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COLONISATION, FRAGMENT RECOVERY, AND DISTURBANCE
IN ZOSTERA MUELLERI BEDS, RAGLAN

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**ABSTRACT**

*Zostera muelleri* is a seagrass currently on the decline in New Zealand. Potential conservation methods rely on accurate information tailored to local sites. This thesis considers the *Z. muelleri* beds at Raglan harbour, New Zealand. It focuses on their mechanisms for acquiring genetic diversity, and their ability to prove resilient to small-scale disturbance.

Extensive sediment sampling at four different study sites within the Raglan harbour has yielded no evidence of a seed bank. *Z. muelleri* reproduction at this site is likely therefore vegetative. Without sexual reproduction to increase the genetic diversity (and therefore the resilience) of the beds, this diversity can be increased by the natural mechanism of seagrass fragments or the deliberate transplantation of seeds, seedlings, or adult plants. This, however, raises conservation questions regarding the tension between “original” and “resilient” environmental states.

Genetic diversity within the seagrass beds may be introduced via the natural dispersal of seagrass fragments. Fragments collected in austral autumn (April) and spring (September) were tested to determine if length of dispersal (floating) time impacted their ability to grow either rhizome length or new shoots. Fragments were randomly assigned to one of five treatments (T0-T4) and left to float for between 0-28 days before planting (T0 = 0 days; T1 = 7 days; T2 = 14 days; T3 = 21 days; T4 = 28 days). After a six-week planting there was no statistically significant difference in rhizome or shoot growth between treatments or between collection times.
The ability of *Z. muelleri* to respond to small-scale disturbance was also assessed. The presence of Canada geese (*Branta canadensis*) has been noted feeding on New Zealand seagrass for the first time, and an experiment mimicking their feeding patterns performed. *Z. muelleri* proved highly resilient to single-event, small scale disturbance at all tidal levels. However, the rapidly increasing population of geese in the Waikato region is expected to increase the level of disturbance to the Raglan beds, and their ability to respond to repetitive, large-scale disturbance may be crucial to their continued survival.
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INTRODUCTION

*Zostera muelleri* is a temperate species present in two of the six global bioregions (Short *et al.* 2007): the Tropical Indo-Pacific and the Temperate Southern Oceans. This thesis is concerned with growth in the latter bioregion; Short *et al.* characterise the seagrasses of this region as growing in “Extensive meadows of low-to-high diversity seagrasses (18 species) often growing under extreme conditions” (7). Given that *Z. muelleri* is an intertidal species, extreme conditions would appear to be part of its natural habitat.

*Z. muelleri* is New Zealand’s only seagrass (Turner and Schwarz 2006; Matheson *et al.* 2009). It performs a similar ecological role here as other seagrasses perform overseas, acting as a nursery species and ecosystem service provider to a wide variety of marine organisms (Matheson *et al.* 2009). A primary factor in its conservation importance is the diversity of organisms it supports. In some communities the presence of *Z. muelleri* may be crucial for individual species – in Hayward *et al.*’s survey (1981) of the Eastern Bay of Islands, the bivalve *Zearcopagia disculus* was not recorded outside of the *Z. muelleri* beds. This does not indicate that *Z. disculus* does not exist outside seagrass beds in other New Zealand coastal communities, but it is potentially indicative of the importance of *Zostera* to the national ecosystem.

Leduc and Probert (2011), when studying meiofaunal community structure in Otago seagrass beds, determined that there was a significant increase in faunal biomass within the beds as opposed to without. They also discovered that the community structures of nematodes differed significantly according to the vegetative level of the seagrass beds, indicating that *Z. muelleri* can support a number of different communities depending on the
density of their beds. Similarly, although fragmented beds contain significantly lower total taxa than continuous beds within inlets, distinct communities were found within each habitat, with shoreline position being the most important variable in determining the composition of microfaunal communities (Mills and Berkenbusch 2009).

Battley et al. (2011) reinforced this finding, after research on the Z. muelleri beds at Farewell Spit. They found that invertebrate biodiversity increased with seagrass cover, and that this diversity could be clustered into four major groups, depending on coverage (one cluster was associated with low coverage and two with high) and other environmental factors (proximity to water channels and low tidal position of the sample sites). This does appear to emphasise the importance of local or immediate environmental conditions not only of seagrass beds as a whole, but within the bed structures themselves.

This associated biodiversity can only be maintained through the preservation – both conservative and restorative – of national seagrass beds.

Restoration of seagrass beds, and the resumption of the ecosystem services these beds provide, is hampered by a number of factors. In the Pacific region, these include habitat loss, high turbidity, lack of economic support and, crucially, poor understanding of current habitats and populations (Paling et al. 2009). The purpose of this thesis is to remedy in some way this last inhibiting factor, specifically regarding the Z. muelleri beds in Raglan harbour, New Zealand. Increased knowledge of the seagrass at that site can only result in more effective, locally-adapted conservation measures in the future.

Of particular interest to this thesis is identification of the mechanisms by which genetic diversity is introduced into this local population, given that such diversity, within the genus,
is positive correlated with increased resilience after disturbance (Hughes and Stachowicz 2004). These mechanisms are explored in three ways:

Chapter One discusses the possibility of a seed bank, indicative of sexual reproduction. Extensive sediment sampling was undertaken in order to determine if a seed bank was present. In the absence of a viable seed bank, adequate understanding of the vegetative properties (and influences on the growth) of Z. muelleri will be crucial in determining any future conservation plan.

In Chapter Two, the viability of Z. muelleri fragments are assessed. Vegetative fragments, when detached from the parent plant, can travel via ocean currents to new sites and attach there, thus introducing the potential for new genetic material to enter into existing beds, or to establish new ones. This chapter determines the viability of fragments after five different periods of floating.

In Chapter Three a pilot experiment attempts to determine the resilience of Z. muelleri after small-scale disturbance. Seagrass is vulnerable to herbivory by large avians, and the Raglan beds have small flocks of Canada geese (Branta canadensis) feeding upon them. This is a stressor likely to increase due to the increasing population of geese in the region, and so the ability of the seagrass to recover after herbivory is potentially of increasing importance.

This thesis can contribute to original knowledge in three ways. Firstly, Raglan harbour had, prior to this thesis, not been thoroughly tested for seed banks. Secondly, this thesis illustrates a trend where the viability rates of fragments from this site are potentially unaffected by season. And finally, this is the first study to note that Canada geese are
feeding on seagrass in New Zealand – previously, the only related studies were on herbivory by black swans (Dos Santos et al. 2012).

**Study sites**

Samples were taken from four different sites within the Raglan harbour (Figure 1). Sites were selected in order to provide a range of harbour environments and patch characteristics.

Figure 1: Study Sites A, C, D, G within Raglan Harbour

Site A (-37.8154, 174.8625), adjacent to the airfield along Marine Parade, is a shallow, sheltered site where the Wainui Stream enters the harbour. There is an extremely sparse
patch of seagrass observable in summer only. Sediment consists primarily of very fine grains (<250 microns).

Site C (-37.7951, 174.8787) is a shallow bay along Wallis Street, adjacent to the silos of the former Cement Factory. Seagrass meadows are extensive and perennial, exhibiting no observable winter dieback. Sediment is mostly relatively fine-grained (>250 microns) but contains high proportions of shellfish and shell fragments.

Site D (-37.7984, 174.8842), in Lorenzen Bay at the base of Daisy Street, is a shallow bay where the population of seagrass is restricted to one thin (<2m) vertical transect (Figure 2). This transect corresponds with a length of rocky substrate that is covered by a thin layer (<2cm) of sediment. Sediment is relatively fine-grained (>250 microns). Seagrass exhibits no winter dieback. Outside of this transect line, thicker layers of sediments contain high proportions of shellfish and shell fragments.

Figure 2: Vertical Band of *Z. muelleri*, Site D.
Site G (-37.8094, 174.8628), at the base of Goodare Road, is a relatively sheltered, steep-banked site deep within the harbour. There is a small (<5m) patch of seagrass that exhibits winter dieback. Sediment consists primarily of clay and small (<2cm) rocks.
LITERATURE REVIEW

The object of this review is to give an overall view of the biological properties of Zostera muelleri, and how its growth and survival may be impacted by its environment.

Supplementing this is an overview of the Zostera genus, as although parallels cannot always be made between representatives of the same genus, it is often useful to look closely related species in order to better understand the responses of the target species.

Zostera muelleri:

Seasonal growth patterns of Z. muelleri are similar to those found in other Zosteraceae species – at least in an Australian environment. Kerr and Strother (1990) found that above-ground biomass correlated with maximum temperature, solar radiation and day length, and that there was subsequently a significant winter minimum and summer maximum relating to growth. The maximum difference in this seasonal variation ranged from a factor of seven to 40 depending on geographic point of sampling. Leaf growth was more strongly correlated with solar radiation and day length than with temperature, however (Kerr and Strother 1989). Similar seasonal growth patterns were also reported by McKenzie (1994).

Similarly, York et al. (2013) found that biomass was dependent on temperature and light: biomass decreased at high temperature (30°C) and also at low light levels. Interestingly, while this decrease was observed when the stressors were tested for individually, the combination of temperature and light stress showed few further complications. The authors concluded that the tested Z. muelleri populations were vulnerable to small chronic changes.
in the tested stressors; changes that are to be expected under predictive climate change models. A similar result – the increase of biomass with increasing light levels – was demonstrated under laboratory conditions by Abal et al. (1994), although the authors were careful to note that laboratory results do not always correspond to those gained under natural conditions.

Absence of *Z. muelleri* was determined by O’Brien et al. (2011) as being influenced by five characteristics of benthic light; the characteristics ranged from light doses to light penetration. They were not wholly indicative, though (the seagrass did not occur in 93% of sites that did not exhibit these criteria, and in just under 50% of sites where all criteria were met). Given the 93% absence variable, however, it would be prudent to establish any prospective restoration site meet these criteria before restoration efforts go ahead.

