tidal state during the observation period and the dashed line indicates the upper limit of seagrass depth distribution. The decrease in swan numbers during the night reflects reduced visibility for counting rather than swan exodus from sites.

Table 2.1: Summary of swan grazing observations from May 2007 to April 2008 at two study sites (Katikati and Omokoroa), and the proportion of time that swans spent grazing on seagrass meadows during the day and night.

Observation scenario	Location	Date	Start time (h:min)	Duration (h)	Time of high or low tide (h:min)	Proportion of time spent grazing (%)	Proportion of time spent grazing per night and day Mean ±SE
Night – High tide	Omokoroa	Dec 07	17.30	12	0.45	60	
	Katikati	Jan 08	19.15	12	0.30	52	
	Omokoroa	Apr 08	18.30	12	0.45	67	**58 ±3**
Night – Low tide	Katikati	Feb 08	13.00	24	1.30	49	
	Katikati	Feb 08	15.15	24	1.30	69	
	Omokoroa	Mar 08	13.15	24	23.30	49	
Day – High tide	Omokoroa	Dec 07	8.00	12	14.15	73	
	Katikati	Feb 08	5.45	12	11.15	67	
	Katikati	Mar 08	6.45	12	11.30	49	
Day – Low tide	Omokoroa	May 07	7.30	12	11.30	50	**59 ±3**
	Omokoroa	May 07	7.30	12	12.15	56	
	Omokoroa	May 07	7.30	12	13.00	58	
	Omokoroa	May 07	7.30	12	13.45	60	
Total mean ±SE						**59 ±2**	

2.3.2. *Defoliated patches*

Two main types of grazing behaviour were observed. The first type consisted of swans remaining relatively static and intensively grazing in one place. This type of grazing was most common when swans were undisturbed and resulted in circular defoliated patches. These patches often took the form of shallow pits in the sediment as swans dug to extract seagrass rhizomes and roots. The second type of grazing behaviour was observed when a disturbance occurred (e.g., boat traffic) and consisted of swans grazing while swimming. In this case, grazing impacts were difficult to quantify; however it was possible to find occasional plants uprooted and defoliated when the seagrass meadow was observed on the subsequent low tide.

Freshly formed defoliated patches varied in diameter from 0.3 to 1.5 m with a mean (± SE) surface area of 0.28 ± 0.02 m^2. Analysis of seagrass biomass remaining in intensively grazed patches compared to ungrazed controls showed that overall 60 % of the total seagrass biomass was removed from the grazed patches including 92 % of the seagrass above-ground and 43 % of the seagrass below-ground biomass. From the below-ground biomass, 25 % of the roots and 99 % of the rhizomes were removed (Table 2.2). The wide variation in seagrass biomass found within the ungrazed control areas suggests that swans probably randomly select feeding patches at the meadow scale (Table 2.2).

Table 2.2: Seagrass biomass in grazed patches compared to ungrazed areas of seagrass meadows, and the proportion of biomass removed by intensive swan grazing creating defoliated patches, at Omokoroa (n = 10 for each date).

Date	Below-ground biomass						Above-ground biomass		
	Rhizome biomass			Root biomass			Shoot biomass		
	Ungrazed area (g m^{-2})	Grazed patch (g m^{-2})	Biomass removed (%)	Ungrazed area (g m^{-2})	Grazed patch (g m^{-2})	Biomass removed (%)	Ungrazed area (g m^{-2})	Grazed patch (g m^{-2})	Biomass removed (%)
19 Mar 08	54	0	99	130	92	26	68	5	92
29 Apr 08	39	0	100	161	133	18	75	10	87
27 May 08	42	1	98	136	89	29	110	2	99
Mean ± SE	45 ± 4	0 ± 0	**99 ± 1** [a]	142 ± 12	105 ± 12	**25 ± 5** [a]	84 ± 11	6 ± 1	**92 ± 2** [a]
Max	95	6	100	331	307	79	280	28	100
Min	11	0	80	45	45	0	30	0	48
Biomass removed (%)	**Below 43 ± 4** [a]						**Above 92 ± 2** [a]		
	Total 60 ± 3 [a]								

[a]significant differences between grazed and ungrazed areas were found for the rhizome, root, shoots, below-ground and total biomasses (paired *t*-test, n = 30, p < 0.01).

2.3.3. *Seagrass consumption rates, calorific value and biomass*

I measured seagrass consumption rates ranging from 140 to 850 g DW swan^{-1} day^{-1} with a mean consumption rate of 394 g DW swan^{-1} day^{-1} (Table 2.3). Seagrass calorific values averaged (± SE) 11 ± 0.4 kJ g^{-1}, and were significantly higher (*t*-test, n = 6, p < 0.01) for the leaves (12.2 ± 0.4 kJ g^{-1}) than the roots and rhizomes (9.9 ± 0.3 kJ g^{-1}). Using the mean consumption rate and the mean seagrass calorific value, I calculated that the black swan calorific intake averaged 4334 kJ day^{-1}.

Table 2.3: Seagrass consumption rates by black swans based on the amount of faeces collected in the observation area and swan numbers (with corrections applied for digestion and tidal/degradation losses).

Date	Location	Collection period (h)	Plot area size (ha)	Mean swan number (n ha^{-1})	Faeces collected (Qf) g DW ha^{-1}	Consumption rate (CR) g DW swan^{-1} day^{-1}
29/30 Jan 08	Katikati	12	1.0	8	460	319
19/20 Mar 08	Omokoroa	24	0.6	19	454	140
28/29 Apr 08	Omokoroa	12	1.0	13	809	345
26/27 May 08	Omokoroa	12	1.0	8	1,228	850
04/05 Feb 09	Katikati	12	1.0	6	341	315
Mean ± SE						**394 ± 120**

2.3.4. *Seagrass biomass and grazing pressure*

Seagrass biomass differed among sites and seasons (Two-way ANOVA, site effect $F_{3, 32}$ = 6.68, p < 0.01, season effect $F_{3, 32}$ = 4.90, p < 0.01). The site with the lowest swan number (Harvey St.) had significantly higher biomass compared to the three other sites (Tukey HSD test, p < 0.05). Although seasonal patterns did not differ across the sites (significant site × season interaction, $F_{9, 32}$ = 1.70, p = 0.13), the biomass at all sites was significantly higher during autumn than in any other season (Tukey HSD test, p < 0.05; Fig 2.4).
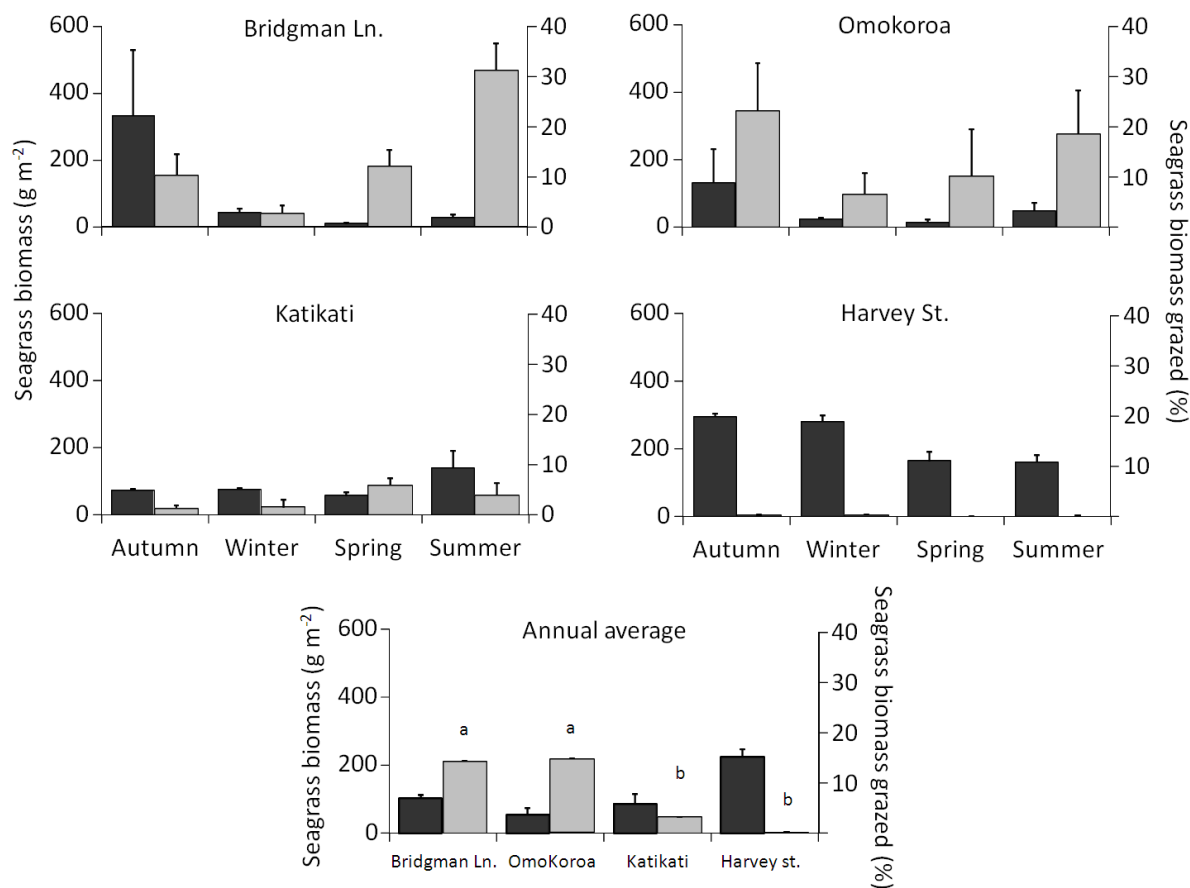
Figure 2.4: Seasonal variations in seagrass biomass and swan grazing pressure (expressed as a % of the biomass standing stock) for each site, and the annual average at study sites in Tauranga Harbour (March 2009 to February 2010). Black columns represent the measured seagrass biomass and grey columns the grazing pressure. Error bars indicate 1 SE of mean values. For grazing pressure columns within each graph those with the same letter are not significantly different at p > 0.05 (Tukey HSD test).

Grazing pressure differed significantly among sites and seasons but the interaction of these two factors was also significant indicating that the seasonal trend in grazing pressure also varied amongst sites (Two-way ANOVA, site effect $F_{3, 32} = 8.78$, p < 0.01, season effect $F_{3, 32} = 3.73$, p < 0.05, site × season effect $F_{9, 32} = 2.26$, p < 0.05). Grazing pressure was consistently higher at the two most swan-populated sites (Bridgman Ln. and Omokoroa sites) in all seasons with the highest pressure (~50 % of biomass) recorded at the Bridgman Ln. site during summer. On an annual basis (i.e.,

performing a one-way anova on pooled seasonal data), I found that grazing pressure was also significantly higher at these two sites (Bridgman Ln., 19 ± 6 % and Omokoroa, 20 ± 6 %) compared to the Katikati (3 ± 1 %) and Harvey St. (0 ± 0 %) sites (Tukey HSD test, p < 0.05; Fig. 2.4).

At the most swan-populated sites, the standing stock of seagrass biomass was considerably lower in the second growing season I monitored (i.e., in February 2010 versus February 2009) (Bridgman 69 % reduction *t*-test, n = 6, p < 0.01; Omokoroa 43 % reduction, *t*-test, n = 6, p = 0.15). At the other two sites (Harvey St. and Katikati) there was minimal change in seagrass biomass (-3 to +1 %) during this time interval (Fig. 2.5).
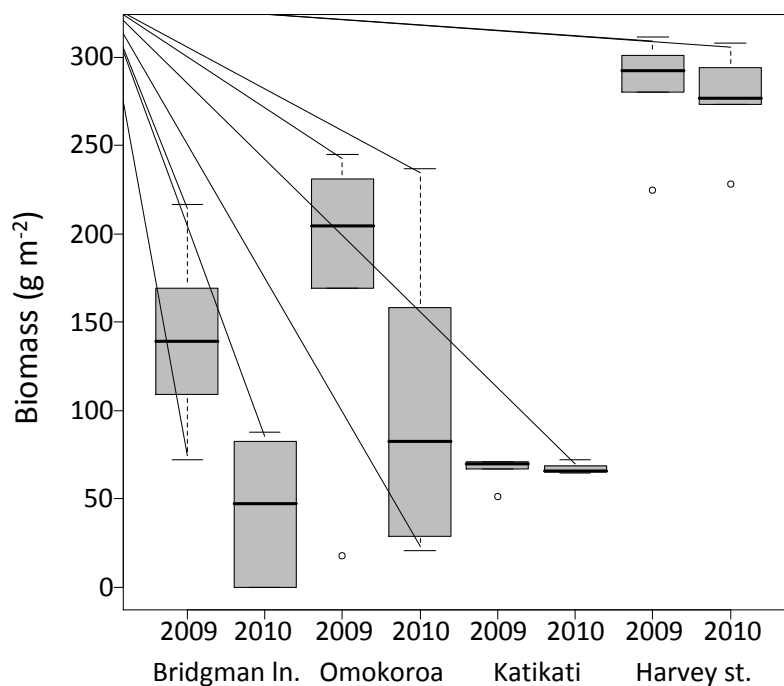
Figure 2.5: Seagrass biomass in February (summer) 2009 and in February 2010 at study sites in Tauranga Harbour. Black bars represent the median, grey columns incorporate the upper to lower quartile range, dashed bars indicate the lowest and/or highest values measured and circles represent outliers.

2.4. Discussion

2.4.1. Swan grazing behaviour and impacts on seagrasses

Results from this study indicate seasonal and spatial patterns in black swan grazing activities. Black swans were most numerous during the autumn period. This coincides with the time of maximum seagrass biomass (Fig. 2.4), consistent with *Z. muelleri* growth in Australia (Kirkman and Cook 1982), and with the time when seagrass rhizomes are likely to be most nutritious as resources are translocated by the plant to over-wintering organs (Dawes and Lawrence 1980). Swans congregated at the sites where the largest seagrass meadows occurred and appeared to randomly graze on different parts of the meadow suggesting that high density seagrass patches do not seem to be preferably targeted by swans. Their distribution was significantly related to seagrass presence which is consistent with observations of black swans in Western Australia (Eklöf et al 2009) and black-necked and coscoroba swans in an Argentinean coastal lagoon (Bortolus et al 1998).

Black swans grazed the meadows predominantly at high tide when the plants were covered by a shallow layer of water (<1m; the length of their necks). It has been suggested that waterfowl feed on seagrass whenever the plant is easily accessible (Percival et al 1996, 1998). However, in this study swans were rarely observed grazing on seagrass while plants were fully exposed at low tide in contrast to other birds such as geese and wigeon (Jacob et al 1981, Percival and Evans 1997). Swans are the largest herbivorous birds that graze on seagrass (Kear 2005). By grazing when plants are immersed in shallow water has some advantages for the swans; (1) seagrass leaves are floating making plants easier to grasp and remove, (2) their energy expenditure is reduced since their body weight is supported by water and they are

better adapted for movement on water than land, possessing webbed feet and relatively powerful legs adapted for propulsion in the water (Hughes and Green 2005).

Swan grazing resulted in the formation of circular devegetated patches where a very high proportion of shoots and rhizomes were removed. When grazing on subtidal seagrass, dugongs are known to create similar-sized scars (~0.39 m^2) impacting the entire plant (Masini et al. 2001). Rhizomes were probably targeted because they typically have a higher sugar and starch content than other seagrass parts (Burke et al. 1996). However shoots often contain more nitrogen than roots (Cebrián and Duarte 1998). In this study I found that *Z. muelleri* shoots had a higher calorific value than below-ground parts which is consistent with other *Zostera* species (Baldwin and Lovvorn 1994, Percival and Evans 1997). Thus, it suggests that the swans will preferentially target both the shoots and rhizomes of *Z. muelleri*. Overall, swans removed on average 60 % of the seagrass biomass in these devegetated patches which is a similar level of biomass removal to that found for dugong grazing scars (68 % biomass removal; Masini et al. 2001).

