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Settlement, retention, growth, and condition in Greenshell™ mussels (*Perna canaliculus*) in the Hauraki Gulf



Rebecca Jayne Smith

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Abstract

The Greenshell™ mussel (*Perna canaliculus*) is the most valuable aquaculture species in New Zealand. A review of the aquaculture development of *P. canaliculus* identified several areas where information was lacking, particularly for the factors that influence the growth, condition and retention, which are critical for effective management of its aquaculture. Over a two year period, both juvenile settlement and adult growth and condition were measured in the Hauraki Gulf to assess the potential addition of Hauraki Gulf-sourced spat to the industry. In order to focus spat collection efforts, settlement rates at two sites were measured and no difference found, although settlement at 11 m depth was consistently higher than at 4 m. Seasonal spat settlement was also consistent between the sites, with considerably higher rates in spring and summer months. Mussels have traditionally been sourced from Ninety Mile Beach, which spawned a month earlier than Hauraki Gulf mussels, and had significant differences in condition during October and November, therefore allowing greater harvesting yield. There was no difference in mussel growth between the two sources. Retention and attachment rates increase our understanding of how it may be possible to selectively seed spat of a certain size range onto farm lines to ensure greater retention rates, and these were found to vary among spat collected from the five locations examined. Attachment rates were found to decrease at a shell length of 5 mm and upwards, whereas retention rates decreased in juvenile mussels from 0.8 to 5 mm in shell length and then increased again from 5 mm upwards. Finally, this research examined how underwater sound affects the growth and retention of spat, with 50 – 500 Hz of white noise resulting in the highest growth and retention rates and lowest mortality for both static and flow-through experiments. Collectively, the results from this study improve our understanding of the biology of *P. canaliculus* in relation to aquaculture conditions. The results also provide a number of avenues for greatly improving the efficiency and commercial production from Greenshell™ aquaculture, not only in the Hauraki Gulf, but potentially in other key growing areas.

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Chapter 1: General Introduction

1.1 Introduction

Global aquaculture production has grown dramatically over the last 50 years, increasing at an average annual growth rate of 6.3 % from 34.6 million tonnes (t) in 2001 to 63.6 million t in 2011 (Bostock et al. 2010, FAO 2014a). By 2016 the global aquaculture production of animals had grown to 80 million t with an estimated value of USD\$243.5 at first sale (FAO 2018), and 30.1 million t of aquatic plants (US\$11.7 billion) were cultured globally in 2014. The growth of this industry is not only due to increased food demands but also the ongoing development of aquaculture technology (Boyd & Schmittou 1999). Due to the plateau in global wild fisheries production observed from 2004-2016, combined with ongoing increases in the human population, aquaculture is helping to meet the growing global demand for both food and economic security (Bostock et al. 2010, FAO 2012, Merino et al. 2012, FAO 2018). World aquaculture production in 2016 consisted of 54.1 million t of finfish, 17.1 million t of molluscs, 7.9 million t of crustaceans, and 0.9 million t of other aquatic animals (FAO 2018).

1.2 Mussel Aquaculture

1.2.1 International Mussel farming

Mussels are thought to be one of the most environmentally sustainable aquaculture species due to the low environmental impact of farms and the absence of a requirement to provide them with feed (Bostock et al. 2010, Naylor et al. 2000). This is unlike the aquaculture of carnivorous finfish species that is most often reliant on fishmeal made from harvested fish. In 2010 marine mussels were the third most important mollusc species produced by aquaculture in the world in terms of total volume (1.9 million t), after clams (5.4 million t) and oysters (5.2 million t) (FAO 2014a). The main species of cultivated mussel around the world are blue (*Mytilus edulis*), Mediterranean (*Mytilus galloprovincialis*), Pacific blue (*Mytilus trossellus*) and green-lipped (*Perna canaliculus*). The largest mussel aquaculture producing countries in

the world are China, Europe, Canada and the USA (FAO 2012). There is a variety of aquaculture techniques used in the production of mussels, including floating rafts, pillars, and Japanese long-line systems, but all techniques essentially rely on suspending mussels in the water column where they are able to feed freely on suspended particulates. The market-ready products derived from mussel aquaculture includes fresh and frozen mussel products (FAO 2012), as well as a variety of processed human and animal health supplements for arthritis and dietary health (Efthimiou & Kukar 2010).

1.2.2 *P. canaliculus* Aquaculture

Aquaculture is one of the fastest growing rural businesses in New Zealand (FAO 2004). In a combined effort, the NZ Aquaculture Council, NZ Seafood Industry Council and the Ministry of Economic Development launched *The New Zealand Aquaculture Strategy* in 2006 (Burrell & Meehan 2006). The strategy aims to increase sales from the New Zealand aquaculture sector to \$1 billion per annum by 2025 with a significant component of this proposed growth coming from expanding the existing mussel aquaculture industry (Fig. 1) (Burrell & Meehan 2006, NZAC 2006, Young 2009). Aquacultured *P. canaliculus* mussels are sold under the registered trade name Greenshell™, and are mostly exported to North America, Europe, with more recently expansion of exports into parts of Asia (NZAC 2010, Alfaro et al. 2012, Seafood New Zealand 2017). The New Zealand Greenshell™ mussel industry produced 97,438 t of green weight in 2014 (FAO 2014b) and *P. canaliculus* is now the single most important seafood export species for New Zealand by value (NZD\$203 million in 2015), and employs the equivalent of 2500 people in the industry (Aquaculture New Zealand 2009, Ministry of Fisheries 2009, Seafood New Zealand 2017). Farming of Greenshell™ mussels began in the 1970's and production has grown exponentially over the last 40 years (Hickman 1976, Dawber 2004).

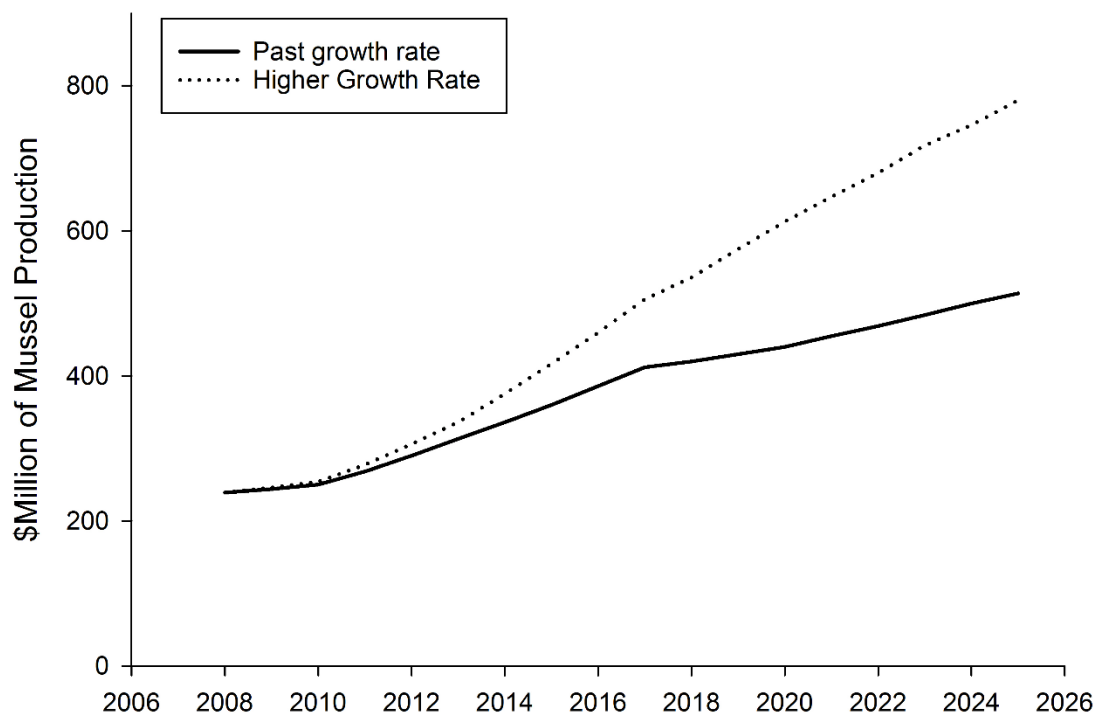


Figure 1. Projections of future value of aquaculture production of *Perna canaliculus* in New Zealand based on the continuation of existing mean growth rate as at 2006 and a higher growth rate associated with meeting the aquaculture sector growth target of a total of \$1 billion of aquaculture production for New Zealand by 2025 (NZAC 2006).

In 2009 there were 1018 authorised mussel farms in New Zealand, concentrated in the Marlborough Sounds and Coromandel areas. The number of farms has not increased significantly since 2009 (Ministry of Fisheries 2009). The Hauraki Gulf is a particularly important region for mussel aquaculture as it is responsible for providing around one third of the total mussel production in New Zealand, with farms located at Great Barrier Island, eastern end of Waiheke Island, around Te Kouma and Coromandel Harbour, Orere Point, and offshore from Wilsons Bay. Warm waters and high phytoplankton productivity help to deliver faster production cycles in mussel aquaculture in most locations in the Hauraki Gulf compared to mussel operations further south in the country (Dawber 2004). The Greenshell™ mussel aquaculture industry is almost entirely reliant on seed mussels collected from the wild, with around 20 % caught on fibrous ropes suspended near mussel farms in the Marlborough Sounds and Golden Bay, and the balance supplied by sporadic strandings of seaweed with

attached mussel seed which is collected from a single beach in northern New Zealand (Alfaro et al. 2004, Gribben 2011, Alfaro et al. 2012).

The commercial harvesting of juvenile green-lipped mussels (which are referred to as spat) for aquaculture began at Ninety Mile Beach on a small scale in the early 1970s and was initially conducted under a fishing permit (Section 63, Fisheries Act 1983) (Ministry of Fisheries 2004). In 2004 mussel spat harvesting was added to the Quota Management System (QMS) (Ministry of Fisheries 2004). Adult wild green-lipped mussels were harvested in a commercial dredge fishery from the 1920s to the 1960s in the Firth of Thames and the inner Hauraki Gulf. This practise ultimately overfished the extensive mussel beds that were once found there (Reid 1968, Greenway 1969). Unfortunately, more than 40 years after the fishery closed the mussel populations have not returned (Morrison et al. 2009, McLeod et al. 2012). There has been some successful establishment of clusters of mussel on the seafloor where shells have dropped from the mussel farms in the area (Morrison et al. 2009). Larval mussel spat supply is not thought to be a problem, with spat being caught by farm operators running experimental spat-collecting lines (Bartrom 1990, Morrison et al. 2009). Larval settlement and growth has been found to be prevented due to the fine sediment composition of the seafloor and absence of substantial surface structure such as dead shells, hydroids and red algae for larvae to settle on, as well as the continuing re-suspension of silt from the seabed during storm events (Morrison et al. 2009, McLeod et al. 2012).

1.2.3 Current Mussel Aquaculture Industry Procedures

Mussel farming in New Zealand was first trialled in the mid 1960s, using pontoon-style (Spanish-raft) cultivation techniques (Bartley 2005). In the mid 1970s, traditional Japanese long-line technology was introduced and since then has been adapted to allow for mechanised farm management practices (Jeffs et al. 1999, Dawber 2004). A typical mussel farm contains approximately 10 long-lines. Each long-line is composed of two backbone ropes (over 100 m in length) and supported by 30–40 large floats (Butler 2003). A continuous mussel grow out rope is attached to the backbone ropes to form vertical loops of rope, 5-15 m in length, that are known as dropper lines, providing a large vertical surface area for growing mussels (Young 2009). Each year around 160 t of drift algae with attached mussel spat is

collected from Ninety Mile Beach when it washes into shallow waters along the beach (Hickman 1976, Alfaro & Jeffs 2002, Alfaro et al. 2004). Ninety Mile Beach is the only location where there are such large amounts of wild spat on detached seaweed and other material that can be conveniently and economically harvested to supply the Greenshell™ mussel industry (Jeffs et al. 2000, Alfaro 2001). *Perna canaliculus* are attached to the algae in densities of up to 1 million spat per kilogram of algae. Once in the surf zone, it is collected either by hand in scoop nets or with a mechanical harvester consisting of a large scoop net mounted on the front of a tractor. The spat material is sorted to remove debris and algae that does not contain mussel spat. Commercial harvesters often return up to 50 % of the unwanted harvested material to the beach after sorting (Ministry of Fisheries 2004). The harvested spat are then mostly shipped in refrigerated trucks to mussel farms around New Zealand (Jeffs et al. 1999). Once at the farms, the algae and spat are placed on farm nursery lines, surrounded by a biodegradable cotton mesh stocking at a density of 1000 to 5000 spat per metre of rope (Fig. 2) (NZAC 2005). Subsequently, the spat migrate onto the rope while the cotton stocking degrades within a few weeks (Alfaro 2004, Carton 2007, Jeffs 1999, Alfaro 2012). When the juveniles are firmly attached to the ropes and have grown to a larger size of 30-40 mm, the mussels are stripped from the lines and transferred to the longline grow-out system at a lower density of around 150–200 mussels per metre (NZAC 2005). This is to reduce densities and maximise production. After approximately one year, once the mussels reach a shell length of 90 to 120 mm, the mussels are ready to be mechanically harvested (Fig. 2) (FAO 2005).

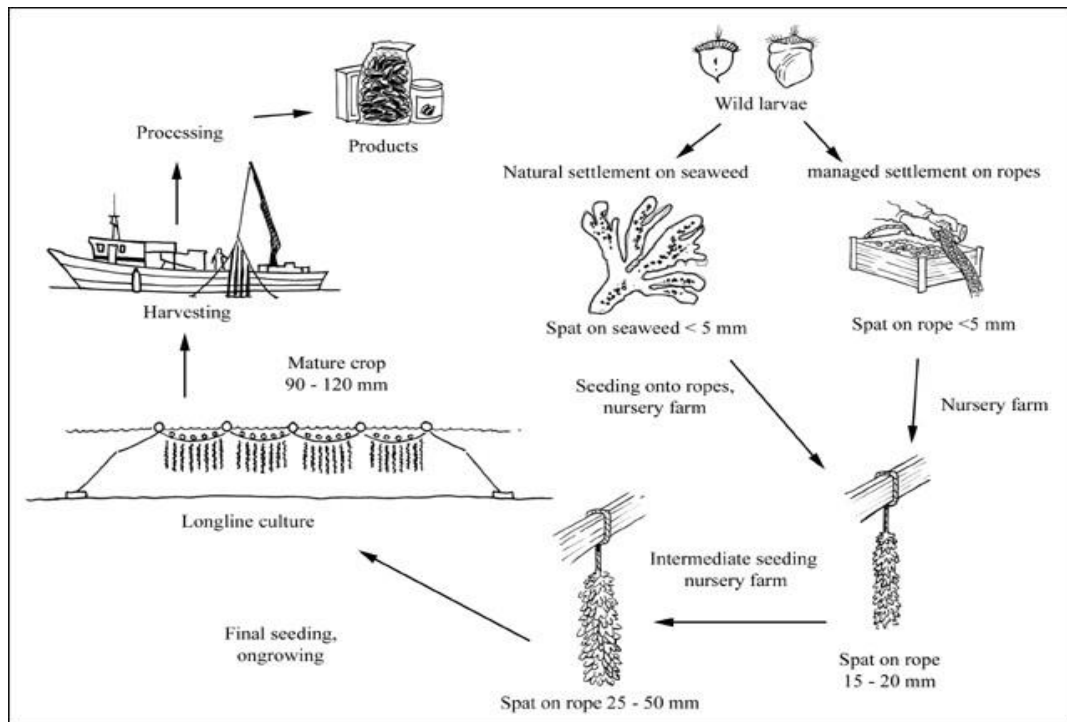


Figure 2: Overview of harvesting and farming procedures of Greenshell™ mussels (FAO 2005).

Perna canaliculus spat is also collected from Golden Bay on spat catching ropes and used to supply aquaculture operations, predominantly in the Marlborough Sounds, in combination with spat from Ninety Mile Beach (Alfaro et al. 2012). The breeding cycles of the cultured mussels derived from spat harvested from these two wild populations appear to be asynchronous. This provides an extended period for harvesting mussels with full gonads which is preferable for improved eating quality and to maximise the harvest weights of mussels. Mussels grown in the Marlborough Sounds from spat sourced from Ninety Mile Beach tend to fatten and spawn from around August until January, whereas mussels grown from Golden Bay spat tend to fatten and spawn later, usually starting around January and ending later in the summer (Alfaro et al. 2012).

The Greenshell™ mussel industry is extremely vulnerable in terms of supply and retention of the mussel seed which the industry relies on. The loss of mussel spat from nursery ropes can be as high as 100 %, but is usually between 50 and 70 % (NIWA 2006, Carton et al. 2007, Sim-Smith 2011). This wasteful use of mussel spat costs the industry between NZD\$6-10 million annually (Alfaro et al. 2012). Very little is known about the actual causes of poor spat retention in *P. canaliculus* although some of this loss is suspected to occur due to poor initial

spat attachment during seeding, predation by fish, high water currents, poor water quality, limited food supply, inclement weather conditions, disease and biofouling (NIWA 2006, Alfaro et al. 2012). The secondary settlement behaviour of *P. canaliculus* is also thought to be responsible for a majority of the loss of spat from seeded mussel farm lines whereby wild spat transferred onto nursery ropes on mussel farms migrates off the ropes, presumably in an effort to find a more desirable habitat (Foote 2003).

1.2.4 Stocks

The Greenshell™ mussel industry is heavily reliant on wild caught spat sourced from Ninety Mile Beach, which can be variable in both quality and quantity (Jefferies et al. 1999). In 2001 a toxic bloom of *Gymnodinium catenatum* saw a ban on any mussel movement and spat collection throughout the Kaitia region, resulting in financial losses of around \$5 million per month to the industry (Irwin et al. 2003). Quality and retention of spat that arrives at the farms can be greatly affected by environmental, collection and transport stress (Foote 2003, Carton 2007, Alfaro et al. 2010). The wild spat supply from Ninety Mile Beach is reliant on hydrodynamic and meteorological patterns for drift algae to be washed on to shore. As a result, the quantity and timing of mussel spat supply from this wild resource is sporadic and unpredictable (Alfaro 2004, Alfaro et al. 2010). For the purposes of the research presented in this thesis, mussels sourced from spat obtained from different areas around the country are referred to by their place of origin, i.e., Hauraki Gulf, Kaitia (Ninety Mile Beach), Marlborough Sounds, Golden Bay and Hauraki Gulf stocks. The use of multiple stocks by the mussel industry is very important as it can even out the supply of spat and minimises the impact of diminished spat availability at one or two of the sites (Hickman 1982, Hickman 1987, Hayden and Kendrick 1992, Hayden 1995). A number of industry groups have developed hatchery production technology for *P. canaliculus*, however, due to the high cost of hatchery production, it is a much more expensive option than the widespread current use of wild caught spat (Alfaro et al. 2012). Development of artificial foods, selective breeding programmes and reliable methods of broodstock mussel conditioning may help reduce these costs in the future (Ragg et al. 2010).

1.3 Biology and Ecology of *P. canaliculus*

1.3.1 Habitat and location

The mussel genus *Perna* differs from the more diverse genus *Mytilus* by its morphological characteristics, including shell colouration, soft tissue morphology, position of muscle scars and geographic distribution (Siddall 1980, Wood et al. 2007). There are three species in the *Perna* genus; *P. canaliculus* (Gmelin 1791) is endemic to New Zealand, while *P. perna* (Linnaeus 1758) is found throughout coastal regions of South America and Africa, and *P. viridis* (Linnaeus 1758) is present in many parts of the Indo-Pacific (Alfaro et al. 2012).

Perna canaliculus is characterised by its large maximum adult size (up to 250 mm in shell height) and distinctive green coloration on its interior shell margin. There is no difference in the mean shell length between males and females of the same age (Alfaro et al. 2001). Adult mussels are found in lower intertidal and sub-tidal (>50 m) locations throughout New Zealand but are most abundant in the warmer waters of the North Island (Powell 1979). Intertidal populations are thought to be limited by aerial exposure (Paine 1971, Marsden & Weatherhead 1998), while sub-tidal populations are limited by predation pressure (Paine 1971). This mussel species typically inhabits waters ranging in temperature from 5.3 – 27°C (MacDonald 1963, Hickman 1991) and is tolerant of salinities from 25-35 ppt (Alfaro 2001, Alfaro 2006). The green-lipped mussel forms dense beds of up to 100 individuals per m² (Flaws 1975, Hickman 1991) on firm substrata such as rocky reefs and wharf piles, but can also be found on sand or mud in the sub-tidal zone (Morton & Miller 1973, Buchanan 1997, Jeffs 1999).

1.3.2 Reproduction and Larval Development

Perna canaliculus has a 1:1 sex ratio and is a dioecious broadcast spawner, the spawning of which normally reaches a peak at some time in late spring to early autumn but is variable among populations (Jenkins 1985, Alfaro 2001). Mussels reach sexual maturity within a year after settling as larvae, which for the majority is at a shell height of 40-50 mm. Immature mussels have cream coloured gonads which, when mature, remain cream in males and are reddish-orange colour in females (Hayden 1994). A female can produce up to 10 million eggs

per season (Buchanan 1999) of about 56-62 μm in diameter, and males can produce an immeasurable number of sperm of about 54 μm in length (Redfearn et al. 1986). Fertilization occurs in the water column, and the zygote develops to a lecithotrophic trochophore larva within hours (Redfearn et al. 1986, Buchanan 1994). At 24 to 48 h after fertilisation, the trochophore larva develops into a D-shaped veliger (Redfearn et al. 1986, LeBlanc 2005, LeBlanc 2008). Within 2-3 weeks a second larval shell grows (prodissoconch II) characterised by a broadly rounded umbo shape and high angular shoulders (Booth 1977). During this time, the larvae undergo rapid growth ($\sim 7 \mu\text{m}$ shell length day) and actively feed on phytoplankton (Jaeckle & Manahan 1989), bacteria (Douillet & Langdon 1993, Moal et al. 1996) and dissolved organic material (Hayden 1995, Buchanan 1999). Veliger larvae have the potential to disperse several hundred kilometres and can remain in the plankton for four to six weeks depending on water temperature, food availability and settlement cues (Redfearn et al. 1986, Jeffs 1999, Alfaro et al. 2010). The development of a pedal organ in the larva marks the transition to a pediveliger and indicates competence to settle, but settlement can be delayed depending on the environmental conditions and the availability of suitable substrate. The settlement of mussel larvae takes place within minutes to 24 h, and is facilitated by chemical and biological cues (Young 2009). *Perna canaliculus* larvae prefer to settle on filamentous substrates, such as fine-branching macroalgae and hydroids (Alfaro & Jeffs 2002, Alfaro & Jeffs 2003, Alfaro et al. 2004). Settlement is completed with attachment to hard substrate by byssal threads secreted by the pedal organ and subsequent metamorphosis to become a plantigrade, or what is commonly known as a spat (Buchanan 1994, Jeffs 1999). Byssopelagic drifting is where mussel larvae produce a long mucus thread that increases the viscous drag on the animal, allowing transport by currents (Sigurdsson et al. 1976, Lane et al. 1985). This allows the larvae to detach and resettle numerous times before finally settling in adult mussel beds, which is known as secondary settlement (NIWA 2006).

A small percentage of *P. canaliculus* mussels spawn throughout the year (Alfaro et al. 2001) and therefore spat settlement takes place year round (Buchanan 1994). Different *P. canaliculus* populations show temporal and spatial variation in spawning that has been linked to local environmental conditions, such as water temperatures and food availability (Alfaro et al. 2001, LeBlanc 2005). These differences have been identified both on small spatial scales and between regions (Alfaro et al. 2003). Differences in mussel spawning are most evident

between North and South Island populations, but geographically close populations can also have differences in spawning times (Alfaro et al. 2003). For example, differences in breeding cycles were observed among mussel populations on Ninety Mile Beach where the most reproductively active populations (two subtidal populations at the southern end and one intertidal population at the northern end of the beach) had a prolonged spawning period between June and December. In contrast the reproductive cycle of the two intertidal populations at the south end of the beach had 2-3 spawning events in the same period (Alfaro et al. 2003). The prolonged spawning period in subtidal mussels compared to adjacent intertidal mussels at Ninety Mile Beach may be due to the larger size of the subtidal mussels.

There are also temporal spawning differences between the North and South Island in wild populations of *P. canaliculus*. Spawning in *P. canaliculus* populations observed at Ninety Mile Beach peak between June and December (Alfaro et al. 2001, Alfaro et al. 2003). However, in the Marlborough Sounds there are two separate spawning periods in early summer and autumn - spring (Flaws 1975, Tortell 1976, Buchanan 1998). These variations have been attributed to regional temperature differences (Alfaro et al. 2001) as gametes can be released at any time during the year although gametogenic activity only occurs at temperatures above 11°C (James & Ross 1997). Water temperatures at Ninety Mile Beach seldom drop below 14°C (Alfaro et al. 2001) while temperatures in Marlborough Sounds are between 10 - 11°C in winter and 20 - 21°C in summer (Tortell 1976, Jenkins 1985).

1.4 Condition cycles

Condition indices (CI) in bivalve shellfish have been used for over half a century for both biological and commercial purposes (Baird 1958). Condition indices in bivalve aquaculture are used to describe the quality of marketed products as well as to illustrate the apparent “health” or physiological activity of the animals (growth, reproduction, etc.) under given environmental conditions (Lucas & Beninger 1985). Meat weight and condition of cultured Greenshell™ at the time of harvest are commercially important as they strongly influence the yield and financial return for farmers (Fox 2003). Many factors are thought to affect the condition indices of marine bivalves. These include food supply, time of year, salinity, temperature, environmental contaminants, size, gonad development and population density

(Lucas & Beninger 1985, Pridmore et al. 1990, Loesch & Evans 1994, Iglesias et al. 1996, Marsden & Weatherhead 1999). The CI response to environmental change is often species-specific (Rainer & Mann 1992, Marsden & Pilkington 1995). Seasonal changes in condition of *P. canaliculus* are caused by the complex interactions of a variety of factors, including food, temperature and salinity, influencing the metabolic activities of the mussel, especially the somatic growth and reproductive processes (Hickman & Illingworth 1980). The condition of farmed *P. canaliculus* has been found to generally follow an annual cycle with high values in autumn and spring and lower values in summer and winter, generally following spawning events (Hickman & Illingworth 1980, Hickman et al. 1991). Mussel condition and meat yields usually reach the lowest levels in mid-winter, if condition declines to very low levels, as it does most winters, then the mussels are no longer suitable to harvest because the meat yield is low and the finished mussel consumer products are inferior (Fox 2003).

The majority of *P. canaliculus* spat used in aquaculture are collected from three locations; Ninety Mile Beach in the North Island and Golden Bay and the Marlborough Sounds in the South Island. Many mussel farmers believe that once grown to harvest size these stocks of mussels gain and lose condition at different times of the year (Fox 1996). Mussels from each of three regions were experimentally grown together in the Pelorus Sounds and were found to have a slightly different condition cycles. In the seven months leading up to mid-winter spawning the Ninety Mile Beach and Marlborough Sounds stocks were in higher condition than the Golden Bay stock. The Ninety Mile Beach stock then exhibited the largest decline in condition of the three stocks during the mid-winter spawning event resulting in the mean condition of the Golden Bay and Marlborough Sounds stocks being 2 % higher than the Ninety Mile Beach stock immediately following spawning (Fox 2003). Spatial and temporal factors (the location and timing of sampling) were also found to be key determinants of mussel condition with changes in condition ranging up to 23 % between sites and 22 % between times within a site (Fox 2003). Harvesting mussel stocks in accordance with different condition cycles could potentially improve the overall mean annual condition index of harvested mussels and minimise the impact of the mid-winter decline in condition. For example, increases in mean annual condition as small as 2 % could be commercially relevant. Improving the mean annual condition of mussels harvested from 38 % to 40 % would increase the meat yield achieved by the Marlborough Sounds mussel industry by 1400 t (Fox 2003). It is likely

that similar asynchronous condition cycles would be present among different stocks of mussels grown in the Hauraki Gulf, although this remains to be established experimentally.

There is large scope for growth of the Greenshell™ mussel aquaculture industry in New Zealand but there are many weaknesses and risks to the production cycle which can be addressed in part through improving our understanding of the biology of this commercially important species. The overall aim of the research presented in this thesis is to begin to tackle some of the production difficulties currently facing the industry in the Hauraki Gulf. This study has the potential to address and mitigate two of the major difficulties; spat supply and the length of harvesting season. It is unwise for an industry to rely on one main source of spat. If *P. canaliculus* spat can start to be commercially harvested in the Hauraki Gulf this could be used as a supplementary supply of spat alongside Ninety Mile Beach, and should anything happen to Ninety Mile Beach spat it could help mitigate the impact to the industry. Additionally the differences in Hauraki and Ninety Mile Beach condition cycles could extend the Hauraki farmers harvesting season and increase their harvesting yield.

1.5 Primary and Secondary Settlement Processes

Primary and secondary settlement of mussel spat was first observed in the European blue mussel, *Mytilus edulis*, where larvae initially settle on filamentous substrata and then move to adult beds once they have grown in size (Bayne 1964). This two-stage settlement process is thought to be a strategy to avoid predation, intraspecific competition and/or ingestion by adult mussels (Bayne 1964). Secondary settlement allows the spat to increase in size before permanent settlement in the highly competitive adult beds (Buchanan 1997) and can occur multiple times until spat settle in the adult beds or reach 6 mm in size, when mucus drifting is thought to be no longer physically possible (Buchanan & Babcock 1997). Primary settlement is thought to be facilitated by many factors, including temperature, light conditions, nutrient availability, water flow, chemical cues, and physical cues of substrata (Young 2009). Larvae tend to settle over a wide range of depths and prefer filamentous substrata such as hydroids, bryozoans, and filamentous red macroalgae (Alfaro & Jeffs 2003, Alfaro et al. 2004, Alfaro et al. 2006, FAO 2008). Primary and secondary settlement processes have been confirmed for *P. canaliculus* through a range of laboratory and field experiments (Buchanan 1994, Buchanan

& Babcock 1997, Alfaro & Jeffs 2002, Alfaro 2006). *Perna canaliculus* larvae do not usually settle directly into adult mussel beds because of the risk of cannibalism and intense intraspecific competition for space (NIWA 2006). Rather, mussels are thought to move into mussel beds at a larger size (>6 mm shell height) using their secondary settlement behavior (Buchanan & Babcock 1997). The rates of secondary settlement of spat have been shown to increase when they encounter environmental stresses such as desiccation and starvation which would be consistent with the behaviour assisting the spat in finding more suitable habitat sites (Buchanan 1997, Foote 2003, Carton 2007). Secondary settlement is essential to aquaculture practices as the re-attachment of spat is essential for the seeding and thinning of the wild collected spat used in commercial aquaculture nursery production. However, secondary settlement is also thought to be the major cause of low spat retention on nursery ropes (Foote 2003). *Perna canaliculus* spat have been found to be very selective when it comes to choosing settlement substrata (Buchanan & Babcock 1997, Alfaro & Jeffs 2002) with the sites for attachment determined not only by the physical structure (Alfaro & Jeffs 2002, Alfaro & Jeffs 2003), but also the chemical composition (Alfaro et al. 2006, Young et al. 2008), and bacterial biofilms (Ganesan et al. 2008, Ganesan et al. 2010) of the substrates as well. These are collectively referred to as settlement cues.

1.6 Genetic Structure of Populations of *P. canaliculus*

Perna canaliculus tend to occur in geographically distinct populations (Jeffs 1999, Gardner 2000, Gardner & Thompson 2001), the genetics of which have been intensively studied (Gardner et al. 1996 a, 1996b, Apte & Gardner 2001). The possible role of genetic differences among populations in driving the observed differences in reproductive activity is uncertain. Several studies using a range of genetic techniques have identified marked genetic discontinuity between populations of *P. canaliculus* in northern and southern New Zealand, as well as genetic diversity at more localised scales in some regions, such as the west coast of the South Island (Smith 1988, Sin et al. 1990, Gardner et al. 1996 a, b, Star et al. 2003, Wei et al. 2013). It was initially proposed that these genetic differences were due to physiological adaptations to different thermal environments and that the water currents may have partially isolated the populations by limiting the movement of larvae (Smith 1988). The subsequent

application of higher resolution genetic techniques indicate that genetic variation across the full distributional range of *P. canaliculus* is best explained by corresponding differences in sea surface temperatures. Hence, it is likely that both genetic and environmental influences play a role in influencing the varied reproductive cycles in populations of this species.

1.7 Spat Retention

Low retention rate of mussel spat after seeding out onto mussel farms is the cause of significant financial losses in the Greenshell™ mussel industry (Alfaro et al. 2012). If spat were able to be assessed for their retention potential before seeding onto farms this would vastly improve the efficiency and reduce costs incurred by the industry. Retention is higher in spat which are in good nutritional condition or have a good feeding history (Foote, 2004). The nutritional health of mussel spat is not the only cause of low retention behaviour of spat. Other non-lethal stressors which have been identified include; desiccation, starvation and temperature fluctuation (Carton et al. 2007). In laboratory experiments starvation was found to reduce spat retention by 42 %, while desiccation reduced spat retention by 38 % compared to the “unstressed” controls. Also, individual spat which attached to the substratum by byssus threads in slow flowing seawater were found to show greater retention behaviour in laboratory experiments. However, the difference in behaviour of spat under these conditions was too small to form the basis for the development of a practical commercial spat sorting method. Mussel spat are likely to experience both starvation and desiccation due to commercial harvesting, transportation and seeding process. Therefore, it is beneficial to handle and move mussel spat under moist conditions and as quickly as possible to reduce these stressors under commercial conditions (Carton et al. 2007).

1.8 Research Aims

The overall aim of this research was to investigate the potential for commercial green-lipped mussel spat collection in the Hauraki Gulf as a means to increase the months spat could be harvested, overall meat yield, and to alleviate spat shortages in the industry. Growing two

stocks of the same commercial mussel species is advantageous as it can increase the number of months mussel can be harvested as well as the overall meat yield. Although there have been many studies looking at the influence of stock on the growth and condition of commercially cultivated *Mytilus* spp. mussels (Dickie et al. 1984, Mallet et al. 1987, Mallet & Carver 1989, Kautsky et al. 1990, Fuentes et al. 1992, Fuentes et al. 1994, Stirling & Okumus 1994, Perez-Camacho et al. 1995, Fox 2003) there is a need for research on *P. canaliculus* stocks. The Hauraki Gulf is a well-established mussel farming area in New Zealand and is therefore an appropriate study site to source an additional stock.