While biomass growth corresponds positively with solar radiation, the photosynthetic rate of *Z. muelleri* decreases with exposure – that is, during low tide when the seagrass is no longer covered by water (Clough and Attiwill 1980). This is likely not a causal factor, however. The authors posit that this could be due to the compaction of leaves without the buoyancy provided by water and changing balance between photosynthesis and higher respiration, but this is something to consider when determining the potential impact of sea level change on the seagrass beds.

The effects of light and temperature on growth rates and biomass have been performed on a tropical population of *Z. muelleri* in an Australian study by Collier et al. (2011) focusing on *Zostera* populations in the tropical north. Substantial decreases in both were observed at temperatures of 33°C (growth rates, for instance, were 0-2% of that observed at a temperature of 27°C). Decreases in growth and biomass increased as temperatures rose but
were more severe when combined with low levels of light. This has implications for future climate change scenarios (a potential outcome also noted by York et al. 2013), with populations of *Z. muelleri* estimated by the authors as possibly contracting as much as 1000km south from the northern Great Barrier Reef. Given *Zostera*’s position in the ecosystem, this may have severe consequences on estuarine habitats and a subsequent economic impact as fisheries are affected.

Photosynthetic rates in this species are impacted by environmental as well as seasonal factors. This is a clear result of *Z. muelleri*’s intertidal position on the shoreline, where it is exposed to sharp variations in temperature, salinity, and insolation. While capable of photosynthetic function at the extremes of each factor – Kerr and Strother (1985) indicate that photosynthesis took place at 15% salinity of normal seawater, and between 3 and 30°C – that same study also indicates decreased function at temperature extremes and as salinity decreases.

Similarly photosynthesis can be affected by chemical contamination resulting from herbicide use. Run-off from surrounding catchment areas during low and moderate river flow events was tested by McMahon et al. (2005). Low river flows in that study caused no observable photosynthetic stress in the seagrass beds, but moderate flows risked exposing the *Z. muelleri* beds to inhibiting concentrations of herbicide. It would be interesting to note if varying levels of herbicidal run-off had different effects on newly germinated *Zostera* seeds instead of healthy adult seagrass.

*In situ* surveys of multi-species seagrass beds, such as that by Ierodiaconou and Laurenson (2002) indicate that when in competition with other seagrass species, *Z. muelleri* flourishes in a high saline, high exposure, medium depth sites. However, field transplant experiments
such as those done by Partridge and Wilson (1988) indicate a limit to the tolerance of Z. *muelleri*. In their fieldwork in an Otago salt marsh, they found that transplanting *Z. muelleri* further up the shoreline than it naturally grew resulted in uniform failure, which suggests that some combination of environmental factors will likely be necessary to ensure not only germination but the survival of newly germinated seagrass. (This upper limit was confirmed by Partridge and Wilson in their following (1989) paper.)

Seed germination can also be affected by seed depth. In their 2007 paper, Bryan *et al.* explored the effect of *Z. muelleri* beds on boundary layers and ocean turbidity. A particularly relevant detail of this research was the conclusion that the presence of waves increased the roughness of the seagrass beds, which has ramifications on the potential for seed dispersal and seed burying.

Water column hypoxia can result in insufficient oxygen transport to plant tissue. This can result in reduced metabolism, protection from phytotoxins, respiration and growth rates. It appears, however, that *Z. muelleri* can counteract this somewhat by modifying rhizospheric conditions in order to ameliorate the effects of hydrogen sulphide in its surrounding sediments (Brodersen *et al.* 2014).

If wave action and turbidity can also be taken as a low-intensity form of disturbance, then restoration experiments focused on *Z. muelleri* recovery after high-intensity disturbance also have implications for the potential efficiency of seed restoration. Macreadie *et al.* (2014) found that *Z. muelleri* recovered quickly after low-intensity shoot loss and that asexual regeneration could eventually recover the beds after high levels of disturbance. However, the study indicated that – at least over the 65 week study period – seeds alone were incapable of recovering the beds after intensive disturbance. The authors concluded
that seed germination was most effective in establishing genetic diversity in existing beds/beds that had recovered through asexual strategies. This seems to indicate that seed-based recovery methods in *Z. muelleri* will be most effective in existing seagrass beds rather than highly disturbed areas where such beds used to exist.

Potentially relevant to this disturbance study is the Jones *et al.* (2008) paper on genetic variability in the New Zealand population of *Z. muelleri*. Genetic differences between populations were clear between the North and South Islands, and between the east and west coasts of each island. Unsurprisingly, intrabed variation was smaller than interbed variation, and there was a genetic correlation to coastal currents. Potential conservation efforts, therefore, will depend on whether or not to increase diversity within a bed, or to restore it to a more original state using local genetic material.

*Zostera genus*

The assumption that having a shared genus means that the species within that genus will have similar characteristics is an uncertain prediction. Not only do the *Zostera* species have very different habitats, but plant size may play a factor in varying productivity and ecology (Duarte 1991). Thus this portion of the literature review is a study in potentials: the wider themes may not cross over, but they might point, in the limitation of the literature on *Z. muelleri*, to a potential path for future research.

The wider ecological potential of the *Zostera* genus has been well documented. Increased *Z. marina* biomass has been correlated with increased fish biomass (Adams 1976) and species-rich ecosystems (Boström and Bonsdorff 1997). Introducing transplants of *Z. japonica* into
new areas has increased faunal diversity in comparison to surrounding, unvegetated habitats (Posey 1988).

Threats to the genus include changing environmental conditions caused by climate change and wasting disease. The latter is correlated with abiotic factors, with decreased light and increased temperature lowering production of the phenolic compounds that help affected species such as *Z. marina* to fight the disease (Vergeer *et al.* 1995). Similar attribution of a multitude of localised causes can be found in den Hartog (1987) though that study is mostly concerned with European populations of the genus. Other observed causes of bed mortality include the eutrophication of coastal waters (den Hartog 1994; Cardoso *et al.* 2004); light reduction caused by that same eutrophication (Koch and Beer 1996); and damage caused by marine animals such as the Cownose ray (Orth 1975).

*Zostera*, like other surveyed species in North Queensland (Lanyon and Marsh 1995) has seasonally dependent growth, with biomass increasing in the wet season and decreasing in the dry. Growth was positively – although not comprehensively – correlated with day length, rainfall, and temperature (*ibid.*). Seasonal variation in growth and/or biomass has also been noted in *Z. marina* by Adams (1976); Thayer *et al.* (1977); Penhale (1977); Aioi (1980); Orth and Moore (1986); Moore and Wetzel (2000); in *Z. japonica* by Harrison (1982b); and in *Z. noltii* by Vermaat *et al.* (1987); Auby and Labourg (1996); Vermaat and Verhagen (1996). In the Mediterranean species *Zostera noltii*, however, life cycle (and thus any concomitant change in biomass) has been linked specifically to temperature instead of season (Buia and Mazzella 1991).

Photosynthetic rates tend to increase with temperature up until an optimum point is reached, at which point both photosynthesis and biomass decline. This optimum in *Z.*
*marina* is variously <29°C in Penhale (1977) and 19°C in Evans *et al.* (1986). Within the boundaries of optimal temperatures, however, growth rates of this species have been observed to be constant at both 10° and 20°C (Zimmerman *et al.* 1989).

Photosynthesis may be relatively unaffected by small to moderate changes in pH levels, although the evidence for this is scant and confined to the study on *Z. noltii* by Invers *et al.* (1997), which showed a significant decrease in rates only at pH 9.

Photosynthetic and growth rates are much more significantly dependent on light, as illustrated by the depth-transplant experiments on *Z. marina* by Dennison and Alberle (1986), where mortality increased as light decreased. They concluded that response to light within a bed is dependent on habitat instead of genetics. Similar results were achieved by Goodman *et al.* (1995), where maximum photosynthesis decreased with reduced light and Zimmerman *et al.* (1995) where transplant survival decreased with depth. In Nielsen *et al.* (2002) the colonisation of *Z. marina* at various depths in a heavily eutrophied environment was studied and as the coastal waters became more transparent, the depth limits increased – presumably due to increased light levels. Other studies that similarly associate *Z. marina* growth with changing light levels are Jacobs (1979); Dennison and Alberle (1982); Kentula and McIntire (1986); Short *et al.* (1995) and Koch and Beer (1996). Orth and Moore (1988) however, caution that localised environmental phenomena such as water clarity can cause considerable variation in depth limits within a species.

Growth rates remain determined by species, however. Harrison (1982a) states that *Z. marina* for instance, is likely to be able to out-compete *Z. japonica* in summer conditions, because the former’s growth rate increases to twice that of the latter’s when temperature and irradiance increases.
While nitrogen fertilisation can stimulate growth, nitrogen limitation in the eelgrass beds appears to be of minimal concern (\textit{Z. marina} in Pedersen and Borum 1993). This result partially conflicts with that of Kenworthy and Fonseca (1992) who stated that nitrogen limitation negatively affected transplants of the same species and recommended nutrient enrichment in order to stimulate the shoot growth of said transplants. It also conflicts with Short (1983) who found that low levels of nitrogen in sediment correlated with higher shoot density and flower abundance for \textit{Z. marina}. Evidence of nitrogen limitation – and the potential benefits of nitrogen enrichment – of that species were also presented in van Lent et al. 1995. Nitrate uptake by roots may also be limited by the presence of high levels of ammonium (Iizumi and Hattori 1982) which could have potential relevance in areas of high fertiliser run-off. Williams and Ruckelshaus (1993) report that fertilisation of \textit{Z. marina} with ammonium increased growth, however, so there is potential for multiple sources of (sometimes competing) enrichment. Yet concentration would appear to be crucial, for \textit{Z. marina} is susceptible to ammonium toxicity, beginning at levels as low as 25μM and exacerbated by temperatures of 20°C (van Katwijk et al. 1997).