In contrast, smaller seagrass herbivores such as sea urchins, fishes and turtles usually graze only on aboveground parts of vegetation (Heck and Valentine 1995, Rose et al 1999, Valentine et al 2000, Moran and Bjorndal 2007, Vergés et al 2008). They tend to crop the leaf blade, without damaging foliar meristems, and plants are able to readily re-generate following short periods of high grazing pressure (Zieman et al 1984, Valentine et al 1997, Kirsch et al 2002, Moran and Bjorndal 2005, Kuiper-Linley et al 2007, Unsworth et al 2007). In the case of larger grazers, such as black swans, that target both above and below-ground parts, plant recovery can be more difficult (Rivers and Short 2007). By damaging foliar and basal meristems the ability to compensate for growth is reduced and this is also compounded by removal of the

photosynthetic material that is needed to support new growth (Huhta et al 2000). In this study I found that swans removed a very high proportion of rhizomes (99 %) in grazed patches which might be expected to hinder plant recovery in these areas. In contrast studies on tundra, trumpeter and bewick swans that feed on fresh water macrophytes have shown that not all of below-ground tubers are removed and thus plants can regenerate the following growing season (LaMontagne et al 2003, Sponberg and Lodge 2005, Beekman et al 1991, Nolet et al 2001). It has been suggested that constraints on energetic profitability limit the ability of swans to fully exploit the tubers, and a sufficient density of tubers is left behind enabling subsequent recovery (Sponberg and Lodge 2005).

2.4.2. Seagrass dynamics in relation to spatio-temporal variations in grazing pressure

I evaluated grazing pressure in Tauranga Harbour by comparing the consumption of seagrass by swans to the biomass of the *Z. muelleri* meadows. The first step in this analysis was the measurement of the consumption rate. I performed these measurements *in situ* based on faecal deposition rate which contrasts with most prior studies that have quantified waterfowl consumption of *Zostera*. Other studies have mostly used enclosure experiments where a food supply is provided to caged birds (Mathiasson 1973) and/or *in situ* exclosures that compare changes in seagrass biomass between grazed and ungrazed areas (Charman 1975, Jacobs et al 1981, Madsen 1988, Percival and Evans 1997). Despite differences in methodology, our average consumption rate (394 g DW swan^{-1} day^{-1}) is consistent with these other studies of bird grazing after taking into account body-weight differences between species (Table 2.4). Moreover, the average daily energy intake (E_I) I calculated, based on this consumption rate and the calorific value of seagrass that I measured (E_I = 4334 kJ

day^{-1}), is comparable to the daily energy requirement (E_R) calculated by using the formula of Nagy (1987) (E_R = 10.9 body mass $^{0.64}$ = 3907 kJ day^{-1}, black swan body mass from Tauranga Harbour averaged 5.0 ± 0.2 kg, n = 22, V. Dos Santos, unpublished data).

Table 2.4: Consumption rates of herbivorous birds grazing on *Zostera* spp.

Bird species	Body weight kg	Diet	Consumption rate g DW [kg bird^{-1}] day^{-1}	Reference
Anas acuta	0.8	*Z. noltii*	84[a]	Jacobs et al 1981
Anas penelope	0.5	*Z. noltii*	90[a]	Jacobs et al 1981
Anas platyrhynchos	1.1	*Z. noltii*	76[a]	Jacobs et al 1981
Branta bernicla	1.4	*Z. noltii*	87[a]	Charman 1975
Branta bernicla	1.4	*Z. noltii*	71[a]	Jacobs et al 1981
Branta bernicla	1.4	*Zostera* spp.	96[a]	Madsen 1988
Branta bernicla	1.4	*Z. noltii*	83[a]	Percival and Evans 1997
Cygnus atratus	5	*Z. muelleri*	79	Present study
Cygnus olor	9	*Z. marina*	77[b]	Mathiasson 1973

[a]consumption rate evaluated *in situ* using exclosure experiment; [b]consumption rate evaluated using cage/enclosure experiment.

By plotting seagrass consumption rates versus bird body weights from the literature and from my study, I found a significant linear relationship (r = 0.999, p < 0.01, n = 9) suggesting that the seagrass intake is proportional to bird body weight (Fig. 2.6). This relationship could potentially be used to estimate seagrass consumption rates for other herbivorous bird feeding on seagrass meadows that have not been previously evaluated.
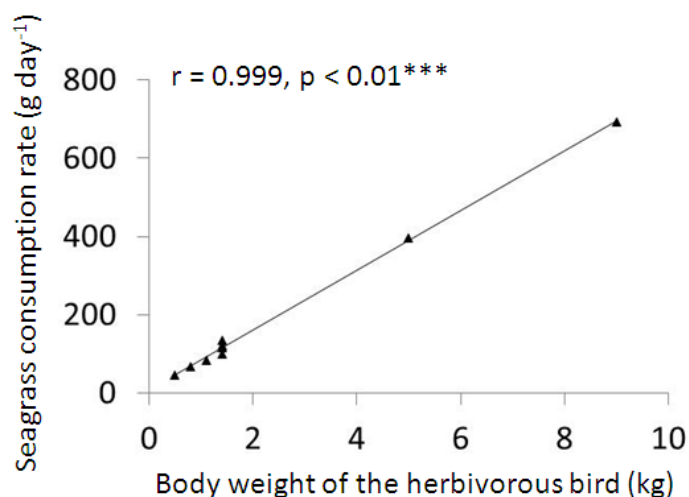
Figure 2.6: Relationship between the body weight of herbivorous birds and seagrass consumption rates. The solid line represents the linear regression (r = 0.999, p < 0.01, n = 9).

Analysis of our site-specific results indicated that sites with the highest grazing pressure, suffered substantial declines in the standing stock of seagrass biomass in the subsequent growing season. Despite the seagrass loss, swans continued to occupy these sites since no significant changes in swan numbers were observed between summer 2009 and 2010. Conversely, at sites where grazing pressure was lower, no change in the standing stock of seagrass biomass was observed. While these results are purely correlative, they do suggest the presence of a potential threshold (19-20 % of mean biomass removed annually) above which seagrass decline may occur as a result of swan grazing. However, this study does not include measurement of other site-specific processes, such as eutrophication, sedimentation and physical disturbances (e.g., removal of biomass by currents, boat anchor/propeller scarring; Fonseca and Bell 1998, Creed and Amado Filho 1999), that may contribute to seagrass loss alone or in interaction with swan grazing. Nevertheless, our results also suggest that grazing pressure is spatially variable around the estuary since some sites had minimal grazing and seagrass loss. Swans seem to prefer sites with large seagrass

meadows and minimal disturbances (e.g., boat traffic). It has been previously reported that human disturbances can decrease the population size of a grazer resulting in a reduction of the grazing pressure (Ranwell and Downing 1959, Jacobs et al 1981). Sites with minor swan grazing pressure close to urban areas (e.g., Harvey St. site) might represent a reserve of floating vegetative propagules and/or seeds that could play a role in the regeneration of nearby impacted meadows (Preen 1995).

Based on the results of this study and Eq. 5, at the estuary-wide scale annual grazing pressure could account for 15 % of the average seagrass biomass, assuming an estuary-wide seagrass area of 29.33 km^2 (Park 1999), an annually averaged number of swans in the harbour of 3700 and a seagrass biomass of 117 g m^{-2} (annual average of the 4 study sites). I estimated the annual estuary-wide swan population by dividing our total monthly counts at the five study sites by 0.08 and averaging. This 0.08 fraction is the proportion of the total harbour count in January (Eastern Region Fish and Game Council, unpublished data) I observed in the same month at our sites and it varied from 0.05-0.09 for the last three annual census dates (Jan 08, 09, 10). This analysis suggests that black swan grazing removes a moderate proportion of the total seagrass biomass at the estuary scale but because the swans are not uniformly distributed the potential exists to remove a greater proportion at sites supporting high swan numbers. At the estuary scale, I estimated that the 20 % removal threshold would be exceeded if the current swan population were to increase by 25 %.

I estimated grazing pressure in relation to the standing stock of seagrass biomass. However another important variable is seagrass production, which if high enough may compensate for grazing. Eklöf et al (2009) estimated that black swan grazing could account for ~ 23 % of intertidal daily above- and below-ground *Halophila ovalis* production (3.1 g (DW) m^{-2} day^{-1}) during autumn in meadows of Western Australia.

They also showed that *H. ovalis* rapidly recovered (within 21 days) following a grazing simulation experiment. I did not measure seagrass production in this study but using the range of summer and winter time *Z. muelleri* above-ground production rates given by Turner and Schwarz (2006b) (from 1.1 to 2 g (DW) m^{-2} day^{-1} respectively), I estimate that black swan grazing in Tauranga Harbour would account for a smaller proportion of the seagrass production during the peak growing season in summer-autumn (from 4 to 9 %). This suggests that the productivity rate of *Z. muelleri* could potentially support the actual grazing pressure exerted by swans during the summer-autumn period. Moreover, it has been previously suggested that when seagrass herbivores remove < 10 % of the plant production the seagrass persists (Mateo et al 2006). However, grazing pressure (intensity and frequency) is highly variable among herbivores and combined with seagrass species/site specific recovery dynamics, generalisations are difficult (Mateo et al 2006).

Studies of geese-grazing pressure on *Zostera* meadows in the Northern Hemisphere have shown that meadows of *Zostera marina* cannot recover the following growing season after very heavy grazing (98 % cover removal) (Rivers and Short 2007). However, in contrast, studies of dugong grazing of tropical seagrass meadows in Australia have shown that multi-species seagrass meadows can tolerate high levels (81-96 %) of annual biomass removal (Preen 1995, Masini et al 2001). It is suggested that these meadows are able to fully recover the following growing season because of the high production and recolonisation rate of one pioneer species (*Halodule ovalis*) and because seed germination of seagrass is stimulated by the abrasive action of dugong grazing. The results of our study suggest that *Z. muelleri* may have a relatively low tolerance to biomass removal, since I have linked a comparatively low level of annual biomass removal (19-20 %) to subsequent decline in the standing

stock of seagrass biomass the subsequent growing season. A low tolerance to black swan grazing by *Z. muelleri* meadows in Tauranga Harbour could be attributed to the targeting and removal of a very high proportion of *Z. muelleri* rhizomes by these birds and the probable lack of significant seed production and germination to facilitate rapid recovery. Flowering and seed production in *Zostera* species in New Zealand and Australia is generally considered rare or infrequent (Bearlin et al. 1999, Turner and Schwarz 2006b).

In conclusion, the results of this study have shown that large grazers, like swans, can exert considerable grazing pressure on the biomass of seagrass meadows. High grazing pressure and removal of reproductive structures may hinder recovery of seagrass meadows in subsequent growing seasons. However, the pressure exerted and impacts can be highly variable at system-wide temporal and spatial scales highlighting the need to account for these scales in study design.

Chapter 3

Influence of nutrient enrichment, sedimentation and herbicide residues on seagrass condition in two contrasting New Zealand harbours

View from Site 1, Aotea Harbour.

3. Influence of nutrient enrichment, sedimentation and herbicide residues on seagrass condition in two contrasting New Zealand Harbours

3.1. Introduction

Seagrass meadows are the most productive ecosystems of the marine environment (Green and Short 2003). They provide essential habitat for globally threatened species (i.e. green turtle, manatee and dugong; Lanyon et al 1989, Hemminga and Duarte 2000, Gell and Whittington 2002) as well as many other motile and sedentary organisms (e.g. fish, shellfish, crabs and shrimps; Kikuchi 1980, McRoy and Helfferich 1980, Odgen 1980, Roblee et al 1991, Zieman 1982). Seagrasses also play a role in maintaining water quality by assimilating nutrients, thus potentially reducing eutrophication and phytoplankton blooms (Short and Short 1984), and by sequestering toxic compounds (e.g. heavy metals) from the water column and sediments (Hoven et al 1999, Ward 1987). They also stabilize sediments through root and rhizome anchorage (Short and Short 1984). However, worldwide many seagrass meadows are in decline primarily as a result of anthropogenic contamination of coastal areas (Short and Willie-Echeverria 1996, Waycott et al 2009).

Nutrient over-enrichment of the water column, that stimulates algal growth and/or the production of organic resuspendable detritus, can vastly reduce water clarity and light available for seagrass photosynthesis impeding growth (Burkholder et al 2007, Duarte 1991, Greve and Krausen-Jensen 2005, Kendrick et al 2002, Sugimoto et al

2007, Williams and Rucklshaus 1993). Other contaminants such as pesticides, and particularly herbicides (*e.g. s*-triazines, substituted ureas), have been reported in seagrass leaves, water and/or sediment (Haynes et al 2000, Lam et al 2005, Macinnis-Ng et Ralph 2004, McMahon et al 2005, Scarlett et al 1999, Shahidul Islam and Tanaka 2004, Zhang et al 2008). These compounds are widely used in agriculture, urban areas and as effective antifouling biocides in paints, and can affect plant growth through direct or indirect inhibition of photosynthesis (Coutris et al 2011).

The origin of seagrass habitat contamination is often attributed to human activities (Kemp et al 1983, Larkum and West 1990, Peters et al 1997, Short and Wyllie-Echeverria 1996). In areas of coastal development, catchment runoff leaches nutrients, pesticides and fine sediment particles into estuaries, a process which is intensified during storm (Blake and Duffy 2010). Impacts of over-enrichment and sedimentation have been well documented (see Burkholder et al 2007). In contrast, less is known about the effects of herbicides, and to our knowledge the cumulative impacts of these anthropogenic contaminants on seagrass ecosystems have not been studied.

In New Zealand, the sole seagrass species, *Zostera muelleri* Irmisch ex Asch. (Zosteraceae), has disappeared in many locations in the last 50-60 years (Inglis 2003). Anthropogenic sedimentation, eutrophication and wasting disease detected in 1960s (Armiger, 1965) are postulated as possible factors responsible for this decline (Inglis 2003, Park 1999). Information on nutrient enrichment, sedimentation and herbicide impacts on *Z. muelleri* is lacking for New Zealand (Matheson and Schwarz 2007, Turner and Schwarz 2006). Thus, work to disentangle the effects of various potential contaminant stressors on seagrass decline is required to better understand the dynamics of this important marine ecosystem. In this study, we surveyed two contrasting harbours, Tauranga and Aotea, which differed in catchment size and land

use. More than one-third of the seagrass has been lost since the 1950's in the more urbanized Tauranga Harbour (Park 1999). We aimed to identify the potential contaminant stressors affecting seagrass by linking seagrass condition with environmental parameters that include nutrient concentrations, sediment grain size characteristics and herbicide residues.

3.2. Materials and methods

3.2.1. Study sites

The study was carried out in two estuaries at similar latitude; Tauranga Harbour (Latitude: -37° 41' 7", Longitude: 176° 9' 58") on the east coast and Aotea Harbour (Latitude: -38° 0' 24", Longitude: 174° 49' 35") on the west coast of the North Island of New Zealand (Fig. 3.1). Tauranga Harbour has a larger surface area than Aotea Harbour (201 and 31 km^2, respectively). However, the percentage of surface area covered by seagrass in the two harbours is similar; 29.33 km^2 or 15 % of surface area in Tauranga Harbour (Park 1999) and 5.33 km^2 or 17 % in Aotea Harbour (Graeme 2005). Five intertidal sites, differing as much as possible in seagrass cover and adjacent land use, and as widely distributed as possible, were selected in each harbour. To analyse relationships between seagrass condition and environmental characteristics we chose a survey approach similar to one used in previous studies (Bradley and Stolt 2006, Matheson and Schwarz 2007).
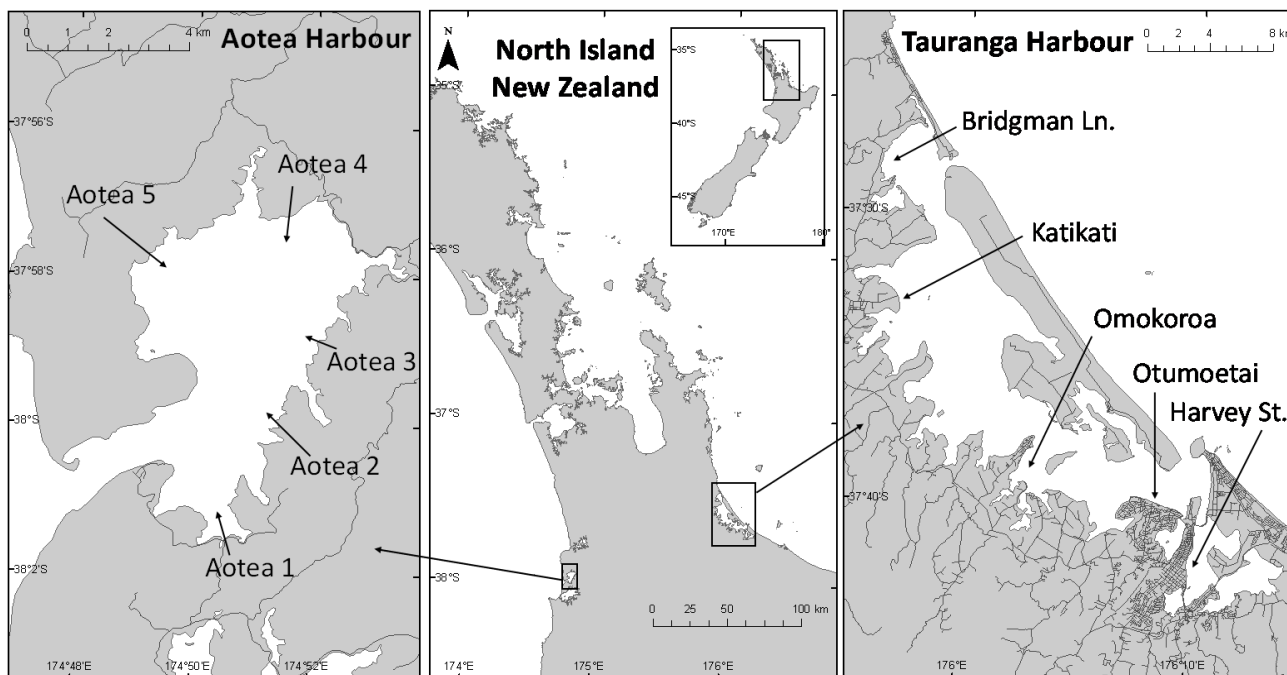
Figure 3.1: Location of the two estuaries, Aotea harbour on the west coast and Tauranga harbour on the east coast of the North Island of New Zealand. The five sites in each harbour are indicated by the arrows. Grey lines within the land represent roads and infrastructure.

