The retention of juvenile *Perna canaliculus* in aquaculture

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Abstract

The green-lipped mussel *Perna canaliculus* is the most important aquaculture species in New Zealand. The poor retention of seed mussels during the early stages of aquaculture is a major problem facing the industry and a constraint to the security of production and sustainable growth of the industry. Few studies have quantified the retention of seed mussels, consequently the timing, magnitude and causes of mussel losses are a considerable research gap that hinders management of this valuable resource. The research presented in this thesis used largely field-based approaches to address the knowledge gap surrounding losses of seed mussels during early aquaculture. Spatial and temporal patterns of mussel losses and biofouling development were assessed in two separate experiments. The retention of Kaitaia spat, the most important source of mussel juveniles in New Zealand, and the effects of the substratum to which the juveniles are attached at seeding were investigated throughout early aquaculture production. The efficacy of placing adult mussels alongside seeded juveniles to prevent subsequent losses of the juveniles was assessed with a separate experiment, which included an assessment of biofouling development in relation to retention of juvenile mussels. Finally, three laboratory-based experiments tested whether losses observed in earlier experimental work could be occurring immediately upon seeding after relay to the mussel farm. Across the field experiments, losses of juvenile mussels were greatest between deployment and the first sampling point (42.9 – 72.9 %, depending on experiment), and in all experiments, losses continued throughout early aquaculture with 64.4 – 84.9 % being lost at the end of early production. Test factors, except duration of deployment, and correlation analyses suggested only small, transient effects of the factors of interest that were tested experimentally in this study. By contrast, secondary settlement behaviour of the juveniles, and to a lesser extent their mortality, was shown to have a major role in mussel losses from aquaculture. Indeed, most mussels detaching from their substratum in the laboratory experiments were alive and re-settled when given the opportunity. Size-analyses indicate that secondary settlement has an inverse relationship with size and the propensity for this behaviour reduces after 1.75 mm in shell-length. The findings of this thesis give insight into the timing, magnitude and causes of losses in the aquaculture of *P. canaliculus* to allow targeted research to further understand the biology of this species and mitigate against its loss from aquaculture to achieve the goal of efficient and sustainable development of the industry.

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Chapter 1.

General introduction

1.1. Mussel Aquaculture

The annual global production of mytilid mussels is around 1.7 million t and is valued at over 3 billion USD (FAO 2016). In many regions of the world mussel aquaculture is an important developing industry. For example, in Chile the export volume of the mussel aquaculture industry grew 34 % per annum between 2000 and 2008 and directly provides 12,000 jobs (Carrasco et al. 2014). Mussel farming in the Galician rías of Spain is valued at 340 million Euros annually and supports over 10,000 jobs. Mussel aquaculture is almost entirely reliant on natural resources such as seed-supply, food provision and sea space that can limit production (Navarro et al. 1991, Smaal 1991, Dankers & Zuidema 1995, Smaal 2002a, Strohmeier et al. 2008, Michler-Cieluch et al. 2009). Spatial and temporal regulatory constraints can also reduce the capacity of mussel aquaculture (Smaal & Lucas 2000, Smaal 2002a). In face of limiting resources and the projected growth in human population and food demand, maximising production efficiencies should be a fundamental goal of sustainable aquaculture development.

Wild-sourced mussel seed-stocks are critically important commodities upon which most mussel industries world-wide are entirely reliant (Smaal 2002a). Variations in spat supply cause significant uncertainty in mussel growing industries because they can dramatically affect the continuity of production (de Vooys 1999, Jeffs et al. 1999, Peteiro et al. 2007a, Alfaro et al. 2010). In mussel aquaculture the loss of seed mussels is a major production issue, the converse of this issue is commonly referred to as ‘retention’ and appears to have a wide variety of causes. For example, losses of the green-lipped mussel, *Perna canaliculus,* in New Zealand have been reported to be greater than 90 % in some instances (Webb & Heasman 2006, Hayden & Woods 2011). The reasons for poor retention

of mussel spat are not well understood, but have been attributed to factors that include predation (Peteiro et al. 2010), desiccation, starvation (Carton et al. 2007), handling during relay to on-growing sites (Webb & Heasman 2006), and secondary settlement processes (Buchanan & Babcock 1997, Carton et al. 2007), and are likely to include factors such as genetic variation and larval condition (Phillips 2002, Dunphy et al. 2013). Only a few studies have specifically addressed the retention of juvenile mussels and the issue is far from understood (Jeffs et al. 1999, Carton et al. 2007, Hayden & Woods 2011). Here, the research pertaining to losses of mytilid mussel seed is reviewed to assess what is known and what aspects are not understood as a route for developing future research priorities. This review pays close attention to retention of the green-lipped mussel in New Zealand, where retention refers to early juvenile mussels remaining on a substratum after recruitment in the wild or after commercial seeding of early juveniles onto growing structures.

1.2. Production cycle in mussel aquaculture

The production cycle for mussel aquaculture typically has two or three distinct stages. Firstly, early juvenile mussels, which are also commonly called spat, are captured and held *in situ* or transported to another site for an initial period of growth. Secondly, juvenile mussels are stripped from their settlement substrata and re-seeded at lower densities. Thirdly, in some production cycles, adult mussels are again stripped and re-seeded for a final grow-out period prior to harvesting (Woods et al. 2012). Not surprisingly, since mussels are usually captured and grown in the natural environment, this cycle closely aligns with the natural life cycle of the mussels (Fig. 1.1). This review concerns the retention of mussels in the first stage of the production cycle (Fig 1.1: grey triangle). Retention is likely

to be influenced by the variety of factors that cause variability in pre-settlement, settlement and post-settlement processes.

Pre-settlement factors include possible effects associated with broodstock influence on larval quality, and subsequent environmental conditions during pelagic larval development. Settlement is the transition from a planktonic larva to a benthic juvenile (plantigrade), and involves metamorphosis which encompasses a significant morphological, physiological and behavioural change. The combination of factors acting during pre-settlement and settlement will determine the number and fitness of spat entering into production, whereas factors influencing post-settlement will modify initial numbers of settlers and determine the number of mussels that continue in aquaculture production cycle, and ultimately can be harvested and sold.

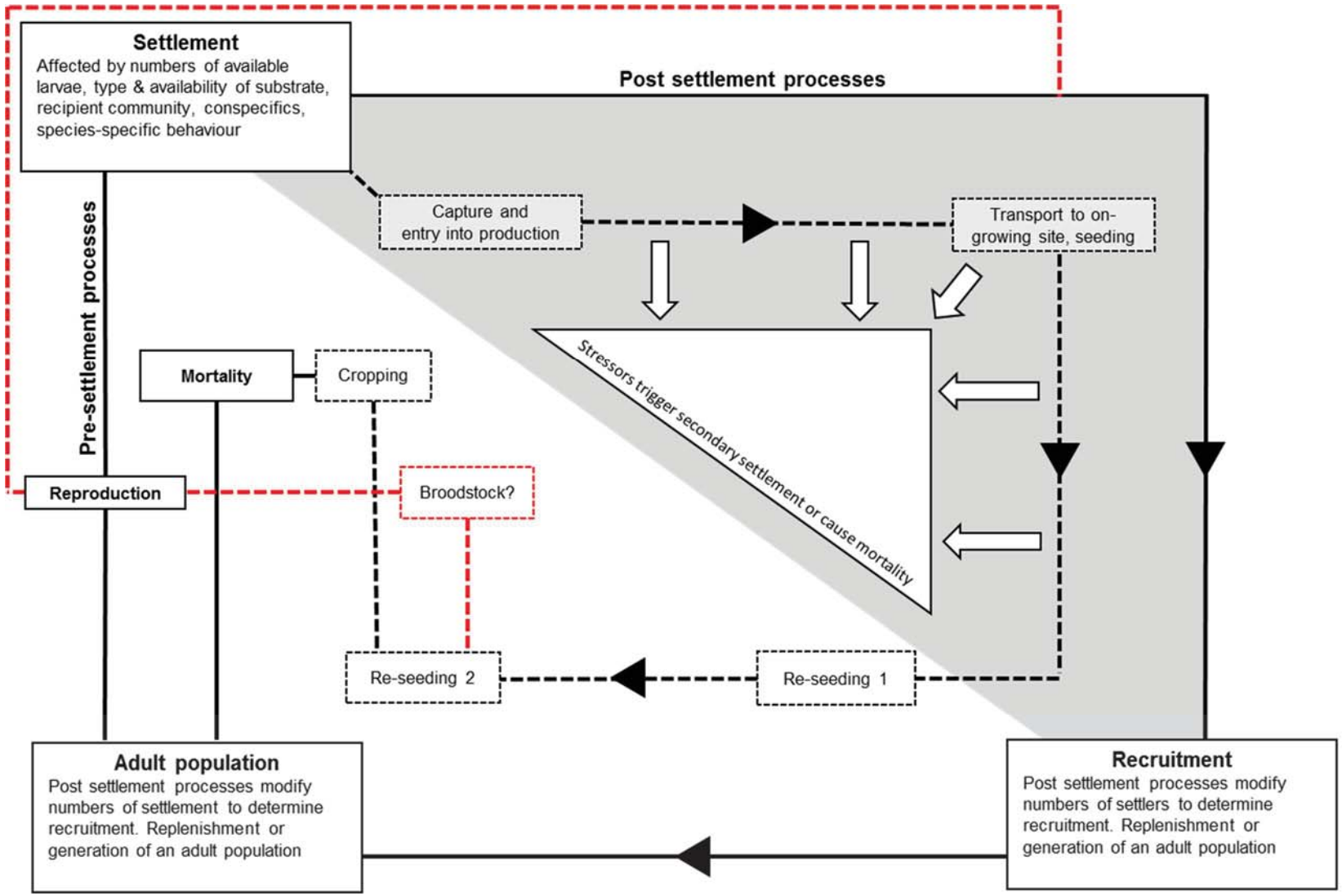
Settlement
Affected by numbers of available larvae, type & availability of substrate, recipient community, conspecifics, species-specific behaviour
Post settlement processes
Pre-settlement processes
Capture and entry into production
Transport to on-growing site, seeding
Mortality
Cropping
Stressors trigger secondary settlement or cause mortality
Reproduction
Broodstock?
Re-seeding 2
Re-seeding 1
Adult population
Post settlement processes modify numbers of settlement to determine recruitment. Replenishment or generation of an adult population
Recruitment
Post settlement processes modify numbers of settlers to determine recruitment. Replenishment or generation of an adult population

Figure 1.1. Diagram showing how a typical aquaculture production cycle (black dashed line) aligns with the life cycle of a mytilid mussel (solid black line). Causes of reduced retention are shown as white arrows. The white triangle is the number of mussels lost during the early production cycle (grey triangle) during which the effects of post-settlement processes are likely to be large. The red dashed line indicates how selective breeding and hatchery-rearing might circumvent reliance on wild sourced spat and better manage early life stages.

1.3. Sources of variation in mussel spat retention

1.3.1. Pre-settlement processes

There are many complex and interacting factors that occur before larvae have settled and metamorphosed (pre-settlement factors) that could potentially determine the performance of mussels after settlement and during their juvenile stages (Pechenik 2006). Whether and how pre-settlement factors influence the subsequent settlement, retention and survival of juvenile mussels is poorly understood, but is likely to be a source of variability in studies of mussel retention.

Genetic variation can account for traits which influence larval survival, behaviour and growth in bivalves (Jones et al. 1996b, Ernande et al. 2004, Dégremont et al. 2010, Brokordt et al. 2012) including mussels (Toro et al. 2004). Non-genetic characteristics of the parental broodstock, such as environmental influences, have been shown to affect the size and survival success of the early life stages of a variety of organisms (McCormick 1998, Marshall & Keough 2004, Marshall & Keough 2005, Sman et al. 2009) including mussels (Bayne 1972, Bayne et al. 1975, Bayne et al. 1978, Baussant et al. 2011). For example, *Mytilus edulis* eggs produced by broodstock that experience increased stress (reduced food rations and higher water temperature) prior to spawning produce smaller eggs that contain less lipid compared to eggs

from mussels under control conditions (i.e., normal temperature and rations) (Bayne 1978). Reduced egg size and lipid content can affect fertilisation success (Marshall et al. 2002) or reduce fitness through resource depletion (Wendt 1998) and increase the likelihood of mortality due to extended pelagic larval duration (PLD) (Pechenik 1999). Maternal effects may ultimately persist through to settlement and beyond and therefore potentially affect retention (O'Connor et al. 2014).

Larval experiences can determine post-settlement performance in marine invertebrates (Pechenik 2006, Podolsky & Moran 2006). Factors including delayed metamorphosis, nutritional limitation and exposure to stressors have been shown to have delayed negative impacts on fitness across life stages for bryozoans (Wendt 1996), polychaetes (Pechenik et al. 2001) and molluscs (Pechenik et al. 2002), including mussels (Phillips 2002, 2004). Larvae of *Mytilus galloprovincialis* receiving more food during development have greater subsequent growth and retention as settled juveniles in the field (Phillips 2002, 2004). Furthermore, larvae that have low food rations (500 microalgal cells ml^{-1}) during their first 20 days followed by high rations (2000 cells ml^{-1}) until settlement, settle later, are smaller and fewer are retained than those with constant high or high then low rations (Phillips 2004). These results are particularly pertinent to studies of mussel retention and indicate that the retention of wild juveniles is likely to be affected by variations in factors influencing the larvae prior to settlement. For this reason, much of this variation is likely to be eliminated in hatchery-raised juveniles where consistent production techniques are used. Therefore, hatchery production of spat offers a unique opportunity to separate pre-settlement and settlement factors from subsequent post-settlement effects. For example, hatchery-raised spat can be used to experimentally test the effects of pre-settlement factors (e.g., differential larval feeding and genotype selection) on subsequent post-settlement performance, such as growth and retention.