This thesis is presented as six chapters, with the four data chapters (2, 3, 4 and 5) written as stand-alone manuscripts for publication. This has resulted in some repetition between chapters. Chapters 1 and 6 provide a synthesis of contextual information and discussion.

1.8.1 Spat Settlement

Being able to reliably predict the timing and quantity of spat arrival in the Hauraki Gulf is vital for efficiently utilising this natural resource. Evaluating spat settlement of *P. canaliculus* mussels using artificial collectors has been implemented in many parts of New Zealand to determine optimal times for collection of mussel settlers for aquaculture (Meredyth-Young & Jenkins 1978, Alfaro & Jeffs 2003). This is the first study to do so in the Hauraki Gulf. Artificial collectors were deployed at two locations and two depths and monitored regularly for two and a half years to establish the settlement pattern of spat in the Hauraki Gulf. Biofouling of mussel lines by *M. galloprovincialis* is a serious problem in the industry (Dawber 2004). Therefore, the settlement of *M. galloprovincialis* was also measured in order to determine if one site or depth consistently had a higher green to blue mussel ratio. This component of the research is presented in Chapter 2 of this thesis.

1.8.2 Growth and Condition

Different mussel stocks can have varying growth rates and condition cycles (Dickie et al. 1984, Mallet et al. 1987, Mallet & Carver 1989, Kautsky et al. 1990, Fuentes et al. 1992, Fuentes et al. 1994, Stirling & Okumus 1994, Perez-Camacho et al. 1995, Fox 2003). Growing

two stocks with asynchronous spawning patterns can increase harvesting time and overall meat yield for farmers (Fox 2003). There has been one study comparing *P. canaliculus* stocks but they did not include Hauraki Gulf stock (Fox 2003). To determine if Hauraki Gulf stock and Ninety Mile Beach stocks have asynchronous spawning patterns and different growth rates, both stocks were grown together in the Hauraki Gulf and monitored for two years. This component of the research is presented in Chapter 3 of this thesis.

1.8.3 Secondary Settlement Behaviour

High losses of spat typically occur shortly after they are placed out on nursery ropes on farms. These losses can be as high as 100 % but are usually between 50 and 70 % (NIWA 2006, Carton et al. 2007, Sim-Smith 2011). These losses of spat from nursery growing ropes are estimated to cost the industry between NZD\$6-10M annually (Alfaro et al. 2012). While there are many suspected causes of this loss, secondary settlement behaviour is thought to be a major one. Secondary settlement is believed to allow the small juvenile mussels to increase in size before migrating to live permanently in the highly competitive adult beds (Bayne 1964, Buchanan 1997). Gaining an understanding of the significance of specific drivers influencing secondary settlement behaviour in the early juveniles will assist in reducing the widespread and costly losses of spat from seeded nursery ropes. Experiments were conducted to determine how the secondary settlement behaviour of wild *P. canaliculus* spat varies with size and natal origin. This component of the research is presented in Chapter 4 of this thesis.

1.8.4 Assessing the Effects of Sound on Secondary Settlement Behaviour and Growth

Settlement of mussel spat has been shown to be influenced by sound. In particular, it was noted that noise from vessels has been shown to increase the density of settlers, reduce settlement time and increase growth rates in marine invertebrates, including mussels which cause biofouling (Wilkens et al. 2012, McDonald et al. 2014, Stanley et al. 2014). This has obvious implications for improving the cost effectiveness of producing hatchery-reared mussel spat. The use of sound in rearing hatchery spat may increase growth, thus shortening time spent in the hatchery which would reduce the cost of producing hatchery spat. In

addition, if sound encourages retention this will reduce losses resulting from poor spat retention. Hatchery spat is currently significantly more expensive than wild caught spat. A range of experiments were conducted to determine if sound increases the growth and retention of *P. canaliculus* spat. These results are reported in Chapter 5 of this thesis.

Chapter Two: Spatial and Temporal Variability in Mussel Spat Arrival in the Hauraki Gulf

2.1 Introduction

The green-lipped mussel, *Perna canaliculus*, is an endemic New Zealand species that is produced from aquaculture and is sold under the trade name Greenshell™. This mussel is mostly exported to North America, Europe and China (NZAC 2010, Alfaro et al. 2012). The Greenshell™ mussel industry produced around NZ\$300M worth of mussels in 2015, making it the second most valuable species of exported seafood from New Zealand (Colin Johnston, Aquaculture New Zealand, pers. comm., NZ Seafood Industry Council, export database). This industry is also a major employer which generates an estimated 2500 jobs, many of which are in rural regions of the country (Aquaculture New Zealand 2009, Ministry of Fisheries 2009). The aquaculture of Greenshell™ began in the 1970's and has grown substantially over the last 40 years (Jeffs et al. 1999, Hickman 1976, Dawber 2004). There are currently over 1000 mussel farms in New Zealand, concentrated mostly in the Marlborough Sounds and the Hauraki Gulf (Ministry of Fisheries 2009).

The Greenshell™ mussel aquaculture industry is almost 100 % reliant on seed mussels collected from the wild with around 20 % caught on fibrous ropes suspended near mussel farms in the Marlborough Sounds and in Golden Bay, and the balance supplied by sporadic strandings of seaweed with attached mussel seed which is collected from Ninety Mile Beach in northern New Zealand (Alfaro et al. 2004, Gribben et al. 2011, Alfaro et al. 2012). Each year approximately 160 t of drift algae with attached mussel spat is collected from Ninety Mile Beach when it washes into shallow waters along the beach (Hickman 1976, Alfaro and Jeffs 2002, Alfaro et al. 2012, Jeffs et al. 2017). Only on Ninety Mile Beach are there such large amounts of wild seed on drifting seaweed material that can be conveniently and economically harvested to supply the Greenshell™ mussel industry with seed (Jeffs et al. 2000, Alfaro 2001). *Perna canaliculus* are attached to the drift seaweed in densities up to 1 million spat per kilogram of seaweed. The wild mussel seed supply at Ninety Mile Beach is reliant on hydrodynamic and meteorological patterns for the drift seaweed to be washed on to shore so both quantity and timing of mussel spat supply from this source is sporadic (Alfaro 2004, Alfaro et al. 2010). Several groups have developed hatchery production technology for *P.*

canaliculus, however, due to the high cost of hatchery production, it is a much more expensive option than the widespread current use of wild-caught seed (Alfaro et al. 2012). Development of artificial foods, selective breeding programmes and reliable methods of broodstock mussel conditioning may help reduce these costs in the future (Ragg et al. 2010).

From the 1920s to the 1970s, a commercial dredge fishery operated in the Firth of Thames and the inner Hauraki Gulf, ultimately overfishing the extensive wild *P. canaliculus* mussel beds which have been estimated to have covered over 1100 km² prior to commencement of commercial fishing (Reid 1968, Greenway 1969). Unfortunately, more than 40 years after the fishery was closed the mussel populations have not returned (Morrison et al. 2009, McLeod et al. 2012). There has been some successful establishment of clusters of live mussels on the seafloor at a small number of locations where shells have dropped from the mussel farms in the area (Morrison et al. 2009). Larval mussel supply is not thought to be the cause of the lack of recovery of the mussel beds, with settling larval mussels being caught by farm operators running experimental spat-collecting lines within the Hauraki Gulf (Bartrom 1990, Morrison et al. 2009). Larval settlement and the growth of juvenile mussels has been found to be inhibited by the fine sediment composition of the seafloor and the widespread absence of substantial benthic surface structure, such as dead shells, hydroids and red algae for larvae to settle on, as well as continuing silt re-suspension from storms (Morrison et al. 2009, McLeod et al. 2012).

Perna canaliculus have a 1:1 sex ratio and is a dioecious broadcast spawner which reaches sexual maturity within a year of settlement which for the majority is at a shell height of 40-50 mm. They have an annual synchronized reproductive cycle and gametogenesis occurs in late autumn/winter which is thought to be due to energy reserves amassed in summer and autumn (Jeffs et al. 1999). At a number of locations in the North Island it has been found that a small percentage of mussels can also be found spawning throughout the year (Alfaro et al. 2001) and therefore spat settlement takes place year round (Buchanan 1994). Different populations of mussels show temporal and spatial variation in spawning that has been linked to local environmental conditions, such as water temperatures and food availability (Alfaro et al. 2001, LeBlanc 2005). These differences have been identified both over highly localised spatial scales (i.e., along a single beach, or intertidal versus subtidal) and between regions (Alfaro et al. 2003). Differences in green-lipped mussel spawning are most evident between

North and South Island populations, but geographically close populations can also have quite marked differences in spawning times (Alfaro *et al.* 2003). For example, mussel populations at Ninety Mile Beach were found to vary significantly in their reproductive activity with two subtidal populations at the south end and one intertidal population at the north end of the beach having a prolonged spawning period between June and December whereas the reproductive cycle of the two intertidal populations at the south end of the beach had 2-3 spawning events in the same period (Alfaro *et al.* 2003). At Ninety Mile Beach, subtidal populations of mussels were found to be larger in size than adjacent populations of intertidal mussels. This is thought to be due to higher growth rates which may help to explain the prolonged spawning period observed in the subtidal beds compared to adjacent intertidal beds.

There are also differences in the temporal pattern of spawning between the North and South Island populations of this mussel species. The peak reproductive activity in *P. canaliculus* populations observed at Ninety Mile Beach are between June and December (Alfaro *et al.* 2001, Alfaro *et al.* 2003), however, in the Marlborough Sounds there are two separate spawning periods in early summer and autumn-spring (Flaws 1975, Tortell 1976, Buchanan 1998). These variations have been attributed to regional differences in seawater temperature (Alfaro *et al.* 2001) as gametes can be released at any time during the year but gametogenic activity only occurs at temperatures above 11 °C (James and Ross 1997). Seawater temperatures at Ninety Mile Beach seldom drop below 14 °C (Alfaro *et al.* 2001) while temperatures in Marlborough Sounds are between 10-11 °C in winter and 20-21 °C in summer (Tortell 1976, Jenkins 1985).

The larvae of *P. canaliculus* can remain in the plankton for four to six weeks and over this time have the potential to disperse several hundred kilometres (Jeffs 1999, Alfaro *et al.* 2010) depending on water temperature, food availability and settlement cues (Redfearn *et al.* 1986, Jeffs 1999). Within three weeks of fertilisation, planktonic green-lipped mussel pediveligers that attain a shell height >240 µm are considered to be competent to settle. The survival and further metamorphosis of these pediveligers to juveniles depends upon their settling and attaching to a solid substratum.

Mytilus galloprovincialis (blue mussels) are a widespread biofouling problem in the Greenshell™ aquaculture industry (Dawber 2004, Atalah et al. 2017, Atalah & Forrest 2017). *Mytilus galloprovincialis* biofouling has many detrimental effects to the industry including; settlement of these mussels onto lines seeded with *P. canaliculus* spat can significantly decrease the retention and production from the Greenshell™ (Carton et al. 2007), obstructing space on *P. canaliculus* spat-catching ropes (Forrest & Atalah 2017). The natural over-settlement by *M. galloprovincialis*, reduces the growth of *P. canaliculus*, through competition for food, by around 5 – 10 % and is estimated to cost this aquaculture industry around US\$ 11.4 M a year in lost production (Forrest & Atalah 2017). *Mytilus galloprovincialis* settlement has been shown to occur at higher rates on mussel ropes seeded with spat sourced from Ninety Mile Beach than ropes seeded with spat from the South Island (Forrest & Atalah 2017). This could be due to spat from Ninety Mile Beach not reaching condition as quickly as South Island-sourced spat (Fox 2003) and thus not being able to compete for space as effectively (Forrest & Atalah 2017). Recent experimental investigations suggest that mussels derived from spat from Ninety Mile Beach show reduced feeding efficiency compared to mussels sourced as spat from the South Island (Forrest & Atalah 2017).

Little is known about the spatial and temporal variability in larval green-lipped mussel settlement in the Hauraki Gulf. Providing specific information about where, when, and the magnitude of spat settlement in the Hauraki Gulf may help to provide the basis for developing a local spat source for mussel farmers in the area. Additionally, if mussels grown from spat sourced from the Hauraki Gulf are found to spawn asynchronously compared to those mussels grown from spat harvested from Ninety Mile Beach, it would have the potential to extend the overall harvesting period and total yield. Therefore, in this study the settlement of *P. canaliculus* and the blue mussel, *M. galloprovincialis* were measured at two depths for two mussel farm sites near Coromandel over a 2.5 year duration to provide information on the timing of peak settlement and depth of settlement.

2.2 Methods

The larval settlement of *P. canaliculus* was measured regularly at two sites in the Hauraki Gulf over a 2.5 year period to determine the time and extent of spat arrival in the area (Fig. 2.1).

Spat catching rope was deployed at two depths as measured from the sea surface (4 and 11 m depth) and at two sites in the Hauraki Gulf, 5.76 km apart (North site -36.898219, 175.408458 and South site -36.945709, 175.433979). The ropes were recovered after a period of three weeks and counts and size of both *P. canaliculus* and *M. galloprovincialis* spat were recorded.

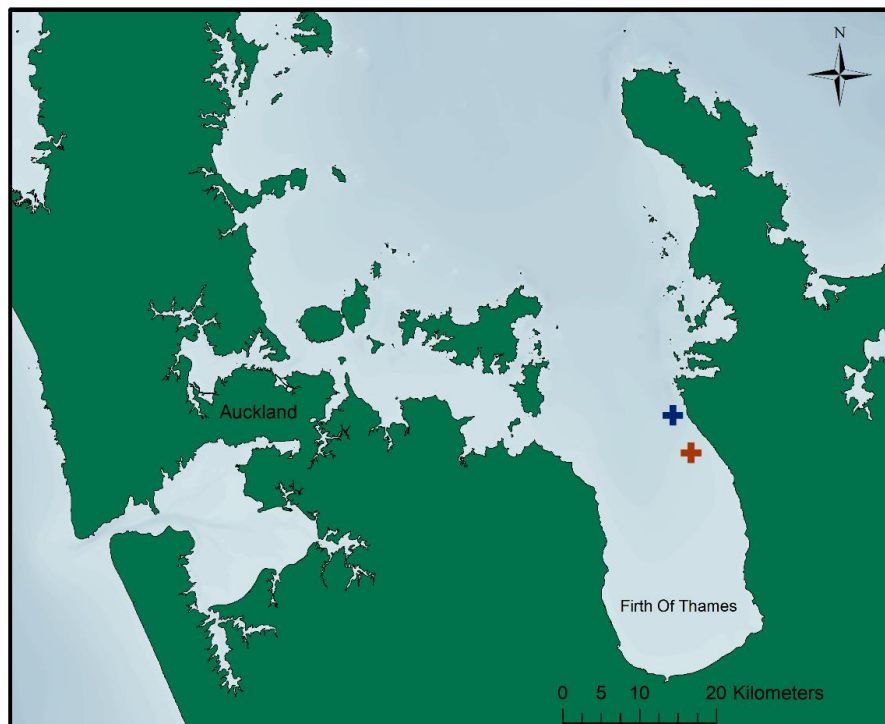


Figure 2.1 Map showing the North (blue) and South (red) sites where spat collection ropes were deployed in the Hauraki Gulf.

2.2.1 Settlement Sampling

An 11 m long dropper of 9 mm diameter polypropylene rope with a 10 kg weight at the end was deployed from a surface float at each site. Each line had three 25 cm lengths of new mussel spat-catching rope (Quality Equipment – 20 mm diameter ‘Spatfinder’ rope) attached to the dropper line with cable ties at both 4 m and 11 m depths down the dropper rope. The spat-catching ropes were recovered approximately every three weeks weather permitting and placed in a labelled plastic bag for return to the laboratory for later analyses. The spat-catching ropes were replaced and the dropper rope re-deployed in the same position.

Occasionally mechanical problems with the boat and inclement weather disrupted the three weekly sampling routine.

After arriving at the laboratory the recovered mussel seed ropes were frozen to await subsequent analyses. To analyse the samples the ropes were firstly thawed and the seed mussels removed from the rope with vigorous agitation in water. Each short length of spat-catching rope was placed in a bucket filled with water and scrubbed forcefully to remove all the attached mussel seed. The water from the bucket was then drained through a 150 µm sieve to recover the dislodged mussels. The scrubbing process was repeated for a second time to ensure the removal of all mussel seed which was confirmed by microscopic examination of the rope after the second scrubbing. Mussel seed are more easily removed from the substrate to which they are attached when the samples have been previously frozen (Alfaro and Jeffs 2003). To give an indication of the age of the spat at settlement and therefore determine if settlement was continuous, the size of the spat was also examined. The material recovered on the sieve was washed through a series of successively finer mesh sieves (1 mm, 500 µm and 150 µm) and placed in labelled plastic containers for counting and identification. The spat were divided into three size classes small (<0.5 mm in shell length), medium (0.5-1.0 mm in shell length) and large (>1.0 mm in shell length) and the numbers in each size class counted under a dissecting microscope. The two mussel species were distinguished by colour, shape and texture as described by Booth (1977). The numbers of blue mussels, *M. galloprovincialis*, was also counted and recorded.

2.2.3 Environmental Data

Water temperature and salinity were provided by a NIWA station buoy positioned 900 m from the South Site (-36.93859, 175.44309) at a depth of 11 m. Measurements were taken every 15 minutes and a daily average taken. Salinity varied little in the study area, from 34-35.5 pp, while temperature showed seasonal change, from a winter low of 13.6°C in July-September, to a summer high of 21.8°C in February-March (Fig. 2.2).

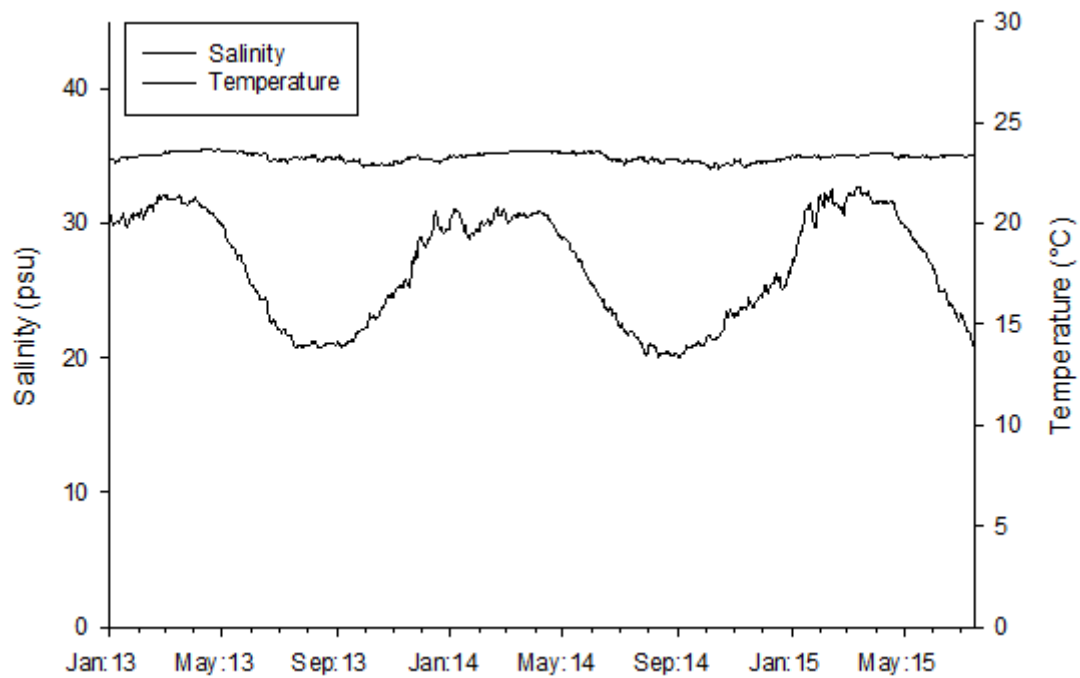


Figure 2.2 Mean daily salinity and water temperature for the Hauraki Gulf from January 2013 to July 2015. Each tick mark on the X axis correspond to the first day of each month.

2.2.4 Statistical analyses

To determine if there were differences in the number of *P. canaliculus* between sites (North versus South), between depths (4 versus 11 m) and among sampling dates a negative binomial generalized linear model (GZLM) with log-link function was fitted to the measured number of mussel spat on the settlement ropes. A GZLM was also used to compare the number of *P. canaliculus* and *M. galloprovincialis* on the settlement ropes. To determine if there was any difference in the size of *P. canaliculus* within settlement ropes and among depths, sites, and sampling dates, a general linear model (GLM) was used.

For each sampling event, the calendar date of recovering the ropes was considered as the sample date for the sake of convenience for graphing and statistical analyses. For all the statistical analyses, adjustments were made for multiple comparisons between means using Sidak's method (Sidak, 1971). The mean values reported from the results of GLMZ and GLM are estimated marginal means. The software SPSS (IBM Corp. Released, 2013) was used to perform all the analyses described above.

To correct for differences in the time that the spat-catching ropes were in the water, and therefore available for larvae to settle onto, the numbers of spat on each rope were divided by the number days the rope was in the water. This enabled data from samples for which recovery was delayed due to logistic constraints, to be standardised and included in the data set.

2.3 Results

From 3 December 2012 to 2 June 2015 a total of 37 sampling events of spat settlement at roughly three weekly intervals were conducted using a total of 444 settlement ropes. Over the entire 2.5 year sampling period over 100,000 *P. canaliculus* spat were collected, while less than 10 % of all spat were *M. galloprovincialis*.

2.3.1 Temporal patterns of *P. canaliculus* spat abundance

There was an overall difference in the mean number of *P. canaliculus* spat found on the settlement ropes among sampling dates ($X^2 = 376.58$, $p < 0.0001$). The abundance of *P. canaliculus* spat followed an annual cycle, peaking in December of both 2013 and 2014 with up to 75 $\text{m}^{-1} \text{d}^{-1}$ of spat on the rope. The lowest abundance of spat was observed from March to July reaching as low as 15 $\text{m}^{-1} \text{d}^{-1}$ in July 2014 and 2015 (Fig. 2.3). Spat in all three size classes were found on every rope for every sample indicating that spat settlement is continuous throughout the three week sampling duration regardless of the time of year. Overall the numbers of spat were highest in 2013 (Fig. 2.4).

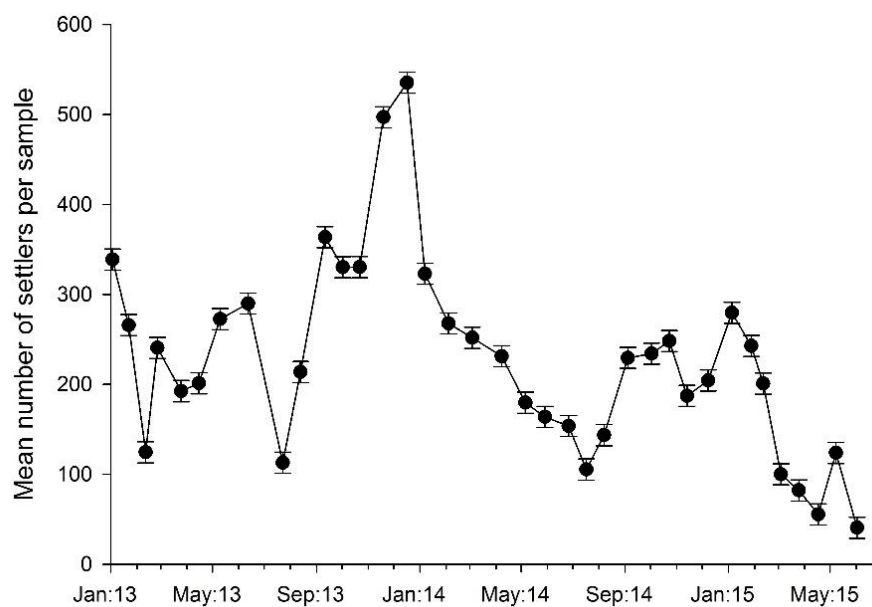


Figure 2.3 Mean number of *P. canaliculus* spat (\pm S.E.) per sampling period regardless of depth or site from January 2013 to June 2015.

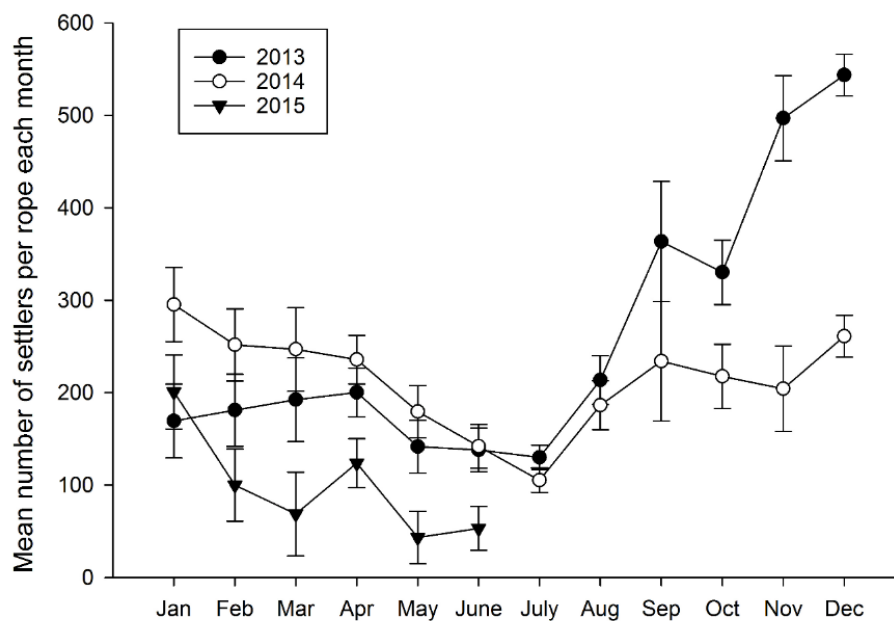


Figure 2.4: Mean number of *P. canaliculus* spat (\pm S.E.) per settlement rope for each month in each of three years of sampling regardless of site and depth of rope deployment.

2.3.3 Spatial patterns of spat abundance

There was no significant difference in the mean number of spat per sample between the two sites after taking into account depth and the time of sampling over 2.5 years ($X^2 = 0.09$, $p = 0.77$) (Fig 2.5).

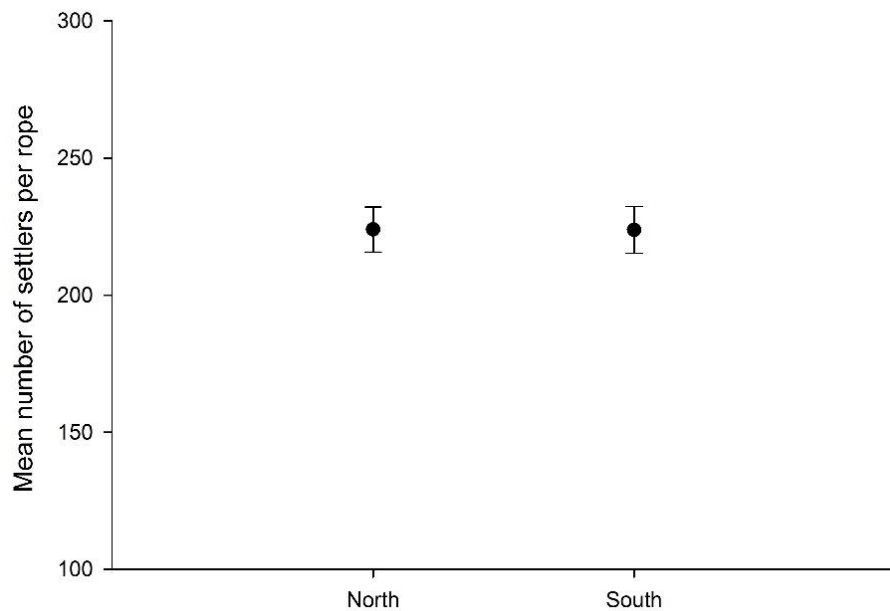


Figure 2.5: Mean number of *P. canaliculus* spat (\pm S.E.) per settlement rope at two locations, North and South, after taking into account depth and the time of sampling over 2.5 years.

2.3.4 Spat abundance at varying depths

There was a significant difference in the mean number of spat per sample between depths (4 m and 11 m) after taking into account site and the time of sampling over 2.5 years. More *P. canaliculus* spat were present at 11 m depth versus 4 m depth ($X^2 = 55.76$, $p < 0.0001$) (Fig. 2.6). No interaction was found between sampling depth and site ($X^2 = 0.20$, $p = 0.66$) (Fig 2.7).

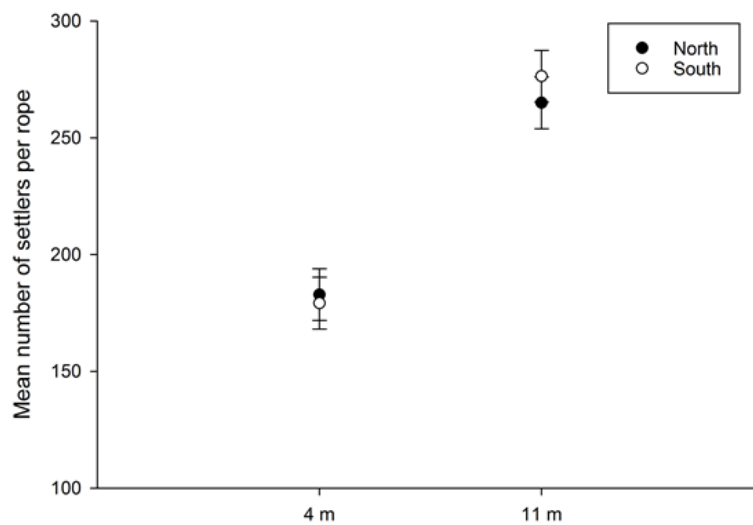


Figure 2.6: Mean number of *P. canaliculus* (\pm S.E.) spat per settlement rope at both 4 m and 11 m depth and North and South locations after taking into account site and the time of sampling over 2.5 years.

2.3.5 Size of spat at settlement

There was an overall significant difference in the size of *P. canaliculus* spat on rope collectors over the 2.5 years of sampling ($F_{(2, 1332)} = 1403.80$, $p < 0.001$) (Fig. 2.7). Spat were predominantly in the smallest size class (mean = 195.82 ± 102.85 μ m in shell length, range 200 - 499 μ m) followed by the medium size class (mean = 24.29 ± 35.35 μ m in shell length, range 500 - 999 μ m) and the lowest number of spat were in the largest size class (mean = 5.78 ± 7.77 μ m in shell length, 1 - 7 mm). There was also a significant interaction between spat size and depth with a higher proportion of small size class spat settling at 11 m versus 4 m depth after taking into account site and the time of sampling over 2.5 years ($F_{(1,1332)} = 52.35$, $p < 0.001$). Medium and large size classes of spat were at similar mean abundance for the two depths (Fig. 2.8).

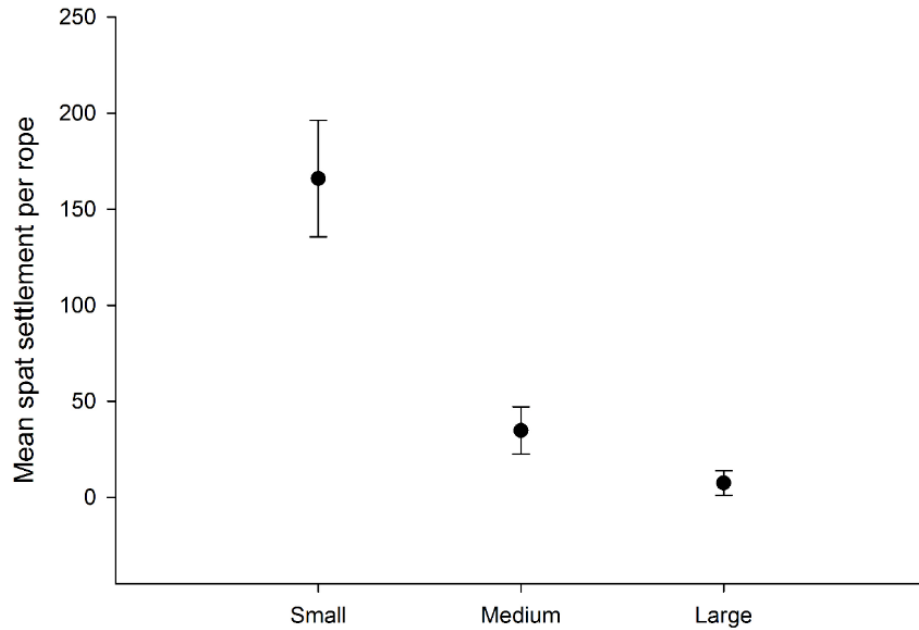


Figure 2.7: Mean number (\pm S.E.) of small (200 - 499 μm), medium (500 – 999 μm) and large (< 1 mm) *P. canaliculus* spat per settlement rope regardless of depth or time of sampling over a 2.5 year period.

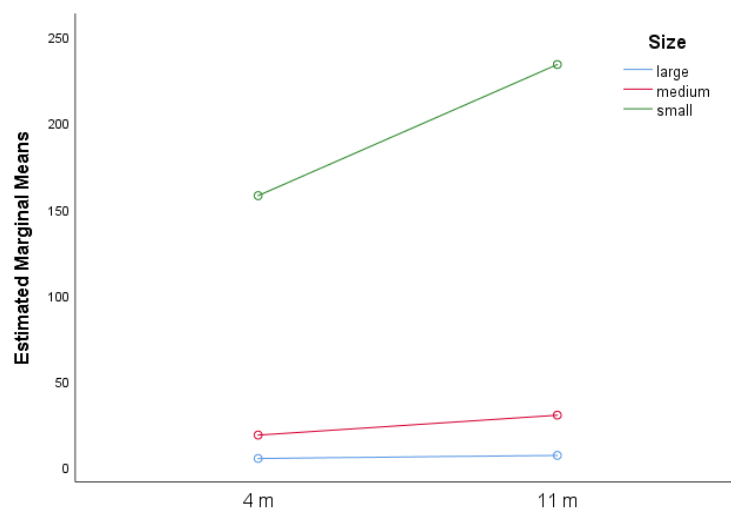


Figure 2.8: Estimated marginal means of counts of *P. canaliculus* spat of three different size classes (small -200 - 499 μm , medium - 500 – 999 μm and large > 1 mm in shell length) at two depths, 4 m and 11 m.