3.2.2. Field surveys

Surveys were performed during the austral summer period (December to February). At low tide, three parallel transects were laid from the shoreward to seaward edges of the seagrass meadows at each site. Transect lengths and distance between transects were scaled to the specific seagrass extent at each site; lengths from 100 to 400 m and distance between transects from 25 to 100 m. Each of the three transects were divided into five equidistant sampling points where seagrass condition metrics and environmental parameters were measured.

At each of the 15 sampling points within a site, the photosynthetic efficiency (F_v/F_m) of one seagrass leaf-blade was measured *in-situ* after a 10-minute dark acclimation period using a dark leaf-clip (DLC-8, Walz) attached to the centre of the leaf-blade with a submersible pulse amplitude modulated (PAM) fluorometer (Diving-PAM, Walz). Seagrass cover was visually estimated within a 0.25 m² quadrat. In this

quadrat, 5 randomly selected leaves were measured (length and width) and a core (8 cm diameter × 10 cm length) sampled in the centre for analysis of seagrass biomass and sediment properties (described below). Additionally, at the mid-point of each transect, 5 cores of sediment (6.5 cm diameter × 5 cm length) were sampled and pooled for herbicide analysis. All independent sediment samples (n = 3 per site) were transported to the laboratory in a cool-box. Adjacent to the mid-point of the middle transect a waterproof light logger (Onset Hobo pendant temp/light UA-002-08) was installed horizontally on a north-facing angled stake just above sediment surface to record intertidal light levels for one month at each site. Water depth at all mid and end-points of each transect was measured once at high tide in order to quantify the typical height of the water column above seagrass. The water depth values were corrected to account for the time of measurement relative to the average high tide level recorded for the harbour in that year.

3.2.3. *Laboratory analyses*

Seagrass collected from the cores was separated in the laboratory into above- and below-ground fractions and dried to constant weight in an oven (70°C for 48 h). Dry weight (DW) of the total, above- and below-ground biomass was determined and the root/shoot ratio (R/S) calculated. Five samples of seagrass above-ground material (from each point of the middle transect at each site) were finely-ground in a mortar and pestle and analysed for particulate nitrogen (PNsg) and particulate phosphorus (PPsg) contents (APHA 4500 N/P (mod)).

Sediment collected from the seagrass biomass cores was analysed for particulate nitrogen (PNsed), after drying (at 60ºC for 48 h) and finely grinding samples, using a CHN analyser (Elementar Vario EL 111, Method 01-1090). Sediment water and organic matter (OM) contents were determined on oven-dried samples (105°C for 24

h) and following loss on ignition in a muffle furnace (450°C for 4 h), respectively. Sediment silt and clay contents (< 63μm grain size) were measured using a particle size analyser (Galai, EyeTech model). Nutrient concentration was measured in the sediment porewater as this generally exhibits a lower temporal variability than the water column and seagrasses typically derive a large proportion of nutrients for growth from the sediment (Romero et al 2006). Sediment porewater was extracted following addition of deionised water (25 ml) by shaking (1 h, 60 rpm) and centrifuging (10 min, 3500 rpm) fresh samples (10 g), with overlying supernatant decanted and analysed for ammonium-N (NH_4-N), nitrate-N (NO_3-N), total dissolved phosphorus (TDP) and dissolved reactive phosphorus (DRP) via flow injection analysis (Lachat 1994). Nutrient concentrations were corrected for dilution associated with the addition of deionised water to samples.

Core samples for herbicide analyses were stored in the dark at low temperature (around 4°C) for a maximum of 24 h before processing. Plant material was removed from each core, and remaining sediment was dried to constant weight in acetone-cleaned glass bowls in an oven at 25°C. The sediment fraction passed through a 2 mm mesh was analysed for *s*-triazine, substituted urea and anilide concentrations without further fractioning as whole sediment (Devault et al 2007). Extraction from sediments was performed with an accelerated solvent extractor (Dionex, ASE 200) following the method described for soils and whole sediments (Concha-Graña et al 2004, Richter et al 1995). Chlorophyll co-extract was removed on a Florisil Sep-Pak cartridge according to Müller et al (2000). The extracts were analysed by gas chromatography coupled to a mass spectrometry detector (Thermo Finnigan, Trace DSQ) under splitless injection and Specific Ion Monitoring conditions (Devault et al 2007, Merlina et al 1994).

Catchment size and land use for each site were determined using a Geographic Information System (ESRI ArcGIS, Land Cover Database). Light logger data was converted from units of lux to photosynthetically available radiation (PAR) according to Thimijans and Heins (1983). In addition to evaluating the overall average 24 h intertidal light availability (Light All) experienced by the seagrass plants, we also evaluated average underwater light availability during the day at high tide only (Light Day HT) as a surrogate for water clarity and the exposed light availability during the day at low tide only (Light Day LT).

3.2.4. *Data analyses*

The software R 2.4.1 (A Language and Environment Copyright, 2006) and the ade4 package (Data Analysis functions to analyse Ecological and Environmental data in the framework of Euclidean Exploratory methods; Chessel et al 2004) were used for all statistical analyses. Some parameters were $\log_{10}$ or $\log_{10}(x + 1)$ transformed to meet the parametric testing assumptions of normality and variance homogeneity prior to analysis. For all parameters, average values obtained in Tauranga versus Aotea Harbours were compared using a Student's *t*-test. Significant differences ($p < 0.05$) for all parameters among sites within each harbour were explored with a one-way analysis of variance (ANOVA). Principal components analysis (PCA) of seagrass metrics and environmental parameters was used to examine spatial differences among and within the harbours. To detect any significant relationships between the environmental parameters and the plant metrics, bivariate linear regression analysis and multiple regression analysis were used. Each bivariate linear regression was plotted and on occasion removal of a single outlier value was warranted. From the significant ($p < 0.05$) bivariate linear regression, the p-value after removing the variability explained by the harbour (*i.e.* Aotea or Tauranga) was also calculated and

indicated. The dataset included a number of correlated explanatory variables and, to take into account this collinearity, multiple regression analyses were performed with all correlated variables included in the model, or with only sub-groups of them. The alternative models were compared and only the best one [with the smallest Akaike information criterion (AIC); *i.e.* explaining the highest proportion of variability with the least number of parameters] was presented.

3.3. Results

A number of the environmental parameters measured differed significantly between the two harbours (Table 3.1a). PCA of all explanatory variables yielded three components that explained 72 % of the variation in the environmental variables and this further emphasises the contrast between the two harbours (Fig. 3.2a). Aotea was characterised by a significantly higher overall 24 h light availability (Light All), day time light availability at high tide (Light Day HT) and at low tide (Light Day LT), and sediment porewater TDP concentration compared to Tauranga (*t*-test, n = 5, p = 0.04, p < 0.01, p = 0.03 and p < 0.01 respectively). Conversely, Tauranga was characterised by a significantly higher concentration of sediment herbicides, sediment OM and had a larger proportion of urban and crop land use in site subcatchments compared to Aotea (*t*-test, n = 5, p = 0.04, p < 0.01, p < 0.01 and p = 0.02 respectively). Of the various herbicide compounds measured, trifluralin had the highest concentration among the anilides, as did cyanazine among the *s*-triazines and chlorotoluron among the substituted ureas (Table 3.2). PCA of all response variables yielded 3 components that explained 81 % of the variation in the seagrass metrics and the contrast between harbours was less visible (Fig. 3.2b). Only the root/shoot ratio and the concentration of particulate nitrogen in the seagrass leaves (PNsg) differed

significantly between the two harbours with the larger ratio and PNsg concentration in Aotea (*t*-test, n = 5, p = 0.03 and p = 0.045 respectively; Table 1b).

Among the environmental parameters, some correlations were found (Table 3.3a) although a number of them were no longer significant after removing the variability explained by the harbour, probably due to the low level of variation of some parameters (i.e. sediment OM, urban and crop land use in subcatchments, see Table 1a) between the sites of Aotea Harbour. Light availability was inversely correlated with sediment OM and to the proportions of urban and crop land use in subcatchments, at day time high tide (light Day HT) (r = -0.69 to -0.86, p = 0.003 to 0.041), at day time low tide (Light Day LT) (r = -0.65 to -0.93, p = 0.001 to 0.043) and overall (Light All) (r = -0.77 to -0.87, p = 0.001 to 0.015; Table 3.3a). Porewater NO_3-N was positively correlated with the proportions of urban and crop land use in subcatchments (r = 0.77, p = 0.010 and r = 0.90, p = 0.001 respectively). These results suggest that a higher proportion of crop land use in subcatchments may enrich the coastal water in NO_3-N decreasing the light availability to seagrass.

Simple linear regression analysis revealed that some of the environmental parameters were good predictors of the seagrass metrics (Table 3.3b). Seagrass leaf length, width, and total, below- and above-ground biomass were positively correlated with sediment porewater NH_4-N (r = 0.70 to 0.86, p = 0.002 to 0.062; Table 3b). Seagrass leaf length was also positively correlated with sediment porewater DRP (r = 0.74, p = 0.023). The multitude of positive relationships found between the seagrass metrics and the sediment porewater NH_4-N suggests that the environment in both harbours may be nitrogen-limited for plant growth. Seagrass cover was negatively correlated with the proportion of urban land use in the subcatchments (r = -0.73, p = 0.025; Table 3.3b).

Table 3.1: Environmental (a) and seagrass (b) characteristics for sites in Tauranga and Aotea harbours. All values are means with the standard error (SE) in parentheses. For harbour averages (bold font) for each parameter values with the same letter are not significantly different between the two harbours at p > 0.05 (*t*-test, n = 5). For sites within a harbour for each parameter values with the same letter are not significantly different at p > 0.05 (one-way ANOVA, n vary from 3 to 30 according to the parameter measured).

(a)

Sites	Sediment				Sediment porewater					
	Herbicide $\mu g\ kg^{-1}$	Silt & Clay %	OM %	PartN $\mu g\ g^{-1}$	NH_4-N $mg\ m^{-3}$	NH_4^+ μM	NO_3-N $mg\ m^{-3}$	NO_3^+ μM	DRP $mg\ m^{-3}$	TDP $mg\ m^{-3}$
Tauranga										
Bridgman Ln.	132 (58) [a]	4.13 (0.26) [b]	1.42 (0.06) [a]	300 (20) [a]	83141 (13419) [bc]	5939 (958)	234 (94) [a]	17 (7)	430 (122) [a]	524 (86) [b]
Katikati	116 (46) [a]	2.04 (0.27) [a]	1.49 (0.20) [a]	270 (20) [a]	16919 (6804) [a]	1208 (486)	10113 (6528) [a]	722 (466)	201 (50) [a]	247 (22) [a]
Omokoroa	98 (16) [a]	2.70 (0.72) [ab]	1.47 (0.11) [a]	280 (60) [a]	35340 (16387) [ab]	2524 (1171)	7360 (1988) [a]	526 (442)	340 (33) [a]	335 (22) [ab]
Otumoetai	122 (29) [a]	1.75 (0.09) [a]	1.11 (0.12) [a]	300 (20) [a]	73031 (18981) [abc]	5216 (1356)	25417 (16755) [a]	1815 (1197)	389 (87) [a]	418 (33) [ab]
Harvey st.	115 (420) [a]	2.24 (0.17) [a]	1.46 (0.10) [a]	380 (20) [a]	96534 (9836) [c]	6895 (703)	164 (94) [a]	12 (7)	213 (77) [a]	328 (42) [ab]
Average (SE)	**116 (5) [b]**	**2.57 (0.42) [a]**	**1.39 (0.07) [b]**	**300 (20) [a]**	**60993 (14999) [a]**	**4357 (1071) [a]**	**8657 (4625) [a]**	**618 (330) [a]**	**314 (46) [a]**	**371 (47) [a]**
Aotea										
Aotea 1	118 (42) [a]	1.56 (0.08) [a]	0.89 (0.06) [a]	300 (0) [a]	45181 (11910) [b]	3227 (851)	676 (490) [a]	48 (35)	411 (81) [b]	1008 (92) [a]
Aotea 2	109 (39) [a]	1.76 (0.28) [a]	1.20 (0.52) [a]	400 (80) [a]	25953 (3506) [ab]	1854 (250)	103 (7) [a]	7 (1)	211 (37) [a]	863 (220) [a]
Aotea 3	69 (33) [a]	1.59 (0.32) [a]	0.72 (0.18) [a]	280 (60) [a]	16035 (3555) [a]	1145 (254)	160 (42) [a]	11 (3)	218 (22) [a]	722 (49) [a]
Aotea 4	54 (6) [a]	2.78 (0.15) [a]	1.15 (0.06) [a]	360 (20) [a]	24807 (2658) [ab]	1772 (190)	86 (19) [a]	6 (1)	271 (34) [ab]	688 (38) [a]
Aotea 5	52 (12) [a]	2.75 (0.69) [a]	1.00 (0.06) [a]	300 (0) [a]	17176 (2629) [a]	1227 (188)	126 (20) [a]	9 (1)	157 (25) [a]	672 (62) [a]
Average (SE)	**80 (14) [a]**	**2.09 (0.28) [a]**	**0.99 (0.09) [a]**	**330 (20) [a]**	**25830 (5227) [a]**	**1845 (373) [a]**	**230 (112) [a]**	**16 (8) [a]**	**254 (43) [a]**	**790 (64) [b]**

(a, continuation)