1.3.2. Settlement

Variations in larval settlement can dramatically affect production in mussel aquaculture and have downstream societal and environmental impacts. For example, low mussel yields following three successive years of poor spatfall led to overexploitation of natural mussel beds and mortality of shorebirds in the Dutch Wadden Sea (de Vooys 1999). The mussel aquaculture industry typically aims to maximise the capture of spat within its regional environmental and legislative constraints, such as deploying settlement material in locations with consistently higher levels of spatfall. Understanding the factors that reduce the retention of spat captured at settlement could inform better management and maximise the efficiency of the mussel industry through greater rates of return for investment.

Larval abundance and their successful settlement are critical for mussel aquaculture because spat are the primary resource for this industry. The number of settlers in a given place might affect subsequent retention through density-dependent processes, such as food competition and self-thinning (Filgueira et al. 2007, Fréchette et al. 2010, Lachance-Bernard et al. 2010) and aggregation (von der Meden et al. 2010), which in turn may influence rates of predation, biofouling or food access. Extensive studies indicate that variation in larval supply is due to a combination of population breeding output and the suitability of subsequent environmental conditions for larval success, including temperature, food availability, predator abundance, spatially advantageous hydrodynamics and the availability of settlement substrate (Underwood & Fairweather 1989, Rilov & Schiel 2006, Rilov et al. 2008, Pineda et al. 2010, Menge et al. 2011). Generally, larval delivery to suitable settlement habitat is driven by coastal oceanography including tidal currents, upwelling and eddies (Menge et al. 2003) and other hydrographic conditions that transport larvae (Pineda et al. 2009). Overall, the processes that

determine the extent and location of larval settlement are highly complex and vary in space and time across an extreme range of scales (Porri et al. 2006, Broitman et al. 2008, Navarrete et al. 2008, Dudas et al. 2009, Porri et al. 2014).

Many studies have shown that characteristics of substrata can affect rates of settlement of mytilid mussels (Bayne 1964, Hunt & Scheibling 1996, Alfaro & Jeffs 2002, Alfaro et al. 2006, Erlandsson et al. 2008, Reaugh-Flower et al. 2010, Gribben et al. 2011). Juvenile mussels have been shown to settle on complex structures such as filamentous algae (Bayne 1964, Moreno 1995, Alfaro & Jeffs 2002, Gribben et al. 2011), seagrasses (Grizzle et al. 1996) and mussel beds (McGrath et al. 1988, Reaugh et al. 2007). Chemical settlement cues exuded by substrata such as algae (Alfaro et al. 2006), their epibiota (Dobretsov 1999, Ganesan et al. 2012a, Ganesan et al. 2012b) and conspecifics (de Vooys 2003), as well as active selectivity for topographic morphology (Gribben et al. 2011), passive selection processes (Eckman 1990) or even settlement among predators as a predator swamping strategy (von der Meden et al. 2015) have been presented as mechanistic explanations of substratum selection in mussels. The vast majority of larval settlement studies quantify settlement as the number of settlers that have arrived after a given period of time, however, that number is likely to be rapidly modified by post-settlement processes prior to eventual sampling and may not reliably measure settlement (South 2016).

Settlement is a complex issue to manage with a view to increasing retention of mussels in aquaculture. For example, wild-sourced mussel spat are commonly allowed to settle at very high densities onto on-growing substrata (long-lines or mussel beds) to ensure sufficient seed mussels are retained (Meder et al. 2005b, Ramsay et al. 2008). However, allowing mussels to settle in high density could have a negative effect on retention as self-thinning processes might

lead to proportionally more spat being lost from densely seeded compared to sparsely seeded substrata (Lachance-Bernard et al. 2010, Fréchette 2012). The optimal settlement density for maximum spat retention of mytilid mussels has not been studied and is, at present, contrary to the typical goal of most spat catching operations, which is to maximise the number of spat captured.

1.3.3. Post-settlement processes

Post-settlement processes can significantly modify initial numbers of settlers and ultimately determine population structure (Hunt & Scheibling 1997, Menge 2000). Post-settlement processes determine how many mussels are retained to be harvested and sold in the aquaculture situation. Importantly, the aquaculture industry can actively manage post-settlement stocks of mussels to promote greater production (Fig. 1.1). However, post-settlement processes are problematic to study, mostly due to the difficulties associated with trying to quantify the abundance and fate of minute organisms in the field. This issue is exacerbated because it has proven difficult to determine the mechanistic cause of reduced retention, in particular the relative contribution of mortality compared to secondary settlement behaviour (Phillips 2004, von der Meden et al. 2012).

Many early juvenile bivalves, including mussels, undertake secondary migrations and settlement among substrata or sites after their primary settlement site (Bayne 1964, Buchanan & Babcock 1997, Alfaro & Jeffs 2002, Navarrete et al. 2015). Three methods of secondary settlement dispersal have been recorded; passive waterborne dispersal, mucus drifting and pedal crawling (Bayne 1964, Sigurdsson et al. 1976, Board 1983, Lane et al. 1985, Davis & Moreno 1995, Buchanan & Babcock 1997, Newell et al. 2010). Passive drifting and byssal drifting can allow juveniles to disperse over relatively large scales (10s to 100s m) while pedal

crawling is more likely to be important at centimetre scales (Cáceres-Martínez et al. 1994, Le Corre et al. 2013). For example, *M. edulis* larvae have been shown to settle on filamentous algae and subsequently re-locate as plantigrades to adult mussel beds via mucus drifting (Bayne 1964). This process has since been termed the 'primary-secondary settlement hypothesis' with supporting (Buchanan & Babcock 1997, Newell et al. 2010) and refuting evidence (Cáceres-Martínez et al. 1994, Lasiak & Barnard 1995, Reaugh et al. 2007, Navarrete et al. 2015) for many mussel species (South 2016). Regardless of the validity of the primary-secondary settlement hypothesis, the post-settlement movement of early juveniles appears to occur in most mussel species and is therefore of critical importance for studies of retention (Erlandsson et al. 2007, Navarrete et al. 2015). Since it is hypothesised that secondary settlement behaviour is responsible for significant losses of spat from aquaculture (Jeffs et al. 1999, Hayden & Woods 2011), understanding the triggers of secondary settlement migrations should be a key goal of studies of mussel retention.

Potentially, there are many causes of secondary settlement in mussels. Waves and currents might dislodge larger settlers from substrata forcing secondary settlement or alternatively causing mortality (Cáceres-Martínez et al. 1994, Erlandsson et al. 2011, Le Corre et al. 2013). Environmental changes in the primary-settlement site might trigger re-locations. For example, the senescence of epiphytic algae in seagrass beds (primary settlement site) and local hydrographic conditions act in concert to deliver secondary settlers of *M. edulis* to adult mussel beds in Maine, USA (Newell et al. 2010). Many studies implicate juvenile size as a major contributing factor to secondary settlement. For example, early juveniles of *P. canaliculus* have an inverse relationship between the degree of branching in macroalgae and the size of attached early juveniles that appears to be due to size-specific site selection rather than differential growth rates (Bayne 1964, Buchanan & Babcock 1997, Alfaro & Jeffs 2002,

Alfaro et al. 2004). In these instances, juvenile mussels might become too large to attach to fine-scale structures, such as bifurcations in the thalli of filamentous algae, and consequently might initiate relocation behaviour.

Secondary settlement could arise out of necessity if a mussel settles in a location unsuitable for recruitment, or it could be a behavioural mechanism that allows juveniles to avoid being consumed by, or competing with, conspecific adult mussels. (Bayne 1964, Alfaro 2006c, Porri et al. 2008). Regardless, mussel larvae that settle outside of adult mussel beds or on unstable substrata require secondary settlement if they are recruit into an adult population. It is unclear whether settlers are attracted by adult conspecifics through environmental cues, such as chemical odours (e.g., de Vooys 2003, Alfaro et al. 2006) or underwater sound (Wilkens et al. 2012), or settle among them as a result of hydrological processes and structural properties of the adult population. Primary settlers of *M. edulis* have been shown to settle in greater abundance among adult conspecifics compared to local canopy-forming algae indicating that there might be some adult-juvenile conspecific attraction for this species (Dobretsov & Wahl 2001). Secondary settlement of the brown mussel, *Perna perna* is greater on substrata that have been experimentally seeded with biofilms and conspecific juveniles compared to un-manipulated controls (von der Meden et al. 2010). The attraction of juvenile mussels to conspecifics and the gregarious behaviour of mussels appear to play an important role in secondary settlement and should be an important focus for studies of retention (Nielsen & Franz 1995, de Vooys 2003, Erlandsson et al. 2008). In the aquaculture situation, retention of juveniles may be reduced by secondary migration away from aquaculture structures in response to an ontogenetic trigger or waterborne cues to attempt to relocate to an adult mussel bed.

The factors that promote successful recruitment to a wild population of mussels are most often absent in aquaculture. For example, long-line mussel farms typically import spat from distant spat catching regions and deploy them in dense, single cohort aggregations that are atypical in natural populations where juveniles recruit among adults (Bayne 1964, Bertness & Grosholz 1985, Alfaro 2006b). These juvenile mussels lack the structural habitat provision of adults that might provide refuge from predation, beneficially modify biogeochemical cycling, and deliver cues that suppress secondary settlement behaviour (Bayne 1964).

Secondary settlement behaviour can be modified by variations in environmental conditions (Eyster & Pechenik 1988, Carton et al. 2007, Hayden & Woods 2011). Sub-lethal stressors such as starvation and desiccation have been shown to affect the retention of *P. canaliculus* (Carton et al. 2007). These findings have important implications for the retention of mussels in aquaculture. Firstly, periods of low food availability and desiccation are potentially common in the wild, before (e.g., intertidal stranding) or during capture (e.g., on intertidal bouchots, or in Kaitaia spat material) or the nursery phase of aquaculture production (e.g., variations in phytoplankton abundance) and might impact subsequent retention of the juvenile mussels. For example, spat might spend periods in the intertidal where they become desiccated, prior to re-immersion, capture and entry into aquaculture where they might succumb more easily to environmental stressors (e.g., variations in salinity, temperature or current velocity) or be less competitive with conspecifics or biofouling organisms due to impaired morphological or physiological factors such as a smaller body size, reduced lipid content or reduced ingestion rates (Sim-Smith & Jeffs 2011). Secondly, variables such as desiccation or food availability can vary once mussels have entered aquaculture production (Carton et al. 2007). Juvenile seed mussels are often transported great distances between catching and on-growing regions (Jeffs et al. 1999, Webb & Heasman 2006). During transport

the mussels might become desiccated, subjected to temperature fluctuations and have no opportunity to feed (Heasman 2013). Periods of desiccation and starvation force bivalves to use internal reserves which could compromise their fitness (Phillips 2004, Webb & Heasman 2006). In addition, desiccation forces the closure of the valves, increasing the accumulation of excretory products and a shift between aerobic and anaerobic respiration reducing overall resilience to stressors (Webb & Heasman 2006, Calderwood et al. 2014).

The hydrodynamic regime into which juvenile mussels are deployed for on-growing can affect spat retention (Eyster & Pechenik 1988, Alfaro 2005, 2006a, Hayden & Woods 2011). For example, there is a positive relationnship between water flow (1, 5, 10 cm s^{-1}) and the attachment success and resilience to increased water velocity (13 cm s^{-1}) of *P. canaliculus* juveniles (Alfaro 2006a). The same species was found to have a similar positive relationship between water velocity and juvenile migration with highest rates of retention and growth at 40 cm s^{-1}, the greatest velocity used by Hayden and Woods (2011). A mechanistic explanation for these results is increased byssus formation that is stimulated by water motion (Eyster & Pechenik 1988). Indeed, individuals of *P. canaliculus* that settle in high water velocity conditions secrete more byssus threads than those settling in more quiescent water (Alfaro 2006a). Another explanation for increased attachment and retention at higher water velocities might be increased delivery of food since mussels can deplete local resources in more quiescent environments (Butman et al. 1994, Dolmer 2000, Hayden & Woods 2011, Babarro & Carrington 2013).

Direct sources of mortality have also been implicated in reducing the retention of juvenile mussels (Hayden 1995, Jeffs et al. 1999, Peteiro et al. 2010). For example, excluding fish predators reduced losses of *M. galloprovincialis* from long-lines in Spain (Peteiro et al.

2010). Predation by diving eider ducks has led to growers initiating a range of bird deterrents to reduce losses of mussels (Dionne et al. 2006). Predators also induce phenotypic anti-predatory responses that could facilitate survivorship or secondary settlement behaviour (Côté 1995, Leonard et al. 1999, Cheung et al. 2004). For example, the green mussel, *Perna viridis*, secretes more byssus threads when exposed to water in which a predator or damaged conspecific has been housed compared to seawater alone allowing it to withstand predatory attack (Cheung et al. 2004). Other sources of direct mortality come from pathogens (Domeneghetti et al. 2014) and environmental stressors such as food depletion (Carton et al. 2007), extreme temperature events (Myrand & Gaudreault 1996), or pollutants (Beaumont & Budd 1984).

Determining the causes of post-settlement losses is a considerable research priority because they cost the mussel aquaculture industry many millions of dollars. Many of the factors that have been shown to affect the distribution of mussels on natural shorelines have not been studied in the context of aquaculture to determine their potential impact on the number of captured mussels that make it to market. While some studies have shown that responses such as secondary settlement behaviour of juvenile mussels, or their deaths, have been triggered by events or conditions earlier in life (Phillips 2002, 2004), the impacts of these are likely compounded by sequential causes of losses. For example, 20 % of out-planted mussels might die or migrate due to desiccation during transport, but a further 60 % might migrate away from the culture substrata later, due to an ontogenetic shift in behaviour. Quantifying the relative effect of such disparate processes as predation and secondary settlement behaviour should be a goal of studies of mussel retention as some factors may have greater impact and be more easily managed than others. More importantly, through the understanding gained from research

that focuses on the mechanistic causes of spat loss, aquaculture might mitigate and manage this costly phenomenon.