2.3.6 Temporal patterns of *Mytilus galloprovincialis* spat abundance

There was an overall significant difference in the mean number of *M. galloprovincialis* spat among the sampling dates ($\chi^2 = 322.48$, $p < 0.0001$). The abundance of *M. galloprovincialis* spat was found to follow an annual cycle, peaking in February in both 2014 and 2015 with an average of up to 92.7 spat g on each rope per sample. The lowest settlement was observed

from April to August reaching as low as 5 per rope per sample in May 2013 and 2015 (Fig. 2.9).

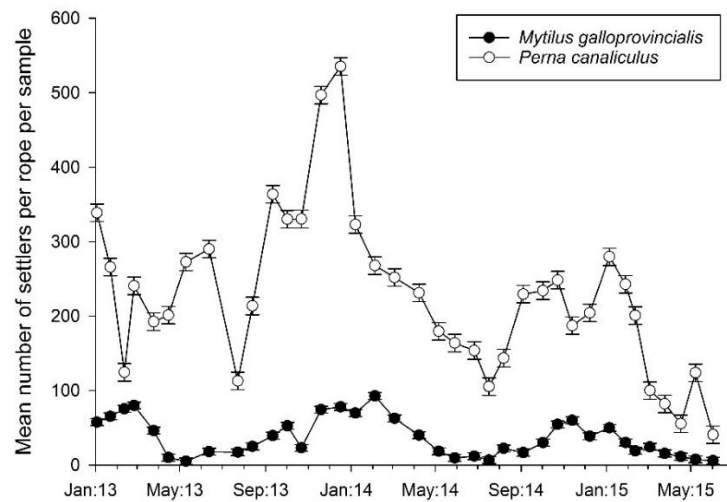


Figure 2.9: Mean number of *M. galloprovincialis* (\pm S.E.) and *Perna canaliculus* settlement per rope per sampling event from January 2013 to June 2015.

2.3.7 Spatial patterns of *Mytilus galloprovincialis* spat abundance

There was no significant difference in the mean number of *M. galloprovincialis* spat per sample between the two sites after taking into account depth and the time of sampling over 2.5 years ($X^2 = 1.39$, $p = 0.24$) (Fig 2.10).

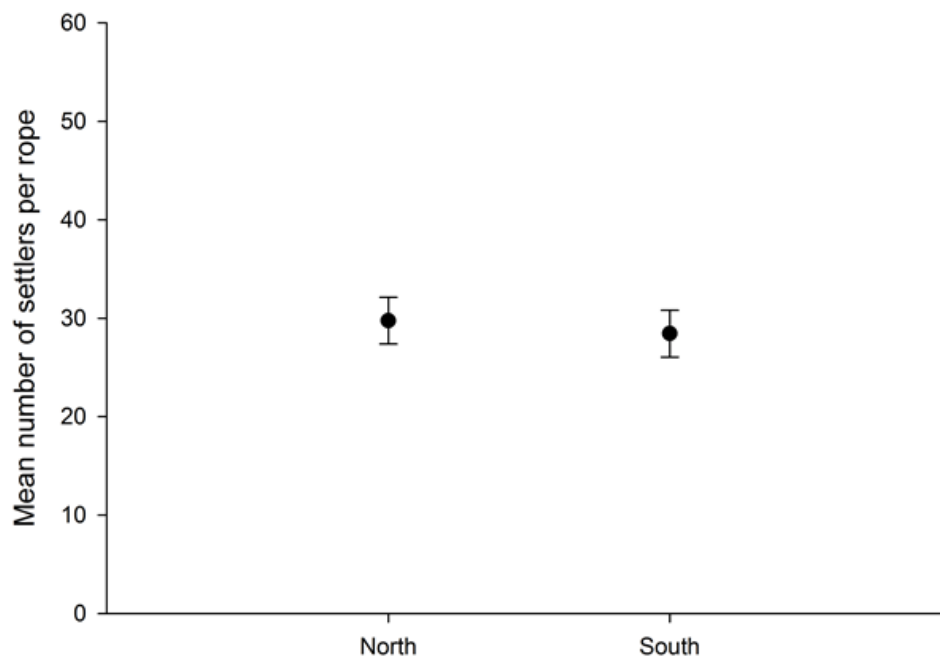


Figure 2.10: Mean number of *M. galloprovincialis* (\pm S.E.) per settlement rope at two locations, North and South, after taking into account depth and the time of sampling over 2.5 years.

2.3.8 *Mytilus galloprovincialis* spat abundance at varying depths

There was a significant difference in the mean number of *M. galloprovincialis* spat per sample between depths (4 m and 11m) after taking into account site and the time of sampling over 2.5 years. More *M. galloprovincialis* spat were at 4 m depth versus 11 m depth ($X^2 = 255.22$, $p < 0.0001$) (Fig. 2.11). No interaction was found between sampling depth and site ($X^2 = 1.15$, $p = 0.29$) (Fig 2.11).

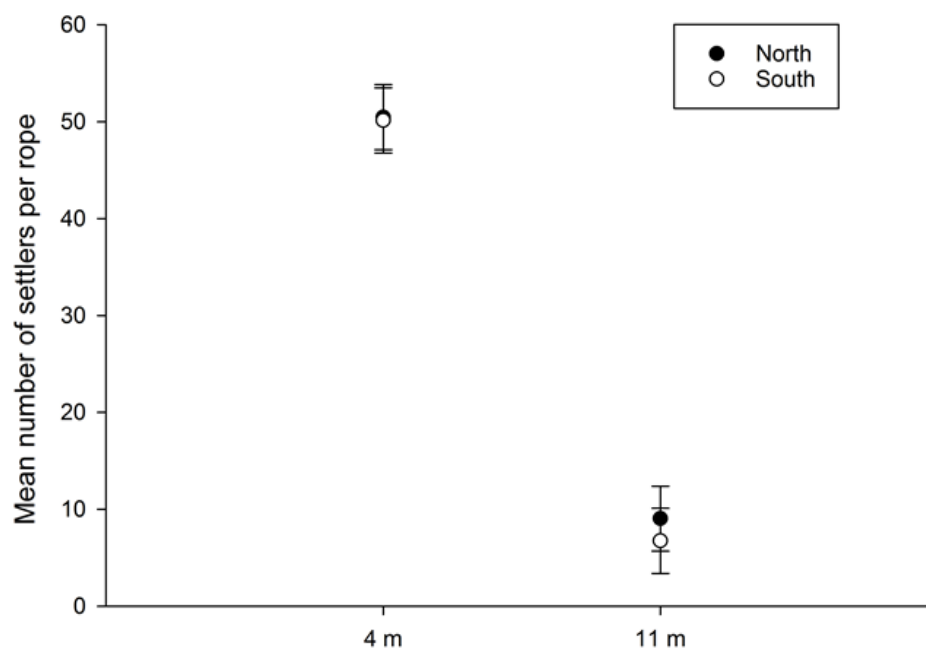


Figure 2.11: Mean number of *M. galloprovincialis* (\pm S.E.) per settlement rope for each depth and site, after taking in account site and the time of sampling over 2.5 years.

2.4 Discussion

In this study the spatial (depth and site) and temporal variation in the abundance of *P. canaliculus* spat was measured in the Hauraki Gulf using spat catching rope that is widely used by the Greenshell™ aquaculture industry. Capture of wild *P. canaliculus* spat for use in aquaculture has become more reliable through the accurate forecasting of peak settlement periods and knowledge of the preferred depth of settlement of the larvae (Meredyth-Young and Jenkins 1978). The patterns of settlement determined by this current study provide valuable guidance to Greenshell™ aquaculture operations for their collection of mussel spat in the Hauraki Gulf.

The seasonal pattern of abundance of *P. canaliculus* spat was consistent between the two sites, being considerably higher in the spring and summer months, making them the most suitable period for focusing any commercial spat collections. Spat abundance was not high enough to provide a commercially feasible source of spat year-round but did reach sufficiently high numbers between October and January. To collect the spat commercially, spat collector ropes will need to be deployed and spat settlement numbers monitored weekly. In the Marlborough Sounds commercial spat collection usually occurs when settlement numbers reach 1000 spat per metre per week (Dan McCall, SpatNZ, pers. comms).

There was no difference found in the abundance of spat between sites for both *P. canaliculus* and *M. galloprovincialis*. The sites in this study were chosen due to anecdotal evidence provided by aquaculture operators that they traditionally have had high spat abundance, with spat commonly found fouling ropes and buoys in the area. These sites are south of the majority of mussel farms in the area and therefore the higher spat settlement could be due to sea surface currents which could carry the larvae south and past the spat collection sites (Black et al. 2000). Spat abundance was significantly higher at 11 m versus 4 m. It has previously been reported that larval settlement in *P. canaliculus* show no depth preference in the Marlborough Sounds (Meredyth-Young and Jenkins 1978). However, a study of settlement ropes deployed off Ninety Mile Beach, found differences in the abundance of *P. canaliculus* spat of different size classes among three depths, i.e., 2, 10 and 18 m depth (Alfaro and Jeffs 2003). Smaller mussels (i.e., <0.99 mm) were more abundant at the two shallower depths, whilst larger spat were more abundant at 18 m depth.

Mytilus galloprovincialis spat had an annual pattern of abundance, peaking in late spring through to late summer with the highest counts in February. This differs slightly from patterns of abundance reported in the Marlborough Sounds which showed a peak in October followed by a smaller peak in abundance on settlement ropes in April (Atalah et al. 2017). In contrast to *P. canaliculus*, significantly more *M. galloprovincialis* spat were present at 4 m versus 11 m in this current study. This distinct depth distribution of settlement of blue mussels has been previously reported for the Marlborough Sounds (Meredyth-Young & Jenkins 1978, Atalah et al. 2017). As *P. canaliculus* larvae appear to preferentially settle in greater numbers at 11 m this makes it easier for aquaculture operators to reduce the amount of *M. galloprovincialis* settling onto grow out ropes and spat collectors if placed at greater depth (Atalah et al. 2017). This shallow depth preference is thought to be explained by the vertical distribution of larvae in the water column as *Mytilus edulis* are known to aggregate in surface waters above the thermocline/halocline (Dobretsov & Miron 2001). On rocky reefs and pylons around New Zealand, the natural zonation of *M. galloprovincialis* is at a higher tidal location than for the distribution of *P. canaliculus* which extends to a deeper subtidal range (Morton and Miller 1973). Interestingly, the settlement of *M. galloprovincialis* has been found to be higher onto nursery ropes seeded with *P. canaliculus* spat from Ninety Mile Beach versus wild caught spat from South Island locations (Forrest & Atalah 2017). This could explain why *M. galloprovincialis* abundance in this current study are relatively low compared to previous reports (Dawber 2004, Atalah et al. 2016, Atalah et al. 2017, Forrest & Atalah 2017).

The greatest number of spat was in the smallest size class (i.e., 200 – 499 μm in shell length) (86%). This is consistent with previous studies, which have also found that the highest abundance of spat was in the 250-300 μm size range (Meredyth-Young and Jenkins 1978, Alfaro and Jeffs 2003). In this study it was assumed based on the size of the spat recovered from the samples that they were the result of only primary settlement of spat with any spat over the size of 300 μm growing to this size after settling as a larva on the collector. While medium (500- 999 μm) and large (> 1 mm) spat were far less common on the spat collectors, it is very unlikely that they were able to grow to this size in only three weeks after settling on the collectors. Therefore, it is highly likely that these larger spat, which made up a much smaller proportion of the total spat catch (i.e., 14%) were secondary settlers. The abundance of *P. canaliculus* spat followed an annual cycle, with the highest abundance in December and

lowest from March – July. This peak in spat abundance is earlier than reported for the peak settlement of larvae of *P. canaliculus* in the Marlborough Sounds, February to April (Meredyth-Young and Jenkins 1978).

While spat sourced from Ninety Mile Beach currently makes up the majority of mussels seeded onto Greenshell™ farms there are many potential benefits to sourcing spat from alternative locations (Fox 2003, Atalah et al. 2017). In the last ten years Golden Bay has become a major area for the gathering of wild spat for South Island mussel farms, with the deployment each year of many kilometres of spat catching rope to gather settling wild Greenshell™ mussel larvae (Atalah et al. 2017). Not only does this provide an alternative source of spat to supplement those sourced from Ninety Mile Beach, but South Island sourced spat also have higher food clearing rates and reach condition faster than mussels grown from spat sourced from Ninety Mile Beach (Atalah et al. 2017) which is extremely beneficial to farmers. Settlement numbers in this study were not high enough to be a commercially feasible source of spat year-round but did reach sufficiently high numbers between October and January. This study provides information which can assist in future-proofing the industry against a short supply of spat by indicating that spat collection in the Hauraki Gulf has the potential to be commercially feasible and deployment of spat collecting material should be concentrated at 11 m depth rather than in shallower water, and is best conducted during Spring and Summer.

Chapter Three: Growth and Condition Cycle of *Perna canaliculus* from Ninety Mile Beach and the Hauraki Gulf

3.1 Introduction

The Greenshell™ industry is heavily reliant on wild-caught spat for initiating the production cycle, with around 99% of the industry's supply coming from the harvesting of wild spat, which can be highly variable in both quality and quantity (Jeffs et al. 1999). The quality of wild spat can be greatly affected by environmental conditions, as well as stresses caused by harvesting and transport to nursery farm sites ready for seeding out (Foote 2003, Carton 2007, Alfaro et al. 2010). Around 80% of the wild spat used in aquaculture is sourced from Ninety Mile Beach where spat attached to drifting debris, including fragments of macroalgae, is brought into shallow water by a combination of hydrodynamic and meteorological processes where it can be harvested (Alfaro 2004, Alfaro et al. 2010). Consequently, the quantity and timing of the supply of mussel spat from this source is highly sporadic. Smaller quantities of spat are caught in Golden Bay and the Marlborough Sounds in the South Island, as well as the Hauraki Gulf and Aotea Harbour in the North Island, on lengths of fibrous rope which are suspended in the water column to attract settling *P. canaliculus* larvae. The use of multiple sources of wild spat by the mussel industry is very important as in combination they help to resolve the uncertainty of spat supply from the individual sources (Hickman 1978, Hayden & Kendrick 1992, Hayden 1995). A number of aquaculture companies have developed hatchery production technology for *P. canaliculus*, however, hatchery production is a more expensive option than the use of wild-caught spat (Alfaro et al. 2012).

Perna canaliculus typically reach sexual maturity within a year after larval settlement, which for the majority is at a shell length of 40 - 50 mm (Jeffs et al. 1999). This mussel is dioecious (1:1 sex ratio) with gametogenesis occurring in late autumn - winter, the timing of which is thought to be due to the energy reserves amassed in summer and autumn which are used for fuelling the subsequent gametogenesis (Alfaro et al. 2001). The species is a synchronised broadcast spawner with peak spawning usually occurring in late spring to early autumn (Jenkins 1985, Jeffs et al. 1999), although a small proportion of mussels can also be found spawning throughout the year (Alfaro et al. 2001) resulting in year-round settlement of larvae

in many locations (Buchanan 1994, Alfaro & Jeffs 2003, Alfaro et al. 2010). Populations of *P. canaliculus* in different locations have temporal variation in spawning that have been linked to direct responses to local environmental conditions, such as water temperatures and food availability, but are also likely to have some basis due to localised adaptation or genetic differences (Alfaro et al. 2001, LeBlanc 2005). These differences in spawning periodicity have been identified both on localised spatial scales as well as between distant regions (Alfaro et al. 2003). For example, spawning in *P. canaliculus* populations at Ninety Mile Beach are at their peak between June and December (Alfaro et al. 2001, Alfaro et al. 2003), whilst in the Marlborough Sounds there are two separate spawning periods in early summer and autumn - spring (Flaws 1975, Tortell 1976, Buchanan 1998). The differences in the timing of spawning between these two locations has been attributed to the response of adult mussels to the different seasonal temperature regimes (Alfaro et al. 2001) because gametogenic activity is thought to only occur in this species at temperatures above 11 °C (James & Ross 1997). Seawater temperatures at Ninety Mile Beach seldom drop below 14 °C (Alfaro et al. 2001), whereas temperatures in Marlborough Sounds are between 10 - 11 °C in winter and 20 - 21 °C in summer (Tortell 1976, Jenkins 1985).

Differences in the timing of mussel spawning are most evident between North and South Island populations, but geographically close populations can also have differences in the timing of peak spawning activity despite synchronous spawning reportedly being a feature of this species (Alfaro et al. 2003). For example, different mussel populations along Ninety Mile Beach were observed to have different spawning periods, with two subtidal populations at the southern end of the beach and one intertidal population at the north end of the beach having a prolonged spawning period between June and December compared with two intertidal populations at the southern end of the beach which had two or three spawning events during the same period. These differences in reproductive activity over relatively small spatial scales are most likely due to adult mussels responding directly to localised differences in environmental conditions, rather than any underlying genetic differences.

This species tends to occur in high concentrations in geographically discrete locations (Jeffs 1999, Gardner 2000, Gardner & Thompson 2001), which are often controlled by localised environmental factors such as depth and wave exposure (Morton & Miller 1973, Menge et al.

2007), temperature regime (Petes et al. 2007), availability of suitable attachment substrate (Buchanan 1994) and food supply (Gardner 2000, Gardner & Thompson 2001, Ren & Ross 2005). Therefore, it is highly likely that some of these same environmental factors that control the localised distribution and abundance in this species also exert a direct influence over the timing of their reproductive activity.

The possible role of genetic differences among populations in driving the observed differences in reproductive activity is uncertain. Several studies using a range of genetic techniques have identified marked genetic discontinuity between populations of *P. canaliculus* in northern and southern New Zealand, as well as genetic diversity at more localised scales in some regions, such as the west coast of the South Island (Smith 1988, Sin et al. 1990, Gardner et al. 1996 a, b, Star et al. 2003, Wei et al 2013). It was initially proposed that these genetic differences were due to physiological adaptations to different thermal environments and that the water currents around parts of New Zealand may partially isolate the populations by limiting the movement of larvae (Smith 1988). The subsequent application of higher resolution genetic techniques indicate that genetic variation across the full distributional range of *P. canaliculus* is best explained by corresponding differences in sea surface temperatures. Hence, it is likely that both genetic and environmental influences play a role in influencing the reproductive cycles in populations of this species.

Temporal differences in the reproductive cycles of *P. canaliculus* are of particular commercial significance in mussel aquaculture, as reproductive condition greatly influences meat yield and the consumer qualities of the harvested product (Çelik et al. 2012). Besides reproductive activity, a range of other factors affect the condition of marine bivalves including food supply, salinity, temperature, environmental contaminants, size, gonad development and population density (Lucas & Beninger 1985, Pridmore et al. 1990, Loesch & Evans 1994, Iglesias et al. 1996, Marsden & Weatherhead 1999).

Condition indices (CI) are a widely used means of measuring the condition of bivalves including for estimating commercial meat yield in aquaculture and as a proxy measure for the apparent “health” or physiological status of the animals under given environmental conditions (Baird 1958, Lucas & Beninger 1985). Greenshell™ aquaculture operators make extensive use of condition indices for dictating the timing of harvesting and processing of

mussels which is often confined to seasonal periods when satisfactory meat yields and financial returns can be achieved (Fox 2003). The condition of farmed *P. canaliculus* has been found to generally follow an annual cycle with high values in autumn and spring and lower values in summer and winter most often following spawning events (Hickman & Illingworth 1980, Hickman et al. 1991). Mussel condition and meat yields usually reach the lowest levels in mid-winter to spring, and if condition declines to very low levels, as it does most winters, then the mussels are unsuitable to harvest (Fox 2003). For Greenshell™ aquaculture operations this is problematic because harvesting equipment, processing plants and associated personnel are unable to be fully utilised during this period. The recent development of premium live mussel export markets for Greenshell™ has also created a demand for year-round supply of mussels in good condition.

The source of mussel stocks is thought to be one of the most important production parameters underlying adult biomass. It is known to affect the average level of growth, levels of mortality and their degree of variation among cohorts (Dickie et al. 1984). There is evidence that mussels grown from geographically separated spat sources have different cycles of condition, providing the potential to extend effective harvesting seasons by utilising mussels with complementary condition cycles (Fox 2003). Mussels grown from spat sourced from Ninety Mile Beach, Golden Bay and the Marlborough Sounds when grown together on a number of farm locations in the Pelorus Sound were found to have a differing overall average condition over a single year, as well as differences in the timing of condition cycles. For example, over one year the mean condition of mussels grown from Marlborough Sounds was 2.5% higher than those grown from spat sourced from Golden Bay, however. Harvesting stocks with different condition cycles could potentially improve the mean annual condition index of harvested mussels and minimise the impact of the mid-winter decline in condition. Increases in mean annual condition as small as 2% are considered to be commercially relevant in Greenshell™ aquaculture (Fox 2003). For example, improving the mean annual condition of mussels harvested from 38% to 40% was estimated to have the potential to increase the overall meat yield achieved by the Marlborough Sounds mussel industry by 1400 t a year (Fox 2003). Given these results it is likely that similar potential differences in the seasonal condition cycles of mussels sourced as spat from different locations may also exist when grown out in the Hauraki Gulf. Such differences would have particular commercial benefit for

the Greenshell™ aquaculture industry in the Hauraki Gulf which produces around a third of the national production of mussels and has recently been pioneering the export of live mussels to markets which require a stable supply year round.

Therefore, the specific aim of this study is to determine whether different mussel stocks have different growth rates and condition cycles when they are grown at the same location in the Hauraki Gulf.

3.2 Methods

The growth and condition of *Perna canaliculus* from two spat sources (i.e., Hauraki Gulf and Ninety Mile Beach) were grown together at the same location at Papakarahi Bay in the Hauraki Gulf and sampled regularly for two years and their condition index determined from samples taken at around three weekly intervals (Fig. 3.1). Around 1000 mussels of 45 - 55 mm in shell length that originated from spat sourced from each of two locations (i.e., Hauraki Gulf within the Coromandel Harbour and Ninety Mile Beach), were grown at Papakarahi Bay over two years. On 2 July 2013 around 1000 mussels from each stock were separately re-seeded onto two 6 m long mussel dropper lines at densities of 170-180 m⁻¹. The two mussel grow out lines were then hung 5 m apart at 1 - 3 m depth on a mussel farm in Papakarahi Bay in the Coromandel Harbour (Fig. 3.1).

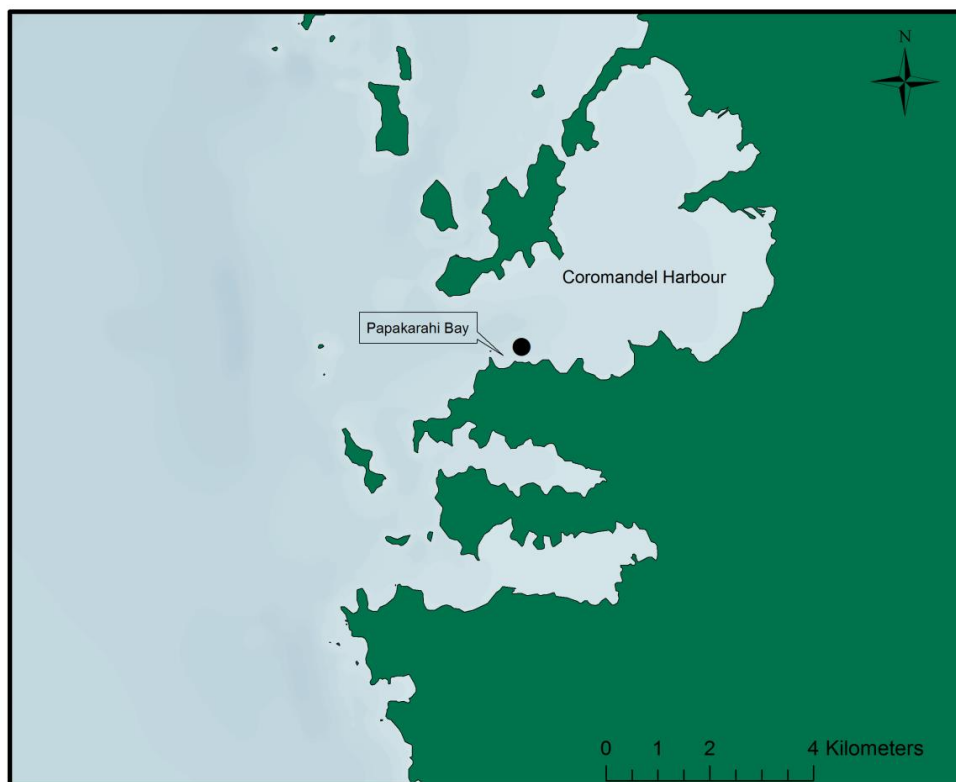


Figure 3.1 Map showing the location of the mussel farm in Papakarahi Bay, Coromandel Harbour, Hauraki Gulf, where the experimental mussel lines were deployed.

3.2.2 Mussel Stock Measurements

Every three weeks 25 mussels from each stock were sampled from their respective growing rope. Occasionally logistical problems, such as inclement weather, disrupted the sampling, in which case the nearest possible sampling time was used. Ten mussels were selected approximately every 2 m along the 6 m dropper line to give a total of 30 mussels removed from each rope. Five extra mussels were needed as any mussel found containing the pea crab *Pinnotheres novaezelandiae* was discarded from sampling because this parasite affects the growth, shell shape, meat weight and condition of mussels (Hickman 1978, Helson 2001, Trottier 2012). The mussels were placed in labelled plastic bags and then stored in a polystyrene container with ice before being shipped in a refrigerated truck to Auckland, where they were processed in the laboratory.

On arrival at the laboratory the mussels were cleaned of any biofouling and placed in holding

buckets with filtered sea water at 18 °C. The mussels were kept in the holding buckets to reduce the stress on the mussels and keep conditions constant, as it took up to 8 h to complete the processing of the sample of mussels. Each mussel was first dried with a paper towel. The shell length was measured using vernier callipers to an accuracy of 1.0 mm. The mussels were then opened, and after removing the flesh from the shell it was placed on a paper towel with another paper towel placed on top and left for two minutes to remove excess moisture. Shells were also dried using paper towels. The whole wet weight (WWW), wet shell weight (WSW) and wet flesh weight (WFW) for each mussel was then measured using a Sartorius LE244S expert analytical balance to the nearest 0.0001 g. The shells and flesh were placed on tin foil lined trays and put in a Gavenkamp IH.200 vacuum oven at 60 °C for 24 h. Once the sampled mussels reached 70 mm in shell length the flesh was dried for 48 hours and the shells for 24 h. Dry flesh weight (DFW) and dry shell weight (DSW) after removing from the drying oven were then recorded. Twenty eight samples at approximately three weekly intervals were taken over the 24 month study.

3.2.3 Condition Indices

A dry weight CI (Lucas & Beninger 1985) was chosen for this study, as it has been evaluated for many bivalves and is known to avoid variation associated with wet weight measurements due to fluctuating moisture content (Hickman & Illingworth 1980). Dry CI = $DFW \times 100 / DSW$ was calculated for each sampled mussel.

A second CI, recommended for use in the Greenshell™ mussel farming industry (Hickman & Illingworth, 1980), was also calculated for comparison, and to provide an indication of how the industry CI differs from the dry weight index. Commercial CI = $WFW / (WWW - WSW) \times 100$.

3.2.4 Statistical Analyses

General linear models (GLM) were used to compare growth and condition of mussels using natal stock origin and time of sampling as factors in the models. Prior to analyses data normality was confirmed with a Shapiro–Wilk’s test and the equality of variances was tested using a Brown–Forsythe test. Where overall experimental factors in the analyses were

significant, pairwise post-hoc comparisons using Tukey's tests were conducted to identify contributing significant differences in the experimental components with Holm-Sidak adjustment made for inflated false discovery rate. For all statistical analyses $\alpha = 0.05$. All analyses were conducted in IBM SPSS Statistics (Version 21).

3.3 Results

3.3.1 Growth

The interactive term from the GLM for natal origin * time of sampling was significant indicating there were differences in the timing of the growth of mussels derived from Ninety Mile Beach and Hauraki mussel stocks ($F_{(27, 1324)} = 2.20$, $P = 0.001$)(Fig. 3.2). Pairwise comparisons of mean shell length between mussels from the two sources for the individual sampling dates identified seven dates when the mean size of mussels from the two stocks were different. The mussels were of a slightly different mean shell length when the experiment was first established on 2 July 2013, 49.6 mm (± 3.3) versus 46.8 mm (± 2.3) for Hauraki Gulf and Ninety Mile Beach mussels respectively. There was no difference in mean mussel shell length for the following sample on 23 July 2013, but for the following four samples from 25 September 2013 and 10 December 2013 the Hauraki Gulf mussels were consistently of a larger mean size. Thereafter, the mean shell length of the mussels remained similar apart from 16 April 2014 when Ninety Mile Beach mussels were 16% larger, and 8 October 2014 when Hauraki Gulf mussels were 21% larger (Fig. 3.2). Time was a significant factor in the GLM reflecting the overall growth of mussels from both stocks over the course of the experiment ($F_{(27, 1324)} = 475.92$, $P = 0.001$). Over the course of the 24 month period the mussels grew from an overall mean shell length of 48.0 mm (± 0.4) to 110.0 mm (± 1.0). Mussel growth tended to be continuous throughout most of the two year study period but declined for a period in spring in both years for both stocks of mussels (Fig. 3.2). Overall, mussel stock was also a significant factor in the GLM ($F_{(1, 1324)} = 6.0$, $P = 0.015$) with Hauraki Gulf mussels showing lower overall growth than those from Ninety Mile Beach. Both stocks of mussels reached a mean harvestable size of 90 mm in shell length in August 2014.

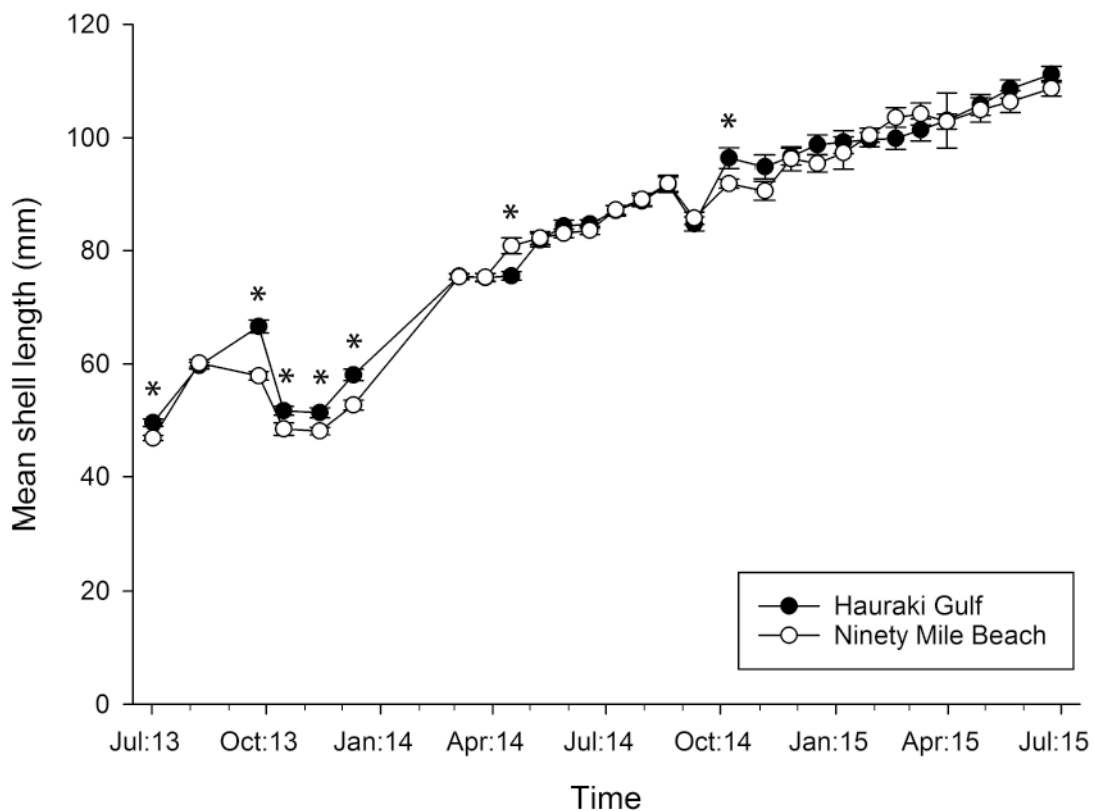


Figure 3.2 Mean shell length (\pm S.E.) of mussels sourced as spat from Ninety Mile Beach and the Hauraki Gulf and subsequently grown at the same mussel farm location in Coromandel, in the Hauraki Gulf, and sampled approximately every three weeks over two years. Statistical differences are denoted with *.

3.3.2 Mussel Condition – Industry CI

Over the course of the 24 month study period mean mussel condition ranged from 67 (\pm 14) in the Hauraki Gulf mussel stock in July 2015 down to 32 (\pm 4) in the Ninety Mile Beach mussel stock in October 2013. Both stocks of mussels reached their highest condition in autumn of 2014 and in autumn - winter of 2015, and were in their lowest condition in spring of 2013 and winter of 2014 (Fig. 3.3). The interactive term from the GLM for natal origin * time of sampling was significant indicating there were differences in the timing of condition of mussels between the two different natal stocks of mussels ($F_{(27, 1324)} = 5.73$, $P = 0.001$). Pairwise comparisons of mean condition between mussels from the two sources for the individual sampling dates identified twelve dates when the mean meat weight of mussels from the two stocks were different (see figure 3.3). The following differences in mean CI were identified

between stocks of mussels for individual sampling events; 02/7/13 HG>NMB by 15%, 08/8/13 HG>NMB by 11%, 25/9/13 HG>NMB by 4%, 15/10/13 HG>NMB by 29%, 13/11/13 HG<NMB by 13%, 10/10/13 HG<NMB by 14%, 05/03/14 HG<NMB by 3%, 26/03/2014 HG<NMB by 8%, 16/04/14 HG>NMB by 4%, 09/05/2014 HG<NMB by 8%, 28/05/14 HG<NMB by 1%, 18/06/2014 HG<NMB by 10%, 09/07/14 HG<NMB by 15%, 30/07/14 HG<NMB by 4%, 20/08/14 HG>NMB by 1%, 10/09/14 HG>NMB by 7%, 08/10/14 HG>NMB by 19%, 05/11/14 HG<NMB by 2%, 26/11/14 HG>NMB by 16%, 17/12/14 HG<NMB by 0.1%, 07/01/15 HG>NMB by 8%, 28/01/15 HG>NMB by 7%, 18/02/15 HG>NMB by 20%, 10/03/15 HG<NMB by 4%, 31/03/15 HG<NMB by 2%, 27/04/15 HG< NMB by 3%, 21/05/15 HG<NMB by 3% and 23/06/15 HG>NMB by 2% (Fig. 3.3). There was no obvious seasonal pattern to the differences in condition identified between mussels from the two sources. For example, while mussels from the Hauraki Gulf consistently had higher mean condition than those from Ninety Mile Beach during winter of 2013, the reverse occurred through much of the following winter in 2014. There was no overall difference in the CI of the mussels from Ninety Mile Beach and Hauraki mussel stocks over the course of the experiment ($F_{(1, 1324)} = 0.01$, $P = 0.92$) (Fig. 3.3). Overall, Time was a significant factor in the condition of mussels from both stocks over the course of the experiment ($F_{(27, 1324)} = 294.02$, $P = 0.001$) as might be expected given the temporal variability in CI for mussels sampled from both stocks over the two year course of the study.