Sites	Water				Catchment land use			
	Light HT $\mu mol\ m^{-2}s^{-1}$	**Light LT** $\mu mol\ m^{-2}s^{-1}$	**Light All** $\mu mol\ m^{-2}s^{-1}$	**Depth** cm	**Urban** %	**Crop** %	**Pasture** %	**Native** %
Tauranga								
Bridgman Ln.	244 (57) [a]	1382 (241) [a]	455 (39) [a]	64 (10) [a]	2.37	13.20	55.33	26.57
Katikati	137 (30) [a]	758 (197) [a]	326 (40) [a]	83 (8) [a]	10.80	40.03	48.34	0.33
Omokoroa	180 (25) [a]	1066 (181) [a]	355 (32) [a]	35 (9) [a]	12.24	20.25	61.92	4.11
Otumoetai	360 (84) [a]	1521 (242) [a]	437 (40) [a]	84 (8) [a]	13.60	11.32	41.55	32.89
Harvey St.	250 (57) [a]	1570 (276) [a]	465 (43) [a]	81 (17) [a]	9.37	12.18	44.23	32.50
Average (SE)	**235 (38) [a]**	**1259 (153) [a]**	**407 (28) [a]**	**69 (9) [a]**	**10 (2) [b]**	**19 (5) [b]**	**50 (4) [a]**	**19 (7) [a]**
Aotea								
Aotea 1	663 (76) [b]	1667 (263) [a]	748 (49) [b]	101 (27) [a]	0	5.03	82.51	0.92
Aotea 2	417 (53) [a]	1494 (250) [a]	569 (46) [ab]	118 (39) [a]	0	0.45	63.33	35.68
Aotea 3	393 (49) [a]	1894 (269) [a]	647 (45) [ab]	105 (43) [a]	0	0	15.04	83.74
Aotea 4	392 (40) [a]	1771 (285) [a]	639 (53) [ab]	76 (8) [a]	0	0.09	53.24	46.15
Aotea 5	246 (39) [a]	1658 (266) [a]	542 (40) [a]	78 (24) [a]	0	6.52	63.91	28.31
Average (SE)	**422 (67) [b]**	**1697 (66) [b]**	**629 (36) [b]**	**96 (8) [a]**	**0 (0) [a]**	**2 (1) [a]**	**56 (11) [a]**	**39 (13) [a]**

(b)

Sites	Seagrass	Seagrass leaves					Seagrass biomass	
	Cover %	Photosynthetic efficiency F_v/F_m	PN µg mg^{-1}	PP µg mg^{-1}	Length cm	Width mm	Total g m^{-2}	Root/Shoot
Tauranga								
Bridgman Ln.	37 (7) [ab]	0.604 (0.050) [a]	16.22 (0.91) [a]	1.80 (0.45) [a]	57 (5) [ab]	1.9 (0.2) [ab]	136 (22) [a]	6.2 (1) [a]
Katikati	19 (8) [a]	0.727 (0.012) [ab]	16.66 (1.47) [a]	1.24 (0.13) [a]	47 (3) [a]	1.4 (0.1) [a]	183 (56) [a]	5.2 (1) [a]
Omokoroa	19 (6) [a]	0.779 (0.006) [b]	11.70 (1.49) [a]	1.17 (0.13) [a]	60 (3) [ab]	1.9 (0.2) [ab]	116 (32) [a]	3.8 (1) [a]
Otumoetai	20 (9) [a]	0.590 (0.073) [ab]	15.36 (1.27) [a]	1.55 (0.21) [a]	55 (5) [ab]	1.3 (0.1) [a]	223 (63) [a]	5.3 (2) [a]
Harvey st.	56 (7) [b]	0.619 (0.046) [a]	16.40 (1.03) [a]	1.28 (0.10) [a]	71 (7) [b]	2.4 (0.2) [b]	234 (33) [a]	2.6 (0) [a]
Average (SE)	**30 (7) [a]**	**0.664 (0.038) [a]**	**15.27 (0.92) [a]**	**1.41 (0.12) [a]**	**58 (4) [a]**	**1.8 (0.2) [a]**	**178 (23) [a]**	**4.6 (0.6) [a]**
Aotea								
Aotea 1	31 (6) [a]	0.398 (0.065) [a]	20.06 (1.39) [a]	0.75 (0.51) [a]	54 (3) [ab]	1.8 (0.2) [a]	170 (38) [a]	8 (2) [a]
Aotea 2	32 (6) [a]	0.608 (0.042) [bc]	15.28 (2.25) [a]	1.28 (0.26) [a]	49 (2) [ab]	1.6 (0.1) [a]	167 (33) [a]	8.3 (3) [a]
Aotea 3	20 (6) [a]	0.510 (0.069) [ab]	18.04 (1.81) [a]	0.58 (0.19) [a]	41 (5) [a]	1.6 (0.1) [a]	83 (18) [a]	9.7 (4) [a]
Aotea 4	33 (6) [a]	0.761 (0.017) [c]	18.16 (1.73) [a]	1.22 (0.20) [a]	55 (2) [b]	1.8 (0.1) [a]	155 (34) [a]	4.4 (1) [a]
Aotea 5	36 (8) [a]	0.654 (0.025) [bc]	19.38 (1.34) [a]	1.44 (0.18) [a]	48 (4) [ab]	1.6 (0.1) [a]	126 (26) [a]	13.2 (3) [a]
Average (SE)	**31 (3) [a]**	**0.586 (0.062) [a]**	**18.18 (0.82) [b]**	**1.06 (0.17) [a]**	**50 (2) [a]**	**1.7 (0.1) [a]**	**140 (16) [a]**	**8.7 (1.4) [b]**

Table 3.2: Herbicide concentrations ($\mu g\ kg^{-1}$) in the sediment for sites in Tauranga and Aotea Harbours[1]. Values for each site are means (n = 3) and values for each herbicide, sub-total group and overall total are not significantly different (p > 0.05) among sites in each harbours (one-way ANOVA). Average value for each group of herbicide are not significantly different (p > 0.05) between the two harbours (*t*-test, n = 5).

Herbicides	Tauranga Harbour					Aotea Harbour					Average
	Bridgman Ln.	Katikati	Omokoroa	Otumoetai	Harvey St.	Aotea 1	Aotea 2	Aotea 3	Aotea 4	Aotea 5	
Aclonifen	1.00	0.67	0.67	1.00	1.00	1.33	0	1.00	0.67	1.00	0.83
Alachlor	0.33	0.33	0.33	0.33	0.33	0	0.33	0.33	0.33	0.33	0.30
Metazachlor	-	-	-	-	-	-	-	-	-	-	-
Metolacholor	-	-	-	-	-	-	-	-	-	-	-
Pendimethalin	0.67	1.00	1.33	1.00	0	0.33	0	0.33	0	0	0.47
Trifluralin	8.00	3.67	3.00	2.00	3.67	3.00	5.33	2.00	1.00	1.67	3.33
Sub-total anilides	**10.00**	**5.67**	**5.33**	**4.33**	**5.00**	**4.67**	**5.67**	**3.67**	**2.00**	**3.00**	**4.93**
Average anilides ± SE			**6.07 ± 1**					**3.80 ± 1**			
Atrazine	-	-	-	-	-	-	-	-	-	-	-
Cyanazine	73.67	56.67	37.33	40.00	49.33	62.33	46.33	0.33	3.67	0.33	37.00
Desethylatrazine (DEA)	0.67	0.33	0	0	0.33	0	1.33	0.33	0	0	0.30
Irgarol	-	-	-	-	-	-	-	-	-	-	-
Sebuthyl-azine	0	0	0.33	0	0	0	0.33	0	0	0	0.07
Simazine	-	-	-	-	-	-	-	-	-	-	-
Terbuthyl-azine	0	0	0	0	0	0	1.33	0	0	0	0.13
Sub-total s-triazines	**74.33**	**57.00**	**37.67**	**40.00**	**49.67**	**62.33**	**49.33**	**0.67**	**3.67**	**0.33**	**37.5**
Average s-triazines ± SE			**51.73 ± 7**					**23.27 ± 13**			
Chlorotoluron	24.33	20.33	21.00	25.67	16.67	27.67	26.33	28.00	45.67	44.33	28.00
Iso-proturon	0.50	0.33	0.33	0.67	0.67	0.33	0.33	0	0	0	0.32
Linuron	1.33	2.67	6.33	3.67	1.67	1.33	1.33	0.67	1.33	1.00	2.13
Metobromuron	14.00	20.67	12.67	15.67	16.67	8.33	16.33	1.00	1.67	2.00	10.90
Metoxuron	7	9.67	14.33	32.33	24.00	13.33	9	35	0	1.40	14.61
Monolinuron	0.33	0	0.33	0	0.33	0	0.33	0	0	0	0.13
Sub-total sub. Ureas	**47.49**	**59.67**	**54.99**	**78.01**	**60.01**	**50.99**	**53.65**	**64.67**	**48.67**	**48.73**	**56.09**
Average sub. Ureas ± SE			**58.60 ± 5**					**53.35 ± 3**			
Overall total	**131.82**	**116.34**	**97.99**	**122.34**	**114.68**	**117.99**	**108.65**	**69.01**	**54.34**	**52.06**	

[1]-, under the detection limit.
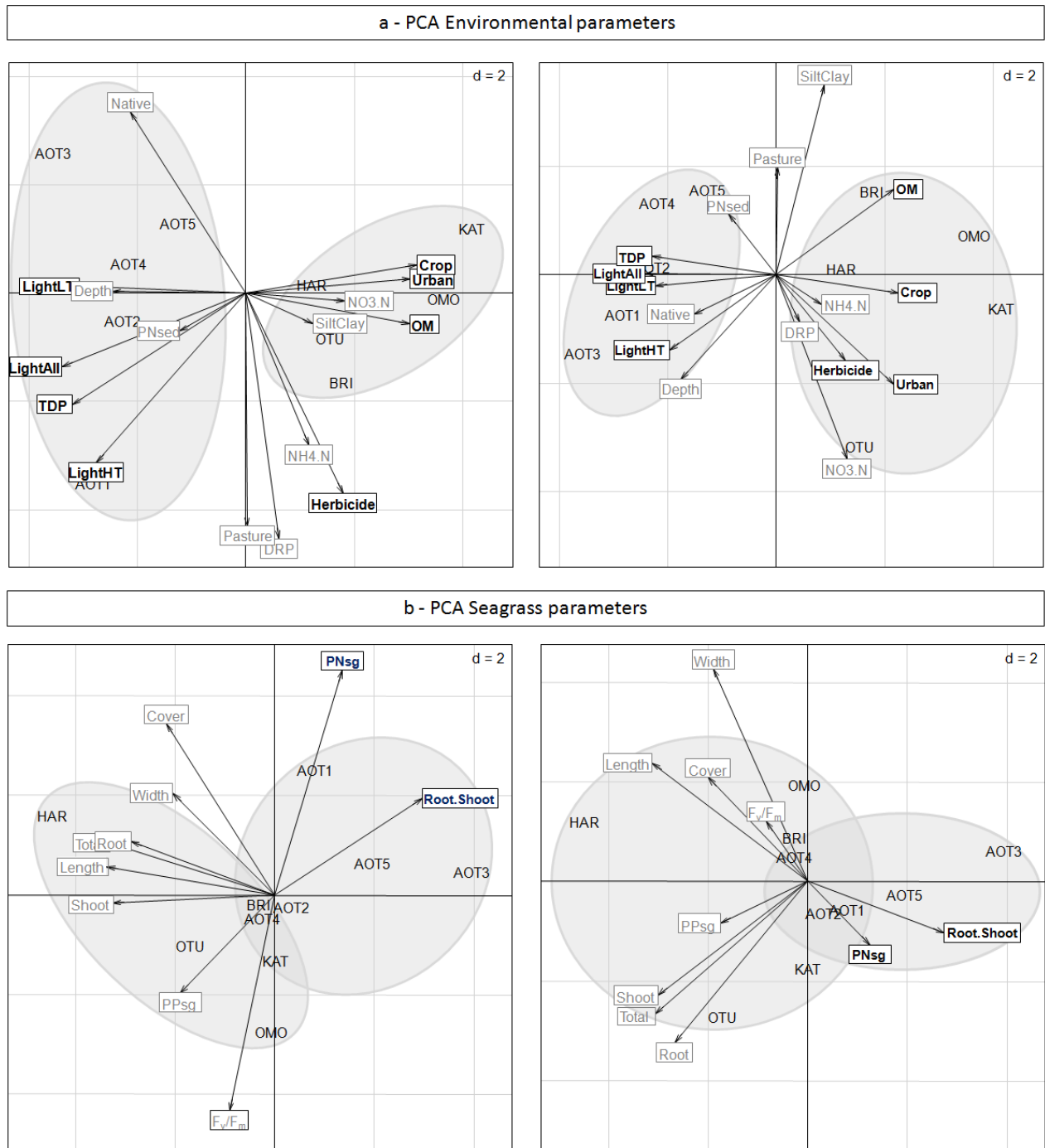
Figure 3.2: Results of two independent Principal Components Analyses (PCA) on environmental parameters (above) and seagrass metrics (below). Figures on the left side represent the axes 1 (explaining 44 and 43 % of the environmental and seagrass variability respectively) and 2 (explaining 16 and 20 % of the environmental and seagrass variability respectively) of the PCA and the right figures the axes 1 and 3 (explaining 13 and 18 % of the environmental and seagrass variability respectively). Sites within a same harbour are grouped

by the circular grey areas. Environmental parameters that differ significantly between the two harbours (*t*-test, n = 5, p < 0.05) are represented in bold.

Table 3.3: Results of bivariate regression analyses (a) among the environmental parameters, and (b) relating seagrass metrics to environmental parameters. P values are calculated without accounting for the harbour effect (p) and after removing the variability explained by the harbour (*i.e.* Aotea or Tauranga) (p^2).

(a)

Environmental parameter	Environmental parameter	n^1	r	F	p	p^2
Crop	Light Day HT	9	-0.86	20.30	0.003**	0.035*
	Light Day LT	10	-0.93	51.73	0.001***	0.002**
	LightAll	10	-0.77	11.36	0.010**	0.320
	$NO_3.N$	9	0.90	30.14	0.001***	0.005**
Urban	Light Day HT	9	-0.69	6.25	0.041*	0.657
	Light Day LT	10	-0.65	5.76	0.043*	0.687
	LightAll	9	-0.87	25.03	0.001**	0.216
	$NO_3.N$	10	0.77	11.35	0.010**	0.033*
OM	Light Day HT	10	-0.70	7.89	0.023*	0.270
	Light Day LT	10	-0.77	11.54	0.009**	0.152
	LightAll	10	-0.74	9.62	0.015*	0.646

'*' p < 0.05; '**' p < 0.01; '***' p < 0.001.
[1], when *n* = 9 one outlier has been removed.

(b)

Seagrass metric	Environmental parameter	n^1	r	F	p	p^2
Cover	Urban	9	-0.73	8.13	0.025*	0.031*
Length	$NH_4.N$	10	0.79	13.49	0.006**	0.040*
	DRP	9	0.74	8.42	0.023*	0.069.
Width	$NH_4.N$	9	0.86	20.59	0.003**	0.009**
Total biomass	$NH_4.N$	9	0.70	6.64	0.037*	0.058.
Below-ground biomass	$NH_4.N$	9	0.64	4.94	0.062.	0.019*
Above-ground biomass	$NH_4.N$	9	0.75	29.41	0.002**	0.036*
Photosynthetic efficiency	LightAll	10	-0.65	5.78	0.043*	0.027*
	SiltClay	9	0.86	17.29	0.006**	0.011*
	Sediment OM	10	0.65	5.80	0.043*	0.065.

'.' p < 0.10; '*' p < 0.05; '**' p < 0.01; '***' p < 0.001.
[1], when *n* = 9 one outlier has been removed.

Seagrass photosynthetic efficiency (F_v/F_m) was positively correlated with silt/clay content and sediment OM (r = 0.86, p = 0.006 and r = 0.65, p = 0.043 respectively; Table 3.3b), and negatively correlated with overall light availability (Light All) (r = -0.65, p = 0.043).

The multiple regression analysis revealed only one further significant relationship (Table 3.4). Sediment OM, total herbicide concentration and sediment porewater NO_3-N together were good predictors of F_v/F_m explaining 96 % of the variability in this metric. Sediment OM and total herbicide concentration together explained 88 % of the variability. We found a positive correlation between F_v/F_m and sediment OM and porewater NO_3-N whereas the correlation was negative between F_v/F_m and the herbicide concentration suggesting a detrimental effect of herbicides on plant physiology.

Table 3.4: Multiple regression results relating seagrass photosynthetic efficiency to environmental parameters; only the best model (i.e., explaining the highest variability with the lowest number of parameters) is presented.

Seagrass metric	×	Predictor	SumSq	F value	P value	Variability (%)
photosynthetic efficiency	×	Sediment OM	0.050	54.59	< 0.001***	42
		Herbicide	0.055	59.96	< 0.001***	46
		NO_3-N	0.009	9.95	0.020*	8
		residual	0.006			

'*' p < 0.05; '**' p < 0.01; '***' p < 0.001.