1.3.4. The effects of biofouling

Mussels in culture must interact with biofouling organisms that are known to impact on condition and survival of adult shellfish (McKindsey et al. 2007, Fitridge et al. 2012). Biofouling can negatively affect shellfish aquaculture production in five key ways: (1) physical damage by boring or encrusting organisms, (2) mechanical interference to shell functioning, (3) competition for resources, (4) environmental modification, such as reduced food availability, and (5) increased weight of biofoulers that increases aquaculture production costs (Fitridge et al. 2012). However, few studies have considered the impact of biofouling organisms during the early mussel production cycle. Since many juveniles are lost during early production, a critical research priority is to determine the cumulative and relative effects of the various factors impacting the retention of juvenile mussels during this period.

The artificial substrata used to collect spat for on-growing provide habitat for a wide range of biota that potentially compete with the juvenile seed mussels for the occupation of space and obtaining food. In mussel aquaculture, spat either settle as part of the successional sequence of colonisation on unoccupied artificial surfaces, such as spat collecting ropes, or are settled en masse by mussel farmers and deployed into the wild. Early colonists in the successional sequence are often microscopic organisms (bacteria and diatoms in biofilms) or propagules of macroscopic taxa (including mussels) that modify the subsequent pattern of settlement and determine community dynamics (Bao et al. 2007, von der Meden et al. 2010, Ganesan et al. 2012b, Wahl et al. 2012, Yang et al. 2014, Lacoste & Gaertner-Mazouni 2015).

Many species recruit to the artificial substrata used for the aquaculture of mussels because these surfaces provide unoccupied space in an environment where this resource is otherwise in limited supply. Consequently the resulting communities can be dynamic in their structure and function (Maki & Mitchell 2003) and attain considerable biomass (Woods et al. 2012). Spat can experience trickle or pulse settlement of other species or conspecifics (Wrange et al. 2010, Sams & Keough 2012, Fletcher et al. 2013a), followed by competition for food and space (Woods et al. 2012, Fletcher et al. 2013b) and modifications to their local environment, such as reduced dissolved oxygen and water-flow, due to increasing biomass of the growing biofouling organisms (De Nys & Guenther 2009). Surprisingly, given the purported high financial impact of biofouling on aquaculture, there appears to have been little focus on the mechanisms by which biofouling organisms affect the juvenile stages of cultured mussels. It is likely that biofouling organisms play a significant role in the 50-90 % losses of spat experienced by some mussel aquaculture operations (Hayden & Woods 2011).

It is not known whether biofouling triggers secondary settlement in mussels, but it can be envisioned that this is a likely response to the settlement and subsequent population growth of a competing biofouling organism (Buchanan & Babcock 1997). Many invertebrates (barnacles, ascidians, hydroids) that are susceptible to overgrowth by colonial ascidians do not settle among their recruits (Grosberg 1981). Since some mytilid mussels can actively select where to settle, it is perhaps likely that they can also actively select when and where to re-locate (Bayne 1964, Gribben et al. 2011).

The stocking density of mussels can affect the rate of biofouling they subsequently experience. For example, individuals of the fouling sea squirt *Ciona intestinalis* are fewer and smaller at medium (~237-246 m^{-1}) and high (~329-527 m^{-1}) stocking densities of *M. edulis*

compared to low stocking densities (~103-116 m^{-1}) although this does not appear to affect mussel retention (Ramsay et al. 2008). There are two key ways in which stocking density of juvenile mussels might affect rates of biofouling. Firstly, sparsely stocked substrata would likely have more primary space creating an opportunity for biofoulers to settle. Secondly, when they are seeded at high density, the weight of growing mussel spat and their biofoulers might become too heavy and fall away from the mussel line, thus more primary space becomes available to opportunistic biofoulers later in the production cycle (Ramsay et al. 2008).

1.4. Retention of *Perna canaliculus* in New Zealand

The New Zealand mussel industry is based on the culture of the endemic green-lipped mussel, *Perna canaliculus* (Jeffs et al. 1999, Dawber 2004, Fig. 1.2). Production of this mussel is ~100,000 t and worth NZ$311 m in export sales and NZ$30 m in domestic sales and accounts for 73 % of the total revenue from aquaculture in New Zealand (Aquaculture New Zealand 2016) . The mussel farming industry is heavily reliant on wild caught spat that wash ashore on Ninety Mile Beach in the far north of the country attached to algae and other debris (Hickman 1976, Alfaro et al. 2010, Fig. 1.2a, b). The algae and other flotsam, to which the juvenile mussels are attached, are collected from the beach, sorted, refrigerated and transported to the mussel farming regions, a process that can take 24 – 72 h. These beach-cast spat account for ~80 % of the mussel seed supply for aquaculture. The remaining ~20 % of total production is sourced from the wild at catching sites near the main mussel farming regions (Jeffs et al. 1999). A small proportion of production (~5 %) is provided by hatchery-reared larvae, although this technology is predicted to grow in importance in the future (Ragg et al. 2010). Once procured, mussel spat are transported to mussel farms for on-growing mostly in the Marlborough Sounds in the South Island and the Hauraki Gulf in the North Island (Fig. 1.2). Scientific research on

P. canaliculus, at least since the late 1970s, has been driven by questions pertaining to its aquaculture and chiefly concern aspects of its physiology and ecology or its use by humans for food and pharmaceuticals (Jeffs et al. 1999, Alfaro et al. 2010, Tuckey et al. 2013, Young et al. 2015, Nguyen et al. 2018).

Retention of juvenile *P. canaliculus* is an enormous problem for the New Zealand aquaculture industry because of great losses from spat lines following deployment (Jeffs et al. 1999, Webb & Heasman 2006, Hayden & Woods 2011), however, the majority of primary data sources documenting this phenomenon remain unpublished and inaccessible (Jeffs et al. 1999). Despite this, the number of studies where the retention of *P. canaliculus* is a response variable or the rationale for studying other variables is greater for this species compared to other commercially cultured mytilids, such as *M. edulis* and *M. galloprovincialis* (Table 1.1). This is likely because the New Zealand mussel industry has now grown to a size at which it is by constrained by its high reliance on one temporally variable seed resource (Alfaro et al. 2004, Alfaro et al. 2010). Furthermore, the collection of spat has recently begun to be regulated under a Quota Management System (QMS) limiting the overall supply from this natural source and thereby making its efficient use of greater importance (Jeffs et al. 2018). The efficient use of spat is also critical to the success of hatchery operations whose production output is limited by hatchery size and the high cost of producing larvae.

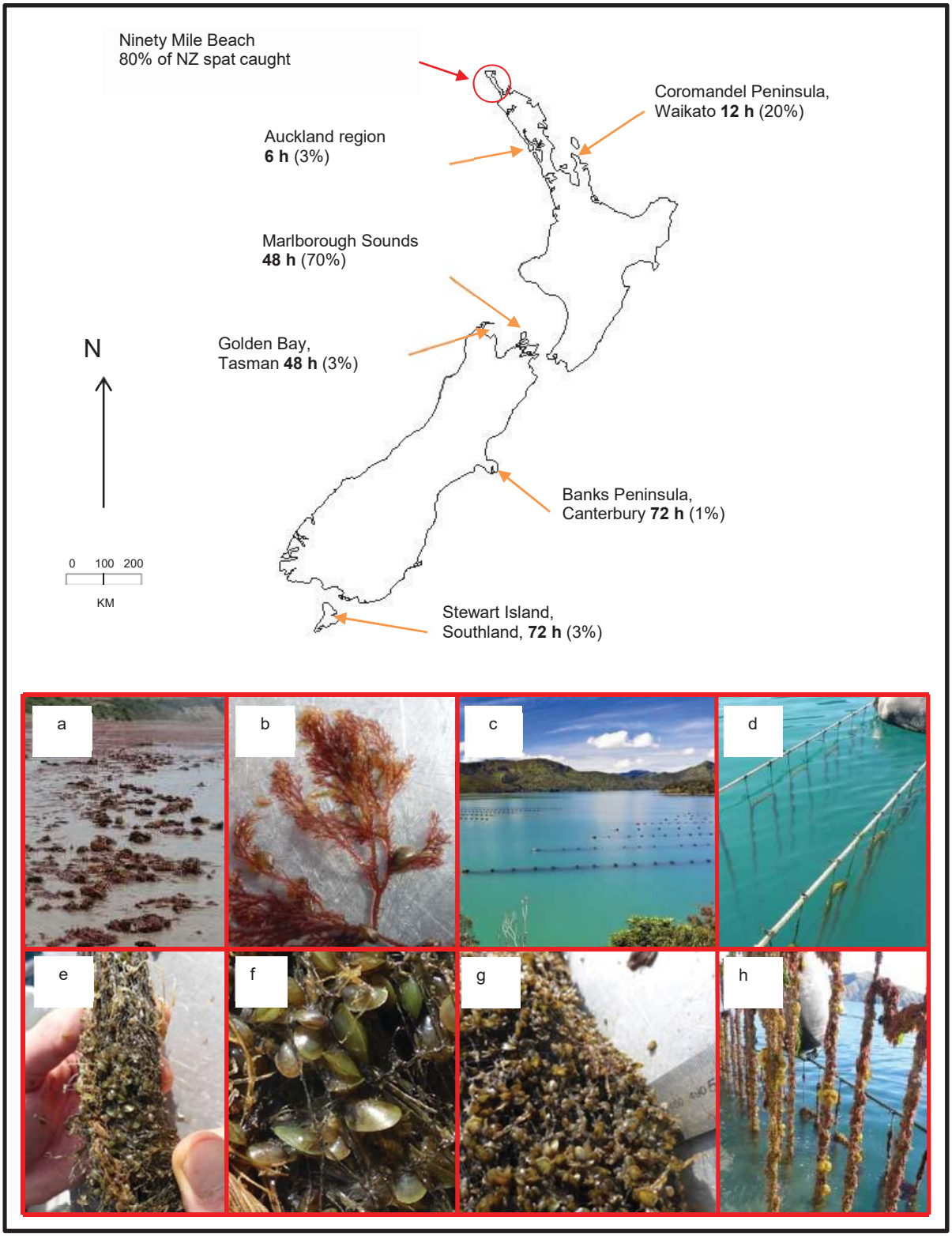
Ninety Mile Beach
80% of NZ spat caught
Coromandel Peninsula,
Waikato **12 h** (20%)
Auckland region
6 h (3%)
Marlborough Sounds
48 h (70%)
N
Golden Bay,
Tasman **48 h** (3%)
Banks Peninsula,
Canterbury **72 h** (1%)
0 100 200
KM
Stewart Island,
Southland, **72 h** (3%)
a
b
c
d
e
f
g
h

Figure 1.2. New Zealand showing the major mussel farming regions (orange arrows) and the primary spat catching region (red circle). The maximum time taken between time of capture at Ninety Mile Beach and deployment in a farming region is emboldened. Numbers in parentheses indicate the regional contribution to total production (Aquaculture New Zealand 2016). The panel shows (a) algal wrack containing spat on Ninety Mile Beach; (b) juvenile *Perna canaliculus* attached to drift seaweed (*Ceramium apiculatum*); (c) an extensive mussel farm in Marlborough Sounds; (d) spat typically deployed on leaded dropper ropes between two backbone lines; (e) hatchery-reared spat on a dropper rope 4 months after deployment (note fibrous settlement rope on the left); (f) a close up of *P. canaliculus* spat (note outer sock used to hold settlement ropes, algae and spat alongside the dropper rope); (g) spat attached to the outer sock; and (h) biofouling on spat dropper ropes.

Determining whether juvenile mussels are lost due to mortality or secondary settlement behaviour is difficult because of the challenges involved in quantifying these processes in the field. However, there is much evidence to support the theory that losses of *P. canaliculus* juveniles might occur through secondary settlement behaviour. Firstly, early and late plantigrades have been shown to be highly mobile post-settlement, employing byssal drifting and pedal walking to move among substrata (Buchanan & Babcock 1997, Alfaro 2005, Carton et al. 2007). Secondly, size-specific affinities of juveniles of this mussel species for substrate characteristics such as surface morphology (Gribben et al. 2011), algal branching pattern (Alfaro & Jeffs 2002), chemical composition (Alfaro et al. 2006) and epibiota (Ganesan et al. 2012a, Ganesan et al. 2012b), imply that migrations among habitats are common and driven by distinctive, advantageous exploratory behaviour (Alfaro 2006a). Thirdly, the conclusions of laboratory (e.g., Gribben et al. 2011) and field-based studies (e.g., Alfaro & Jeffs 2002) are strongly supported by studies that show that large, post-metamorphic *P. canaliculus* settle onto newly deployed mussel lines or artificial collectors (Alfaro & Jeffs 2003, South 2016).

Presumably, these juveniles must be undergoing secondary settlement and have likely transferred from intermediary substrata. Therefore, there is a strong case for research into the triggers of secondary settlement behaviour in *P. canaliculus*.

The process of seeding mussel ropes uses mesh socks to hold either the natural substrate material (mostly macroalgae) or the fibrous coir rope used in hatcheries that contain the spat against a leaded dropper rope that includes in numerous plastic filaments onto which the juvenile mussels are intended to relocate. The outer socks as well as the original substrate to which the spat are attached to when deployed degrade after a matter of months leaving a stock of mussels attached to the remnants of the sock and the dropper rope (Fig. 1.2d – g). The notion that secondary settlement behaviour is detrimental to the New Zealand mussel industry is paradoxical because nearly all transfer of spat, whether from beach-cast algae or hatcheries, requires out-planted juveniles to migrate from their initial settlement site or their site at capture

(e.g., algae or fibrous coir rope to the dropper rope on which they will subsequently be on-grown). If spat remain attached to either natural substrate (wild spat) or coir rope (hatchery spat) they are vulnerable to loss. For example, a juvenile *P. canaliculus* attached to a coir rope will be lost if it does not reattach following the disintegration of the coir. Alternatively, if that mussel migrates away from the seaweed to the outer sock, it is again vulnerable to loss along with the degradation of the sock (Fig. 1.2g). This is a highly complex problem to manage. Optimising secondary settlement to on-growing ropes requires knowledge of the triggers of secondary settlement and the determinants of substrate selection.

