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Population dynamics of restored green-lipped mussel (*Perna canaliculus*) beds in the Hauraki Gulf, New Zealand

By

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A thesis submitted in fulfilment of the requirements for the degree of

Doctorate of Philosophy in Marine Science

Leigh Marine Laboratory

University of Auckland, New Zealand

April 2017

Abstract

The widespread degradation of biogenic habitats created by bivalve beds has spurred numerous restoration initiatives worldwide. The success of those restoration initiatives depends on the persistence of restored populations which in turn relies on robust and frequent assessments of population dynamics to identify potential limitations to the persistence of those populations. The research presented in thesis aimed to examine the potential for restoration of the nearly extirpated green-lipped mussel in the Hauraki Gulf, New Zealand and to develop a foundation for best practice methods in restoration of this, and other mussel species. With the deployment of seven experimental mussel beds and multiple field and laboratory experiments, this body of research addresses the questions of; 1) whether transplanted mussels are persistent in restored mussel beds, 2) to what extent sea star predation contributes to mortality of adult and juvenile mussels, 3) whether the addition of attachment substrate enhances the persistence of adult and juvenile mussels, and 4) whether recruitment at the restored mussel bed site is limited by the amount of available settlers. Experimental mussel beds exhibited high mortality, with only 26.2% survival of originally estimated abundance after 25 months. This decline was attributed to unsustainable levels of mortality of adult mussels combined with a near absence of recruiting mussels. Predation by sea stars was estimated to have removed 30.1% of the mussels over the 25 month study, contributing to 40% of the overall mortality observed for experimental mussel beds. However, the large sea stars inhabiting the beds did not preferentially select for juvenile mussels. Providing attachment substrate was found to not enhance the persistence of transplanted adult mussels. However, juvenile mussels preferentially attached to adult mussels and had higher survival in the presence of a sea star predator compared to either mussel shell or unmodified substrate. The settlement of mussels on artificial collectors within the restoration site was greater for collectors within mussel beds than on the soft-sediment but

was lower than previously observed on artificial collectors placed in the vicinity of natural populations of green-lipped mussels elsewhere. The overall findings of this thesis suggest that sea star predation and lack of recruitment will limit the success of future restoration efforts. Therefore, further investigation and development of techniques for overcoming these limitations will be necessary for enabling effective restoration of sustainable beds of green-lipped mussels to the Hauraki Gulf in the future.

Acknowledgements

I would like to take this space to thank all the people who have been instrumental in the completion of this thesis.

I would like to thank my supervisor, Dr. Andrew Jeffs, for his guidance and support throughout the development and writing of this thesis. He was always available to aid in any logistical complications with both my field and laboratory work. I would also like to thank my co-supervisor, Dr. Shane Kelly, in particular for his help with the logistics of deploying and development of the sampling protocols for the experimental mussel beds.

I would also like to thank the staff and students at the Leigh Marine Laboratory for their help in the field and in the laboratory. I would like to thank Errol Murray for always taking the time out of whatever he was doing to answer any question I had, no matter how minute; he always knew just where to find whatever I needed for my laboratory experiments. I would like to thank Peter Browne, who not only helped to get my signed off on my diving but also helped out on some diving and boating when volunteers were scarce. Thanks to both Peter and Errol for their help in constructing equipment. I would like to thank Brady Doak who made every trip to Rotoroa Island pleasant and relatively stress free, despite some logistically difficult situations. I would particularly like to thank Jan Hesse, Arie Spyksma, Richard Bulmer, Paul Caiger, and Matt Pine who always seemed to make themselves available to volunteer their time to help me with both diving and boating. I would also like to thank Max Schofield, Evan Brown, Leo Zamora, Sydney Harris, Gus Pawson and Nichols Lao for their help with diving and laboratory experiments. I would also like to thank Kathy Ruggiero from

the University of Auckland statistical consulting centre for her guidance through the statistical analyses and her help learning R.

I would like to thank the Revive our Gulf group that supported my research. In particular, I would like to thank John Laurence who helped to orchestrate the deployment of the mussel beds with North Island Mussels Ltd as well as the acquisition of mussels for my small scale experiments. I would like to thank John Newton who helped to arrange for all the boating and diving aspects of the mussel settlement monitoring which would not have been possible without his help. I would also like to thank Shaun Lee for this help with diving and his constant enthusiasm for the project.

I would also like to thank the J.S. Watson Trust for their support in the purchasing of equipment vital to the monitoring of mussel beds and the University of Auckland for their financial support throughout my research.

Lastly, I would like to thank my family for their constant support, especially given the immense distance. I would especially like to thank my wife, Mary-Ellen, for putting up with me, letting me vent when I needed to, and sharing in the triumphs. Thank you for all your patience as I finished up my writing.

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Chapter 1

General introduction

1.1 Ecosystem services and functions of bivalve mollusc beds

Many species of bivalve molluscs, such as mussels and oysters, form large congruent populations within coastal ecosystems in many places around the world. These bivalve beds maintain their form through attachment to hard substrates, conspecifics in particular, either permanently cementing to those structures (e.g., oysters) or temporarily attaching via byssal threads (e.g., mussels). The cluster and attachment of the bivalves in this manner creates a complex three dimensional structure (a process known as bioengineering) that is often of greater complexity than the surrounding benthic environment, especially on soft-sediment environments. This complex structural habitat created by the presence of these bioengineering bivalve species is known as biogenic habitat, and is usually capable of supporting diverse and abundant communities of organisms (Bahr & Lanier, 1981; Hall-Spencer & Moore, 2000; Luckenbach et al., 2005; Commito et al., 2008; McLeod et al., 2014) that utilise the numerous cracks and crevices among the bivalves. The organisms associated with the biogenic habitat benefit from increased foraging potential for both resident and transient species (Lee & Kneib, 1994; Jiang & Carbines, 2002; Grabowski & Powers, 2004) as well as reduced predation (Lee & Kneib, 1994; Kamenos et al., 2004) as predators must either increase search effort to locate the prey or may not be able to access them. The biogenic habitats created by bivalve species tend to increase the biodiversity, abundance, and the total biomass of organisms relative to the surrounding habitats (Coen et al., 1999; Commito et al., 2008; Stunz et al., 2010; McLeod et al., 2014) making them an

important habitat in many benthic environments. Furthermore, they are frequently identified as nursery habitats for ecologically and commercially important species, such as coastal demersal fishes, which move out of these habitats once they have become established (Peterson et al., 2003).

Most bivalve species feed by filtering suspended particles out of the water column, and the enormous filtering capacity of an entire bed or reef of bivalves provides a number of benefits. Beds of bivalves are capable of filtering through vast quantities of seawater (Newell, 1988; Broekhuizen et al., 2002) which can reduce suspended particles in the water column. Bivalves have been shown to be able to reduce phytoplankton biomass in the water column by as much as 74% (Asmus & Asmus, 1991; Prins et al., 1998; Norén et al., 1999; Dolmer, 2000). This can exert a strong top-down control of the phytoplankton community in shallow waters and has been suggested as a potentially important buffer for eutrophication of coastal waters (Officer et al., 1982; Alpine & Cloern, 1992). The suspension feeding of these bivalves in turn moves pelagic productivity down to the benthic community where it would otherwise be largely inaccessible, a process known as benthic-pelagic coupling (Fréchette et al., 1989; Dame et al., 1991; Norkko et al., 2001). The captured pelagic nutritional and energy resources become available at the sea floor through the direct consumption and turnover of the bivalve species, as well as through the utilisation of bivalve biodeposits which alone can support up to 31% of the energy demands of small mobile invertebrates (Norling & Kautsky, 2007). Benthic-pelagic coupling can have a major influence on the fundamental structuring of coastal ecosystems by determining whether they are dominated by pelagic consumers or benthic consumers (Newell, 2004) such as the decapod crustaceans and demersal fish that constitute many coastal fisheries.

Biogenic bivalve habitats also provide a number of anthropogenic benefits. They frequently provide an important food resource to coastal communities. These bivalve

populations also support larger benthic consumers that are of commercial and recreational importance (Coen et al., 1999; Peterson et al., 2003; Newell, 2004). The three dimensional structures bivalves create also serve to harden shorelines and protect them from erosion (Piazza et al., 2005; Grabowski & Peterson, 2007; Scyphers et al., 2011). Bivalve filtering can also serve as a buffer for eutrophication as a result of terrestrial derived nutrients and assist with the removal of suspended sediment derived from land run off (Officer et al., 1982; Alpine & Cloern, 1992).

1.2 Loss of bivalve habitat

Around the world many of these highly important coastal biogenic habitats created by bivalve beds are in decline, having been completely removed or highly degraded by anthropogenic disturbance, such as overharvesting (de Jonge et al., 1993; Rothschild et al., 1994; Service & Magorrian, 1997; Cranfield et al., 1999; Kennedy & Roberts, 1999). The resulting effects of the loss of these bivalve habitats on the ecosystem functioning and the magnitude of the loss of ecosystem services are not well understood (Coen et al., 2007). The losses of populations of bed-forming bivalves in coastal waters is readily observable, however, the removal of the habitat also results in negative impacts on populations of species normally associated with these biogenic habitats (Jiang & Carbines, 2002; Bordehore et al., 2003; Carbines et al., 2004; Carbines & Cole, 2009; Du Preez & Tunnicliffe, 2011). Reduction in bivalve populations reduces the filtering capacity and consequently the capture of pelagic production by the benthos (Jorgensen, 1990) which may lead to a shift in the food web toward greater domination of the nutrients and energy provided by phytoplankton being utilised by pelagic consumers. This reduction of production passing into benthic communities can impact the

productivity of associated species which are often of commercial and recreational importance (Newell, 2004).

1.3 Bivalve restoration

Bivalve restoration in the form of restocking populations depleted by harvesting has a long history in many parts of the world, most often with the aim of eventual harvesting of the resultant populations. More recently, increasing knowledge of the lost ecosystem services and functions provided by these bivalves has also spurred restoration initiatives with the goal of improving ecosystem health and functioning. Regardless of the motivation for conducting restoration, the strategies utilised in these initiatives focus on overcoming limitations to the natural recovery of the targeted species, especially around issues of larval recruitment (Arnold, 2001; Brumbaugh et al., 2006; Brumbaugh & Coen, 2009; Kennedy et al., 2011). The larvae of most bivalve species are planktonic and will settle to the benthic environment on particular substrates where they will metamorphose and grow into juveniles. If there is inadequate supply of larvae or settlement substrate, then populations are unlikely to recover naturally. The issue of low larval supply can be tackled by bolstering the effective breeding population to increase larval output. Such an approach to restoration is referred to as a "recruitment limited strategy" (Brumbaugh & Coen, 2009). This approach to restoring larval supply often involves transplanting either juveniles or adult bivalves from either wild or hatchery-reared stocks into areas of existing populations or where they were historically present. The issue of limited settlement substrate can be tackled by the supply of additional substrate to the benthic environment to enhance settlement. Such an approach to restoration is referred to as a "substrate limited strategy" (Brumbaugh & Coen, 2009) and has typically

ranged from the small scale addition of bagged bivalve shells to the mass dumping to the seabed of various larval settlement substrates.

The ultimate success of restoration efforts relies on the establishment and persistence of the restored bivalve bed regardless of whether the goal is harvesting for human consumption or improving ecosystem health. Therefore, understanding population dynamics and the many factors that affect recruitment and mortality is critical to the success of any restoration initiative for bivalve beds. Much of the research to date has focussed on the process of re-establishment of bivalve beds, examining such factors as the effects of substrate characteristics (Bartol et al., 1999; Nestlerode et al., 2007; Fariñas-Franco et al., 2013; van der Heide et al., 2014) and larval dispersal (Barnes et al., 2007; Gregalis et al., 2008; Elsäßer et al., 2013) on recruitment to restoration sites, as well as substrate complexity (Frandsen & Dolmer, 2002; Christensen et al., 2015) and deployment density (Capelle et al., 2014) on the survival of transplanted bivalves. There are numerous other factors (i.e., environmental conditions, disease, predation, etc) that can affect the survival of settling, recruiting, and adult bivalves with the potential to pose limitations on the persistence of restored bivalve beds. Robust and ongoing assessment of the population dynamics of bivalve restoration initiatives has frequently been lacking (Mann & Powell, 2007) but is critical not only to determine if the populations are persistent but also to identify potential stressors that might be limiting recruitment and/or adult survival. Improving our understanding of the importance of the many factors that influence the establishment and persistence of restored beds will help to develop best practice methods for individual bivalve species that maximize recruitment and survival, providing the greatest chance for successful restoration outcomes.

1.4 Green-lipped mussel

The green-lipped mussel (*Perna canaliculus*) is an endemic species to New Zealand that forms beds of mussels on rocky and soft-sediment substrates throughout much of the coastal waters around the country (Jeffs et al., 1999; Alfaro, 2006b; Alfaro et al., 2008; Morrison et al., 2010; McLeod et al., 2014; Paul-Burke, 2015). The larvae of this species settle preferentially onto filamentous structures, such as macroalgae (Buchanan & Babcock, 1997; Jeffs et al., 1999; Alfaro, 2006b; Alfaro et al., 2006; Gribben et al., 2011; Young et al., 2015), and predominantly recruit to mussel beds through secondary migration from this primary settlement substrate (Buchanan & Babcock, 1997; Alfaro, 2006b). At Ninety Mile Beach in northern New Zealand, filamentous macroalgae can become inundated with mussel settlers (Alfaro et al., 2004; Alfaro et al., 2010), much of which becomes beach-cast and is collected from the beach in large quantities each year to provide seed for stocking most of the mussel aquaculture industry throughout the country (Jeffs et al., 1999).

Adult mussels are capable of filtering up to 350 l day^{-1} (Broekhuizen et al., 2002), providing a potentially important driver of benthic-pelagic coupling within New Zealand coastal waters. The biogenic habitat created by beds of these mussels is highly productive and supports diverse and abundant communities of small mobile invertebrates and fish (McLeod et al., 2014). Commercially important species, such as snapper (*Pagrus auratus*) are also known to be associated with this type of habitat (Thrush et al., 2002).

Within the Hauraki Gulf, on the northeastern coast of the North Island, green-lipped mussels were once common on the soft-sediment sea floor, particularly in the Firth of Thames and Coromandel (Figure 1.1)(Greenway, 1969; Reid, 1969). These populations supported a dredge fishery from 1910 until the close of the fishery in 1969 due to diminishing returns (Paul, 2012). Despite the closure of the fishery for nearly half a century, there is little

evidence of natural recovery of these populations (Morrison et al., 2010; McLeod et al., 2014) and it is unclear what may be limiting this recovery. A recent study in the Firth of Thames has shown that adult mussels are still capable of surviving in the present environmental conditions (McLeod et al., 2012) with a 68% survival of caged adult mussels over 18 months. However, recruitment was not observed within remnant beds of adult mussels and larval settlement was nearly absent on settlement collectors except for six individual settlers over six collectors found on a single sampling date during a 18 month study (McLeod, 2009). This suggests that mussel bed restoration may be possible and would require the implementation of a recruitment limited strategy to increase the breeding population and overcome a potentially inadequate supply of mussel larvae in the system.

1.5 Study aims

The overall aim of this research was to determine the feasibility of green-lipped mussel restoration on the soft-sediments of the Hauraki Gulf as a means to return some of the lost ecosystem services and functions resulting from their widespread extirpation last century. Although there are guidelines available for best practice methods for the restoration of bivalve populations (Brumbaugh et al., 2006), there are very few published examples of large scale restoration through transplanting mussels aside from the bottom culture practices of the Dutch Wadden Sea (Dolmer & Frandsen, 2002). To date, research into the ecological restoration of marine mussel beds has only ever involved small scale experiments (Fariñas-Franco et al., 2013; van der Heide et al., 2014; de Paoli et al., 2015) and has not been conducted on the scale of deploying entire beds of adult mussels. In the case of green-lipped mussels, no previous restoration efforts have been implemented with this species prior to this

study other than traditional Māori practices of transplanting small quantities of bivalves among accessible coastal locations (Waitangi Tribunal, 1988).

1.5.1 Persistence of restored mussel beds

Assessing population dynamics in restored populations is an essential component of any restoration initiative (Brumbaugh et al., 2006), not only to determine if the populations are persistent but also to identify stressors that might limit persistence. The mass transplantation of adult green-lipped mussels from suspended aquaculture operations to the soft-sediment sea floor, in an effort to re-establish mussel beds, has not previously been attempted. Unlike the previous study that demonstrated the survival of small clumps of adult mussels held in cages (McLeod et al., 2012), in the current study the transplanted mussels would be exposed to predation and arranged in a large mussel bed where individual mussels would potentially be subject to density-dependent factors, both of which could lead to high rates of mortality of adult mussels. Given the lack of settlement of mussels in the Firth of Thames (McLeod, 2009), there could also be a lack of sufficient recruitment into the restored beds to offset the mortality of transplanted adult mussels. The establishment of a set of seven replicate experimental mussel beds at the study site adjacent to Rotoroa Island in the Hauraki Gulf allowed for regularly repeated sampling over a period of 25 months to assess the mortality and recruitment of mussels within the restored mussel beds and allow for the subsequent evaluation of their potential for long term persistence. The sampling, combined with regular and long term observations of the experimentally restored mussel beds also allowed for the identification of potentially inhibiting factors, such as sea star predation, that warranted further examination as a potentially major cause of mussel mortality. This component of the

research, examining the population dynamics of the restored beds, is presented in Chapter 2 of this thesis.

1.5.2 Sea star predation

Predation by sea stars can strongly influence the distribution and abundance of mussel populations (Paine, 1966; Paine, 1971; Paine et al., 1985). In some cases this predation can lead to the mass removal of mussels (Sloan & Aldridge, 1981; Dare, 1982). The transplanting of mussels for bottom culture in the Dutch Wadden Sea has shown that sea star predation is capable of completely consuming entire mussel beds after they have been transplanted into new locations (Kristensen & Lassen, 1997). Predatory sea stars are known to be associated with green-lipped mussels (Inglis & Gust, 2003; Paul-Burke, 2015) and have contributed to losses of up to 88% of the mussels in a natural mussel bed (Paul-Burke, 2015). Predation of transplanted mussels by sea stars migrating onto restored mussel beds has the potential to impact the persistence of the beds. Therefore, sampling and additional experimentation was undertaken to identify the contribution of sea star predation to overall mortality within the experimental mussel beds and to determine whether the selection of juvenile mussels by predatory sea stars may reduce recruitment into the restored mussel populations. This component of the research is presented in Chapter 3 of this thesis.

1.5.3 Importance of attachment substrate

The additional provision of attachment substrate is a common restoration practice when restoring oyster populations (Brumbaugh et al., 2006; Brumbaugh & Coen, 2009), however, the importance of attachment substrate for the successful restoration of mussel beds in soft-

sediment environments is undetermined. Mussels do not typically attach byssal threads to sand or mud (Bayne, 1964; Commito, 1987; Commito et al., 2005) likely due to small grain sizes providing little anchorage for the mussel. Mussel beds often contain shell and cobbles that the mussels attach to as well as to conspecifics (Commito et al., 2014). Previous studies with other mussel species suggest that these attachment substrates are of little importance to adult mussels (Fariñas-Franco et al., 2013; de Paoli et al., 2015) but may be of greater importance to juvenile mussels (Fariñas-Franco et al., 2013; van der Heide et al., 2014). Therefore, experiments were conducted to determine whether the provision of attachment substrate enhances the persistence and survival of juvenile and adult green-lipped mussels on soft-sediment. This component of the research is presented in Chapter 4 of this thesis.

1.5.4 Assessing limitations to recruitment

Determining whether a lack of natural recovery is due to inadequate larval availability to restoration sites or a lack of available settlement substrates is fundamental to determining the appropriate restoration strategy (Brumbaugh et al., 2006; Brumbaugh & Coen, 2009). Assessing larval settlement of green-lipped mussels using artificial collectors has been implemented in many parts of the country to determine optimal times for collection of mussel settlers for aquaculture (Meredyth-Young & Jenkins, 1978; Alfaro & Jeffs, 2003). Using these artificial collectors, a previous study in the Hauraki Gulf has indicated that larval supply could be inadequate to maintain benthic populations of green-lipped mussels (McLeod, 2009). The assessment of benthic mussel settlement in the Hauraki Gulf is limited to this single study and given that settlement of mussel larvae is known to vary both spatially and temporally (Hunt & Scheibling, 1998; Alfaro & Jeffs, 2003; Alfaro, 2006b) there was a need to determine if there is adequate larval supply to the restored mussel beds and

potentially corroborate whether the Hauraki Gulf system is indeed recruitment limited. Therefore, sampling of larval settlement was undertaken at the experimental mussel beds and on the surrounding soft-sediment habitat over a one year period. This component of the research is presented in Chapter 5 of this thesis.

This thesis is presented in a journal manuscript format and whilst the chapter elements do include some redundancy in content they help to present the individual elements of this research project, the conclusions of which are brought together in the final chapter, the General Discussion – Chapter 6.

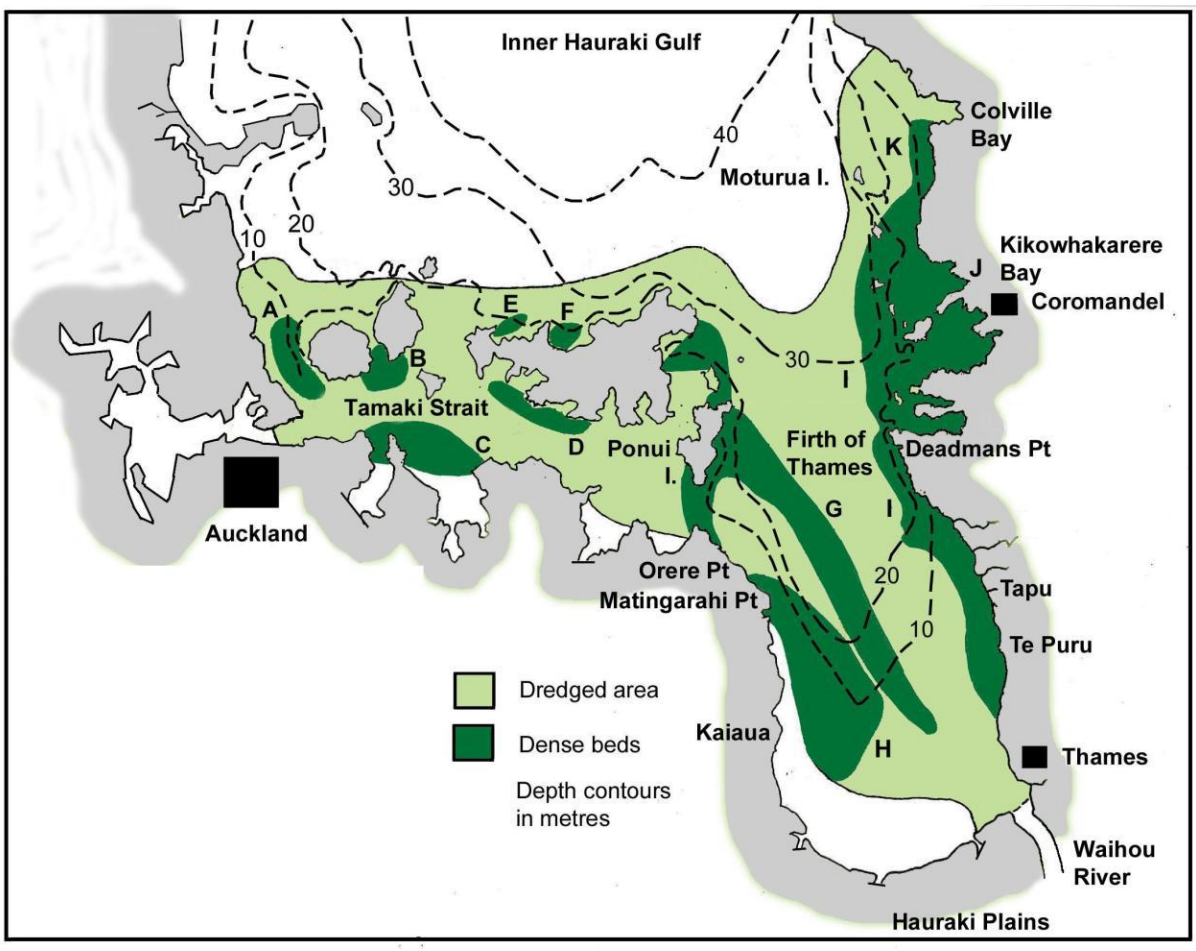

Figure 1.1 Historical mussel dredging areas within the Hauraki Gulf from Paul (2012), which was redrawn from Reid (1969).

Chapter 2

Restoring mussel beds onto soft-sediment using transplanted adults

2.1 Introduction

Bed-forming marine bivalves, such as mussels and oysters, are an integral component of many coastal ecosystems, altering nutrient and energy dynamics and also providing three dimensional structure which supports abundant and diverse communities of associated organisms (Hall-Spencer & Moore, 2000; Meyer & Townsend, 2000; Coen et al., 2007; Commito et al., 2008; Trigg et al., 2011; McLeod et al., 2014). These habitats provide increased foraging potential for both resident and transient species and provide refuge from predation (Lee & Kneib, 1994; Jiang & Carbines, 2002; Grabowski & Powers, 2004). The bivalves that constitute these beds can reduce the phytoplankton biomass in the water column by as much as 74% and effectively control the phytoplankton community in shallow waters, providing a potential buffer for eutrophication (Officer et al., 1982; Alpine & Cloern, 1992; Norén et al., 1999; Dolmer, 2000). The result of this enormous filtering potential is a transfer of pelagic productivity to the benthos, known as benthic-pelagic coupling (Dame et al., 1991; Loo & Rosenberg, 1996; Norkko et al., 2001). Along with providing three-dimensional structure, it is this exchange of energy from the pelagic to the benthic environment that makes these biogenic habitats such important sources of diversity and productivity in the marine environment.