3.4. Discussion

3.4.1. Effects of nutrients, light availability and sedimentation on seagrass condition

Nutrients for seagrass growth are available from both the sediment porewater and the water column (Harlin 1981, McRoy et al 1972, Short and McRoy 1984). Both NH_4-N and NO_3-N are used by seagrasses (Burkholder et al 2007), but NH_4-N is usually the predominant form in sediment pore waters (Romero et al 2006) and the preferred inorganic nitrogen source (Alexandre et al 2010). In N-limited environments, N enrichment can increase seagrass growth (Agawin et al 1996, Bulthuis et al 1992, Lee and Dunton 1999, Udy and Dennison 1997) which is consistent with our results. We found that sediment pore water nitrogen concentration (NH_4-N) was the parameter most strongly related to seagrass biomass and morphometry metrics, suggesting that *Zostera muelleri* growth in these two harbours may be regulated by N-availability. The average sediment pore water NH_4-N concentrations found at sites in this study (1,145 to 6,895 μM) appear to be within growth-regulating range. Our pore water concentrations are higher than typical water column concentrations (Brun et al 2002, Burkholder et al 1992, Van Katwijk et al 1997) but are of similar magnitude to pore water concentrations found in other intertidal sediments (up to ~7,000 μM; Hanson and Kristensen 1998, Hopkinson et al 1999, Lohrer et al 2010, Murray et al 1978). Seagrass plants appear able to tolerate much higher nitrogen concentrations in sediment pore waters than in the water column. Nitrogen toxicity has been recorded at relatively low water column concentrations (16 μM, van Katwijk et al 1997, Brun et al 2002) whereas concentrations above 30,000 μM (10,000 μM NH_4-N + 20,000 μM NO_3-N) are required in sediment pore waters to induce detrimental effects on plant physiology

(for *Zostera marina*; Peralta et al 2003). The nitrate plus ammonium concentrations in our sediment pore waters were generally well below this threshold. The ability of roots to tolerate higher N concentrations than leaves in the external media has previously been documented (Peralta et al 2003, van Katwijk et al 1997). This is attributed to (1) the NH_4-N uptake rate being lower via roots than leaves, and roots being better able to regulate nitrogen uptake (e.g. via reduction of root hairs); (2) generally lower pH levels in sediment compared to the water column, lowering NH_4-N uptake rates; and (3) release of oxygen from plant roots facilitating nitrification and lowering NH_4-N concentrations in the immediate vicinity of roots (van Katwijk et al 1997).

Nutrient enrichment in coastal waters is often attributed to developed land use and increased terrestrial runoff (Kemp et al 1983, Vitousek et al 1997). In this study, higher porewater NO_3-N concentrations were associated with a greater proportion of urban and crop land use in subcatchments. Both urban and crop land use were inversely correlated with day time light availability at high tide (Light Day HT). This suggests that urban and crop land use may have indirect detrimental effects on light availability at high tide by contributing to NO_3-N enrichment, and thus presumably to the stimulation of phytoplankton growth. We found that urban land use in subcatchments was negatively correlated with seagrass cover. However, since cover is only a visual representation of the area colonized by seagrass leaves and does not account for below-ground parts or any layering of seagrass leaves, it is probably a less reliable measure of seagrass abundance than the other metrics measured in this study (e.g. biomass). Seagrass biomass was not correlated with urban land use in subcatchments therefore indicating no clear detrimental impact of urbanisation on seagrass abundance.

In terms of plant physiology (specifically photosynthetic efficiency, F_v/F_m), light availability, sediment organic matter content, total herbicide and porewater nitrate concentration were the best predictors of this metric. As an intertidal species, *Z. muelleri* is exposed to high irradiation at low tide. The day time light availability at low tide measured in this study (~1300 and ~1700 μmol m^{-2} s^{-1} for Tauranga and Aotea Harbours, respectively) is well above light-saturating levels for this species (> 350 μmol m^{-2} s^{-1}) and also above levels considered photo-inhibitory (~1100 μmol m^{-2} s^{-1}) (Flanigan and Critchley 1996). When plants were fully submerged during the day at high tide they received on average ~240 and ~420 μmol m^{-2} s^{-1} for Tauranga and Aotea Harbours, respectively. This suggests that the plants at most sites, particularly in Aotea Harbour, have an adequate, and often over-, supply of light, especially when exposed. Thus, under these circumstances, factors that reduce excess light supply may benefit the plant. Interestingly, it was found that the *Z. muelleri* photosynthetic efficiency increased as sediment organic matter contents, silt/clay contents and porewater NO_3^- concentrations increased. Dissolved and particulate organic carbon and humic substances, as well as inorganic particulate substances and suspended organisms, are known to be absorbers of solar radiation in waters (Arts et al 2000). If we assume that the quantity of sediment organic matter, silt/clay and NO_3^- also reflects the abundance of these compounds in the overlying water, then these particles either suspended in the water, deposited on plant leaves or as stimulants for algal growth, presumably play a role in excess light absorption and mitigation of photo-inhibition effects.

3.4.2. *Herbicide effects*

In this study we found a negative relationship between the photosynthetic efficiency of the plant (F_v/F_m) and sediment total herbicide concentration which suggests that herbicide contamination has a detrimental impact on seagrass physiological performance. This relationship only appears once the effect of sediment OM has been controlled by using this parameter as a covariate in multiple regression analysis. Herbicide compounds are known to be bound to particles of OM through their lipophilicity (Shea 1989) and thus, highly organic sediments may reduce plant exposure to herbicides.

In the present study, herbicide components found in the sediment were *s*-triazines, substituted ureas and anilides. In other estuarine habitats in Australia, China and Europe, *s*-triazines molecules such as Irgarol 1051 and atrazine have been detected in waters and/or sediments (Gough et al 1994, Lam et al 2005, Readmand et al 1993, Scarlett et al 1997, 1999, Tolosa et al 1996, Zhang et al 2008, Zhou et al 1996). Also, the substituted urea diuron was detected in sediments, coastal waters and seagrass in Australia (Haynes et al 2000, McMahon et al 2005). In our study, Irgarol and atrazine molecules were not detected in the sediment and diuron was not measured; however the presence of anilides and other substituted ureas and *s*-triazines molecules indicates some contamination of seagrass habitats in New Zealand, particularly in estuaries with more developed catchments (e.g. Tauranga Harbour).

Cyanazine was present in the highest concentration compared to all other *s*-triazines compounds measured (37 µg kg^{-1}). To our knowledge this compound has not previously been detected in seagrass habitats. This compound is used as an herbicide in various crop cultures (beans, cereal grains, onions, peas, potatoes, pulses, sweet corn), which could explain its presence in Tauranga Harbour where there is a

moderately high abundance of crop land use (19 % of catchment). Of the various substituted ureas measured in this study we detected high concentrations of chlorotoluron in the sediment. This compound is used for weed control in carrot, parsnip, and wheat cultivation, and has not been recorded previously in estuarine sediments although it was present in higher concentrations in this study (28 µg kg^{-1}) compared to other substituted ureas such as the diuron detected in Australian estuarine sediments (from 0.1 to 10.1 µg kg^{-1}; Haynes et al 2000, McMahon et al 2005).

Anilides in sediment have previously been measured in European freshwater river sediments where the compounds metolachlor and metazachlor were found in high concentrations (up to 680 and 910 µg kg^{-1} respectively; Devault et al 2007). In our study these compounds were not detected and only minimal concentrations of other anilides as nitro derivatives were found compared to *s*-triazines and substituted ureas. Among them, trifluralin was found in the highest concentrations (3.33 µg kg^{-1}). Trifluralin is an herbicide widely used (especially in the United States) to prevent root development of weeds through interruption of mitosis. Rapid degradation of this molecule in aquatic systems minimizes its risk to seagrass and to other aquatic macrophytes. Nevertheless regular input through water runoff of trifluralin may pose a threat to aquatic plant communities (Yockim et al 1980).

To our knowledge, the sediment concentrations above which the predominant herbicide compounds found in this study (cyanazine, trifluralin and chlorotoluron) detrimentally affect seagrass have not yet been determined. Based on our results, seagrass photosynthetic efficiency is reduced in the cumulative presence of these herbicides. However, no relationships were found between herbicide concentrations and other seagrass condition metrics (cover, biomass and morphometry). This suggests that the higher levels of herbicide contamination detected in this study (up to

a total of 132 μg kg^{-1}) have a detrimental effect on seagrass physiology, reducing F_v/F_m values from 0.78 (excellent condition) down to 0.40 (severe strain) (Mohammed et al 2003), but do not affect the overall abundance of seagrass. Although clear differences in contaminant stress were observable between the two harbours, with generally higher levels of contamination evident for Tauranga (higher sediment herbicide and organic matter concentration, higher proportions of developed land use and lower light availability), no large distinction among the seagrass parameters measured was detected between the two harbours. Our study results therefore do not provide conclusive evidence that these contaminants have contributed to the historical decline of seagrass meadows in Tauranga harbour. However, our findings do demonstrate that anthropogenic activities can contaminate estuarine waters and sediments to the potential detriment of seagrass meadow condition.

Chapter 4

Seagrass resilience to waterfowl grazing at sites differing in anthropogenic contaminants and other environmental characteristics

Black swan grazing at Katikati site, Tauranga Harbour

4. Seagrass resilience to waterfowl grazing at sites differing in anthropogenic contaminants and other environmental characteristics

4.1. Introduction

Plant-herbivore interactions play a major role in the structure and functioning of terrestrial, freshwater and marine systems (Crawley 1983). Damage caused by herbivores can vary according to species and population size, the frequency of grazing, and the parts of the plant that are removed. The effects of herbivory can therefore range from minor changes in plant cover to formation of gaps to complete destruction of the plant habitat (Crawley 1983). The selective foraging behaviour of various herbivore species disturbs the structure of plant habitats in different ways. For instance, large herbivores (e.g., elephants, dugongs) often remove whole plants while smaller herbivores (e.g., caterpillars, fish, sea urchins) often remove only leaf material (Crawley 1983, Lodge 1991, Preen 1995, Pamo and Chamba 2001, Alcoverro and Mariani 2002). Leaf removal causes loss of vital photosynthetic material which directly impacts the growth rate of the plant (Rosenthal and Kotanen 1994, Honkanen et al 1999, Millard et al 2001). Root and rhizome removal weakens plant physical structure and reduces capacity for nutrient uptake (Crawley 1983, Grayston et al 2001). Herbivory can also damage the foliar and basal meristems (i.e., the locations where growth is initiated), which may impede plant regeneration affecting recovery dynamics (Rivers and Short 2007).

After grazing, plant recovery can occur via in-growth from the edge of the grazed area, elongation of remaining rhizomes and roots and/or germination of seeds. The existence of grazing refuges (parts of the habitat undisturbed by herbivory) may facilitate the regeneration of grazed areas (Preen 1995, Milchunas and Noy-Meir 2002) by providing new propagules for recolonisation and/or functioning as a propagule bank (Kalamees and Zobel 2002, Santamaría and Rodríguez-Gironés 2002). Mechanisms of compensation also play an important role in recovery from herbivory. For instance, increases in relative growth rates or enhancement of seed production have been observed in response to grazing (McNaughton 1983, Zieman et al 1984, Belsky 1986, Richards 1993, Valentine et al 1997, Moran and Bjorndal 2007). Energy stores (e.g., nitrogen reserves) in plant below-ground organs may also be important for some plant species to regenerate from herbivory (Vergés et al 2008, Cherry and Gough 2009).

In many instances natural grazer and plant populations have evolved together and can readily coexist (Breedlove and Ehrlich 1968). However, under unusual intense grazing pressure (e.g., due to an unprecedented increase in the grazer population or the introduction/ invasion of a new herbivore species), impacts can be long-lasting or even permanent. In terrestrial forests, the introduction of mammal herbivores has been shown to greatly reduce the seedling abundance of dominant tree species which, in the longer term, can hinder the natural forest regeneration process (Vázquez 2002). In seagrass ecosystems, atypical intensive grazing (> 80 % removal of plant cover) due to an increase in the migrating waterfowl population has been shown to hinder plant recovery in subsequent growing seasons (Rivers and Short 2007).

Globally, plant communities are increasingly affected by anthropogenic activities which can adversely affect plant condition and may reduce plant resilience to

herbivore damage. Examples include air pollution and acid rain that cause lesions and damage to leaf surfaces of terrestrial and fresh water plants (Cape 1993, Liken et al 1996, Larssen et al 2006) and pesticides and herbicides leached from agricultural or urban land that detrimentally affect the growth systems of non-target plants, such as aquatic macrophytes (Courtris el al 2011). Another pervasive influence on the condition of aquatic plant communities in waters adjacent to areas of developed land use is contamination with fine sediment and nutrient runoff which leads to smothering of plants, algal blooms and reduced light availability for growth (Lotze et al 2006).

Within the marine environment, seagrasses constitute one of the most productive ecosystems, providing habitat for many organisms and threatened species and supporting a wide range of herbivores that vary in size from small invertebrates (e.g., sea urchins) to large mammals (e.g., dugongs) (Green and Short 2003). Unfortunately, seagrass decline has been reported in many parts of the world (Short and Willie-Echeverria 1996, Waycott et al 2009). Anthropogenic contaminants are postulated to detrimentally disturb this ecosystem via nutrient over-enrichment, (eutrophication) causing algal blooms, and via sedimentation, both of which decrease light availability for seagrasses, and via other contaminants, such as herbicides, that are directly harmful to seagrass physiological systems (Lotze et al 2006). While in some instances seagrass meadows may be impacted by only one of these contaminant stressors (see Burkholer et al 2007), in many cases all of these stressors are linked, typically being leached from developed land use, and operate simultaneously. Thus, the interactive effect of these multiple contaminant stressors, combined with the additional impact of grazer populations, may exacerbate and cause long-lasting damage to seagrass meadows. However, only a limited number of studies have attempted to assess the impact of multiple stressors on seagrass meadows (Ibarra-Obando et al 2004, Eklöf et

al 2009). These studies indicate that seagrass is more sensitive to light and nutrient limitation than to grazing. However, Eklöf et al (2009) suggested that seagrass already exposed to poor light availability (resulting from experimental shading) may have its resilience to grazing reduced.

In New Zealand, black swan grazing on temperate seagrass (*Zostera muelleri* Irmisch ex Asch. (Zosteraceae)) meadows results in the formation of devegetated patches (~ 0.28 m^2) where a high proportion of seagrass biomass is removed (99 % of rhizomes and 92 % of leaves, Chapter 2). By creating devegetated patches swans can make plants on the edge of these patches more vulnerable to erosive forces (Fonseca and Bell 1998), thus potentially facilitating expansion of the grazing scars and destabilisation of the meadow structure. To date, no study had assessed whether devegetated patches resulting from grazing are subject to expansion and so this warrants further consideration. Where grazing pressure is high, and with a high proportion of regenerative organs removed within grazing scars, annual recovery of meadows from grazing appears to be hindered (Chapter 2), as has also been observed for Canada geese grazing on *Zostera marina* meadows in the USA (Rivers and Short 2007). *Zostera muelleri* is the sole seagrass species in New Zealand (Jacobs et al. 2006, Jones et al 2008) and its decline has been documented in estuaries throughout the country (Park 1999, Inglis 2003, Reed et al 2004). In some locations, grazing by black swans together with anthropogenic sedimentation and eutrophication (Park 1999, Inglis 2003) are postulated as factors responsible for this decline (Park 1999). Evaluating how black swan grazing together with other environmental stressors can affect seagrass meadow condition and resilience is essential to better understand these disturbance effects and the recovery dynamics of these meadows.

In this study, I simulated, *in situ,* black swan grazing at four contrasting sites in Tauranga harbour that differed in environmental characteristics and exposure to potential anthropogenic contaminants (see Chapter 3). The black swan population in the harbour has steadily increased in recent decades (from 1900 to 5100 birds in mid-summer between 1979 and 2010; Eastern Region Fish and Game Council, unpublished data). I hypothesised that seagrass resilience to, and recovery from, grazing would vary among sites due to their differences in exposure to contaminant levels. To test this hypothesis, I simulated high and low grazing intensity in late summer, the period of peak swan grazing pressure, and monitored the regeneration of the simulated grazed patches for a year at the four sites. I also monitored a wider area surrounding each grazed patch to determine whether patch formation affected the integrity of the adjacent meadow area, making it more vulnerable to disturbance from erosive forces (i.e., from currents and wave wash).