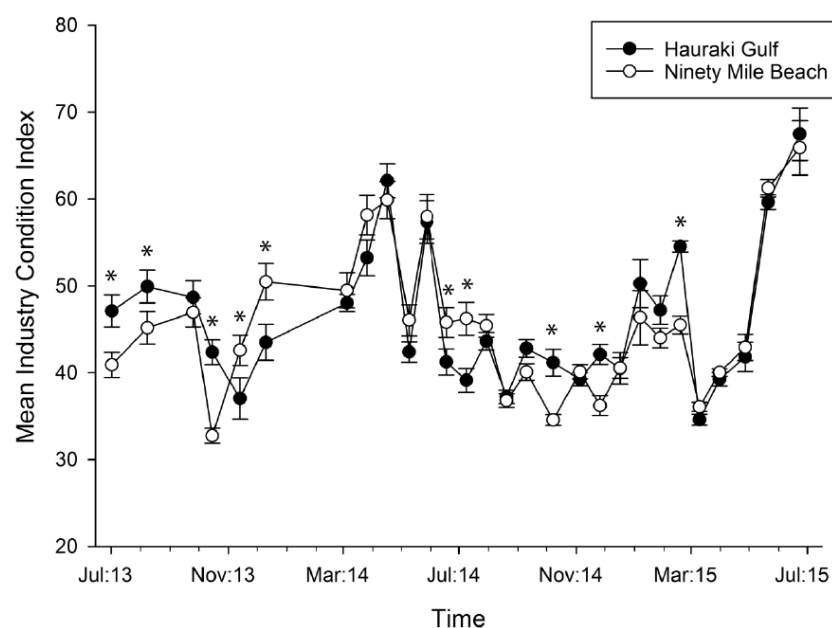


Figure 3.3 Mean Industry CI (\pm S.E.) of mussels sourced as spat from Ninety Mile Beach and the Hauraki Gulf grown at the same mussel farm location in Coromandel, Hauraki Gulf, and sampled approximately every three weeks over two years. Statistical differences are denoted with *.

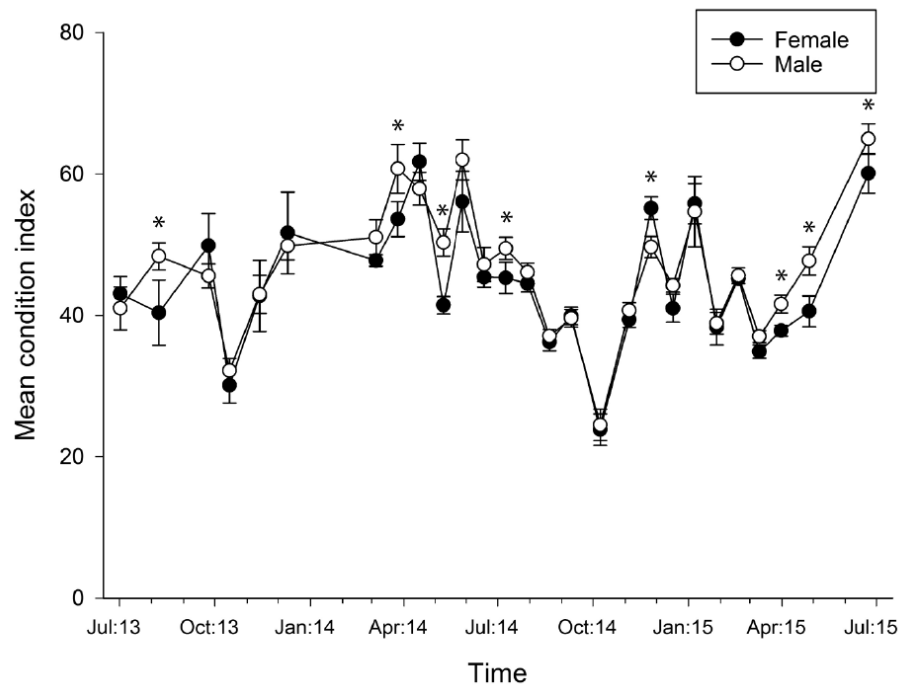


Figure 3.4: Mean Industry CI (\pm S.E.) of male and female mussels sourced as spat from Ninety Mile Beach grown in Coromandel, Hauraki Gulf, and sampled approximately every three weeks over two years. Statistical differences are denoted with *.

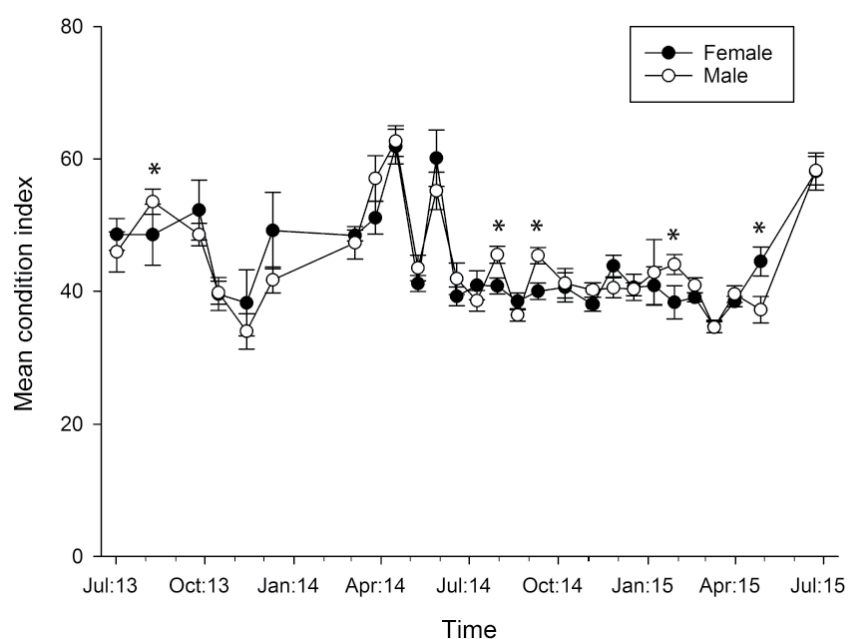


Figure 3.5: Mean Industry condition index (\pm S.E.) of male and female mussels sourced as spat from the Hauraki Gulf and grown in Coromandel, Hauraki Gulf, and sampled approximately every three weeks over two years. Statistical differences are denoted with *.

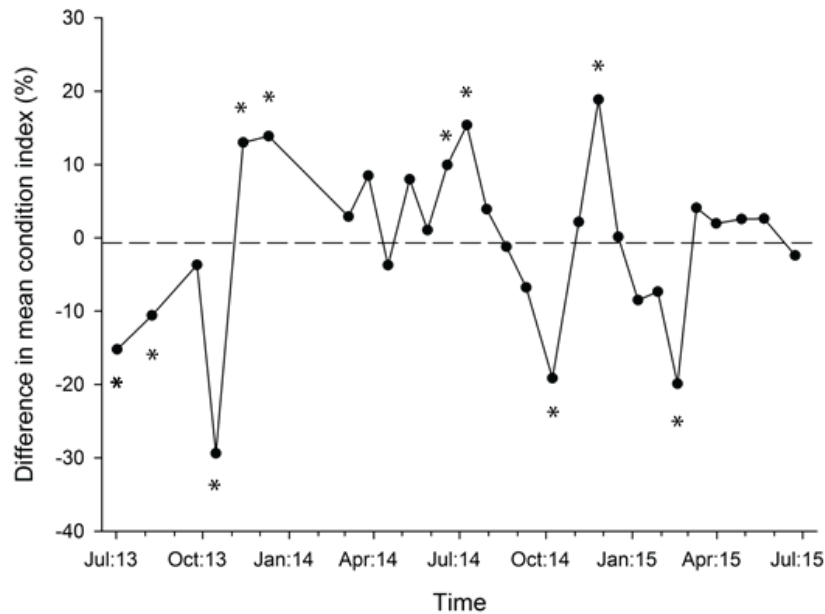


Figure 3.6. Difference in mean commercial CI condition of mussels sourced as spat from Ninety Mile Beach and Hauraki Gulf grown at the same mussel farm location in Coromandel, Hauraki Gulf, and sampled approximately every three weeks over two years. Positive values denote that Ninety Mile Beach mussels have higher condition than Hauraki Gulf mussels and negative values indicate the opposite. Statistical differences are denoted with *.

3.3.1 Mussel Condition - Dry Index

For dry CI measures the interactive term from the GLM for natal origin * time of sampling was significant indicating there were differences in the timing of condition of mussels between the two different natal stocks of mussels ($F_{(27, 1324)} = 3674$, $P = 0.001$). Pairwise comparisons of mean condition between mussels from the two sources for the individual sampling dates identified ten dates when the mean meat weight of mussels from the two stocks were different (see figure 3.8). The following differences in mean CI were identified between stocks of mussels for individual sampling events; 02/7/13 HG>NMB by 20%, 08/8/13 HG>NMB by 15%, 25/9/13 HG>NMB by 7%, 15/10/13 HG>NMB by 36%, 13/11/13 HG<NMB by 17%, 10/10/13 HG<NMB by 20%, 05/03/14 HG<NMB by 3%, 26/03/2014 HG<NMB by 10%, 16/04/14 HG>NMB by 7%, 09/05/2014 HG<NMB by 10%, 28/05/14 HG<NMB by 4%, 18/06/2014 HG<NMB by 14%, 09/07/14 HG<NMB by 25%, 30/07/14 HG<NMB by 5%,

20/08/14 HG>NMB by 8%, 10/09/14 HG>NMB by 10%, 08/10/14 HG>NMB by 28%, 05/11/14 HG<NMB by 5%, 26/11/14 HG>NMB by 25%, 17/12/14 HG<NMB by 4%, 07/01/15 HG>NMB by 10%, 28/01/15 HG>NMB by 10%, 18/02/15 HG>NMB by 23%, 10/03/15 HG<NMB by 13%, 31/03/15 HG<NMB by 3%, 27/04/15 HG< NMB by 6%, 21/05/15 HG<NMB by 8% and 23/06/15 HG>NMB by 5% (Fig. 3.8). There was no obvious seasonal pattern to the differences in condition identified between mussels from the two sources. For example, while mussels from the Hauraki Gulf consistently had higher mean condition than those from Ninety Mile Beach during winter of 2013, the reverse occurred through much of the following winter in 2014. There was no overall difference in the CI of the mussels from Ninety Mile Beach and Hauraki mussel stocks over the course of the experiment ($F_{(1, 1324)} = 0.01$, $P = 0.68$) (Fig. 3.7). Overall, Time was a significant factor in the condition of mussels from both stocks over the course of the experiment ($F_{(27, 1324)} = 306.02$, $P = 0.001$) which reflected the extent of the overall temporal variability in the condition of the mussels during the course of the study.

The dry condition index and commercial condition index delivered very similar results (see figure 3.7), however, dry CI produced a greater difference in condition than the industry index but followed the same pattern (Fig. 3.8). The greatest difference between CI results occurred in July 2014 when the industry condition difference between stocks was 15% and the dry condition difference was 25%. The variance between the two stocks ranged from 0.4% (March 2013) to 10% (July 2014) (Fig. 3.8).

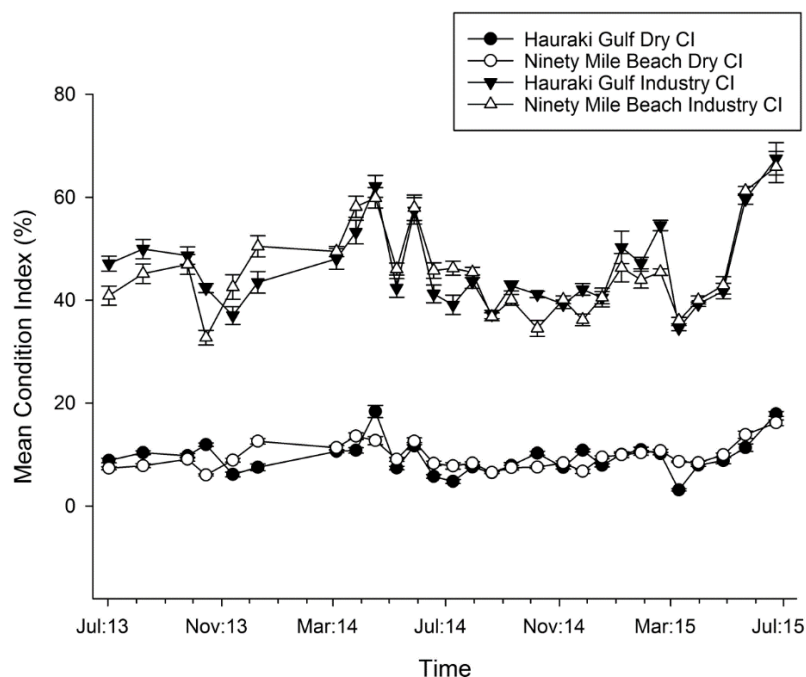


Figure 3.7. Mean industry condition index and dry condition index of mussels sourced as spat from Ninety Mile Beach and Hauraki Gulf grown at the same mussel farm location in Coromandel, Hauraki Gulf, and sampled approximately every three weeks over two years.

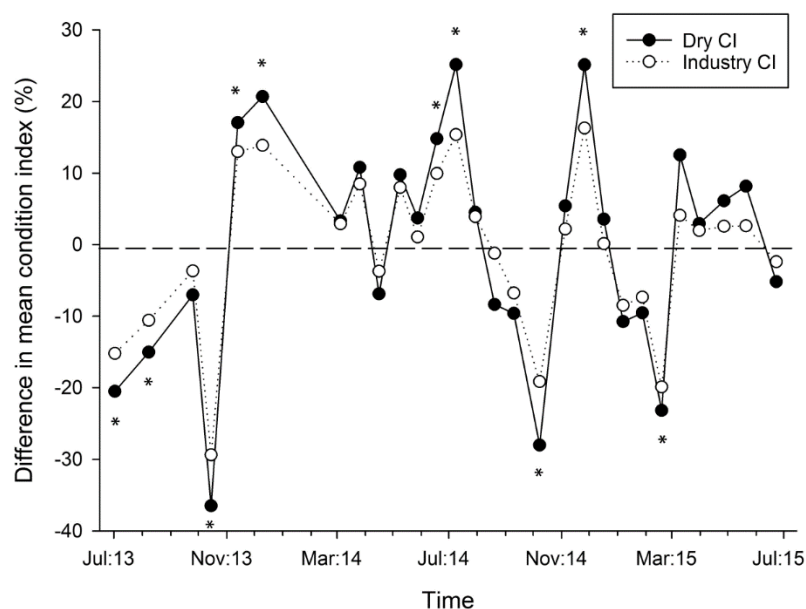


Figure 3.8. Mean industry condition CI and dry condition CI of mussels sourced as spat from Ninety Mile Beach and Hauraki Gulf grown at the same mussel farm location in Coromandel, Hauraki Gulf, and sampled approximately every three weeks over two years. Positive values denote that Ninety Mile Beach mussels have higher condition than Hauraki Gulf mussels and negative values indicate the opposite. Significant dry CI differences denoted with *.

3.3.2 Meat Weight

The interactive term from the GLM for natal origin * time of sampling was significant indicating there were differences in the timing of the meat weight of mussels derived from Ninety Mile Beach and Hauraki mussel stocks ($F_{(27, 1324)} = 4.83, P = 0.001$) (Fig. 3.9). There was no overall difference in the meat weight of the mussels from Ninety Mile Beach and Hauraki mussel stocks over the course of the experiment ($F_{(1, 1324)} = 0.06, P = 0.94$) (Fig. 3.9). Overall, Time was a significant factor in the condition of mussels from both stocks over the course of the experiment ($F_{(27, 1324)} = 112.47, P = 0.001$) as might be expected given the temporal variability in meat weight for mussels sampled from both stocks over the two year course of the study. Pairwise comparisons of meat weight between mussels from the two sources for the individual sampling dates identified eight dates when the mean meat weight of mussels from the two stocks were different (see figure 3.9).

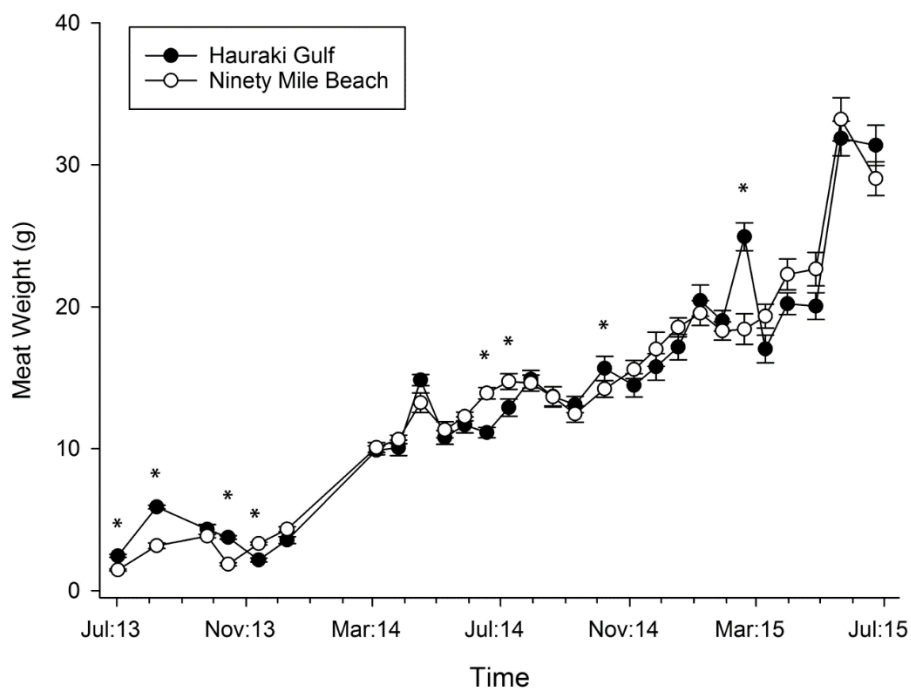


Figure 3.9. Mean wet meat weight of mussels sourced as spat from Ninety Mile Beach and Hauraki Gulf grown at the same mussel farm location in Coromandel, Hauraki Gulf, and sampled approximately every three weeks over two years. Statistical differences are denoted with *.

3.4 Discussion

3.4.1 Growth

Mussel spat taken from Ninety Mile Beach and the Hauraki Gulf and grown together at the same location in Coromandel for two years had only small differences in shell length that were of only short duration. In spring of both years the mussels from Ninety Mile Beach temporarily had smaller shell length than those from the Hauraki Gulf, whereas in both years during autumn the Hauraki Gulf mussels tended to be larger. These small differences in seasonal growth between the mussels from two natal stocks are likely to reflect minor differences between their genetically predetermined response to the environmental conditions in terms of energy allocation to growth and reproduction. However, the overall concordance of the growth of the two stocks of mussels at the Coromandel site over the two years indicates that growth is most likely to be driven by environmental rather than genetic differences between mussels from the two different stocks. The transplantation of mussels to a new environment has the potential to influence their subsequent patterns of growth (Lee 1986, Kautsky et al. 1990). This is due to mussels being able to modify their feeding behaviour to compensate for reduced food quantity and quality and therefore maximise their energy intake (Bayne et al. 1984, 1987, Widdows et al. 1984, Marsden & Weatherhead 1999). It is widely reported that changes in growth patterns of bivalve shellfish are predominately environmentally mediated (Dickie et al. 1983, Widdows et al. 1984, Mallet et al. 1987b, Kautsky et al. 1990). For example, the experimental transplant of adult blue mussels, *Mytilus edulis*, from an estuarine environment into a sub-littoral environment found that changes in the fundamental physiology of the mussels (i.e., clearance rates, absorption efficiency, respiration, excretion rates, O : N and scope for growth) continued to occur over a two month period toward those of the resident mussel population (Widdows et al. 1984). However, clearance rates, scope for growth and growth efficiency continued to remain out of step with resident mussels after two months which together indicates that these physiological parameters are both environmentally and genetically mediated. Similar studies transplanting *M. edulis* between the Baltic and North Seas, and to different locations within the Atlantic Ocean concluded that the growth is predominantly regulated by non-genetic factors, however stock differences were responsible for mortality effects (Dickie et al. 1983, Kautsky et al. 1990). The overall effect on biomass and potential yield was approximately equally attributable to site and stock

influences (Dickie et al. 1983). When mussels 0.8 – 3.2 mm in size sourced from three locations (Ninety Mile Beach, Golden Bay, Marlborough Sounds) were grown together in the Marlborough Sounds their subsequent patterns of growth over a period of one year were similar (Fox 2003). However, when larger mussels of 64 – 73 mm in size were sourced from the same three locations and then grown together at the same location in the Marlborough Sounds there were marked differences in their subsequent patterns of growth. It was concluded that the growth differences observed in these larger mussels were due to the early growing environment significantly influencing the subsequent growth of mussels rather than a result of the expression of genetic differences among the three stocks of mussels or the result of a direct response to environmental conditions (Fox 2003). When looking to optimise growth rates there is no advantage in sourcing spat from the Hauraki Gulf over the already used Ninety Mile Beach as there was no significant differences in growth rates between the two stocks.

3.4.2 Condition

The condition indices of mussels grown from spat sourced from Ninety Mile Beach and the Hauraki Gulf were different for six of the 25 regular sampling events taken over the two years indicating that they ripened and spawned at different times and intensities. The results show that mussels sourced from Ninety Mile Beach spawned in October in both years, one month earlier than the mussels from Hauraki Gulf. During the period of October to December the CI between the two mussel stocks differed by up to 30%. The spawning of mussels sourced from Ninety Mile Beach had a greater effect on mussel condition and appeared to occur only once per year during spring. In contrast, Hauraki Gulf mussels appeared to have one large spawning event during November in 2013 but three smaller spawning events in 2014 in July, September and November. Researchers have speculated that genetic differences among stocks of mussels are responsible for the observed seasonal differences in their condition cycles (Fox 2003, Camara & Symonds 2016). Similar variations in condition cycles were also observed for *P. canaliculus* sourced from several geographic locations and grown together at the same location in the Marlborough Sounds (Fox 2003). Spat generated by spawning broodstock from different natal populations that were raised in identical hatchery conditions had the same variations in condition cycles, thus providing further evidence that the differences were

genetically mediated (Fox 2003). If differences in the timing of spawning are genetically predetermined among different mussel stocks then there is the potential to exploit this feature in using hatchery methods to produce spat with different seasonal condition cycles which would have the potential to extend harvesting seasons and the overall meat yield for farmers. This is because the best time to harvest mussels is just before they spawn as they have a higher meat weight and are more pleasing to eat. For farmers in the Marlborough Sounds improving the mean annual condition of mussels harvested from 38% to 40% was estimated to have the potential to increase the overall meat yield achieved by the Marlborough Sounds mussel industry by 1400 t a year (Fox 2003). In November 2014, December 2014 and February 2015 mussels sourced from Hauraki Gulf had an average CI 19%, 16% and 20% higher than that of mussels sourced from Ninety Mile Beach. This equated to an overall average of 10 g more meat wet weight per mussel at harvest. At typical industry levels of mussel production of 150 mussels per metre of grow out rope and 3500 m of grow out rope per farm, the difference in meat yield amounts to around 5 t for a typical mussel farm. With a current domestic market value of NZ\$5 per kilo (Hawkes Bay Seafood) for whole live mussels this equates to NZ\$25,000. Current United Kingdom market value for half shell frozen mussels is around NZ\$23 per kilo (The Fish Society UK). As one shell makes up approximately 18% of a mussels whole weight this would equate to NZ\$94,300. It is important when considering the mussel industry that differences between stocks are due to true stocks effects rather than being environmentally mediated. This is because environmental conditions are unable to be controlled or predicted and therefore any gains from using two stocks could not accurately be predicted year to year. The genetic differences between Hauraki Gulf and Kaitaia populations has not been sufficiently investigated. Previous studies on *Perna canaliculus* populations have not found a difference between Kaitaia and Hauraki Gulf populations (Star et al. 2003) but genetic testing procedures have improved greatly and it warrants re-testing.

3.4.3 Male and female condition

The CI of male and female mussels was generally similar over the two years of the study with both stocks. The most notable difference in condition between the two sexes occurred one to two months before spawning when females of both stocks had lower condition than males.

This is most likely due to the greater somatic resources required by female mussels during gametogenesis compared with males (due to the smaller energetic demands of spermatogenesis with no requirement for provisioning with yolk reserves (Kautsky 1982, Bagamein et al. 2004). Hauraki Gulf mussels tended to show less synchronicity in CI between the sexes compared with mussels sourced from Ninety Mile Beach. This could be a result of the apparent multiple smaller spawning events which resulted in less reduction of CI compared to Ninety Mile Beach mussels, and consequently both male and female Hauraki Gulf mussels were able to recover their reproductive condition more quickly.

Intense spawning events during high temperatures can result in mortality (Dickie et al. 1984, Mallet et al. 1987a, Myrand et al. 2000). Due to a depletion of reserves after spawning mussels are particularly vulnerable to stressful environmental conditions such as high water temperatures (Bayne 1975, 1984, Newell & Thompson 1984). In February 2015 seawater temperatures were around 22°C compared to 19°C in February 2014 in the Hauraki Gulf therefore Hauraki Gulf mussels may have lowered spawning effort in order to conserve resources. Physiologically flexible and genetically variable populations are more likely to have lower mortality rates (Mallet et al. 1987b, Kautsky et al. 1990). Eleven stocks of juvenile *Mytilus edulis* mussels transplanted along the coast of Nova Scotia exhibited different levels of mortality. Stocks originating from more stressful environments tended to exhibit lower mortalities than those originating from less stressful environments (Mallet et al. 1987b). Due to the large scale of this trial measuring mortality was not feasible. It would be wise to investigate this in the future as mortality may vary between Hauraki Gulf and Ninety Mile Beach stocks, especially during periods of greater environmental variability. This could have a large effect on the overall yield for mussel farmers in the Hauraki Gulf area.

3.4.4 Dry Condition Index

The use of both the dry condition index and industry condition index in this study provided the opportunity to examine the accuracy of the current industry CI. The industry CI and dry CI produced the same results with matching statistically significant samples. However the dry CI provided stronger results of differences between stocks CI with higher percent difference. This difference could be attributed to the extra bias produced by the water weight thus

making dry CI the more accurate measurement (Crosby & Gale1990). The industry CI is much more practical to use in the industry as it takes considerable less time and is able to measure on the boat. It is therefore very pleasing to note that the industry CI is powerful enough to detect the same differences as the dry CI. In terms of the power of condition indices to identify significant differences in meat weight, there was a success rate of 80%. For the samples on 10 December 2013 and 26 November 2014 both dry and industry CI had significant differences between stocks, however there was no significant difference in meat weight between stocks. All other significant differences in CI correlated to a significant difference in meat weight. There were no instances of a difference in meat weight but no difference in CI. Therefore CI is a fairly accurate indicator of differences in meat weight.

3.5 Conclusions

The results of this study indicate that mussels sourced from Ninety Mile Beach and the Hauraki Gulf have subtle differences in the timing of their condition cycles and reproductive behaviour when grown together in the Hauraki Gulf. These differences are likely to be the result of genetic differences between these two stocks, and testing of other stocks sourced from elsewhere around New Zealand may result in greater differences in the timing of condition which could potentially confer greater commercial benefits. These results provide further evidence that growing multiple stocks together could be financially beneficial for mussel farmers. As the mussels used in this study were all from one cohort it would be advisable to repeat this study on a larger scale to include several year classes to conclusively determine that the differences observed in this study are a true stock effect. While the benefits gained from growing Hauraki Gulf stock and Ninety Mile Beach stock together may not be overly impressive it is important to understand the potential growth, spawning and condition cycles of any potential new stock. For the industry to grow it is imperative that the dependence on Ninety Mile Beach source spat is reduced; either through increasing hatchery spat production or through the identification of other wild spat stocks. This study highlights how *P. canaliculus* stocks can vary in spawning behaviour, both timing and intensity and condition cycles.

Chapter Four: Secondary Settlement Behaviour in *Perna canaliculus* Spat

4.1 Introduction

Greenshell™ (*Perna canaliculus*) aquaculture was first developed in New Zealand in the 1970s and the value of production has grown to around \$300M per annum in 2015 with the vast majority going to export sales (\$260M in 2015) (Colin Johnston, Aquaculture NZ, pers. comm, NZ Seafood Industry Council, 2016). Despite the rapid growth in this industry to date, the supply of mussel seed or “spat” is a continuing constraint to increasing consistent aquaculture production. The Greenshell™ aquaculture industry is heavily reliant on harvesting wild spat for initiating its production cycle. The vast majority of the spat that supplies the industry, around 80 %, is harvested from Ninety Mile Beach in northern New Zealand where it is cast ashore attached to seaweed and other debris (Jeffs et al. 1999, Alfaro et al. 2010, Jeffs *et al.* 2018). The balance of wild spat is caught on fibrous ropes placed in areas with high natural settlement of larval mussels, such as in Golden Bay, parts of the Marlborough Sounds, Aotea Harbour and the Hauraki Gulf. These natural sources of wild mussel spat are unpredictable and there is some evidence of decline in some of these sources of wild spat. For example, in the Hauraki Gulf, where spat fall has been monitored for the last 6 years, there has been a steady decline which has resulted in reduced commercial spat catches (Tom Hollings, Coromandel Marine Farmers’ Association, pers. comm.).

In addition to the difficulty of sourcing sufficient mussel spat to maintain Greenshell™ aquaculture production, high losses of spat typically occur shortly after they are placed out on nursery ropes on farms. These losses can be as high as 100 % but are usually between 50 and 70 % (NIWA 2006, Carton et al. 2007, Sim-Smith 2011, South et al. 2017). These losses of spat from nursery growing ropes are estimated to cost the industry between NZD\$6-10M annually (Alfaro et al. 2012). Low retention of mussel spat seeded out onto nursery ropes on mussel farms requires farmers to excessively overstock the nursery ropes with spat in order to ensure sufficient seed mussels are retained for subsequent on-growing. This solution is only feasible where an abundance of relatively low cost wild spat is readily available, which is not often the case, given the constraints to the harvest of wild spat. More costly spat can be

sourced from hatchery production, but it is not economically viable to greatly overstock nursery ropes with hatchery-reared spat in anticipation of spat losses. Improving the retention of mussel spat will ultimately reduce the harvesting pressure on wild spat by making more efficient use of the existing harvested resource, while also increasing economic returns from Greenshell™ aquaculture, and increase the economic feasibility for commercial application of hatchery-reared spat (Meder et al. 2004).

Little is known about the causes for poor spat retention in *P. canaliculus*, although some potential causes include; poor initial spat attachment during seeding of nursery ropes, predation by fish, high water currents, poor water quality, low nutritional condition of spat, limited food supply, inclement weather conditions, disease, poor handling and biofouling (NIWA 2006, Alfaro *et al.* 2012, Foote 2003, Carton 2007, Alfaro et al. 2010, South *et al.* 2017). However, the secondary settlement behaviour of spat is suspected to be responsible for the majority of the loss of spat from seeded mussel nursery lines (Foote 2003, South *et al.* 2017).

The process of secondary settlement is when juvenile mussels detach their byssal threads and either crawl or disperse via byssopelagic migration (mucous drifting) to secondary sites where they attach to the substrate with byssal threads (Martel 1991, Buchanan 1997, Alfaro 2005). Using their pedal organ, plantigrade mussels of up to 5 - 6 mm of shell length are able to secrete a buoyant mucous strand of up to 25 cm in length which can create sufficient buoyancy and drag in water currents to slow the descent of the spat in the water column and facilitate transport of the spat to another location (Buchanan 1994, Buchanan and Babcock 1997). When the drifting mucous thread comes into contact with a rough surface it easily becomes entangled, thereby anchoring the mussel, which is then able to crawl up the mucous thread and examine the suitability of the new site for more permanent attachment. The mussel will then attach itself to the substratum via byssal threads if the site is suitable, or if the site is unsuitable, the mussel will detach the old mucous thread and secrete another to facilitate drifting to another potential settlement site.

Secondary settlement behaviour of mussel spat was first observed in the blue mussel, *Mytilus edulis*, where larvae were observed to initially settle on filamentous substrata and then the subsequent plantiger would later move, or secondary settle, into adult beds once they had grown in size (Bayne 1964). This two-stage settlement process is thought to be a strategy to

avoid predation, intraspecific competition and/or ingestion by adult mussels (Bayne 1964). Secondary settlement is thought to allow the small juvenile mussels to increase in size before migrating to live permanently in the highly competitive adult mussel beds (Bayne 1964, Buchanan 1997), although the evidence in support of this proposition is equivocal given that primary settlers have also been found to occur in beds of adult mussels of several species, including *P. canaliculus* (Lasiak and Barnard 1995, McGrath et al 1988, Buchanan and Babcock 1997). However, in the case of *P. canaliculus*, the larvae are thought to mostly avoid settling into adult mussel beds because of the demonstrated risk of cannibalism and intraspecific competition for space (Zeldis et al. 2004, Alfaro 2006, NIWA 2006). Rather, juvenile mussels at one study site have been shown to move into mussel beds at a larger size (0.5 – 5.5 mm) through secondary settlement (Buchanan and Babcock 1997). The cues for initiating and suppressing secondary settlement behaviour are largely unknown, although secondary settlement has been shown to increase when spat of *P. canaliculus* encounter environmental stresses such as desiccation and starvation (Buchanan 1997, Foote 2003, Carton 2007). There is some evidence from laboratory experiments involving the release of juveniles of *P. canaliculus* of different sizes into a vertical column of still seawater which indicated that secondary settlement by mucous thread drifting ceases by the time the juveniles reach 6 mm in shell length (Buchanan and Babcock 1997). This may be due to changes in their pedal glands responsible for producing the mucous threads used for byssal-pelagic drifting (Lane et al. 1982), or an inability to detach their byssal threads (Board 1983). Unfortunately, the reporting on the initial experiments of Buchanan and Babcock (1997) on the mucous drifting behaviour of *P. canaliculus* spat was highly descriptive with no structured investigation of the effect of spat size on the expression of mucous drifting behaviour. Likewise, there has been no published research that experimentally examines the propensity of *P. canaliculus* spat of different sizes to undergo secondary settlement. This is despite the potential significance of understanding the phenomenon and finding a possible solution to the spat losses affecting commercial aquaculture production.