Despite the importance of these bivalve habitats, anthropogenic disturbance such as decreased water quality and overharvesting have led to the degradation and loss of many

bivalve habitats around the world (de Jonge et al., 1993; Rothschild et al., 1994; Service & Magorrian, 1997; Cranfield et al., 1999). The increasing knowledge and awareness of the numerous benefits provided by bivalve beds and the consequences of their loss have spurred recent efforts to restore degraded bivalve habitats. These restoration initiatives aim to establish a persistent population such that there is sufficient recruitment to offset the many sources of mortality to the adult population. Despite the critical importance of assessing the population dynamics in bivalve restoration, the monitoring of recruitment and mortality of restored populations has received relatively little attention (Mann & Powell, 2007). This lack of monitoring makes it difficult to determine the ultimate success of these initiatives or to identify the ecological processes, such as recruitment limitation, which may be impinging on the persistence of the restored population.

The green-lipped mussel, *Perna canaliculus*, is endemic to New Zealand where it is found throughout the country, often forming extensive beds in shallow coastal waters (Jeffs et al., 1999; Morrison et al., 2010). These mussels once covered more than 1300 km^2 of soft-sediment sea floor in the Hauraki Gulf, a large coastal embayment in the northern North Island, (Greenway, 1969; Reid, 1969) before being nearly extirpated by intensive commercial dredge fishing from 1910 - 1969 and subsequent poaching until 1978 (Paul, 2012). Only a few small remnant mussel beds remain totalling around 0.64 km^2 (McLeod, 2009). Experiments into the survival of small quantities of caged adult mussels transplanted into these soft-sediment environments revealed that the lack of population recovery was not due to adverse environmental conditions inhibiting the survival of adults that were experimentally placed on the seabed in cages to protect them from predators (McLeod et al., 2012). However, the survival of these caged mussels does not reflect the potential survival in the presence of predation or incorporate the effects of density-dependant factors that mussels would experience as part of a larger mussel bed. It is unclear whether beds of adult mussels

are capable of persisting in the current natural environment of the Hauraki Gulf which has changed markedly over the last 50 years (HGF (Hauraki Gulf Forum), 2014) and what potential factors might inhibit or help to promote the long term persistence of restored mussel beds. Therefore, this study aims to determine if mussel beds can be re-established on the soft-sediment environment in the Hauraki Gulf and to identify any potential limitations to their subsequent persistence. Seven experimental mussel beds were established by transferring large quantities of adult mussels from aquaculture onto soft-sediments at a restoration site and four of these beds were subsequently assessed regularly over two years for changes in population size and the size structure of mussels. The results of this study will help contribute to the development of best practice methods for future mussel restoration initiatives.

2.2 Materials and methods

2.2.1 Site and mussel bed deployment

Cable Bay (S 36º 48' 32", E 175º 11' 37"), off the northern tip of Rotoroa Island, in the Hauraki Gulf was selected as a suitable site for deploying adult mussels (Figure 2.1). This bay has a large expanse of soft-sediment in shallow water (3-12 m below chart datum) with low tidal currents and reasonable water clarity (diver visibility of 1.5 - 3 m). The seabed substrate in the area of deployment of the mussels consisted of a layer of about 5 cm of fine mud overlyinge a more stable mix of fine mud, sand, and shell hash. Preliminary surveys showed no signs of mussels within the study site, and the rocky foreshore was dominated by oysters, with no existing mussel beds present within the surrounding Cable Bay. On 28 November 2013 seven mussel beds were created using adult mussels (70 - 100 mm shell length, SL) from mussel aquaculture (North Island Mussels Ltd) and deployed from the

company's barge. Each mussel bed was formed from approximately 1 t of freshly-harvested adult mussels that were released from the barge at the water's surface and allowed to sink onto the sea floor. Each mussel bed was defined by a distinct margin between the predominantly contiguous bed and the surrounding benthic environment. Where mussels aggregated into clumps, these were included as part of the bed when clumps comprised five or more mussels. These clumps of mussels were never greater than 0.25 m from one another and no other clumps of mussels were observed to be closer than 3 m from the defined bed margin. The distance between the seven adjacent mussel beds ranged from 9 - 35 m at depths of 3.9 - 5.1 m below chart datum. Due to logistic constraints of deploying SCUBA divers, only the four westernmost mussel beds, hereafter referred to as the experimental mussel beds, were monitored for changes in their population. These beds were labelled I-IV running west to east (Figure 2.1).

2.2.2 Population estimates

Sampling began two months after deployment (February 2014). Mussel density and size were assessed by divers using four quadrats (0.0625 m^2) haphazardly placed on each experimental mussel bed. Haphazard placement of the quadrats involved divers dropping the square quadrat frames from a height greater than 1 m above the mussel bed and maintained a minimum of 0.5 m from the bed margin. Quadrats were spread across the length of the bed (>1 m from adjacent quadrats) such that the processing of quadrats by divers did not unduly reduce the visibility within quadrats of adjacent divers. This helped to reduce potential sampling biases and provide a more accurate representation of mussels across the entire bed. Within each quadrat, mussels were removed, enumerated, and measured (SL) *in situ* before being replaced back into the bed. Particular attention was paid to locating any recruiting

juvenile mussels (<60 mm SL) within the quadrats that would not have been dropped initially. The reduced underwater visibility due to processing the quadrats was most pronounced up to 0.5 m above the bed and divers measured mussels above the resuspended sediment enabling them to reliably observe any mussel recruits at least 10 cm SL or larger. To assess the area occupied by each mussel bed, a transect was run across the longest axis of the mussel bed to measure the bed length. Along this transect the corresponding perpendicular width of the bed was measured at 1 m intervals. The measured margins of the bed were later mapped and then summed in order to estimate the total bed area. Sampling for this study was conducted five times over 25 months with an interval of approximately six months between sampling dates (September 2014, February 2015, October 2015, and March 2016). The abundance of mussels within each bed at each sampling date was estimated as the product of bed size and mean mussel density. Percent loss was calculated as the differences in abundance of mussels between successive sampling dates (hereafter referred to as the sampling interval) divided by the initial abundance for that sampling interval. The percent loss was adjusted to a per month loss (30 day) for ease of comparisons between sampling intervals of unequal lengths of time.

To determine the potential loss associated with the initial deployment of the mussels and their subsequent loss during the first two months after deployment an estimate of the initial number of mussels was calculated for comparison to the first sampling dates. Although exact measures of total mussels were not available prior to deployment, several assumptions were made to calculate the number of mussels deployed. Firstly, the weight of the bags were unknown and almost certainly varied, however, a weight of 1 t of harvested mussels was assumed based on the typical weight for the size of mussel harvesting bag utilised. Secondly, a mean mussel size of 87.3 mm SL (s^2 = 76.6, n = 308) was measured during the first survey. A mean size of 85 mm SL was assumed for the harvest size of the mussels at the time of their

deployment to the seabed. Using allometric relations between weight and size (SL) (Hickman, 1979), a mean mussel size of 85 mm SL would correspond with a weight of approximately 50 g. The total number of mussels deployed was therefore estimated to be 20,000 mussels per 1 t bag.

2.2.3 Data analyses

The assumption of normality and heterogeneity of variance were confirmed firstly by visually inspecting quartile-quartile plots and plots of residuals versus fitted values (respectively) of models prior to running analyses. Abundance data were log transformed to meet the assumption of heterogeneity of variance. Abundance and density measures were fitted to a linear model (LM) and tested for differences using a two-way ANOVA with mussel bed and sampling date as fixed effects. Percent loss was also fitted to a linear model and tested for differences using a two-way ANOVA with mussel bed and sampling interval as fixed effects. Measurements for shell length during the first sampling date were measured in size bins of 10 mm SL for logistic reasons, rather than to the nearest mm which was used in all subsequent sampling dates. For analyses, mussels were assigned a median shell length corresponding with their size bin (e.g., 85 mm for a mussel measured 80 – 90 mm). Shell length measures were then fitted to a linear mixed effect model (LMM) and analysed using a two-way ANOVA with mussel bed and sampling date as fixed factors. The individual quadrat from which each mussel length originated was incorporated into the model as a random effect. Differences in mean rate of growth were also fitted to a linear model and tested for differences using a two-way ANOVA with mussel bed and sampling interval as fixed effects. Any differences within overall significant factors for ANOVA analyses were compared using pairwise *t*-tests ("predictmeans" function in R) with a false discovery rate correction for

multiple comparisons ("fdr" function in R). All statistical tests were computed in R version 3.2.3 and RStudio version 0.99.879 using the R packages ggplot2, lme4, predictmeans, and car.

2.3 Results

2.3.1 Mussel bed structure

Within a few weeks after deployment the adult mussels in each of the experimental beds had mostly congregated into contiguous mussel beds with a few smaller clumps of mussels on the margins of the main bed. This was more pronounced at two of the mussel beds (beds I & IV), for which the southern side of the bed was characterized by clumps of mussels rather than a discrete margin. These clumps were most likely due to the last of the mussels that were shaken out of the 1 t transporting bag at the end of the deployment for each experimental bed. The fine mud substrate over which the mussel beds were established was not always firm enough to support the weight of the mussels on the surface of the sediment. This resulted in the partial or complete sinking of some of the deployed mussels, particularly in areas where mussels settled upon other mussels. Throughout the study, this surface layer of mud around the site was eroded and transported away, leaving the compacted mud and shell hash exposed as the surface layer while the layer of fine mud was largely retained within the boundaries of the mussel bed. Over the course of the study each of the experimental mussel beds maintained defined margins with little sign of fragmentation. However, fragmentation was observed by volunteer divers in the other three mussel beds which were not subjected to regular sampling, with the two easternmost beds being completely dispersed by wave action during a storm event. The initial size of the four experimental mussel beds, as measured in

February 2014, ranged from 24.4 m^2 to 45.2 m^2 and varied little over the 25 month study period (Figure 2.2). The only exception was on September 2014 when bed IV exhibited a temporary contraction and bed I which exhibited a temporary expansion in October 2015 . Both beds resumed a similar area on the following sampling date. At the conclusion of the study, there were still well defined bed margins, however, mussels no longer formed a contiguous population. Mussels instead formed dense clumps within the boundary of the mussel bed that were interspersed with numerous shells from dead mussels.

2.3.2 Mussel density

Analyses of mussel density estimates over the course of the study showed significant effects for both mussel bed ($F_{3,60} = 3.76$, $p = 0.015$) and sampling date ($F_{4,60} = 42.99$, $p < 0.001$) as well as the interaction of both factors ($F_{12.60} = 2.34$, $p = 0.016$) indicating that trends in density were not independent for either factor (Table 2.1). Multiple comparisons of density showed consistently significant decreases in the density of mussels from the beginning of the study to that of the final density for all mussel beds. Pooled mean density for the four experimental beds over the course of the study dropped from 637 ± 90 (SE) mussels m^{-2} in February 2014 to 144 ± 23 mussels m^{-2} in March 2016 (Figure 2.3).

2.3.3 Mussel Abundance

Analysis of the mean abundance of mussels in the four beds showed significant effects for sampling date ($F_{4,60} = 28.70$, $p < 0.001$) (Table 2.1). Overall mean abundance of mussels within the four mussel beds decreased throughout the study, from 20549 ± 3301 mussels in February 2014 to 5307 ± 1102 mussels in March 2016. This change in the mussel abundance

over the 25 months equates to a mean survival of 26.2% (± 4.6). Multiple comparisons indicated that the abundance of mussels showed no difference between sampling dates for a given year, but significant differences between sampling dates of different years (Figure 2.4). The lack of significant interaction ($F_{12, 60} = 1.42$, $p = 0.18$) indicates that these differences were consistent for all of the four mussel beds. Analysis of mean abundance also showed a significant effect of mussel bed ($F_{3,60} = 4.76$, $p = 0.005$) (Table 2.1) which represents an initial difference in the abundance of mussels that were deployed, a difference that remained consistent throughout the study as indicated by the lack of significant interaction term in the analyses.

2.3.4 Percent loss and sources of mortality

The percent loss ($month^{-1}$) showed significant effects of sampling interval ($F_{3, 240} = 18.28$, $p < 0.001$) and mussel bed ($F_{3, 240} = 3.72$, $p = 0.012$) as well as their interaction ($F_{9,240} = 5.31$, $p < 0.001$) (Table 2.1). This indicated that percent loss of mussels was neither consistent for time or mussel bed. Further analyses did isolate some individual differences among particular sampling intervals or mussel beds, however, there were no obvious or consistent overall patterns in the percent loss associated with either mussel beds or sampling intervals (Figure 2.5). For example, there was no indication of any seasonal patterns in percent loss of mussels corresponding with sampling intervals covering either the spring-summer or fall-winter. An analysis on the absolute number of live mussels lost between sampling intervals showed a similar lack of overall pattern as the analysis of the percent loss data (data not shown). On two occasions, the mortality was negative, which would have indicated an increase in population size. On both occasions, these negative mortalities were associated with a compression of the bed size. Although immigration of mussels from other beds is not

impossible, these negative mortalities are most likely due to sampling error where quadrats were placed in densely packed areas of mussels which predominated the bed at those sampling dates. The estimated mean loss of mussels from the four experimental beds for the two month interval between deployment and the initial sampling of mussels on February 2014 was calculated as 1.4 ± 8.2 % of mussels month^{-1}.

Throughout the study, divers made several observations on mortality and identified several sources of mortality for the mussels. A large number of the deceased mussels remained within the boundary of the mussel beds as evident by the large number of shells within that boundary. From the initial survey in February 2014 it was apparent that the burial of mussels, either by sinking into the soft-sediment or sedimentation, had resulted in some mortality. This most likely was the result of mussels being pushed into the sediment by the initial piling up of conspecifics during deployment. However, the burial of live mussels in sediment was not commonly observed by divers over the duration of the study. The predatory sea star *Coscinasterias muricata*, ranging in size from 9.0 - 35.0 cm (as measured from the tip of the longest arm to the opposite side of the oral disc) and in abundances ranging from 7 - 32 sea stars bed^{-2} were observed feeding on mussels throughout the study. Predatory gastropods were also present in low abundances. No fish or crustaceans of sufficient size to prey upon the adult mussels were present during any of the surveys, but this does not exclude the possibility of transient predators utilising the mussel beds. Although there were some signs of broken shell that might be consistent with fish or crustacean predation, the mussel shells within the beds were predominantly intact which suggested that this type of predation was not a prominent feature.

2.3.5 Mussel size

Mussel size showed a significant effect for sampling date ($F_{3, 60} = 76.10$, $p < 0.001$) with the mean size of mussels increasing significantly on each successive sampling date. Mussel size as estimated from measurements of shell length pooled from across all beds increased from an initial mean of 86.0 ± 0.48 mm in February 2014 to 109.4 ± 1.9 mm on the final sampling date in March 2016, 25 months later (Figure 2.6). This pattern was consistent across all four mussel beds as indicated by the lack of a significant interaction term in the analysis ($F_{9, 60} = 1.12$, $p = 0.36$) (Table 2.1). Mussel size differed among the four mussel beds ($F_{3, 60} = 9.26$, $p < 0.001$) which likely represents initial differences in the size of the mussels deployed from each bag, a difference which was maintained throughout the 25 months of sampling given the non-significant interaction term in the analysis. The error around the mean mussel size increased over the course of the study. This was most likely due to the decreasing sample size of mussels measured from each quadrat rather than an increase in the variability of mussel sizes. Since only the mussels measured in the four quadrats were utilised in this analysis, the sample size decreased over time as densities decreased. The mean rate of growth in mussel size (SL) showed significant effects of mussel bed ($F_{3, 240} = 3.49$, $p = 0.016$), sampling interval ($F_{3, 240} = 6.35$, $p < 0.001$), and their interaction ($F_{9, 240} = 3.88$, $p < 0.001$) (Table 2.1). However, there were no consistent patterns of difference in the mean rate of mussel growth associated with time, seasonality, or mussel bed. The maximum shell length encountered during sampling dates also increased throughout the study period from 120 mm to 136 mm SL. Mussels less than 60 mm SL were only observed once in February 2015, when three mussels recruits were observed within bed II (26 and 32 mm SL) and bed I (45 mm SL).

2.4 Discussion

2.4.1 Persistence of mussel beds

All four experimental mussel beds persisted until the end of the study, 27 months after they were deployed to the soft-sediment sea floor of the Hauraki Gulf. The persistence of these restored mussel beds showed at least an equivalent length of time to the persistence of mussels deployed in bottom culture practices in the Dutch Wadden Sea (Ysebaert et al., 2009), from which the deployment methods in this study were adapted. Despite the persistence of the experimental mussel beds in this current study, there were significant declines in the abundance of mussels across all beds throughout the study. Only 26.2% of the estimated abundance of mussels from February 2014 survived to the end of the study and at this rate of loss, it is reasonable to predict that these beds will cease to exist by 2017.

The methods used to initially establish these beds were clearly successful regardless of whether the beds persisted long term. The mean percent mortality between the deployment in November 2013 and the first initial sampling in February 2014 was estimated at 1.4% $month^{-1}$ which was lower than the mean rate of mortality in the beds (3.3% $month^{-1}$) observed throughout the remainder of the study. The variability around the estimated mean percent loss was quite high among the beds which was likely due to differences in weight of bags and initial size (and by extension, the weight) of mussels in those bags compared to the assumed weight of bags and length. The estimated mortality during this period and after deployment is likely to be low, however, it is difficult to determine whether this high variability represents actual differences in mortality across beds or is the result of the many assumptions used to estimate initial numbers deployed. Regardless, the mortality of mussels as a result of being buried under the weight of conspecifics during deployment could possibly be avoided by

deploying mussels in lower densities. Transplanting mussels at lower densities has already been shown to increase survival in *M. edulis* (Capelle et al., 2014) and could further reduce this initial mortality in future deployments of *P. canaliculus*.

Not all of the mussel beds that were deployed were persistent throughout the study. Two of the three additional experimental mussel beds were fragmented by storm waves early in the study during 5 - 8 June 2014 with the mussels being dispersed over a wider area. Therefore, it would be prudent for future mussel restoration initiatives to avoid deployments in shallow water.

2.4.2 Recruitment

The decline in these restored mussel beds is due in part to a lack of recruitment. A total of only three mussel recruits were observed on only one of the five sampling dates across the entire 25 month study, which indicates that there was little to no recruitment for most beds. This could be due to insufficient larval supply to the restored mussel beds given that previous studies have shown little to no larval settlement within the nearby Firth of Thames area of the Hauraki Gulf, with only six settlers found on a set of six larval settlement collectors over an 18 month period of sampling (McLeod, 2009). Although there are sources of larvae in the Hauraki Gulf, most notably from the numerous adult mussels in large scale aquaculture operations, dispersal of larvae may not be supplying the restoration site. A lack of settlement substrate could also be contributing to the low levels of recruitment as green-lipped mussel settlement is enhanced in the presence of both filamentous structures and chemical cues (Buchanan & Babcock, 1997; Alfaro et al., 2006; Gribben et al., 2011; Ganesan et al., 2012), associated with their preferred settlement substrate, filamentous macroalgae (Buchanan & Babcock, 1997; Alfaro et al., 2004; Alfaro, 2006b; Alfaro et al., 2010). In areas with well

established green-lipped mussel populations, such as Ninety Mile Beach, filamentous macroalgae can be inundated with settling mussel larvae at certain times of year (Buchanan & Babcock, 1997; Alfaro et al., 2004). Filamentous macroalgae are often lacking in soft-sediment environments due to a lack of substrate for attachment and were not observed within the experimental mussel beds or on the surrounding soft-sediment throughout the entire study period. Post-settlement mortality could also be contributing to the near lack of recruitment. A community of small mobile invertebrates and small demersal fish rapidly became established in the experimental mussel beds, some of which may prey upon recruiting mussels. The adult mussels themselves are also known to cannibalise settling larvae and even ingest early post-settlers, resulting in mortality due to being wrapped in mucus and smothered in pseudofaeces (Buchanan & Babcock, 1997; Alfaro, 2006a; Porri et al., 2008). The relative importance of the availability of larval supply, settlement substrates, and post-settlement mortality to the low recruitment observed in this study require further investigation if restoration practices are to be modified to overcome this critical limitation.

2.4.3 Mortality

Although the magnitude of mortality observed within the restored mussel beds is clearly greater than recruitment, it does not appear to be greater than that observed in some natural populations of this species. Measurements of the size of the green-lipped mussel population at the Waimangu Point in the Hauraki Gulf showed average declines in the population as high as 11.8 % month^{-1} (McLeod, 2009), which was comparable to the maximum recorded decline of 12.3 ± 1.2% month^{-1} for the restored mussel beds in this study. At three other natural green-lipped mussel beds along Ninety Mile Beach, the recapture of marked mussels of a single cohort resulted in estimated rates of loss of 3.1%, 5.4%, and 6.8% month^{-1} over the one

year study (Alfaro et al., 2008). The estimated mortality rate across the restored mussel beds was largely comparable to the aforementioned mortality rates from previous studies, with a mean mortality rate of 3.3 ± 2.6% month^{-1}. The mortality observed in these restored mussel beds may not represent an elevated level of mortality, however, in the absence of recruits, even natural levels of mortality will be a limitation to the persistence of restored mussel beds.

There are a number of potential contributors to the mortality of mussels within the beds which warrant further investigation. Mortality appeared to occur evenly across the experimental mussel beds, with the overall extent of the beds remaining broadly consistent over the course of the study while the density of mussels declined. Loss of mussels due to bed fragmentation and some sources of predation (e.g., many fish predators) are typically characterised by the loss of mussels around the margins of the bed where mussels have the fewest attachments to conspecifics (Burch & Seed, 2000). Similarly, harvesting for human consumption by divers is an unlikely cause of the loss of mussels as it could be expected to result in the complete removal of patches of mussels rather than the selection of individual mussels across the bed. Had this type of removal been occurring it would either have reduced the bed area or left lasting bare patches within the bed which would have been obvious to research divers during the regular sampling. The rate of mussel loss showed no consistent pattern of change either over time or among the four mussel beds. The fact that the percent of mussels lost between sampling intervals did not change despite significant reductions in the population density strongly suggests that density-dependent factors were not responsible for this mortality. Furthermore, measured mussel densities within this study, which ranged from 100 ± 34 to 760 ± 90 mussels m^{-2} were well within those found in wild populations of this species. For example, the density of remnant beds in the Hauraki Gulf and Bay of Plenty ranged from 170 to 1200 mussels m^{-2} (McLeod, 2009) while the densities of mussel beds at Ninety Mile Beach, which also contained large numbers of small recruits, were all greater

than 22,400 mussels m^{-2} (Alfaro, 2006b). There were no temporal patterns in the rate of mussel loss from the mussel beds among the sampling intervals that would indicate either seasonal or episodic events. Rates of predation of sessile organisms are often characterized by seasonal changes in the feeding behaviour of their predators and the observed lack of seasonality in mussel mortality suggests predation may not be a major contributor to the overall mortality. Harmful toxic microalgae blooms and some types of disease and parasitism often tend to be episodic in their impacts on organisms by causing very high rates of mortality (Perkins, 1976; Shumway, 1990), neither of which were observed in the mussel beds during this study. Green-lipped mussels are capable of living for at least 4 years and for one natural population at Ninety Mile Beach, the adult mussel population was comprised of mussels that were on average 2.5 years in age (Alfaro et al., 2008). Based on growth rates for aquaculture mussels (Jeffs et al., 1999), the deployed mussels were between 6 and 18 months of age which would make them 2.75 - 3.75 years old at the end of the study. In addition, the maximum size of mussels at the restored beds was also relatively small (136 mm SL) compared to historic subtidal mussel beds in Pelorus Sound (>150 mm SL)(Stead, 1971) and dead mussel shells retrieved from within the soft-sediments at the restoration site (>150 mm SL, personal observation), suggesting that these mussels were capable of reaching greater sizes and potentially greater ages. Although the mussels were still relatively young at the conclusion of the study, if mussels were approaching senescence, the mortality rate could have been expected to increase towards the end of the study, which it did not.

Although patterns of mortality do not suggest predation to have contributed greatly to mortality, the presence of the sea star, *C. muricata*, in relatively high abundances on all four mussel beds throughout the study was the only directly observed cause of either predation or mortality. Sea stars are known predators of bivalves and their ability to impact population dynamics has been well studied (Paine, 1966; Paine, 1969; Dayton, 1971; Paine, 1971).

Predator-prey interactions between these two species have not been well studied making it difficult to predict the potential impact of sea star predation on these mussel populations . In Ōhiwa Harbour, in the Bay of Plenty, New Zealand, high densities of sea stars were observed to be feeding on the local mussel population (Paul-Burke, 2015). Predation by these sea stars was thought to have contributed considerably to the observed loss of 88% of the green-lipped mussels over four years. Predation by *C. muricata* on another bivalve species, the pipi, *Paphies australis*, has shown sea stars to consume an average of one pipi per day, regardless of prey or predator size (Cook, 1989). The pipis used in this previous study were smaller than the average size of mussels within the experimental mussel beds, but if the predation rate is even half that observed for pipis, the high abundance of sea stars throughout the study (mean of 16 ± 1 sea stars bed^{-1}) could have resulted in a significant amount of predation. Greater investigation into the feeding ecology of these sea stars on green-lipped mussels is needed in order to determine their contribution to the observed mussel mortality.

Environmental conditions at the restoration site may also be contributing to mortality among mussels in the experimental beds. The mussels transplanted from aquaculture grow out ropes were grown in suspended culture in the Hauraki Gulf, but derived from seed mussels originally sourced from Ninety Mile Beach at the northern tip of the North Island some 450 km away by sea. Efforts to enhance natural marine populations have shown that not all environments are optimal for the survival of individuals from either non-native wild or hatchery-reared stocks (Munro & Bell, 1997; Arnold, 2001; Le Vay et al., 2007; Arnold, 2008). In some instances efforts to transplant mussels into non-natal areas have been shown to result in elevated mortality for the mussels (Mallet et al., 1987; Stirling & Okumuş, 1994). The known populations of mussels in the Ninety Mile Beach area grow on hard substrate surfaces in areas with high wave exposure and water turbulence (Alfaro, 2006b; Alfaro et al., 2008) and may not be suited to the soft-sediments and more benign conditions of the Hauraki

Gulf. Furthermore, the morphology and behaviour of the adult mussels transplanted to the experimental beds will have developed under high growth conditions in suspended culture which may prove to be unsuitable for post-transplant existence on soft-sediments. The possible contribution of environmental conditions to the mortality of mussels warrants greater investigation given that the only available stock of adult mussels suitable for restoring mussel populations comes from commercial suspended aquaculture operations. If these stocks are not suitable for long term survival in this environment, then alternative sources of mussels for transplant will need to be identified before any further restoration can be initiated.