4.2. Materials and methods

4.2.1. Study sites

The study was carried out in Tauranga harbour (Latitude: -37° 41' 7.1298", Longitude: 176° 9' 58.0134") located on the east coast on the North Island of New Zealand (Fig. 4.1). Tauranga harbour has a surface area of 201 km^2 and the seagrass meadows cover 29.33 km^2, representing 15% of the harbour (Park 1999). Four sites spread throughout the harbour were chosen. The sites differed in exposure to potential contaminants, environmental characteristics and swan grazing pressure (see Fig. 4.2, Chapters 2 and 3). Bridgman Ln. site has higher sediment herbicide levels, silt and clay contents, porewater P concentrations and higher swan numbers than all other sites. Harvey St. site has higher sediment total N and porewater NH_4-N levels, higher

light availability and a higher proportion of the catchment in native land use compared to the other sites. Katikati and Omokoroa sites have higher sediment organic matter levels, higher porewater NO_3-N levels and a higher proportion of the catchment in crop and urban land use relative to the other sites. The experiment was performed on the intertidal seagrass, *Zostera muelleri*, and was set up in February (late summer) 2009 with monitoring occurring monthly for a year until February 2010.
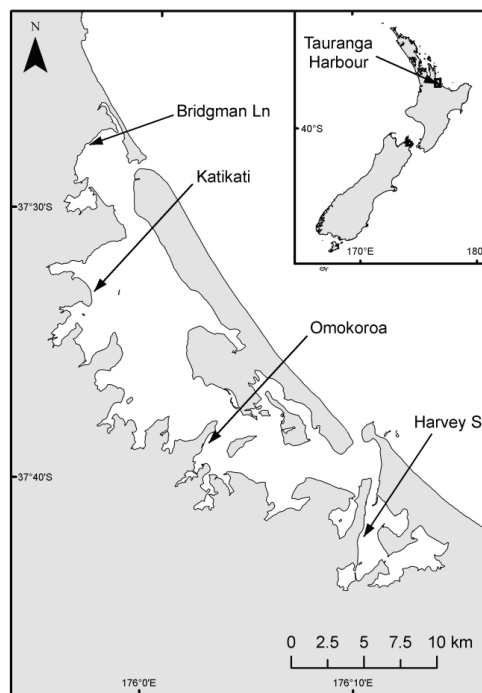
Figure 4.1: Location of the four study sites in Tauranga harbour, North Island, New Zealand.
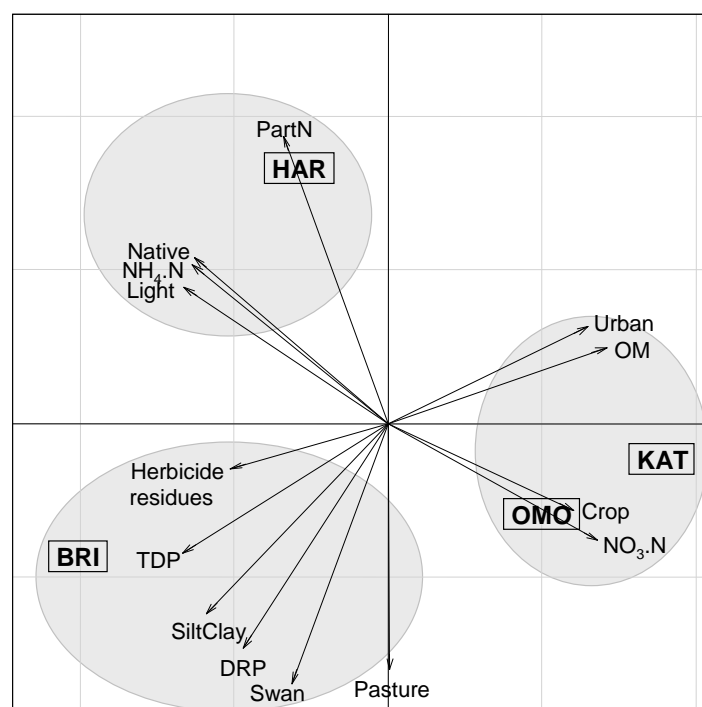
Figure 4.2: Principal components analyses (PCA) on environmental and potential contaminant parameters in Tauranga harbour (from Chapter 2). The PCA yields two components that explain 88 % of the variation in the environmental variables measured (axes 1 and 2 expressing 60 and 28 % of the environmental variability respectively). Sites are grouped by the circular grey areas ("BRI" Bridgman Ln. "HAR" Harvey St., "OMO" Omokoroa and "KAT" Katikati sites). "Native", "Urban", "Crop" and "Pasture" is the type of the land-use, expressed as the % of the catchment area. "Light" is the overall average intertidal light availability to seagrass. "PartN" (particulate nitrogen), "OM" (organic matter), "SiltClay" (silt and clay content) and "Herbicide residues" were measured in the sediment. "NH_4-N" (ammonium-N), "NO_3-N" (nitrate-N), "TDP" (total dissolved phosphorus) and "DRP" (dissolved reactive phosphorus) were measured in the sediment pore-water (Chapter 3). "Swan" represents the annual average number of swans (Chapter 2).

4.2.2. *Grazing simulation experiment*

A block experimental design was used to assess the effects of two grazing scenarios. At each site, 6 blocks were randomly chosen within the seagrass meadow and marked with a wooden stake. At each of the 6 blocks, 4 × 0.25 m^2 square plots, representing 2 different grazing treatments, one control, and one initial (to define

initial seagrass characteristics) were marked out with small coloured marker pegs at a distance of 3 m around the stake and equidistant from each other. The size of the experimental plots (0.25 m^2) was similar to the average scar size created by black swan grazing (0.28 m^2, Chapter 2). The four plot types represented (1) high grazing intensity simulation plots where ~100 % of seagrass above and below-ground biomass was removed (corresponding to the most common grazing behaviour observed, see Chapter 2), (2) low grazing intensity simulation plots where ~40 % of seagrass biomass was removed (the 0.25 m^2 quadrat was divided into 25 squares among which the seagrass content of 10 squares (0.01 m^2) randomly selected was removed; (corresponding approximately to a second type of grazing behaviour observed when swans were subject to intermittent disturbance, see Chapter 2), (3) a non-treated control plots where 0 % of the seagrass biomass was removed, and (4) non-treated initial plots where seagrass biomass was destructively sampled and leaf size was measured prior to the simulation (these plots were not used any further).

The percentage cover of seagrass in each plot was estimated prior to and immediately after grazing treatments (February 2009) and at monthly intervals thereafter for one year (post-grazing February 2009 to February 2010). To examine the possibility of subsequent expansion of the devegetated plots following grazing simulation due to erosive forces, the % cover of a larger area (0.75 m^2) surrounding each plot was also monitored. Cover estimation was performed by laying a 0.25 m^2 quadrat over each plot then a larger 1m^2 quadrat over this, with the plot in the centre of this area. Using a step ladder a photograph was taken from above and the seagrass cover of the plot and the surrounding area (not including the plot) was determined after laboratory analysis. On a computer, a 10×10 square grid was overlain on each quadrat area (0.25 and 1m^2) and the number of squares with more than 50 % cover of

seagrass were counted and summed to provide an estimate of seagrass cover. To estimate the percentage cover of the surrounding area only (a_s, 0.75m^2) the following formula was applied:

$$a_s = \frac{\left(a_t * 100\right) - \left(a_p * 25\right)}{75}$$

Where a_t is the cover of the 1 m^2 quadrat and a_p the cover of the 0.25 m^2 quadrat.

Seagrass biomass of plots was measured at the start (February 2009, in initial plots only) and end of the experiment (February 2010, in both grazed (high and low intensity) and control plots). Two cores (8 cm diameter × 10 cm length), one from the middle and one from the edge of each 0.25 m^2 plot, were sampled to determine the dry weight (DW, 70°C for 48 h) of total, above and below-ground biomass. I sampled cores at the middle and edge of the plots to examine the possibility of unequal recolonisation of seagrass across the plot area. From the fresh above-ground biomass, the size (length and width) of 5 randomly selected seagrass leaves was measured with a vernier caliper (Promark® 150 mm). For the high grazing intensity plots I calculated the net regeneration rate (g m^{-2} day^{-1}) of the biomass (total, below and above) over the experimental period and the % of seagrass biomass that recovered relative to the control plots.

4.2.3. Data analyses

Statistica Version 8 (StatSoft, Inc. Oklahoma, USA) was used for all statistical analyses. Data were log (x+1) transformed to conform to normality and homogeneity of variance where necessary.

Analyses of seagrass cover were done separately for the 0.25 m^2 simulation plots and for the larger 0.75 m^2 areas surrounding them. I applied Repeated Measures ANOVA, and post-hoc Tukey HSD multiple comparison tests if applicable, to test for

(1) significant differences ($p < 0.05$) in seagrass cover for all plots (treatment and control) between the beginning (February 2009, post-grazing) and the end (February 2010, a year after grazing) of the experiment, where site, year and interactive effects of these two factors were tested; and (2) to better quantify the timing of potential recovery, significant differences ($p < 0.05$) in the monthly seagrass cover were explored where site, month, grazing treatment and interactive effects were tested.

I applied factorial ANOVA and Post-hoc Tukey HSD multiple comparison tests if applicable to (1) detect any natural significant ($p < 0.05$) variation (i.e., not due to the grazing treatment) in seagrass biomass (total, above and below-ground) and leaf size (length and width), between the initial (February 2009) and control plots (February 2010), related to site, core sampling location (middle or edge), year and interactive effects; and to (2) test whether the seagrass biomass (total, above and below-ground) and the leaf size (length and width) measured at the end of the experiment (February 2010) were influenced by site, core sampling location (middle or edge), grazing simulation treatment and the interactive effects. I used the student *t*-test to detect any significant difference ($p < 0.05$) in the % recovery and regeneration rate of above-ground versus below-ground biomass.

4.3. Results

4.3.1. Seagrass cover

Significant changes in the seagrass cover of control plots over the course of the experiment were not detected at most sites, except for Bridgman Ln., where cover decreased significantly (by 58 %) from February 2009 (pre-grazing) to February 2010 (Repeated measures ANOVA, time × site effect $p = 0.048$, Tukey HSD test, $p < 0.05$; Table 4.1 and Fig. 4.3). Seagrass cover in high grazing intensity plots was

significantly lower than control plots at all sites from February 2009 (immediately following grazing) to September 2009 (Repeated measures ANOVA, month × treatment effect, $p < 0.001$, Tukey HSD test, $p < 0.01$; Table 4.1 and Fig. 4.3). From October 2009, (i.e., 9 months after grazing simulation) to the end of the experiment (February 2010), no significant differences in seagrass cover were found between the high grazing intensity plots and control plots at all sites (Tukey HSD test, $p > 0.05$) indicating that the cover of the high grazing intensity plots had been restored to the level of the controls within 9 months. Seagrass cover in low grazing intensity plots was not significantly different than control plots from February 2009 (post-grazing) to the end of the experiment (Tukey HSD test, $p > 0.10$) at all sites. Analyses of the monthly cover of the high grazing intensity plots indicated gradual regeneration from the edge of the plot suggesting recovery predominantly by vegetative spread as opposed to any seed germination.

The percentage cover of the areas surrounding all plots (control, high and low grazing intensity) was significantly lower in February 2010 (one year after grazing) than in February 2009 (post-grazing) (Repeated measures ANOVA, year effect $p = 0.004$, Tukey HSD test, $p < 0.05$; Table 4.1 and Fig. 4.3) and was significantly lower for Bridgman Ln. and Omokoroa sites compared to Katikati and Harvey St. sites (Repeated measures ANOVA, treatment × site effect $p = 0.040$, Tukey HSD test, $p < 0.05$). No significant differences were detected among treatments for the monthly covers of the areas surrounding the experimental plots (Repeated measures ANOVA, treatment effect, $p = 0.643$, month × treatment effect, $p = 1$; Table 4.1 and Fig. 4.3).

Table 4.1: Results of repeated measures ANOVA on seagrass cover of the experimental plots and surrounding areas that compare (1) cover in February 2009 (immediately post-grazing) to cover in February 2010 (a year after grazing) of each treatment, and (2) the monthly cover measurements from February 2009 to February 2010. P-values in **bold** indicate significant effects.

	Experimental plots			**Surrounding areas**		
Effects	*df*	*F*	*p-value*	*df*	*F*	*p-value*
(1) Cover						
Year	3	43.73	**<0.001**	3	5.26	**0.004**
Site	9	3.38	**0.001**	9	1.59	0.134
Year × Site	9	2.01	**0.048**	9	1.67	0.328
(2) Cover						
Treatment	2	177.50	**<0.001**	2	0.40	0.643
Site	3	112.70	**<0.001**	3	100.4	**<0.001**
Month	12	5.5	**<0.001**	12	7.10	**<0.001**
Treatment × Site	6	3.50	**0.002**	6	2.20	**0.040**
Treatment × Month	24	6.00	**<0.001**	24	0.30	1
Site × Month	36	3.50	**<0.001**	36	3.10	**<0.001**
Treatment × Site × Month	72	0.00	1	72	0.20	1
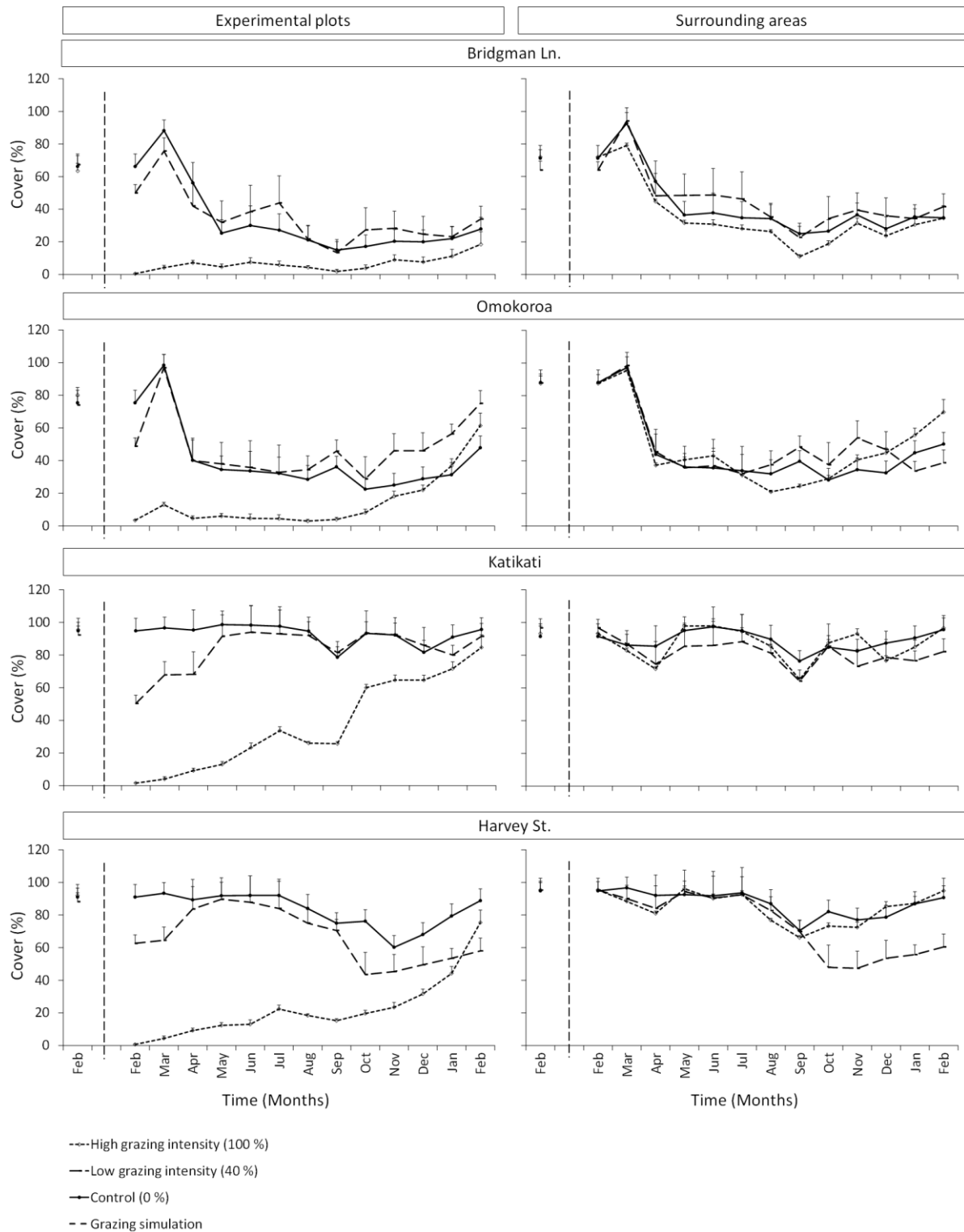
Figure 4.3: Monthly seagrass cover pre- (February 2009) and post- (February 2009 to February 2010) grazing simulation for the different experimental treatment plots and the surrounding areas for each site. The short dashed line represents the cover of the high grazing intensity plots, the long dashed line represents the cover of the low grazing intensity plots, the solid line represents the cover of the control plots and the vertical dashed line indicates the time when the grazing simulation occurred. Error bars indicate 1 SE of mean values.