Table 1.1 Studies of secondary settlement for commonly studied mytilid mussels in the wild and in aquaculture. The retention column indicates whether "retention" was the focus or rationale of study.

Genus	Species	Range	# studies	Aquaculture	Wild	Retention+
Mytilus						
	edulis	Europe, USA	7		7	
	galloprovincialis	Australia, Europe, NZ, South Africa, USA	5	1	4	1
	spp.	USA*	1		1	
Perumytilus						
	purpuratus	Chile, Peru, Argentina	1		1	
Semimytilus						
	algosus	Chile, Peru, Argentina	1		1	
Perna						
	canaliculus	NZ	10	6	4	5
	perna	South Africa	8		8	
	viridis	Asia-Pacific region, Japan, Americas	1		1	

Retention+ indicates whether understanding retention issues was rationale for study
* Settlers of different species within *Mytilus* are difficult to separate using morphological characteristics

Predation by fish is a potentially significant cause of poor retention of spat. For example, mussel farmers in the Hauraki Gulf do not deploy spat in late summer due to the predation impacts of snapper (Steve Greene, North Island Mussels Ltd, pers. comm.). The spotted wrasse, *Notolabrus celidotus*, has been observed to completely strip mussel farm ropes seeded with > 5 mm juvenile mussels in the Marlborough Sounds (Hayden 1995). Predators might also have more indirect negative effects on mussel retention. For example, odours from crabs and starfish have been shown to reduce retention of *P. canaliculus* on experimental substrata in the laboratory (Meder et al. 2005a). It is not known whether cues from predators are a significant trigger of secondary settlement during early aquaculture production.

The goal of optimising spat management practices has driven much of the research pertaining to retention of *P. canaliculus* (Table 1.2). Many factors (e.g., desiccation, starvation, temperature spikes) could impact retention and can occur either during early life stages or transport to on-growing sites (Webb & Heasman 2006, Carton et al. 2007). Impacts from early stressors can manifest themselves physiologically as reductions in bodily glycogen reserves (Sim-Smith & Jeffs 2011) and reduced activity (Webb & Heasman 2006), and alterations to metabolic profiles (Young et al. 2015). As a result, work has focused on methods of assessing larval or spat health with the aim of determining the likely viability of a mussel crop and developing best management practices through grading and pricing (Webb & Heasman 2006, Sim-Smith & Jeffs 2011). Determining condition at the time of seeding would perhaps allow better management practices, such as feeding nutritionally poor spat prior to seeding, improving transport practices, or avoiding out-planting compromised spat which would produce poor retention (Carton et al. 2007). It seems more likely that, since spat supply continues to be a limiting factor in the New Zealand mussel industry, spat fitness at seeding might be better used to predict losses and establish baseline viability data to allow more accurate estimates of retention due to factors occurring post-seeding.

1.5. Conclusions

Despite considerable research into the biology and ecology of mytilid mussels, factors that might affect the retention of mussels in aquaculture remain poorly understood largely because of few focused studies and the complexity of the phenomenon. The complex life history of mytilid mussels introduces inherent problems for their management that are beyond the direct control of mussel farmers but could potentially determine the ultimate level of retention of mussel spat. Surprisingly, more focus appears to have fallen on pre-settlement and settlement

factors, perhaps due to the research into hatchery-rearing capabilities or the reliance on high numbers of spat to supply the aquaculture industry. A research focus on the retention of spat on farms might offer insights into the timing, magnitude and causes of mussel loss to inform more effective approaches to managing seed mussels and reducing their losses.

1.6. Research aims

Understanding the factors affecting mussel retention in the early production cycle is critical to improving the overall efficiency of aquaculture industries based on mytilid mussels. The research presented in this thesis aims to assess losses of juvenile mussels in the field throughout the early stages of mussel aquaculture to determine when losses occur, how many mussels are lost, and the underlying causes. There are four experimental research chapters that assess losses of mussels in the context of wider research questions that are described below.

Research presented in Chapter 2 aims to quantify losses of seed-mussels and the development of biofouling assemblages at different spatial and temporal scales to determine the role of the recruitment and development of the biofouling organisms in the retention of seed mussels. Hatchery-reared mussels were used in Chapter 2 to eliminate the possible effects of wide-variations in size, fitness and other factors that are associated with wild spat from confounding results and to provide field-based assessments of the efficiency of hatchery-reared juveniles.

Research presented in Chapter 3 aims to assess losses of Kaitaia spat – the primary source of seed mussels in New Zealand – in the early aquaculture situation. Kaitaia spat are attached to a wide variety of substrata therefore experiments assessed retention among substrata with different characteristics (fine, coarse and mixed macroalgae and typical deployments of Kaitaia spat). The effect of the degradation of these substrata, and the

remaining attached mussels on the chemical environment close to the mussel ropes was assessed by bringing the ropes from the field into the laboratory where they could be monitored. Possible relationships between the conditions near the ropes and retention were assessed, as was retention in the laboratory situation.

Research presented in Chapter 4 aims to test hypotheses about the relationship between adult and juvenile mussels by deploying adult mussels alongside hatchery-reared juveniles to determine whether this seeding approach could be used as a tool to mitigate against spat-losses. The effects of density of adult mussels, including whether living mussels or just their shells were seeded alongside the juveniles, on the retention of the juveniles and the accumulation of the biofouling assemblage were tested. The results of this chapter have been published as "Differential effects of adult mussels on the retention and fine-scale distribution of juvenile seed mussels and biofouling organisms in long-line aquaculture" in Aquaculture Environment Interactions, Volume 9: 239 – 256, 2017.

Research presented in Chapter 5 aims to assess whether the losses of mussels recorded in earlier chapters could occur immediately at the time of deployment into the aquaculture environment. Three experiments using Kaitaia spat (Experiments 1 & 2) and hatchery-reared juveniles (Experiment 3) used a short period of immersion (2-min) to assess the quantity of mussels that can detach from their substrata in typical seed-deployments. The size and status of the retained and detached mussels was quantified and used to assess patterns of secondary settlement vs mortality in each of the three experiments.

Each of the chapters in the thesis which includes the results of original research (i.e., Chapters 2, 3, 4 & 5) is presented as a self-contained manuscript to facilitate ongoing publication of the work in peer reviewed scientific journals. The work presented in each of the

chapters is linked by the common research mission which has been introduced in this current chapter and the significance of the collective research findings are discussed in the General Discussion (Chapter 6).

Table 1.2. Table of studies pertaining to the retention of *Perna canaliculus*

Author(s)	Research aims	Retention studied?	Mussel size	Test Factors (levels)	Response variables	Key finding(s)
Hickman (1976)	Investigate the possibility of using beach-cast spat attached to algae material as a source of seed for mussel aquaculture	Yes	2 mm at seeding	Different aquaculture substrata. Polypropylene ropes, coir, trays. Two sites	Length-frequency data	High mobility noted. Mussels did not attach to aquaculture substrata, mostly they attached to each other or drifted away. Over-crowding reduces growth.
Hayden (1995)	1. Temporal and spatial variation in larval abundance. Effects of wind, temperature and salinity on larval distribution	No	0.2 - 0.3 mm	Years (n = 4), days (n = > 21), sites (n = 4 - 6), depths (n = 2)	Larval abundance 1000 L-1	Positive effect of depth on larval abundance, increased variability and larger mussels at depth. Site-site variation with environmental conditions. No consistent patterns among years, larvae appear aggregated, rather than homogeneously dispersed. Daily weather patterns important.

2. Spatial and temporal variation at settlement, effects of spacing of spat catching ropes, and recruitment (settled mussels > 1.5 mm after 8 and 12 wks)	No	Settlers < 1.5 mm Recruits > 1.5 mm	Years (n = 4), days (n = 7), weeks (n = 4), depths (5 - 20 m in 5-m increments). Spacings = 1 m, 130 mm and 65 mm	Mean number 20 cm^{-1} mussel rope	Consistent settlement among years, 0 -235.5 spat 20 cm^{-1} rope. High variability among sites/depths. Greater settlement on ropes placed closer together. Recruitment ranged from 0.6 - 2179.3 spat 20 cm^{-1} rope after 8 weeks. High variability among years sites and depths.
3. Determining relationships between larvae, settlers and recruits	No	Settlers < 1.5 mm Recruits > 1.5 mm	Correlation analyses of number of larvae, settlers and recruits per site/depth	Number 20 cm^{-1} mussel rope	Near consistent positive relationships between larval abundance and settlement among years across sites. Finer-scale analysis indicates site, depth temporal variability in relationship between larvae and settlement. Frequent strong relationships between settlement and recruitment at 8 weeks, but not at 12 weeks.

	4. Effects of fish predation and operational artefacts (moving ropes and handling) on the retention of juveniles	Yes	9.35 mm, 14.5 mm, 0.86 mm, 1.2 mm, 0.5 - 5 mm	Caged vs uncaged and cage controls. Disturbed (removed from the water and left on deck for 5-minutes) vs undisturbed. Multiple sites were assessed	Number 20 cm^{-1} mussel rope	Reduced numbers of mussels after 24 h due to migrations to cages. Big mussels > 5 mm strongly affected by predation. Big mussels within them eaten first. No effect of predation on small mussels. No effects of disturbance across sites.
Buchanan and Babcock (1997)	1. Substrate affinities of primary and secondary settlers	No	< 0.5, 0.5-5, > 5 mm	Substratum type (algae, hydroids, adult mussels), site (rock or sand) Size-frequency distributions within each substratum	Number /size-class (%)	More < 0.5 mm settlers on filamentous algae/hydroids. More > 0.5 mm settlers on mussels.
	2. Sinking velocity of settling mussels	No	0.6-8, 1.5-2mm	State (dead, alive and active, non-active), Size-classes	Sinking time (s^{-1})	Mucus drifting slows descent by 50 %. Mucus drifting occurred in mussels sized up to 5 - 6 mm.
	3. Settlement selection of primary settlers	No	~250-300 µm	Substratum type (*Amphisbetia bispinosa*, *Corallina officinalis*, *Laurencia botryoides*, *Melanthalia abscissa* and *Pachymenia himantophora*)	Settlement (#) g^{-1} substratum	Primary settlers prefer filamentous substrata.

Alfaro and Jeffs (2002)	Effects of substratum morphology on rates of settlement	No	< 0.5, 0.5 1, 1-1.5, 1.5-2, > 2 mm	Substratum type (coarse, medium, fine branching) for natural and artificial substrata; Size frequency distributions within each substratum	Settlement (#) cm^{-2} substratum	More < 0.5 mm settlers on fine-branching substrata. More > 0.5 mm settlers on coarse branching substrata.
Foote (2003)	Effect of nutrition history (1 month) on retention	Yes	0.5 - 3 mm	Feeding regime (14500, 29000 and 58000 cells d^{-1})	Retention (# & %)	Low (14500 cells) feeding reduced retention.
Alfaro and Jeffs (2003)	Variability in mussel settlement onto ropes placed in the wild	No	<490, 500-990, >1000 µm	Site (2), Depth (2, 10 18 m), Month (Jul, Aug, Sept 2000) Analyses repeated for each size class	Settlement (#) 0.5 m^{-1}	Strong spatial-temporal variability. Mussels >1mm settle deeper.
Alfaro et al. (2004)	The spatial and temporal variability in density and distribution of mussel larvae and spat associated with bottom-drifting algae	Indirectly	(< 0.49, 0.5–0.99, 1.0–1.49,1.5–1.99, > 2.0 mm	Month, Across-shore (in-shore and offshore), and Along-shore (S=south, M=Mid, and N=North)	Number mussels kg^{-1} algae (ww), size distribution (percent) among size-classes	Consistent pattern of increasing mussel size and increasing degree of branching in algal substrata in samples collected progressively northward along Ninety Mile Beach, and through time.

Meder et al. (2005a)	Effects of chemical odours from predators on retention	Yes	1.5 - 3 mm	Predator (control, conspecific, crab and starfish)	Retention (%),	Negative effects of crab and starfish odours.
Alfaro (2005)	Effect of water flow and oxygen concentration on settlement of larval (hatchery reared) and juvenile (from subtidal reefs and surf zones) *P. canaliculus*	No	0.5 - 3 mm	3 water flow regimes (~1, 5, and 10 cm/s) and 3 oxygen concentrations (~6, 9, and 12 mg/L)	Settlement (%), Mortality (%)	Mortality greatest at low water flows and decreased with increasing water flows for larvae (~55–5 %) and juveniles (~70–50 % for subtidal, 23–4 % for surf zone mussels), increasing oxygen decreased the mortality of the larvae, but not the juveniles. Larval settlement increased with water flow. Larval counts on filaments suggest that exploratory behaviour occurs at low and medium water flows, but not at high water flows. Oxygen concentration did not affect juvenile settlement.
Meder et al. (2005b)	Effects of starvation on retention	Yes	0.4 - 1 mm	Starvation (0, 4 & 8 d^{-1} starvation pre-experiment). Starvation (0 and 8 days feeding starvation during experiment)	Retention (%), growth	No effects of starvation or feeding regime on retention, 8 days starvation pre- and during experiment reduced growth.