2.4.4 Growth

Although the experimental mussel beds exhibited declines in abundance, the mean size, as measured by SL, of the remaining mussels continually increased throughout the study. This increase in mean mussel size could be the result of either individual growth of mussels and/or increased mortality of smaller-sized mussels. The corresponding increase in the maximum size of mussels over the study suggests that this increase in shell length is largely due to growth. The rate of growth for mussels on the experimental beds (11.7 mm $year^{-1}$) was comparable to that of the growth rates previously recorded on natural and experimentally deployed benthic mussel beds. For example, at Ninety Mile Beach growth rates of small mussels (20 - 30 mm SL) across three intertidal beds ranged from 7.2 ± 1.2 mm $year^{-1}$ to 45.6 ± 3.6 mm $year^{-1}$ (Hickman, 1979; Alfaro et al., 2008). Growth rates of green-lipped mussels deployed in the experimental plots in the nearby Firth of Thames in the Hauraki Gulf also exhibited low but comparable rates of growth at 14.6 mm $year^{-1}$ for mussels of a comparable size to the present study (McLeod et al., 2012). This continuing growth rate of mussels in the experimental beds suggests that environmental conditions in the experimental beds were

sufficient to maintain growth. This would suggest that environmental conditions may not have been the direct cause of mussel mortalities. The lack of an increase in the rate of growth as the density of mussels within each bed declined also suggests that density-dependent factors were not adversely affecting the growth of mussels.

2.5 Conclusions

Restoration of mussel beds has only recently received attention and unlike oysters, the practices for their restoration are not well developed. This study has successfully demonstrated that the adaptation of common bottom culture practices for deploying mussels to the soft-sediment is an effective means of initially establishing green-lipped mussel beds. These mussels are clearly capable of surviving and forming coherent mussel beds on soft-sediment and are in sufficient condition to allocate resources to somatic growth. The lack of observed density-dependent effects suggests that modifications to reduce the density of mussels upon deployment are unlikely to increase the long term survival of transplanted mussels. However, the long term persistence of these restored beds is limited by insufficient recruitment to offset the rate of mortality. Understanding and addressing the factors which are contributing to this lack of recruitment will be critical to developing effective mussel restoration techniques. The absence of preferred natural larval settlement substrate from the restored mussel beds is an obvious starting point for further investigation. Although mortality may be at natural levels in the restored populations, isolating the relative contribution of the various sources of mortality may help to identify potential modifications to restoration practice to decrease mussel mortalities. Sea star predation and the biological suitability of the source of mussels used for transplanting are also key issues that with further research are likely to deliver improved outcomes for future restoration efforts. The success of future

restoration efforts for green-lipped mussels will rely on advancing the effectiveness of restoration practices if restored beds are to have any chance of being sustainable.

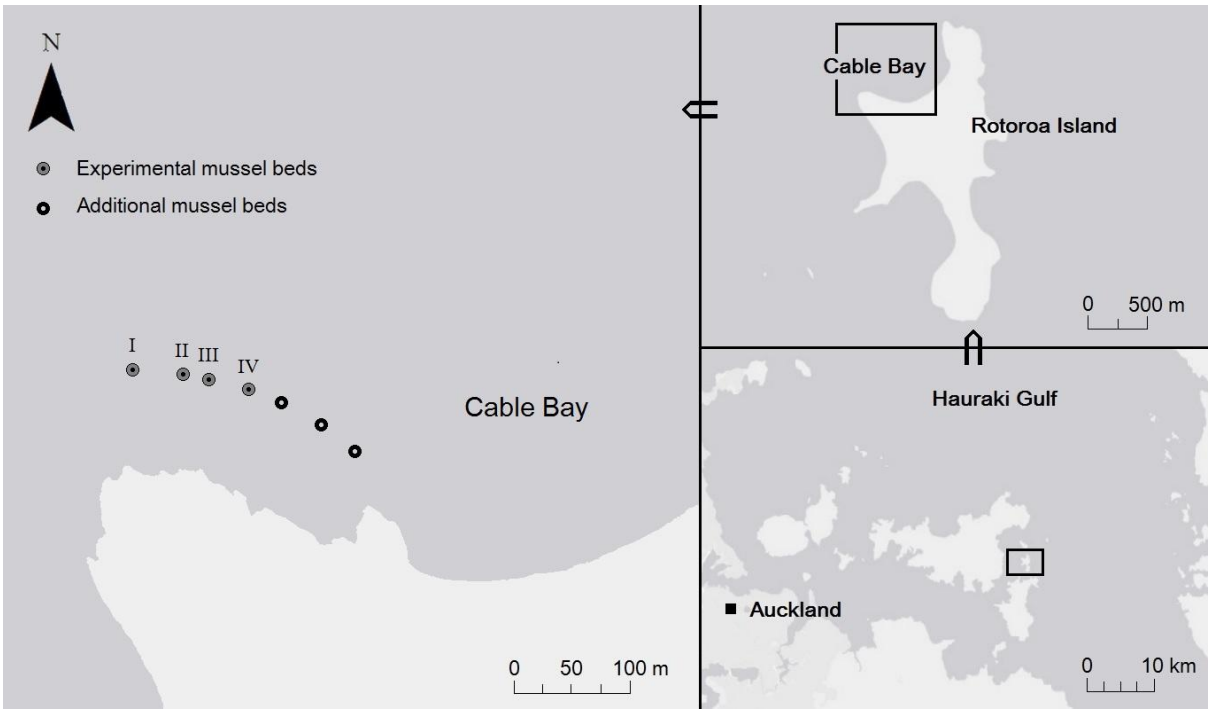

Figure 2.1 Map of restoration site with deployed bed locations indicated by roman numerals.

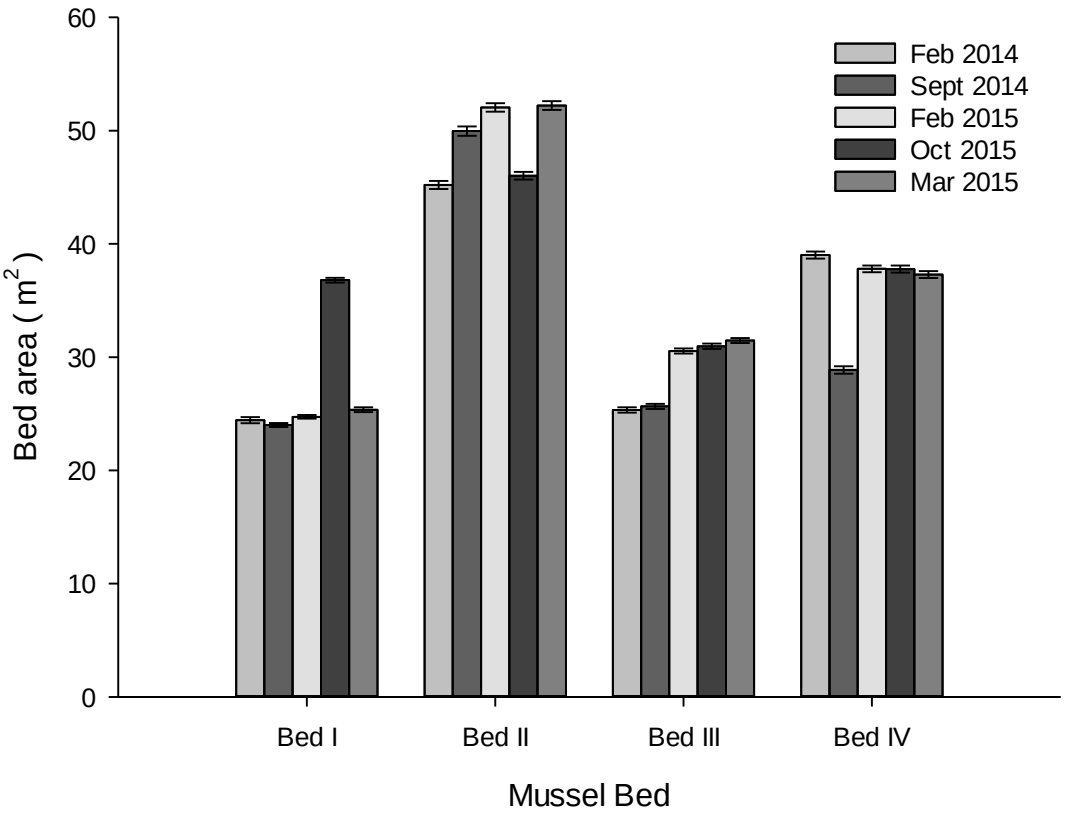

Figure 2.2 Bed area for each of four experimental mussel beds on each sampling date over two years. Error bars for bed area denote propagation of error in the precision of the measurements.

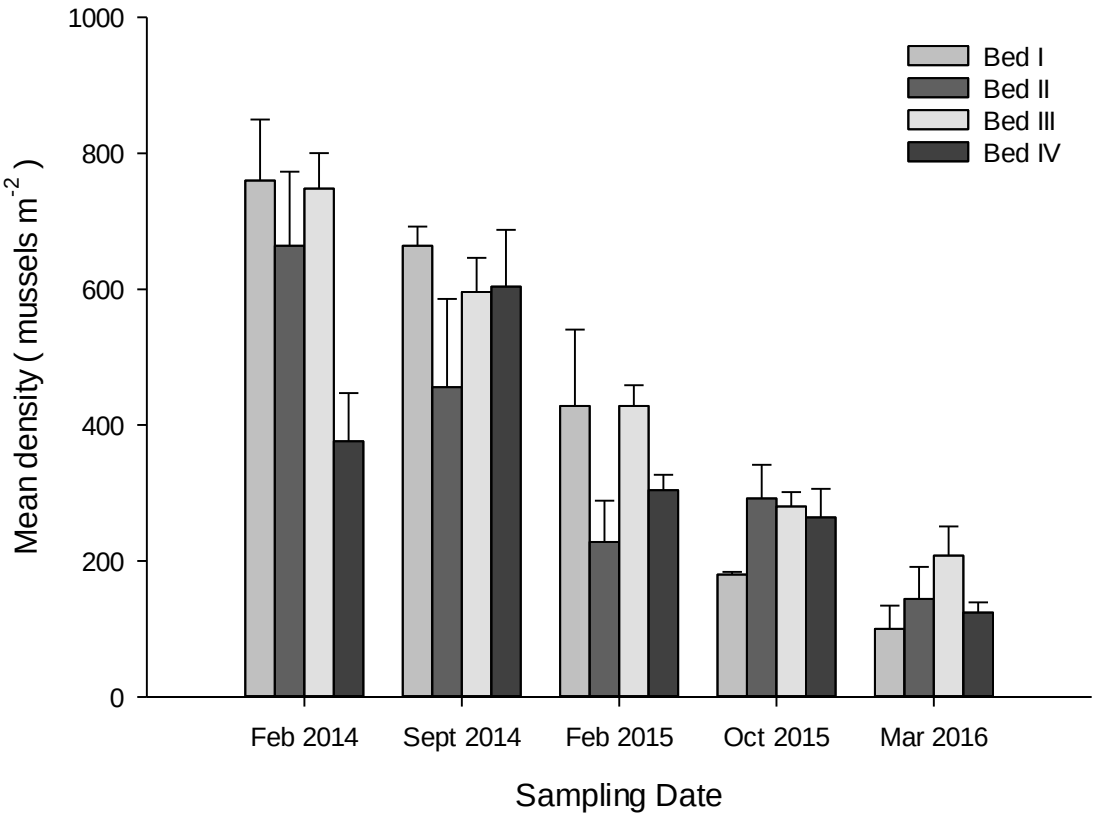

Figure 2.3 Mean (±SE) density of mussels for each of four experimental mussel beds for each sampling date.

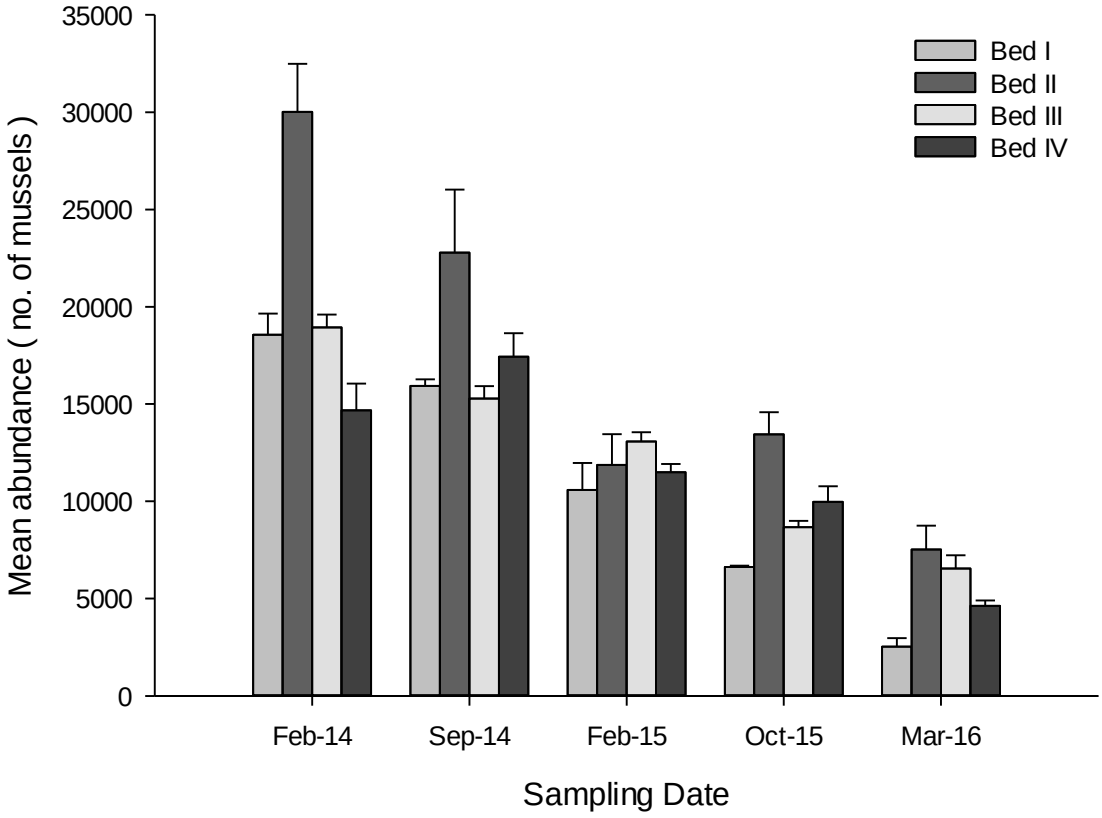

Figure 2.4 Mean (±SE) abundance of mussels within each of four experimental mussel beds for each sampling date.

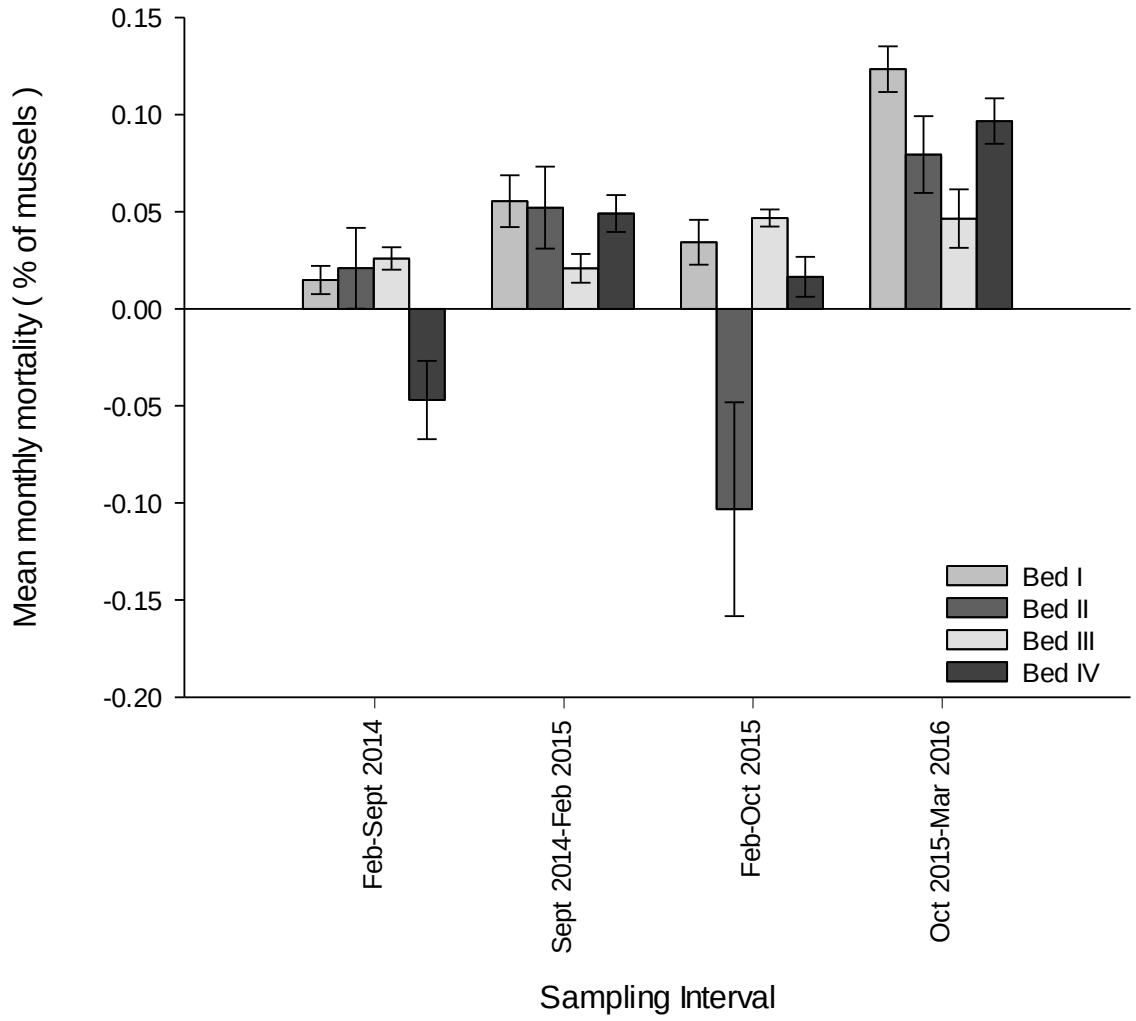

Figure 2.5 Mean (±SE) rate of mussel mortality for each of four mussel beds over each sampling interval.

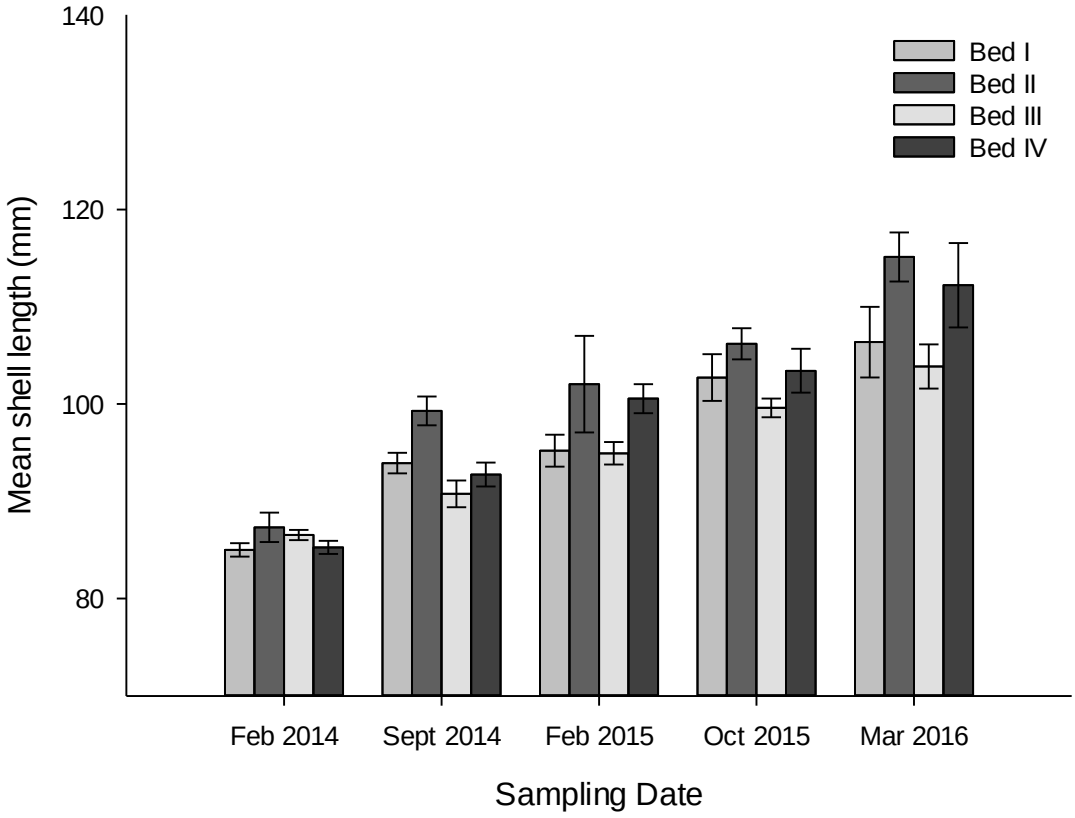

Figure 2.6 Mean (±SE) mussel shell length for each of four experimental mussel beds for each of the sampling dates.

Table 2.1 Results of ANOVA comparisons of means for the variables; density, abundance, loss rate, growth rate and shell length for four adult mussel beds placed on soft-sediment in the Hauraki Gulf and measured over two years. Significance was determined at an α of 0.05.

	Source of Variation	DF	MS	F value	P value
Density	Mussel Bed	3	62263	3.76	0.015
	Sampling Date	4	711765	42.99	<0.001
	Mussel Bed * Sampling Date	12	38709	2.34	0.016
	Residual	60	16557		
Abundance	Mussel Bed	3	0.95	4.76	0.005
	Sampling Date	4	5.73	28.70	<0.001
	Mussel Bed * Sampling Date	12	0.28	1.42	0.181
	Residual	60	0.20		
Percent Loss	Mussel Bed	3	0.02	3.72	0.012
	Sampling Interval	3	0.11	18.28	<0.001
	Mussel Bed * Sampling Interval	9	0.03	5.31	<0.001
	Residual	240	0.006		
Growth Rate	Mussel Bed	3	3.01	3.49	0.016
	Sampling Interval	3	5.47	6.35	<0.001
	Mussel Bed * Sampling Interval	9	3.34	3.88	<0.001
	Residual	240	0.86		

		Num DF	Den DF	F	p
Shell Length	Mussel Bed	3	60	9.26	<0.001
	Sampling Date	4	60	76.10	<0.001
	Mussel Bed * Sampling Date	12	60	1.12	0.3622
	Intercept	1	1896	46434.20	<0.001

Chapter 3

Impacts of sea star predation on restored mussel beds

3.1 Introduction

Predatory sea stars can play a major role in structuring intertidal and subtidal benthic communities, especially those dominated by sedentary bivalves, such as mussels (Paine, 1966; Paine, 1974; Menge et al., 1994) where predation often limits the abundance of these populations and influences their distribution (Dayton, 1971; Paine, 1971; Paine, 1974; Navarrete & Menge, 1996; Robles et al., 2009). Although many species of sea stars coexist with mussel populations (Paine et al., 1985), sea star predation can also result in elevated levels of mortality within mussel populations. For example, the sea star *Asterias rubens* forms huge aggregations on beds of the blue mussel, *Mytilus edulis* (Sloan & Aldridge, 1981). Such aggregations can consume up to 50 ha of natural beds of mussels in three months, a quantity equivalent to 3500 t of juvenile mussels (20 mm shell length) (Dare, 1982). Predation by sea stars can also result in the consumption of entire beds of relayed mussels for aquaculture purposes, such as was observed for *A. rubens* which consumed an entire bed of 100 t worth of *M. edulis* (Kristensen & Lassen, 1997).The enormous potential predation pressure from sea stars can also impose a significant limitation to the success of bivalve restoration efforts. The persistence of re-established bivalve populations relies on recruitment to be either equal or greater than the amount of natural mortality, including predation. Therefore, understanding the magnitude of mortality in restored populations of bivalves from sources such as sea star predation is a critical component to determining the success of restoration efforts by identifying limitations to the survival of the bivalves.

Chapter 3

Predation by sea stars poses a potential limitation to the restoration of depleted populations of green-lipped mussels, *Perna canaliculus*, on the soft-sediment seabed of the Hauraki Gulf, New Zealand (Greenway, 1969; Reid, 1969; McLeod, 2009), which were previously removed by overfishing (Paul, 2012). Previous studies have shown sea star predation can influence the abundance of green-lipped mussels (Paine, 1971; Paine et al., 1985). Sea stars, such as *Coscinasterias muricata*, are known to aggregate in high densities within natural beds and beneath farms of green-lipped mussels, feeding on mussels that have presumably dropped from the farms above (Inglis & Gust, 2003; Paul-Burke, 2015). Higher densities of these sea stars appear to be associated with concentrations of live adult mussels even within natural beds of mussels (Inglis & Gust, 2003; Paul-Burke, 2015). *Coscinasterias muricata* is commonly found throughout the Hauraki Gulf and the establishment of the first restored beds of green-lipped mussels in 2013 (see Chapter 2) quickly resulted in a high abundance of sea stars on these beds. However, there is limited information on the feeding ecology of *C. muricata* on *P. canaliculus* with which to begin to determine their potential impact via predation upon the transplanted adult mussels. Given that previous studies have suggested that restored mussel beds in the Hauraki Gulf system are likely to be recruitment limited due to a near lack of observed settling mussels over a 18 month period (McLeod, 2009), it will be critical to determine whether predation by sea stars will preferentially target the potentially small number of recruiting mussels. The current study therefore aims to; 1) describe patterns of sea star abundance across experimental beds of adult mussels restored into the Hauraki Gulf, 2) develop a quantitative estimate of mortality of mussels due to sea star predation to determine the relative contribution to overall mortality for these experimental beds, and 3) determine if these sea stars preferentially select for juvenile and post-settling mussels rather than the restored adult mussels.