4.3.2. *Seagrass biomass*

The total, below- and above-ground biomass in control plots at most sites were not significantly different in February 2010 than in initial plots sampled in February 2009. The only exception was the Bridgman Ln. site where the biomass was 65 % lower in February 2010 (Three-way ANOVA, year, site and year × site effect: p = 0.003, p = 0.012 and p = 0.001 respectively, Tukey HSD test, p < 0.001; Table 4.2 and Fig. 4.4).

The total, below- and above-ground biomass measured in the high grazing intensity plots in February 2010 (one year post-grazing) was significantly lower (by 70, 73 and 55 % respectively) than in the control plots measured at the same time (three-way ANOVA, treatment effect: p = 0.003, p = 0.005 and p = 0.003 respectively, Tukey HSD test, p < 0.01; Table 4.2 and Fig. 4.4) but the above-, below-ground and total biomass of low grazing intensity plots did not differ significantly from controls. In the high intensity grazing plots, regeneration of above-ground biomass (45 ± 4 %) relative to the control plots, was significantly higher than regeneration of below-ground biomass (27 ± 4 %) (*t*-test, p = 0.006, n = 4). The average net regeneration rate of above-ground biomass (0.12 ± 0.04 g m^{-2} day^{-1}) was lower than that of the below-ground biomass (0.22 ± 0.10 g m^{-2} day^{-1}) although they were not significantly different (*t*-test, p = 0.27, n = 4). On average for the four sites, 30 % of the total biomass recovered within a year and at a net regeneration rate of 0.34 g m^{-2} day^{-1}. The below- and above-ground biomass measured in all plots at Bridgman Ln. site a year after the grazing simulation was significantly lower than in Katikati and Harvey St. sites but not the Omokoroa site (three-way ANOVA site effect: p < 0.001 for both below- and above-ground biomass, Tukey HSD test, p < 0.05), and was lower than all other sites for total biomass (three-way ANOVA site effect: p < 0.01, Tukey HSD test p < 0.001). Overall, at Bridgman Ln. site, a

significant decrease in biomass (total, below and above) from the beginning (pre-grazing) to the end of the experiment was observable.

I detected no differences in the total, above- and below-ground biomass of cores collected in the middle of plots versus those at the edge of plots amongst grazing treatments, sites, or time of sampling (see Table 4.2).

Figure 4.4: Seagrass biomass prior to the grazing simulation (initial) and one year after grazing simulation for the different experimental treatment plots (control, high (High Gr.) and low (Low Gr.) grazing intensity) for each site. Black columns represent the below-ground biomass, grey columns the above-ground biomass width and the vertical dashed line indicates the time when the grazing simulation occurred. Error bars indicate 1 SE of mean values.

Table 4.2: Results of (1) a three-way ANOVA of seagrass biomass (total, below and above-ground) of the initial plots in February 2009 compared to control plots in February 2010, and (2) a three-way ANOVA of biomass (total, below and above-ground) among grazing treatments one year after grazing simulation (February 2010). P-values in **bold** indicate significant effects.

Effects	Total biomass			Below-ground biomass			Above-ground biomass		
	df	*F*	*p-value*	*df*	*F*	*p-value*	*df*	*F*	*p-value*
(1) Biomass									
Year	1	11.03	**0.001**	1	9.50	**0.003**	1	13.20	**0.001**
Site	3	9.54	**<0.001**	3	8.25	**<0.001**	3	13.45	**<0.001**
Location	1	0.05	0.816	1	0.05	0.816	1	0.12	0.734
Year × Site	3	5.09	**0.003**	3	3.94	**0.012**	3	6.55	**0.001**
Year × Location	1	0.09	0.767	1	0.12	0.736	1	0.00	0.978
Site × Location	3	0.02	0.997	3	0.03	0.994	3	0.09	0.964
Year × Site × Location	3	0.25	0.864	3	0.51	0.676	3	0.03	0.994
(2) Biomass									
Treatment	2	6.14	**0.003**	2	5.68	**0.005**	2	6.07	**0.003**
Site	3	10.48	**<0.001**	3	7.59	**<0.001**	3	18.73	**<0.001**
Location	1	1.89	0.173	1	2.27	0.136	1	0.54	0.465
Treatment × Site	6	0.73	0.623	6	1.03	0.413	6	0.37	0.897
Treatment × Location	2	2.62	0.079	2	2.35	0.102	2	2.30	0.107
Location × Site	3	0.10	0.962	3	0.27	0.845	3	0.04	0.991
Treatment × Site × Location	6	0.18	0.983	6	0.30	0.932	6	0.13	0.992

4.3.3. Seagrass leaf size

There were no significant differences in seagrass leaf length and width between initial plots measured in February 2009 and control plots measured in February 2010 (three-way ANOVA, year effect: $p = 0.4$ and $p = 0.6$ respectively; Table 4.3 and Fig. 4.5). Results show a significant site × year effect in seagrass leaf length for the control plot in February 2010 versus initial plots in February 2009 (three-way ANOVA, site × year effect: $p = 0.022$), although the Tukey HSD post-hoc testing did not detect any further significant differences ($p > 0.05$).

Seagrass leaf length measured in control and initial plots was smaller at Bridgman Ln. site compared to Harvey St. site only (three-way ANOVA, site effect: $p < 0.001$, Tukey HSD test, $p < 0.001$), although seagrass leaf width measured in these plots was smaller at Bridgman Ln. compared to all other sites (three-way ANOVA, site effect: $p < 0.001$, Tukey HSD test, $p < 0.05$).

Three-way ANOVA detected a significant grazing treatment effect on seagrass leaf size (length and width) ($p = 0.040$ and $p = 0.035$ respectively; Table 4.3 and Fig 4.5), but further post-hoc testing did not substantiate this effect ($p > 0.05$). However there were significant differences in seagrass leaf size for low and high intensity grazing plots among sites (three-way ANOVA, site effect: $p < 0.001$ for both length and width). Leaf size (length and width) was smaller at Bridgman Ln. in high and low grazing intensity plots compared to all other sites (Tukey HSD test, $p < 0.05$).

I detected no significant differences in seagrass leaf length and width for cores collected in the middle of plots versus those at the edge among grazing treatments, sites, or time of sampling (see Table 4.3).
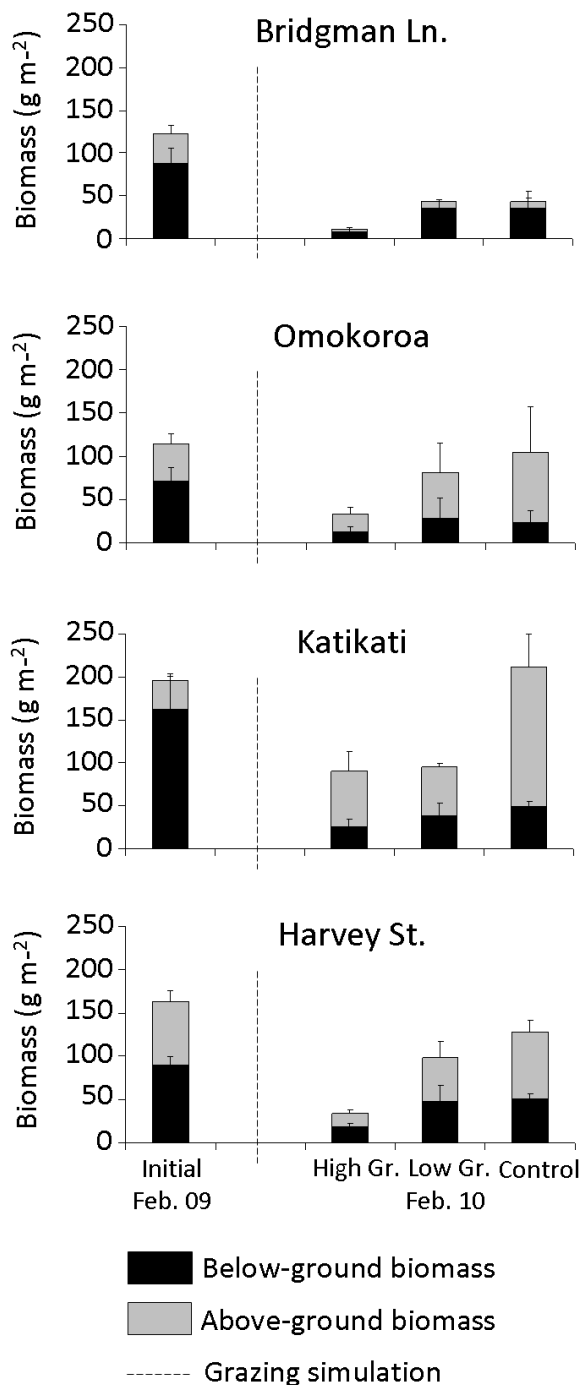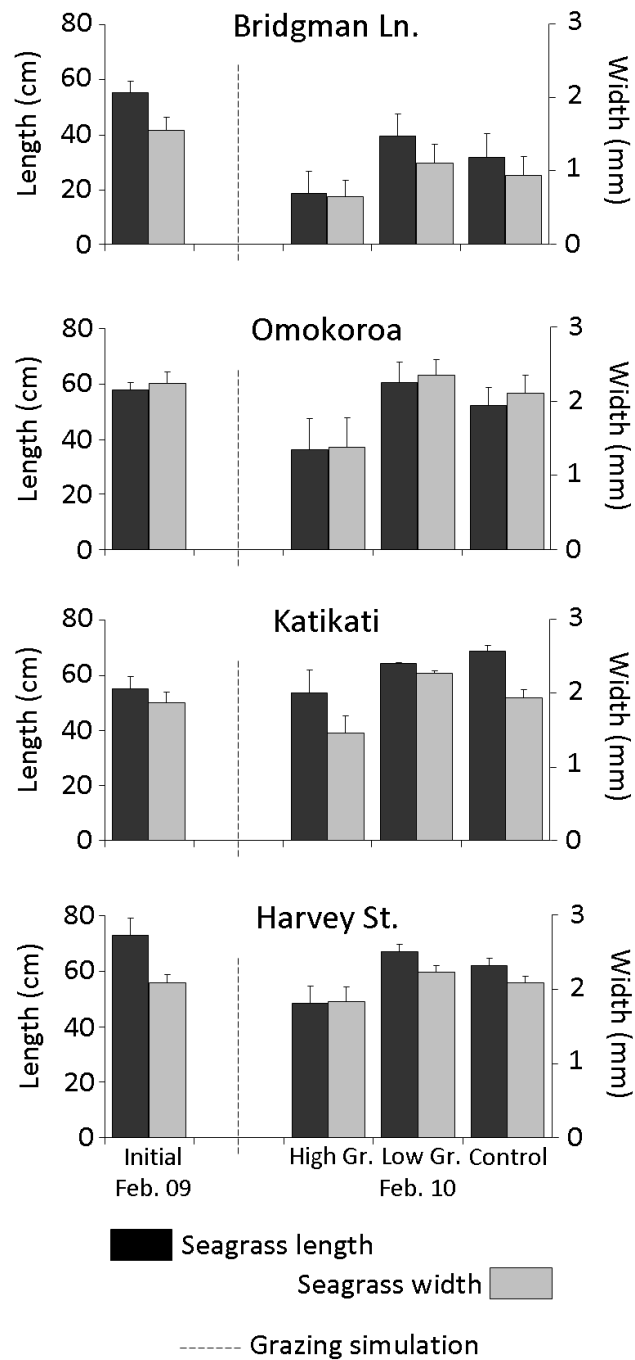
Figure 4.5: Seagrass leaf size before the grazing simulation (initial) and one year after grazing simulation for the different experimental treatment plots (control, high (High Gr.) and low (Low Gr.) grazing intensity) for each site. Black columns represent the seagrass length, grey columns the seagrass width and the vertical dashed line indicates the time when the grazing simulation occurred. Error bars indicate 1 SE of mean values.

Table 4.3: Results of (1) a three-way ANOVA of seagrass leaf size (length and width) comparing initial plots in February 2009 before grazing simulation to control plots in February 2010 one year after grazing simulation, and (2) a three-way ANOVA of seagrass leaf size (length and width) between grazing treatments one year after grazing simulation (February 2010). P-values in **bold** indicate significant effects.

Effects	Length			Width		
	df	*F*	*p-value*	*df*	*F*	*p-value*
(1) Leaf size						
Year	1	0.87	0.353	1	0.31	0.577
Site	3	7.00	**<0.001**	3	11.64	**<0.001**
Location	1	2.03	0.159	1	0.66	0.418
Year × Site	3	3.41	**0.022**	3	0.30	0.822
Year × Location	1	0.03	0.871	1	0.15	0.700
Site × Location	3	0.96	0.416	3	0.56	0.642
Year × Site × Location	3	0.37	0.779	3	0.23	0.877
(2) Leaf size						
Treatment	2	3.37	**0.040**	2	3.54	**0.035**
Site	3	12.93	**<0.001**	3	23.08	**<0.001**
Location	1	0.19	0.662	1	1.53	0.221
Treatment × Site	6	0.66	0.685	6	0.61	0.722
Treatment × Location	2	0.87	0.425	2	0.08	0.925
Location × Site	3	1.86	0.145	3	0.17	0.915
Treatment × Site × Location	6	0.21	0.971	6	0.44	0.852

4.4. Discussion

4.4.1. Effect of grazing intensity

In this study, black swan grazing was simulated at high and low intensity levels at four contrasting estuarine seagrass meadow sites and the recovery response of the meadows was monitored through time. High and low grazing intensity had no effect on seagrass leaf size (length or width). For the low grazing intensity plots (~40 % biomass removed) seagrass percentage cover and biomass (the latter measured only after one year) at all sites was never significantly different to undisturbed control plots, suggesting that at this grazing intensity level, seagrass meadows were not significantly impacted by herbivory, even in the short-term. However, for the high

grazing intensity plots (~100 % biomass removed), while seagrass cover was restored within 9 months of disturbance at all sites, only 30 % of the total biomass had recovered a year after grazing, indicating only a partial regeneration. The lack of complete seagrass biomass recovery in the devegetated patches after one year suggests that persistent high intensity grazing can cause long-lasting damage to *Z. muelleri* meadows. These results emphasize the importance of grazing intensity to seagrass grazing scar recovery rates.

4.4.2. *Effect of variation in environmental conditions and contaminants*

Despite differences in environmental characteristics and potential contaminant levels among the contrasting sites I selected, seagrass regeneration from grazing was similar for all sites. This suggests that seagrass resilience in this harbour is not affected by the current levels of environmental heterogeneity and exposure to potential contaminants. It could also suggest that the environmental conditions and exposure to contaminants that characterised each site may not be distinct enough to detect any seagrass resilience dissimilarities between the sites or that the regeneration rates do not accurately reflect the amount of biomass that will ultimately be attained at sites upon complete recovery from grazing. However, the overall contamination level in Tauranga Harbour may be responsible for the incomplete recovery, although I found no obvious detrimental effects of these contamination levels on seagrass biomass or morphometry in Chapter 3. The expansion of this study to other harbours and sites that contrast more widely in environmental characteristics and exposure to potential contaminants is needed to more rigorously examine the levels and variation of these parameters that affect seagrass regeneration rates. At one site (Bridgman Ln.) although partial seagrass recovery was observed, equivalent to that of other sites, the cover and the biomass of the control plots decreased significantly between February

2009 and 2010 indicating an overall decrease in seagrass abundance at this site. The conditions that distinguish this site from others in the harbour (i.e., higher grazing pressure, higher sediment herbicide concentrations, higher sediment silt/clay content and higher porewater P concentrations, Fig. 4.2) may be factors that have contributed to this decline, particularly the high swan grazing pressure which I linked to biomass decline at this site in Chapter 2.