Nutrient loading can result in the proliferation of phytoplankton, macroalgal, and epiphytic organisms which shade the seagrass beds, limiting light and therefore growth. Morris et al. (2007) experimented with nutrient loading at three Australian \textit{Z. muelleri} sites and concluded that the beds were sensitive to even low levels of loading. They were not able to determine threshold levels, however. The presence of nutrient loading is of course site-dependant, and may have effects on the validity of long-term restoration plans in the absence of a pollution management plan.
Nutrient loading and the resulting decrease in light has been seen to cause decline in seagrass beds by reducing shoot density rather than growth rate (Z. marina in Hauxwell et al. 2003). Decrease in shoot density as opposed to growth rate indicates potential problems with recruitment and survival of shoots.

Population decline in Z. marina due to algal population increase after nutrient loading has also been observed by Short et al. (1995). Nutrient enrichment was also counteracted by the increased presence of grazers in the Z. marina population studied by Neckles et al. (1993).

Other potential limitations may be phosphorus and carbon, sourced primarily in Z. marina from the water rather than the sediment (Penhale and Thayer 1980).

Bed recovery via transplants have been shown to be fatally compromised by substantial turbidity, irrespective of light levels, as shown in the Z. marina studies of Moore et al. (1997). Low levels of turbidity should be survivable, however, as the shear and roughness of water currents over a seagrass bed increases with bed area (Fonseca et al. 1982) and this is clearly no obstacle to the continuing global presence of the seagrasses. Indeed, Z. marina beds have been shown capable of withstanding currents of 120-150cm/s (Fonseca et al. 1983). As an underwater pollinator, currents can be crucial to the reproduction of this species (Ackerman 1986) but localised effects of currents at particular research sites need to be studied to determine how greatly they influence sexual reproduction at those sites. A genetic examination of three North Sea populations of Z. marina, for instance, indicates that currents have played an effective role in exchanging genetic material (Reusch et al. 2000).
A study of note here might be Olesen and Sand-Jensen (1994) who determined that mortality of fragmented *Z. marina* beds strongly declined with fragment or patch age and size. This may have consequences for conservation decisions, with older beds with larger patches yielding potentially greater results for minimal investment.

Sexual reproduction has been shown to be affected by predation. Fishman and Orth (1996) assessed a potential loss to predation of up to 65% of *Z. marina* seeds, materially affecting the (sexual) reproductive capability of seagrass beds. Seed production in this species appears to take place in late winter and spring along latitudinal gradients (Silberhorn *et al.* 1983; Phillips *et al.* 1983a).

Seed presence within the sediment of seagrass beds has been shown to decrease with depth (*Z. noltii*); however seed presence did not translate to viability as the *Z. marina* populations on the same site had a 50% germination rate and a 13% seedling survival rate (Harrison 1993). Seedling mortality, Harrison concluded, was dependent on elevation rather than density. In other studies, however, mortality appears season-dependent (Churchill 1983) although this is likely to be more a function of low temperature or light, given that none of the Churchill seedlings, growing in New York, survived the winter/spring to the summer.

Seed germination in *Z. marina* is correlated with low temperature – observed when temperature decreased to 15°C (Moore *et al.* 1993); when temperature increased to between 0-10°C in spring conditions (Orth and Moore 1983). A different result was reported by Hootsmans *et al.* (1987): it was observed that maximum germination took place in both *Z. marina* and *Z. noltii* at 30°C and 1% salinity, with germination decreasing with lower temperatures and higher salinities. This is a substantial difference. The seedlings of this
study survived best – in both species – at 10°C, however, which seems to be more in line with other studies.

There appears to be no correlation between germination and salinity in the study of *Z. marina* by Orth and Moore (1983), although Phillips et al. (1983b) show a strong correlation between germination and salinity in the same species (and no correlation with temperature!). It has been observed, however, that seedlings from different *Zostera* species survive better at different salinities (Hootsmans *et al.* 1987). It may be possible, in the absence of experimental error, that location and/or genetic variation within the species is responsible for these conflicting results.

Seed germination also appears to be delayed by the presence of oxygen (Moore *et al.* 1993), while vegetative reproduction and biomass in *Z. marina* is increased by carbon dioxide enrichment (Palacios and Zimmerman 2007). This last may be particularly useful when it come to restoring seagrass in a climate changing world.

*Final note:*

Because seagrass provides ecosystem services to the wider coastal ecology, it can be useful – especially from a financial perspective – to leverage knowledge of these existing services into future healthy ecosystem models. Bearlin *et al.* (1999), for instance, modelled future *Z. muelleri* populations as key indicators of coastal health. If indicators like this are accepted at a state or government level, long-term funding for coastal management will necessarily include funding for seagrass monitoring. The authors are perhaps less strident in this
Ecosystem restoration is a dynamic process (Yap 2000). Moberg and Rönnbäck (2003) make a similar point in that species restoration does not occur in a vacuum and that large-scale processes such as interactions between major vegetation types are not always adequately considered. Establishment – or re-establishment – of declining seagrass beds will have effects on the surrounding environment that are not wholly predictable and an ongoing, flexible management plan is recommended. Yap stresses that for this management plan to be successful, local communities must be active participants. This is a reasonable assessment, especially considering the Finn et al. (2010) study concluding that seagrass data collected by trained community volunteers was highly correlated with the accuracy of that obtained by research scientists. Furthermore, locals are more likely to be earlier aware of environmental changes than regional or state services and their information gathering and communication is a vital part of monitoring any conservation programme. Broadening the research base by including more stakeholders can also provide unexpected benefits. On a macro-level, establishing an international forum such as the European Seagrass Restoration Workshop allowed multiple stakeholders a chance to informally review and compare their various reports, upon which it became clear that an unintentional bias towards short-monitoring periods of less than a year was present, according to Cunha et al. (2012).

Although wider studies of community engagement are not always frequent, those that do exist – like Yap and Cunha (op. cit.) – appear to uniformly encourage engaging with a wide variety of restoration-interested parties.
CHAPTER ONE:

SEED BANKS AND THE POTENTIAL FOR TRANSPLANTION IN THE RAGLAN ZOSTERA BEDS

Introduction

As a nursery species that promotes biodiversity (Hayward et al. 1981; Battley et al. 2011; Leduc and Probert 2011), *Zostera muelleri* is an important indicator of the health of coastal ecosystems (Turner and Schwarz 2006) and its national decline (Matheson et al. 2011; de Lange et al. 2012) puts these ecosystems at risk. While conservation strategies can include both restoration of existing beds and the establishment of new ones, Irving et al. (2011) argues that the priority of ameliorating potential climate change effects means that expensive projects to establish new beds should be decreased in favour of the preservation of existing habitats.

If the existing *Z. muelleri* beds in Raglan harbour are to be conserved, their ability to respond to disturbance and shifting environmental factors is crucial. The presence of a seed bank, indicating the potential for sexual reproduction, could therefore prove advantageous. Within the genus, recolonisation of a completely destroyed *Zostera marina* bed occurred quickly and thoroughly due partly to a high-quality seed bank (Plus et al. 2003). However, if the anoxic crisis that prompted the original mortality were to reoccur then the authors state that a second recolonisation would take considerably longer due to the depleted state of the seed bank. This indicates that continual disruptive events have an increasingly high cost to remedy, encouraging environmental disaster prevention ahead of cure. Within *Z. muelleri* beds, however, seed banks have proved a greater aid to genetic diversity than recovery.
disturbance (Macreadie et al. 2014) although again, the effects of disturbance on a seed bank will affect the ability of the bank to increase genetic diversity.

Thus, if present, the ability of a seed bank to withstand both single and multiple disturbance effects needs to be assessed. If absent, alternate means of increasing genetic diversity within the existing meadows may need to be explored.

Z. muelleri production of seeds has been described as “rare or episodic” in New Zealand environments (Matheson et al. 2009). Flowering occurs over the summer months, and seeds have been observed over-wintering and germinating in spring, although at very low levels (Ramage and Schiel 1998). Jarvis et al. (2015) reports over 60% of seeds were located a depth >2cm at their Port Curtis study site, and that over 50% of seeds found were located at a 5-10cm depth.

The presence of a seed bank in Raglan harbour has yet to be determined. The objective of this experiment is to establish if such a seed bank exists at this location, as the answer will necessarily impact any conservation plans developed in the future.

Method

Sediment samples from all four study sites were taken in autumn, winter and spring. Sample sizes were n=20 for each season at sites A, D, G and n=40 at site C. Sampling methods depended on the particular characteristics of the site.

Core samples (10cm depth, 3cm width) were taken at sites A and C.
At Site A, samples were taken along every metre of a 10m low tide horizontal transect. Samples were also taken along every metre of a 10m mid tide transect. The few observable seagrass plants observed at this site (summer only) were at low tide and the relevant transect went through their former position, although no trace of them was visible in the sampled seasons.

The extensive seagrass population at all tidal levels at Site C was the cause of the higher sample numbers at this site. Samples were taken along every metre of horizontal transects of 10m each (two transects at high tide, two transects at low tide).

Sites D and G had only a thin layer of sediment over a hard rocky layer and so adequate core samples could not be taken. Sediment samples were scraped instead.

At site D, samples were taken every metre along three vertical transects. One transect of eight metres was taken along the centre line of the seagrass patch, and the two remaining transect lines were taken along the edges of the seagrass patch. All samples were taken in the mid-low tide region, as this was where the vertical distribution of the seagrass was most abundant.

At site G, samples were taken every metre along a low tide horizontal transect, as the steep bank of the site severely limited the width of the intertidal zone. This transect went through the existing seagrass patch.

At point of collection for all samples, large articles (for example shellfish, crabs, rocks) within each sample were manually discarded.
Within a laboratory environment, samples were sieved by a PsS laboratory test sieves of 2mm and then 250 microns. The filtered samples were then weighed and examined under a microscope for *Z. muelleri* seeds.

**Results**

Table 1 records the mean filtered sample weight and seed count of the collected sediment samples. The striking result is the total absence of seeds, indicating the lack of viable seed banks (or indeed any seed banks) within any of the sampled sites.