3.2 Materials and methods

3.2.1 Mussel abundance and size within experimental mussel beds

Seven experimental mussel beds were deployed on 28 November 2013 in a sheltered embayment on the northern side of Rotoroa Island in the Hauraki Gulf, New Zealand (S 36º 48' 32", E 175º 11' 37") (see chapter 2). Each mussel bed consisted of approximately 1 t of mussels (70 - 100 mm shell length) placed on the seabed in shallow water between 3.9 - 5.1 m below chart datum. The seafloor sediment in this experimental area initially consisted of a 5 cm surface layer of fine mud which eroded away during the study, leaving the underlying firmer mud, sand, and shell hash as the predominant sediment. Mussel beds ranged in size from 24.4 m^2 to 45.2 m^2 and were separated by 9 - 35 m. Four of the experimental mussel beds were sampled approximately every six months for population size and the size frequency of resident mussels. At each sampling date four 0.0625 m^2 quadrats were placed haphazardly across each mussel bed and the density of mussels within each quadrat was measured as well as the shell length of each mussel. Mussel size as measured by shell length was sampled to quantify the number of recruiting mussels. Recruiting mussels were identified as being <60 mm in shell length (SL), a size that would have been smaller than any of the initially deployed mussels. Bed area was determined by first running a transect line across the longest axis of the mussel bed to measure the length of the bed. At each marked 1 m interval of the transect line, the width of the bed was measured. The measured margins of the bed were later mapped and the resulting areas summed in order to estimate bed area. Population size of each bed was then calculated as the product of bed area and mussel density for each bed on each sampling date. Mortality of mussels during intervals between sampling dates

(sampling intervals) were calculated as the difference between initial and final estimates of mussel abundance for each bed.

3.2.2 Sea star abundance and size within experimental mussel beds

On each sampling date, the total number of sea stars within each mussel bed was counted. On 1 October 2015 the size of individual sea stars on the mussel beds was also measured. The density of sea stars was calculated for each bed at each sampling date separately. The data were inspected for deviations from normality and heterogeneity of variance firstly by visually inspecting quartile-quartile plots and plots of residuals versus fitted values (respectively) of models prior to running the analysis. The data were fitted to a linear mixed effect model (LME) and tested for differences in density among sampling dates using a repeated measures ANOVA with mussel bed as the subject. In the event of significance, pairwise *t*-tests ("predictmeans" function in R) were conducted using a false detection rate correction ("fdr" function in R). In addition, correlation between sea star density and mussel density for each bed and each sampling date was investigated using Pearson's product-moment correlation ("corr.test" function in R).

3.2.3 Consumption rates

Consumption rate of adult mussels by sea stars was measured using a field-based feeding experiment deployed in Leigh Harbour, New Zealand (S 36º 17' 14", E 174º 48' 37") on 1 April 2015. A total of 18 cages of 0.15 × 0.5 × 0.5 m were constructed of coarse plastic mesh (20 mm openings) and deployed at a depth of 3.2 m below chart datum, in an area of sea floor composed of sand substrate. The plastic cages were held in place by a frame made from

lightweight stainless steel (5 mm diameter) which extended into the seabed. Cages were arranged so that they were no greater than 1 m apart. The cages were designed to prevent large mobile predators, such as fish and lobster, from removing the mussels placed inside the cage, while not unduly restricting water flow to the mussels and sea stars within. Each cage was stocked with 25 adult mussels (80 - 100 mm SL). In 12 randomly selected cages one adult sea star ranging in size from 14.5 - 20.0 cm was added. These sea stars were collected at random from the mussel restoration site on mussel beds away from those beds being regularly sampled for changes in mussel abundance and transported back to the Leigh Marine Laboratory prior to deployment in the field. The six remaining cages were controls that were not subjected to sea star predation. The adult mussels were provided from a local aquaculture operation and several months prior to the experiment had been transplanted to the seabed in a local embayment to later be moved into the experiment. On 30 April, 19 May, and 30 June 2015, divers opened each cage, counted the number of surviving mussels in both sea star and control cages, and restocked the number of mussels back to 25 individuals. A HOBO temperature data logger was attached to one of the cages and measured seawater temperature every 15 min during the study period. These data were compared to temperature data gathered by a HOBO temperature logger placed within the restoration site to ensure the decreasing seawater temperatures experienced in the present feeding study during the fall to winter season largely encompassed the range of temperatures experienced at the restoration site. The consumption rate of mussels by sea stars over each sampling interval was standardised to a daily rate and further adjusted to account for natural mortality by subtracting the mean mortality rate of control cages from the consumption rate. The data were inspected for deviations from normality and heterogeneity of variance firstly by visually inspecting quartile-quartile plots and plots of residuals versus fitted values (respectively) of models prior to running the analysis. Mussel consumption data were then fitted to a linear

mixed effects model (LME) with sampling interval and size as fixed effects and individual sea stars as a random effect within size. Repeated measures ANOVA was then used to determine differences in consumption rate due to either sea star size or sampling interval. In the event of significance, pair-wise *t*-tests ("predictmeans" function in R) were conducted using a false detection rate correction ("fdr" function in R). Although relationships between body size and consumption rate have been observed for sea stars (Gooding & Harley, 2015; St-Pierre & Gagnon, 2015), previous studies with *C. muricata* have shown no significant differences in the consumption rate of the pipi, *Paphies australis*, regardless of the size of sea star or pipi offered (Cook, 1989). Therefore, size was included in order to confirm this lack of relationship between body size and consumption rate in *C. muricata*. All statistical tests were computed in R version 3.2.3 and RStudio version 0.99.879.

3.2.4 Estimating predation in experimental mussel beds

Estimated predatory impact was calculated using the modified equation from Dempster (1960) and is as follows:

$$N = \sum_{i=1}^{n} C\left(\frac{P_i + P_{i+1}}{2}\right) t_{i,i+1}$$

where N is the total number of mussels consumed summed over all sampling intervals, C is the consumption rate of mussels per sea star derived from the consumption rate experiment, P_i is the initial number of sea stars within the mussel bed for the sampling date, P_{i+1} is the number of sea stars within the mussel bed for the following sampling date, and $t_{i,i+1}$ is the amount of time between sampling dates or the sampling interval.

The equation makes several assumptions in order to produce this quantitative estimate. The first is that consumption rate will be relatively constant. Sea stars are known to exhibit high and low periods of food consumption associated with factors such as temperature, level of satiation, and reproductive cycle (Menge, 1972). The effects of varying temperature on consumption rate are accounted for in the range of temperatures experienced during the consumption rate experiment which was similar to the range of temperatures experienced at the restoration site. Satiation is accounted for in the length of sampling intervals (i.e., months) within the consumption rate experiment. Sea stars are likely to experience both hunger and satiation over such time periods. However, there was insufficient information to incorporate any differences that may exist in sea star consumption rate related to their reproductive cycle.

The second assumption is that sea stars consume primarily mussels within the mussel beds and thus the consumption rate will reflect rates determined in the consumption rate experiment. Although the exact composition and densities may not be the same, epibionts (algae, sponges, bryozoans, barnacles, tunicates) were present on the mussels and numerous other organisms (hermit crabs, triplefin fish, gastropods) were seen utilising the structure created by the mussels within the experimental cages in the feeding experiment. Therefore, it is likely that sea stars had similar access to alternative prey as was available on the experimental mussel beds.

Estimated predatory impact was calculated for each bed at each sampling interval and compared to estimated overall abundance and mortality of mussels within the experimental mussel beds.

3.2.5 Prey size selection

On 8 October 2015, 12 sea stars (*C. muricata*) were collected haphazardly from the mussel restoration site from the three mussel beds that were not subject to regular sampling for changes in mussel abundance. Sea stars were transported in seawater back to the Leigh Marine Laboratory where they were held in flow-through seawater tanks at ambient temperatures (14.1 - 22.7 °C) and provided with adult green-lipped mussels as a source of food. The sea stars ranged in size from 10.5 - 23.5 cm as measured from the tip of the longest arm to the opposite side of the oral disc. Mussels collected from Pakiri Beach (S 36º 15' 31", E 174º 45' 4") were classified into three size groups based on shell length (SL): small (8 - 20 mm), medium (30 - 50 mm), and large (60 - 80 mm). The experimental setup consisted of six 129 l black rectangular plastic tanks supplied with flow-through sea water at ambient temperatures. Sets of six mussels of each size class were placed into each of the experimental tanks with care taken to ensure that mussels within each size group within a tank covered the full range of that size group. A single adult sea star was placed in five of the six tanks while the sixth tank was used as a control treatment. After one week, surviving mussels in each tank were measured and the number of consumed mussels of each size class were counted. Due to the high variability in consumption rates of sea stars over a one week period, ranging from no mussels to all the mussels provided, only sea stars that had consumed between 3 - 15 of the mussels were included in subsequent analyses and these sea stars were replaced in their tanks with fresh individuals. In the event that a sea star had not consumed 3 - 15 mussels during the one week period, they were retained to be re-run in the following week. All tanks were supplied with fresh mussels, including the control tank, and the experiment was run again. This continued until all sea stars had consumed between 3 - 15 mussels in a one week period and could be used in the analysis. The experiment was conducted from 14 October 2015 to 24

December 2015, over which a total of seven trials were run. Only one sea star did not eat over this time interval. Differences in the frequency of consumption of mussels from each size class was analyzed first using a heterogeneity chi-square test to determine if there were differences between sea stars before performing a chi-square test using the pooled data. In the event of significance, the contribution of mussel size groups to that difference were determined using standardized residual analysis.

3.3 Results

3.3.1 Mussel abundance and size within experimental mussel beds

High mortality of adult mussels was estimated to be occurring in all four experimental mussel beds over the 25 months of observation. At the end of the study the mean survival across all experimental beds was 5307 ± 1102 mussels representing 26.2 ± 4.6% of the initially estimated abundance in the four mussel beds in February 2014 (Figure 3.1). However, the size of each bed remained relatively constant over the entire study. The mean loss of mussels from individual beds for individual sampling intervals ranged from 217 ± 115 mussels month^{-1} to 1819 ± 538 mussels month^{-1}. In February 2015 a total of three recruits with shell lengths of 26, 32, and 45 mm were observed in only two of the four mussel beds. No other recruits were observed on any other sampling date.

3.3.2 Sea star abundance and size within experimental mussel beds

Initial mean sea star abundance in February 2014, approximately two months after the deployment of the mussel beds was 8.25 ± 0.95 sea stars bed^{-1} and quickly grew to 17.50 ±

3.10 sea stars bed^{-1} by September 2014 (Figure 3.2). Mean abundance of sea stars remained relatively high until the end of the study in March 2016. Density of sea stars within the mussel beds over the 25 month study ranged from 0.21 - 1.25 sea stars m^{-2} of mussel bed. Sea star density showed a significant difference among sampling dates ($F_{4,12} = 3.62$, $p = 0.04$) (Table 3.1) which was due to a significantly lower density of sea stars on the first sampling date. There were no significant differences among any of the other sampling dates (all $p > 0.93$). This initial difference is likely to represent an early period of sea star immigration from the surrounding soft-sediment habitat and rocky foreshore. Abundances of sea stars on individual experimental mussel beds rarely decreased between sampling dates and it is likely that many of the sea stars remained within the boundary of the mussel beds throughout the study. Qualitative surveys of the soft-sediment before and after deployment indicated very low densities of sea stars, and no sea stars were ever observed on the soft-sediment between mussel beds. There was also no significant correlation detected between densities of sea stars and density of mussels within each bed (correlation coefficient $r = -0.254$, $t = -1.1$, $p = 0.28$). The size of the sea stars measured on 1 October 2015 ranged from 9.0 - 35.0 cm with a mean of 21.9 ± 0.4 cm, which was characteristic of the size of sea stars observed on the experimental mussel beds throughout the study (Figure 3.3).

3.3.3 Consumption Rate

Due to the loss of several cages on the final sampling date, consumption rate was only available for seven sea stars cages and mortality for only five control cages. There was a significant effect of sampling interval on the daily rate of consumption of mussels by the caged sea stars ($F_{2,10} = 15.31$, $p < 0.001$) and the non-significant interaction term indicates that these differences were consistent for all sea star sizes ($F_{2,10} = 0.50$, $p = 0.62$) (Table 3.2).

Mean consumption rate was highest during the third sampling interval (0.55 ± 0.05 mussels day^{-1}) which was significantly higher than both previous sampling intervals (Figure 3.4, both $p < 0.008$). However, the mean consumption rate was similar between the first (0.35 ± 0.04 mussels day^{-1}) and second (0.25 ± 0.04 mussels day^{-1}) sampling dates ($p = 0.09$). Sea star size had no effect on consumption rate ($F_{1,5} = 5.50$, $p = 0.07$). Mean temperature decreased as the experiment progressed, with a range of 13.4 - 21.3 ºC which largely corresponded with the range observed at the restoration site of 12.3 - 24.1 ºC.

3.3.4 Estimating predation in experimental mussel beds

Estimates of sea star predation upon adult mussels within the experimental mussel beds used a consistent consumption rate across all sampling intervals (0.41 ± 0.03 mussels day^{-1}) which was derived from the consumption rate experiment. This consisted of the total consumption of mussels over the entire experiment. In so doing, any differences in consumption rate of sea stars that could be due to seawater temperature or satiation were included in the overall estimate of consumption rate. Sea stars were also pooled to derive the consumption rate given the lack of difference associated with sea star size. Estimations of predation indicated that the estimated mean overall number of mussels consumed by sea stars over the 25 month study was 5863 ± 853 mussels (Figure 3.1). This represents 30.1 ± 5.5% of the initially estimated abundance of mussels in February 2014. The relative contribution of this estimated predation to the overall mortality of mussels in the experimental beds over the 25 month period was 40.4 ± 6.3%. The causes for the remaining 59.6% of the mortality of mussels over this period were not identified.

3.3.5 Prey size selection

The frequency of mussels from each of the three size groups that was consumed by sea stars was statistically similar among all of the sea stars tested (Table 3.3) and thus there were no differences associated with the experimental range in the size of sea stars. The pooled data revealed a statistically significant difference in the frequency of mussels consumed among the three size categories of mussels (Table 3.3). The frequency of mussels consumed by all 11 sea stars was 9, 37, and 38 mussels for the small, medium, and large size classes of mussels, respectively (Figure 3.5). Analysis of the standardized residuals found the lower consumption of mussels in the small size class to be the only statistically significant contributor ($z = 3.59$, $p < 0.001$) to the observed differences in consumption among mussel size classes. Throughout each weekly trial, mussels remained largely clumped in one or several small groups, with less than two solitary mussels per tank at the completion of each trial. Mussels in the small size class were predominantly seen attached to the larger conspecifics even as the larger mussels were being consumed. Both live and dead mussels of the small size class were observed attached to discarded mussel shells. Control tanks showed very little mortality with the pooled frequency of dead mussels across all seven control tanks being two mussels for each of the small, medium, and large size classes. This mortality equates to a mean (±SE) natural mortality of 0.86 ± 0.26 mussels week^{-1} overall or 0.29 ± 0.08 mussels week^{-1} within each category. No adjustments were made to the frequency of mussels consumed by sea stars given the small and even mortality across all size classes of mussels in the control tanks.

3.4 Discussion

3.4.1 Sea star abundance in experimental mussel beds

Only a few months after transplanting adult mussels onto the sea floor, the mussel beds had attracted high abundances of sea stars, likely from surrounding habitats. The density of sea stars continued to gradually increase until approximately 11 months after deployment of the adult mussels. Although long term tagging of the sea stars was not feasible due to the sea stars removing any form of tag provided within days (personal observation), the lack of decreasing density of sea stars within individual mussel beds within most sampling dates suggests that the sea stars that immigrated to the beds remained within those beds. Sea stars are known to locate prey through odour plumes (Rochette et al., 1994; Drolet & Himmelman, 2004), and the lack of appreciable immigration beyond September 2014 may suggest that the beds had attracted most of the sea stars from the surrounding areas of soft-sediment habitat early in the study. The deployment of a further 63 t of adult mussels in September 2014 about 100 - 500 m away considerably increased the availability of mussel beds within the area and may also have resulted in decreased localised immigration of sea stars to the four experimental beds that were the focus of this study.

The distribution of sea stars among the experimental mussel beds appears to be unrelated to the density of mussels. This is in contrast to observations on natural beds of this mussel species in Ōhiwa Harbour, in northeastern New Zealand, where sea star abundance was found to decline as the abundance of mussels in beds declined (Paul-Burke, 2015). Sea star density was positively correlated with mussel coverage of the substrate suggesting a possible relationship between predator and prey abundance. The densities of sea stars on the experimental mussel beds in the present study were considerably lower than those observed

for Ōhiwa Harbour. Despite declining densities of mussels within the restored mussel beds, the abundance of mussels may still have provided sufficient food sources to support these sea stars throughout the study.

3.4.2 Sea star predation in experimental mussel beds

The mean percent mortality of mussels within mussel beds over the 25 months was 73.8% and predation by the sea star, *C. muricata*, appears to be a major contributor to that mortality. The mean estimated predation was 30.1% of the original population, accounting for 40.4% of the total mortality. This represents a significant source of mortality for the experimental beds and a potential limitation to future restoration initiatives. Even in natural populations of green-lipped mussels such as that in Ōhiwa Harbour sea stars can gather in high densities resulting in significant amounts of predation for the mussels (Paul-Burke, 2015). The abundance of sea stars was found to be 1.2 million on extensive natural mussel beds in Ōhiwa Harbour in 2009 and subsequent sampling of mussel populations showed that the number of mussels had decreased by 88% (approximately 14 million mussels) between 2009 and 2013. Although the loss of mussels was not directly attributed to sea star predation it was highly likely they were a major contributor as they were observed actively feeding on mussels in the bed, despite a nearby abundance of alternative prey, being the bivalve, *P. australis*.

The presence of sea stars within natural green-lipped mussel beds varies spatially with high densities of sea stars in some mussel beds while others exhibited negligible amounts of sea stars (McLeod, 2009). Even when relaying mussels for bottom culture, similar to the methods used to restore beds in the current study, sea star predation is not consistent across those mussel beds (Kristensen & Lassen, 1997). The deployment of four *M. edulis* mussel beds, each of approximately 100 t in a Danish fjord, resulted in only one of the mussel beds

being completely consumed by *A. rubens* within three months of relaying while the other three mussel beds persisted throughout the 25 month study. Overcoming limitations of sea star predation to the persistence of relayed mussel beds may therefore be possible by selecting restoration sites that contain relatively low abundances of sea stars.

Predation by sea stars, however, varies not only spatially but also temporally, which may also be associated with swarming behaviour which has the potential to result in particularly high levels of mortality among prey populations. Sea stars, such as *A. rubens*, are known to form high density aggregations that are capable of causing mass mortality among populations of bivalve prey (Sloan & Aldridge, 1981; Dare, 1982). The high abundance of sea stars observed in Ōhiwa Harbour in 2009 was localized to a particular area within the mussel bed where densities were an average of 34 sea stars m^{-2} (Paul-Burke, 2015). In 2013 sea star abundance dropped to 4.3 sea stars m^{-2} and was dispersed throughout the mussel bed rather than concentrated in a particular area. This suggests that *C. muricata* may also exhibit this temporally variable swarming behaviour, which could result in intermittent impacts on prey populations.

For the effective restoration of mussel beds, some form of remediation from sea star predation may be necessary in the event that suitable restoration sites that are free of sea stars cannot be located or episodic increases in sea star abundance are subsequently observed within mussel restoration sites. A number of different methodologies have been developed to remove sea stars from seabed culture mussel beds (Barkhouse et al., 2007) which could be applied to alleviate mussels from sea star predation. In the Hauraki Gulf, the removal of sea stars could be particularly effective given that sea star density did not differ after a rapid initial colonization and the absence of any smaller recruiting sea stars on the beds. Episodic surges in sea star abundance are likely to cause greater mussel mortality and prevention

would be reliant on continual monitoring followed by implementation of sea star remediation techniques when an outbreak is detected.

3.4.3 Predation of juvenile and post-settlement mussels

Sea stars of the size occupying the mussel beds were shown to prefer to consume adult mussels and not small juvenile or early post-settlement mussels (<20 mm SL). Larger sea stars are known to select for larger mussels, which may not necessarily be of optimal size, but would provide higher nutritional returns than these small juveniles and post-settling mussels (Tokeshi, 1989; Hummel et al., 2011). However, these sea stars are not completely discriminatory when selecting prey and despite these previous studies showing that larger sea stars typically select for larger mussels, smaller and less nutritionally rewarding mussels are also consumed. In the case of the sun sea star, *Heliaster helianthus*, which consumes entire clumps of mussels, the smaller mussels in the clump are likely to be consumed as a result of being attached to larger, more rewarding mussels (Tokeshi, 1989). Within the selection experiment in the current study, both living and dead mussels of the smaller size class were observed attached to the shells of mussels which had been consumed by the sea stars and discarded. This suggests that those small mussels that were consumed were unlikely to be directly selected as prey but rather were bycatch of consuming the attached adult, and that attached juvenile mussels are not necessarily consumed when adult mussels are selected. Although these sea stars are capable of consuming recruiting mussels, it is unlikely that they are responsible for the near lack of juvenile mussels observed within the experimental mussel beds throughout the 25 month study.

3.5 Conclusion

Efforts to restore mussel beds in the Hauraki Gulf are constrained by high mortality among transplanted adult mussels and estimations of predation by sea stars in the current study suggest that they contribute a considerable amount to that mortality. Predation by these sea stars, however, does not appear to be contributing to the lack of mussel recruits within the mussel beds. The long term persistence of these restored adult mussel beds and the future success of restoration efforts will be dependent on modifying best practice methods to reduce sea star predation by identifying restoration sites that are low in sea star abundance, removing initially attracted sea stars, and continually monitoring restoration sites for episodic increases in sea star abundance.

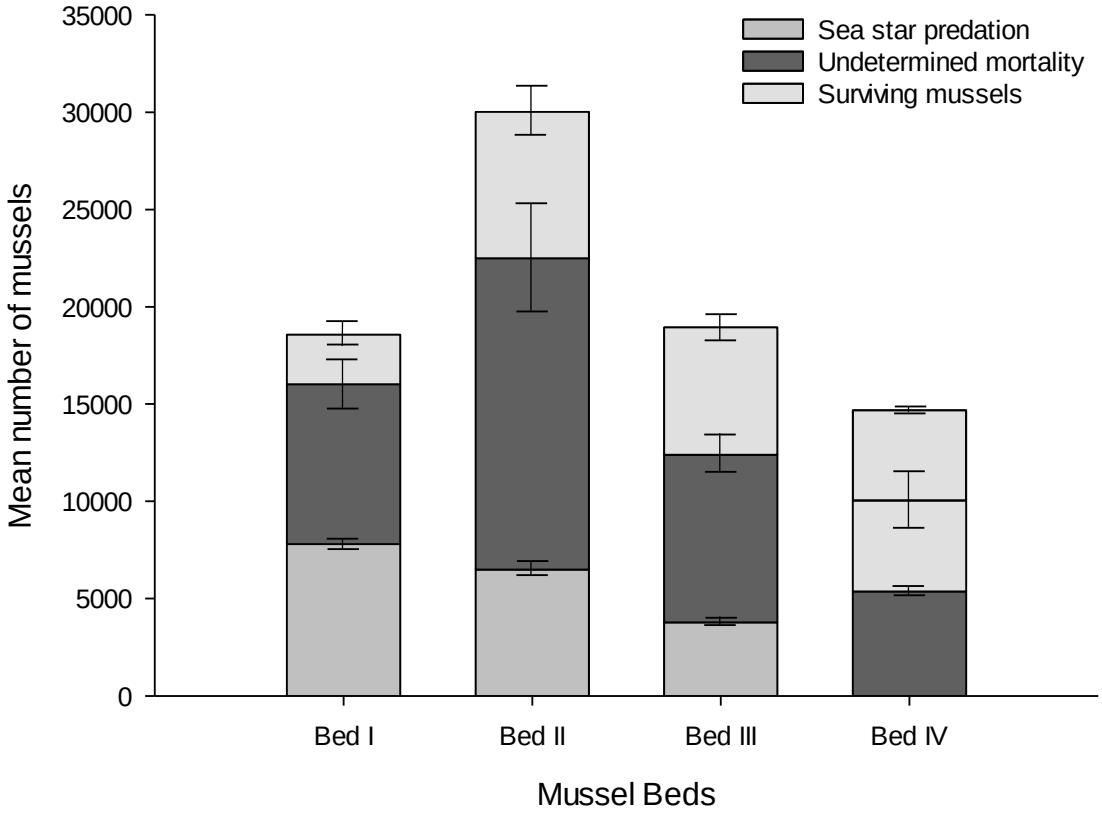

Figure 3.1 Estimated mean numbers of adult mussels (±SE) within each of the four experimental mussel beds that survived, died of undetermined sources, and were consumed by sea stars over a 25 month period.