4.4.3. *Effect of grazing on surrounding meadow integrity*

The seagrass cover of areas surrounding the experimental plots was not affected by either of the two grazing intensity treatments suggesting that plants adjacent to the devegetated, grazed patches caused by swan grazing do not become more vulnerable to subsequent disturbance from erosive forces (e.g., currents and wave wash). In fact, this ungrazed reserve (Noy-Meir 1975) presumably plays an important role in the recovery of adjacent grazed patches through lateral vegetative elongation of rhizomes. Studies often monitor the recovery of devegetative patches (e.g., Peterken and Conacher 1997, Rollon et al 1999) but, to our knowledge, their possible expansion has not previously been examined.

4.4.4. *Recovery mechanisms*

4.4.4.1. *Recovery from seeds*

In terrestrial and freshwater systems studies have shown that presence of seed banks can play a major role in the recolonisation of open gaps in vegetation (Abernethy and Willby 1999, Combroux et al 2001, Kalamees and Zobel 2002, Weerasinghe et al 2008). However, in marine environments, less is known about the viability and the extent of seagrass seed banks (Orth et al 2000), particularly those of poorly studied species. In some cases seagrass regeneration from seed is known to

contribute significantly to meadow recovery as shown for multi-species meadows in eastern Australia and in fact seed germination here is actually stimulated by the abrasive action of herbivore (dugong) grazing (Preen 1995). Conversely, it appears that seagrass seed banks and regeneration of meadows via seed germination may be limited in some areas and/or for some species. For example, in Australasia, seagrass flowering and production of viable seeds is considered rare in *Zostera* species (Bearlin et al. 1999, Turner and Schwarz 2006b) suggesting a limited recovery via seed germination and a prominent role of vegetative regeneration in these meadows. Indeed, the monthly cover analyses of the high grazing intensity plots in this study strongly suggested vegetative regeneration from the edge to the centre of the plot with little evidence for germination from seeds.

4.4.4.2. Recovery via clonal regrowth

There was no difference in seagrass biomass between the middle and edge of the plots at the end of the experiment (one year after grazing) suggesting an even, but low density, recolonisation of the plot area, relative to the level of the undisturbed meadow. This recolonisation strategy presumably also allowed the seagrass to reach an above-ground cover similar to those seen in the ungrazed control plots (as I found no difference in the cover of treatment plots one year after grazing) yet biomass had not fully developed in high grazing intensity plots. This strategy may also explain the rapid and full biomass recovery of the low grazing intensity plots since the devegetative patches were smaller and more closely surrounded by seagrass. Cover is a visual representation of the area occupied by seagrass leaves which does not take into account (1) the below-ground biomass and (2) layering of seagrass above-ground material as plants age; thus through time, seagrass biomass may increase but not necessarily cover. This difference in results based on use of cover versus biomass

highlights the superiority of biomass measurements for accurate assessment of seagrass meadow recovery; however, the advantage of cover measurements is their non-destructive nature.

Plant growth rate presumably has an important influence on the speed of meadow regeneration from any ungrazed reserve material and this growth rate can vary both inter- and intra-specifically (Duarte and Chiscano 1999), the latter depending on specific site conditions and resources available for growth (Hiesey et al 1942, Nicotra and Rodenhouse 1995). In terms of inter-species differences, pioneer species such as *Halophila ovalis* appear to have a relatively high leaf production rate (3.1 g m^{-2} day^{-1} during autumn; Eklöf et al 2009) compared to other species (e.g., *Zostera muelleri*, from 1.1 g m^{-2} day^{-1} during winter to 2 g m^{-2} day^{-1} during summer; Turner and Schwarz 2006b) which may facilitate more rapid meadow recovery following grazing. Indeed, in Thailand, the high productivity rate of *H. ovalis* is considered responsible for its fast recovery after dugong grazing that cause similar damage to seagrass meadow as black swans (Nakaoka and Aioi 1999, Chapter 2). I did not measure leaf production rates in this study but the relatively low net biomass regeneration rate determined in this study (0.34 g m^{-2} day^{-1}, average for all sites) for *Zostera muelleri*, may have contributed to the incomplete recovery from high grazing intensity.

In the year following high intensity grazing, recovery of the above-ground biomass relative to controls (45 %) greatly exceeded that of the below-ground biomass (28 %). This suggests that development of above-ground biomass and production of photosynthetic material is important, at least in the early stages of regeneration.

4.4.5. Management implications

In this study, I have shown that only 30 % of the total biomass recovers in large (~0.25 m^2), devegetated patches created by swan grazing within a year. Thus, on the assumption that the regeneration rate remains constant through time, complete regeneration of these devegetated patches would take more than 3 years (i.e. 3.3 years, 40 months) to occur. If the dominant type of swan grazing activity results in the formation of these large patches, then this result implies that should grazing remove more than 30% of biomass in a meadow on an annual basis, then the meadow is unlikely to fully regenerate and may suffer long-term decline. This 30 % threshold for biomass removal, based on plant regeneration rates, exceeds the 19-20 % threshold identified in chapter 2 based on a correlation because swan abundance and significant annual change in seagrass biomass, but together these results may provide a useful threshold range. The current swan population has been shown to graze 15 % of the annual seagrass biomass in Tauranga harbour (Chapter 2). Thus to reach the threshold range of 19 to 30 % of annual seagrass biomass removed by grazing, the current swan population needs to increase by a factor ranging from 1.25 to 2 (i.e. ~ 4630 to 7400 swans). However, it is possible that swans may prefer to graze new emerged shoots as opposed to older, more established biomass. It has been shown that green turtle, dugong, and brant geese repeatedly graze the same area since young seagrass leaves contain more fibre and less lignin than older leaves which facilitates digestion (Bjorndal 1980, Thayer et al 1984). Thus, older, less palatable patches of seagrass may act as a permanent ungrazed reserve to assist seagrass regeneration. The preference of black swans for new versus old biomass material was not examined in this study but this would be a useful avenue of investigation for future research to enable better understanding of seagrass recovery dynamics from swan grazing.

Seagrass meadows have important economic and ecological values (i.e., providing habitat for important commercial and recreational fisheries (Hemminga and Duarte 2000, Costanza et al 2007) and food and habitat for threatened species such as dugong, turtle and sea-horses), thus effective resource management that regulates factors contributing to seagrass decline is needed. The results of this study suggest that some regulation of the swan population in Tauranga harbour may be required in the future, should numbers continue to increase, to ensure the persistence of this valuable habitat.

Chapter 5

General conclusions

Black swan grazing at Bridgman Ln. site (mangroves in the foreground)

5. General conclusions

5.1. *Summary of chapter conclusions*

Seagrass meadows are an important ecological and economically valuable marine system. The need to disentangle the reasons that contribute to their decline is essential. This thesis work examined how herbivory by black swans, anthropogenic contaminants and their interactions affect seagrass meadows in New Zealand.

In Chapter 2 of this thesis, observations showed that black swans foraged primarily at high tide (day and night) and were more numerous at sites with larger meadows, particularly during autumn. Grazing created circular devegetated patches with a high amount of above and below-ground biomass removed. I measured an average seagrass consumption rate of 394 g dry weight (DW) swan^{-1} day^{-1}. Results showed a substantial decline (43-69 %) in plant biomass in the subsequent growing season at the sites where grazing was most intense, and suggest that black swan grazing could contribute to seagrass decline when the proportion of annual biomass removed by grazing exceeds a threshold of 19-20 %. Moreover, I estimated that, at harbour scale, a 25 % increase in swan numbers (i.e. an average swan population > 4630 birds) during mid-summer is required to reach this threshold.

The survey conducted in Chapter 3 suggested that the seagrass in both harbours (Tauranga and Aotea) was nitrogen limited. An increasing proportion of urban and crop land use in the catchments appeared to play a role in decreasing water clarity

through nutrient enrichment with nitrate (NO_3-N), although there was no evidence of seagrass light limitation. Herbicide contamination of estuarine sediments was detected, particularly in the most developed harbour, and this was associated with a decrease in plant photosynthetic performance. However, no herbicide effects were detected on seagrass biomass or morphometry.

The grazing simulation experiment conducted in Chapter 4 showed that grazing intensity and the spatial pattern of grazing at small scales (i.e. large patches versus small patches) affects the regeneration rate within seagrass meadows. At low grazing intensity (~40 % of biomass removed, or 10×0.01 m^2 small patches within a 0.25 m^2 plot) seagrass cover and biomass were not significantly different from controls throughout the one year experiment suggesting that this grazing behaviour does not detrimentally affect seagrass meadow. At high grazing intensity, (~100 % of biomass removed within a 0.25 m^2 plot), the cover of these plots was restored within 9 months although only 30 % of the total biomass regenerated after a year. This suggests that full recovery of larger grazing scars created by swans may take several years (>3 years, assuming a constant regeneration rate). Presumably, the large size of the scars created by swan grazing increases the time necessary for regeneration via vegetative ingrowth from the edge. This demonstrates that persistent high intensity grazing creating large devegetated patches has the potential to contribute to reduced seagrass biomass at sites from one year to the next. As resilience responses to the different grazing treatments were similar across all sites, this suggests that the current levels of site variation in environmental characteristics and potential contaminants within the seagrass meadows of this harbour do not affect seagrass regeneration rates from swan grazing pressure which supports the results found in the previous chapter.

5.2. *Overall conclusion*

Seagrass meadows are declining worldwide and many stressors are postulated to contribute this decline (see Fig. 1.1). In the case of Tauranga Harbour, more than one-third of the seagrass meadows disappeared between 1959 and 1996 (Park 1999). This study contributes to the understanding of this decline by assessing the impacts of black swan grazing pressure combined with potential anthropogenic contaminant effects on seagrass meadows. It provides crucial information about black swan feeding behaviour in estuaries and contributes to our knowledge of plant/herbivore interactions by highlighting the importance of herbivory in temperate seagrass systems, which has often been underestimated. It also provides important information concerning the potential impact of coastal development on the marine environment with evidence of nutrient enrichment and herbicide contamination of seagrass habitat.

From these results, I cannot conclude that the anthropogenic contaminants measured in this study or the black swan grazing pressure are by themselves responsible for the seagrass meadow decline in Tauranga Harbour. However, the study results do indicate that black swans have the potential to detrimentally affect seagrass meadows when grazing pressure is high; specifically when grazing creates many, large devegetated patches. In addition, the concentration of sediment herbicide was associated with detrimental effects on seagrass physiology. Although there was no apparent impact of herbicides on plant biomass or morphology, effects on physiology could be a precursor to such impacts should contamination levels increase in the future.

Since these results may have an important bearing on resource management decisions for Tauranga Harbour, it is recommended that the limitations of this study

are duly recognised and that further investigations are performed to corroborate these results. Indeed (1) calculations and extrapolations at the harbour scale were based on a seagrass coverage estimated in 1996 that needs to be updated, (2) the measurement of the contaminant parameters was performed only once and other useful parameters, such as seagrass growth rate and water column nutrient, suspended sediment and herbicide concentrations, could also be measured and (3) the biomass removal threshold determined in Chapter 4 is based on the assumption that the dominant type of grazing impact in the harbour results in the formation of large, devegetated grazing scars and that the regeneration rate for plant recolonizing these scars remains constant through time. It would be useful to validate these assumptions in the future.

5.3. Suggestions for future research

The study limitations outlined above are considered useful topics for future research. In addition to these, other more general topics are suggested below.

My results from making observations and measurements at individual sites within the harbour suggest that black swans feed randomly on the seagrass within a variable meadow and do not appear to target high plant density areas (Chapter 2). However, little is known about site selection and movements of this large, mobile grazer at the entire harbour scale. Thus, further observations using blimps (floating airships) with mounted cameras and/or aerial/satellite photography are recommended to improve understanding of the swan population dynamics and habitat choice at the harbour scale.

From my field-based measurements and analysis of correlations between seagrass condition metrics and harbour environmental/contaminant parameters (Chapter 3), some important relationships emerged and warrant further attention. So far, little is

known about the impact of the different herbicide components found in this study on the marine ecosystem. It has been postulated that the toxicity of herbicides may be more detrimental when absorption occurs through leaves than via root uptake (Ralph et al 2003). Moreover, herbicide toxicity varies according to the seagrass species and the time of exposure. Although, bio- and photo-degradation in marine waters can reduce the half-life of toxic compounds (Solomon et al 1996, Scarlett et al 1999), some studies have shown that it does not necessarily reduce the damage since the degradation product can remain toxic (Okamura et al 2000, Zhang et al 2008). Thus, further studies are needed to: (1) identify the nature and amount of herbicides present in the water column, (2) understand how herbicide compounds degrade in estuarine water and sediment; and (3) evaluate the concentration levels at which these toxic compounds and their respective degradation products detrimentally affect different seagrass species.

This study has shown that activities linked to human development are closely related to NO_3-N enrichment of estuarine water and that seagrass condition may be strongly regulated by porewater NH_4-N availability and the presence of herbicide compounds in the sediments (Chapter 3). Thus the effects of these contaminants on seagrass meadows warrants further investigation using laboratory or in situ experimental manipulations that test the growth responses and sensitivity of Zostera muelleri to a range of contaminant concentrations both individually and interactively. In situ nutrient experiments could take the form of addition experiments using slow-release fertilisers buried in the sediment (e.g. Worm et al 2000, Udy et al 1999, Kenworthy and Fonseca 1992). This study provides information on the levels of contaminants likely to be encountered in situ which will be a useful guide for appropriate treatment levels to be used in such controlled experiments.

In addition to the black swan grazing pressure and anthropogenic contaminant effects assessed in this study, other stressors can also contribute to seagrass decline (see Fig. 1.1) and probably warrant further study, particularly in Tauranga Harbour where the cause of the historical seagrass decline was unable to be confirmed. Other stressors not examined here include wasting disease, other contaminants (e.g. petrochemicals, heavy metals), physical damage from coastal developments and recreational activities and extreme natural disturbances (e.g. large storms).

From the range of other stressors that can potentially impact seagrass (see Fig. 1.1), global climate change, as a result of human activities, is expected to lead to increased water temperatures and sea levels, which could detrimentally affect seagrass physiology and biomass. It has been shown that fluctuations in water level, temperature, and salinity can affect seagrass distribution, growth, biomass, and enzymatic activity, and increase seagrass vulnerability to disease (Short and Neckles 1999, Masini and Manning 1997, Fletcher and Fletcher 1995, Kamermans et al. 1999, Zieman et al. 1999, Burdick et al. 1993). Although global climate change is unlikely to have contributed to historical seagrass decline it may affect seagrass regeneration from natural and anthropogenic disturbances, such as those investigated in this study, in the future. Thus, evaluating how global climate change affects seagrass condition alongside other more commonly studied stressors would be an interesting avenue of future research.

Recently concern has been raised in northern New Zealand about the expansion of mangrove beds in coastal areas and associated reduction in areas suited to recreation. Thus, in some places, local management agencies have allowed the removal of this important ecosystem. Mangroves grow in muddy sediments so their expansion is linked to accelerated sedimentation of estuaries as a result of anthropogenic catchment

developments. The mangrove system forms an important habitat for fish and other marine biota (Barbier 2000, Chaves and Bouchereau 2000) and they are known to play a role in filtering coastal runoff water by trapping fine sediment particles that potentially smother seagrasses and corals (Victor et al 2004). Currently little is known about the impacts of mangrove expansion and/or removal on adjacent habitats such as seagrass meadows. This information would assist resource managers and scientists to weigh up the costs and benefits of mangrove removal activities.

Finally, this thesis examined in detail the impact of black swan grazing pressure combined with anthropogenic contaminants, on seagrass meadows, and results can support decisions to maintain seagrass meadows in Tauranga Harbour. From a broader point of view, this study also emphasizes the importance of a multi-factorial approach *in situ* to identify the factors affecting seagrass meadow dynamics. It provides empirical data about herbivory and anthropogenic contamination that could usefully serve to support modelling approaches. Models that incorporate all factors affecting seagrass dynamics in order to predict and anticipate seagrass loss may further improve our understanding of this ecosystem and so its management.

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