Alfaro (2006b)	1. Mussel concentrations in seawater	No	< 0.25, 0.25-0.5, > 0.5 mm	Site (n = 3), Date (n = 4) within each size - class	Number 20 L^{-1} seawater	High variability among sites and dates. Large (> 0.5 mm) mussels found in water column.
	2. Short-term (daily) effects of natural and artificial substrata on settlement and recruitment.	No	< 0.5, 0.5-2, > 2 mm	Site (n = 3), Date (n = 5), Substrate (algae, artificial (mesh), bare rock), Habitat (low shore algal zone, upper shore mussel zone) within each size-class	Mussels (#) cm^{-2} substratum	Majority of primary settlers (<0.5 mm) settle away from adult mussel beds High variability among sites and dates, but not substrata for large (> 0.5 mm) mussels.
	3. Long-term (monthly) effects of natural and artificial substrata on settlement and recruitment.	No	< 0.5, 0.5-2, > 2 mm	Site (n = 3), Date (n = 5), Substrate (algae, artificial (mesh), bare rock), Habitat (low shore algal zone, upper shore mussel zone) within each size-class	Mussels (#) cm^{-2} substratum	Low rates (15 cm^{-2} d^{-1}) of secondary settlement (> 0.5 mm) in mussel beds.
Alfaro (2006a)	Effect of water motion and air bubbles on attachment	Indirectly	3-5 mm	Water-flow (1, 5 & 10 cm s^{-1}), Air bubbles (+/-), Time (before/after high flow period)	Attachment (#), Byssal threads (#)	Positive relationship between flow-rate and attachment. Both increased flow and air bubbles increased number of byssal threads.

Alfaro et al. (2006)	Influence of chemical cues from macroalgae in the settlement of *P. canaliculus* larvae	No	Not stated	Field experiment - extracts from algae: *Scytothamnus australis*, *Melanthalia abscissa*, *Corallina officinalis*, *Carpophyllum maschalocarpum, Plocamium costatum*, *Osmundaria colensoi*, *Gigartina alveata* and control. Laboratory experiment - extracts from *S. australis*, *M. abscissa* and control plates	Number of mussel settlers plate^{-1}	Field experiment - differences between control and blank plates versus treatment plates, but no differences among the five algal extract plates. Laboratory experiment - no difference between *S. australis* and *M. abscissa* extracts plates, but differences between *S. australis* and control plates and *M. abscissa* and control plates.
Webb and Heasman (2006)	1. Effects of lethal & sublethal agents	Indirectly	0.7 - 3 mm	(1) Agents (seawater, 10 % ethanol & 10 % ethanol + 30 mins seawater), (2) Agents (seawater, 10 % ethanol & heat (+60°C), freshwater)	Proportion stained	Increased stress increases staining - less resilience implied.
	2. Effects of stagnant & nutrient loaded water		0.7 - 3 mm	Exposure time (4, 8, 16, 24, 36, 48 ,56, 69 h; controls at 0 and 69 h)	Proportion stained	Increased stress increases staining - less resilience implied.
Carton et al. (2007)[1]	1. Retention of plantigrades with different attachment histories	Yes	0.5 - 3 mm	Attached vs not attached following flume trial	Retention (%)	Greater retention of plantigrades that had previously attached.

	2. Retention of plantigrades with different migration histories		0.5 - 3 mm	Walked* vs not walked following walking trial	Retention (%)	No differences between groups that had and had not shown walking behaviour prior to the experiment.
	3. Effects of starvation on retention		0.5 - 3 mm	Feeding regime (6, 4, 2 and 0 days a priori)	Retention (%)	Starvation had negative effects on retention that reduced as rations were increased.
	4. Effects of temperature and desiccation on retention		0.5 - 3 mm	Temperature (control, 30 °C temperature spike [2 hours]) and desiccation (0, 5 hours)	Retention (%)	Desiccation reduced retention compared to control, but not 30 °C temperature spike.
Van de Ven (2007)	Effects of *Notomithrax minor* (crab) on survival, growth and byssus of *P. canaliculus*	Yes	4.86 mm,16.8 mm	Density (12 & 3), sex (m/f) and size (small/large) of crabs. 8 and 12-week deployments. 2-sites were assessed	Retention (#)	Reduced retention and greater byssus production with increased numbers of crabs. Decreased retention more pronounced in small spat. Crabs ate 56.43 spat $crab^{-1}$ h^{-1}.

Alfaro et al. (2010)	Temporal patterns of beach-cast spat/algae	No	Various	Wind speed, wind direction, tidal range water temperature, swell height. Data over a 9-yr period	Spatfall events and amounts	Offshore winds increase probability of spatfall events, increased wave height reduced this effect. 1 - 16 spatfall events mo^{-1} and 480 - 103340 kg mo^{-1}. Inter annual variation driven by ENSO.
Gribben et al. (2011)	1. Effects of non-polar cues on settlement	No	200-230 µm	Species (extracts from 8 algae and 1 hydroid), Concentration (2, 20 µg cm^{-2})	Settlement cm^{-2}	No effect of non-polar cues.
	2. Effects of polar cues on settlement		200-230 µm	Species (extracts from 8 algae and 1 hydroid), Concentration (2, 20 µg cm^{-2})	Settlement cm^{-2}	No effect of polar cues.
	3. Effects of non-polar cues and time on settlement		200-230 µm	Species (extracts from 4 algae), Concentration (5, 25 & 50 µg cm^{-2}) and Time (6 & 24 h-1)	Settlement cm^{-2}	No effect of non-polar cues, more settlement after 24 hours.

	4. Effects of non-polar cues and surface morphology		200-230 µm	Surface (rough & smooth), Species (4 algae) and Time (6 & 24 h^{-1})	Settlement cm^{-2}	Significantly increased settlement with surface complexity. Significant negative effects of some algae that disappeared over time.
Hayden and Woods (2011)	Effect of water velocity on retention	Yes	3-4 mm	Velocity (40, 10, 4, 1 cm s^{-1})	Retention (#), growth	Lower migration and higher retention at 40 cm s^{-1}.
South (2016)	Effects of timing, duration and place of deployment on numbers of mussels in artificial units of habitat (AUH)	Yes	< > 500 µm	Timing; days 1 - 7 during a 7-day period. Duration; 1 - 7 days, Location; 0.5 - 2 m apart on a rocky reef	Number of mussels AUH^{-1}, Mussels AUH^{-1} day^{-1}	High variability in settlement between days and durations. Mussels > 500 µm can settle and out-migrate from AUHs at small (daily) temporal scales.

(South et al. 2017)	Effects of adult mussels on juvenile retention, distribution and biofouling	Yes	~ 1mm - ~30 mm	Treatment (+/- 5 or 20 live or shells of adult mussels and a standard dropper rope as a control	Number of retained mussels rope^{-1}	Adding mussels or shells didn't enhance juvenile retention, which was less than 20 % over 5 months. Most losses (46 %) had occurred after 1 month. Adult mussels did increase the number of many potentially problematic biofouling organisms, and the number of seed mussels moving to dropper ropes.

[!]These experiments are also presented in Foote (2003), [*]Walked = juveniles that exhibited pedal-crawling (walking behaviour) prior to experimentation

Chapter 2.

The role of biofouling in the loss of seed mussels in aquaculture

2.1. Introduction

Mussel aquaculture is a globally important industry that is expanding in many parts of the world, with total global production of mussels increasing by over 10 % in the last decade to 1,975,093 t in 2015 (FAO 2016). Shellfish aquaculture relies on the successful seeding and subsequent survival of juveniles. Managing stocks of juveniles can be highly inefficient, with many individuals being lost during the early stages of production (Hayden & Woods 2011, Capelle et al. 2016a). One cause of losses of juveniles could be the development and growth of assemblages of biofouling organisms that compete for resources, such as attachment space and particulate food material (Lesser et al. 1992, Woods et al. 2012, Fletcher et al. 2013b), cause dislodgements due to excessive biomass on the aquaculture structures (Forrest 2017), or mechanically damage the shellfish and reduce their value (Dunham & Marshall 2012).

In New Zealand, the mussel aquaculture industry is entirely based on the culture of the endemic green-lipped mussel, *Perna canaliculus* Gmelin 1791, and is the most valuable seafood export sector accounting for > 70 % of total export revenue from aquaculture (Aquaculture New Zealand 2016). Biofouling is a major issue for mussel aquaculture in New Zealand. For example, the natural over-settlement by wild blue mussels, *Mytilus 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 (Woods et al. 2012, Forrest & Atalah 2017). The invasive colonial ascidian *Didemnum vexillum* can reduce the density of larger juvenile *P. canaliculus* (20 – 40 mm) via direct impacts such as displacement or mortality (Fletcher et al. 2013b). Biofouling can also affect production by reducing the efficiency of harvesting. For example, the green macroalga,

Cladophora ruchingeri, can clog mussel harvesting equipment delaying the harvesting process (Pochon et al. 2015). Most studies have focused on the effects of biofouling development on adult mussels. However, the deployments of seed mussels can experience significant development of biofouling assemblages although their possible impacts on seed mussels early in the production cycle are not well understood (South et al. 2017).

Massive losses of juvenile mussels, which are commonly referred to as poor 'retention', have been reported early in aquaculture production (< 6 months) and potentially relate to a variety of factors including transport-induced stress (Carton et al. 2007), water velocity in the early production site (Hayden & Woods 2011), fitness (Webb & Heasman 2006), nutritional quality (Sim-Smith & Jeffs 2011) and predation (Hayden 1995). Given the small size of *P. canaliculus* in early production it is possible that many components of the biofouling assemblage will have adverse effects through competition for food, predation, pre-emption of primary space, direct physical damage, or adverse modifications to the local environment. Biofouling colonisation and development is a successional process (Scheer 1945, Wahl 1989, Bloecher et al. 2013). Consequently, early colonisers tend to be transitory and will eventually be replaced by a more persistent assemblage of organisms (Scheer 1945, Connell & Slatyer 1977). Therefore, it is likely that the identity of biofouling organisms that might be important during early production differs from the more commonly studied taxa that tend to occur later in the production cycle, such as ascidians, hydroids and mussels (Ramsay et al. 2008, Fitridge & Keough 2013, Fletcher et al. 2013b, Forrest & Atalah 2017). For example, early colonists on suspended mussel ropes can be dominated by amphipods (South et al. 2017).

Sea-based culture of *P. canaliculus* in New Zealand uses long-line techniques with dropper-ropes suspended from a string of surface floats (Jeffs et al. 1999). While dropper-ropes provide significantly increased surface area for the attachment of mussels, they also provide for the settlement and recruitment of biofoulers into a mussel farm (Lesser et al. 1992). Furthermore, the substrata used to hold juvenile mussels can modify patterns of biofouling due to their composition and complexity (Filgueira et al. 2007, Carl et al. 2012a, South et al. 2017). It is possible that as biofouling builds up on the dropper rope it decreases the retention of juveniles via direct negative interactions such as predation and smothering, or by triggering secondary settlement behaviour, which is considered to be an important cause of mussel losses in New Zealand aquaculture (Jeffs et al. 1999).

In this study, two separate deployments of seed *P. canaliculus* were regularly assessed to determine the development of the biofouling assemblage and the corresponding retention of seed mussels.

2.2. Materials and methods

2.2.1. Study site and source of juvenile mussels

This study was undertaken at two mussel farms located around 1 km apart, in outer Pelorus Sound, Marlborough, New Zealand (40° 57' 18"S, 174° 3' 39"E). The juvenile mussels used were hatchery-reared by SpatNZ Ltd (Nelson, New Zealand). Hatchery-reared juveniles have shared developmental histories, such as common parentage, *ad libitum* access to food and managed densities, and are therefore less likely to show wide variation in their response to experimental conditions than wild juveniles. In the hatchery, larval mussels were settled onto

fibrous coir ropes (coconut fibre ropes c. 10 mm in diameter) that are transported to the field and deployed by suspending them in the water column from surface floats, after the coir has been held alongside a lead-cored polypropylene nursery dropper rope with a mesh outer sock, forming a nursery rope. Juvenile seed mussels are usually deployed for around four to six months and are reseeded into grow-out sites once they reach > 10 mm in shell length. This study assessed retention of the juvenile seed mussels and biofouling development during two such deployments of seed mussels.

2.2.2 Experimental Design

To quantify the retention of juvenile *P. canaliculus* and the development of biofouling during the early production cycle, a section of nursery rope was obtained immediately after seeding, on-board a commercial seeding vessel, and cut into 190 short lengths (45 cm) of rope, hereafter termed 'experimental ropes'. Ten experimental ropes were haphazardly sampled following the seeding process to provide an estimate of the density and size of the juvenile seed mussels at the outset of the experiment. Fifteen replicate experimental ropes were attached about 10 cm apart to each of 12 frames that were then haphazardly assigned to the two mussel farm sites (i.e., six frames per mussel farm site). The frames (100 × 90 cm) consisted of two wooden vertical rods (20 × 10 × 90 mm) intersected horizontally at the top, middle and bottom by three cylindrical nylon rods (10 mm in diameter × 100 mm in length). Three of the six frames at each site were haphazardly assigned to either 2 m or 5 m depth treatments. One 2 m and one 5 m frame were deployed in pairs at each of three 'areas' within each site, at the end of three consecutive mussel farm lines that were spaced about 10 m apart. These areas were included to assess possible within-site variations in retention. The 5 m frames were attached below the

2 m frames. Frames were weighted to align them vertically in the water column, so that the positioning of the experimental ropes was equivalent to typical production nursery ropes.