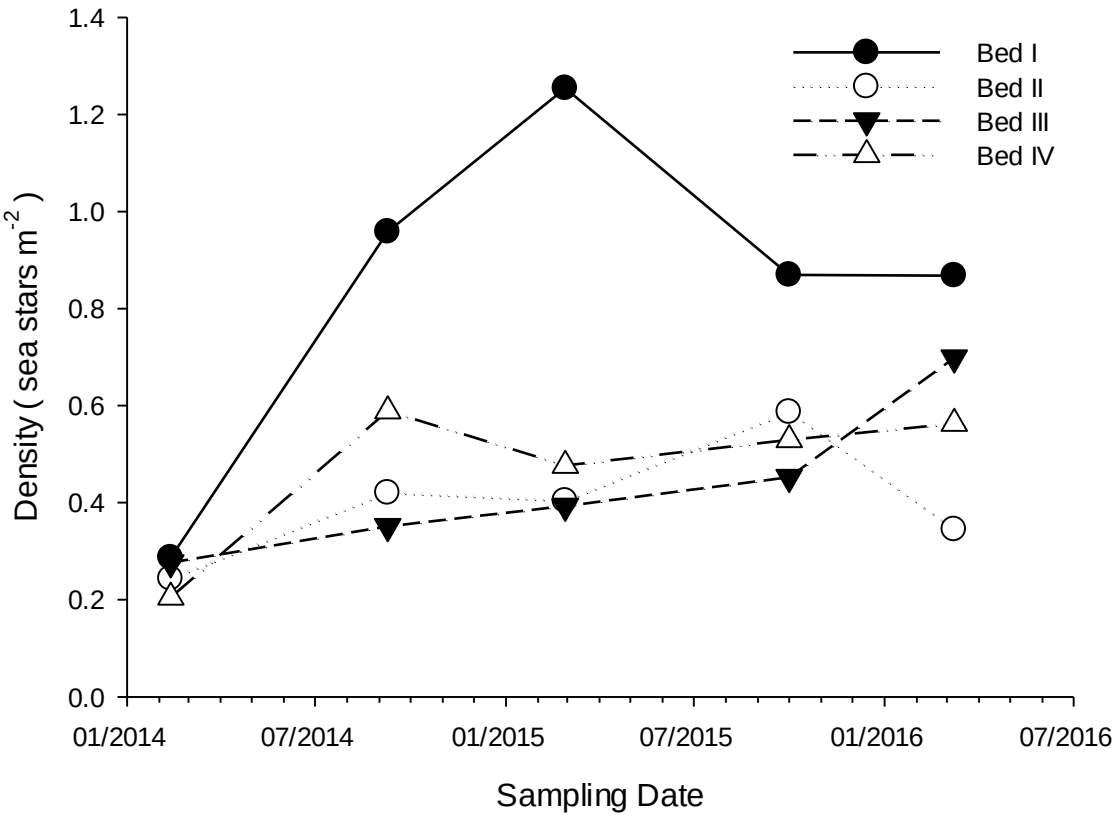

Figure 3.2 Sea star density observed in the four experimental mussel beds throughout the 25 month study.

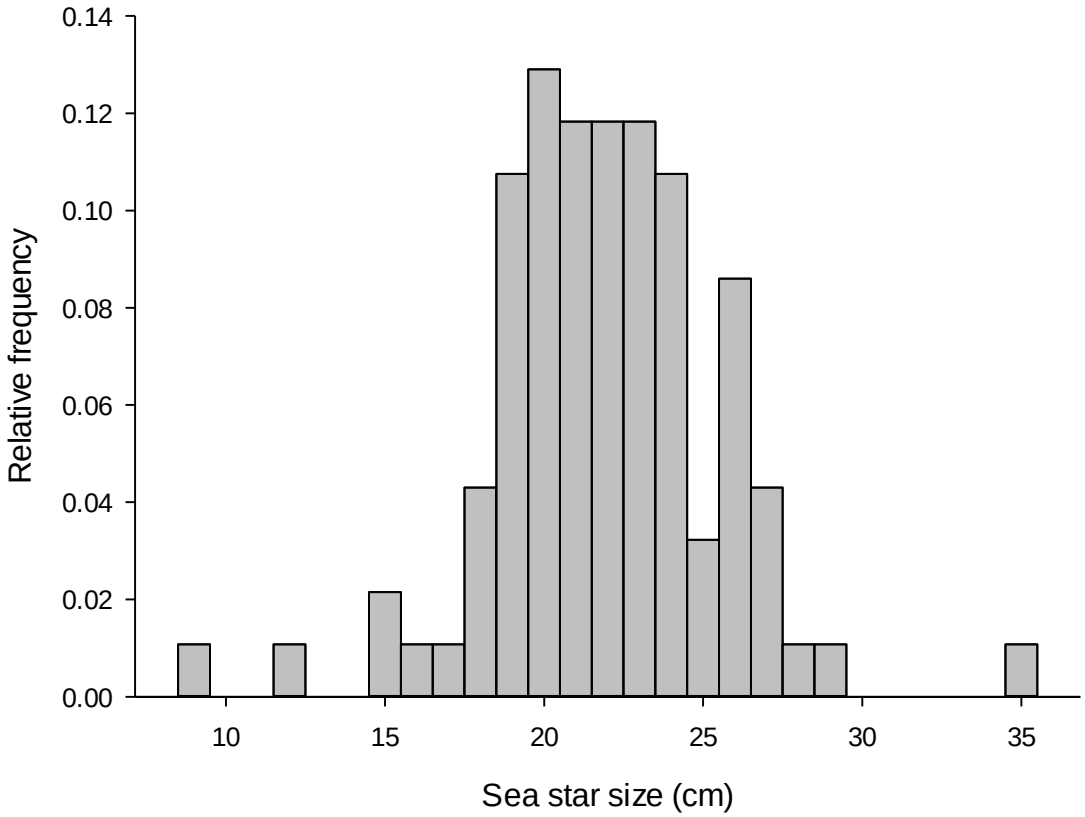

Figure 3.3 Relative size frequency of sea stars in the four experimental mussel beds on 1 October 2015.

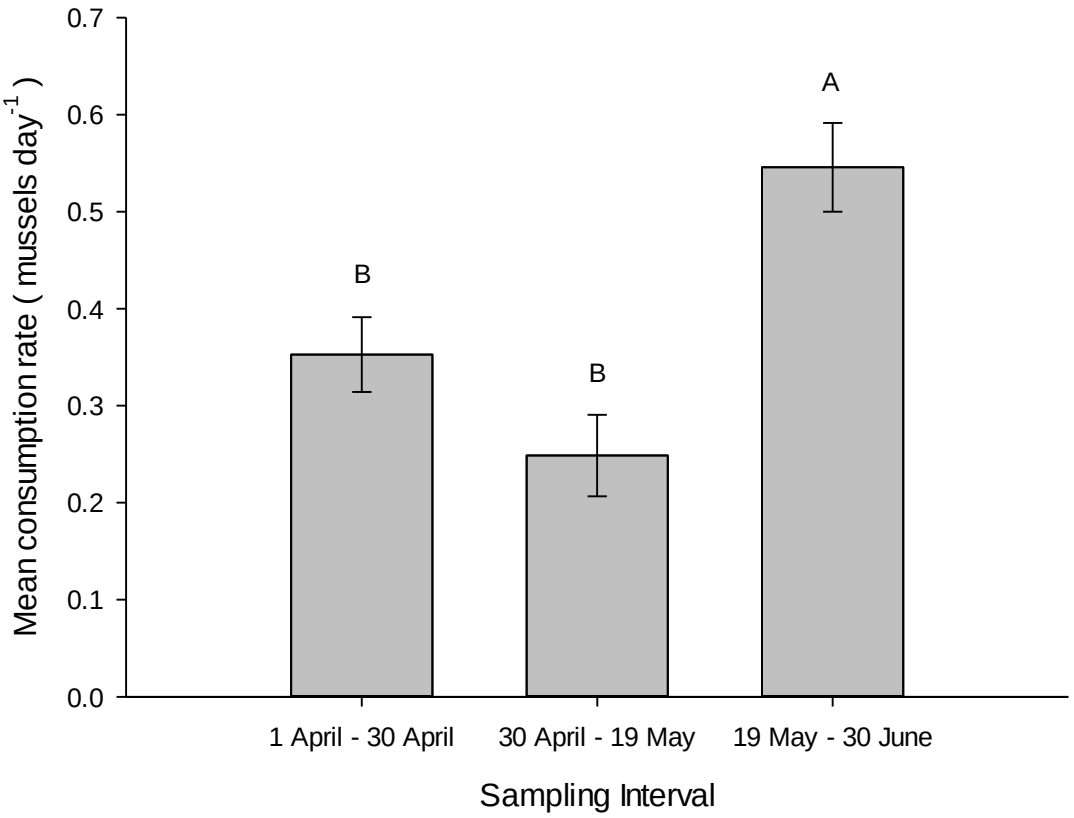

Figure 3.4 Mean consumption rate (±SE) of caged adult mussels in the field by individual sea stars. Significant differences between sampling intervals are indicated by different letters above the bars ($p < 0.05$).

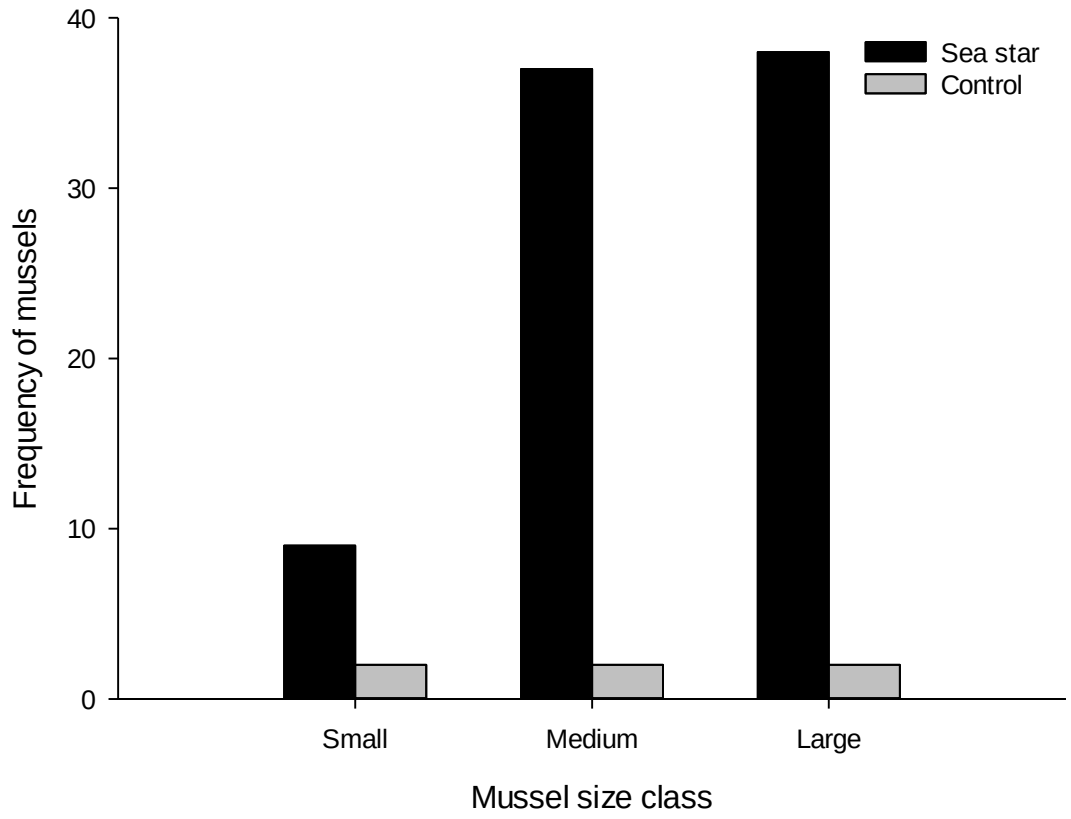

Figure 3.5 Frequency of mussels consumed by eleven individual sea stars and mortality of mussels within seven control tanks over seven days for three size classes of mussels, small (8 - 20 mm SL), medium (30 - 50 mm), and large (60 - 80 mm).

Table 3.1 Results of ANOVA comparison of mean sea star density within four experimental mussel beds placed on soft-sediment in the Hauraki Gulf and measured over two years. Significance was determined at an α of 0.05.

Source of variation	Num DF	Den DF	F value	P value
Intercept	1	12	26.79	<0.001
Sampling date	4	12	3.62	0.04

Table 3.2 Results of ANOVA comparison of mean consumption rate of mussels by caged sea stars of two size classes over three months. Significance was determined at an α of 0.05.

Source of variation	Num DF	Den DF	F value	P value
Intercept	1	10	310.34	<0.001
Sampling date	2	10	15.31	<0.001
Size	1	5	5.50	0.07
Sampling date * Size	2	10	0.50	0.62

Table 3.3 Results of chi-square tests examining proportion of small (8 - 20 mm), medium (30 - 50 mm), and large (60 - 80 mm) mussels (SL) consumed by a total of 11 sea stars over a one week period. Significance was determined at an α of 0.05.

Chi-square test	DF	χ^2 value	P value
Heterogeneity	18	13.37	0.77
Proportion consumed	2	18.03	<0.001

Chapter 4

Does the provision of attachment substrate in restoration initiatives enhance persistence of mussels on soft-sediment substrate?

4.1 Introduction

Epifaunal bivalves such as oysters and mussels attach to hard substrates to reduce the risk of dislodgement and to maintain group cohesion. These hard substrates can include natural cobbles and bedrock, anthropogenic structures, such as seawalls and wharf pilings, as well as other organisms, such as mangroves and most commonly, conspecifics. The gregarious nature of many epifaunal bivalves often leads to the formation of extensive contiguous populations, known as beds, which occur both intertidally and subtidally within coastal ecosystems. The degradation of coastal habitats throughout the world has frequently resulted in the loss of bed-forming bivalve species, as well as much of the attachment substrate once available to these organisms (de Jonge et al., 1993; Rothschild et al., 1994; Service & Magorrian, 1997; Kennedy & Roberts, 1999; Coen & Luckenbach, 2000; Cranfield et al., 2004). This lack of suitable substrate in turn frequently contributes to the slow recovery of these depleted bivalve populations once anthropogenic impacts have been addressed. Therefore, one of the most common practices for restoration of bivalve beds is the provision of substrate to augment the available surface area for natural larval settlement and subsequent attachment and retention of juveniles and adults (Brumbaugh & Coen, 2009).

The provision of substrate is frequently an integral component of oyster restoration initiatives given that settling oysters attach permanently to the substrate. Furthermore, the

characteristics of the provided substrate can influence their subsequent effectiveness for promoting natural oyster larval recruitment. For example, providing substrates with high relief can positively influence oyster settlement (Gregalis et al., 2008), while increased structural complexity of added substrates can increase survival and growth of oyster settlers (Bartol et al., 1999). The type of substrate material provided may also vary in effectiveness, with the provision of oyster versus surf clam shell cultch resulting in a greater survival and growth of oyster recruits (Nestlerode et al., 2007). Identifying effective substrates and incorporating them into bivalve restoration initiatives has the potential to greatly increase the likelihood of successful restoration.

Unlike oysters, mussels do not permanently attach during larval settlement as they retain some mobility even as adults, but like oysters they retain a dependence on hard substrates to anchor themselves within their environment with detachable byssal threads. This is particularly important on soft-sediment habitats where mussels rarely attach to the primary sediment (Bayne, 1964) as the byssal threads are often unable to attach to the small grain sizes, and if they were able to attach, it would not provide any anchoring. Instead, many mussels species rely on rocks, shells, and predominantly conspecifics for attachment in habitats dominated by soft-sediment (Commito et al., 2014) with experiments showing that recruiting mussels primarily use these attachment materials rather than bare soft-sediment (Commito et al., 2014; van der Heide et al., 2014). Correspondingly, this would tend to suggest that the provision of some form of substrate would be critical to the success of any efforts to restore mussel populations on soft-sediment substrates. However, adults of the northern horse mussel, *Modiolus modiolus*, showed no increase in survival when transplanted onto shell cultch of either high or low relief when compared to soft-sediment (Fariñas-Franco et al., 2013). Likewise, the survival of transplanted adult blue mussels, *Mytilus edulis*, was not higher on natural fibre mats made of coir compared to those transplanted directly onto

soft-sediment (de Paoli et al., 2015). In contrast, larval recruitment of both northern horse mussels and blue mussels were both higher in the presence of adults compared with any other available substrate including shell cultch (Fariñas-Franco et al., 2013; Commito et al., 2014). The survival of seed mussels was also shown to be greatest when provided with more complex substrates (Frandsen & Dolmer, 2002). This suggests that on soft-sediment, the re-establishment of adult mussel beds by transplanting adults may not require the additional provision of attachment substrate, but the provision of attachment substrate may provide critical habitat for transplanted juvenile and recruiting mussels.

Extensive mussel beds of the green-lipped mussel, *Perna canaliculus*, covering over 1300 km^2 on soft-sediment in the Hauraki Gulf, New Zealand, were nearly extirpated by dredge fishing during the last century (Greenway, 1969; Reid, 1969) and subsequently led to the removal of much of the available hard substrate in the form of mussels and attached shells. Although it is unclear why these populations have not recovered since the closure of the fishery in 1969 (Paul, 2012), the loss of suitable benthic attachment substrates may have contributed to the lack of recovery in this population. Consequently, the aim of this study was to determine whether the provision of additional attachment substrate is critical to the success of restoration efforts now commencing in this mussel population in the Hauraki Gulf. These restoration initiatives currently have two potential sources of mussels for establishing restored mussel beds, wild juvenile mussels and aquaculture-reared adult mussels. In the field, the persistence of both transplanted juvenile and adult mussels onto soft-sediment substrate was assessed when provided with additional substrate of either mussel shells or adult conspecifics. For juvenile mussels, further laboratory tests were conducted to assess substrate attachment preferences and the potential for reduced predation by a sea star predator when provided with these alternative attachment substrates, both of which will influence the persistence of transplanted mussels in the field.

4. 2 Materials and Methods

4.2.1 Study site

The Hauraki Gulf is located on the north-eastern coast of the North Island of New Zealand. Field experiments were conducted in a sheltered coastal embayment on the northern section of Kawau Bay, in the Hauraki Gulf (S 36º 22' 47", E 174º 49' 02"). All experimental plots were situated at a depth of 4.1 to 4.9 m below chart datum on fine sand substrate. Although the sediment was not representative of sediments throughout the Hauraki Gulf which certainly varies in geochemistry and grain size, the small grain sizes of soft sediment similarly provide no structural support for establishing mussels which was the primary focus of this investigation.

4.2.2 Mussel sources

Wild juvenile mussels (*P. canaliculus*) are regularly found attached to drifting algae (Alfaro et al., 2010) and were collected from Ninety Mile Beach (35º 02' 08" S, 173º 10' 05" E) and Muriwai Beach (S 36º 50' 05", E 174º 27' 59") in northern New Zealand. Juvenile mussels (1 - 5 mm shell length (SL)) were housed within the laboratory in aquaria supplied with ambient seawater with aeration until utilized in field and laboratory experiments. Adult mussels were most easily obtained from the extensive aquaculture operations in the Hauraki Gulf, where juvenile mussels originating from wild sources at Ninety Mile Beach in northern New Zealand, are grown on suspended long lines until they reach commercial size (Jeffs et al., 1999). The adult mussels (80 - 100 mm SL) utilized in these experiments were cleared of all fouling organisms and kept outdoors in covered aquaria with flow-through seawater until

deployment. Mussel shell was obtained either from aquaculture industry or from deceased mussels from experiments. These mussel shells were cleaned of any attached organisms or remnant mussel flesh prior to use in experiments.

4.2.3 Use of substrate by adult mussels in the field

On 26 November 2013, twenty 0.25 m^2 (0.5 × 0.5 m) plots were established by divers *in situ* arranged in five rows, each containing four plots. Each plot was separated by a distance of 1.5 m and marked with a subsurface float. The crossed experimental design consisted of a substrate level of either unmodified soft-sediment or the addition of 60 clean adult mussel shells (80 - 100 mm SL) which was crossed with a predator exclusion or access level, with a total of five replicates per treatment. Predator exclusion plots were enclosed in a lightweight stainless steel frame covered with coarse plastic mesh (20 mm openings). The plastic mesh prevented large mobile predators, such as fish and lobster, from removing and consuming mussels from the plots, while not unduly restricting water flow to the mussels inside. Forty live adult green-lipped mussels were then transplanted into each plot. After 50 days, the number of surviving mussels in each plot was enumerated by divers.

4.2.4 Use of substrate by juvenile mussels in the field

On 26 November 2013, a total of fifteen 1.5 m^2 circular plots were established by divers in three rows of five plots at the field site and marked with subsurface floats. The experiment consisted of three substrate treatments; 1) unmodified soft-sediment substrate, 2) addition of ≈250 adult mussel shells (80 - 100 mm SL) and, 3) addition of ≈1200 live adult mussels. The three substrate treatments were each randomly allocated to five of the plots with no more than

two of the same substrate treatment per row. Six days later, 0.318 kg (±0.013 SE) of macroalgae covered in juvenile mussels (5298 mussels ±353 SE, from Ninety Mile Beach) that was contained in a biodegradable mesh sock (5 - 10 mm mesh size) were prepared in the laboratory and then secured to the centre of each of the 15 experimental plots with a stainless steel pin driven 10 cm into the sediment by divers. The mesh sock helped to maintain the pre-measured quantities of juvenile mussels during transport and ensure the macroalgae with attached juvenile mussels remained within the positioned plot. These mesh socks are commonly used for deploying juvenile mussels on seaweed in aquaculture operations (Jeffs et al., 1999). The mesh size used did not unduly inhibit the movement of the juvenile mussels into and out of the sock, allowing the juvenile mussels to freely disperse onto the plot. After a period of 44 days each plot was surveyed by haphazardly placing four 0.0625 m^2 quadrats and quantifying the number of remaining juvenile mussels (1 - 5 mm SL) within each quadrat.

4.2.5 Substrate selection by juvenile mussels in the laboratory

Within the laboratory, nine round 60 l plastic tubs (320 mm diameter base, 500 mm high) were filled evenly across their base with natural sediment (sieved to a grain size <1 mm, from Leigh Harbour) to a depth of 10 mm. The tubs were then filled with 20 l (depth of 150 mm) of filtered seawater (5 µm) which was aerated via a single air-stone placed in the centre above the sediment. The bottom of each tub was sectioned into three concentric areas arranged by distance from the centre of the tub; 1) central area (0 - 5 cm diameter), 2) intermediate area (5 - 10 cm) and, 3) outer area (beyond 10 cm and including the walls of the tub) (Figure 4.1). The three treatments consisted of three modifications to the intermediate area only; either unmodified natural sediment substrate, adult mussels (nine mussels in

groups of three), or mussel shell (18 clean adult mussel shells). Three replicates per substrate treatment were used in each of two trials on 23 and 25 May 2015 for a combined total of six replicates per treatment using fresh mussels for each trial. For each replicate, 40 wild juvenile mussels of 1 - 5 mm shell length sourced from Ninety Mile Beach were placed within 2 cm of the central area of each tub. After 24 hours the juvenile mussels in each of the designated areas were enumerated.

4.2.6 Predation refuge provided by substrates in the laboratory

Six 2 l square containers (18 cm base, 12 cm height) were filled evenly across the base with natural sediment (sieved to a grain size <1 mm, Leigh Harbour) to a depth of 10 mm. Each container was supplied with flow-through (10 l h^{-1}), filtered seawater (5 µm) which was aerated via a single air-stone. Water escaped through the screened (0.25 mm) outlets to the containers which were located 3 cm below the lip of the container, maintaining a dry surface where mussels were unable to escape. The crossed experimental design consisted of a substrate factor of either unmodified soft-sediment, mussel shell (20 clean adult mussel shells), or adult mussels (10 adults in a single group) which was crossed with a predation factor, i.e., presence or absence of a small eleven-armed sea star, *Coscinasterias muricata* for a total of six experimental treatments. Each of the six containers was set up with one of the six experimental treatments which constituted a single block. Those treatments containing a predator were supplied with small sea star, ranging in size from 3.5 - 5 cm as measured from the tip of the longest arm to the opposite side of the oral disc, which are known to consume mussels. Into each container 100 juvenile mussels (1 - 5 mm SL, from Muriwai Beach) were placed and the number of surviving mussels was assessed after one week. A total of five

blocks were run consecutively, spanning a five week period which commenced on 12 November 2015 and concluded on 24 December 2015.

4.2.7 Statistical analyses

The data were inspected for deviations from normality and heterogeneity of variance firstly by visually inspecting quartile-quartile plots and plots of residuals versus fitted values (respectively) of models prior to running the analysis. For the experiments on substrate use by adult mussels, the data deviated from normality and were therefore fitted to a Poisson distribution using a general linear model (GLM) and tested using a Wald chi-square test to determine if differences in the number of surviving mussels were due to either the substrate and/or access by predators. For the experiment on selection of substrate by juvenile mussels, the data were normally distributed and thus were fitted to a Gaussian distribution using a linear model (LM) and tested using an ANOVA for differences in the percent of mussels among substrate treatments within each area separately. For the experiment assessing potential predation refuge from sea star predators, the data deviated from normality and were therefore fitted to a Poisson distribution using a general linear mixed-effects model (GLMER) incorporating blocks as a random effect. A Wald chi-square test was utilised to determine if differences in mortality were attributed to the substrates and/or presence of predators. Significance was further examined using pairwise *t*-tests ("predictmeans" function in R) with a false discovery rate correction for multiple comparisons ("fdr" function in R). For the field experiment on survival of juvenile mussels, results are presented using descriptive statistics only because there was very low recovery of juvenile mussels across all plots. All statistical tests were computed in R version 3.2.3 and RStudio version 0.99.879.

4.3 Results

4.3.1 Use of substrate by adult mussels in the field

There were no significant effects of the provision of mussel shell as attachment substrate, the access or exclusion of predators, or their interaction on the survival of adult mussels across plots (Table 4.1). Survival rates across all replicates was high (mean survival 93.1 ± 0.9% SE) and no mussels out of the 40 mussels that were initially deployed into the plots were found outside of the plots indicating that no emigration occurred during the experiment.

4.3.2 Use of substrate by juvenile mussels in the field

There was very low recovery of the juvenile mussels transplanted into each plot, with only two individuals being the highest recorded number of juveniles in any sampled quadrat. No juvenile mussels were found in any plots of the unmodified soft-sediment treatment. A total of three juvenile mussels were found across all plots in the mussel shell treatment, resulting in a mean density of 2.4 ± 1.7 mussels m^{-2} (SE). These juvenile mussels were only found attached to the adult mussel shell which provided a very low relief (1-3 cm above the sediment) and had mostly become buried by sediment. Only four juvenile mussels were found across all plots in the adult mussel treatment, resulting in a mean density of 3.2 ± 1.5 mussels m^{-2} (SE). Unlike the mussel shells, the adult mussels provided higher relief (5-16 cm above the sediment) for attachment of the juveniles, with all juvenile mussels being found at least 10 mm above the sediment.