**Table 1: Seasonal Sediment Mean Filtered Sample Weights and Seeds**

<table>
<thead>
<tr>
<th>Season</th>
<th>Sample Site</th>
<th>Sample Size</th>
<th>Mean Filtered Weight (g)</th>
<th>Seed Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autumn</td>
<td>A</td>
<td>20</td>
<td>11.98</td>
<td>0</td>
</tr>
<tr>
<td>Autumn</td>
<td>C</td>
<td>40</td>
<td>15.36</td>
<td>0</td>
</tr>
<tr>
<td>Autumn</td>
<td>D</td>
<td>20</td>
<td>24.11</td>
<td>0</td>
</tr>
<tr>
<td>Autumn</td>
<td>G</td>
<td>20</td>
<td>33.62</td>
<td>0</td>
</tr>
<tr>
<td>Winter</td>
<td>A</td>
<td>20</td>
<td>42.24</td>
<td>0</td>
</tr>
<tr>
<td>Winter</td>
<td>C</td>
<td>40</td>
<td>32.33</td>
<td>0</td>
</tr>
<tr>
<td>Winter</td>
<td>D</td>
<td>20</td>
<td>21.84</td>
<td>0</td>
</tr>
<tr>
<td>Winter</td>
<td>G</td>
<td>20</td>
<td>18.74</td>
<td>0</td>
</tr>
<tr>
<td>Spring</td>
<td>A</td>
<td>20</td>
<td>26.60</td>
<td>0</td>
</tr>
</tbody>
</table>
Discussion

The absence of a seed bank in Raglan harbour has consequences for any potential conservation plan for the seagrass populations in that harbour.

It indicates that *Z. muelleri* reproduction at Raglan is primarily vegetative. The possibility of low numbers of viable seeds existing at this location remains, but they are unlikely to be large contributors to the population.

Consequently, the introduction of genetic diversity resulting from sexual reproduction is likely to be low. This effect can be ameliorated somewhat by the introduction of genetic material via fragments (see Chapter Two), and there is some indication that genetic mixing within the local beds has taken place (Jones 2008), possibly via this mechanism.

Genetic variation can also be introduced into the Raglan *Zostera* beds through seed or seedling transplantation. *Z. muelleri* seeds might be introduced into the Raglan environment from other New Zealand sites, for instance, but for several reasons this may not be advisable. Firstly, the national scarcity of *Z. muelleri* seeds makes sourcing these seeds problematic, and the removal of any located seeds may impact heavily on the meadow ecology of the source environment. Secondly, seed germination does not imply seedling viability – germination of *Z. muelleri* seeds was observed on the Kaikoura peninsula (Ramage

<table>
<thead>
<tr>
<th>Spring</th>
<th>C</th>
<th>40</th>
<th>21.48</th>
<th>0</th>
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<tbody>
<tr>
<td>Spring</td>
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<tr>
<td>Spring</td>
<td>G</td>
<td>20</td>
<td>13.54</td>
<td>0</td>
</tr>
</tbody>
</table>
and Schiel 1998) but the resulting seedlings did not survive subsequent wave disturbance (Ramage and Schiel 1999).

This lack of efficiency has been reflected in other conservation efforts involving Zostera seed transplants. Natural restoration has proved to be limited in Z. marina beds where physical and biological factors (narrow inlet mouths and limited seed availability such as at Raglan) have prevented the propagation of seeds between beds. In one such case however, Orth et al. (2006) were able to intervene by harvesting over a period of years 24 million seeds and transplanting them into areas where the seagrass beds were declining. This restoration effort proved successful, even though only 5-10% of the transplanted seeds successfully germinated. If there were a means of triggering germination in a greater proportion of the seeds, then the restoration programme could be conducted more efficiently. As it is, the collection and transfer of up to 21.6 million seeds had no result. Some efficiency was gained through the use of low-impact mechanical harvesters, although mechanical planters did not increase seedling germination (Marion and Orth, 2010b). In a New Zealand environment, however, where millions of seeds are simply unavailable, a similar multi-year effort would be both expensive and likely futile.

Seed predation is concluded to be a significant factor in the non-establishment of seedlings (Marion and Orth, 2010a) but it should be noted that in this study, set over two study sites, seed burial had mixed success. Harwell and Orth (1999) had more significant success in protecting Z. marina seeds from predation by using burlap bags. This technique improved survival by a factor of three, and might be a useful technique for Z. muelleri if an adequate seed source is discovered. Predation levels would have to be established first, however, for that seagrass and under local conditions, before the utility of bags could be established.
More efficient results have been observed when transplanting seedlings (as opposed to seeding sites and hoping for germination) but these have not always been effective in the intertidal zone (Davis and Short, 1997).

This success of transplanted seedlings may be relevant in that, if germination proves insufficient at a particular site due to predation or other influences, laboratory germination may be required. If germination rates can be improved via a thorough understanding of germination triggers for that species, eventual transplant of the subsequent seedlings may be a more efficient means of restoration. Relying on vegetative growth instead of sexual reproduction may be counterproductive, as Reynolds et al. (2012) indicate that, in their studied populations of Z. marina, genetic diversity was highly correlated with increased capacity of the seagrass to provide ecosystem services.

Alternately, in the continued absence of a viable seed source – as seems likely, in the New Zealand context – it may be more effective to substitute the transplanting of seeds with that of whole plants.

Moore and Short (2006) encourages four main steps in the restorative process: site selection, with an understanding of local environmental conditions that may affect the seagrass – such as the suitability assessment demonstrated by Zimmerman et al., (1991); the identification of source (planting) stock; identification of the most reliable method of planting; and a monitoring programme designed to identify if a successful outcome has been achieved. This approach is more focused on whole plant transplant than germination, but the final stage – appropriate for both germination and transplant approaches – will necessarily alter according to the intentions of the actors.
There is often little differentiation from researchers concerning natural and artificial recovery (Thorhaug 1986). While this has changed in the decades since publication (current papers frequently reference both laboratory and in situ rates of recovery) this is representative of a wider issue where questions on the value of an artificial versus a natural ecosystem (should it be restored to the original state or a more resilient state, for example) are ongoing. This debate, illustrated in papers such as Fonseca (2011) is especially crucial in areas where restoration is actually impossible. An example of this can be seen in the former Z. marina beds of the Wadden Sea described in van der Heide et al. (2007) where the collapse of the seagrass eco-system led to a change in turbidity such that recovery can no longer take place. In such cases, conservation biologists must decide if no action is preferable to an action which increases biodiversity without restoring an original state. This is an issue which does not as yet apply to the Z. muelleri Raglan beds (although the extremely fragmentary state of Site A may draw parallels) but it is a debate which any ongoing conservation plan should consider. If the Raglan meadows decline at the rate of the national prediction (Matheson et al. 2011), then the tension between originality and resilience will have to be addressed.

Conclusion

The lack of a discernible Z. muelleri seed bank in Raglan harbour indicates that future conservation plans may need to include a mechanism for increasing genetic diversity at that site. While this could happen naturally via fragments, the manual transplantation of seeds or whole plants is also possible. Given the lack of adequate seed sources in New Zealand, seed transplantation is unlikely to be a viable or efficient alternative. Transplants of
seedlings or mature plants may provide increased diversity, but this will also alter the “natural” state of the Raglan meadows, and this should be taken into account in future restoration plans.
CHAPTER TWO

VIABILITY AND RESILIENCE OF ZOSTERA MUELLERI VEGETATIVE FRAGMENTS OVER MULTIPLE TIME PERIODS

Introduction

The reproductive strategies of seagrass are both vegetative and sexual (Vermaat and Verhagen 1996; Turner and Schwarz 2006; Matheson et al. 2009) and contribute to the long-term stability of seagrass beds (Turner and Schwarz 2006; Macreadie et al. 2014). In the absence of observable seed banks, vegetative reproduction is critical in not only maintaining existing beds, but in creating and establishing new ones.

One such vegetative strategy is that of the seagrass fragment, which can be dispersed via oceanic currents. The viability of the fragment – how long it can survive while drifting in the current – and the ability of the fragment to remain viable when it reaches an appropriate environment is critical (Weatherall et al. 2016) as it may result in genetic transfer between beds in the absence of sexual reproduction. *Zostera muelleri* fragments have a relatively long period of buoyancy once dislodged from the substrate. For example, in sub-tropical Australian conditions, *Z. muelleri* fragments have an average floatation time of 21 days (Weatherall et al. 2016). This draft period may be dependent upon temperature, which as it increases also increases settling velocity (Weatherall et al. 2016).

As environmental factors such as temperature may affect dispersal, so too might the state of the source material (Hall et al. 2006). Seasonal variation in biomass of *Zostera spp.* – a variation that is frequently exhibited in winter dieback followed by a subsequent spring/summer increase in plant matter – has been observed in the United States of

This variation has been observed in above-ground shoot density (Harrison 1993; Vermaat and Verhagen 1996), biomass (Penhale 1977), leaf detritus (Thayer et al. 1977), leaf area (Aioi 1980) and leaf growth (Vermaat et al. 1987; Kerr and Strother 1989); in both above- and below-ground biomass (Aioi 1980; Aubry and Labourg 1996; Jacobs 1979; Kerr and Strother 1990; Lanyon and Marsh 1995; McKenzie 1994; Orth and Moore 1986).

This seasonal variation has implications for both the growth and viability of seagrass fragments. Fragments dispersed during summer, for example, may have detached from plants exhibiting higher growth rates or increased productivity and robustness. Those fragments dislodged from a population undergoing winter dieback, however, may have a parent plant entering senescence and thus may be less likely to both survive prolonged floatation, or be able to effectively recruit upon settlement.

A benefit to fragment creation in winter is that winter die-back typically results in a decrease in shoot density resulting in an increase in potential fragment recruitment sites. The increased shoot density of the summer months may inhibit fragment ability recruit and establish.