The experiment was carried out on two occasions to assess possible seasonal differences in biofouling. The first experiment (Experiment 1) was deployed on 7 July 2015 and retrieved on 30 December 2015, a duration of 176 days (ca. 25 weeks), and the second experiment (Experiment 2) was deployed on 30 December 2015 and retrieved on 16 May 2016, a duration of 136 days (ca. 20 weeks). It was initially planned to deploy the frames into their designated sites and areas on the same day, but this was impossible in Experiment 1 due to poor sea conditions. Therefore, all frames were deployed 1 m apart within one area at Site 1, but at their assigned depths. After 15 days (when it was possible to access the farm site again) the frames were retrieved, and three replicate experimental ropes were taken from each of three frames at both depths to assess initial biofouling and retention (18 ropes in total). The experimental ropes were then distributed among the originally intended spatial treatments (i.e., sites, areas and depths). In Experiment 2, poor weather and logistical constraints reduced the number of sample occasions from an intended five to three. At the end of the experiments an additional three extra ropes for each frame were sampled, where their remaining numbers were sufficient (see Table 2.1 for a summary of the number of experimental ropes collected).

The recovery of three experimental ropes from each frame was undertaken on five occasions for experiment 1 at 15, 44, 84, 136 and 178 days and for experiment 2 on three occasions at 8, 77 and 138 days. On each sampling occasion, experimental ropes were retrieved from each frame by removing the frames from the sea and cutting the cable ties on three

haphazardly selected ropes. Individual experimental ropes were then stored in plastic bags, chilled and returned to the laboratory for analysis.

Both experiments experienced frame losses due to severe weather conditions and possibly damage from farm maintenance operations over the experimental period. Experiment 1 lost 7 % of the total number of frames available for sampling (pooled over durations) and Experiment 2 lost 33 % of available frames. For example, for Experiment 2, one set of 2 and 5 m frames was lost at Site 2 between January and March, and another set between March and May 2016. Three additional replicate experimental ropes, where they were available, were collected from each of the remaining frames at the end of the experiment (see Table 2.1 for a summary of the number of frames and experimental ropes collected).

In the laboratory biofouling organisms were removed from each experimental rope over a 250 μm sieve. Biofouling organisms were identified to the highest possible taxonomic resolution (usually to family level or higher) and grouped into 'mobile invertebrates', 'algae' and 'sessile invertebrates'. Mobile invertebrates were counted whereas the dry weight (g dw after 48 h at 50 °C) of algae and sessile invertebrates were quantified. Sessile serpulid worms were difficult to remove from the experimental ropes and were therefore counted and analysed with the mobile invertebrates. When some mobile invertebrates were highly abundant (i.e., > 1000) they were subsampled using a Fulsom sample-splitter, while for less abundant taxa all individuals were counted. All wild settlers of *P. canaliculus* (identifiable by smaller size than the hatchery juveniles) were also counted.

2.2.3. Statistical analyses

Retention at the beginning of mussel aquaculture

Initial losses of seed mussels over the shortest deployment durations in this study were assessed using ANOVA to compare the mean number of mussels at the start of the experiment to the mean number remaining attached to the ropes after 15 days for Experiment 1 and after 8 days for Experiment 2. The starting density of mussels, as well as the density at each site and depth combination, were considered as separate levels of the analyses. The assumptions of normality and homogeneity of variance of the data were tested with Shapiro-Wilks' and Levene's tests, respectively. Data were log (x + 1) transformed where necessary to meet the assumptions of ANOVA. Tukey's tests were used to determine pairwise differences among means following significant results in the overall ANOVAs.

Duration	Site 1		Site 2	
(days)	2 m	5 m	2 m	5 m
		Experiment 1		
15	3 (9)	3 (9)		
44	3 (9)	3 (9)	3 (9)	3 (9)
84	3 (9)	3 (9)	3 (9)	3 (9)
136	3 (9)	3 (9)	2 (6)	2 (6)
178	3 (15)	3 (15)	2 (9)	2 (9)
		Experiment 2		
8	3 (9)	3 (9)	3 (9)	3 (9)
77	2 (6)	1 (3)	2 (6)	2 (6)
138	2 (12)	1 (6)	1 (6)	1 (6)

Table 2.1. Sampling schedule for Experiments 1 & 2 showing number of frames and replicates (in brackets) collected after each duration.

Retention throughout early production

The mean numbers of juvenile mussels that remained on the lines over the full duration of the two field experiments were compared with factorial permutational analysis of variance (PERMANOVA). Two analyses were run for Experiment 1, because day 15 lacked replication at the site scale. First, a model testing the effects of Duration (random; 15, 44, 84, 136 and 178 days) and Depth (fixed; 2 levels 2 & 5 m) was used at Site 1 only. Second, day 15 was omitted, to balance the design allowing variation among sites to be tested. The effects of Duration (random; 44, 84, 136 and 178 days), Site (fixed: Sites 1 & 2) and Depth (fixed: 2 & 5 m) on the number of juvenile *P. canaliculus* were tested. Together, this sequential approach allowed the early retention of juveniles, and then the effects of sites to be assessed. The full model, Duration (random: 8, 77 and 138 days), Site (fixed: Sites 1 & 2) and Depth (fixed: 2 & 5 m), was used in Experiment 2. Finally, within-site spatial effects on the mean number of mussels were assessed when full datasets were available (i.e., days 44 and 84 in Experiment 1, and day 8 in Experiment 2) with nested PERMANOVAs testing for the fixed effects of Site (Sites 1 & 2) and Depth (2 m & 5 m), and the random effects of Area (Areas 1 -3), nested within sites.

The assumption of homogeneity of variance of the data was tested with permutational analysis of multivariate dispersions (PERMDISP) using distances to the centroid of the data cloud, a procedure that is equivalent to Levene's test (Anderson et al. 2008). Data were log (x + 1) transformed where necessary to meet the assumptions of homogeneity. Analyses were done on similarity matrices based on Euclidean distance using 9999 permutations to obtain p-values. Type III sums of squares were used to address differences in the number of

experimental ropes among and within factors (Table 1). Pairwise PERMANOVAs were used to determine differences among levels of a factor, following significant main tests.

Development of the biofouling assemblage

The development of the biofouling assemblage in terms of variations in taxonomic richness (all taxa, mobile invertebrates, algae and sessile invertebrates) and structure of assemblages of mobile invertebrates (square root transformed data), algae and sessile invertebrates (no data-transformations) were assessed using the PERMANOVA designs described above via univariate and multivariate models, based on matrices of Euclidean distances and Bray-Curtis similarities, respectively. The assemblage structures of algae and sessile invertebrates were assessed only at Days 84, 136 and 178 for Experiment 1, and Days 77 and 138 for Experiment 2 due to their almost complete absences earlier in the experiments that led to many undefined similarities in the Bray-Curtis matrices. Both experiments experienced losses of experimental ropes resulting in unbalanced designs (see 2.2 and Table 2.1 for details) that can affect the power of PERMANOVA analyses if data dispersion varies among levels of a factor (Anderson & Walsh 2013). Therefore, PERMDISP was used to assess data-dispersion when significant differences in assemblage structure were detected by PERMANOVA (Anderson et al. 2008). Two-dimensional multi-dimensional scaling (MDS) plots were used to visually assess and present the multivariate data-sets. Similarity of Percentages (SIMPER) was used to identify taxa making important contributions to differences among factor-levels.

Development of dominant biofouling taxa

The distributions of four key biofouling taxa were assessed due to their importance in driving differences in assemblages (the amphipods Ischyroceridae, *Paradexamine* spp., and *Caprella* spp.) and, in the case of *Mytilus galloprovincialis*, because it is considered to be a problematic fouling organism in the region (Forrest & Atalah 2017, South et al. 2017). The total biomasses of algae and sessile invertebrates were also analysed individually. The abundances and dry weights of key biofouling taxa were assessed using PERMANOVA and pairwise comparisons.

Biofouling as a predictor of **Perna canaliculus** *retention*

Canonical analysis of principal coordinates (CAP) was used to predict the abundance of juvenile *P. canaliculus* as a function of the biofouling assemblage for each duration separately. CAP uses principle coordinate axes to characterise multivariate assemblages (in the form of a Bray-Curtis dissimilarity matrix) into a single axis (CAP 1), which is then correlated to the variable of interest (*P. canaliculus* abundance). The number of principal coordinate axes (m) used in each analysis was selected to avoid over-parameterisation of the model. Analyses were done on square-root (counts of mobile invertebrates) or untransformed data (dry weights of algae or sessile invertebrates). Dispersion weighting was applied to sites, or sites and depths, based on where PERMANOVA indicated significant variation in the response variables relating to assemblage structure. Where CAP analyses were significant, the data were sorted into three groups corresponding to low, medium and high CAP values. SIMPER was used to assess the species contributing 50 % of the differences among assemblages associated with low and high CAP values to give an indication of the identity of important taxa and the direction of their relationship with *P. canaliculus* abundance. In addition, the number of *P. canaliculus*

remaining on experimental ropes was correlated to the number of the key biofouling taxa using Spearman's rank correlations. Explained variation in terms of numbers of mussels was estimated by multiplying the standard deviation of *P. canaliculus* data by the r^2 value following significant CAP or correlation analyses.

2.3. Results

2.3.1. Retention of juvenile Perna canaliculus

Retention in the initial stages of early production

The mean (± SE) number of juvenile *Perna canaliculus* deployed at the start of the field experiments was 805.9 ± 82.9 45 cm $^{-1}$ in Experiment 1, and 1122.4 ± 158.3 45 cm^{-1} in Experiment 2 (Fig. 2.1). There were significant losses of juveniles after the shortest deployments in this study, with reductions of 42.9 in Experiment 1 after 15 days ($F_{2, 25} = 9.72$, $p = 0.0008$), and 49.1 % in Experiment 2 after 8 days, respectively ($F_{3, 41} = 10.20$, $p = 0.0001$, Fig. 2.1). On average, these losses of juveniles amounted to 2.9 and 6.1 % per day across treatments for Experiments 1 and 2, respectively.

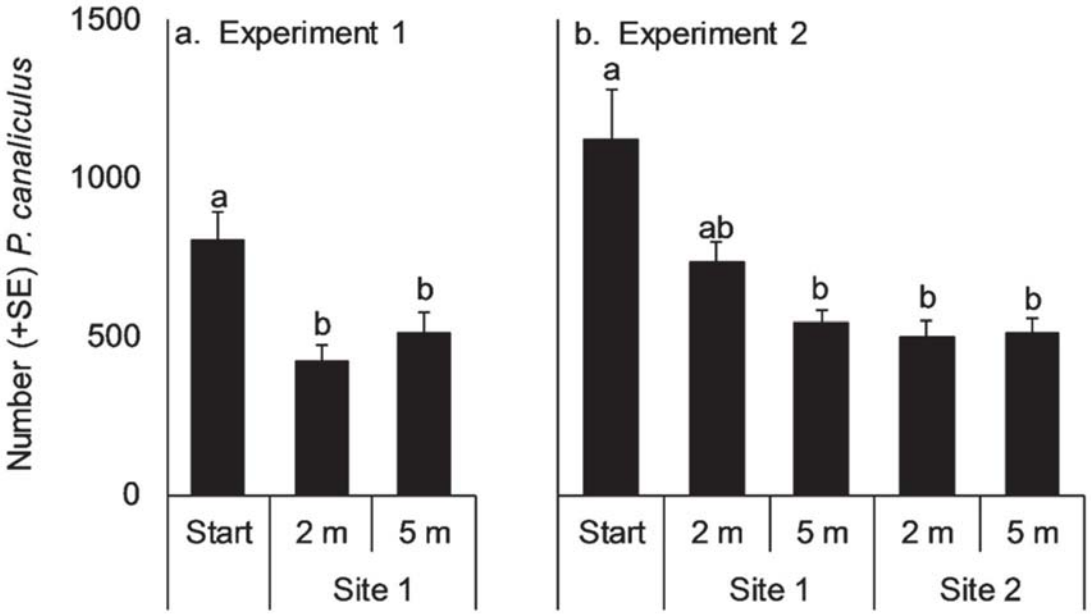

Figure 2.1. The retention (mean number per 45 cm of experimental rope + SE) of juvenile *Perna canaliculus* in Experiments 1 (a) and 2 (b) after deployments at two depths (2 and 5 m) for periods of 15 and 8 days, respectively. Start = mean number of mussels at the start of the experiment (n = 10). In Experiment 1, n = 9 for each depth. In Experiment 2, n = 9 for each site-depth combination. The same letters above the bars indicate homogenous groups following significant one-way ANOVAs and Tukey's post-hoc tests.

Retention throughout early production

The number of *P. canaliculus* in Experiment 1 declined by 22.5 % from 460.2 ± 41.9 per rope section at 15 days, to 321.0 ± 24.2 at 84 days (0.3 % per day), after which retention stabilised for the remainder of the early production period ($F_{3,\ 134} = 4.82$, p = 0.0029, Fig. 2.2a, Table 2.2). The mean number of retained mussels at the end of Experiment 1 was 287.0 ± 19.0. In Experiment 2, the number of juvenile *P. canaliculus* declined across sites and depths by 38.4 % from 571.5 ± 28.8 at 8 days, to 351.5 ± 25.7 at 77 days (0.5 % per day), and again by 30.8

% to 243.3 ± 17.2 at 138 days (0.2 % per day; $F_{2, 75} = 51.65$, p = 0.0001, Fig. 2.2b, Table 2.2). Compared to the starting density, 64.4 and 78.3 % of seeded mussels were lost over the entire duration of Experiments 1 and 2, respectively. There was also some variation between sites in Experiment 2, with more *P. canaliculus* being retained on experimental ropes at Site 1 (639.3 ± 32.9 vs. 503. 7 ± 42.2) after 8 days and more at Site 2 (273.1 ± 21.0 vs. 198.5 ± 24.7) after 136 days. There were no effects of depth on the mean number of *P. canaliculus* that were retained on the experimental ropes over the course of the experiment. The mean number of retained *P. canaliculus* did not vary among areas within sites when durations with full data-sets were analysed for either of the two experiments.