4.3.3 Substrate selection by juvenile mussels in the laboratory

Overall, the majority (58.6%) of the juvenile mussels remained in the central area after 24 h (Figure 4.2), often attached to other juvenile mussels, and the proportion of mussels remaining in the central area did not differ among the treatments (Table 4.2). A minority of juvenile mussels (9.8%) moved into the outer area after 24 h and the proportion of mussels in this outer area was different among the three treatments, with a significantly smaller proportion of mussels occupying this area in the adult mussel versus soft-sediment control treatments (t=3.07, DF =15, p = 0.01) as well as the mussel shell versus soft-sediment control treatments (t=2.97, DF=15, p= 0.01), but there was no significant difference in the proportion of mussels between the adult mussel and mussel shell treatments (t=0.11, DF=15, p=0.92). There were also significant differences in the proportion of juvenile mussels moving into the three different treatments in the intermediate area. The proportion of juveniles found on adult mussel substrate was greater than both the proportion of mussels located on mussel shell substrate (t=2.43, DF=15, p=0.03) as well as the soft-sediment control substrate (t=4.88, DF=15, p<0.001). The proportion of juveniles found on the mussel shell was in turn also greater than the proportion of mussels on the soft-sediment substrate (t=2.45, DF=15, p=0.03). Juvenile mussels that had moved into the mussel shell treatment areas were always found attached to shells while juveniles in the adult mussel treatment were always found attached to the live adult mussels, either on their shells or among their byssal threads. Adult mussels did not move from their designated areas during the course of the experiment.

4.3.4 Predation refuge provided by substrates in the laboratory

There were significant differences in the mortality of green-lipped mussels for both the substrate (χ^2 = 39.8, DF = 2, p <0.001) and predation factors (χ^2 = 321.1, DF = 1, p <0.001) as well as their interaction (χ^2 = 13.3, DF = 2, p = 0.001) (Table 4.3). All experimental combinations without a predator had consistently low levels of mortality of juvenile mussels (mean = 23 mussels ± 4) and were not different among the three types of substrate (All p values >0.6) (Figure 4.3). The mortality of juvenile mussels in treatment combinations without a predator was also consistently lower than every treatment combination where a predator was present (All p values <0.001). The experimental combinations with a predator and either the unmodified substrate or the shell substrate had similar high levels of mortality of juvenile mussels (means of 78 ± 13 and 71 ± 8 mussels, respectively, *t* = 2.41, p = 0.216). However, the mortality of juvenile mussels in the experimental combination containing a predator and adult mussels as substrate (mean of 46 ± 8 mussels) was significantly lower than those containing a predator and either unmodified soft-sediment substrate or the shell substrate (p values <0.001). There was no mortality of the adult mussels provided as substrate within this experiment and thus the lower mortality of the juvenile mussels was not due to the sea stars consuming the adult mussels instead of the juveniles. Sea stars were seen actively consuming juvenile mussels and all mussels were accounted for in every tank at the end of the experimental run. It was observed that juvenile mussels in the unmodified substrate treatment were either dispersed over the soft-sediment or clumped together whereas juvenile mussels in shell and adult mussel treatments were predominantly observed to be attached to the hard structures of the shells and byssal threads of adult mussels.

4.4 Discussion

The provision of additional larval settlement and attachment substrate is a proven method for many oyster restoration efforts (Luckenbach et al., 1999; Brumbaugh et al., 2006). However, the value of providing additional substrate for establishing mussels on soft-sediments appears to differ between adults and juveniles. In this study, adult green-lipped mussels showed no increase in survival on soft-sediment whether they were provided with additional hard substrate for attachment (i.e., mussel shell material) or transplanted directly onto the unmodified soft-sediment. For *P. canaliculus*, as well as other bed-forming mytilid species, attachment to live conspecifics appears to be of primary importance for anchoring and subsequent retention in soft-sediment habitats (Fariñas-Franco et al., 2013; de Paoli et al., 2015). The clumping of adult mussels facilitated by mutual byssal attachment to surrounding live mussels likely provides greater anchorage on soft-sediments when compared to attaching to lighter mussel shell. When in natural soft-sediment beds, individual *M. edulis* were shown to form the greatest number of byssal attachments to conspecifics despite the presence of other solid substrates, such as pebbles and shell hash (Commito et al., 2014). Therefore, it is unlikely that the supply of additional substrate is required for improving the attachment and survival of adult green-lipped mussels transplanted onto soft-sediment habitats.

In contrast, the provision of attachment substrate appears to be important for restoration initiatives relying on transplanting juvenile green-lipped mussels. In the laboratory, significantly greater numbers of juvenile green-lipped mussels placed on soft-sediment moved and attached to adult mussels and mussel shells. In addition, juvenile mussels moving into areas containing mussel shell or adults were always observed attached to these hard structures. A similar pattern was observed in laboratory predation experiments, with juvenile mussels found predominantly attached to these hard structures (>70%, personal

observation) rather than remaining on the soft-substrate. Likewise, in the field experiment the juvenile green-lipped mussels were only observed attached to these hard substrates after being transplanted onto soft-sediment, with none located on the soft-sediment. In *M. edulis*, naturally recruiting mussels in soft-sediment habitats also attached preferentially to hard substrates such as adult mussels and coir ropes (Commito et al., 2014; van der Heide et al., 2014). Soft-sediments offer no structural support to transplanted small juvenile mussels and thus any attempts to restore mussel beds with mussels of this smaller size will require the addition of some form of substrate to maximize their retention and survival.

The provision of adult mussels as an attachment substrate for juvenile mussels was shown to reduce predation by sea stars on the juvenile mussels. In contrast, the survival of juvenile mussels was not improved by the provision of mussel shell as an attachment substrate when compared to bare soft-sediment. However, the mechanism responsible for this difference in survival of mussels between the three substrates is unclear. One possibility is that the complex matrix of adult mussels that are tightly bound by byssal threads may restrict the predatory probing of sea stars and increase the amount of search effort they expend in order to locate juvenile mussels. Blue mussels, *M. edulis*, exhibit decreased mortality from green crab predation, *Carcinus maenas*, when transplanted onto structurally more complex shell or adult mussel substrates when compared to mussels transplanted onto structurally simple substrates (Frandsen & Dolmer, 2002). This difference can be at least partly attributed to a greater time spent by green crabs searching for their prey on these more structurally complex habitats.

Observations of the field experimental plots also suggested that the vertical elevation provided by attachment substrate may have enabled the remaining juvenile mussels to avoid smothering by sediment. However, close observations of the experiment in the field indicated the lower elevation of the mussel shells above the sediment may have been insufficient, as

most of the surviving mussels were nearly buried by sediment. Providing greater vertical relief using live adult mussels may therefore be important to the survival of transplanted and potentially recruiting juvenile mussels and warrants further investigation.

For juvenile *P. canaliculus* on soft-sediment, both adult mussels and mussel shell may offer suitable attachment substrate when compared to soft-sediment, however, live adult mussels provide some protection from sea star predation, are preferred by juvenile mussels as attachment substrate, and potentially reduce the risk of juveniles being smothered by sediment. Many mussel species, including *P. canaliculus*, are known to settle primarily into mussel beds (McGrath et al., 1988; Lasiak & Barnard, 1995; Alfaro, 2006b; Reaugh et al., 2007; Commito et al., 2014), however, adult mussels of many species are known to be cannibalistic of both larvae and plantigrade mussels (MacIsaac et al., 1991; MacIsaac et al., 1995; Davenport et al., 2000; Zeldis et al., 2004; Alfaro, 2006a; Porri et al., 2008). This cannibalism can be significant, with larvae and plantigrade mussels constituting 70% of the total counts of planktonic organisms ingested by mussels at some times of the year (Alfaro, 2006a). Comparisons of levels of cannibalism by adult mussels to natural primary settlement have shown that cannibalism can lead to a reduction of as much as 77% of competent larvae available to settle (Porri et al., 2008). This not only poses a risk to subsequent recruitment of mussels to restored beds but also poses a risk to transplanted juveniles. Adult *P. canaliculus* were observed to have consumed bivalves up to 2.4 mm in shell length (Alfaro, 2006a) and a study of *M. edulis* has shown that they are capable of ingesting particles up to 6 mm (Davenport et al., 2000). Organisms that were ingested by adult mussels but not consumed, such as small crustaceans, were mucus bound and discarded through pseudofaeces, which also mostly resulted in their mortality. Although there are benefits to providing adult mussels as attachment substrate for transplanted juvenile mussels, mortality as a result of being

ingested by adult mussels, may also lead to decreased survival of recruiting mussels to this habitat as well as for juvenile mussels transplanted in restoration initiatives.

4.5 Conclusion

Maximizing the survival of transplanted and subsequently recruiting mussels into the breeding population is essential for establishing sustainable beds. The low retention of juvenile mussels in the field experiments of this study after only 44 days emphasizes this importance. The loss of natural mussel beds and their lack of recovery over the last half century has left the Hauraki Gulf devoid of much of the historically available attachment substrate in the form of adult mussels. This research presents evidence to support the hypothesis that the loss of adult beds as substrate has contributed to the lack of population recovery in the green-lipped mussel, an effect that is likely to have occurred in other species of mytilid where beds have been damaged by anthropogenic impacts. The restoration of adult mussel beds using only transplanted adult mussels will provide not only suitable attachment for additional transplanted adult mussels but will be critical for the retention of both transplanted and naturally recruiting juvenile mussels.

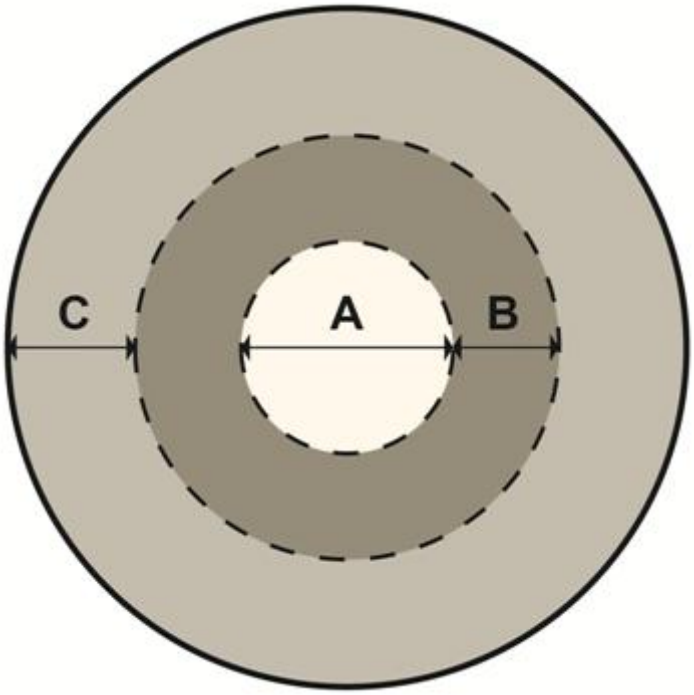

Figure 4.1 Experimental laboratory setup of circular containers used in juvenile substrate selection experiments, depicting the (A) central, (B) intermediate, and (C) outer areas of the floor of the tank. Mud substrate was provided evenly across all areas, but different substrates were experimentally placed in the intermediate area, i.e., addition of live adult mussels versus addition of mussel shells versus no modifications (soft-sediment).

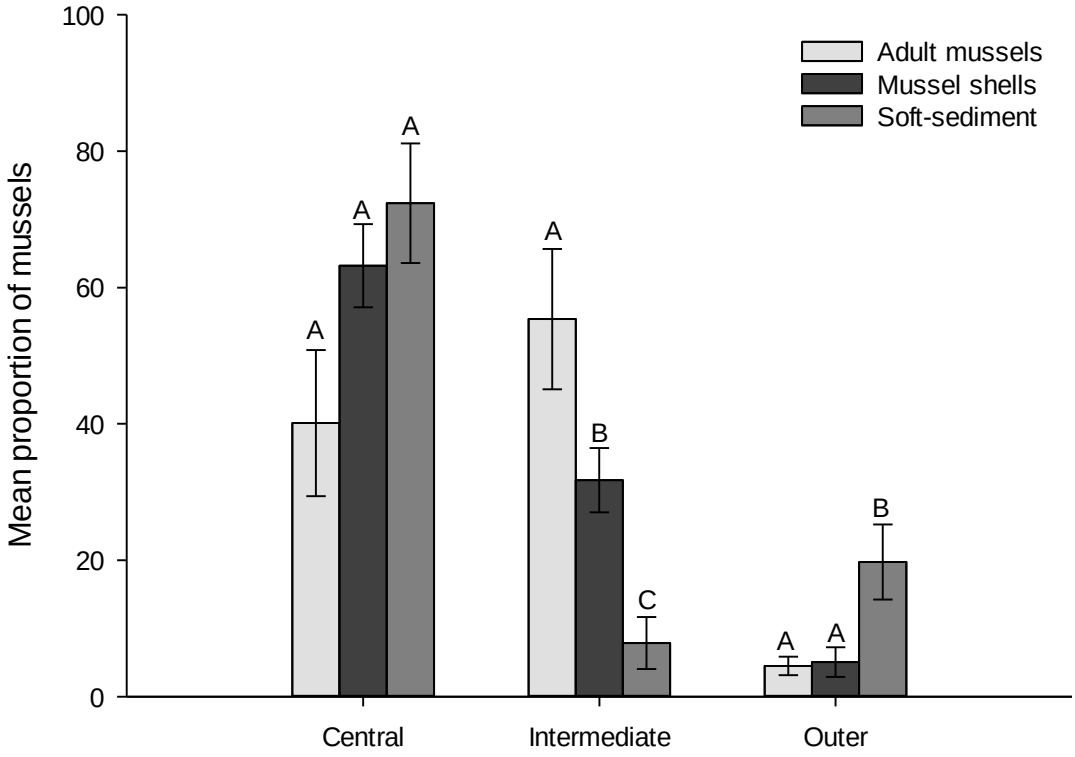

Figure 4.2 Proportion of juvenile mussels (±SE) occupying the central, intermediate, and outer areas of the juvenile substrate selection experiment when live adult mussels mussel shell or no additional substrate was provided in the intermediate area of the experimental tank (see Figure 1). Significant differences between the three substrate treatments within each area of the tank are indicated by different letters above the bars.

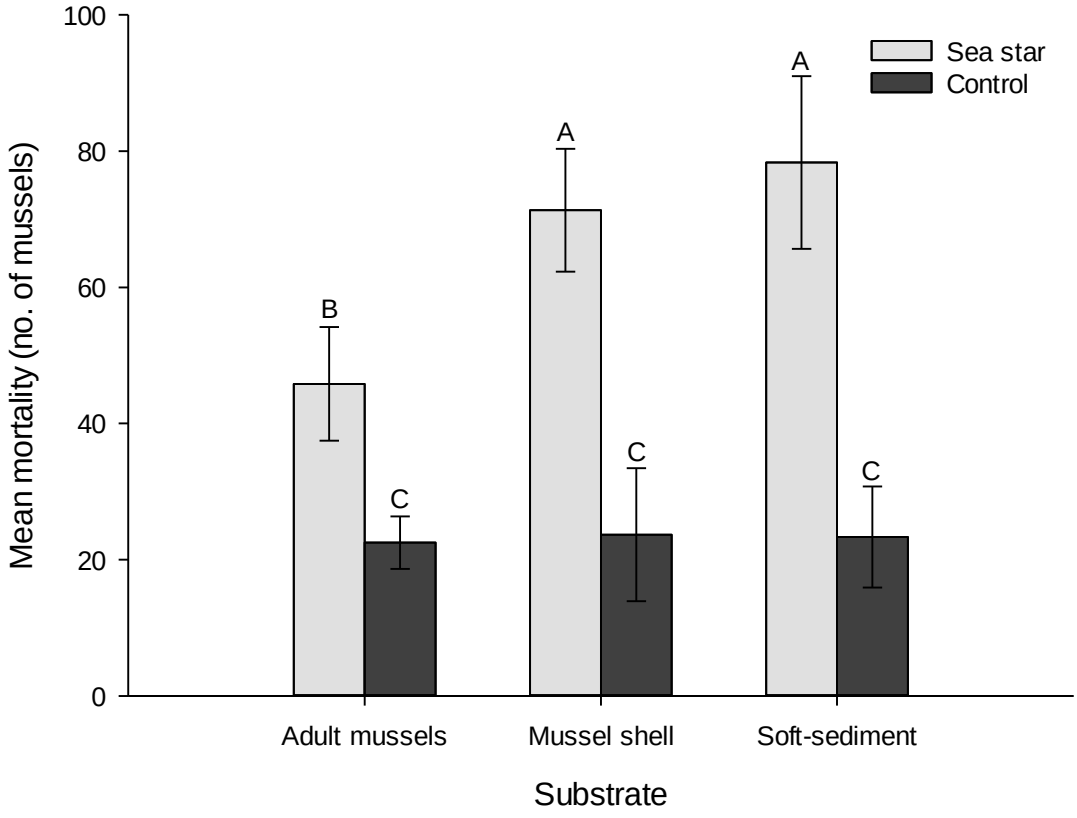

Figure 4.3 Mean mortality of juvenile mussels (±SE) in predation experiment provided with either adult mussels, mussel shells, or unmodified substrate as available attachment substrates in both absence (control) and presence (predator) of a sea star predator. Significant differences between all treatment combinations are indicated by different letters above the bars.

Table 4.1 Summary of statistical test for differences in survival of transplanted adult mussels in the presence/exclusion of predators and either provided addition attachment substrate or transplanted to the bare soft-sediment. Significance was determined at an α of 0.05.

	Chi square	DF	P value
Substrate	0.001	1	0.97
Predation	1.462	1	0.23
Substrate*Predation	0.387	1	0.53

Table 4.2 Summary of statistical tests for differences in proportion of mussels within designated areas when provided different attachment substrates. Significance was determined at an α of 0.05.

Area	Source of Variation	DF	Mean square	F vlaue	P value
Centre Area	Substrate	2	0.166	3.62	0.052
	Residual	15	0.046		
Substrate Area	Substrate	2	0.339	11.89	<0.001
	Residual	15	0.028		
Outer Area	Substrate	2	0.045	6.09	0.012
	Residual	15	0.007		

Table 4.3 Results of chi-square tests examining mortality of juvenile mussels in the presence/absence of a sea star predator when provided different attachment substrates. Significance was determined at an α of 0.05.

Wald chi-square test	DF	χ^2 value	P value
Substrate	2	39.76	<0.001
Predation	1	321.12	<0.001
Substrate*Predation	2	13.31	0.001

Chapter 5

Larval settlement within a restored mussel bed site: enhanced settlement in the presence of adult conspecifics

5.1 Introduction

In many parts of the world, the highly productive and diverse communities created by oyster and mussel beds are in decline (de Jonge et al., 1993; Rothschild et al., 1994; Service & Magorrian, 1997; Kennedy & Roberts, 1999; Coen & Luckenbach, 2000; Cranfield et al., 2004) and the lasting success of restoration efforts is dependent on maintaining larval recruitment to these bivalve populations. If recruitment to restoration sites is too low then populations will either dwindle or fail to establish. Insufficient larval recruitment in bivalve populations has two principal causes, insufficient larval supply and/or inadequate larval settlement substrate, with each cause requiring different approaches for implementing effective restoration (Brumbaugh & Coen, 2009). In the case of insufficient larval supply, it is frequently necessary to enhance the size of the effective adult breeding populations and ensure there are larval pathways to restoration sites in order to increase larval supply. Whereas, when there is inadequate larval settlement substrate it is necessary to provide sufficient suitable substrate that is free of competing species. Hence, the determination of the adequacy of larval supply versus larval settlement substrate is a key early step in developing bivalve restoration initiatives which relies on some understanding of larval settlement behaviour and patterns for the targeted species within restoration sites.

Larval dispersal and settlement in bivalves involves a complex interaction of a number of biotic and abiotic factors that is critical to maintaining existing populations

through ongoing recruitment as well as for the establishment of new populations. Dispersal from source populations to potential restoration sites is dependent on factors such as currents, wind patterns, larval duration, and water temperature (Rodríguez et al., 1993) which vary both spatially and temporally (Hunt & Scheibling, 1997; Alfaro & Jeffs, 2003; Porri et al., 2006). In addition, larval swimming behaviour influences the vertical distribution of larvae, allowing bivalves to avoid tidal currents that would potentially transport them away from desirable habitat (Knights et al., 2006; Robins et al., 2013). The spatial distribution of settling larvae within the restoration site is often largely dependent on the available settlement substrate, with larvae often actively choosing to metamorphose in the presence of specific chemical (Pawlik, 1992; Anderson, 1996; Alfaro et al., 2006; Bao et al., 2007; Ganesan et al., 2012) and structural cues associated with the substrate (Snelgrove et al., 1998; Snelgrove et al., 1999), or even auditory cues (Wilkens et al., 2012). This process of substrate selection is particularly important for species, such as oysters, for which the settling larvae attach permanently to the chosen settlement substrate. Mussels are sedentary but unlike oysters, they are not sessile and are capable of moving after settlement. The primary settlement substrates where mussels first settle and metamorphose, are often filamentous macroalgae (Buchanan & Babcock, 1997; Dobretsov & Wahl, 2001; Alfaro et al., 2004). However, once settled the juvenile mussels will often undergo a secondary settlement into the structurally complex habitat created by the dense adult populations (Bayne, 1964; Erlandsson & McQuaid, 2004; Commito et al., 2014). This settlement strategy has historically been considered the predominant form of recruitment. Alternatively, larval mussels are also known to settle directly into adult mussel beds and there is growing evidence that this strategy can be the predominant form of recruitment for some mussel species in some locations (McGrath et al., 1988; McGrath & King, 1991; Lasiak & Barnard, 1995; Snodden & Roberts, 1997; Erlandsson & McQuaid, 2004; Reaugh et al., 2007). In soft-sediment environments where

available macroalgae is often either scarce or absent, primary settlement into adult mussel beds may be a greater contributor to recruitment than secondary settlement.

The green-lipped mussel, *Perna canaliculus*, which once covered extensive areas of the soft-sediment sea floor of the Hauraki Gulf, New Zealand, has nearly been extirpated by commercial dredge fishing (Greenway, 1969; Reid, 1969) and exists today only in small isolated populations (McLeod et al., 2014). Although it is uncertain as to why these populations have not recovered since the fishery closed in 1969 (Paul, 2012), both larval supply and absence of settlement substrate may have contributed to the lack of recovery. In terms of larval supply, efforts to describe larval settlement patterns at three sites in the Firth of Thames in the inner Hauraki Gulf by deploying artificial settlement material found negligible larval settlement over an 18 month period (McLeod, 2009) which would suggest that the widespread removal of the *P. canaliculus* population in the Hauraki Gulf may have diminished the larval supply to the point where populations cannot re-establish. A lack of appropriate settlement substrates may also be involved in limiting recruitment in this environment. Filamentous macroalgae, a known preferential settlement substrate of green-lipped mussels in other areas (Buchanan & Babcock, 1997; Alfaro et al., 2004; Alfaro et al., 2006), are generally absent in many soft-sediment environments due to factors such as reduced light levels, sedimentation, and most notably, a lack of hard substrates for attachment and growth. Alternatively, the loss of >99% of the mussel beds within the Hauraki Gulf may also represent a lack of settlement substrate if mussels in this area settle primarily into mussel beds. Therefore, understanding whether there is larval supply to this system and the substrate preferences of these larvae will be critical to the success of restoration initiatives for this species and will provide some insight into the lack of recovery of green-lipped mussel populations within the Hauraki Gulf. This study aims to assess settlement and recruitment of green-lipped mussels at a restoration site over a one year period to determine whether the

Hauraki Gulf larval supply or available settlement substrate may be limiting recruitment. This will be accomplished through the monthly assessment of larval settlement on artificial collectors placed both within restored mussel beds as well as in adjacent soft-sediment habitat.

5.2 Methods

5.2.1 Study Site and Experimental Setup

The study site is located in Cable Bay (S 36° 48' 35", E 175° 11' 31"), a sheltered embayment on the northern side of Rotoroa Island in the Hauraki Gulf, northern New Zealand (Figure 5.1). Seven experimental mussel beds were established on 2 December 2013, ranging in extent from 24.4 m^2 to 45.2 m^2 in area and each bed consisted of approximately 20000 adult green-lipped mussels (80 - 100 mm, shell length -SL). Each mussel bed was separated from adjacent beds by 9 - 35 m of soft-sediments at a water depth of 3.9 - 5.1 m, running roughly parallel to the shoreline. The benthic sediment within the site originally consisted of a layer of about 5 cm of fine mud which eroded away during the study, leaving the underlying firmer mud, sand, and shell hash as the predominant sediment, which provided minimal hard substrate for the settlement and attachment of mussels.

On 23 April 2014 larval settlement collectors were deployed to monitor settlement of larval *P. canaliculus* within the study site for a one year period (Figure 5.2). The settlement collectors used in this study consisted of a 30 cm length of polypropylene larval settlement rope (Loop Spat Rope, Quality Equipment Group Ltd.) which has been successfully used to capture settling green-lipped mussel larvae commercially in many parts of the New Zealand (Alfaro & Jeffs, 2003). Each settlement collector was clipped at both ends to a line that was

anchored to the sea floor with a steel peg and held upright in the water column by a plastic net float at the opposite end. These lines were suspended between 0.2 and 0.8 metres above the sediment, depending on the amount of debris that accumulated around the steel peg between collections. Three replicate settlement collectors (a cluster) were placed into each of three of the restored mussel beds (i.e., clusters M1, M2, M3)(Figure 5.1), arranged such that each collector in each cluster was more than 1 m from the outer edge of the mussel bed and at least 1.5 m distant from an adjacent collector. This arrangement ensured that collectors were placed well within the mussel bed rather than near the edge but far enough apart so that they were unlikely to tangle. Three control clusters, each comprising three collectors (clusters C1, C2, C3) were also deployed on the soft-sediment located approximately 40 m from each of the mussel beds in water of similar depth (3-6.2 m below chart datum)(Figure 5.1). The collectors comprising each control cluster were separated by 1.5 m with each cluster separated by 22 m. The loss of two of the mussel beds (M1 and M2) which were dispersed by storm action was observed during sampling on 13 July 2014. By September 2014, there were only several small clumps of mussels (20 - 50 mussels) remaining at these two locations, which were all located more than 1 m from any of the settlement collectors that were previously within the confines of the mussel bed. For all periods following the 13 July 2014 sampling date, mussel bed clusters M1 and M2 were reclassified as removed mussel beds (R1 and R2, respectively), which provided a unique opportunity to observe how the magnitude of settlement changes when the mussel bed was removed. During the sampling on 12 October 2014, the destroyed mussel bed cluster R2 was removed and two additional clusters of settlement collectors were deployed into two of the additional mussel beds that were not previously monitored for settlement (clusters M4 and M5)(Figure 5.1).