Also, if growth is inhibited by temperature and insolation, for example, then seagrass fragments attaching over the autumn or winter months might be expected to have a
decreased growth rate in comparison with fragments that have the opportunity to attach and become viable during the spring or summer months. Alternately, spring or summer recruitment may more quickly exhaust the limited carbohydrate stores of the fragment rhizome.

As seeds have not been detected at the study sites (see Chapter One), the meadows are unlikely to be maintained by sexual reproduction. It is therefore reasonable to assess the ability of *Z. muelleri* fragments as an alternative mechanism for maintaining existing beds or creating new ones. This experiment aims to determine if the duration of time the fragment drifts affects the potential recruitment ability of the fragment. As a secondary aim, this experiment hopes to determine if there is a difference in fragment potential depending on the season of detachment.

**Method**

Monthly mean sea surface temperature for Raglan has been taken from the Surf Forecast website, using records dating from 1984. These are shown in Table 1, and indicated a relatively minimal range of temperature change (5°C, from minimum to maximum).

<table>
<thead>
<tr>
<th></th>
<th>Jan</th>
<th>Feb</th>
<th>March</th>
<th>April</th>
<th>May</th>
<th>June</th>
<th>July</th>
<th>Aug</th>
<th>Sept</th>
<th>Oct</th>
<th>Nov</th>
<th>Dec</th>
</tr>
</thead>
<tbody>
<tr>
<td>°C</td>
<td>19</td>
<td>20</td>
<td>19</td>
<td>18</td>
<td>17</td>
<td>16</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>16</td>
<td>17</td>
</tr>
</tbody>
</table>

Fragments were collected from an intertidal study site C, at Raglan (see thesis Introduction), New Zealand, in austral autumn (April – mean temperature 14.6°C Raglan, 14.9°C Hamilton,
Seagrass fragments were obtained by being uprooted with a trowel and collected. Excess sediment was gently removed via washing with seawater, before the fragments were placed in buckets containing seawater gathered from the site and transported back to the research laboratory at the University of Waikato, in Hamilton. The time before collection and arrival at the laboratory averaged 180 minutes.

For the experimental work, fragment length was standardised to rhizomes with three shoots only. Rhizome length for 100 randomly selected fragments was recorded for each season (average autumn length = 4.82 cm ± 0.19 cm) and these fragments were randomly assigned to one of five different treatments (20 fragments per aquaria).

Experiments occurred in outdoor aquaria (55 L) containing a 5cm deep layer of washed silica sand as the substrate. Each aquarium was filled with manufactured saltwater (34-36 ppt) and left for two days for the substrate to settle before fragments were added (Figure 1). The aquaria remained in ambient temperature conditions, which were be similar to the intertidal environments the seagrasses would face in their normal coastal setting.

Five experimental treatments were used. Treatment 1 acted as a control and all fragments (n=20) were planted into the aquaria at the start of the experimental period (see Figure 2). Treatments 1-4 had fragments floating for defined periods of time (day 7, 14, 21, and 28 respectively) with fragments subsequently planted. During the experimental period evaporation was compensated for by adding fresh water to the aquaria.

The experimental duration for each planting was 6 weeks. At experiment end, fragments were gently uprooted from the sediment, with rhizome length and number of shoots...
recorded. For the purpose of this study, shoot growth was achieved if at the end of the experiment there were more than 3 shoots present per rhizome length.

This experiment was repeated in austral spring (September – mean temperature 11.5°C Raglan, 12.7°C Hamilton), with another 100 fragments collected from the same site. The average spring rhizome length = 4.73 cm ± 0.16 cm). There is no statistically significant difference between the initial mean rhizome lengths of autumn and spring rhizomes (F(1,198) = 0.117, p = 0.73), which is to be expected as both samples were standardised to a rhizome length containing 3 shoots. Similarly, there is no statistically significant difference between the autumn initial rhizome lengths of each treatment (F(4,95) = 0.180, p = 0.95) or between the spring initial rhizome lengths of each treatment (F(4,95) = 0.201, p = 0.94).

Data was analysed using a one-way ANOVA, with a p-level of 0.05.
Figure 1. Experimental Aquaria Set-up.

Figure 2. Planted *Zostera muelleri* Fragments.
Results

Tables 2 and 3 summarise the number of fragments still floating before planting for each treatment (T0-4). Notable is the fact that initial sinking could be counter-acted, and that fragments could rise to the surface over time.

Table 2: Number of floating autumn fragments before planting (n = 20 / Treatment)

<table>
<thead>
<tr>
<th></th>
<th>0 Days</th>
<th>7 Days</th>
<th>14 Days</th>
<th>21 Days</th>
<th>28 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment 0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Treatment 1</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Treatment 2</td>
<td>6</td>
<td>6</td>
<td>17</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Treatment 3</td>
<td>11</td>
<td>11</td>
<td>17</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Treatment 4</td>
<td>9</td>
<td>7</td>
<td>17</td>
<td>17</td>
<td>20</td>
</tr>
</tbody>
</table>

Table 3: Number of floating spring fragments before planting (n = 20 / Treatment)

<table>
<thead>
<tr>
<th></th>
<th>0 Days</th>
<th>7 Days</th>
<th>14 Days</th>
<th>21 Days</th>
<th>28 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment 0</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
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<td>3</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Treatment 2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Treatment 3</td>
<td>6</td>
<td>2</td>
<td>3</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>Treatment 4</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

The mean rhizome length of the final autumn and spring fragments, after treatments T0, T1, T2, T3, and T4 are shown in Figure 3. There is no statistically significant difference between the post-treatment autumn rhizome lengths (F(4,95) = 0.184, p = 0.95). Nor is there a statistically significant different between post-treatment spring rhizome lengths (F(4,95) = 0.965, p = 0.43).
Figure 3: The Final Mean Rhizome Length for Treatments T0-T4

Figure 4: Number of Fragments Exhibiting Shoot Growth in Treatments T0-T4.
Comparing the autumn and spring rhizome lengths for each treatment, there is no statistically significant difference between any of the treatments: T0 (F(1,38) = 0.123, p = 0.73); T1 (F(1,38) = 0.305, p = 0.58); T2 (F(1,38) = 0.105, p = 0.75); T3 (F(1,38) = 1.039, p = 0.31); T4 (F(1,38) = 2.635, p = 0.11).

The number of fragments from all autumn and spring treatments exhibiting shoot growth is shown in Figure 4. There is no significant statistical difference within the autumn treatments (F(4,95) = 0.885, p = 0.48) or within the spring treatments (F(4,95) = 0.386, p = 0.82).

There is also no significant statistical difference between the respective autumn and spring treatments: T0 (F(1,38) = 0.422, p = 0.52); T1 (F(1,38) = 0.369, p =0.55); T2 (F(1,38) = 0.877, p = 0.35); T3 (F(1,38) = 1.900, p = 0.18); T4 (F(1,38) = 0, p = 1).

Discussion

The aims of this research were to determine if the duration of time a fragment drifted had an effect on its subsequent potential for recruitment; also to determine if there were a difference between autumn and spring detachments. It was determined that neither the duration of drift nor the detachment season had a statistically significant effect. This suggests both that fragments remain capable of growth after 28 days drifting (7 days more than that reported by Weatherall et al. 2006), and that light levels were adequate to sustain photosynthesis – as indicated by the refloating fragments: this is likely a result of photosynthetic rate exceeding respiration, resulting in air bubble which cause the fragments to float.
It should be noted that in this experiment, the *Z. muelleri* fragments were, after planting, existing in what is essentially a consistently subtidal environment, despite it being a largely intertidal species. The fragments were not exposed to the potential stressor of desiccation, for instance, but neither were they exposed directly to light once planted as they might be in, for instance, a high tide position. Photosynthetic ability in *Z. muelleri* is however decreased by temperature extremes and low salinity (Kerr and Strother 1985), so a subtidal environment may increase photosynthetic rates. In assessing *Z. muelleri* reaction to extreme temperature York et al. (2013) established that at an upper temperature limit of 32°C tolerance sharply declined; this is not a temperature typical of Raglan spring or autumn seasons, and so high temperature is unlikely to be a growth or photosynthetic limiting factor.

A lack of a pattern between the spring and autumn fragment shoot growth was evident in this study. I note however, that this experiment is not extensive enough to determine seasonal changes, for which an experiment length covering >3 years is more appropriate. This initial study suggests that fragment shoot growth appears unaffected by the season of collection.

The observed patterns may also be a result of the collection site. The Raglan seagrass bed in which the fragments were collected exhibited little winter die-back (personal observations). It is noted that winter die-back has been observed in other Raglan sites, where the seagrass occurs mainly in small patches, but the meadows of the fragment collection site C are more extensive.

Responsibility for the winter minima has been associated with temperature and rainfall (Lanyon and Marsh 1995) but the primary causative factor appears to be daylight hours and
insolation (Aioi 1980; Jacobs 1979; Clough and Attiwill 1980; Kerr and Strother 1989). This is supported by Dennison and Alberte (1982), who noted that leaf production rates decreased with decreased light levels. Similarly the 1986 vertical transplant experiment by the same authors indicated that seagrass growth is substantially light-limited, a finding supported by the San Francisco transplants described in Zimmerman et al. (1995). Associated with this is photosynthetic ability, which has been shown in Zostera spp. to both be dependent on daily light levels (Goodman et al. 1995), and which increases (subject to an upper limit) at higher temperatures (Evans et al. 1986). This indicates that, conversely, photosynthetic ability can decrease at lower (winter) temperatures, and it is plausible that this is the case with fragments as well as whole plants.

Response to light is strongly species dependant, however, and is frequently linked to other environmental conditions such as shore position (Harrison 1982a), temperature (Collier et al. 2011), water transparency (Nielsen et al. 2002; Orth and Moore 1988), eutrophication (Short et al. 1995) and carbon dioxide (Palacios and Zimmerman 2007), ammonium (van Katwijk et al. 1997) and nitrogen (van Lent et al. 1995) concentrations.