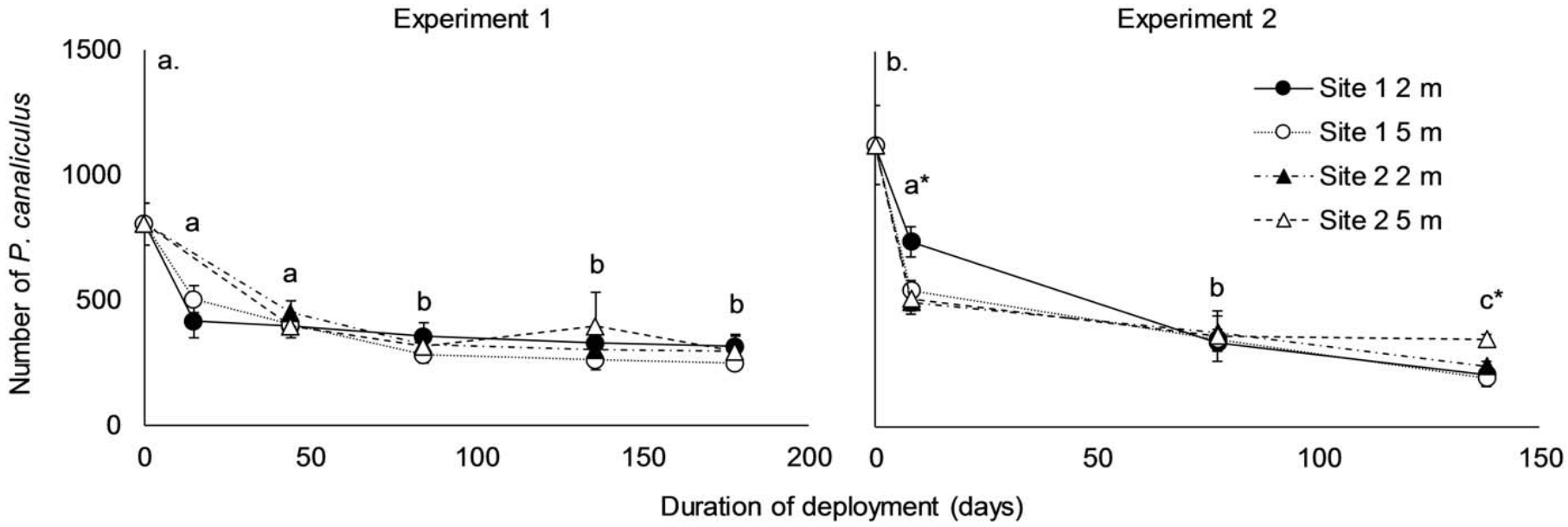

Figure 2.2. The retention (mean number per 45 cm of experimental rope + SE) of juvenile *Perna canaliculus* in Experiments 1 (a) and 2 (b) at two depths (white symbols) at two sites (Site 1 = circles, Site 2 = triangles) in the outer Marlborough Sounds sampled at varying intervals over 178 and 136 days in Experiments 1 and 2, respectively. Abundance at the start of the experiments is shown, but not incorporated in the statistical analyses of these data (see 3.1 and Fig. 2.1). The same letters above the symbols indicate homogenous groups following significant effects of Duration regardless of site or depth. Differences between sites at individual sampling durations are indicated by an asterisk.

Table 2.2. Summary of p–values from PERMANOVA statistical tests performed in this study. Full results tables are provided in Appendix 2.

			Taxonomic richness					Assemblage structure			Key biofouling taxa					
	Source	f/r	*Perna*	All taxa	Mob.	Algae	Ses.	Mob.	Algae	Ses.	Isch	Para	Cap	*Mytilus*	Algae	Ses.
								Experiment 1								
Sites 1 & 2	Duration	r	**0.0029**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
	Site	f	0.1405	0.7024	0.5017	0.0577	0.1296	0.0743	0.1016	0.0728	**0.0032**	0.5045	0.2037	0.0953	0.1924	0.1565
	Depth	f	0.1512	0.8944	0.6958	0.3672	0.8402	0.3206	0.1123	0.2405	0.3708	0.7065	0.8113	0.1488	0.7246	0.1403
	Du×Si	r	0.906	0.1529	0.2887	0.2872	0.8084	**0.0001**	**0.0021**	**0.0001**	0.8661	**0.0001**	**0.0001**	0.0511	**0.0093**	0.044
	Du×De	r	0.9038	**0.0107**	0.0894	0.2213	0.1577	**0.0204**	**0.0022**	0.5368	0.8709	0.0126	**0.0205**	0.0375	0.0773	0.6422
	Si×De	f	0.2392	0.5449	0.0056	0.8719	0.8399	0.2553	**0.0437**	0.2766	0.1134	0.1152	0.2664	0.0118	0.0742	0.5549
	Du×Si×De	r	0.5488	0.5211	0.9999	0.6874	0.1878	0.288	0.8418	0.459	0.8301	0.5978	0.1885	0.7422	0.5139	0.9848
Site 1	Duration	r	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**			**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
	Depth	f	0.4921	0.5467	0.7667	0.5318	0.5493	0.6623			0.1109	0.5141	0.594	0.9235	0.0605	0.2671
	Du×De	r	0.2845	0.3313	0.44	0.2835	0.8166	**0.0129**			0.8473	0.1323	**0.03**	0.2356	0.2972	0.6321
								Experiment 2								
Sites 1 & 2	Duration	r	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
	Site	f	0.8177	0.4953	0.713	0.1911	0.9901	**0.035**	0.4353	0.3456	0.2562	0.7726	0.3472	0.2736	0.6437	0.328
	Depth	f	0.8274	0.4632	0.8826	0.216	0.4945	0.9644	0.4655	0.6019	0.2241	0.2364	0.6913	0.1366	0.3248	0.5601
	Du×Si	r	**0.0021**	**0.0004**	**0.0063**	0.0177	0.1327	**0.0001**	0.1735	**0.004**	0.0107	**0.0001**	**0.0002**	0.4574	0.0509	0.0343
	Du×De	r	0.2095	**0.0437**	0.331	0.1826	**0.0447**	**0.0086**	0.3917	0.3056	0.1055	0.4168	**0.0018**	0.9676	0.7901	0.5816
	Si×De	f	0.142	0.7216	0.3096	0.6831	0.3471	0.7272	0.6649	0.3332	0.2086	0.362	0.5401	0.1881	0.6939	0.4505
	Du×Si×De	r	0.5267	0.2325	0.4113	0.7378	0.6273	0.1099	0.5675	0.8132	0.2185	0.3574	0.1693	0.9783	0.6571	0.907

f/r: fixed/random. **Bold** text indicates significance at $p < 0.05$ or < 0.01 where variances were heterogeneous

Duration is the number of days experimental ropes and juvenile seed mussles were deployed in the field.

Mob. = mobile invertebrates, Ses. = sessile invertebrates, Isch = Ischyroceridae, Para = *Paradexamine* spp., Cap = *Caprella* spp.

2.3.2. Biofouling in early **Perna canaliculus** *aquaculture*

Development of the biofouling assemblage

Overall, 89 taxa (86 in Experiment 1, 61 in Experiment 2) were found on the experimental ropes recovered from the two sites. Abundant taxa included amphipods, ascidians, bryozoans, mussels and algae (Appendix 1). The effect of Duration was an important source of variation in all analyses compared to the spatial factors Site and Depth that only occasionally had significant effects. Total taxonomic richness of biofouling increased over time, plateauing in Experiments 1 and 2 after durations of 136 days (33.0 ± 0.7 taxa) and 77 days (26.7 ± 0.6), respectively (Fig. 2.3a & b, Table 2.2). Mobile invertebrates accounted for 70 and 79.5 % of the taxa found in Experiments 1 and 2, respectively, with their richness plateauing after 134 and 77 days in Experiments 1 and 2 (Fig. 2.3c & d, Table 2.2). Algae comprised 14.67 and 7.16 % of total taxonomic richness and exhibited significant rises and falls in their occurrence during both experiments (Fig. 2.3e & f, Table 2.2). Algal richness peaked in the middle of the Experiment 1 (136 days) and Experiment 2 (77 days). The richness of sessile invertebrates accounted for 15.3 % (Experiment 1) and 13.3 % (Experiment 2) of the total taxonomic richness and generally increased through time, plateauing after 134 (6.3 ± 1.3) and 77 (4.7 ± 1.5) day durations in Experiment 1 and 2, respectively (Fig. 2.3g & h, Table 2.2).

2. The role of biofouling in seed-loss

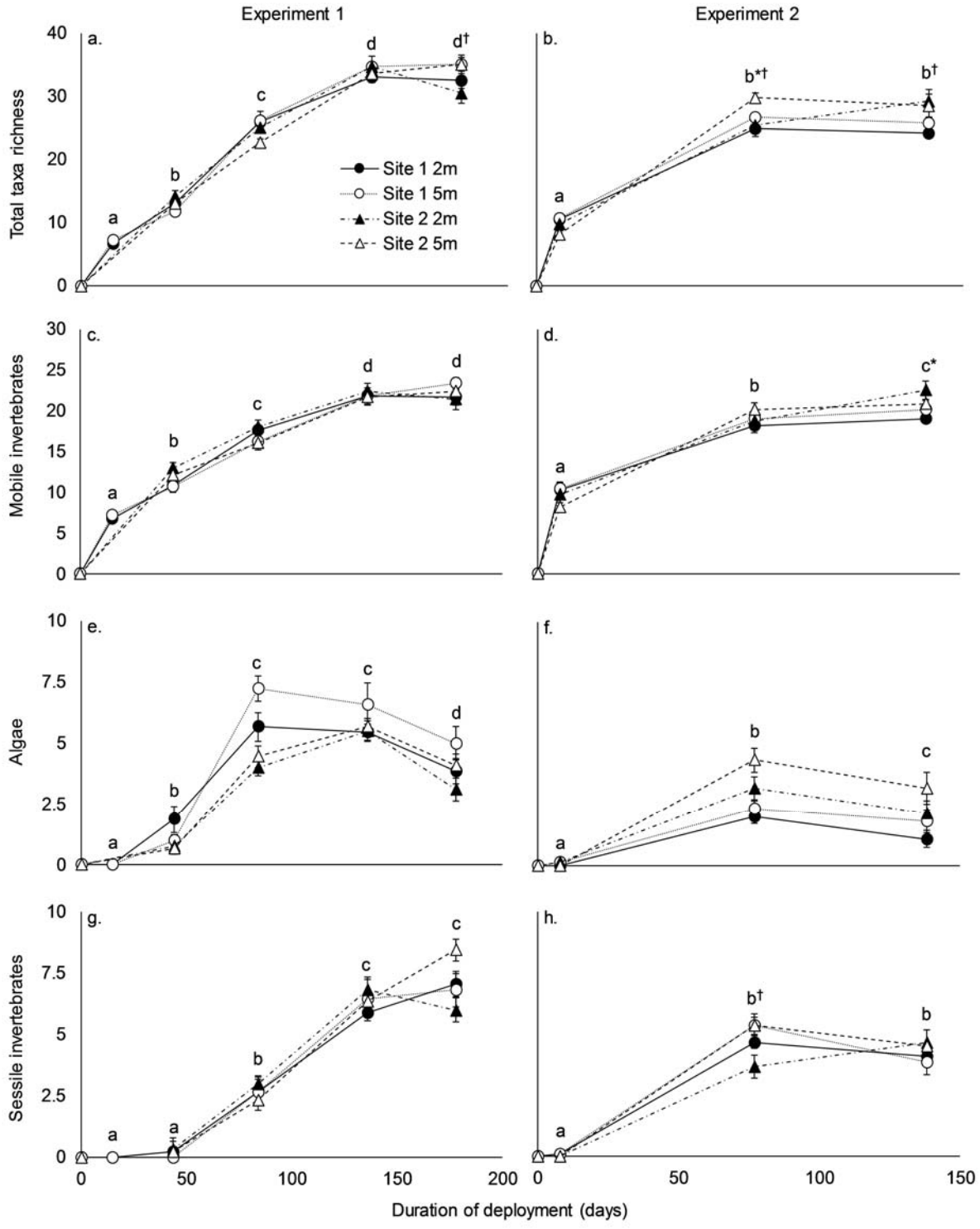

Figure 2.3. Taxonomic richness (mean number ± SE) of taxa (a & b), mobile invertebrates (c & d), algae (e & f) and sessile invertebrates (g & h) in Experiments 1 (left panel) and 2 (right panel) at two depths (2 m = black and 5 m = white symbols) and two sites (Site 1 = circles, Site 2 = triangles). The same letters above the symbols indicate homogenous groups following significant effects of the factor Duration regardless of site and depth. Differences between sites and depths within individual sampling durations are indicated by 1 or 2 asterisks, respectively.

The structure of the mobile invertebrate biofouling assemblages showed significant temporal and spatial variation across sites and depths in Experiments 1 & 2 with the effect of Duration being the most important source of variation in all analyses (Fig 2.4a & b, Table 2.2). The mobile invertebrate assemblage in Experiment 1 was different among all durations ($F_{3, 134}$ = 145.27, p = 0.001, Fig. 2.4a, Table 2.2), with the amphipods Ischyroceridae, *Paradexamine* spp. and *Caprella* spp. accounting for 34.6 – 73.2 % of the pairwise dissimilarities. The above taxa, and occasionally the amphipod *Gamaropsis typica* and Tanaidacea, were important determinants of between-site and occasional between-depth variations. In Experiment 2, the assemblage of mobile invertebrates varied through time when Ischyroceridae was the most important taxon in all pairwise contrasts (17.39 – 55.58 %). Assemblages of mobile invertebrates were consistently different between sites, whereas the effects of depth were only important after a duration of 77 days in Experiment 2.