5.2.2 Collection and Processing

Settlement collectors were retrieved and replaced approximately every four weeks depending on weather conditions facilitating diver operations. Divers detached each collector and sealed them within a plastic bag before attaching a clean settlement collector to the line. Bagged settlement collectors were returned to the surface where they were placed in an insulated container and transported back to the laboratory and frozen. Freezing samples prior to processing facilitates the removal of mussels from the settlement collectors (Alfaro & Jeffs, 2003). The settlement collectors were later thawed and each collector cleaned by washing gently with fresh water within a bucket before all contents were paced through a series of sieves (1, 0.5, and 0.2 mm). The recovered material from the three sieves was examined under a dissecting microscope and all green-lipped mussel settlers were counted and classified into three size groups (0.2 - 0.5, 0.5 - 1.0, and >1.0 mm). A thorough examination under a dissecting microscope of a subsample of the washed collectors across all sampling dates (three control and three mussel bed collectors) confirmed that the washing method reliably removed all attached mussels.

5.2.3 Recruitment to mussel beds

In an effort to relate settlement patterns to recruitment, four of the mussel beds were also regularly sampled for recruiting mussels (see Chapter 2). These four beds did not include the two that were destroyed by storm action so were able to be sampled throughout the study to detect the arrival of any recruits. Approximately every six months for a two year period (i.e., five sampling dates) four 0.0625 m^2 quadrats were placed haphazardly within the boundary of each mussel bed and the quadrat systematically searched for recruiting mussels measuring

<60 mm in shell length (SL), which was the minimum size of mussels deployed at the outset to form the restored beds.

5.2.4 Data Analyses

Due to the loss of the two mussel beds containing settlement clusters, there was unequal replication within treatments for some sampling intervals (the time between deployment of clean settlement collectors and their subsequent retrieval), resulting in an unbalanced design that could not be analysed using a single model. In addition, on several sampling dates, divers were unable to recover some of the control collectors and thus the specific control clusters for the missing collectors were not incorporated into analyses for those dates. Therefore, the unbalanced data set required separate analyses to assess the effects of the substrate treatments on the magnitude of settlement within the restoration site as well as any seasonal differences. In both cases visual inspections of the data for deviations from normality and heterogeneity of variance using quartile-quartile plots and plots of residuals versus fitted values (respectively) of models indicated deviations from normality, and thus settlement was fitted to a Poisson distribution using a General Linear Model (GLM) which is more appropriate for this type of count data. Due to the large number of zeros in the data set and the use of a log-link in the GLM function for Poisson distribution, settlement predictor variables were coded by the addition of one to the total settlement per collector. All statistical tests were computed in R version 3.2.3 and RStudio version 0.99.879.

To test whether the presence of adult mussel beds affects the magnitude of settlement in soft-sediment habitats, differences in the amount of settled larvae on collectors were analysed for each sampling interval separately. Due to unequal replication of clusters for each substrate treatment across sampling intervals, differences among all clusters regardless of

substrate placement were analysed rather than nesting within substrate. A Wald chi-square test was used to assess differences in magnitude of settlement and in the event of significant differences further pair-wise *t*-tests ("predictmeans" function in R) were conducted using a false discovery rate correction for multiple comparisons ("fdr" function in R). Differences between treatments were then assessed based on these multiple comparisons. For the final two sampling intervals, there was insufficient replication of collectors within all control settlement clusters on soft-sediment. Following a lack of significant differences among all comparisons of control clusters prior to these sampling intervals, all collectors from control clusters were pooled to provide an estimate of settlement for comparison to mussel bed clusters within these two sampling intervals.

The assessment of seasonal differences in the magnitude of settlement required a consistent data set from clusters of collectors. Unfortunately, this was only available for the single mussel bed cluster (M3). Again, given the lack of difference among all comparisons of control clusters, the settlement for these collectors was pooled for each sampling date to provide a comparison to this single mussel bed cluster. Due to unequal times between successive sampling dates, the data were standardised to a 28 day period to facilitate comparisons prior to fitting the model. Potential differences in settlement among sampling intervals were detected using Wald chi-square test with pairwise *t*-tests ("predictmeans" function in R) using a false discovery rate correction for multiple comparisons ("fdr" function in R).

5.3 Results

5.3.1 Size distribution of larvae

Distinction between primary settlement and secondary migration is difficult to ascertain based on mussel size, however, as has been used in previous studies, mussels <0.5 mm SL were considered to have settled directly onto the collector as larvae, whereas those >0.5 mm SL could be the result of either primary settlement or secondary migration (Hunt & Scheibling, 1998; Alfaro, 2006b). The largest juvenile mussel on any collector was 17 mm SL . Observations of line-grown mussels have shown growth rates as high as 0.3 mm day^{-1} at certain times of the year (Hickman, 1979). Given the sampling intervals used in the present study, it is most likely that this mussel was not a settler during that sampling interval but rather a recruit that had migrated onto the line from elsewhere. Throughout the study the size of mussels on the collectors were predominantly between 0.2 and 0.5 mm SL. The overall proportion of mussels across all collectors that were greater than 0.5 mm SL ranged from 0 - 5%. These results indicate that the vast majority of mussels on the collectors were the result of larval settlement, rather than secondary migration.

5.3.2 Effects of substrate on settlement

The clusters of collectors within mussel beds exhibited significantly greater mussel settlement than all control clusters of collectors on soft-sediment for the five sampling intervals up to 7 September 2014 (Figure 5.3) and the three sampling intervals after 11 January 2015 (Figures 5.4). With the exception of the sampling interval covering 10 August - 7 September 2014, which was characterized by low mean (±SE) settlement across all

collectors (1.5± 0.7 mussels collector^{-1}) (Figure 5.3), all of the aforementioned sampling intervals were characterized by relatively high mean settlement across all collectors (ranging 5.4 ± 2.0 to 165.1 ± 34.5 mussels collector^{-1}). The two sampling intervals between 12 October 2014 and 7 December 2014 showed significantly greater mean settlement between mussel bed clusters M4 and M5 and all soft-sediment control clusters (Figures 5.3 and 5.4). However, mussel settlement on the cluster of collectors in mussel bed M3 did not differ from soft-sediment control clusters for these two sampling intervals. These two sampling intervals were characterized by relatively low mean settlement across all collectors (<4.3 mussels collector^{-1}). Low settlement (<0.7± 0.2 mussels collector^{-1}) characterized the sampling intervals covering 7 September - 12 October 2014 and 7 December 2014 - 11 January 2015 with no differences in settlement detected among any of the settlement clusters (Figures 5.3 and 5.4).

5.3.3 Effects of removed mussel beds

Prior to the loss of mussel beds M1 and M2 due to storm damage, both collector clusters on these beds showed significantly greater mussel settlement than control clusters (Figure 5.3). However, once these two mussel beds were destroyed by wave action, which transported the adult mussels away from the fixed collectors, the numbers of mussels settling on these clusters decreased in comparison to the only remaining intact mussel bed (M3) after 14 June 2014 (Figures 5.3 and 5.4). Cluster R1 (formerly M1) had similar numbers of settling mussels as the soft-sediment control clusters for the remainder of the study. While cluster R2 (formerly M2) differed from soft-sediment control clusters in July - August 2014 but not thereafter.

5.3.4 Seasonal differences in settlement

Mean mussel settlement differed among the 12 sampling intervals between April 2014 and April 2015 for M3, which was the only cluster on a mussel bed that remained intact for the entire study (χ^2 = 1389.7, DF = 11, p <0.001). In April 2014, mean settlement was relatively high at 55 ± 9 mussels collector^{-1} and decreased through until September 2014 to relatively low levels (mean of 3 ± 1 mussels collector^{-1}) which were then maintained through to January 2015 (Figure 5.5). Mean mussel settlement at M3 for all sampling intervals between 7 September 2014 and 11 January 2015, when the mussel settlement was at its lowest, did not differ among the sampling intervals. Mean settlement at M3 subsequently increased through to the end of the study in April 2015 when it reached its highest level of 144 ± 45 mussels. Although there was only one year of sampling, the results suggest a seasonal pattern of settlement which is higher between the months of March to August with little settlement between August and March. The mean settlement of mussels on the pooled control collectors on soft-sediment also differed over the one year sampling period (χ^2 = 661.2, DF = 11, p <0.001), with the only significant difference being the settlement over the interval of 23 March - 21 April 2015 (50 ± 15 mussels collector^{-1}) being higher than all other sampling intervals. Mean settlement across all other sampling intervals was consistently low (1 ± 0 mussels collector^{-1}) compared to the collectors in the mussel beds.

5.3.5 Recruitment to mussel beds

Mussel recruitment was nearly absent throughout the 25 months of quadrat sampling the four restored mussel beds. Only three recruiting mussels (26, 32, and 45 mm SL) were observed on a single sampling date (February 2015) within two of the mussel beds.

5.4 Discussion

5.4.1 Seasonality in settlement

To exploit potential opportunities to enhance settlement for the restoration of green-lipped mussel populations, it may be advantageous to target the deployment of settlement material in periods known to deliver high larval settlement. Seasonality in larval settlement, however, is not consistent across the geographic range of this species. Settlement was shown to be greatest during July to January with additional peaks of settlement in August - September at Ninety Mile Beach in far northern New Zealand (Alfaro, 2006b), whilst in the Marlborough Sounds in central New Zealand, peaks of settlement occurred during April for some areas, whilst other areas exhibited no observable peaks (Meredyth-Young & Jenkins, 1978). While determining seasonality of larval settlement was not the specific aim of this relatively short study, the results did indicate that settlement was largely concentrated during the months of March to August in the Hauraki Gulf, with the highest settlement in April - May. During these periods settlement on collectors in mussel beds were orders of magnitude higher than during the remainder of the year. Therefore, efforts to enhance settlement in this area would be best initially targeted towards this period until the seasonality of settlement can be more tightly defined for the Hauraki Gulf.

5.4.2 Increased settlement above mussel beds

Settlement of mussels on collectors within the restored mussel beds was generally higher than settlement collectors over soft-sediment. Furthermore, mussel settlement at fixed collector sites decreased following the removal of adult mussels from around the site by storm action.

These results strongly suggest that the adult mussels provide some form of enhancement that attracts and/or promotes the settlement of larval mussels. Both chemical (Pawlik, 1992; Anderson, 1996; Alfaro et al., 2006; Bao et al., 2007; Ganesan et al., 2012) and auditory cues (Wilkens et al., 2012) have been shown to enhance larval settlement in this species and may be responsible. The enhanced settlement could also be the result of the filtering capabilities of the adult population drawing a greater amount of water across the mussel beds, increasing the quantity of mussel larvae available for settlement. Another possible cause for the enhanced settlement could be the increased boundary layer as a result of both filtering and surface roughness (van Duren et al., 2006) resulting in mussel larvae becoming trapped in the turbulent water above the mussel bed. Although the mechanisms causing this enhancement are unknown, a lack of these mussel beds and the enhancement processes they provide could explain the low settlement previously observed on settlement collectors deployed over soft-sediment in the inner Hauraki Gulf (McLeod, 2009). The low settlement observed in this previous study is consistent with settlement levels observed on the control collectors over soft-sediment and away from adult mussel beds in this current study. The extensive scale of loss of mussel beds throughout the Hauraki Gulf has reduced this potentially important settlement habitat, which is likely contributing to a reduction in overall recruitment success.

5.4.3 Larval supply

Although there are larvae available for recruitment to restored mussel beds, the results of this study suggest that larval supply in the Hauraki Gulf is currently limited. The mean settlement rate throughout the year at the restoration site for settlement collectors within mussel beds was 36 ± 8 mussels month^{-1} with a maximum observed settlement on any collector of 418 mussels month^{-1}. Relative to settlement observed throughout other areas within the

geographic range of the green-lipped mussel, the magnitude of the settlement at the restoration site is comparatively low. For the Marlborough Sounds in the South Island of New Zealand, mussels are known to settle on similar collectors at more than 1000 individuals week^{-1} (Meredyth-Young & Jenkins, 1978). At Ninety Mile Beach in northern New Zealand, where huge quantities of mussel spat attached to bottom-drifting algae are collected to supply much of the aquaculture industry for this species (Alfaro et al., 2004), settlement levels on collectors of a similar design to this study were also much greater than the levels observed in this study, approximately 1800 mussels month^{-1} throughout most of the year (Alfaro & Jeffs, 2003). Along this same coast, settlement collectors of a different composition but of similar or lesser surface area, also exhibited greater levels of settlement than this current study (Alfaro, 2006b). Collectors placed directly into mussel beds as well as in adjacent algal habitat exhibited mean settlement rates ranging from 56 - 2447 mussels month^{-1} for much of the year. Although it is difficult to relate settlement levels on artificial collectors to recruitment of natural beds, the larval supply in these areas are known to be of sufficient magnitude to sustain wild populations of mussels (Alfaro, 2006b). The comparatively lower settlement rates measured in this current study provide further evidence to support the hypothesis that larval supply in the Hauraki Gulf may be limited and thus of insufficient magnitude to sustain restored mussel populations.

5.4.4 Settlement Substrate

Limitations in substrate availability may also be contributing to the lack of recruitment to the experimental mussel beds within the Hauraki Gulf. Whilst recently settled larval mussels (<0.5 mm SL) were consistently found on the collectors over most of the one year of sampling for mussel settlement, very few recruits (3 out of 1976 mussel sampled) were

observed in any of the four restored mussel beds during the five sampling dates over the 25 month study. This almost complete lack of recruitment into the restored beds, despite the availability of larval settlers on the artificial collectors, could be due to limited availability of natural settlement substrates on the mussel beds. The filamentous structures of some key macroalgal and hydroid species are known to be important to settling mussels (Bayne, 1964; Lasiak & Barnard, 1995; Buchanan & Babcock, 1997; Dobretsov & Wahl, 2001; Alfaro et al., 2004; Alfaro, 2006b; Gribben et al., 2011), many of which also provide chemical cues that promote settlement (Alfaro et al., 2006). Recruitment as a result of primary settlement onto these filamentous natural settlement substrates followed by a secondary settlement into adult mussel beds has been shown to be the predominant source of recruitment in this species (Buchanan & Babcock, 1997; Alfaro et al., 2004; Alfaro et al., 2006). Divers sampling the mussel beds and surrounding areas of soft-sediment over the 25 month period of the study never observed any of the filamentous macroalgae and hydroids previously identified as important natural settlement substrates for this species (Buchanan & Babcock, 1997; Alfaro et al., 2006) despite many of them being known to occur in the Hauraki Gulf (Lao, 2016). Therefore, the absence or the spatial separation of these important settlement substrates from adult mussel beds in the Hauraki Gulf may be restricting the scale of primary settlement and subsequent secondary migration resulting in an overall lack of recruitment to the adult mussel beds.

5.4.5 Predation of settlers

High mortality of primary and secondary settlers could also be contributing to the observed lack of recruitment to the restored mussel beds. A diverse community of organisms was observed to quickly establish within the experimental mussel beds including mobile

gastropods, crustaceans, and small demersal fish, any of which may have preyed upon settling mussels. Adult conspecifics are also known to cannibalize settling larvae and migrating post-settlers arriving at beds of adult mussels (Buchanan & Babcock, 1997; Alfaro, 2006a; Porri et al., 2008). This mortality can account for 77% of competent larvae available for settlement when comparing estimated numbers of larvae ingested by adult mussels to that of successful settlers in experimental plots without adult mussels (Porri et al., 2008). At certain times of the year the cannibalism of larvae can constitute up to 70% of the total ingested plankton from the water column by adult mussels (Alfaro, 2006a). The role of predation and conspecific cannibalism of primary and secondary settlers arriving in the experimental adult mussels beds need to be further investigated as a possible cause of the lack of recruitment in the experimental beds.

5.5 Conclusion

The arrival of reasonable numbers of mussel settlers on the artificial settlement collectors placed within the experimental mussel beds over much of the year and consistently for all experimental mussel beds indicated the presence of an ongoing larval supply. However, the lack of natural larval settlement substrate such as filamentous algae at the experimental mussel beds and on the adjacent soft-sediment sea floor of the Hauraki Gulf is a likely cause for the lack of recruitment observed for these beds. High levels of predation and/or cannibalism of mussels recruiting into adult mussel beds could be an alternative explanation for the scarcity of recruits in the beds, although settling mussels on the artificial settlement material persisted in the presence of mobile predators associated with the beds. With a lack of recruitment, natural levels of mortality will eventually lead to the loss of the entire beds of transplanted adult mussels, and the failure of the restoration initiative. The provision of

filamentous larval settlement substrate, whether artificial or natural, in association with the deployment of adult mussels to the sea floor may help to ensure sufficient ongoing mussel recruitment to sustain restored mussel beds. Future mussel restoration efforts should determine the feasibility and effectiveness of also restoring filamentous macroalgae and hydroids that are known to attract high rates of natural settlement of larvae of this mussel species.

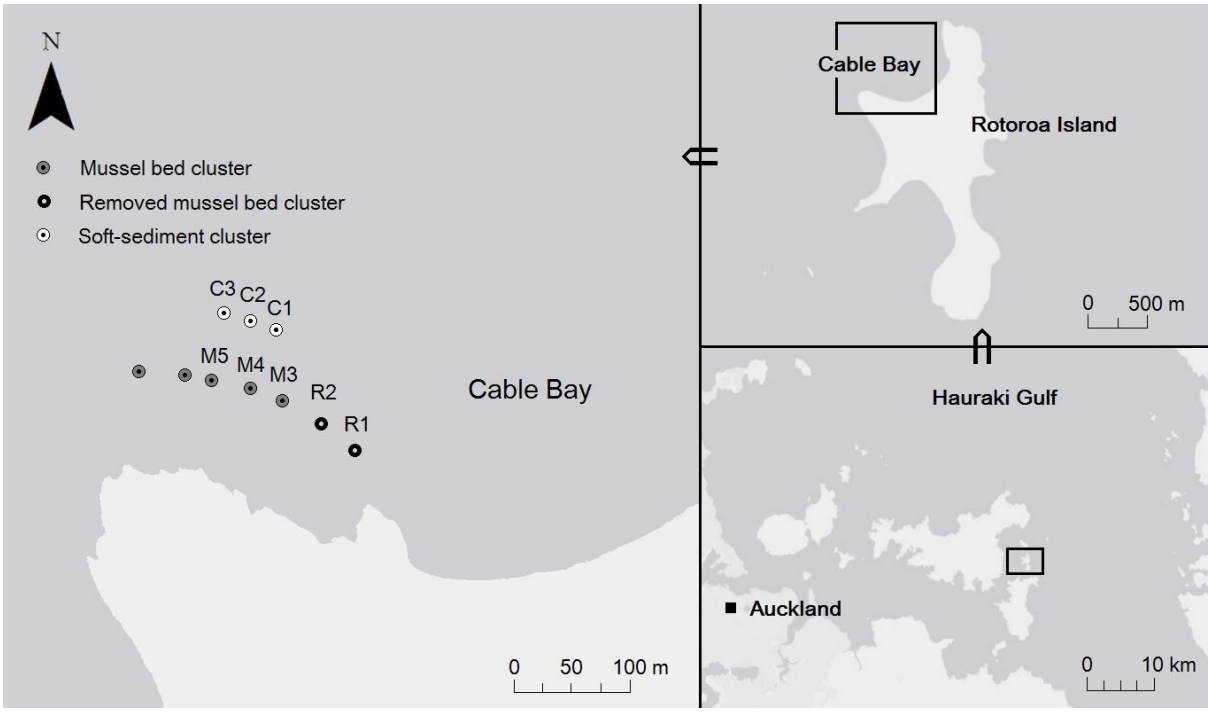

Figure 5.1 Map of restoration site indicating placement of mussel bed (M), control soft-sediment (C), and the destroyed mussel bed (R) clusters.

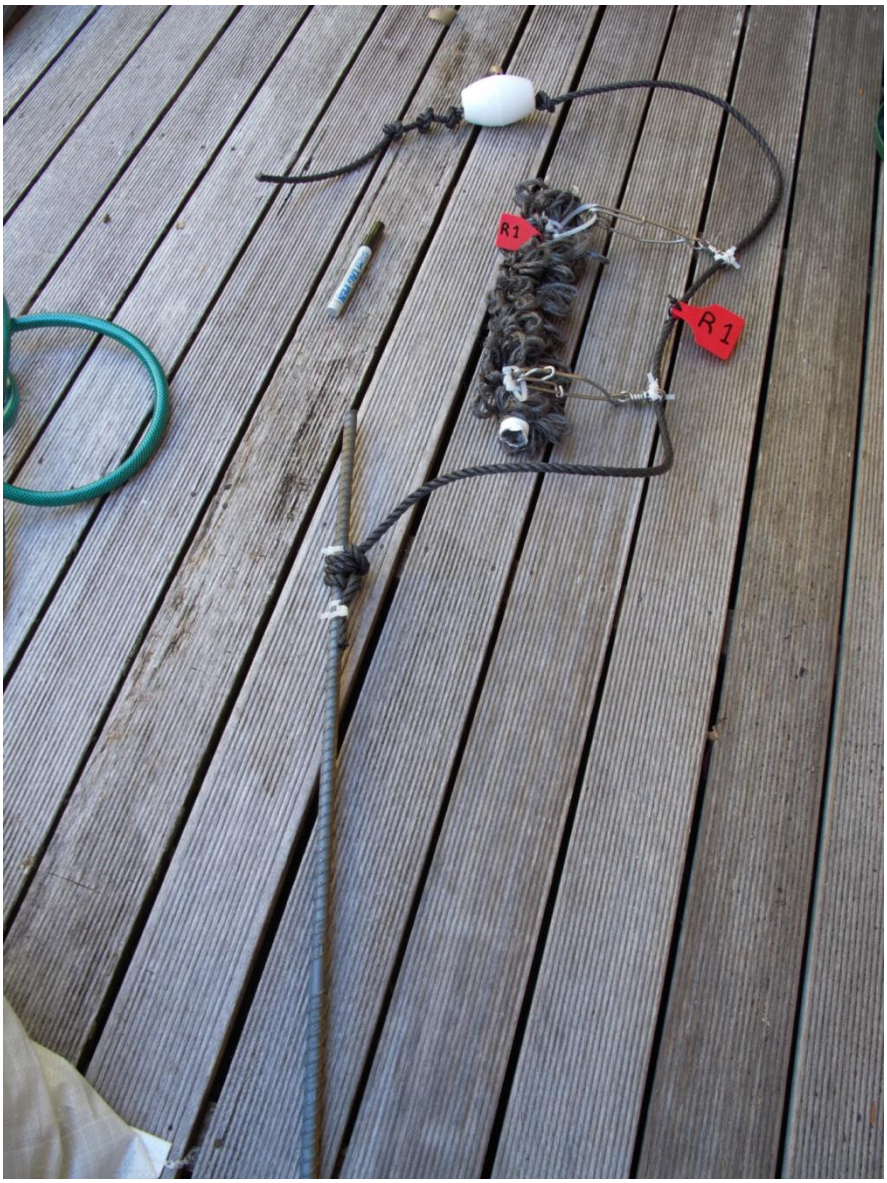

Figure 5.2 Settlement collector setup showing artificial collector (looped rope) attached by stainless steel shark clips to anchoring line which is tied to a steel rod hammered into the sediment.

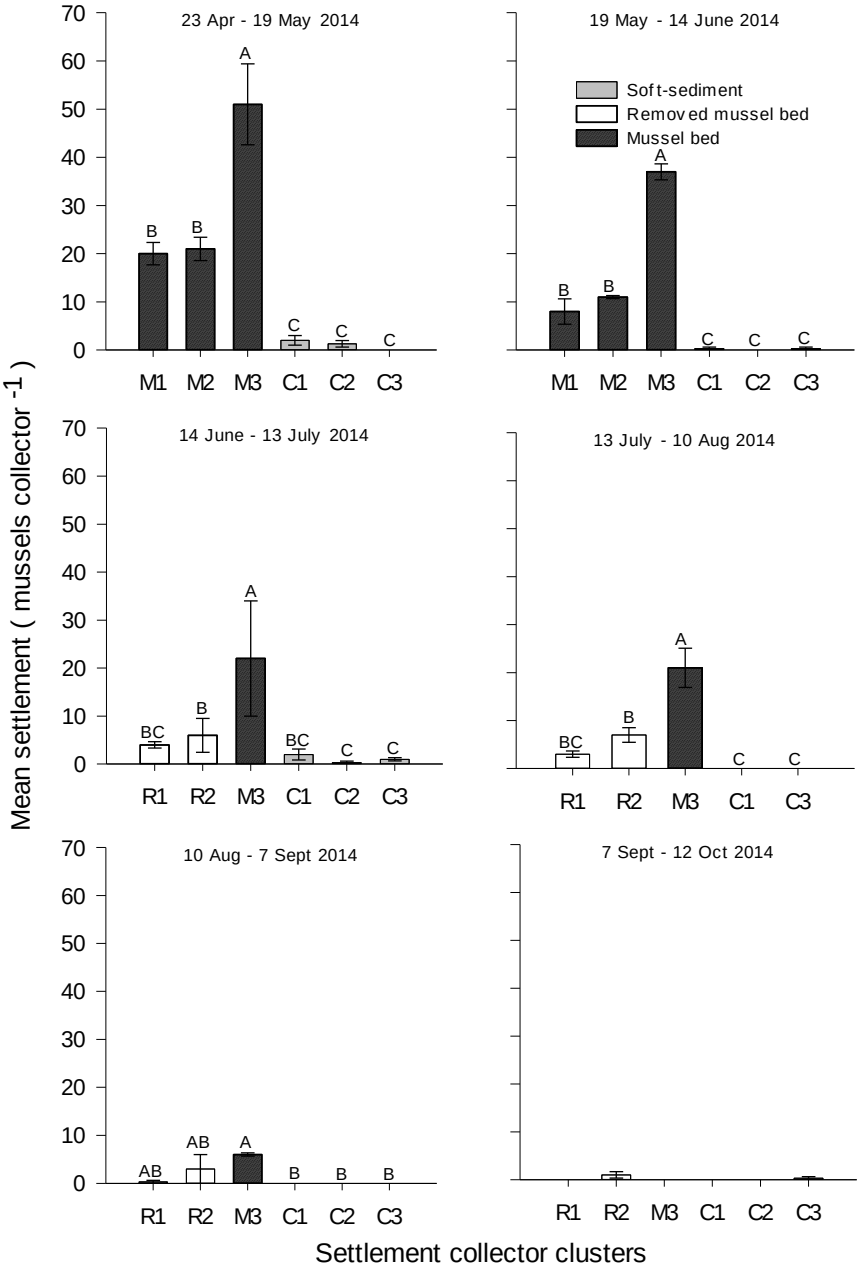

Figure 5.3 Monthly comparisons of mean (±SE) mussel settlement on collectors placed in triplicate (a cluster) among mussel beds (M1, M2, M3), adjacent soft-substrate (C1, C2, C3, Control), and removed mussel bed (R1, R2) areas between 23 Apr - 12 Oct 2014. Significant differences between pairs of clusters of collectors within a single sampling interval are indicated by different letters above the bars.