Anecdotal reports from Greenshell™ aquaculture operators suggest that wild *P. canaliculus* spat harvested from different locations show some consistency in the extent of their subsequent losses following seeding out on nursery lines. These observations indicate that the extent of secondary settlement behaviour may be influenced by the geographic origin of

spat, or driven by differences in environmental conditions between the wild spat harvesting locations and the mussel farm destination of the spat. Both juvenile and adult mussels are known to exhibit some differences in behaviour, such as strength of byssal attachment and valve gaping behaviour, that are characteristic of their natal geographic location, and which continue to manifest themselves when the mussels are translocated (Christensen et al. 2015, Zardi et al. 2015). Therefore, it is likely, but remains to be determined, that wild *P. canaliculus* spat harvested from different locations express consistent differences in their secondary settlement behaviour.

The aims of this study were to determine how the secondary settlement behaviour of wild *P. canaliculus* spat varies with size and natal origins. A greater understanding of the significance of specific drivers of the secondary settlement behaviour in the early juveniles of this mussel species will ultimately assist in tackling the widespread and costly losses of spat from seeded nursery ropes.

4.2 Methods

4.2.1 Spat Size versus Secondary Settlement Behaviour

Wild spat material harvested from Ninety Mile Beach on 3 November 2014 was transported immediately to the laboratory in an insulated container at ambient seawater temperature at the time of harvest. Upon arrival in the laboratory the spat were removed from the associated material they were attached to with fine tweezers. The spat were then sieved and only those mussels that were able to pass through a 0.5 mm sieve were retained by placing them in a 36 L tank containing aerated 18 °C seawater into which a 10 m length coir twine was suspended as a series of loops onto which the juvenile mussels could attach. The spat were fed a mixture of axenically cultured microalgae (*Isochrysis galbana* and *Chaetoceros muelleri* 1:1) at a concentration of 200,000 cells per spat per day (~1.3 million algal cells ml⁻¹). This feeding ration was increased by 30 % each week to account for the increase in spat size and to ensure feeding of the mussels was maintained at satiation. The holding tank was cleaned every three days by draining and rinsing down with fresh seawater before refilling. After three days a subsample of around 150 spat were taken from the holding tank by randomly selecting a

section of the coir twine and removing all the attached mussels with fine tweezers. The size of the mussels was measured by photographing the mussels under a microscope and image analyses was then used to measure the shell length of each mussel. The 150 mussels were randomly divided into three replicates of 50 mussels each and transferred into three 1 L upright conical tanks, each with a 10 cm length of coir twine, and subjected to vigorous aeration. After 12 hours any mussels remaining in the upright tanks that had not attached to the coir twine were removed and counted, and by deduction the number of spat remaining on the coir was calculated as a measure of the attachment behaviour of spat. The aeration in the three replicate upright tanks was then turned off and after 12 hours any mussels in the upright conical tanks that were no longer attached to the coir twine were removed and counted, and by deduction the number of spat remaining on the coir was calculated as a measure of the retention behaviour of spat. The spat remaining in the 36 L tank that were not used for experimentation were reared under the previously described laboratory conditions and at roughly two weekly intervals for six months 150 spat were randomly selected from the holding tank and the procedure for assessing mussel spat attachment and retention behaviour was repeated. In so doing the attachment and retention behaviour of the same cohort of spat was measured over a range of sizes ranging in mean shell length from 0.5 mm to 10 mm for a total of 15 times over the six month period. The 15 sizes classes were; 0.25 – 0.50 mm, 0.501 – 0.8 mm, 0.801 – 1.5 mm, 1.51 – 2.0 mm, 2.01 – 2.5 mm, 2.51 – 2.75 mm, 2.751 – 3.0 mm, 3.01 – 5 mm, 5.01 – 5.5 mm, 5.51 – 5.8 mm, 5.81 – 6.0 mm, 6.01 – 7.0 mm, 7.01 – 8.0 mm, 8.01 – 9.0 mm, 9.01 – 10.0 mm.

4.2.2 Spat Natal Origins

Wild and hatchery-raised *P. canaliculus* spat were sourced from five locations that are commonly used as sources of spat for seeding commercial mussel farms, i.e., Ninety Mile Beach, Hauraki Gulf, Muriwai Beach, Aotea Harbour and a commercial hatchery in Nelson. Spat were removed from drift macroalgae which was collected from Ninety Mile Beach on 11 November 2014 and again on the 19 April 2015. Hauraki Gulf spat were collected on spat catching ropes suspended in the water column in Coromandel Harbour and recovered on the 13 November 2014 and again on 8 December 2014. Mussel spat from Muriwai Beach were collected from macroalgae growing on intertidal rocks on 18 April 2015 and 30 June 2015. Mussel spat from Aotea Harbour were collected on spat catching ropes suspended in Aotea

Harbour and removed on 20 April 2015 and again on 30 June 2015. Hatchery spat were sourced from SpatNZ Ltd hatchery in Nelson on 18 March 2015. All sampled spat was placed in insulated polystyrene boxes at ambient temperature at collection and transported immediately to the laboratory.

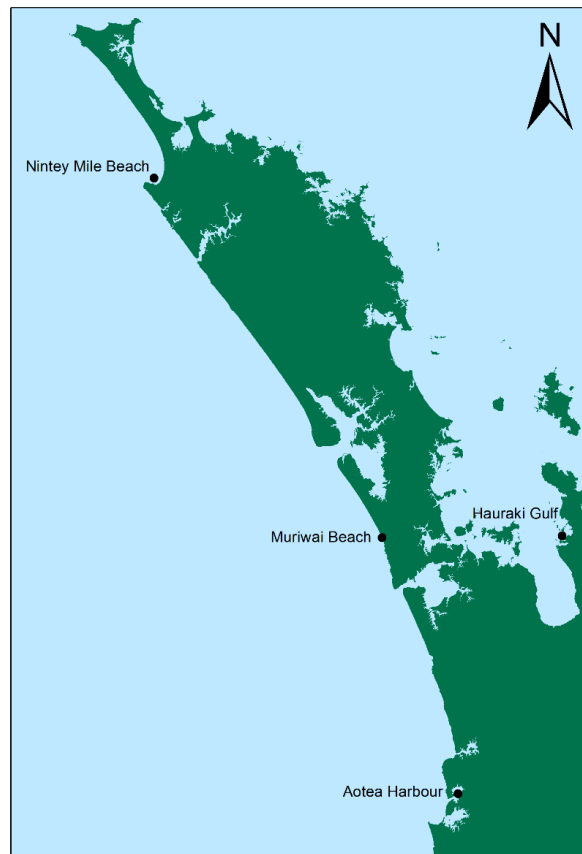


Figure 4.1. Locations for collecting samples of wild spat used for experimentally comparing attachment and retention of spat from different locations. Hatchery-reared spat were also included in the experiment which came from SpatNZ Ltd's hatchery in Nelson.

Once in the laboratory spat were removed from their attachment substrate with fine tweezers and transferred to a 36 L holding tank with 5 μm filtered seawater at 18 °C. To remove any possible differences among spat due to differences in nutritional condition the spat were fed a mixture of axenically cultured microalgae (*Isochrysis galbana* and *Chaetoceros muelleri* 1:1) at a concentration of 200,000 cells per spat per day (~1.3 million algal cells ml⁻¹).

After three days the spat were removed from the holding tank. To ensure that the wild spat from different sources that were used in the experiments were of a similar size for the experimental comparisons of attachment and retention, the spat were passed through a series of sieves to separate out spat of 1.2 - 2.5 mm in shell length because this size class consistently had the highest numbers of spat for all spat sources and spat of this size are known to undertake secondary settlement (Buchanan and Babcock 1997). For each sample of wild spat five replicate groups of 50 spat were randomly selected from the holding tank. The spat in each replicate were then measured by photographing them with a reference measure and then shell lengths for individual juvenile mussels were measured from the digital images using Image J software (Rasband 2014). The spat in each replicate were then seeded onto a 40 cm length of coir twine suspended in a 1 L conical tank with 18 °C seawater with an air stone at the bottom with an air flow rate 0.4 L min⁻¹ (Fig. 4.2). The spat were left in the tanks for two hours. The twine was then carefully removed and any unattached spat counted and removed from the tank. The twine was then put back in the tank and the air bubblers turned to low airflow of 0.1 L min⁻¹. Spat were fed every 24 hrs with the addition of cultured microalgae which provided 200,000 cells per spat per day. After 5 days the number of spat not attached to the twine were recovered and counted. Spat attachment for each replicate was calculated as the proportion of spat remaining attached to the coir twine after the initial 2 hrs, while spat retention was calculated as the proportion of spat that were initially seeded onto the coir twine that were remaining attached at the end of the experiment (i.e., after 5 days). The mortality for each replicate was calculated by identifying how many spat had died in each replicate. Mortality of individual mussel spat was determined by examining the mussels in a dish of seawater closely under a binocular microscope to determine permanent gaping of the valves and the lack of a response by the mussel with touching with fine tweezers.

4.2.3 Sinking Velocity of Spat

Sinking velocity of *P. canaliculus* spat of different shell lengths was determined by timing their descent in a column of seawater. On 15 November 2016 wild spat of a wide range of sizes attached to macroalgae were collected at Muriwai Beach and transported immediately to the laboratory. The spat were separated from the macroalgae and then ten mussels for each of the following size classes were randomly selected from the sample; 1.01 - 2.0 mm, 2.01 – 3.0 mm, 3.01 – 4.0 mm, 4.01 – 4.5 mm, 4.51 – 5.0 mm, 5.01 – 5.5 mm, 5.51 – 6.0 mm, 6.01 – 6.5

mm, 6.51 – 7.5 mm. Individual spat were cleaned with a paper towel to remove any mucous threads and other adherent material, weighed and then carefully placed with fine tweezers 10 cm below the surface of a 5 m vertical column of 5 µm filtered seawater at 16° C and a salinity of 33 pp held in a transparent acrylic pipe (500 mm Ø). Each mussel was released from the tweezers and the time of the descent of each mussel over 1.9 m down the tube was timed using a digital timer and the mussel then recovered from the base of the tube. The spat were then placed in a container with 10 % neutral buffered formalin (NBF) to kill them without causing the valves to open. The time of descent for each dead mussel was then measured again for comparison. For all experimental mussels, an average sinking velocity of the length of the water column was calculated as the distance travelled down the water column by the measured time of descent.

4.2.4 Data analyses

Binary logit general linear models were used to compare measures of attachment and retention among spat of different sizes and spat from different natal origins. Prior to analyses, the normality of the data was confirmed with a Shapiro–Wilk’s test and the equality of variances was tested using a Brown–Forsythe test. Where overall experimental factors in the analyses were significant, pairwise post-hoc comparisons using Tukey’s tests were conducted to identify contributing significant differences in the experimental components with Holm–Sidak adjustment made for inflated false discovery rate. Mean measured sinking velocities were compared among alive and dead spat of different sizes using a two-way ANOVA. For all statistical analyses $\alpha = 0.05$. All analyses were conducted in IBM SPSS Statistics (Version 21).

4.3 Results

4.3.1 Spat Size versus Secondary Settlement Behaviour

The mean proportion of initial attachment of spat to the coir twine was significantly different among the 15 size classes of spat measured over the three month period ($F_{(8, 44)} = 2.3$, $P = 0.04$). The seven size classes of spat of 4.51–5.0 mm and smaller all had a significantly higher proportion of initial attachment to the coir twine than spat in the seven size classes that were

larger (Tukey HSD, $P < 0.05$) (Fig. 4.2). The mean initial attachment of spat decreased from 90 % for spat of 3.01 – 5.0 mm in shell length to 25 % in spat at 9.01 – 10.0 mm of shell length (Fig. 5.2). The majority of the spat were observed to be attaching to the coir twine within the first five minutes.

Overall, the mean proportion of retention of spat on the coir twine was different among the 15 size classes of spat ranging from 0.5 – 10 mm in shell length ($F_{(8, 44)} = 9.52$, $P = 0.001$). Spat of 3.01 – 5.0 mm in shell length had significantly lower retention than all other spat size classes. Retention of spat at 0.51 - 0.8 mm in shell length were significantly higher than spat of 1.5 – 5 mm in shell length, but did not differ from spat of 5.51- 10 mm shell length (Tukey HSD, $P < 0.05$) (Fig. 4.2). The mean retention of spat tended to decrease from 0.5 to 5.0 mm and thereafter immediately increase, but stabilising at around 90 % from 5.01 – 10.0 mm. No detachment from the coir twine was observed to occur within the first hour after initiating the retention phase of the experiment, however, the majority of the detachment of mussels occurred over the first three days with mussels dropping off the coir twine and sinking to the base of the conical tank. Subsequently, pedal crawling behaviour was commonly observed in the detached spat, which either crawled to the water line at the top of the tank or clumped together by attaching to one another at the bottom of the conical tank.

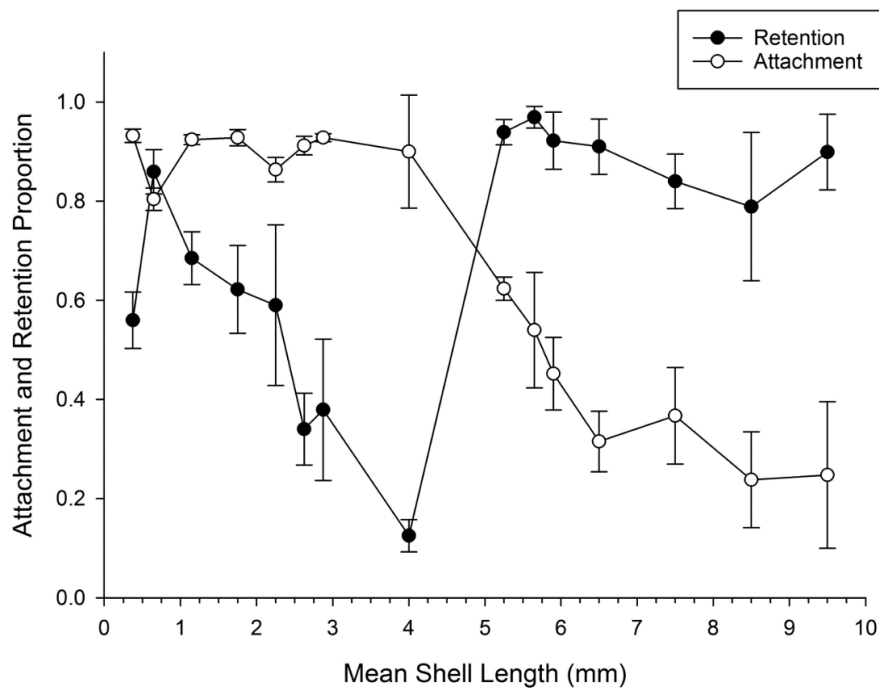


Figure 4.2. Mean proportion of initial attachment and subsequent retention (\pm S.E) of *Perna canaliculus* spat for 15 different size classes of shell length. Data are plotted on the x-axis at the mean shell length for each size class of spat.

A linear regression showed a significant relationship between initial attachment of spat and subsequent retention regardless of spat size class ($F_{(1, 66)} = 17.867$, $p < 0.001$). However, the regression explained less than half of the variability in the relationship ($R^2 = 0.462$).

4.3.2 Spat Natal Origins

Independent t-tests were run to determine if there were any differences between samples taken from the same location on different days. No significant differences were found for any location.. Overall, the mean initial proportion of attachment of spat to the coir twine was found to be significantly different among the nine samples of spat that came from the five geographically separated sources ($F_{(8, 36)} = 2.89$, $p = 0.014$) (Fig. 4.3). The initial attachment of both Muriwai Beach spat samples was significantly lower than for the first Ninety Mile Beach spat sample (NMB1 - 11 November 2014) but there were no other significant differences between pairs of sites (Tukey HSD, $P < 0.05$). The two samples of Ninety Mile Beach spat had the two highest proportions of initial attachment at 93 % and 87 %. The two Muriwai Beach samples had the lowest proportions of initial attachment at 64 % and 62 %. Samples of spat

from the Hauraki Gulf, Aotea Harbour and the hatchery had mean initial proportions of initial attachment between 67 % and 76 %.

Overall, the proportion of retention of spat to the coir twine was found to be significantly different among the nine samples of wild spat ($F_{(8, 36)} = 5.58$, $p = 0.001$)(Fig. 4.4). The retention of spat sourced from Muriwai Beach and Hauraki Gulf was lower than for spat sourced from Ninety Mile Beach, the hatchery and Aotea Harbour (Tukey HSD, $P < 0.05$). There was no significant difference between the mean retention of spat sampled from Muriwai Beach and the Hauraki Gulf, and likewise for the retention of spat sampled from Ninety Mile Beach, Aotea Harbour and the hatchery (Tukey HSD, $P < 0.05$) (Fig. 4.3).

There was no significant difference found in the proportion of mortality among the nine samples of spat coming from five different sources ($F_{(8, 36)} = 0.63$, $p = 0.751$). There were only four individual mussels that died during the entire experiment. One mussel each from the first and second Hauraki Gulf spat samples, one mussel from the first Aotea Harbour sample and the one was from the first Muriwai Beach sample.

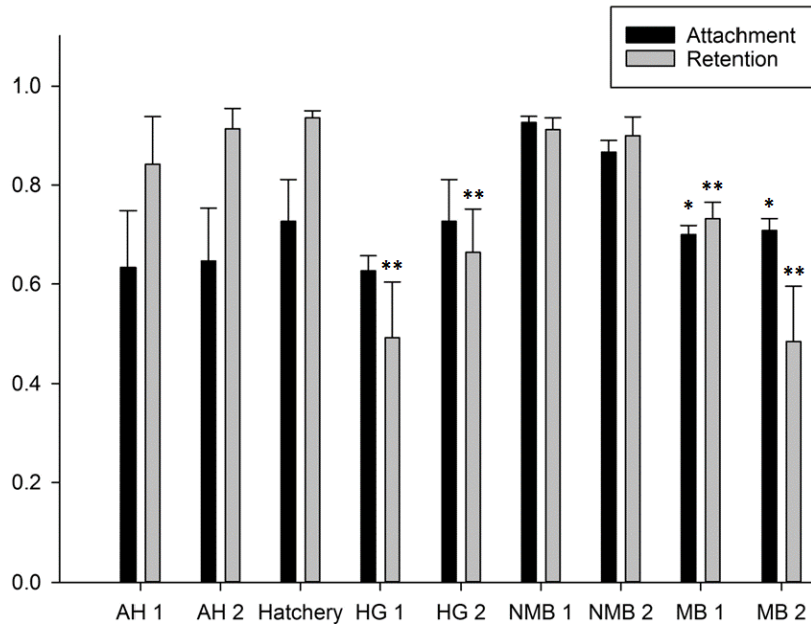


Figure 4.3. Mean proportions of initial attachment and subsequent retention (\pm S.E) of nine samples of *Perna canaliculus* spat sourced from five different locations; Aotea Harbour (AH), Hatchery, Hauraki Gulf (HG), Ninety Mile Beach (NMB) and Muriwai Beach (MB). Significantly different means among sets of means of attachment and mean proportions of retention denoted as follows; * = significant difference compared to NMB 1, ** = significant difference compared to NMB 1, NMB 2, AH 1, AH 2 and Hatchery.

4.3.3 Sinking Velocities of Spat

There was an overall significant difference in the mean wet weight of the spat from the nine different size classes ($F_{(8, 88)} = 42.49$, $p = 0.001$), with wet weight of mussels increasing in proportion to their increasing shell length (Fig. 4.5). There was no statistically significant difference in sinking velocity between the spat that were dead or alive for all size classes ($F_{(8, 162)} = 1.70$, $p = 0.102$). However, there was a statistically significant difference in the mean sinking velocity for spat of different sizes ($F_{(8, 88)} = 42.49$, $p = 0.001$). The sinking velocity of the smallest two size classes, 1.01-2 mm and 2.01-3 mm in shell length, were significantly lower compared to all other groups. Mean sinking velocity in mussel spat of 1.01-2 mm shell length ($9.6 \pm 0.2 \text{ cm s}^{-1}$) was 10 % lower than those of 2.01-3 mm, which in turn had a 16 % lower mean sinking velocity ($10.7 \pm 0.3 \text{ cm s}^{-1}$) than the next largest size class 3.01-4.0 mm ($12.8 \pm 0.3 \text{ cm s}^{-1}$). The mean sinking velocity in the next three larger size classes of spat (3.01-4.0 mm, 4.01-4.5 mm and 4.51-5 mm) were not significantly different from each other but did

differ significantly from the four larger size classes of spat (i.e., 5.01-5.5 mm, 5.51-6 mm, 6.01-6.5 mm and 6.51-7.5 mm) (Fig. 4.6).

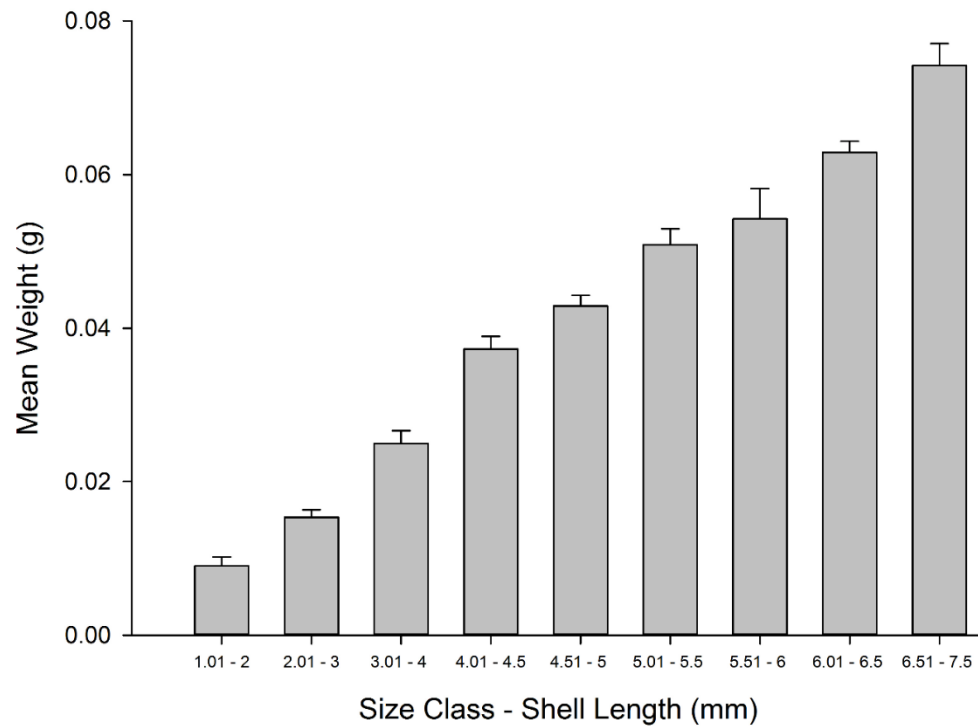


Figure 4.5 Mean wet weight (\pm S.E) of *Perna canaliculus* spat in nine different size classes.

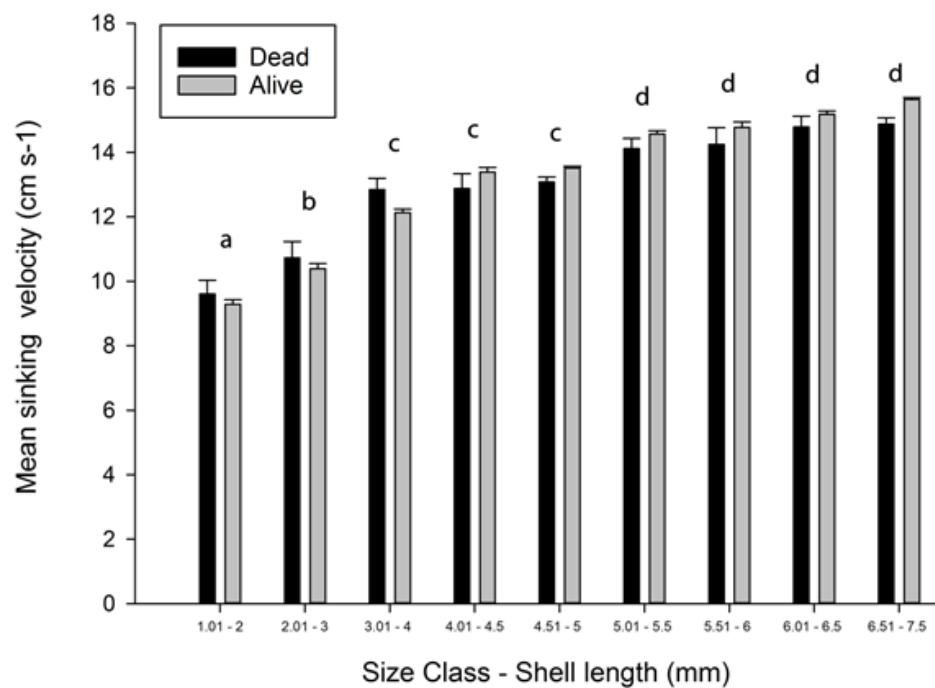


Figure 4.6. Mean sinking velocity (\pm S.E) of alive and dead *Perna canaliculus* spat in nine size classes. Significantly different mean mean sinking rates among spat of different size ranges regardless of whether alive or dead have different letters above the bars. There were no overall differences in mean sinking rates for spat for spat that were alive versus those that were dead.

4.4 Discussion

The poor retention of Greenshell™ spat deployed onto mussel farms is a major inefficiency and production vulnerability for this significant aquaculture industry. Furthermore, a lack of knowledge of the underlying causes of the losses of mussel spat from farms makes it difficult for the industry to take steps to begin to resolve this issue. The results of the current study indicate that wild spat of the same size sourced from different locations and at different times had different rates of initial attachment and retention when tested under standardised laboratory conditions. For example, wild mussel spat sourced from Ninety Mile Beach on two separate occasions had significantly higher initial attachment rates than those sourced from four other locations, including hatchery-raised spat. Likewise, the measured retention rates of wild spat sourced on two occasions from both Muriwai Beach and the Hauraki Gulf were found to be significantly lower than for spat sourced from Ninety Mile Beach, Aotea Harbour and for hatchery-raised spat. The attachment and retention of spat sampled from each of four sites at two different times was not different. If these results are indicative of a consistent pattern of behaviour of spat it would suggest that some sources of mussel spat may provide benefits to aquaculture production by way of higher retention following seeding onto mussel farms. However, the laboratory standardisation process used for this study may mask other factors which may contribute to attachment and retention behaviour of spat. For example, all spat were fed to satiation prior to being used in the experiments, and it has previously been shown that compromised nutritional condition greatly reduces the retention behaviour of spat (Foote 2003, Meder *et al.* 2005, Carton *et al.* 2007). Likewise, harvested wild spat are highly variable in the sizes of spat they contain (Jeffs *et al.* 2018) which can be expected to also greatly influence their subsequent attachment and retention onto nursery growing ropes according to the further results of this current study.

Mussel spat are known to be highly selective when choosing where to settle and deciding whether to stay (Caceres-Martinez *et al.* 1994, Carl *et al.* 2012, von der Meden *et al.* 2015). Water temperature, food availability, predators, conspecifics and substrate type have all been

identified as factors influencing the initial larval settlement and subsequent attachment of mussel spat (Alfaro and Jeffs 2002, Gribben et al. 2011). Mussel larvae have been shown to express different settlement preferences within varying areas of the same embayment (von der Meden et al. 2015). Chemical cues from conspecifics have both been found to deter and attract the settlement of larvae of *Perna perna* and *Mytilus galloprovincialis* at different sites (von der Meden et al. 2015). Larval settlers of both species actively avoided conspecifics whereas secondary settlers were found to favour plates with another settler already present (von der Meden et al. 2010). This site-dependent variation in behaviour could be due to unmeasured site differences such as background signals from the surrounding biota or the presence of predators not included in the study (von der Meden et al. 2015). Gaping behaviour and attachment strength in adult mussels have both been shown to be genetically mediated adaptive responses in two populations of *Perna perna*, suggesting that variation in behaviour such as secondary settlement, may also have some genetic basis (Zardi et al. 2015).

In the current study, mussel spat for experimentation were collected from Muriwai Beach from macroalgae attached to rocks within the surf zone which were uncovered at low tide. This habitat has extremely high wave action and aeration, unlike the experimental conditions. On collection, spat from Ninety Mile Beach were attached to drift macroalgae and other debris that was washed along the seafloor until they were beachcast (Alfaro and Jeffs 2002). Therefore, it is likely that the Ninety Mile Beach spat was fully submerged and drifting just above the seafloor until it was beached upon the shore and is used to constant submersion. Spat collected from Aotea and Hauraki Harbours were collected on suspended subtidal spat collecting rope positioned within relatively sheltered harbours with little to no wave action, but considerable tidal movement of waters. Hatchery spat were reared in tanks with vigorous aeration. Therefore, we would have expected the hatchery spat to have relatively high attachment and retention rates given its prior holding conditions. Hatchery spat were found to have a higher retention rate although this was not significantly higher than the retention rates for wild spat from Ninety Mile Beach or Aotea Harbour. Hatchery spat was found to have a retention rate of 94 ± 0.1 % after 3 days in experimental conditions in this current study. In contrast, spat from the same hatchery, but of a smaller size range (0.29 to 1.76 mm with a mean length of 1.01 ± 0.01 mm) deployed into a mussel farm had retention of 53.6 % after 1 month, and only 18.5 % after 5 months (South et al. 2017). This finding suggests that

the secondary settlement behaviour is likely to continue to express itself beyond the 5 day experimental period used in this current study. Furthermore, once seeded onto nursery lines on a mussel farm the spat are exposed to many environmental factors that are known to decrease their retention including; food availability (Foote 2003, Meder et al. 2005, Carton et al. 2007), predators (Hayden 1995, Peteiro et al. 2010), biofouling (Fitridge et al. 2014), water conditions (Hayden and Woods 2011), and handling stress during transport from spat source to nursery farm site (Webb and Heasman 2006, Carton et al. 2007). Juvenile mussel size had a major influence on the experimentally measured initial attachment and subsequent retention rates. The initial attachment rates of spat significantly decreased with increasing shell length of spat, dropping from 93 % attachment at a spat shell length of 0.5 mm down to 25 % for mussels of 10 mm. There was no significant difference in the initial attachment rates for spat within the seven size classes for mussels tested between 5.5 to 10.0 mm in size. This lower overall initial attachment rate in mussels greater than 5.5 mm in shell length may be due to the loss of the ability to migrate via byssal thread drifting. Spat are thought to lose the ability to secrete the mucous threads, which enables mucous thread drifting behaviour at around 5 mm in shell length (Buchanan and Babcock 1997). This could mean that spat become more selective in their attachment as they increase in size as a result of mucous thread drifting migration becoming more difficult as they approach 5 mm in shell length. Also, as determined in this study, the sinking rates of juvenile mussels above 5 mm in shell length becomes significantly higher which could be expected to further limit any remaining ability they may have for pelagic mucous thread drifting.

The retention of mussel spat decreased with increasing shell length from 0.5 mm (56 %) up to 5.0 mm (92 %) before increasing to around (94 %) in spat up to 10.0 mm. As shell length increases in juvenile mussels their sinking rates were found to increase concomitantly, which is probably due to their increasing specific gravity (Sigurdsson 1976, Buchanan and Babcock 1997). It is likely that the reduced effectiveness of dispersal by mucous rafting in juvenile mussels of increasing size results in the behaviour being curtailed once they reach a size where it is no longer effective due to increased sinking, or the result of an inability to produce the prerequisite mucous threads (Buchanan and Babcock 1997). Therefore, as spat grow it could be expected to see an increase in retention as spat have a decreasing dispersal potential making it more risky for them to detach from their existing site. A relatively high rate of

retention was observed for small juvenile mussels of 0.51 - 0.80 mm in shell length (85.9 %), a level of retention which was only matched by mussels greater than 5.0 mm in shell length (5.0 - 5.5 mm, 94 %, 5.51 - 5.8 mm, 96.9 %, 5.81 – 6.0 mm 92.2 %, 6.01 – 7.0 mm 91 %, 7.01 – 8.0 mm 84 %, 9.01 – 10.0 mm 90 %). Plantigers of 0.51 - 0.8 mm in shell length are recent primary settlers and may go through a period of greater retention behaviour to help ensure their initial establishment, prior to commencing secondary settlement behaviour in order to seek superior settlement locations. *Perna canaliculus* spat prefer fine branching substrata when they are <0.5 mm in size and coarse branching substrata when 1.5-2.0 mm in size indicating changing preferences in the attachment substrate and the active movement for spat of increasing size (Alfaro and Jeffs 2002). This active secondary settlement behaviour appears to become increasingly pronounced for juvenile mussels of increasing size, reaching its highest level in juveniles of 5.01 – 5.5 mm in shell length (12.5 % retention). Beyond this size (i.e., 5.0 - 5.5 mm in shell length, 94 % retention) the retention rates increased dramatically.

This is consistent with previous observations that mucous thread drifting behaviour in *P. canaliculus* is thought to stop at 5 - 6 mm in shell length due to an anatomical change of the pedal glands which prevents the secretion of the mucous threads (Buchanan and Babcock, 1997). However, retention rates for spat > 5.5 mm in shell length averaged 88 % suggesting that byssal thread drifting may continue beyond this size, or that juvenile mussels of this size may be prepared to leave their settled location without mucous drifting. This later behaviour was observed in a small proportion of spat of 5.5 – 10 mm in shell length which were releasing byssal threads and dropping to the bottom of the tank without producing mucous threads. Adult *Perna canaliculus* mussels have been shown to have a high rate of cannibalism through consuming juveniles of up to 2.4 mm in length (Alfaro 2005). Conspecific larvae and juveniles are thought to be a significant part of an adult mussel's diet with the ability to filter out spat up to 2.4 mm in shell length which have been found within the stomach contents of adult mussels (Alfaro, 2005). This is commonly used as an explanation for the existence of the pronounced secondary settlement behaviour in *P. canaliculus* spat compared to other mussel species. For example, in *Mytilus edulis*, secondary settlement behaviour is largely confined to spat of a much smaller size as the production of mucus threads ends at around 1 – 2.5 mm in shell length associated with the reduction of the pedal glands involved in the secretion of

mucous used in this behaviour (Lane *et al.* 1982). In *P. canaliculus* the settlement on larvae away from the adult beds, on to filamentous algae gives the spat time to grow to a sufficient size whereby the risk of cannibalism is reduced. It should therefore be safe for spat that have reached 5 mm in shell length to settle in to adult mussel beds (Bayne 1964). This could explain why some secondary settlement behaviour continues in larger spat ranging from 5.5 – 10.0 mm. By dropping from the line the spat are able to sink to the sea floor and crawl via pedal walking. If they encounter a mussel bed or hard substrata they are of a sufficient size to attach with a lower risk of predation.