A lack of a substantial winter minima at the collection site indicates that changes in light and temperature may not be stressors for the seagrass at that site, and indeed the monthly variation in sea surface temperature is, as shown above, minimal. Similarly, the lack of a statistically significant difference between autumn and spring rhizome lengths and shoot growth indicates that floating is not a stressor for the fragments.

To more fully determine any seasonal effects on Raglan seagrass fragments, this experiment could be repeated each year through to 2018. Similarly, a more controlled environment could be used to examine temperatures, light effects in order to investigate the effects of
individual environmental variables on *Z. muelleri* fragments. The presence of seagrass fragments capable of growing shoots in the Raglan area may potentially increase the stability of the beds if the source of the fragments is sufficiently genetically distinct, even though their own growth, once established, would also be vegetative. This is especially the case as the distance that an individual fragment is transported may also contribute to dispersal across large distances (Reusch et al. 2000) and between local populations (Hall et al. 2006).

It has been observed in *Z. marina* that individual populations may have developed genotypes adapted to the temperature of their location (Phillips et al. 1983a). This selective adaptation, if apparent in *Z. muelleri* as well as *Z. marina*, may limit the potential of the fragment to adequately adapt to an environment significantly different to that in which it originated. Genotypic analysis has shown that sexual reproduction may mitigate this differentiation in North Sea colonies that are relatively close to each other (Reusch et al. 2000), but this is a reproductive strategy that relies upon both the existence of a viable seed bed and successful transport of those seeds between colonies. As there is little potential for sexual reproduction at Raglan, potential source locations of fragments may need to share significant similarities with the Raglan environment to be successfully recruited there.

Genetic analysis of *Z. muelleri* beds in New Zealand indicates a low level of genetic transfer between sites (Jones et al. 2008). Given this, it is unsurprising that genetic similarity is greatest within each site, although some sites, such as that at Raglan, exhibit genotypic mixing within the local estuary beds (*ibid.*). The lack of established seed banks (see Chapter One) would appear to indicate, however, that this mixing may not be the result of sexual reproductive strategy. Vegetative growth or fragment colonisation is therefore likely to be
responsible. The extent of this is unknown, however, and is dependent on successful recruitment of fragments, which is itself dependant on a range of factors such as site availability and competition.

Competition for attachment sites is not limited to that of the fragment’s own species. Ocean currents may transport fragments to sites other than that in which they originated, and at these new sites fragments may have to compete with different – often non-seagrass – species. This is particularly relevant in regions where existing seagrass beds are already being out-competed by other organisms. Den Hartog (1994), for example, describes the destruction of a mixed *Z. marina* and *Z. noltii* bed in the United Kingdom by the blanketing incursions of the chlorophyte *Enteromorpha radiatia*. Cardoso *et al.* (2004) note that the increasing eutrophication of coastal ecosystems can result in the replacement of seagrass beds by macroalgal blooms, a result which is supported by Hauxwell *et al.* (2003). If increased eutrophication from run-off encourages the establishment of competing non-seagrass species in Raglan, the ability of *Z. muelleri* fragments to re-attach may be significantly compromised.

Other factors than competition may also affect the ability of fragments to attach and thrive. Harrison (1982b) notes that seedlings from *Zostera japonica* opportunistically colonise a Canadian site on a yearly basis, but that the success of seedling establishment is strongly weather-dependant. It is possible that this is can be used as a partial analogue for seagrass fragments, which may be less likely to successfully root and colonise if weather is particularly damaging. Tidal position can also affect growth rate of whole plants, with higher tidal position typically resulting in lower growth rates (Kentula and McIntire 1986) due to added environmental stressors, and it is not unreasonable to infer that the attaching
position of any fragment along the vertical shoreline may have similar effects on the survival of that fragment. Increased turbidity has also been shown to negatively affect the viability of seedling transplants (Moore et al. 1997). Patch size of seagrass beds is also a factor, with patch mortality in Z. marina sharply increasing as patch size decreased (Olesen and Sand-Jensen 1994), which may have a substantial effect on the long-term survival of any fragments deposited in thinly colonised environments.

These environments may be thinly colonised due to the absence of existing seagrass beds, the decline due to environmental factors present in the existing beds, or there may be patches of sparse shoot density in an otherwise stable meadow due to disruption events such as those resulting from large herbivores. The potential increase in large herbivores (specifically Canada geese, see Chapter Three) may also compromise the ability of fragments to attach and become grow.

A suggested next step for this research would be hydrodynamic modelling, in order to determine possible dispersal paths for fragments, with Raglan both as a source and destination. Genetic comparison between source and destination beds could then be undertaken in order to confirm the modelled findings.

Conclusion

A relatively substantial proportion (4-8 of n=20 fragments/treatment, or 20-40%) of Z. muelleri fragments are able to produce new shoots after floating for as much as a month before reattachment. This indicates that floating is not a sufficient stressor to prevent shoot growth, and may explain the documented genotypic diversity in the Raglan seagrass beds.
The shoot growth of autumn fragments appears to be statistically similar to that of spring fragments. However there is not yet enough evidence to say that there is no seasonal effect such as that experienced by Hall et al. (2006); this result is as yet only indicative of a pattern and may be explained by the Raglan environmental conditions and the subsequent state of the local seagrass beds.

However, the ability of fragments to survive within the Raglan environment – as opposed to the experimental aquaria – may be subject to change. Stressors such as changing environmental conditions resulting from climate change, eutrophication, and herbivory by large avian creatures may substantially reduce fragment survival and growth. Any subsequent conservation plan for the seagrass in the region will have to take into account these risk factors, or determine an alternate means of introducing genotypic diversity within the local beds.
CHAPTER THREE:  
RESPONSE TO GRAZING-TYPE SMALL SCALE DISTURBANCE IN ZOSTERA MUELLERI  
SEAGRASS BEDS, RAGLAN.

Introduction

Seagrass populations are subject to a number of disturbance factors, one of which is grazing by large avian herbivores. Grazing can alter the productivity of seagrass, interrupt the detritus cycle, and affect the digestibility and nutrient contents of the plants (Thayer et al. 1984). This grazing can be constant or seasonal, affected by migration (Ganter 2000; Seymour et al. 2002) and climate (Clausen et al. 2012), and the interaction between birds and beds can have long-term implications for the conservation of those beds. Disturbance of this sort may even damage seagrass meadows beyond immediate recovery (Short et al. 2006), and the growing pressure of bird populations on ever more limited beds means that the ability of seagrass to recover from grazing disturbance is crucial to the continuing survival of both the seagrass and the birds.

Brent geese (Branta bernicla) for example, rely heavily on seagrass as a food source, and the decreasing availability of seagrass due to climate change, pollution, and other environmental factors has had significant effect on their body mass (Clausen et al. 2012) and population abundance at feeding grounds during migrations (Seymour et al. 2002). The declining global population of seagrass therefore affects more than the seagrass itself; the potential impact of grazers on seagrass beds is mirrored by the impacts of decreasing seagrass on the grazers. Subsequent conservation plans, in regions where seagrass beds are
a food source for large herbivores such as geese, will need to reflect this dual relationship. That relationship is governed by a number of factors.

Feeding behaviour varies between species, but Jacobs et al. (1981) observed five different strategies by avian herbivores: pecking at the above-ground vegetation; the removal of whole plants via trampling; grubbing with beaks for whole plants; digging for rhizomes; swimming above the beds and tearing up leaves or whole plants. Feeding strategy therefore has subsequent consequences for reproduction. A feeding strategy that prioritised digging for rhizomes and the consumption of meristematic tissue, for instance, would potentially affect the potential for vegetative reproduction more than a strategy that prioritised pecking at above-ground vegetation. Birds may also use more than one strategy, leading to different rates of decline in above and below ground vegetation – for instance brent geese who preferentially target seagrass shoots but will graze on rhizomes when shoots have been reduced (Madsen 1988). Furthermore seagrass meadows may be grazed by different species using different strategies at one time, such as at the Exe estuary in England where brent geese and wigeons (Anas penelope) forage differently, the geese tending to eat whole plants while the wigeons targeted floating, above-ground vegetation (Fox 1996).

The intensity and duration of the grazing period is also a factor in the ability of seagrass to recover from grazing disruption. Continuous grazing by birds has been observed to cause long-term destruction of beds, as in the Dutch Wadden Sea. Jacobs et al. (1981) observed that brent geese, wigeon, pintail (Anas acuta) and mallard (Anas platyrhynchos) when grazing on Terschilling Island from September-December 1974, resulted in the above-ground vegetation almost totally disappearing.
Frequently seagrass beds are able to recover from short intense seasonal grazing patterns, for instance those of migrating geese (Ganter 2000). This is not always the case, however. *Zostera marina* beds on Fishing Island (Portsmouth Harbour, USA) were grazed upon by a flock of nearly 100 Canada geese (*Branta canadensis*) over-wintering from January-April 2003. No such grazing had been observed for a period of 20 years prior to this – it is thought that an unusually cold winter contributed to the change in grazing sites – so the subsequent disruption on the Fishing Island beds can be described as both sudden and significant. Biomass loss was recorded as reaching 680 g m\(^{-2}\) and cover loss from 98% to <1% was observed along one transect. The beds were monitored until July 2003, and did not recover via vegetative or sexual strategies during this time (Rivers and Short 2007). Furthermore, over a three year monitoring period, density and percent cover showed significant and continued decline post-grazing (Short *et al.* 2006).

Similarly, long-term grazing by black swans (*Cygnus atratus*) on *Zostera muelleri* beds at Tauranga Harbour, New Zealand, suggests that high-intensity grazing by the swans – up to 20% annual removal of average seagrass biomass – has consequences on the subsequent *Z. muelleri* growing season. Plant biomass declined up to 69% in the following season, indicating that the meadows are unable to fully recover from the presence of the swans. Particularly relevant is the feeding strategy of *C. atratus*: 99% of rhizomes were removed, along with 92% of shoots and 25% of roots (Dos Santos *et al.* 2012). This can only have a substantial effect on vegetative regrowth within the meadows.