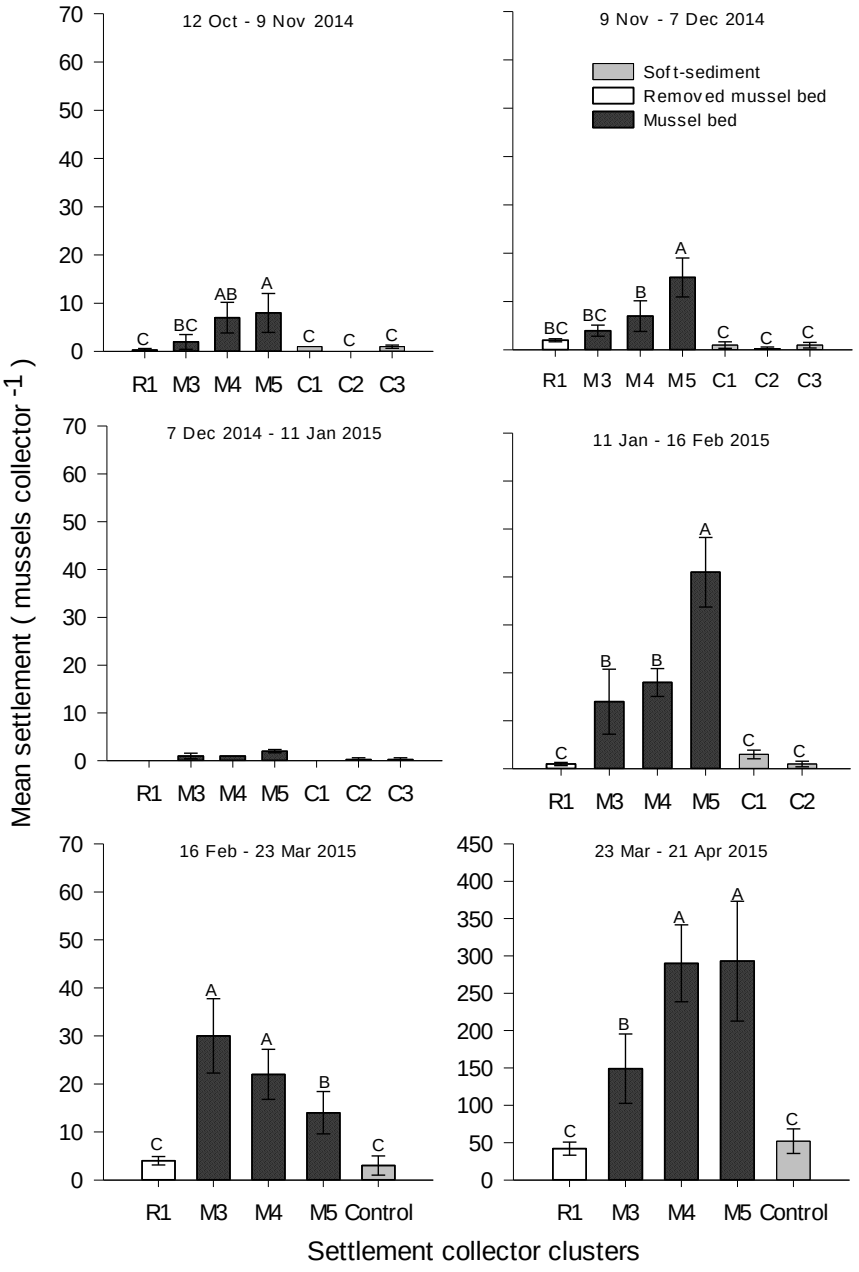

Figure 5.4 Monthly comparisons of mean (±SE) mussel settlement on collectors placed in triplicate (a cluster) among mussel beds (M3, M4, M5), adjacent soft-substrate (C1, C2, C3, Control), and removed mussel bed (R1) areas between 12 Oct 2014 - 21 Apr 2015. Significant differences between pairs of clusters of collectors within a single sampling interval are indicated by different letters above the bars. Note the different scales on the final sampling interval.

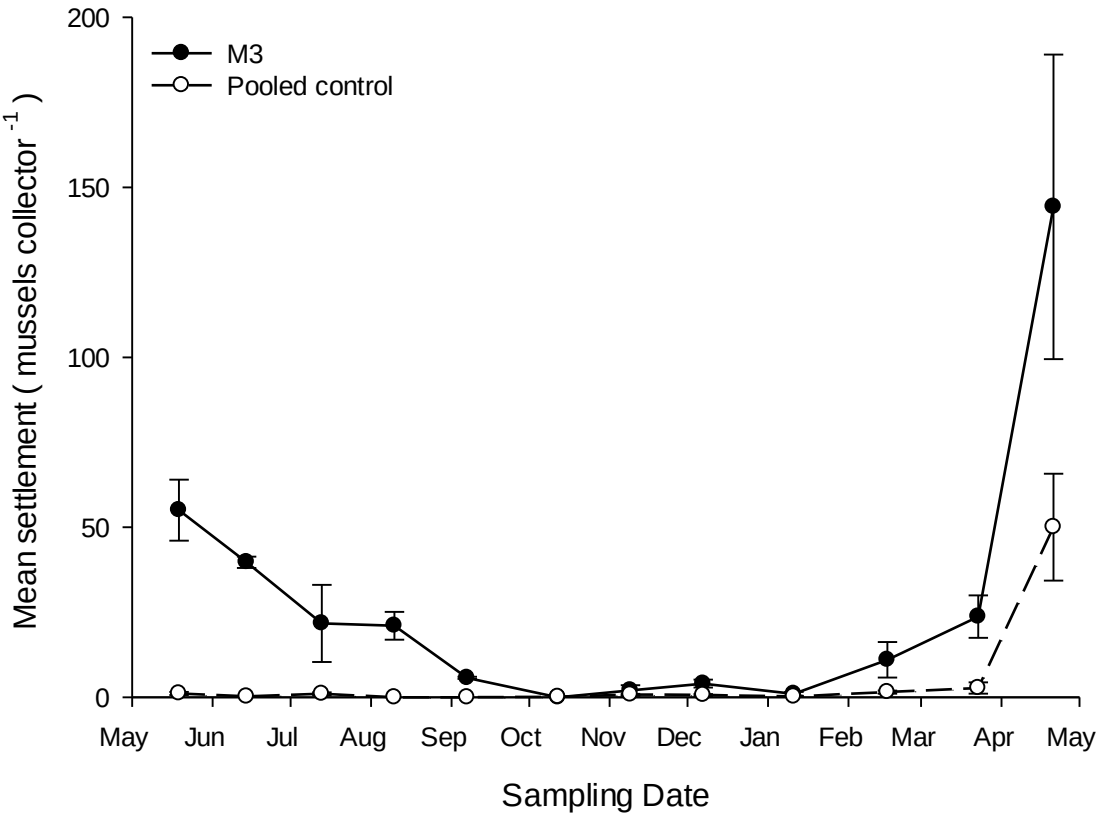

Figure 5.5 Monthly mean (±SE) settlement of *Perna canaliculus* on three settlement collectors within an experimental mussel bed (M1) and nine adjacent control collectors on areas on soft-substrate.

Chapter 6

General Discussion

6.1 Recruitment pathways in mussels

The settlement of mytilid mussels on filamentous macroalgae as a primary settlement substrate, followed by secondary migration of the juveniles to adult habitats is known as primary-secondary settlement and has long been considered the predominant source of recruitment into mussel beds (Bayne, 1964; Buchanan & Babcock, 1997; Erlandsson et al., 2011). Species of filamentous macroalgae favoured by the settling larvae of many species of mussels provide both structural and chemical cues that greatly increase larval settlement (Pawlik, 1992; Alfaro et al., 2006; Gribben et al., 2011). Adult mussels are known to cannibalise settling mussel larvae (Buchanan & Babcock, 1997; Alfaro, 2006a; Porri et al., 2008) and thus direct primary settlement within mussel beds poses an inherent risk of predation. Utilising a primary-secondary settlement strategy greatly reduces this risk as larvae can settle, metamorphose and grow to larger sizes at which they are less likely to be consumed by adult mussels whilst undertaking secondary migration into the mussel beds. However, there is evidence that the primary-secondary settlement strategy may not always be the predominant source of recruitment in mytilid mussel species, and that primary settlement directly to mussel beds can be the dominant recruitment process (McGrath et al., 1988; McGrath & King, 1991; Lasiak & Barnard, 1995; Reaugh et al., 2007). For example, entire cohorts of mussels that had directly settled to mussel beds were observed for several populations of *Mytilus edulis* in the United Kingdom (McGrath et al., 1988; McGrath & King, 1991). For the brown mussel *Perna perna*, some sites along the south African coast

exhibit predominantly greater quantities of recently settled mussels in adult mussel beds than on turfing algae (Lasiak & Barnard, 1995; Reaugh et al., 2007). Direct larval settlement into mussel beds could be expected to reduce the risk of mortality and the uncertainty of locating a mussel bed associated via secondary migration. This mortality during secondary migration has been demonstrated to be as high as 50% (Reaugh et al., 2007) but will vary according to the spatial separation of the filamentous macroalgae to that of the mussel bed.

Most previous research examining the efficacy of settlement substrates for the larvae of mytilid mussels have omitted the use of adult mussels as a potential substrate largely due to the widespread acceptance of the primary-secondary settlement hypothesis. However, more recent studies have shown that the mussel larvae of some species will settle preferentially into the complex network of byssal threads when compared to artificial filamentous substrates (Ompi, 2010). Furthermore, settlement-competent larvae have been shown to utilise both macroalgal and adult mussel odours by exhibiting directed swimming behaviour toward these cues (Morello & Yund, 2016). In the current study, the overall settlement of green-lipped mussels was markedly higher on artificial collectors placed within adult mussel beds (Chapter 5) compared with collectors placed on bare soft-sediment habitat adjacent to the mussel beds. This indicates that there are some factors associated with the adult green-lipped mussels that may help to enhance mussel settlement in the vicinity of mussel beds. The exact mechanism is unknown, however, chemical cues, auditory cues, the filter feeding adults drawing greater larvae towards the bed, and/or an increased boundary layer trapping greater larvae above the mussel bed could be responsible. It is also uncertain whether this enhanced settlement may act in synergy with macroalgae as a primary settlement substrate. Filamentous macroalgae preferred by settling larvae are frequently found growing in association with natural populations of green-lipped mussels (Buchanan & Babcock, 1997; Alfaro, 2006b; McLeod, 2009). The combination of settlement cues from macroalgae and the

enhanced settlement in the presence of adult mussels may promote settlement of mussel larvae onto filamentous macroalgae in the vicinity of a suitable secondary settlement site (i.e., mussel beds), and in so doing reduce the risk of mortality associated with this post-settlement migration into mussel beds. The factors responsible for this enhanced settlement within adult mussel beds may also assist post-settling larvae undergoing secondary migration from spatially separated, primary settlement structures to the mussel beds.

Further experimentation is necessary to determine which factors associated with the established mussel beds are responsible for the observed enhanced settlement, and its corresponding potentially synergistic interaction with settlement cues from filamentous macroalgae. Filamentous macroalgae were absent from the transplanted adult mussel beds and surrounding soft-sediment habitat in the current study and may have been a significant factor in the absence of recruitment of juvenile mussels to the beds, despite the availability of primary settlers on the artificial collectors located within the mussel beds.

Rather than being two discrete and competing recruitment strategies in mytilid mussels, primary-secondary settlement and direct primary settlement strategies are more likely to be complementary, and the predominant strategy responsible for recruitment is likely situational. For instance, in situations when larval settlement is low, cannibalism by adult mussels may lead to substantial mortality among settling larvae and thus primary-secondary settlement may be a more viable settlement strategy. Alternatively, increasing spatial separation of mussel beds from sources of filamentous macroalgae will result in increased mortality associated with secondary migration, making direct primary settlement a more viable settlement strategy. Direct primary settlement could be of greater importance in soft-sediment environments where filamentous macroalgae are potentially scarce, such as in the current study. The predominantly small mussels (<500 μm) observed on artificial collectors in this study could suggest that there was very little secondary migration to the

mussel beds (Chapter 5) and could indicate that primary settlement was the predominant strategy for recruiting mussels. The lack of recruiting mussels within the mussel beds (Chapter 2) could be due to cannibalism of the few settling larvae but could also be due to a lack of secondary migration of mussels as a result of an absence of filamentous macroalgae from both the mussel beds and the surrounding soft-sediment. Understanding the relative contributions of these two settlement strategies to mussel recruitment relative to available larval supply and the spatial availability of settlement substrates warrants further investigation. This could be addressed through the experimental transplant of filamentous macroalgae into adult mussel beds and adjacent soft-substrate areas using recently developed novel transplant techniques (Lao, 2016). Demonstration of the complementary role of adult mussels and macroalgae for recruitment to mussel beds may ultimately result in the necessity to transplant both sets of organisms in order to establish mussel beds with sufficient ongoing recruitment to maintain the beds.

6.2 Lack of recovery in green-lipped mussels in the Hauraki Gulf

The need for restoration comes from the fact that the natural recovery of destroyed habitat does not always occur when the original stressor that led to the habitat destruction has been removed. In the Hauraki Gulf, the overharvesting of green-lipped mussels is considered to be largely responsible for the loss of vast areas of mussel beds (Paul, 2012). Despite a lack of fishing pressure on mussel populations in the Hauraki Gulf for nearly half a century there has been no signs of any natural recovery (McLeod et al., 2012; Paul, 2012) with only a few small populations of mussels still remaining (McLeod, 2009). Previous work has demonstrated a lack of mussel settlement on artificial collectors placed on areas of bare soft-sediment sea floor (McLeod, 2009) which suggested that the Hauraki Gulf may have

inadequate larval production to re-establish mussel beds. There are sources of green-lipped mussel larvae available within the Hauraki Gulf from both the remnant mussel beds as well as the numerous aquaculture operations. Despite these available larval sources the results of this current study corroborates the suggestion that much of the Hauraki Gulf may be recruitment limited (McLeod, 2009), as indicated by the relatively low levels of larval settlement found at the restored mussel beds compared to other locations around New Zealand where mussel populations are well established (Chapter 5) as well as a lack of recruiting mussels into the experimentally transplanted adult mussel beds (Chapter 2). The northern horse mussel, *Modiolus modiolus*, exhibits predominantly local retention of larvae at source populations, suggesting that these populations are maintained by self-seeding (Elsäßer et al., 2013). Patterns of larval dispersal in the green-lipped mussel are unknown for the Hauraki Gulf. However, the low settlement and lack of recruitment in the current study could be the result of dispersal being dominated by processes that retain larvae locally. The restored beds were not located in close proximity to any sources of larval mussels other than the restored beds themselves. A small mussel farm is located on the opposite side of the Waiheke channel (2 km distant) and would represent the most likely source of non-locally retained larvae, however, the greatest sources of mussel spat would be from aquaculture operations in Coromandel (20 km distant) which would be an unlikely source should local larval retention be the predominant strategy for supplying larvae to populations. The loss of the extensive mussel beds throughout the Hauraki Gulf has not only greatly reduced the breeding population and larval supply but may also be restricting the spatial availability of larvae for recruitment and population recovery. The use of hydrodynamic models has been previously employed to examine dispersal patterns from source populations (Elsäßer et al., 2013) and could be utilised in conjunction with settlement collectors to determine if local retention is the predominant dispersal pattern in the Hauraki Gulf.

The historic adult mussel beds in the Hauraki Gulf would have provided critical habitat for recruiting mussels. Both primary and secondary settlers are known to preferentially settle to areas containing adult conspecifics (Fariñas-Franco et al., 2013; Commito et al., 2014) and in soft-sediment habitats mussel settlers have been shown to attach predominantly to hard structures such as artificial structures and conspecifics (van der Heide et al., 2014). The current study has shown that juvenile green-lipped mussels will preferentially attach to live adult mussels versus the shells of dead mussels, and transplanted juveniles were exclusively recovered attached to either adult mussels or dead mussel shell in a field experiment (Chapter 4). Therefore, the removal of adult mussel beds in the Hauraki Gulf is likely to have greatly reduced the available attachment substrate for recruiting mussels. Furthermore, predation of juvenile mussels by small sea stars was found to be reduced when juvenile mussels were attached to adult mussels compared to when they were provided with either dead mussel shell or unmodified soft-sediment for attachment (Chapter 4). The complex matrix of byssal threads and tightly packed adult mussels found within an established bed of mussels are likely to provide a significant barrier to probing by predators of juvenile mussels. Post-settlement mortality plays an important role in structuring populations of marine invertebrates (Hunt & Scheibling, 1997) and the complex habitat created by the adult mussel beds likely reduces the risk of mortality for recruiting mussels.

Mortality by predators such as sea stars could also be limiting recovery of the mussel beds in the Hauraki Gulf. The deployment of 7 t of adult mussels in the current study quickly attracted sea stars in high abundance that resided and preyed on the mussels in the restored beds for the duration of the study (Chapter 3). Research on sea star feeding ecology has demonstrated that predation on mytilid mussels can have a major impact on the distribution and abundance of bivalve populations (Paine, 1966; Paine, 1971; Paine et al., 1985). The eleven-arm sea star *Coscinasteris muricata,* is commonly found in high densities on subtidal

green-lipped mussel beds (Inglis & Gust, 2003; McLeod, 2009; Paul-Burke, 2015). Although densities of sea stars varies throughout the Hauraki Gulf, including on remnant mussel beds, any recovering or re-establishing mussel bed may be quickly overwhelmed and removed by sea star predators migrating from adjacent soft-sediment habitats. This study estimated that the magnitude of the sea star predation would be sufficient to ultimately remove the mussel beds. Losses of up to 88% of green-lipped mussels over four years have been observed in a natural mussel population in Ōhiwa Harbour, of which the high densities of sea stars were certainly a major contributor (Paul-Burke, 2015). Sea stars are also known to swarm in immense densities and migrate into mussel beds to feed sometimes leading to the removal of entire mussel beds (Dare, 1982; Kristensen & Lassen, 1997). Although the sea stars did not reach densities close to those measured in previous studies, in the current study the mortality of adult mussels from sea star predation after 25 months accounted for 30.1% of the initially estimated mussel population (Chapter 3). This still represents a considerable source of predation and especially in an environment where recruitment is limited, even a small amount of predation can result in the failed establishment or complete removal of recovering mussel beds.

6.3 Best practice methods for restoration of green-lipped mussel beds

The criteria for success in any bivalve restoration effort includes both the establishment and persistence of the restored population. The robust and frequent assessment of population dynamics is a fundamental aspect of bivalve restoration, not only to determine the ongoing success of these efforts, but also to identify those factors that might limit or promote the sustainability of those restored populations. Experimental investigations of those limiting and promoting factors, along with observations during assessments of restored populations, can

then be incorporated into best practice methods that provide a foundation for future restoration efforts and help to direct further research. The current study is the first recorded instance of mass transplantation of green-lipped mussels from suspension aquaculture to the soft-sediment sea floor for the purposes of restoration. The deployment methods were similar to that employed in sea floor mussel aquaculture practices in Europe where juvenile mussels are released from the surface to settle to the sea floor and aggregate into a mussel bed (Ysebaert et al., 2009; Dolmer et al., 2012). The low estimated mortality associated with the deployment technique in the current study, which was comparable to subsequent mortality rates throughout the remainder of the study, suggests that this method of deploying adult mussels is a viable means to establish mussel beds with this species (Chapter 2). The high density of mussels deployed to the sea floor can result in decreased subsequent survival (Capelle et al., 2014) and in the current study, the high density of mussels initially deployed was observed to result in mortality due to the burial of mussels in the soft-sediment under the weight of settling conspecifics (Chapter 2). By reducing densities of the mussels deployed from the surface or by divers rearranging the mussels following deployment, the initial mortality could be further reduced. Previous studies have shown that the provision of attachment substrate in the form of shell hash does not enhance the persistence of transplanted mussels (Fariñas-Franco et al., 2013; de Paoli et al., 2015) and the results of this study on green-lipped mussels has confirmed that persistence of adult mussels would not be increased by providing attachment substrate prior to transplanting onto soft-sediments.

Despite the successful establishment of the mussel beds in the Hauraki Gulf, the persistence of these beds is unlikely. Over the 25 months of the study the mean mortality of mussels was 74% of the initially estimated mussel abundance (Chapter 2). The pattern of relatively steady and ongoing decline of all of the experimental mussel beds over the course of the study period suggests that the mussel beds will cease to exist at some point in 2017.

Having ample recruitment to offset mortality is critical to the maintenance of restored mussel populations. However, the restored mussel beds exhibited insufficient recruitment of juvenile mussels to offset the mortality observed (Chapter 2). Levels of settlement to artificial collectors in the current study were comparatively lower (Chapter 5) than those observed at natural mussel beds where recruitment is at sufficient levels to maintain their respective populations (Meredyth-Young & Jenkins, 1978; Alfaro, 2006b). The low larval supply, lack of settlement substrates, and mortality of settlers are likely contributing to this lack of recruitment, however, the relative importance of each contributor needs to be determined in order to develop best practice methods to overcome these limitations. For instance, constrained larval supply could be overcome by greatly increasing the local breeding stock if there is a high degree of larval retention at the restoration site. Alternatively, future restoration sites could be selected on the basis of either being within close proximity to existing larval sources or at sites to which larvae regularly disperse. A greater understanding of larval dispersal patterns in this mussel species in the Hauraki Gulf will be necessary to adequately understand and address these issues. Similarly, the relative importance of the availability of settlement substrates (both filamentous algae and adult mussel beds) and the subsequent contribution of settlers from those settlement substrates to recruitment can be experimentally examined as outlined previously.

Sea star predation is also a major constraint to the persistence of restored mussel populations in the Hauraki Gulf. Predation by these sea stars accounted for 40.1% of the mortality observed on the experimental mussel beds over the 25 month study (Chapter 3). Although abundances of these sea stars may vary both spatially and temporally, they are common throughout the Hauraki Gulf and are likely to pose a potential limitation to any restoration effort. In the event that there is low larval supply or recruitment, the predation from sea stars will have a greater impact on those restored populations. Overcoming this

limitation to the persistence of restored mussel beds may require the selection of restoration sites that exhibit low sea star abundance, or the subsequent removal of sea stars that migrate or recruit into restored mussel beds utilising one of the many methods developed for sea star removal in seabed aquaculture practices of mussels (Barkhouse et al., 2007). In the case of the current study the removal of sea stars that colonized the restored mussel beds shortly after their establishment (Chapter 3) was likely to have been effective given there was relatively little subsequent arrival of sea star migrants or recruits.

The final major limitation to the persistence of the restored mussel beds examined in this study is the remaining 60% of the mortality of adult mussels which remains unexplained. Although many potential sources of mortality were ruled out as possible prime contributors, the effect of the local environmental conditions on the transplanted mussels is unknown. Transplanting mussels from hatchery or wild stock into non-natal environments has been shown to result in higher mortality than using mussels from wild stock originating from the local environment (Mallet et al., 1987; Stirling & Okumuş, 1994). Mussels supplied from aquaculture in the current study originate from seed mussels gathered at Ninety Mile Beach in northern New Zealand where populations are exposed to high wave action and grow on rock substrate. In addition, once grown out in suspended aquaculture they exhibit different morphologies and behaviours related to the culture conditions. Whether the environmental conditioned encountered by adult mussels placed on soft-sediment in the Hauraki Gulf is less conducive for the survival of these mussels sourced from aquaculture versus the remnant population is unknown. The growth of mussels in this study (Chapter 2) indicated that mussels in the restored beds are allocating resources to somatic growth, however, this does not preclude the possibility that the condition of the mussels could have been poor, possibly leaving the mussels susceptible to other forms of mortality such as predation or disease. The effects of transplanting adult mussels derived from the aquaculture of non-natal juveniles

onto the soft-sediment sea floor of the Hauraki Gulf could easily be examined by the experimental transplant of natal and non-natal adult mussels, both aquacultured and wild, into this situation.

6.4 Conclusion

This research has provided greater insight into the processes affecting adult green-lipped mussels once deployed to soft-sediment habitats, as a potential approach to restoring the once extensive beds of this mussel species that were found in the Hauraki Gulf. While the method of deployment in itself is effective in rapidly creating benthic beds of adult mussels, high ongoing mortality, partly due to sea star predation, combined with almost no recruitment will ultimately result in the loss of the restored mussel beds. Study of these restored mussel beds indicate that a combination of constrained larval supply, a lack of preferred larval settlement substrates, and sea star predation are likely major contributors to the lack of natural recovery following the closing of the green-lipped mussel fishery in the Hauraki Gulf over 50 years ago. Although the mussel beds established in this study are unlikely to persist, the knowledge gained from them is likely to greatly assist in future mussel restoration efforts in the Hauraki Gulf.

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