Poor nutritional condition of spat has been identified as one of the major causes of secondary settlement behaviour and therefore the consequent poor retention in *P. canaliculus* spat (Foote 2003, Carton *et al.* 2007, Sim-Smith and Jeffs 2011). Spat in poor nutritional condition have been found to have significantly lower retention after seeding out (Carton *et al.* 2007). The retention of *P. canaliculus* spat was 42 % lower for spat that were unfed for 6 days before seeding out compared with those fed continuously (Carton *et al.* 2007). Throughout the experiment in this current study, the spat were fed continuously at a rate of satiation to eliminate nutrition as a contributing factor. As high levels of secondary settlement behaviour was still present we can infer that there are other factors involved triggering secondary settlement behaviour in this species.

4.5 Conclusions

Overall, there was moderate and significant relationship between the initial attachment and subsequent retention rates for spat of sizes from 0.5 mm – 10.0 mm ($R^2 = 0.462$, $p < 0.001$). As initial attachment rate decreased the subsequent retention rate tended to increase regardless of spat size class. Smaller spat may initially be less selective when deciding to settle as it is relatively easy for them to release their byssal thread and secrete a new one to aid their migration. Spat of 0.5 – 4.99 mm have been observed to secondary settle multiple times before permanently settling (Buchanan and Babcock, 1997). This would account for the relatively high attachment rates and lower retention rates observed in the spat in this size range (Fig. 5.3). Conversely, juvenile mussels of > 5 mm in shell length had much lower initial attachment rates and higher retention rates implying more sedentary behaviour.

The results of this current study show that the size and/or the age of spat of *P. canaliculus* at the time of seeding out is an important factor for ensuring their initial attachment and subsequent retention. As the use of hatchery spat increases within the Greenshell™ industry, the retention rates will become an important factor for maintaining the efficiency of seeding hatchery spat on to farm lines. The results from this laboratory study indicate that the best size to seed spat on to lines is at 0.8 mm in shell length as they have both a high initial attachment rate (80 %) and subsequent retention rate (86 %). However, these results need to be confirmed for the field situation. Alternatively, raising spat to beyond 5 mm in shell length in a land-based nursery situation prior to seeding out, could be used to greatly increase retention and the efficient use of spat in this mussel aquaculture industry, provided the initial attachment of these larger spat could be facilitated prior to transfer to farms.

Chapter Five: Using Sound to Increase *Perna canaliculus* Spat Retention and Growth

5.1 Introduction

The Greenshell™ mussel aquaculture industry in New Zealand is extremely vulnerable in terms of supply and retention of the early juvenile mussels, known as spat, which are used to initiate the aquaculture production cycle of mussels. The loss of mussel spat from aquaculture nursery ropes can be as high as 100%, but is typically between 50 and 70% (NIWA 2006, Carton et al. 2007, Sim-Smith 2011). This inefficient use of mussel spat is estimated to cost the industry between NZD\$6-10 million annually (Alfaro et al. 2012) and recently led to the loss of 232 jobs from New Zealand's largest Greenshell™ producer, Sanford Ltd (NZ Herald, 9 April 2015). Greenshell™ aquaculture operations typically grossly overstock nursery ropes with spat in order to overcome their subsequent low retention on the ropes, thereby ensuring sufficient seed mussels are retained for subsequent production. This is viable where an abundance of relatively low cost wild spat is readily available. However, for more expensive hatchery spat, the losses due to poor retention of spat becomes an expensive impost on commercial production. Improvement in the retention rates of mussel spat will reduce the harvesting pressure on wild spat, increase production efficiency and economic returns, and increase the economic feasibility for the commercial application of hatchery-reared spat (Meder et al. 2004).

Very little is known about the actual causes of poor spat retention in *P. canaliculus*, although some of this loss is suspected to occur due to poor initial spat attachment during seeding, predation by fish, high water currents, poor water quality, limited food supply, inclement weather conditions, disease and biofouling (Hayden 1995, NIWA 2006, Carton et al., 2007, Alfaro et al. 2012, South et al. 2017). However, the majority of the loss of spat from seeded mussel farm lines is thought to be the result of the secondary settlement behaviour of *P. canaliculus*, whereby the spat release their attachment to the substrate and secrete mucous strands that enable them to drift to a new location in the water column (Buchanan and Babcock 1997, Foote 2003, Martel 1991, Buchanan 1997, Alfaro 2005). The initiation of secondary settlement behaviour and therefore their loss from mussel farms appears to be strongly associated with the prior exposure of the spat to environmental, collection and transport stresses (Foote 2003, Carton et al., 2007, Alfaro et al. 2010). For example, rates of

secondary settlement have been shown to increase when wild spat encounter environmental stresses, such as desiccation and starvation, in the time between harvest and eventual seeding onto farms (Buchanan 1997, Foote 2003, Carton et al. 2007), with retention of spat decreasing by nearly a third when experimentally exposed to desiccation (Carton et al. 2007). Likewise, the retention of Greenshell™ spat could be increased by almost a third if they were in good nutritional condition or had been provided with a good recent feeding history (Carton et al. 2007). Wild mussel spat frequently experience both starvation, desiccation, and large temperature fluctuations due to the commercial harvesting methods, followed by packing, transportation and seeding processes that are involved in the utilisation of spat for seeding mussel farms (Webb & Heasman 2006, Sim-Smith and Jeffs 2011, Heasman 2013).

More recently, underwater sound has been found to promote the larval settlement and/or post-settlement growth of a wide range of benthic sedentary marine invertebrate taxa, including hard coral, bryozoans, calcareous tubeworms, barnacles and bivalves (Vermeij et al. 2010, Wilkens et al. 2012, McDonald et al. 2014, Stanley et al. 2014, Lillis et al. 2013, Lillis et al. 2015). For example, *P. canaliculus* larvae settled much more quickly when exposed to underwater sound from a motorised vessel (Wilkens et al. 2012), and recently-settled Pacific oysters, *Crassostrea gigas*, grew faster in the presence of vessel sound (Stanley et al. 2014). Oyster larval recruitment has also shown to be significantly higher on larval collectors exposed to the sounds of oyster reefs compared to control treatments without the addition of sound (Lillis et al. 2015). Given that these previous results show that an early stage in the lifecycle of *P. canaliculus* is responsive to underwater sound cues it is possible that Greenshell™ spat may also show some useful responses to exposure to underwater sound. Therefore, the aims of this study was determine whether underwater sound may stimulate the growth and retention of Greenshell™ mussel spat in laboratory experiments.

5.2 Methods

5.2.1 Spat preparation

Greenshell™ spat material harvested from Ninety Mile Beach on 3 November 2014 and 10 February 2015 was transported immediately to the laboratory in an insulated container at ambient seawater temperature at the time of harvest. Upon arrival in the laboratory, spat within the range of 1 – 5 mm were removed from the associated material they were attached to with fine tweezers. The manually removed spat were then placed in a 36 L tank containing aerated 18°C seawater and 10 m of suspended loops of coir twine on which the juvenile mussels could attach. The spat were fed a mixture of axenically cultured microalgae (*Isochrysis galbana* and *Chaetoceros muelleri* at a ratio of 1:1) at a concentration of 200,000 cells per spat per day (~ 1.3 million algal cells ml^{-1}). This feeding ration was increased by 30% each week to account for the increase in spat size and to ensure feeding of the mussels was maintained at feeding saturation levels. The holding tank was cleaned every three days by draining and rinsing down with fresh seawater before refilling. Growth of spat was measured by taking digital images of the spat immediately prior to them being seeded on the coir twine and then measuring individual shell length at the conclusion of the experiment using Image J software (Rasband 2014). Any spat that did not attach were then photographed and measured again using Image J and their measurement was then removed from the starting measurements.

5.2.2 Sound treatments

Six treatments were used in two experiments: Treatment 1. White Noise 1 (50 – 500 Hz); Treatment 2. White Noise 2 (501 – 1500 Hz); Treatment 3. White Noise 3 (1501 – 10,000 Hz); Treatment 4. White Noise 4 (10,001 – 20,000 Hz); Treatment 5. Ship Hull Noise (see Wilkens et al. 2012); Treatment 6. No Sound. The silent treatment was used as a control for the effects of the five other sound treatments. The ship hull noise was provided as a potential positive control, given that *P. canaliculus* larvae have previously been shown to respond to this sound (Wilkens et al. 2012). The four white noise treatments were used to broadly divide up the audible frequency spectrum into ranges within which mussel spat may respond differently. The white noise within four specific frequency bands was generated using sound software (MatLab R2014a, MathWorks) and recorded as digital sound files which were later replayed in the experimental treatments using amplifiers connected to underwater sound projectors which best matched their frequency output ranges (Lubell Labs Inc., Columbus, OH; LL964,

frequency response 0.2–20 kHz or J9 Transducer, Underwater Sound Reference Division, frequency response 0.04–20 kHz).

2.3 Static Experiment

In the first experiment mussel spat, collected on 3 November 2014 were held in static seawater whilst they were exposed to the sound treatment because static conditions are known to promote secondary settlement behaviour in *P. canaliculus* spat (Alfaro 2005, 2006). For this experiment mussel spat were randomly selected from the holding tank and assigned into 30 groups, each group containing 70 individual mussel spat. The size of the individual mussels in each group was measured by photographing the mussels under a microscope and image analyses was then used to measure the shell length of each mussel. The same method was used to measure the size of the mussels at the conclusion of the experiment.

The 30 groups, each of 70 spat, were transferred into 1 L upright conical tanks filled with filtered seawater (UV, 5 μ m), each with a 20 cm length of coir twine suspended in the centre of the tank. After placement in the conical tank the spat were subjected to vigorous aeration from the base of the tank for 12 h which promoted the attachment of most spat to the coir twine. Any spat not attached after this time were removed, measured, and subtracted from the group total. The coir twine with attached spat were then carefully transferred into 1 L acoustically transparent containers (194 mm H x 108 mm W x 108 mm D) with screw top lids, with the coir twine attached to the lid so it hung vertically in the container, not touching the sides. Five containers were then randomly allocated as replicates for each of the six different experimental sound treatments. Each treatment had temperature controlled (18°C) water baths with a sound projector (Lubell Labs Inc., Columbus, OH; LL964, frequency response 0.2–20 kHz and J9 Transducer, Underwater Sound Reference Division, frequency response 0.04–20 kHz) playing one of the six sound treatments continuously at a sound level of 126 dB re 1 μ Pa RMS, except for the silent treatment for which the sound projectors were not activated. The 1 L acoustically transparent containers were placed into individual acoustic water baths so that they continued to retain the spat within the tank whilst exposing the mussel spat to the sound treatment through the walls of the container while also maintaining constant temperature. The spat were fed a mixture of axenically cultured microalgae (*Isochrysis galbana* and *Chaetoceros muelleri* 1:1) at a concentration of 200,000 cells per spat per day

(~1.3 million algal cells ml⁻¹). The seawater in the containers was changed every two days and the inside walls of the containers scrubbed clean to avoid biofilm build up. The experiment ran for 25 days. Mussel spat retention and spat mortality was measured on four separate occasions during the course of the experiment (days 1, 6, 9 & 25) by counting the number of spat remaining on each coir strand by observing how many spat were on the bottom and sides of each container. Spat retention was then calculated as the proportion of spat that were initially seeded onto the coir twine that remained attached at the time of observation. The mortality of spat was determined by the presence of permanent gaping of the valves of spat and the lack of a response by the mussel when the mantle was touched with fine tweezers.

5.2.4 Flow-Through Experiment

In the second experiment mussel spat, collected on 10 February 2015 were held in flow through seawater whilst they were exposed to the sound treatment as water flow is known to influence the secondary settlement behaviour of spat (Alfaro 2005, 2006). A total of 540 mussel spat were selected from the 36 L conical holding tank and randomly allocated among 36 upright conical 1 L tanks filled with filtered seawater (UV, 5 µm) each with a 30 cm length of coir twine suspended in the centre of the tank (i.e., 15 spat per tank). Each group of 15 spat were photographed to determine shell length and then seeded onto the coir twine using the methods previously described for the static experiment.

For each of the six sound treatments (see Section 5.2.2) there were two 30 l experimental tanks (480 L x 340 W x 280 mm H) which had free-flowing seawater, at 30 l h⁻¹, filtered to 200 µm. This flow rate was slow enough to prevent spat from being able to be carried from one coir twine to a neighbouring coir twine. Each tank contained a sound projector (Lubell Labs Inc., Columbus, OH; LL964, frequency response 0.2–20 kHz or J9 Transducer, Underwater Sound Reference Division, frequency response 0.04–20 kHz) playing one of the six sound treatments continuously at a sound level of 126 dB re 1 µPa RMS, except for the silent treatment for which the sound projectors were not activated. Three replicate lengths of coir with attached mussel spat were suspended in each of the experimental tanks, equally spaced 150 mm apart and 80 mm off the bottom of the tank to prevent reattachment of spat after they released from the coir twine. Plastic mesh (80 µm) was placed over the outflow pipe to prevent any spat from leaving the tank. The tanks were scrubbed clean every three days. After

cleaning the tanks were refilled with seawater and an equal quantity (~ 1.3 million algal cells ml^{-1}) of cultured microalgae (*I. galbana* and *C. muelleri*) was added into each tank and left for 30 min before the seawater flow-through was resumed. The experiment was run for 14 days with retention, growth and mortality of the spat only measured at the conclusion of the experiment. All spat attached to the coir twine at the end of the experiment were measured for shell length as per the methods described previously for the static experiment.

5.2.7 Data analyses

Static and Flow-Through Experiments

The mean shell length of mussels at days 1 and 25 of the experiment for each replicate length of coir twine were used to calculate a mean growth rate for each replicate. This estimate of growth assumed that individual spat that departed the coir twine during the experiment did so at random. The mean growth of the mussels was compared among treatments using a one-way ANOVA after firstly confirming data normality and equal variances by performing a Shapiro–Wilk’s test and a Brown–Forsythe test respectively. Where the results of the ANOVA identified an overall difference among the treatment means, pairwise Dunn’s tests were conducted to isolate the differences among individual treatment means.

The retention of the mussel spat for each experiment was calculated as the proportion of mussels that were remaining attached to the coir twine in each replicate at day 25 compared to the number on the twine at day 1. Proportional mussel retention data were arc-sine transformed, checked for normality and equality of variances, and then mean retention of mussel spat was compared among treatments using a one-way ANOVA. Proportional mussel retention did not conform to the assumption of equality of variances for the flow through experiment so a comparison was made using a non-parametric Kruskal-Wallis test. If an overall significant result was identified then paired comparisons were made using a Welch’s test.

The mortality of mussels over the course of the experiment was calculated as proportion of the mussel spat that were dead in each replicate at day 25 compared to the total number of live spat on the twine at day 1. Proportional mortality of mussels did not conform to the assumption of equality of variances for either of the two experiments, so comparisons among treatments for each experiment was made using a non-parametric Kruskal-Wallis test. If an

overall significant result was identified then paired comparisons were made using a Welch's test.

5.3 Results

5.3.1 Static Experiment

The average shell length of the spat at the start of the experiment for each treatment were: treatment 1- White Noise (50 – 500 Hz) 2.6 ± 0.1 mm; treatment 2 - White Noise (501 – 1500 Hz) 2.4 ± 0.1 mm; treatment 3 - White Noise (1501 – 10,000 Hz) 2.4 ± 0.1 mm; treatment 4 - White Noise (10,001 – 20,000 Hz) 2.4 ± 0.1 mm; treatment 5 - Ship Hull Noise 2.4 ± 0.1 mm; and treatment 6 - No Sound, 2.5 ± 0.1 mm. There was no significant difference in size among groups at the start of the experiment ($F_{29,2100} = 1.1$, $P < 0.328$).

5.3.1.2 Growth

There was a significant difference in mean growth of the mussels as measured by change in shell length among the six sound treatments ($F_{5,29} = 6.7$, $P < 0.001$) (Fig. 5.1). Treatment 1 (white noise 50 – 500 Hz) had the highest growth (1.1 ± 0.1 mm, 44.2 % increase in shell length), followed by Treatment 2 (white noise 501 – 1500 Hz; 0.9 ± 0.1 mm, 38.8 %), Treatment 5 (ship hull; 0.8 ± 0.1 mm, 34.4 %), Treatment 4 (white noise 10,001 – 20,000 Hz; 0.8 ± 0.1 mm, 32.1 %), Treatment 3 (white noise 1501 – 10,000 Hz; 0.7 ± 0.1 mm, 26.6 %), and Treatment 6 (no sound) having the smallest mean growth (0.5 ± 0.1 mm, 20.8 %). Mean growth of mussels was significantly higher in Treatment 1 than Treatments 3, 4, 5 and 6 (Tukey Test; $P < 0.05$).

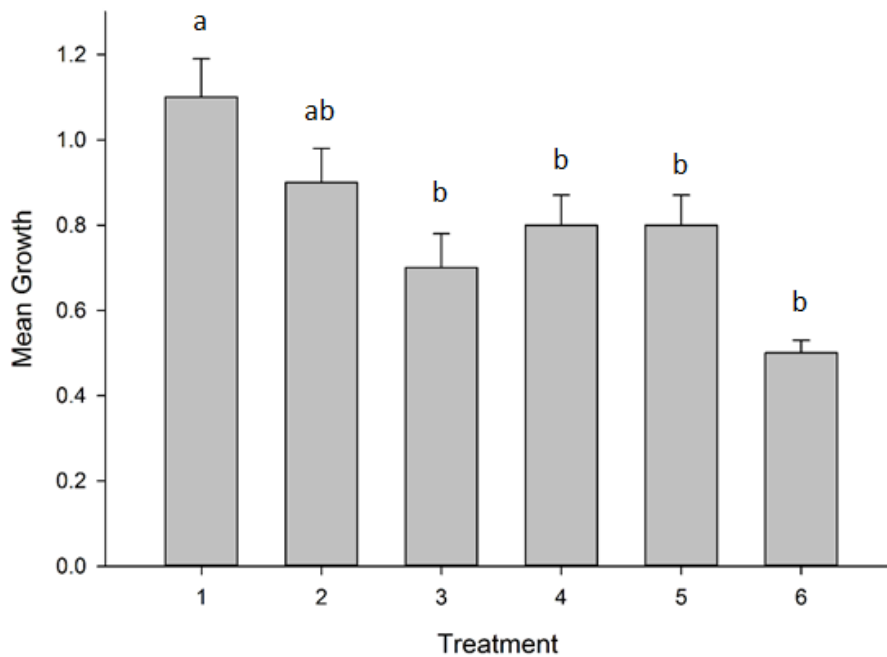


Figure 5.1 Mean growth (\pm S.E.) as measured as a change in shell length of mussel spat over 25 days for six treatments: 1 - White Noise (50 – 500 Hz), 2 - White Noise (501 – 1500 Hz), 3 - White Noise (1501 – 10,000 Hz), 4 - White Noise (10,001 – 20,000 Hz), 5 - Ship Hull Noise and 6 - No Sound. Letters indicate statistically significant groupings.

5.3.1.1 Retention

Treatment 6 (No Sound) consistently tended to have lower numbers of mussel spat retained on the coir twine at all four sampling events over the course of the experiment, i.e., days 1, 6, 9 and 25. At the conclusion of the experiment Treatment 1 (white noise 50 – 500 Hz) had the highest mean number of spat retained on the twine ($80.4 \pm 2.12\%$), followed by Treatment 2 (white noise 501 – 1500 Hz; $77.7 \pm 1.99\%$), Treatment 4 (white noise 10,001 – 20,000 Hz; $76.5 \pm 1.85\%$), Treatment 5 (ship hull; $73.7 \pm 2.01\%$), Treatment 3 (white noise 1501 – 10,000 Hz; $70.8 \pm 2.33\%$), and Treatment 6 (no sound) had the lowest average number of spat retained on the twine (68.1 ± 2.11). However, despite the possible trends in the data, there was no significant difference in mean mussel spat retention among the treatments ($F_{5,29} = 6.4$, $P = 0.27$) (Fig. 5.2).

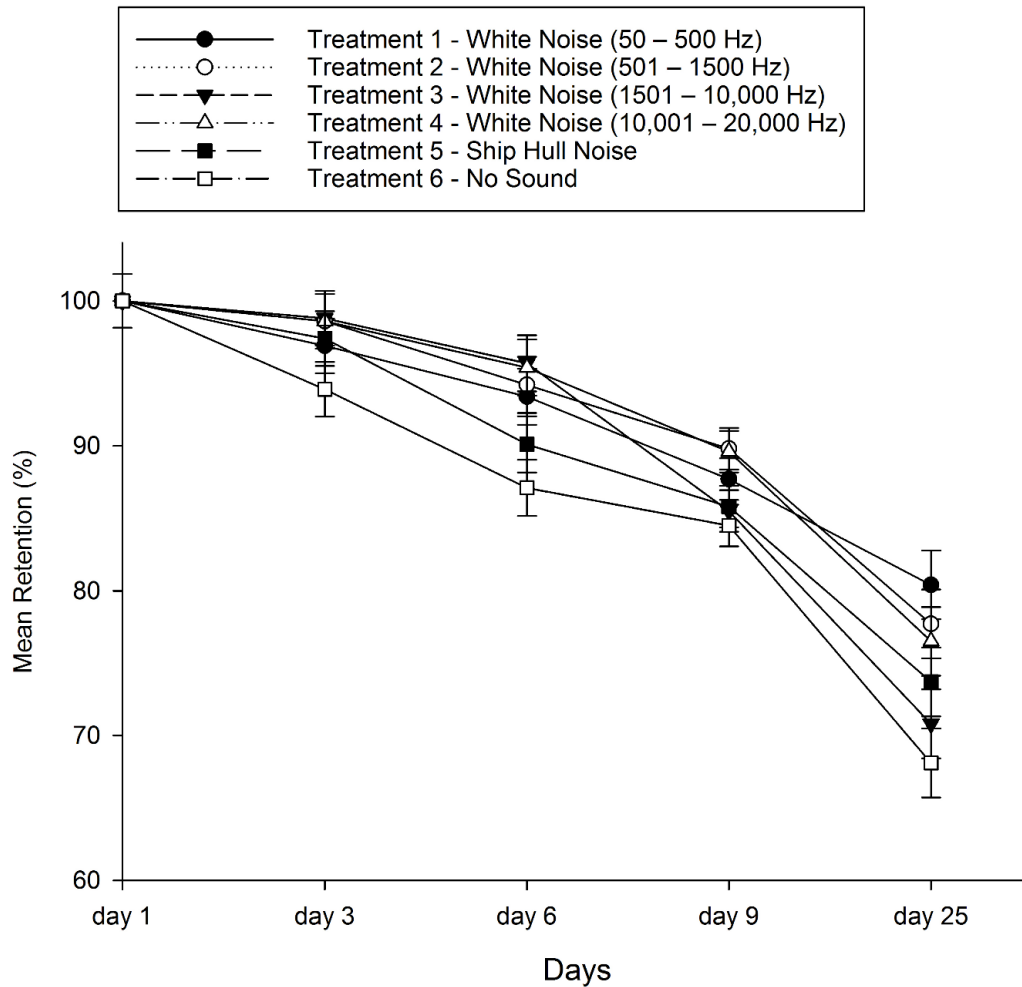


Figure 5.2 Mean spat retention (\pm S.E.) as observed after 3, 6, 9 and 25 days for six treatments; 1 - White Noise (50 – 500 Hz), 2 - White Noise (501 – 1500 Hz), 3 - White Noise (1501 – 10,000 Hz), 4 - White Noise (10,001 – 20,000 Hz), 5 - Ship Hull Noise, and 6 - No Sound.

5.3.1.3 Mortality

The mortality of mussel spat was significantly different among the six sound treatments ($H = 17.8$, $P = 0.003$), with Treatment 6 (no sound) having higher mortality compared to both Treatments 1 and 2 (white noise 50 – 500 Hz and white noise 501 – 1500 Hz) (Welch's Test; $P < 0.05$; Fig. 5.3). Treatments 1 and 2 had the lowest mortality during the experiment (12.1 and 15.1%, respectively), while Treatment 6 had the highest mortality (28.6%). Treatments 3, 4 and 5 were intermediate (25.8, 18.6 and 22.2%, respectively).

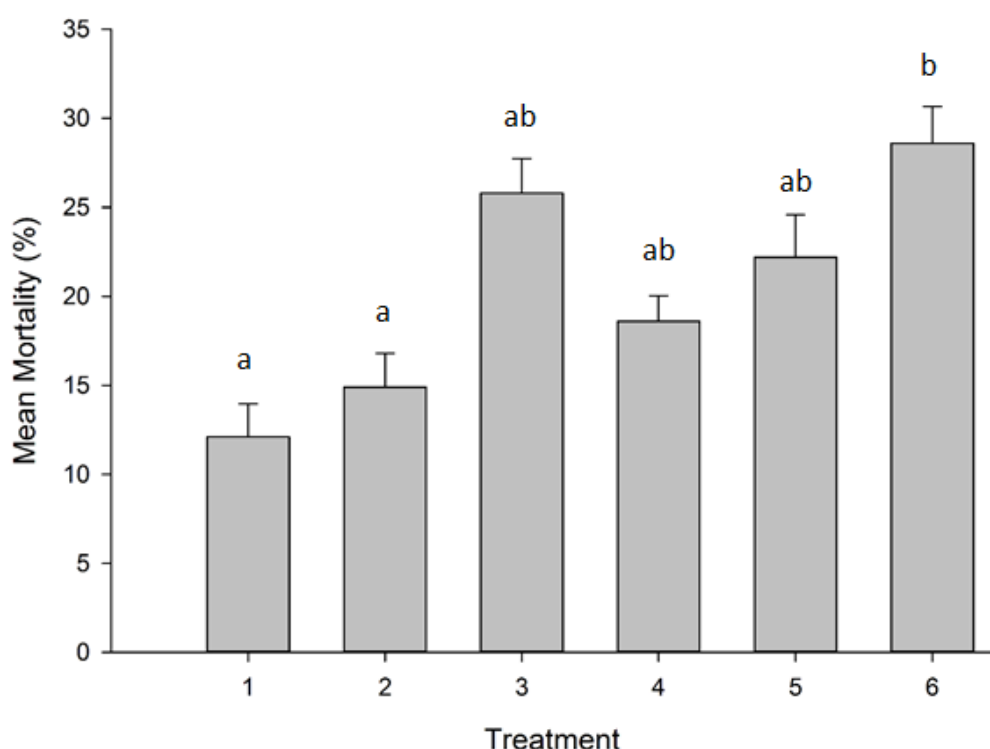


Figure 5.3 Mean percent mortality (\pm S.E.) at the end of 25 days for the six sound treatments; 1 - White Noise (50 – 500 Hz), 2 - White Noise (501 – 1500 Hz), 3 - White Noise (1501 – 10,000 Hz), 4 - White Noise (10,001 – 20,000 Hz), 5 - Ship Hull Noise and 6 - No Sound. Letters indicate statistically significant groupings.

5.3.2 Flow-Through Experiment

The mean shell length of the spat at the start of the experiment for each treatment was:

treatment 1- White Noise (50 – 500 Hz) 2.7 ± 0.1 mm; treatment 2 - White Noise (501 –

1500 Hz) 2.8 ± 0.1 mm; treatment 3 - White Noise (1501 – 10,000 Hz) 2.7 ± 0.1 mm;

treatment 4 - White Noise (10,001 – 20,000 Hz) 2.7 ± 0.1 mm; treatment 5 - Ship Hull Noise

2.7 ± 0.1 mm; treatment 6 - No Sound, 2.7 ± 0.1 mm. There was no significant difference in size among groups at the start of the experiment ($F_{35,504} = 0.933$, $P < 0.581$).

5.3.2.1 Growth

The mean growth of mussels as measured by increasing shell length (mm) was different among the six sound treatments ($F_{5,30} = 3.0$, $P < 0.026$) (Fig. 5.4). Mean growth of mussel spat was significantly higher in Treatment 1 than Treatments 3, 4 and 6 (Tukey's test; $P < 0.05$).

Treatment 1 had the highest mean growth (0.6 ± 0.1 mm, 22.2% increase in shell length), followed by Treatment 5 (0.5 ± 0.1 mm, 17.5%), Treatment 2 (0.4 ± 0.1 mm, 14%), Treatment

3 (0.3 ± 0.1 mm, 11.9%), Treatment 4 (0.3 ± 0.1 mm, 10.9%), and Treatment 6 had the smallest mean growth (0.2 ± 0.0 mm, 7.1%).

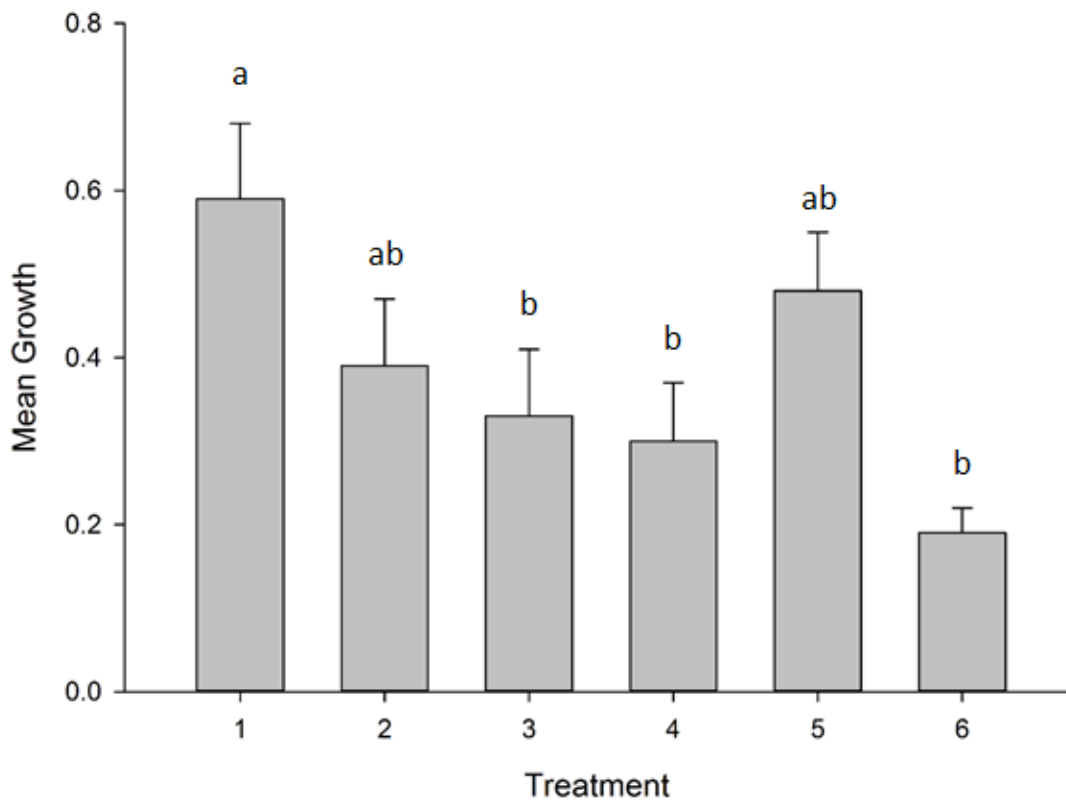


Figure 5.4 Mean growth (\pm S.E.) as measured as change in shell length of mussel spat over 14 days for six treatments: 1 - White Noise (50 – 500 Hz), 2 - White Noise (501 – 1500 Hz), 3 - White Noise (1501 – 10,000 Hz), 4 - White Noise (10,001 – 20,000 Hz), 5 - Ship Hull Noise and 6 - No Sound. Letters indicate statistically significant groupings.

5.3.2.2 Retention

The retention of mussel spat differed significantly among the six sound treatments ($H = 19.4$, $P < 0.002$)(Fig. 5.5). At the conclusion of the experiment, Treatment 1 had the highest mean number of spat retained on the twine ($74.4 \pm 4.6\%$), followed by Treatment 5 ($49.8 \pm 7.2\%$), Treatment 3 ($44.7 \pm 6.5\%$), Treatment 2 ($42.5 \pm 5.9\%$), Treatment 4 ($32.5 \pm 4.7\%$), and Treatment 6 had the lowest mean number of spat retained on the twine ($26.5 \pm 4.6\%$). Mean retention of mussel spat was significantly higher in Treatment 1 than Treatments 2, 3, 4 5 and 6. Treatment 6 had significantly lower mean retention than Treatments 2, 3 and 5 (Tukey's test; $P < 0.05$).