The Dos Santos *et al.* study, however, states that *C. atratus* is the only large intertidal grazer of *Z. muelleri* in New Zealand. This is incorrect; the impetus for this chapter was the repeated observation of a small flock of Canada geese (*Branta canadensis maxima*) feeding
on *Z. muelleri* beds at Raglan. A biosecurity factsheet (June 2015) supplied by the Waikato Regional Council states that these geese, while not currently classified as pests, are nonetheless a “potential nuisance animal”. The geese are widely distributed in the region, particularly on coastal farms and wetlands. The observed grazing flock numbered less than six individuals, but rising goose population is likely to see increased grazing on the seagrass beds of the region.

There are indications that *Z. muelleri* can recover rapidly from low-intensity shoot loss, and eventually recover – via vegetative growth – from high levels of disturbance (Macreadie et al. 2014). Low species diversity is, however, often correlated with a decreased recovery from disturbance in seagrass (Hughes and Stachowicz 2004), and so the effects of grazing on *Z. muelleri* are particularly relevant to establishing conservation strategies on both a national and local level. That Dos Santos et al. claimed the black swan as the only large herbivore grazing on intertidal *Z. muelleri* populations indicates that herbivory by *B. canadensis maxima* may be at present minimal, and the small grazing flock observed supports this. The increasing population of geese, however, promises increasing rates of herbivory, and the Raglan seagrass beds are likely to be impacted by this in future. Given this, the potential recovery rates of *Z. muelleri* both in the Waikato region and in New Zealand as a whole may benefit from further investigation, in order to provide useful information for the formulation of future conservation plans for when the impacts of the rising goose population are more advanced.

Given the observed small flock of grazing geese, it is impractical at this time to repeat experiments such as that performed by Dos Santos et al. who had a much larger avian population impact to assess. A pilot study mimicking the effects of small scale grazing
disturbance is more practicable, however, and offers both a baseline of recovery and
resilience in the Raglan *Z. muelleri* beds and an indicator for potential future experiments at
that site.

*Methods*

The experiments took place at site C (see Introduction), Wallis Street, Raglan.

Ten 33cm quadrats were used to make one continuous horizontal transect along the high
tide line in May 2016, and *Z. muelleri* shoot density within each quadrat was recorded. This
transect recording count was repeated at mid and low tide lines. The experiment was
repeated along the same transect lines in June and August 2016 to give baseline shoot
density counts at each tide level.

In May 2016, three additional quadrats were randomly placed at each tide level and their
position recorded. Each of these nine quadrats had their *Z. muelleri* shoot density recorded,
and then all shoots within the quadrats were clipped off at surface level in order to mimic
surface feeding by avian herbivores. Shoot density within the “clipped” quadrats was
recorded again in August 2016. For purposes of comparison, three control quadrats were
randomly assigned from within the belt transect.

Also in May 2016, another three additional, separate quadrats were randomly placed at
each tide level. Their position was recorded, as was quadrat shoot density. Subsequently the
seagrass within the quadrats was removed entirely both above and below-ground to mimic
whole-plant feeding by avian herbivores. Shoot density within the “removed” quadrats was recorded again in August 2016. For purposes of comparison, three control quadrats were randomly assigned from the belt transect quadrats not used as “clipped” controls.

Statistical analysis was performed using one-way ANOVA and t-tests with a p-level of 0.05.

Results

Figure 1 illustrates the mean shoot density along the transect quadrants at all tide levels over time.

There is a significant statistical difference \( F(2,27) = 8.588, p=0.001 \) between the high tide means. The statistical difference is primarily found in the initial May sample. Comparing the mean with the high June sample yields two-sample \( t(18) = 3.645, p = 0.002 \); comparing it with the high August sample yields two-sample \( t (18) = 3.000, p = 0.008 \). The high tide June and August means show no statistical difference: two-sample \( t(18) = 0.847, p = 0.41 \).

There is no significant statistical difference between the mid tide means \( F(2,27) = 2.723, p = 0.08 \) or the low tide means \( F(2,27) = 0.232, p = 0.79 \) over time.

Figures 2a and 2b show the change in shoot density between the high tide clipped and control clipped quadrats. After 3 months there is no significant statistical difference between mean shoot density in the clipped (two-sample \( t(4) = 0.949, p = 0.40 \)) or control quadrats (two-sample \( t(4) = 2.21, p = 0.09 \)).

Figures 3a and 3b show the change in shoot density between the mid tide clipped and control clipped quadrats. After 3 months there is no significant statistical difference
between mean shoot density in the clipped (two-sample \(t(4) = 0.181, p = 0.87\)) or control quadrats (two-sample \(t(4) = 1.965, p = 0.12\)).

Figures 4a and 4b show the change in shoot density between the low tide clipped and control clipped quadrats. After 3 months there is no significant statistical difference between mean shoot density in the clipped (two-sample \(t(4) = 0.038, p = 0.97\)) or control quadrats (two-sample \(t(4) = 0.087, p = 0.93\)).

Figures 5a and 5b show the change in shoot density between the high tide removed and control removed quadrats. After 3 months there is no significant statistical difference between mean shoot density in the removed (two-sample \(t(4) = 0.221, p = 0.84\)) or control quadrats (two-sample \(t(4) = 2.091, p = 0.11\)).

Figures 6a and 6b show the change in shoot density between the mid tide removed and control removed quadrats. After 3 months there is no significant statistical difference between mean shoot density in the removed (two-sample \(t(4) = 0.655, p = 0.55\)) or control quadrats (two-sample \(t(4) = 0.352, p = 0.74\)).

Figures 7a and 7b show the change in shoot density between the low tide removed and control removed quadrats. After 3 months there is no significant statistical difference between mean shoot density in the removed (two-sample \(t(4) = 0.225, p = 0.83\)) or control quadrats (two-sample \(t(4) = 0.742, p = 0.50\)).
Figure 1: Mean Shoot Density of Transect Quadrats at all Tide Levels.
Figure 2a: Delta Shoot Density in High Tide Clipped Quadrats.

![Figure 2a](image)

Figure 2b: High Tide Quadrats, Treatment 1 (Clipped)

May August

Figure 2b: Delta Shoot Density in High Tide Clipped Control Quadrats.

![Figure 2b](image)

Figure 2b: High Tide Quadrats (Clipped Control)

May August
Figure 3a: Delta Shoot Density in Mid Tide Clipped Quadrats.

Figure 3b: Delta Shoot Density in Mid Tide Clipped Control Quadrats.
Figure 4a: Delta Shoot Density in Low Tide Clipped Quadrats.

![Figure 4a: Low Tide Quadrats, Treatment 1 (Clipped)](image)

Figure 4b: Delta Shoot Density in Low Tide Clipped Control Quadrats.

![Figure 4b: Low Tide Quadrats (Clipped Control)](image)
Figure 5a: Delta Shoot Density in High Tide Removed Quadrats.

![Figure 5a: High Tide Quadrats, Treatment 2 (Removed)](image)

May
August

Figure 5b: Delta Shoot Density in High Tide Removed Control Quadrats.

![Figure 5b: High Tide Quadrats (Removed Control)](image)

May
August
Figure 6a: Delta Shoot Density in Mid Tide Removed Quadrats.

Figure 6b: Delta Shoot Density in High Tide Removed Control Quadrats.
Figure 7a: Delta Shoot Density in High Tide Removed Quadrats.

![Graph showing Delta Shoot Density in High Tide Removed Quadrats](image1)

Figure 7b: Delta Shoot Density in High Tide Removed Control Quadrats.

![Graph showing Delta Shoot Density in High Tide Removed Control Quadrats](image2)
Discussion

The quadrat transects indicate that the high tide growth of *Z. muelleri* appears more vulnerable to winter conditions and/or die-back than the mid or low tide growths – although this is evidenced only at the beginning of winter, as shoot density appears to stabilise between the June and August samples. At mid and low tide levels, however, there is no evidence of a significant decrease in shoot density. This indicates that winter die-back may not be a significant factor at Site C except at the very top of the tidal range.

The rapid recovery of the “grazed” quadrats to pre-disturbance levels is encouraging. Most noticeable is that recovery to pre-treatment levels is independent of treatment. It was expected that clipped quadrats would more easily recover, as it involved the removal of the visible, above-ground sheath only. The below-surface growing stem remained. Yet vegetative growth appears remarkably resilient, and the recovery of the removed quadrats to their pre-treatment levels can be said largely to rely upon the vegetative growth of the surrounding seagrass. It is certainly possible that floating fragments may have colonised the disturbed area, but the growth rate of fragment rhizomes and shoots (see Chapter Two) does not appear likely to account for the entire recovery. More probable is that vegetative growth from the surrounding plants is largely responsible, although the attachment of previously floating fragments may well be supplemental. This is something that can only be confirmed via genetic analysis, however, which is beyond the scope and budget of this thesis.

The demonstrated resilience of the beds does prompt further questions, however: the reproductive strategy behind the shoot recovery, the ability of this study to act as a proxy for avian herbivory, and the implications for seagrass conservation at the Raglan beds.
It must be acknowledged that this study is not sufficiently statistically robust. With only three replicates for each treatment at each tidal level, the results – although perhaps indicative of a potential trend – cannot be relied upon. It is recommended that this experiment be used as a pilot study and, if repeated, that treatment replication on every tidal level is substantially increased.

Sexual reproduction within seagrass beds has been shown to be unaffected by grazing (Jacobs et al. 1981). No evidence of recovery via sexual propagation after grazing disruption was noted by Rivers and Short (2007). It must be remembered, however, that sexual reproduction is not always a factor in specific seagrass beds, so the effects of grazing on sexual reproduction – as with vegetative – should be observed in specific locations before conservation strategies for those locations are made. The lack of an observable seed bed in the Raglan study sites, including site C (see Chapter 1), indicate that sexual propagation is unlikely to be responsible for the rapid regrowth of the “grazed” quadrats.