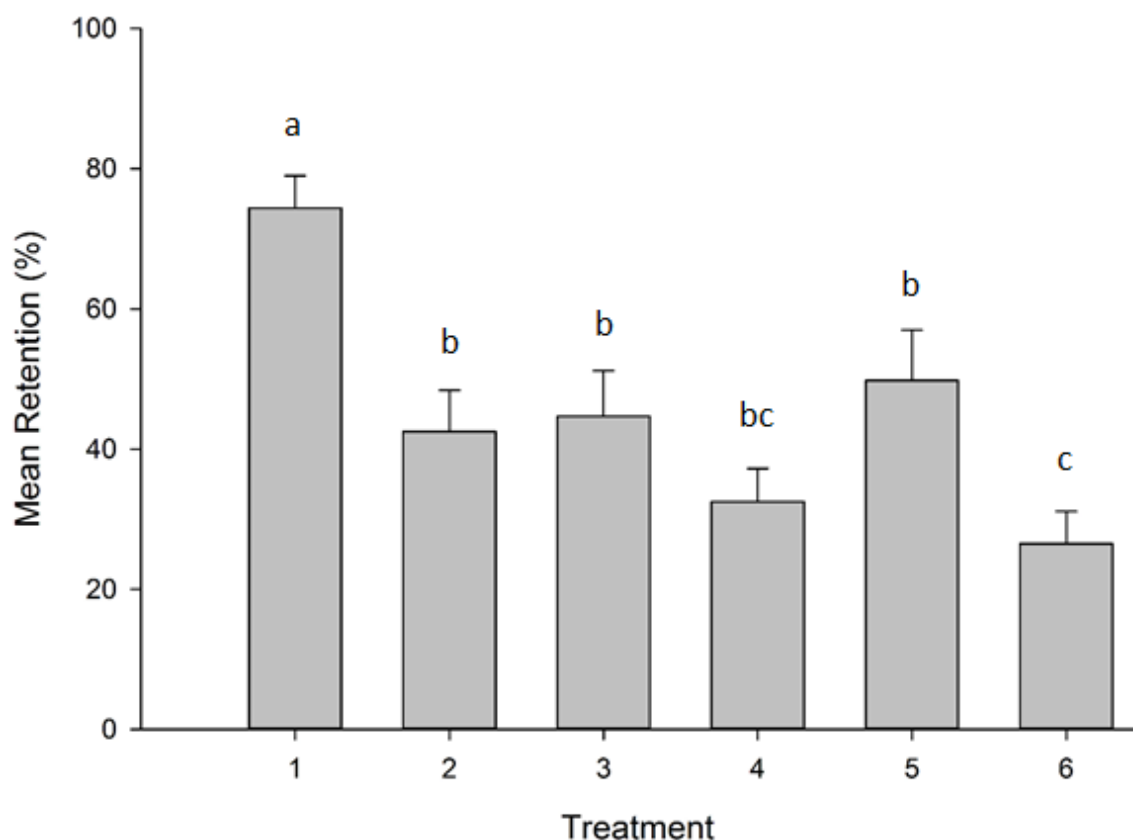


Figure 5.5 Mean spat retention (\pm S.E.) as observed after 14 days for six treatments; 1 - White Noise (50 – 500 Hz), 2 - White Noise (501 – 1500 Hz), 3 - White Noise (1501 – 10,000 Hz), 4 - White Noise (10,001 – 20,000 Hz), 5 - Ship Hull Noise and 6 - No Sound. Letters indicate statistically significant groupings.

5.3.2.3 Mortality

The mortality of mussel spat was significantly different among the six sound treatments ($H = 14.6$, $P = 0.004$; Fig. 5.6), with Treatment 6 having higher mortality compared to both Treatments 1 and 2 (Welch's Test; $P < 0.05$). Treatment 1 had the lowest mortality during the experiment ($10.1 \pm 1.3\%$), followed by Treatment 2 ($12.0 \pm 1.0\%$), Treatment 4 ($15.6 \pm 1.3\%$), Treatment 5 ($20.4 \pm 2.3\%$), Treatment 3 ($15.9 \pm 1.5\%$), and Treatment 6 had the highest mortality ($27.4 \pm 2.1\%$).

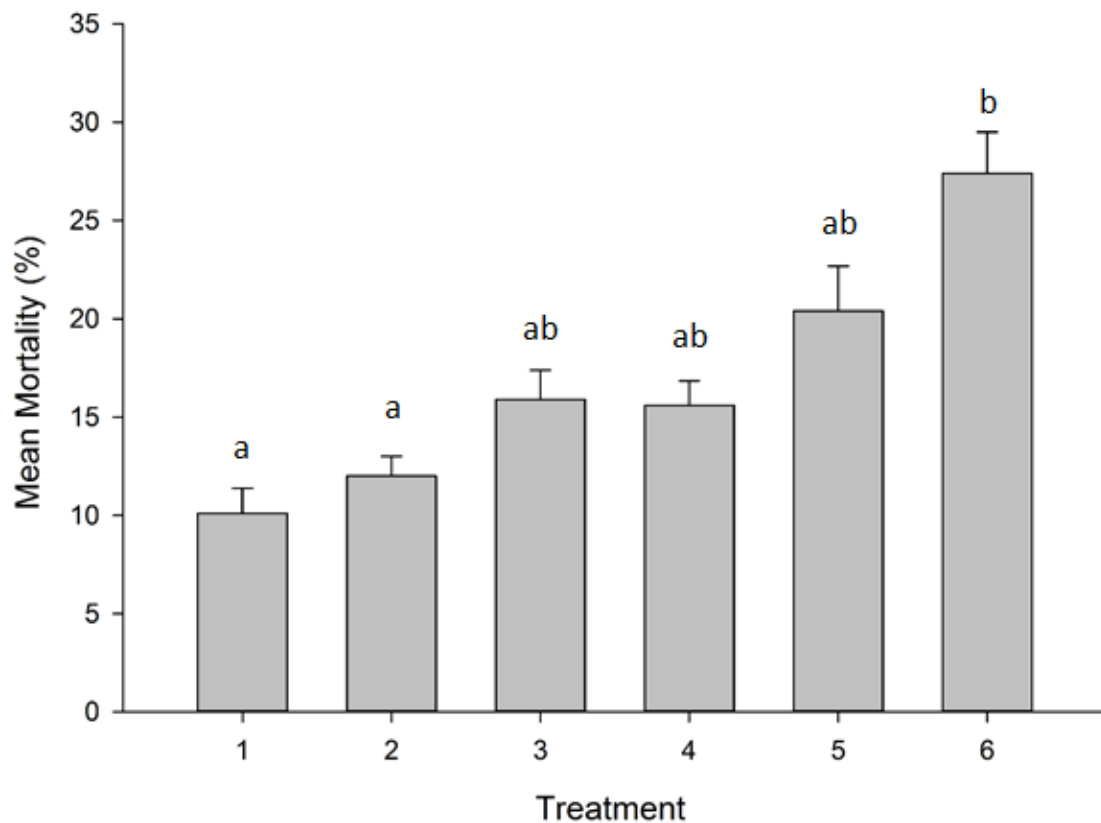


Figure 5.6 Mean percent mortality (\pm S.E.) at the end of 14 days for the six sound treatments: 1 - White Noise (50 – 500 Hz), 2 - White Noise (501 – 1500 Hz), 3 - White Noise (1501 – 10,000 Hz), 4 - White Noise (10,001 – 20,000 Hz), 5 - Ship Hull Noise and 6 - No Sound. Letters indicate statistically significant groupings.

5.4 Discussion

Many marine invertebrate taxa possess mechanosensory structures capable of serving as sound receptors (Rogers and Cox 1988, Budelmann 1992, Zhadan and Semen'kov 1984, Zhadan 2005), and there is growing evidence of underwater sound mediating the behaviour, especially settlement, in larval echinoderms, cnidarians, crustaceans and molluscs (Mooney et al. 2010, Pine et al. 2012, Stocks et al. 2012, Wilkens et al. 2012).

Underwater acoustic signals could be a useful settlement cue for larvae that have the potential to be effective over a large spatial scale (metres to kilometres) by helping the larvae to determine, or improve, their position relative to the sound source, ultimately increasing their ability to encounter their preferred settlement substrate (Wilkens et al. 2012, Lillis et al. 2013). Larvae often travel long distances from their initial spawning location to their eventual settlement habitat. Therefore, there is evolutionary benefit in the ability of larvae to use

effective long range cues for locating appropriate settlement habitat (Lillis et al 2013). Vessel noise has been shown to induce settlement in *P. canaliculus* larvae (Wilkens et al. 2012). Larvae exhibited a graded settlement response in relation to the intensity of the vessel noise with larvae exposed to the high intensity vessel noise settling earlier (Wilkens et al. 2012). Ambient sources of underwater sound has also been shown to induce settlement in oyster larvae, with increased settlement occurring in the presence of sound from reef habitat versus sound from soft benthic habitats and no sound (Lillis et al. 2013). This results indicate that underwater sound associated with different benthic habitats influences the settlement behaviour of marine bivalve larvae. Given the presence of this behaviour in larvae, it is also possible that underwater sounds may have a flow-on effect of increasing retention, as individuals that have previously settled will have positive sensory cues that they are in a desirable settlement location.

In the current study there was some evidence that underwater sound may influence the retention of juveniles, which had previously settled as larvae. In the flow-through experiment, treatment 1 (50 – 500 Hz) had the highest retention of mussel spat, while Treatment 6 (No Sound) had the lowest. In the static experiment there was a consistent trend for lower retention of spat to be found in Treatment 6 (No Sound) at all four sampling events (i.e., days 3, 6, 9 & 25) compared to Treatments 1 to 5 with replayed underwater sound. The overall rates of spat retention in the static seawater experiment were high compared to the flow through experiment. Consequently, these conditions may not be optimal for determining differences in mussel spat retention among treatments. For example, in the flow-through experiment there was an overall significant difference in the retention of mussel spat among the six sound treatments, with Treatment 1 (50 – 500 Hz) having the highest spat retention; three times more on average than the silent treatment (63% versus 21% spat retained). Treatments 2, 3, and 5 had no difference in their relative ability to retain spat. Treatments 4 (10,001 – 20,000 Hz) and Treatment 6 (No Sound) had the least ability to retain spat.

There is evidence from this study that underwater sound can dramatically increase the growth of Greenshell™ mussel spat and that this varies in relation to sound frequency. Both the static and flow-through experiments found differences in the increase in shell length of the mussel spat among the six sound frequency treatments. In both experiments Treatment 1 (50 – 500 Hz) produced the greatest increase in mean shell length, while Treatment 6 (No Sound)

consistently resulted in the least shell growth. In the static experiment the difference between the growth of mussels was nearly double in Treatment 1 (50 – 500 Hz) versus treatment 6 (No Sound). The results were even more pronounced in the flow-through experiment, where the growth of mussels in Treatment 1 was roughly double those in sound Treatments 3 and 4 (1501 – 10,000 Hz, & 10,001 – 20,000 Hz), and triple the growth of mussels in Treatment 6. The pattern of increasing growth rate with the increasing proportion of low frequency sound strongly suggests that while the growth of mussels responds to a wide range of sound frequencies, lower frequencies provide the best results (i.e., especially 50 – 500 Hz). The possible mechanism for this increase in growth in relation to exposure to underwater sound is uncertain and has only been observed previously in a range of sessile marine invertebrates (i.e., species of barnacles, bryozoans and oysters) growing on artificial settlement surfaces exposed to vessel noise compared to identical surfaces without noise (Stanley et al. 2014). The common appearance of this marked growth response among a variety of taxa suggests it has some fundamental biological basis. It has been suggested that the vibrational effect of low-frequency sound may assist in reducing the boundary layer around the spat which may facilitate the rate of food capture (Vedel 1998; Taylor et al. 2010, Stanley et al. 2014). This hypothesis would perhaps be supported by the findings that lower sound frequencies, which produce longer wavelength vibrations in water, also produced the most pronounced growth effects. However, in the current study the enhancement of growth was also quite pronounced in the flow-through experiment, whereas the water flow included in this experiment could have been expected to have diminished any boundary layer effects on feeding if they were present.

Mortality was found to be significantly higher in Treatment 6 (No Sound) than in Treatments 1 (50 – 500 Hz) and 2 (501 – 1500 Hz) for both the static and flow-through experiments. The basis for this apparent improvement in survival is unclear, but may be associated with the apparent stimulatory growth effect of underwater sound on the mussels.

A low frequency range of 100-1000 Hz has been shown to be important for the attraction, settlement and metamorphosis of many marine invertebrate larvae (Montgomery et al. 2006; Götz et al. 2009; Jeffs et al. 2011). It is also the dominant frequency of the ambient sound produced in many underwater habitats, including macroalgae-dominated and urchin-dominated rocky reefs (Radford et al. 2010; Simpson et al. 2011). It may well be that sound

that is consistent with natural reef habitats, which are commonly colonised by mussels, provides a cue that promotes behaviour consistent with rapidly establishing in the habitat (i.e., fast growth, retention and survival).

Overall, these laboratory experimental results indicate that low frequency sound has the potential to provide increased retention, growth and reduced mortality in wild-caught mussel spat. In particular, low frequency white noise of 50 – 500 Hz produced a marked increase in the growth of mussel spat in both the static and flow-through experiment. It remains to be demonstrated whether these marked improvements in the performance of spat in response to stimulation by low frequency underwater sound can also be produced in a commercial hatchery or field situation, such as a spat nursery growing area. Such experiments are a logical progression from this initial laboratory study.

Chapter Six: General Discussion

An understanding of the factors that influence the growth, condition and retention of *P. canaliculus* is critical for effective management of the aquaculture of this species. A review of research on the aquaculture development of *P. canaliculus* for this species (Chapter One) identified a number of areas where information was lacking and in need of further research. These findings were used to guide the specific aims for the remainder of the research presented in this thesis. Consequently, in the following four research chapters of this thesis new information regarding the settlement behaviour, growth and condition of *P. canaliculus* in the Hauraki Gulf is presented and discussed in some detail within the conclusions of each chapter. In summary, the body of research presented in the thesis provides evidence of the commercial potential for the Hauraki Gulf to serve as a new a source of *P. canaliculus* spat (Chapters Two and Three). This new information also increases the understanding of the larval settlement and subsequent growth of this species in the Hauraki Gulf. The change in the secondary settlement behaviour of *P. canaliculus* spat in regards to size and natal origins was also evaluated (Chapter Four). These finding increase our understanding on how it may be possible to selectively seed spat of a certain size range on to farm lines to ensure greater retention. Finally, this research examined how underwater sound affects the growth and retention of spat and how this may be applied in mussel farms to encourage greater retention of seeded spat on farm lines (Chapter Five).

6.1 Spat settlement in the Hauraki Gulf

Constraints in the supply of mussel spat has led to Greenshell™ aquaculture operators looking to new locations and means for gathering wild spat for seeding mussels farms. For example, in the last ten years Golden Bay has become a major area for the gathering of wild spat for South Island mussel farms, with the deployment each year of many kilometres of spat catching rope to gather settling wild Greenshell™ mussel larvae. Aquaculture operators in the Hauraki Gulf have also become interested in the potential to catch mussel spat locally as an alternative source of spat for seeding their farms, and have been making use of a wider range of sources of spat, including spat caught from Aotea Harbour and offshore from Opotiki (Tom

Hollings, Executive Director Coromandel Marine Farmers' Association, pers. comm.). Therefore, an initial study was made of the potential for spat catching in the eastern Hauraki Gulf by measuring spatfall monthly at two depths (4 and 11 m) at two sites a kilometre apart over a period of two years. It was found there was no difference in the overall numbers of spat settling at the two sites for the study period. However, for both sites the settlement of Greenshell™ at 11 m depth was consistently much higher than at 4 m depth, while for blue mussels the reverse was the case. The seasonal pattern of spat settlement was consistent between the two sites and was considerably higher in the spring and summer months and would be the most suitable period for focusing any commercial spat collections. Settlement numbers were not high enough to be a commercially feasible source of spat year-round but did reach sufficiently high numbers between October and January. This study provides information which can assist in future-proofing the industry against a short supply of spat by indicating that spat collection in the Hauraki Gulf has the potential to be commercially feasible and deployment of spat collecting material should be concentrated at 11 m depth rather than in shallower water, and is best conducted during Spring and Summer.

6.2 Growth rates of *Perna canaliculus* sourced the Hauraki Gulf

This thesis presents the first research examining the growth rates of *Perna canaliculus* sourced as spat from the Hauraki Gulf in comparison to spat sourced from Ninety Mile Beach (Chapter 3). There was no difference in the growth of mussels from the two sources over a two year period. Small variations in seasonal growth between the mussels from the two natal stocks are likely to reflect minor differences resulting from their genetically predetermined response to the environmental conditions in terms of energy allocation to growth and reproduction. However, the overall concordance of the growth of the two stocks of mussels at the Coromandel site over the two years indicates that growth is most likely driven by environmental rather than genetic differences between mussels from the two different stocks. It is widely reported that changes in growth patterns of bivalve shellfish are predominately environmentally mediated (Dickie et al. 1983, Widdows et al. 1984, Mallet et al. 1987b, Kautsky et al. 1990). For example, the experimental transplant of adult blue mussels, *Mytilus edulis*, from an estuarine environment into a sub-littoral environment found

that changes in the fundamental physiology of the mussels (i.e., clearance rates, absorption efficiency, respiration, excretion rates, O : N and scope for growth) continued to occur over a two month period as they became more closely aligned with those of the resident mussel population (Widdows et al. 1984). However, clearance rates, scope for growth and growth efficiency continued to remain out of step with resident mussels after two months, indicating that these physiological parameters are both mediated by environmental and genetic factors. Similar studies transplanting *M. edulus* between the Baltic and North Seas and to different locations within the Atlantic Ocean, concluded that the growth is predominantly regulated by non-genetic factors, however, stock differences were responsible for differential mortality effects (Dickie et al. 1983, Kautsky et al. 1990). Unfortunately mortality was unable to be measured in this current study due to the large number of mussels seeded onto the grow-out rope which made it logistically impossible to keep track of exact numbers of mussels and their fate. This is an area which needs to be investigated in the future to determine if the two stocks have the same survivorship and response to environmental changes and weather events.

6.3 Condition cycle of *Perna canaliculus* sourced the Hauraki Gulf

After a two year study assessing the condition cycle of spat sourced from, and grown out in the Hauraki Gulf alongside spat sourced from Ninety Mile Beach, it was found that stocks did not follow a consistent annual pattern of condition. However, it appears that mussels sourced from Ninety Mile Beach spawned a month earlier than Hauraki Gulf mussels with a significant difference in condition occurring during October and November in both years of the study. During this period the CI between the two mussel stocks differed by up to 30%. The spawning of mussels sourced from Ninety Mile Beach had a greater effect on mussel condition and appeared to occur only once per year during spring. In contrast, Hauraki Gulf mussels appeared to have one large spawning event during November in 2013 but three smaller spawning events in 2014 in July, September and November. Researchers have speculated that genetic differences among stocks of mussels are responsible for the observed seasonal differences in their condition cycles (Fox 2003, Camara & Symonds 2016). If mussels that are sourced from spat caught in the Hauraki Gulf and Ninety Mile Beach are able to be grown together they might be able to be harvested at different times for potentially at least one

month of the year, with an increased yield of up to 30% for this period, thus greatly increasing the overall yield of commercial farming operations in the Hauraki Gulf.

Temporal differences in the reproductive cycles of *P. canaliculus* are of particular commercial significance in mussel aquaculture, as reproductive condition greatly influences meat yield and the consumer qualities of the harvested product (Çelik et al. 2012). There is evidence that mussels grown from geographically separated spat sources have different cycles of condition, providing the potential to extend effective harvesting seasons by utilising mussels with complementary condition cycles (Fox 2003). For example, mussels grown from spat sourced from Ninety Mile Beach, Golden Bay and the Marlborough Sounds when grown together at a number of farm locations in the Pelorus Sound were found to have differing overall average condition over a single year, as well as differences in the timing of their condition cycles (Fox 2003). Over one year the mean condition of mussels grown from Marlborough Sounds was 2.5% higher than those grown from spat sourced from Golden Bay. Harvesting stocks with different condition cycles could potentially improve the mean annual condition index of harvested mussels and minimise the impact of the mid-winter decline in condition. Increases in mean annual condition as small as 2% are considered to be commercially relevant in Greenshell™ aquaculture (Fox 2003). It is also worth further investigating the genetic differences between the two populations to determine if the differing condition cycle is a genetically premediated response. Genetic testing has improved greatly since the last study on *P. canaliculus* population genetics was conducted in 2003 (Star et al. 2003).

6.4 Secondary settlement behaviour in *Perna canaliculus* spat

In addition to the difficulty of sourcing sufficient mussel spat to maintain Greenshell™ aquaculture production, high losses of spat typically occur shortly after they are placed out on nursery ropes on farms. While there are many possible causes for this low retention of mussel spat, their secondary settlement behaviour is suspected to be responsible for a majority of the loss of spat from seeded mussel nursery lines (Foote 2003). Anecdotal reports from Greenshell™ aquaculture operators suggests that wild *P. canaliculus* spat harvested from different locations show some consistency in the extent of their subsequent losses following seeding out on nursery lines. These observations indicate that the extent of

secondary settlement behaviour may be influenced by the geographic origin of spat, or be driven by differences in environmental conditions between the wild spat harvesting location and the mussel farm destination of the spat. Both juvenile and adult mussels are known to exhibit some differences in behaviour, such as their strength of byssal attachment and valve gaping behaviour, which continue to manifest themselves when the mussels are translocated (Christensen et al. 2015, Zardi et al. 2015). However, it remains to be determined whether wild *P. canaliculus* spat harvested from different locations express consistent differences in their secondary settlement behaviour. This study looked at the changes in secondary settlement behaviour of *P. canaliculus* spat sourced from five distinct locations (Hauraki Gulf, Ninety Mile Beach, Muriwai, Aotea Harbour and a commercial hatchery) as they were grown to larger juveniles. The secondary settlement behaviour was found to change with the size of juveniles, with both attachment and retention varying in relation to the changing size of the mussels. Attachment rates (i.e., the extent to which spat readily attached to spat rope) of spat were found to decrease at a shell length of 5 mm and upwards, whereas experimentally determined retention rates decreased in juvenile mussels from 0.8 to 5 mm in shell length and then increased again from 5 mm upwards. Secondary settlement behaviour also varied among the five source locations of spat, with spat sourced from Muriwai and Hauraki Gulf spat having lower retention rates than Ninety Mile Beach, Aotea Harbour and hatchery-raised spat. Spat from Muriwai also had lower attachment rates than spat collected from Ninety Mile Beach. There was a moderate relationship observed between attachment and retention rates, as spat retention rates tended to increase proportionately as attachment rates decreased. With the recent development of an effective commercial hatchery, this source of spat is likely to become more widely used in the industry in the near future. By seeding spat at 0.8 mm instead of the current 1.01 – 3.0 mm (Jeffs et al. 2018), more spat should successfully attach and be retained on farm lines. A greater understanding of the significance of specific drivers of the secondary settlement behaviour in the early juveniles of this mussel species is required to ultimately assist in tackling the widespread and costly losses of spat from seeded nursery ropes.

6.5 The effect of sound on the retention and growth of *P. canaliculus* spat

Underwater sound has been found to promote the larval settlement and/or post-settlement growth of a wide range of benthic sedentary marine invertebrate taxa, including bivalves (Vermeij et al. 2010 Wilkens et al. 2012, McDonald et al. 2014, Stanley et al. 2014, Lillis *et al.* 2013). For example, *P. canaliculus* larvae settled much more quickly when exposed to underwater sound from a motorised vessel (Wilkens *et al.* 2012), and recently-settled Pacific oysters, *Crassostrea gigas*, grew faster in the presence of vessel sound (Stanley et al. 2014). Given that these previous results show that an early stage in the lifecycle of *P. canaliculus* is responsive to underwater sound cues it is possible that Greenshell™ spat may also show some useful responses to exposure to underwater sound. This current study looked at the growth and retention rates of juvenile *P. canaliculus* spat exposed to six different sound treatments (Treatment 1. White Noise 1 (50 – 500 Hz); Treatment 2. White Noise 2 (501 – 1500 Hz); Treatment 3. White Noise 3 (1501 – 10,000 Hz); Treatment 4. White Noise 4 (10,001 – 20,000 Hz); Treatment 5. Ship Hull Noise; Treatment 6. No Sound), in both a static and flow through experimental set-up. The results found that the white noise treatment between 50 – 500 Hz had the highest growth rate and retention rates and lowest mortality for both the static and flow-through experiments. The no sound treatment had the lowest growth rate and retention rates and the highest mortality for both static and flow-through experiment. Overall, the laboratory experimental results indicated that low frequency sound has the potential to provide increased retention, growth and reduced mortality in wild-caught mussel spat. There is evidence from this study that underwater sound can dramatically increase the growth of Greenshell™ mussel seed, with Treatment 1 (Low Frequency White Noise 50 – 500 Hz) nearly doubling the mean increase in shell length of mussel spat compared with Treatment 6 (No Sound) in both the static and flow through experiment. The pattern of increasing growth rate with the increasing proportion of low frequency sound strongly suggests that while the growth of mussels responds to a wide range of sound frequencies, lower frequencies are likely to provide the best results (i.e. 50 – 500 Hz).

These results are very promising and provide an excellent start point for investigating the role of sound in the hatchery production of shellfish. These results need further research to understand the biological basis of the effect of the sound on juvenile mussels and whether or

not these results can be transferred to an applied situation, either in the hatchery or on a long-line mussel farm.

6.6 General Conclusions

Overall, the body of research presented in this thesis will provide valuable information for the commercial development of *P. canaliculus* seed harvesting within the Hauraki Gulf. It also offers new insights into how spat retention may be improved with the use of sound and size sorting of spat that are relayed onto nursery ropes on farms. Spat sourced from the Hauraki Gulf will be a welcome addition to the already limited supply available for use by the Greenshell™ aquaculture industry. Mussel spat harvested from the Hauraki Gulf offers the same growth rate as spat sourced from Ninety Mile Beach, and its use has the potential to increase the overall harvested yield of mussels due to the distinct and consistent differences in the timing of the spawning season. Transferring Hauraki Gulf spat to mussel farms within the Marlborough Sounds has the potential to be beneficial to farmers looking to extend their harvesting season and warrants further investigation.

In order to understand the true potential of sound as a tool for promoting increased growth and retention rate of *P. canaliculus* spat additional field studies need to be undertaken. Exploring the use of sound as both an attractant near spat collectors and as a promoter of retention alongside newly seeded lines should be the next step. This use of sound may increase the settlement of other biofouling organisms and thereby aggravate the already problematic biofouling experienced on mussel farm lines. The frequency of sound may need to be investigated to optimise and target the sound used for *P. canaliculus* if possible.

Collectively the results from this study improve our understanding of the biology *P. canaliculus* in relation to aquaculture conditions. The results also provide a number of avenues for greatly improving the efficiency and commercial production from Greenshell™ aquaculture, not only in the Hauraki Gulf, but potentially in other key growing areas.

Literature cited

Alfaro, A. C. (2001). Ecological dynamics of the green-lipped mussel, *Perna canaliculus*, at Ninety Mile Beach, northern New Zealand. (Doctoral dissertation, University Auckland), Auckland.

Alfaro, A. C. (2005). Effect of water flow and oxygen concentration on early settlement of the New Zealand green-lipped mussel, *Perna canaliculus*. *Aquaculture*, 246(1-4), 285-294.

Alfaro, A. C. (2006a). Evidence of cannibalism and benthic-pelagic coupling within the life cycle of the mussel, *Perna canaliculus*. *Journal of Experimental Marine Biology and Ecology*, 329(2), 206-217.

Alfaro, A. C. (2006b). Byssal attachment of juvenile mussels, *Perna canaliculus*, affected by water motion and air bubbles. *Aquaculture*, 255, 357-361.

Alfaro, A. C., Copp, B. R., Appleton, D. R., Kelly, S., & Jeffs, A. G. (2006). Chemical cues promote settlement in larvae of the green-lipped mussel, *Perna canaliculus*. *Aquaculture International*, 14(4), 405-412.

Alfaro, A. C., Jeffs, A. G., & Hooker, S. H. (2001). Reproductive behavior of the green-lipped mussel, *Perna canaliculus*, in northern New Zealand. *Bulletin of Marine Science*, 69(3), 1095-1108.

Alfaro, A. C., & Jeffs, A. G. (2002). Small-scale mussel settlement patterns within morphologically distinct substrata at Ninety Mile Beach, northern New Zealand. *Malacologia*, 44, 1-16.

Alfaro, A.C., & Jeffs, A.G. (2003). Variability in mussel settlement on suspended ropes placed in Ahipara Bay, Northland, New Zealand. *Aquaculture*, 216, 115-126.

Alfaro, A. C., Jeffs, A. G., & Creese, R. G. (2004). Bottom-drifting algal/mussel spat associations along a sandy coastal region in northern New Zealand. *Aquaculture*, 241(1), 269-290.

Alfaro, A., Jeffs, A. G., Gardner, J. P. A., Breen, B. B., & Wilkin, J. (2011). Green-lipped Mussels in GLM 9. New Zealand Fisheries Assessment Report, 48, 80.

Alfaro, A. C., Jeffs, A. G., & Hooker, S. H. (2003). Spatial variability in reproductive behaviour of green-lipped mussel populations of northern New Zealand. *Molluscan Research*, 23, 223-238.

Alfaro, A. C., McArdle, B., & Jeffs, A. G. (2010). Temporal patterns of arrival of beachcast green-lipped mussel (*Perna canaliculus*) spat harvested for aquaculture in New Zealand and its relationship with hydrodynamic and meteorological conditions. *Aquaculture*, 302(3-4), 208-218.

Alfaro, A. C., Young, T., & Ganesan, A. M. (2011). Regulatory effects of mussel (*Aulacomya maoriana* Iredale 1915) larval settlement by neuroactive compounds, amino acids and bacterial biofilms. *Aquaculture*, 322, 158-168.

Ansell, A. D. (1975). Seasonal changes in biochemical composition of the bivalve *Astarte montagui* in the Clyde Sea area. *Marine Biology*, 29(3), 235-243.

Apte, S. & Gardner, J. P. A. (2001). Absence of population genetic differentiation in the New Zealand greenshell mussel *Perna canaliculus* (Gmelin 1791) as assessed by allozyme variation. *Journal of Experimental Biology and Ecology*, 258, 173-194.

Aquaculture New Zealand. (2009). New Zealand aquaculture: farm facts. http://www.aquaculture.org.nz/userfiles/PDFs/NZ_Aquaculture_Farm_Facts.pdf. Cited 12 October 2012.

Atalah, J., Rabel, H., & Forrest, B. M. (2017). Modelling long-term recruitment patterns of blue mussels *Mytilus galloprovincialis*: a biofouling pest of green-lipped mussel aquaculture in New Zealand. *Aquaculture Environment Interactions*, 9, 103-114.

Bagamian, K. H., Heins, D. C., & Baker, J. A. (2004). Body condition and reproductive capacity of three-spined stickleback infected with the cestode *Schistocephalus solidus*. *Journal of Fish Biology*, 64(6), 1568-1576.

- Baird, R. H. (1958). Measurement of condition in mussels and oysters. *ICES Journal of Marine Science*, 23(2), 249-257.
- Barlow, L. A. (1990). Electrophysiological and behavioral responses of larvae of the red abalone (*Haliotis rufescens*) to settlement-inducing substances. *Bulletin of Marine Science*, 46(2), 537-554
- Barnes, H., Barnes, M., & Finlayson, D. M. (1963). The seasonal changes in body weight, biochemical composition, and oxygen uptake of two common boreo-arctic cirripedes, *Balanus balanoides* and *B. balanus*. *Journal of the Marine Biological Association of the United Kingdom*, 43(01), 185-211.
- Bayne, B. L. (1964). Primary and secondary settlement in *Mytilus edulis* L. *Journal of Animal Ecology*, 33, 513-523.
- Bayne, B. L. (1975). Reproduction in bivalve molluscs under environmental stress. *Physiological ecology of estuarine organisms*. University of South Carolina Press, Columbia, 259-277.
- Bayne, B. L. (1984). Aspects of reproductive behaviour within species of bivalve molluscs. *Advances in Invertebrate Reproduction*, 3, 357-366.
- Beninger, P. G., & Lucas, A. (1984). Seasonal variations in condition, reproductive activity, and gross biochemical composition of two species of adult clam reared in a common habitat: *Tapes decussatus* L. (Jeffreys) and *Tapes philippinarum* (Adams & Reeve). *Journal of Experimental Marine Biology and Ecology*, 79(1), 19-37.
- Black, K. P., Bell, R. G., Oldman, J. W., Carter, G. S., & Hume, T. M. (2000). Features of 3-dimensional barotropic and baroclinic circulation in the Hauraki Gulf, New Zealand. *New Zealand Journal of Marine and Freshwater Research*, 34(1), 1-28.
- Board, P. (1983). The settlement of post larval *Mytilus edulis* (settlement of post larval mussels). *Journal of Molluscan Studies*, 49, 53-60.
- Booth, J. D. (1977). Common bivalve larvae from New Zealand: Mytilacea. *New Zealand Journal of Marine and Freshwater Research*, 11: 407-440.

- Bostock, J., McAndrew, B., Richards, R., Jauncey, K., Telfer, T., Lorenzen, K., Little, D., Ross, L., Handisyde, N., Gatward, I., & Corner, R. (2010). Aquaculture: Global status and trends. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365(1554), 2897-2912.
- Boyd, C. E., & Schmittou, H. R. (1999). Achievement of sustainable aquaculture through environmental management. *Aquaculture Economics and Management*, 3(1), 59-69.
- Buchanan, S. J. (1994). The settlement behaviour and recruitment of *Perna canaliculus*, (Masters Thesis, University of Auckland), Auckland.
- Buchanan, S. J. & Babcock, R. (1997). Primary and secondary settlement by the greenshell mussel *Perna canaliculus*. *Journal of Shellfish Research*, 16(1), 71-76.
- Buchanan, S. J. (1998). Spat production of the Greenshell mussel, *Perna canaliculus* in New Zealand, (Doctoral dissertation, University of Auckland), Auckland.
- Buchanan, S. J. (2001). Measuring reproductive condition in the Greenshell™ mussel *Perna canaliculus*. *New Zealand Journal of Marine and Freshwater Research*, 35(5), 859-870.
- Budelmann, B. U. (1992) Hearing in nonarthropod invertebrates. In *The Evolutionary Biology of Hearing* (pp. 141–155). Springer, New York, NY.
- Burrell, M., & Meehan, L. (2006). Aquaculture New Zealand strategy. Creative Design Advertising Ltd. Accessed October 15, 2012 from http://www.nzmic.co.nz/Assets/Content/Publications/sector_strategy_final_low_resolution.pdf
- Caceres-Martinez, J., Robledo, J. A. F., & Figueras, A. (1994). Settlement and post-larvae behaviour of *Mytilus galloprovincialis*: field and laboratory experiments. *Marine Ecology Progress Series*, 112, 107-117.
- Cai, W., Dunford, N. T., Wang, N., Zhu, S., & He, H. (2016). Audible sound treatment of the microalgae *Picochlorum oklahomensis* for enhancing biomass productivity. *Bioresource Technology*, 202:226-230.

- Camara, M. D., & Symonds, J. E. (2014). Genetic improvement of New Zealand aquaculture species: programmes, progress and prospects. *New Zealand Journal of Marine and Freshwater Research*, 48(3), 466-491.
- Carl, C., Poole, A. J., Sexton, B. A., Glenn, F. L., Vucko, M. J., Williams, M. R Whalan, S., & De Nys, R. (2012). Enhancing the settlement and attachment strength of pediveligers of *Mytilus galloprovincialis* by changing surface wettability and microtopography. *Biofouling*, 28(2), 175-186.
- Carton, A. G., Jeffs, A. G., Foote, G., Palmer, H., & Bilton, J. (2007). Evaluation of methods for assessing the retention of seed mussels (*Perna canaliculus*) prior to seeding for grow-out. *Aquaculture*, 262(2-4), 521-527.
- Çelik, M. Y., Karayücel, S., Karayücel, İ., Öztürk, R., & Eyüboğlu, B. (2012). Meat yield, condition index, and biochemical composition of mussels (*Mytilus galloprovincialis* Lamarck, 1819) in Sinop, South of the Black Sea. *Journal of Aquatic Food Product Technology*, 21(3), 198-205.
- Christensen, H. T., Dolmer, P., Hansen, B. W., Holmer, M., Kristensen, L. D., Poulsen, L. K., Stenberg, C., Albertsen, C.M & Støttrup, J. G. (2015). Aggregation and attachment responses of blue mussels, *Mytilus edulis* —impact of substrate composition, time scale and source of mussel seed. *Aquaculture*, 435, 245-251.
- Colin Johnston, Aquaculture New Zealand, pers. comm., NZ Seafood Industry Council, export database
- Dawber, C. (2004). Lines in the water: a history of Greenshell mussel farming in New Zealand. River Press for NZ Marine Farming Association.
- Douillet, P. A., & Mangdon, C. J. (1993) Effects of marine bacterial on the culture of axenic oyster *Crassostrea gigas* (Thunberg) larvae. *Biological Bulletin*, 184: 36-51.
- Dickie, L. M., Boudreau, P. R., & Freeman, K. R. (1984). Influences of stock and site on growth and mortality in the blue mussel (*Mytilus edulis*). *Canadian Journal of Fisheries and Aquatic Sciences*, 41(1), 134-140.

- Eckwert, H., Alberti, G., & Köhler, H. R. (1997). The induction of stress proteins (hsp) in *Oniscus asellus* (Isopoda) as a molecular marker of multiple heavy metal exposure: I. Principles and toxicological assessment. *Ecotoxicology*, 6(5), 249-262.
- Efthimiou, P., & Kukar, M (2010). Complementary and alternative medicine use in rheumatoid arthritis: proposed mechanism of action and efficacy of commonly used modalities. *Rheumatol International*. 30, 571–586.
- Flaws, D. E. (1975). Aspects of the biology of mussels in the Cook Strait area (Doctoral dissertation, Victoria University of Wellington), Wellington.
- Fitridge, I., Dempster, T., Guenther, J. & de Nys, R. (2012). The impact and control of biofouling in marine aquaculture: a review. *Biofouling*, 28, 649–669
- Fitt, W. K., Coon, S. L., Walch, M., Weiner, R. M., Colwell, R. R., & Bonar, D. B. (1990). Settlement behavior and metamorphosis of oyster larvae (*Crassostrea gigas*) in response to bacterial supernatants. *Marine Biology*, 106(3), 389-394.
- Fisheries and Aquaculture topics. The state of World Fisheries and Aquaculture (SOFIA). (2012) Retrieved from: <http://www.fao.org.ezproxy.auckland.ac.nz/fishery/sofia/en>
- Fleck, J., & Fitt, W. K. (1999). Degrading mangrove leaves of *Rhizophora mangle* Linne provide a natural cue for settlement and metamorphosis of the upside down jellyfish *Cassiopea xamachana* Bigelow. *Journal of experimental marine biology and ecology*, 234(1), 83-94.
- Food and agriculture organization of the United Nations (FAO) (2004) Fishery and Aquaculture Country profiles. New Zealand. Fishery and Aquaculture Country Profiles. In: FAO Fisheries and Aquaculture Department [online]. Rome. Updated 5 August 2004.
- Food and agriculture organization of the United Nations (FAO) (2004). The hatchery culture of bivalves: a practical manual. Retrieved from: <http://www.fao.org/docrep/007/y5720e/y5720e00.htm>

Food and agriculture organization of the United Nations (FAO) (2005). Cultured Aquatic Species Information Programme. *Perna canaliculus*. Cultured Aquatic Species Information Programme. Retrieved from:

http://www.fao.org/fishery/culturedspecies/Perna_canaliculus/en

Food and agriculture organization of the United Nations (FAO) (2010) Fishstat Database. Retrieved from: <http://www.fao.org/faostat/en>

Foot, G. (2003). Characterisation of the viability of Greenshell™ mussel spat, *Perna canaliculus* with respect to hatchery production and growout retention, (Masters thesis, University of Auckland), Auckland.

Forrest, B. M., & Atalah, J. (2017). Significant impact from blue mussel *Mytilus galloprovincialis* biofouling on aquaculture production of green-lipped mussels in New Zealand. *Aquaculture Environment Interactions*, 9, 115-126.

Fox, S. P. (2003). The growth of cultured *Perna canaliculus* in Pelorus Sound, New Zealand: the importance of spat origin, environment and time of harvest (Doctoral dissertation, University of Canterbury), Christchurch.

Fusetani, N. (2004). Biofouling and antifouling. *Natural product reports*, 21(1), 94-104.

Ganesan, A. M., Alfaro, A. C., Brooks, J. D., & Higgins, C. M. (2010). The role of bacterial biofilms and exudates on the settlement of mussel (*Perna canaliculus*) larvae. *Aquaculture*, 306: 388-392.

Gardner, J. P. A. (2000). Where are the mussels on Cook Strait (New Zealand) shores? Low seston quality as a possible factor limiting multi-species distributions. *Marine Ecology Progressive Series*, 194, 123-132.

Gardner, J. P. A., Eyles, R. F., & Pande, A. (1996b). Biochemical-genetic variation in wild and a cultured population of greenshell mussel, *Perna canaliculus*. *New Zealand Journal of Marine and Freshwater Research*, 30, 435-441.

- Gardner, J. P. A & Palmer, N. L. (1998). Size-dependant, spatial and temporal genetic variation at the leucine aminopeptidase (LAP) locus among blue mussels (*Mytilus edulis*) populations along a salinity gradient. *Marine Biology*, 132, 275-281.
- Gardner, J. P. A., Pande, A., Eyles, R. F. & Wear, R. G. (1996a). Biochemical genetic variations among populations of the greenshell mussel *Perna canaliculus* from New Zealand. *Biochemical Systematics and Ecology*, 24, 763-774.
- Gardner, J. P. A. & Thompson, R. J. (2001). Naturally low seston concentration and the net energy balance of the greenshell mussel (*Perna canaliculus*) at Island Bay, Cook Strait, New Zealand. *New Zealand Journal of Marine and Freshwater Research*, 35, 457-468.
- Götz, T., Hastie, G., Hatch, L. T., Raustein, O., Southall, B. L., Tasker, M., & Thomsen, F. (2009). Overview of the impacts of anthropogenic underwater sound in the marine environment. London: OSPAR Commission; p. 134.
- Greenway, J. P. C. (1969). Surveys of mussels (Mollusca: Lamellibranches) of Thames, 1961–67. *New Zealand Journal of Marine and Freshwater Research*, 3: 304–17.
- Gribben, P. E., Jeffs, A. G., de Nys, R., & Steinberg, P. D. (2011). Relative importance of natural cues and substrate morphology for settlement of the New Zealand Greenshell™ mussel, *Perna canaliculus*. *Aquaculture*, 319, 240–246.
- Hadfield, M. G., & Paul, V. J. (2001). Natural chemical cues for settlement and metamorphosis of marine invertebrate larvae. *Marine chemical ecology*, 431-461.
- Harder, T., Lam, C., & Qian, P. Y. (2002). Induction of larval settlement in the polychaete *Hydroides elegans* by marine biofilms: an investigation of monospecific diatom films as settlement cues. *Marine Ecology Progress Series*, 229, 105-112.
- Hayden, B. J. (1994). What do we know about greenshell mussel spat? Part, 1, 45-47.
- Hayden, B.J. (1995). Factors affecting recruitment of farmed greenshell mussels, *Perna canaliculus* (Gmelin) 1791, in Marlborough Sounds, (Doctoral dissertation, University of Otago), Dunedin.

Hayden, B. & C. Woods. (1997). Current speed has an effect on mussel spat retention. *Aquaculture Update*, 19, 9.

Hayden, B. J., & Woods, C. M. C. (2011). Effect of water velocity on growth and retention of cultured Greenshell™ mussel spat, *Perna canaliculus*. *Aquaculture International*, (19), 957–971.

Heasman, K. G. (2013). Temperature and humidity of Kaitia weed during harvesting, storage and transport and suggested influences on spat viability, Cawthron Report No. 2428., Prepared for Marine Farmers Association, 18pp. plus appendices

Helson, J. (2001). An investigation into the absence of mussels (*Perna canaliculus*, *Aulacomya maoriana* and *Mytilus galloprovincialis*) from the south coast of Wellington, New Zealand, (Doctoral dissertation, Victoria University of Wellington), Wellington.

Hickman, R. W. (1976). Potential for the use of stranded seed mussels in mussel farming. *Aquaculture*, 9, 287-293.

Hickman, R. W. (1978). Incidence of a pea crab and a trematode in cultivated and natural green-lipped mussels. *New Zealand Journal of Marine and Freshwater Research*, 12(2), 211-215.

Hickman, R. W., & Illingworth, J. (1980). Condition cycle of the green-lipped mussel *Perna canaliculus* in New Zealand. *Marine Biology*, 60(1), 27-38.

Ibarrola, I., Hilton, Z., & Ragg, N. L. (2017). Physiological basis of inter-population, inter-familiar and intra-familiar differences in growth rate in the green-lipped mussel *Perna canaliculus*. *Aquaculture*, 479, 544-555.

Iglesias, J. I. P., Camacho, A. P., Navarro, E., Labarta, U., Beiras, R., Hawkins, A. J., & Widdows, J. (1996). Microgeographic variability in feeding absorption, and condition of mussels (*Mytilus galloprovincialis* LMK): a transplant experiment. *Journal of Shellfish Research*, 15, 673-680.

Irwin, A., Hallegraeff, G. M., McMinn, A., Harrison, J., & Heijnis, H. (2003). Cyst and radionuclide evidence demonstrate historic *Gymnodinium catenatum* dinoflagellate populations in Manukau and Hokianga Harbours, New Zealand. *Harmful Algae*, 2(1), 61-74.

Jaeckle, W. B., & Manahan, D. T. (1989). Feeding by a “nonfeeding” larva: uptake of dissolved amino acids from seawater by lecithotrophic larvae of the gastropod *Haliotis rufescens*. *Marine Biology*, 103(1), 87-94.

James, M. R., & Ross, A. (1997). Sustainability—how many mussels can we farm? *Aquaculture Update*, 18, 1.

Jeffs, A., G (1999). Overview and bibliography of research on the greenshell, *Perna canaliculus*, from New Zealand waters. *Journal of Shellfish Research*, 18, 347-360.

Jeffs, A. G., R. C. Holland, S. H. Hooker & B. J. Hayden. (1999). Overview and bibliography of research on the greenshell mussel *Perna canaliculus* from New Zealand waters. *Journal of Shellfish Research*, 18, 347–360.

Jeffs, A.G., Delorme, N.J., Stanley, J., Zamora, L.N., & Sim-Smith, C. (2018). Composition of beachcast material containing green-lipped mussel (*Perna canaliculus*) seed harvested for aquaculture in New Zealand. *Aquaculture*, 488, 30-38.

Jeffs, A. G., Sim-Smith, C., & Alfaro, A. C. (2005) Development of the green-lipped mussel spat resource in northern New Zealand. National Institute of Water & Atmospheric Research Ltd, NIWA Client Report: AKL2005-06, NIWA Project: MIC05101, Auckland.

Jeffs, A., & Meder, A. (2005) Detecting mussel spat condition using biochemical staining. National Institute of Water & Atmospheric Research Ltd. NIWA Client Report: AKL2005- 012, Auckland.

Jenkins, R. J. (1985). Mussel Cultivation in the Marlborough Sounds (New Zealand). N.Z. Fishing Industry Board, Wellington.

Johnson, C. R., Sutton, D. C., Olson, R. R., & Giddins, R. (1991). Settlement of crown-of-thorns starfish: role of bacteria on surfaces of coralline algae and a hypothesis for deepwater recruitment. *Marine ecology Progress series*, 143-162.

- Johnston, I. A., & Goldspink, G. (1973). Some effects of prolonged starvation on the metabolism of the red and white myotomal muscles of the plaice *Pleuronectes platessa*. *Marine Biology*, 19(4), 348-353.
- Kautsky, N. (1982). Quantitative studies on gonad cycle, fecundity, reproductive output and recruitment in a Baltic *Mytilus edulis* population. *Marine Biology*, 68(2), 143-160.
- Kautsky, N., Johannesson, K., & Tedengren, M. (1990). Genotypic and phenotypic differences between Baltic and North Sea populations of *Mytilus edulis* evaluated through reciprocal transplantations. *Marine Ecological Progress Series*, 59, 203-210.
- Lane, D. J. W., J. A. Nott, & Crisp, D. J. (1982). Enlarged stem glands in the foot of the post-larval mussel, *Mytilus edulis*: adaptation for bysso-pelagic migration. *Journal of the Marine Biological Association of the United Kingdom*, 62(4), 809-818.
- Lasiak, T. A., & Barnard, T. C. E. (1995). Recruitment of the brown mussel *Perna perna* onto natural substrate: a refutation of the primary/secondary settlement hypothesis. *Marine Ecology Progress Series*, 120, 147-153.
- LeBlanc, N., Landry, T., Stryhn, H., Tremblay, R., McNiven, M., & Davidson, J. (2005). The effect of high air and water temperature on juvenile *Mytilus edulis* in Prince Edward Island, Canada. *Aquaculture*, 243(1-4), 185-194.
- LeBlanc, N., Tremblay, R., Davidson, J., Landry, T., & McNiven, M. (2008). The effect of selection treatments on *Mytilus edulis*, modifications of genetic and physiological characteristics. *Marine Biology*, 153(6), 1141-1152.
- Lee, S. Y. (1986). Growth and reproduction of the green mussel *Perna viridis* (Bivalvia: Mytilacea) in contrasting environments in Hong Kong. *Asian Marine Biology*, 3, 111-127.
- Lillis, A., Eggleston, D. B. & Bohnenstiehl, D. R. (2013). Oyster larvae settle in response to habitat-associated underwater sounds. *PLoS ONE* 8, e79337.
- Loesch, J. G., & Evans, D. A. (1994). Quantifying seasonal variation in somatic tissue: surfclam *Spisula solidissima* (Dillwyn, 1817)—a case study. *Journal of Shellfish Research*, 13, 425-431.

- Lucas, A., & Beninger, P. G. (1985). The use of physiological condition indices in marine bivalve aquaculture. *Aquaculture*, 44, 187-200.
- Maki, J. S., Rittschof, D. M. O. S., Samuelsson, M. Q., Szewzyk, U., Yule, A. B., Kjelleberg, S. & Mitchell, R. (1990). Effect of marine bacteria and their exopolymers on the attachment of barnacle cypris larvae. *Bulletin of Marine Science*, 46(2), 499-511.
- Mann, R., (1978). A comparison of morphometric, biochemical and physiological indexes of condition in marine bivalve molluscs. *Energy and environmental stress in aquatic systems*, 484-497.
- Mann, R., & Glomb, S. J. (1978). The effect of temperature on growth and ammonia excretion of the Manila clam *Tapes japonica*. *Estuarine and coastal marine science*, 6(3), 335-339.
- Mallet, A. L., Carver, C. E. A., Coffen, S. S., & Freeman, K. R. (1987a). Winter growth of the blue mussel *Mytilus edulis* L.: importance of stock and site. *Journal of Experimental Marine Biology and Ecology*, 108(3), 217-228.
- Mallet, A. L., Carver, C. E. A., Coffen, S. S., & Freeman, K. R. (1987b). Mortality variations in natural populations of the blue mussel, *Mytilus edulis*. *Canadian Journal of Fisheries and Aquatic Sciences*, 44(9), 1589-1594.
- Marsden, I. D., & Pilkington, R. M. (1995). Spatial and temporal variations in the condition of *Austrovenus stutchburyi* Finlay, 1927 (Bivalvia: Veneridae) from the Avon-Heathcote estuary, Christchurch. *New Zealand Natural Sciences*, 22: 57-67.
- Marsden, I. D., & Weatherhead, M. A. (1998). Effects of aerial exposure on oxygen consumption by the New Zealand mussel *Perna canaliculus* (Gmelin, 1791) from an intertidal habitat. *Journal of Experimental Marine Biology and Ecology*, 230(1), 15-29.
- Marsden, I. D., & Weatherhead, M. A. (1999). Shore-level induced variations in condition and feeding of the mussel *Perna canaliculus* from the east coast of the South Island, New Zealand. *New Zealand Journal of Marine and Freshwater Research*, 33(4), 611-622.

- Martel, A. (1991). Drifting and dispersal of small bivalves and gastropods with direct development. *Journal of Experimental Marine Biology and Ecology*, 150(1), 131-147.
- Mayzaud, P. (1976). Respiration and nitrogen excretion of zooplankton. IV. The influence of starvation on the metabolism and the biochemical composition of some species. *Marine Biology*, 37(1), 47-58.
- McGrath, D., P. A. King, & Gosling, E. M. (1988). Evidence for the direct settlement of *Mytilus edulis* larvae on adult mussel beds. *Marine Ecology Progress Series*. 47(1), 103-106.
- McLeod, I. M., Parsons, D. M., Morrison, M. A., Le Port, A., & Taylor, R. B. (2012). Factors affecting the recovery of soft-sediment mussel reefs in the Firth of Thames, New Zealand. *Marine and Freshwater Research*, 63(1), 78-83.
- Meder, M., A. G. Jeffs, J. Bilton, & H. Palmer. (2005). The influence of condition and feeding on the retention of mussel spat. SEA03104, NIWA Client Report.
- Menge, B.A., Daley, B.A., Sanford, E., Dahlhoff, E.P., & Lubchenco, J. (2007). Mussel zonation in New Zealand: An integrative eco-physiological approach. *Marine Ecology Progress Series*, 345, 129-140.
- Meredyth-Young, J., & Jenkins, R. (1978). Depth of settlement of two mussel species on suspended collectors in Marlborough Sounds, New Zealand. *Marine and Freshwater Research* 12(1): 83–86.
- Merino, G., Barange, M., Blanchard, J. L., Harle, J., Holmes, R., Allen, I., Allison, E.H., Badjeck, M.C., Dulvy, N.K., Holt, J., & Rodwell, L. D. (2012). Can marine fisheries and aquaculture meet fish demand from a growing human population in a changing climate? *Global Environmental Change*, 22(4), 795-806.
- Ministry of Fisheries. (2004). Introduction of seaweeds into the Quota Management System on 1 October 2005 Consultation Document. 76pp.
- Ministry of Fisheries. (2009). Green-shell mussels. Retrieved November, 2012, from <http://fs.fish.govt.nz/Page.aspx?pk=122>.

- Moal, J., Samain, J. F., Corre, S., Nicolas, J. L., & Glynn, A. (1996) Bacterial nutrition of great scallop larvae. *Aquaculture International*. 4: 215-223.
- Montgomery, J.C., Jeffs, A. G., Simpson, S., Meekan, M., & Tindle, C. (2006). Sound as an orientation cue for the pelagic larvae of reef fish and decapod crustaceans. *Advances in Marine Biology*, 51:143-196.
- Mooney, T. A., Hanlon, R. T., Christensen-Dalsgaard, J., Madsen, P. T., Ketten, D. R., & Nachtigall, P. E. (2010). Sound detection by the longfin squid (*Loligo pealeii*) studied with auditory evoked potentials: sensitivity to low-frequency particle motion and not pressure. *Journal of Experimental Biology*, 213(21):3748-3759.
- Morrison, M. A., Lowe, M. L., Parsons, D. M., Usmar, N. R., & McLeod, I. M. (2009). A review of land-based effects on coastal fisheries and supporting biodiversity in New Zealand. Ministry of Fisheries.
- Morse, A. N., & Morse, D. E. (1984). GABA-mimetic molecules from *Porphyra* (Rhodophyta) induce metamorphosis of *Hafotis* (Gastropods) larvae. *Hydrobiologia*, 116(1), 155-158.
- Morton, J. E., & Miller, M. (1973). The New Zealand Sea shore. Collins & Sons, London.
- Myrand, B., Guderley, H., & Himmelman, J. H. (2000). Reproduction and summer mortality of blue mussels *Mytilus edulis* in the Magdalen Islands, southern Gulf of St. Lawrence. *Marine Ecology Progress Series*, 197, 193-207.
- National Institute of Water and Atmospheric Research (2004) Managing New Zealand's climate; a resource and a hazard. NIWA Information Series.
- National Institute of Water and Atmospheric Research (NIWA). (2006). Greenshell™ mussels: Solving the case of the disappearing spat. *Water & Atmosphere*, 14(3), 16–17.
- Naylor, R. L., Goldburg, R. J., Primavera, J. H., Kautsky, N., Beveridge, M. C. M., Clay, J., Folke, C., Lubchenco, J., Mooney, H., & Troell, M. (2000). Effect of aquaculture on world fish supplies. *Nature*, 405(6790), 1017-1024.
- Newell, R. I. E., & Thompson, R. J. (1984). Reduced clearance rates associated with spawning in the mussel, *Mytilus edulis* (L.) (Bivalvia, Mytilidae). *Marine biology letters*, 5(1), 21-33.

New Zealand Aquaculture Council (NZAC), (2006). The New Zealand Aquaculture Strategy, The New Zealand Aquaculture Strategy, Nelson.

NZ Seafood Industry Council (NZAC), (2016). Seafood export database.

<http://www.seafoodnewzealand.org.nz/publications/export-information/>

Paine, R. T. (1971). A short-term experimental investigation of resource partitioning in a New Zealand rocky intertidal habitat. *Ecology*, 52(6), 1096-1106.

Pearce, C. M., & Scheibling, R. E. (1990). Induction of metamorphosis of larvae of the green sea urchin, *Strongylocentrotus droebachiensis*, by coralline red algae. *The Biological Bulletin*, 179(3), 304-311.

Peteiro, L. G., Filgueira, R., Labarta, U. & Fernández-Reiriz, M. J. (2010). The role of fish predation on recruitment of *Mytilus galloprovincialis* on different artificial mussel collectors. *Aquaculture Engineering*, 42, 25–30.

Petes, L.E., Menge, B.A., & Murphy, G.D. (2007). Environmental stress decreases survival, growth, and reproduction in New Zealand mussels. *Journal of Experimental Marine Biology and Ecology*, 351 (1-2):83-91.

Petes, L. E., Menge, B. A., & Harris, A. L. (2008). Intertidal mussels exhibit energetic trade-offs between reproduction and stress resistance. *Ecological Monographs*, 78(3), 387-402.

Phillips, N. E. (2002). Effects of nutrition-mediated larval condition on juvenile performance in a marine mussel. *Ecology*, 83(9), 2562-2574.

Pine, M. K., Jeffs, A. G., & Radford, C. R. (2012). Turbine sound may influence the metamorphosis behaviour of estuarine crab megalopae. *PloS ONE*, 7(12):e51790.

Powell, A, W. B. (1979) New Zealand Mollusca: Marine land and freshwater shells. Collins, Auckland.

Pridmore, R. D., Roper, D. S., & Hewitt, J. E. (1990). Variation in composition and condition of the Pacific oyster *Crassostrea gigas*, along a pollution gradient in Manukau Harbour, New Zealand. *Marine Environmental Research*, 30(3), 163-177.

- Qian, P. Y., Lau, S. C., Dahms, H. U., Dobretsov, S., & Harder, T. (2007). Marine biofilms as mediators of colonization by marine macroorganisms: implications for antifouling and aquaculture. *Marine Biotechnology*, 9(4), 399-410.
- Radford, C. A., Jeffs, A. G., Tindle, C. T., & Montgomery, J. C. (2008a). Resonating sea urchin skeletons create coastal choruses. *Marine Ecology Progress Series*, 362:37-43.
- Radford, C. A., Jeffs, A. G., Tindle, C. T., & Montgomery, J. C. (2008b). Temporal patterns in ambient noise of biological origin from a shallow water temperate reef. *Oecologia*, 156:921–929.
- Radford, C. A., Stanley, J. A., Tindle, C. T., Montgomery, J. C., & Jeffs, A. G. (2010). Localised coastal habitats have distinct under-water sound signatures. *Marine Ecology Progress Series*, 401:21–29.
- Ragg, N. L. C., King, N., Watts, E., & Morrish, J. (2010). Optimising the delivery of the key dietary diatom *Chaetoceros calcitrans* to intensively cultured Greenshell™ mussel larvae, *Perna canaliculus*. *Aquaculture*, 306(1-4), 270-280.
- Rahim, S. A. K. A., Li, J. Y., & Kitamura, H. (2004). Larval metamorphosis of the sea urchins, *Pseudocentrotus depressus* and *Anthocardia crassispina* in response to microbial films. *Marine Biology*, 144(1), 71-78.
- Rainer, J. S., & Mann, R. (1992). A comparison of methods for calculating condition index in eastern oysters *Crassostrea virginica* (Gmelin, 1791). *Journal of Shellfish Research*, 11: 55-58.
- Rasband, W. S. (2014). Image J, US National Institutes of Health, Bethesda, Maryland, USA.
- Redfearn, P., Chanley, P., & Chanley, M. (1986) Larval shell development of four species of New Zealand mussels: (Bivalvia, Mytilacea). *New Zealand Journal of Marine and Freshwater Research*, 20: 157-172.
- Reid, B. (1968). Mussel survey Hauraki Gulf and Firth of Thames 1958. Fisheries Technical Report. No. 34. 24 p. New Zealand Marine Department.

- Ren, J.S., & Ross, A.H. (2005). Environmental influence on mussel growth: A dynamic energy budget model and its application to the greenshell mussel *Perna canaliculus*. *Ecological Modelling*, 189 (3-4):347-362.
- Rogers, P. H., & Cox, M. (1988) Underwater sound as a biological stimulus. In *Sensory biology of aquatic animals*. In *Sensory Biology of Aquatic Animals* (pp. 131-149). Springer, New York.
- Sarvaiya, N., & Kothari, V. (2015). Effect of audible sound in form of music on microbial growth and production of certain important metabolites. *Microbiology*, 84(2), 227-235.
- Seed, R. (1976) Ecology. In: Bayne BL (ed) *Marine mussels: their ecology and physiology*. Cambridge University Press, Cambridge, p 13–66.
- Shafee, M. S. (1981). Seasonal changes in the biochemical composition and calorific content of the black scallop *Chlamys varia* (L.) from Lanveoc, Bay of Brest. *Oceanologica Acta*, 4(3), 331-341.
- Siddall, S. E. (1980). A clarification of the genus *Perna* (Mytilidae). *Bulletin of Marine Science*, 30: 858-870.
- Sigurdsson, J, B. (1976). The dispersal of young post-larval bivalve molluscs by byssus threads. *Nature*, 262, 386-387.
- Simpson, S. D., Radford, A. N., Tickle, E. J., Meekan, M. G., & Jeffs, A. G. (2011). Adaptive avoidance of reef noise. *PLoS ONE*, 6:e16625.
- Sim-Smith, C. J., & Jeffs, A. G. (2011). A novel method for determining the nutritional condition of seed green-lipped mussels, *Perna canaliculus*. *Journal of Shellfish Research*, 30(1), 7-11.
- Sin, F., Khoo J. & Parker, G. (1990). Enzyme variation between littoral and sublittoral populations of the green-lipped mussel *Perna canaliculus*. *Comparative Biochemistry and Physiology*, 95B, 419-422.

- Smith, P. J. (1988). Biochemical-genetic variation in the green-lipped mussel *Perna canaliculus* around New Zealand and possible implications for mussel farming. *New Zealand Journal of Marine Freshwater Research*, 22, 85-90.
- Soares, A. R., da Gama, B. A., da Cunha, A. P., Teixeira, V. L., & Pereira, R. C. (2008). Induction of attachment of the mussel *Perna perna* by natural products from the brown seaweed *Styopodium zonale*. *Marine Biotechnology*, 10(2), 158-165.
- South, P.M., Floerl, O., & Jeffs, A.G. (2017). Differential effects of adult mussels on the retention and fine-scale distribution of juvenile seed mussels and biofouling organisms in long-line aquaculture. *Aquaculture Environment Interactions*, 9, 239-256.
- Stanley, J. A., Wilkens, S. L., & Jeffs, A. G (2014). Fouling in your own nest: Vessel noise increases biofouling. *Biofouling*, 30: 837-844.
- Star, B., Apte, S., & Gardner, J.P.A. (2003). Genetic structuring among populations of the greenshell mussel *Perna canaliculus* revealed by analysis of randomly amplified polymorphic DNA. *Marine Ecology Progress Series*, 249, 171-182.
- Steinberg, P. D., de Nys, R., & Kjelleberg, S. (2001). Chemical mediation of surface colonization. *Marine chemical ecology*, 335-387.
- Stocks, J. R., Broad, A., Radford, C., Minchinton, T. E., Davis, A. R. (2012). Response of marine invertebrate larvae to natural and anthropogenic sound: A pilot study. *Open Marine Biology Journal*, 6:57–61.
- Taylor, A. C., & Venn, T. J. (1979). Seasonal variation in weight and biochemical composition of the tissues of the queen scallop, *Chlamys opercularis*, from the Clyde Sea area. *Journal of the Marine Biological Association of the United Kingdom*, 59(3), 605-621.
- Toonen, R. J., & Pawlik, J. R. (1996). Settlement of the tube worm *Hydroides dianthus* (Polychaeta: Serpulidae): cues for gregarious settlement. *Marine Biology*, 126(4), 725-733.
- Tortell, P. (1976) Investigation into settlement behaviour of mussels and the hydrology of a selected area with a view to farming the shellfish. (Doctoral dissertation, Victoria University of Wellington), Wellington.

Trottier, O., & Jeffs, A. G. (2012). Biological characteristics of parasitic *Nepinnotheres novaezelandiae* within a *Perna canaliculus* farm. *Diseases of Aquatic Organisms*, 101(1), 61-68.

Turner, E. J., Zimmer-Faust, R. K., Palmer, M. A., Luckenbach, M., & Pentcheff, N. D. (1994). Settlement of oyster (*Crassostrea virginica*) larvae: effects of water flow and a water-soluble chemical cue. *Limnology and Oceanography*, 1579-1593.

Vedel, A. (1998). Phytoplankton depletion in the benthic boundary layer caused by suspension-feeding *Nereis diversicolor* (Polychaeta): grazing impact and effect of temperature. *Marine Ecology Progress Series*, 163:125-132.

von der Meden, C. E., Porri, F., McQuaid, C. D., Faulkner, K., & Robey, J. (2010). Fine-scale ontogenetic shifts in settlement behaviour of mussels: changing responses to biofilm and conspecific settler presence in *Mytilus galloprovincialis* and *Perna perna*. *Marine Ecology Progress Series*, 411, 161-171.

von der Meden, C. E., Cole, V. J., & McQuaid, C. D. (2015). Do the threats of predation and competition alter larval behaviour and selectivity at settlement under field conditions? *Journal of Experimental Marine Biology and Ecology*, 471, 240-246.

Walne, P. R. (1976). Experiments on the culture in the sea of the butterfish *Venerupis decussata* L. *Aquaculture*, 8(4), 371-381.

Walters, L. J., Hadfield, M. G., & Smith, C. M. (1996). Waterborne chemical compounds in tropical macroalgae: positive and negative cues for larval settlement. *Marine Biology*, 126(3), 383-393.

Wear, R. G., & Gardner, J. P. (2001). Biological effects of the toxic algal bloom of February and March 1998 on the benthos of Wellington Harbour, New Zealand. *Marine Ecology Progress Series*, 218, 63-76.

Webb, S. C. & Heasman, K. G. (2006). Evaluation of fast green uptake as a simple fitness test for spat of *Perna canaliculus* (Gmelin, 1791). *Aquaculture*, 252, 305-316.

- Wei, K., Wood, A.R., & Gardner, J.P.A. (2013). Population genetic variation in the New Zealand greenshell mussel: Locus-dependent conflicting signals of weak structure and high gene flow balanced against pronounced structure and high self-recruitment. *Marine Biology*, 160(4):931-949.
- Widdows, J., Donkin, P., Salkeld, P. N., Cleary, J. J., Lowe, D. M., Evans, S. V., & Thomson, P. E. (1984). Relative importance of environmental factors in determining physiological differences between two populations of mussels (*Mytilus edulis*). *Marine ecology progress series*. Oldendorf, 17(1), 33-47.
- Wilkens SL, Stanley JA, & Jeffs AG. (2012). Induction of settlement in mussel (*Perna canaliculus*) larvae by vessel noise. *Biofouling*, 28, 65–72.
- Wood, A. R., Apte, S., MacAvoy, E. S., Gardner, J. P. A. (2007) A molecular phylogeny of the marine mussel genus *Perna* (Bivalvia: Mytilidae) based on nuclear (ITS1&2) and mitochondrial (COI) DNA sequences. *Molecular Phylogenetics and Evolution*, 44, 685-698.
- Wright, J. R., & Boxshall, A. J. (1999). The influence of small-scale flow and chemical cues on the settlement of two congeneric barnacle species. *Marine ecology progress series*, 183, 179-187.
- Young, T. (2009). Pharmacological induction of larval settlement in the New Zealand mussel *Perna canaliculus*, (Masters thesis, Auckland University of Technology), Auckland.
- Young, T. J., Alfaro, A. C., & Robertson, J. (2008) Settlement cues for the green lipped mussel. 8th Larval Biology Symposium, Lisbon, Portugal
- Zardi, G. I., Nicastro, K. R., McQuaid, C. D., Castilho, R., Costa, J., Serrão, E. A., & Pearson, G. A. (2015). Intraspecific genetic lineages of a marine mussel show behavioural divergence and spatial segregation over a tropical/subtropical biogeographic transition. *BMC Evolutionary Biology*, 15(1), 100.
- Zeldis, J., Robinson, K., Ross, A., & Hayden, B. (2004). First observations of predation by New Zealand Greenshell mussels (*Perna canaliculus*) on zooplankton. *Journal of Experimental Marine Biology and Ecology*, 311(2), 287-299.

Zhadan, P. M., Semen'kov, P. (1984). An electrophysiological study of the mechanoreceptory function of abdominal sense organ of the scallop *Patinopecten yessoensis* (Jay). Comparative Biochemistry and Physiology Part A: Physiology, 78 (4):865–870.

Zhadan, P. M. (2005) Directional sensitivity of the Japanese scallop *Mizuhopecten yessoensis* and Swift scallop *Chlamys swifti* to water-borne vibrations. Russian Journal of Marine Biology, 31(1):28-35.

Zhao, B., Zhang, S., & Qian, P. Y. (2003). Larval settlement of the silver-or goldlip pearl oyster *Pinctada maxima* (Jameson) in response to natural biofilms and chemical cues. Aquaculture, 220(1), 883-901.