Vegetative reproduction within seagrass beds has been shown to be negatively affected by grazing (Jacobs et al. 1981; Rivers and Short 2007; Dos Santos et al. 2012). This is not observably the case site C. However, avian herbivory at that site is currently minimal, and the experimental design was not repetitive. It measured recovery from a single disturbance only. It is possible that if the “grazed” quadrats were subject to repeated disturbances their ability to return to pre-disturbance levels would be compromised. Given the likely increase in the Waikato wild goose population, and the probable increase in herbivorous behaviour concomitant with that, experiments to establish the recovery of the Z. muelleri beds after repeated or ongoing disruption are suggested. The current results are encouraging, but are likely not adequate proxies for possible future bird populations. There is always the
possibility that increased goose herbivory (especially repeated grazing at any one location) may cause a decrease in the ability of the seagrass beds to regenerate. In which case, measures must be taken to conserve the seagrass population by limiting the impact of the geese. This may be achieved by limiting the goose population via cull.

Excessive grazing such as that observed on Fishing Island would have to be curtailed if the beds are to be preserved. While intensive grazing during the winter of 2003 was responsible for 96% of the seagrass loss at that location since that time, the continued grazing presence of Canada geese has raised the possibility of a reduction in their population through increased hunting opportunities (Short et al. 1999). Thayer et al. (1984) argue that the pressure of large grazing herbivores such as birds is likely to be less than in the past due to increased pressure on bird populations and the subsequent reduction on their populations, but if this is true on a global, historical level then as Rivers and Short (2007) show, it is certainly not so on small or localised scales, and pressures such as climate change may increase grazing pressure on individual seagrass beds such as those in Raglan in the future.

Alternately, introduced seagrass species have been shown to provide adequate food for grazing birds. Baldwin and Lovvorn (1994) refer the Zostera japonica beds of British Columbia, first documented in the 1950s and showing a 17-fold increase in coverage between 1970 and 1991. Migrating birds – including B. bernicla, A. platyrynchos, A.acuta, the American wigeon (Anas Americana) and the green-winged teal (Anas crecca) – fed preferentially on Z. japonica over the native Zostera marina. The exotic Z. japonica was assessed as providing 4.6 million use days by flocks of up to 80,000 birds in December 1991. The deliberate introduction of a non-native species, however, may have negative effects on
the existing ecosystem. It is therefore suggested that the culling of geese is a preferential option if their herbivory becomes too much for the beds to sustain.

It should also be noted that increased genetic diversity within a species can increase resistance to grazing by geese and decrease time needed for meadows to recover from grazing to pre-disturbance levels, as observed by Hughes and Stachowicz (2004) in their study on Z. marina. If Z. muelleri can be shown to respond in the same way then it might prove to be a conservation advantage. Alternately, if shoot recovery is shown to be linked to intra-bed diversity then increasing the diversity of Z. muelleri beds, tending as they do to disparate homogeneities (Jones et al. 2008) is to be encouraged.

It is possible, however, that regular grazing may be beneficial to the long-term health of seagrass beds. Jacobs et al. (1981) posit that such grazing keeps sedimentation and erosion in equilibrium, and that without regular grazing silt would accumulate such that the beds would eventually rise above the high water mark and subsequently decline. Nacken and Reise (2000) report that Zostera noltii blade density is lower in enclosed sites than grazed sites in the season after grazing by B. bernicla and A. penelope, indicating that grazing has had a positive effect on revegetation. Ferson (2007) reports that leaf growth, shoot biomass, shoot density, and rhizome biomass increased in goose-grazed California populations of Z. marina as compared to ungrazed populations. Valentine and Heck (1999) argue that grazing may stimulate growth and primary production, although this is of course potentially dependent on the type of grazing and the reproductive strategy of the grazed population. For instance, Z. noltii beds in the Zandkreek estuary of the Netherlands rely upon shoot growth to survive the winter, as opposed to rhizome fragments or the minimal contribution of seed banks (in this they are apparently similar to the Raglan seagrass beds).
When geese and ducks graze heavily upon the Zandkreek shoots, then Dutch beds suffer massive biomass loss (Vermaat and Verhagen 1996) and their long-term survival is threatened.

**Conclusion**

*Z. muelleri* proves itself resilient to small scale single disturbance, recovering to pre-disturbance levels of shoot density within four weeks. This response is highly likely to be purely vegetative. Overall shoot density also proves remarkable consistent, within site C, to seasonal variation, although there is a small but significant decrease in density at high tide level at the beginning of winter.

Vegetative response to the removal of both above-ground vegetation and all vegetation in the “grazed” quadrats indicate that Valentine and Heck may be correct in their assessment of grazing stimulating primary production. In which case, the risk factor of silt to the Raglan seagrass beds needs to be established so that if/when the local goose population increases enough that the seagrass requires a specific conservation plan against avian herbivory, the cost-benefit analysis of substantial geese presence may be accurately assessed.
CONCLUSION

The purpose of this thesis was to determine potential factors that may affect the long-term viability of the *Z. muelleri* meadows at Raglan, New Zealand. Given the significant global decline in seagrass populations, the development of conservation plans – particularly local plans adapted to specific environments – is crucial to the resilience of existing beds and the restoration of absent ones.

It is not within the scope of this thesis to determine every potential environmental factor that may affect *Z. muelleri* at this site. For instance, eutrophication levels and nitrogen run-off from coastal farms have not been determined, nor the potential effect of these on competitive organisms such as the *Chlorophyta*. Neither have the potential effects of climate change been taken into account. These are stressors that have yet to be explored at this site.

This thesis has been primarily concerned with the means of establishing genetic variation within the Raglan meadows. Increased genetic diversity is positively correlated with the ability of a population to successfully respond to stressors, and so the potential mechanisms by which the Raglan beds can maintain or increase genetic diversity is an important consideration for any subsequent conservation plan.

Reproduction at the Raglan site has been determined to be primarily vegetative. Extensive sampling has yielded no evidence of a seed bank. The potential for genetic mixing through sexual reproduction at this site is therefore limited.

It is possible that further sampling may locate evidence of an existing seed bank at Raglan. Given that *Z. muelleri* has not produced viable seed banks at other New Zealand sites,
however, this national trend should be expected to hold at the Raglan site and the data gathered for this thesis supports this conclusion.

An alternate means of introducing genetic diversity is through the mechanism of seagrass fragments. Previous research (Weatherall et al. 2016) indicated the ability of *Z. muelleri* fragments to float for several weeks, which would allow for the transfer of material via ocean current both within the local ecosystem and, potentially, between regional ecosystems. These fragments are a source of potential genetic diversity, able to reproduce via vegetative growth after attachment at a new site.

The viability of *Z. muelleri* fragments after prolonged floating has been assessed. When viability is defined as the ability to grow new shoots after post-floating planting, a substantial proportion (20-40%) of fragments were assessed as viable after different lengths of floating treatment. This indicates that fragments are potentially able to increase genetic diversity within their attachment-meadow, but this potential is dependent upon the fragment to actually attach. A fragment that does not attach is incapable of growing into a viable plant.

There is indication of some genetic variability within Raglan harbour. Given the absence of a seed bank this would seem to indicate that fragments are attaching and viable within the harbour environment. It is arguable that this variability could be increased by the deliberate planting of fragments from an outside source. Experiments on other *Zostera* species indicate that individual plants may adapt strongly to their local sites, however, and this would encourage transplanting from regional more than national sources. Yet if transplantation is determined to be advisable, the transplant of whole plants rather than fragments would likely give a greater chance of success.
The ability of fragments to attach within an existing bed may be affected by disturbance. They may simply be out-competed by the vegetative growth of the established plants. A small-scale pilot experiment, designed to mimic the grazing of resident Canada geese, indicated that shoot density is highly resilient to small-scale disturbance. While it is possible that the disturbed patches were colonised by fragments only, it is significantly more likely that vegetative growth is responsible.

Given that the Waikato population of Canada geese is increasing, the level of disturbance to the beds from their herbivory is likely to increase in future. As disturbance level rises, the ability of the seagrass to compensate for plant loss via vegetative growth may be compromised. This may increase the chance of fragment attachment, but it would also leave fragments vulnerable to herbivory.

This grazing experiment is a pilot study only. The sample size is insufficient to give statistically robust results, and the effects of prolonged grazing are not accounted for. Repetition of the experiment, on a larger scale, and including multiple grazing events over time is recommended. Genetic testing of the post-grazing seagrass within the grazed quadrants is also recommended, in order to establish whether the resultant growth is vegetative or fragmentary.

Taken together, these results establish a population of seagrass characterised by vegetative growth and some local genetic mixing that possibly results from fragment transfer. The Raglan population is subject to minor disturbance from Canada geese, albeit a disturbance that is likely to increase in severity as the goose population sharply increases. In order to increase the chances of meadow resilience to grazing, an increase in genetic diversity (as well as the possible population control of geese) is advisable. This diversity may come from
fragments, but a pro-active approach consisting of cross-planting between local sites may help to somewhat future-proof the Raglan meadows against future stressors.

It is not within the scope of this thesis to develop or implement such a plan, whether on its own or as part of a broader conservation initiative. Further study is needed in some areas, and stakeholder engagement must be sought. Clear communication between scientists, regional institutions and local citizens must be established, with monitoring programmes established. The Raglan beds are extensive and healthy but they will require good future management in order to remain that way, so the continued study of local stressors (both existing and potential) is needed in order to ensure this.
REFERENCES


Hayward, Bruce W. and Roger V. Grace, Fred J. Brook. “Soft-bottom Benthic Macrofaunal Communities of the Eastern Bay of Islands, Northern New Zealand.” Tane. 27 (1981): 103-122.


Williams, Susan L. and Mary H. Ruckelshaus. “Effects of Nitrogen Availability and Herbivory on Eelgrass (Zostera marina) and Epiphytes.” *Ecology.* 74.3 (1993): 904-918.