Algal assemblage structure varied among durations, sites and depths in Experiment 1, but only among durations in Experiment 2 (Fig. 2.4c & d, Table 2.2). The green alga *Ulva* sp. and the red alga *Polysiphoina abscissoides* were the most important taxa through time in Experiment 1, together accounting for > 78.2 % of the differences among durations. The biomass of *Ulva* sp., *P. abscissoides* and *Colpomenia* sp. contributed to site-site differences in

Experiment 1 (Fig. 2.4c). In Experiment 2, *P. abscissoides* (69.5 %) and the brown alga, *Colpomenia* sp. (15.7 %) contributed most to the differences through time (Fig. 2.4d).

The assemblage structure of sessile invertebrates varied among durations and sites in Experiments 1 and 2 (Fig. 2.4e & f, Table 2.2). The arborescent bryozoans *Bugulina flabellata* and *Bugulina stolonifera* were the most important taxa driving differences among durations in Experiment 1. *B. stolonifera* (34.0 %), the compound ascidian *Aplidium phortax* (26.0 %) and the solitary ascidian *Ciona* robusta. (21.0 %) were important in Experiment 2. The two sites were consistently different in Experiment 1 (t = 1.77 – 2.87, $p < 0.014$) and Experiment 2 (t = 2.87 & 1.77, $p < 0.0189$).

PERMDISP analyses indicated that data dispersion was typically different among durations. It is therefore possible that some analyses are either more or less conservative, depending on the number of samples in the factor-levels (Anderson & Walsh 2013). However, the effect of duration was strongly significant in all analyses and was therefore considered to reflect real differences in assemblage structure among durations. Data dispersion was less variable, with significant pairwise differences following significant main or interactive effects among sites or depths (within a duration) in three out of the 50 possible pairwise contrasts. Therefore, these analyses were robust and suggest that differences among sites or depths is due to actual differences in biofouler assemblage structure, rather than variation among experimental ropes.

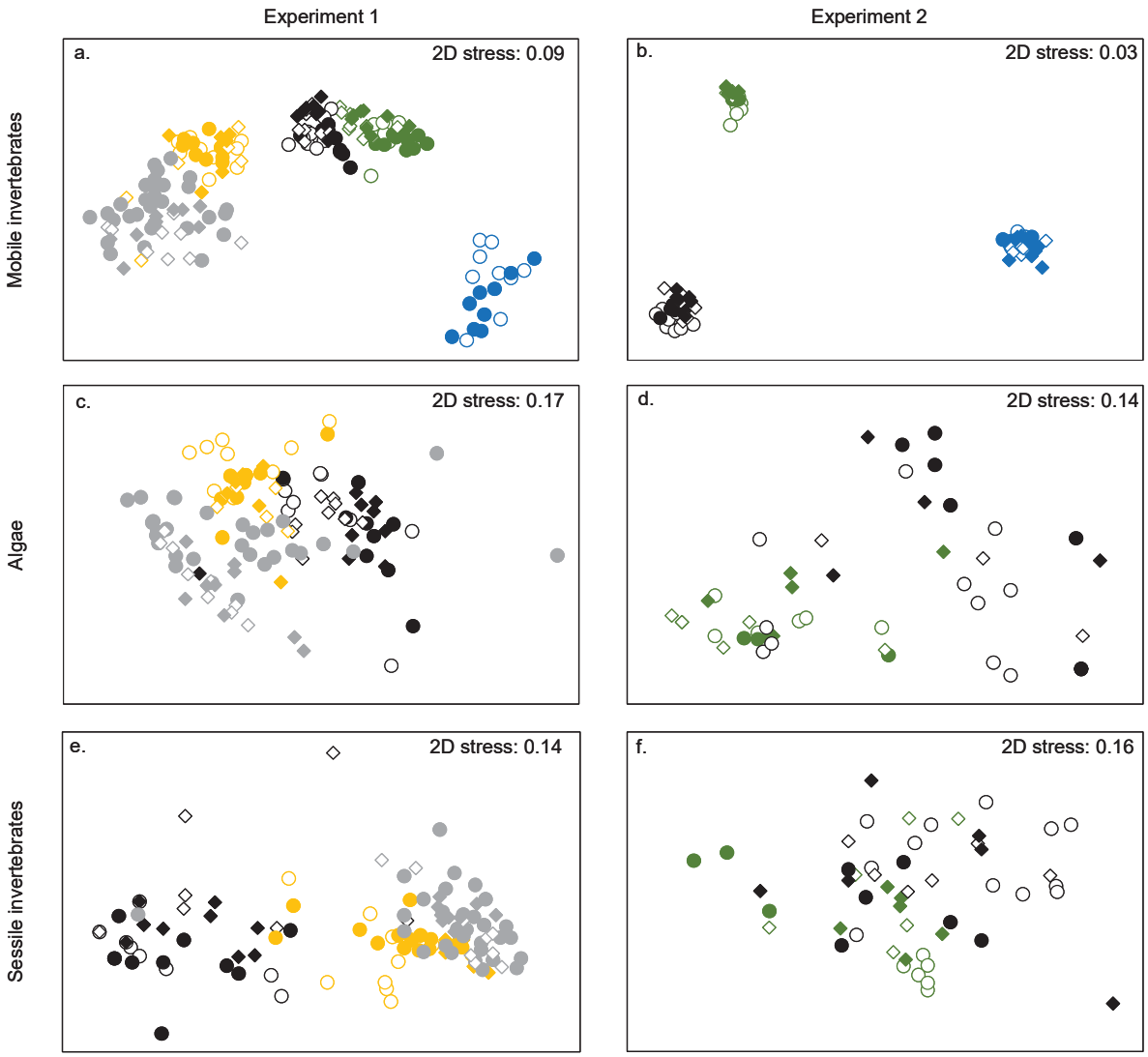

Figure 2.4. MDS plots showing the effects of Duration (coloured symbols), Site (Site 1 = circles, Site 2 = triangles) and Depth (2 m = open symbols, 5 m = filled symbols) on the structure of biofouling assemblages of mobile invertebrates, algae and sessile invertebrates. In Experiment 1, blue = 15, green = 44, black = 84, yellow = 136 and grey = 178-day durations. In Experiment 2, blue = 8, green = 77 and black = 138-day durations.

Development of key biofouling taxa

All the key biofouling taxa examined varied strongly among durations in Experiments 1 and 2 with fewer and smaller spatial differences (Fig. 2.5, Table 2.2). The tube-building amphipods, Ischyroceridae, were early colonists, and the most abundant taxon in this study. The amphipods *Paradexamine* spp. and *Caprella* spp. were the next taxa to recruit in high abundances in Experiment 1 whereas, in Experiment 2, *Paradexamine* spp. were in lower abundances throughout the experiment. Both taxa had declined in abundance at the end of the experiments (Fig. 2.5c-f, Table 2.2). The number of *M. galloprovincialis* on the experimental ropes increased through time in Experiment 1 (Fig. 2.5g & h, Table 2.2) attaining a maximum mean abundance of 397.6 ± 19.9, whereas in Experiment 2 the abundance of this species dropped after 77 days. The total biomass of algae fluctuated over time in Experiment 1, peaking at 6.1 g dw ± 0.5 after 136 days. The abundance of algae was much lower throughout Experiment 2. The biomass of algae had declined from its maximum at the end of both experiments (Fig. 2.5i, Table 2.2). By contrast, the biomass of sessile invertebrates increased over time in Experiment 1 and plateaued in Experiment 2 after 77 days (Fig. 2.5kl, Table 2.2).

2.3.3. Biofouling as a predictor of **Perna canaliculus** *retention*

CAP analyses indicated significant relationships between the assemblages of mobile invertebrates and the number of *P. canaliculus* after 136 ($r^2 = 0.57$, $p = 0.004$) and 178 ($r^2 = 0.37$, $p = 0.005$) days in Experiment 1 explaining variation of 92.0 and 48.8 mussels, respectively (Table 3). Fewer *P. canaliculus* juveniles were associated with greater numbers of the amphipods *Paradexamine* spp., *Caprella* spp., Ischyoroceridae and *Apocorophium acutum*, and fewer *Parawaldeckia* sp. after 136 days in the field. Similar patterns were found after 178

days except greater numbers of *Paradexamine* spp. and *M. galloprovincialis*, and fewer Serpulidae were associated with lower numbers of *P. canaliculus.* There was also a significant relationship between the assemblage of sessile invertebrates and that explained variation of 79.4 *P. canaliculus* at 178 days ($r^2 = 0.43$, $p = 0.003$) with greater biomass of *B. stolonifera*, the encrusting bryozoan *Watersipora subtorquata*, and the colonial ascidian *Aplidium phortax* being associated with fewer *P. canaliculus* juveniles.

Assemblages with greater numbers of *Paradexamine* spp. and Ischyroceridae were associated with greater numbers of *P. canaliculus* and explained variation of 67.4 *P. canaliculus* juveniles at 8 days in Experiment 2 ($r^2 = 0.34$, $p = 0.04$). By the end of the Experiment 2 (138 days), variation of 72.0 mussels was explained by assemblages where more *Paradexamine* spp., Tanaidacea, *Caprella* spp., Nematoda, Ischyroceridae and *Gammaropsis typica*, and fewer *Parawaldeckia* sp. ($r^2 = 0.39$, $p = 0.015$) were associated with lower abundance of *P. canaliculus* juveniles. At 77 days, variation of 28.2 mussels was explained by sessile invertebrate assemblages ($r^2 = 0.24$, $p = 0.016$) that were characterised by lower biomass of the sponge *Sycon ciliatum* and greater biomass of *Diplosoma listerianum* where there were fewer *P. canaliculus* juveniles.

2. The role of biofouling in seed-loss

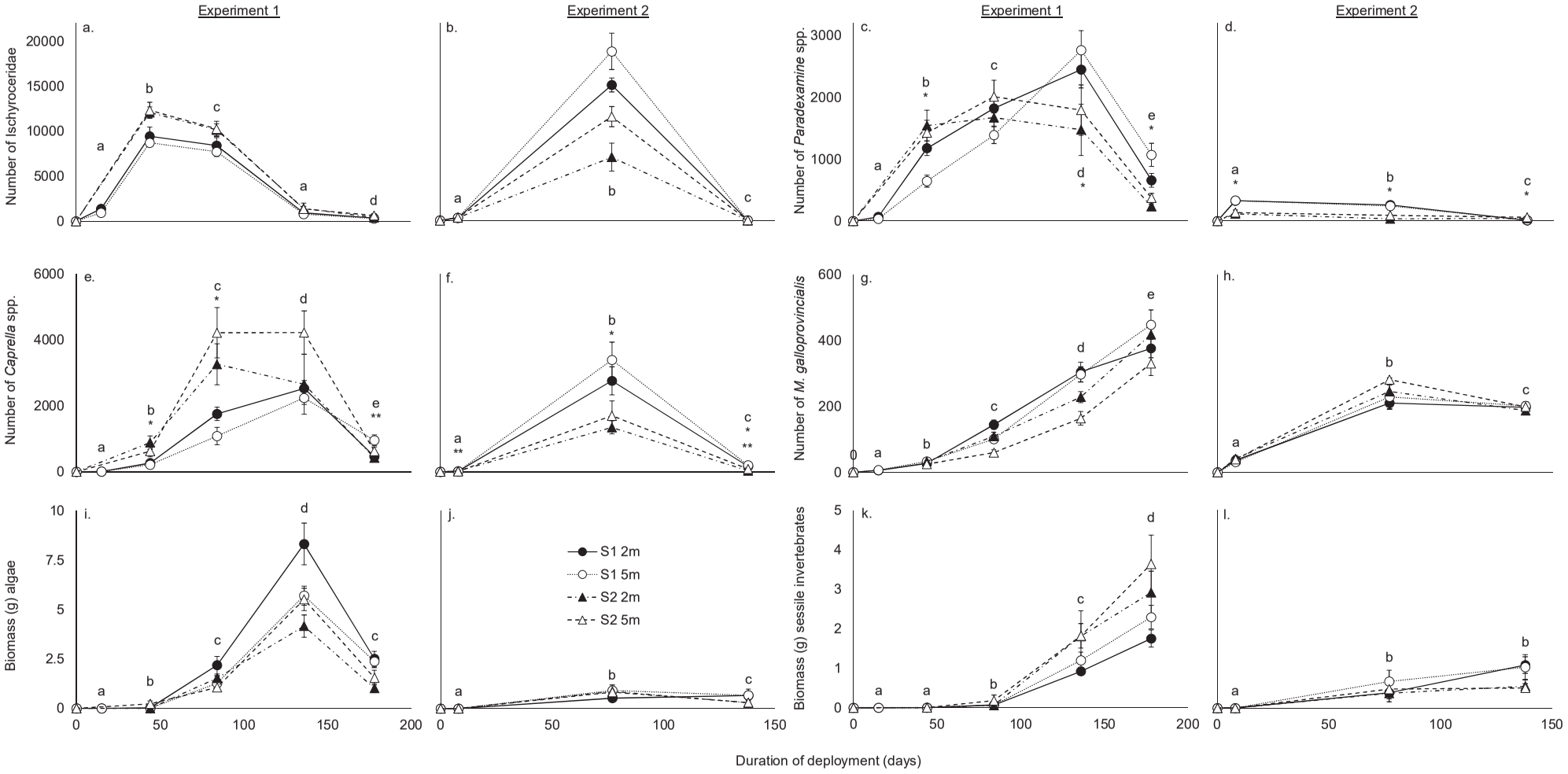

Figure 2.5. The effects of Duration of deployment (days), Site (Site 1 = circles, Site 2 = triangles) and Depth (2 m = black and 5 m = white symbols) on the mean (± SE) abundance of key biofouling taxa on 45 cm experimental ropes in Experiments 1 and 2. The same letters above the symbols indicate no pairwise differences between durations following significant effects of the factor Duration regardless of site and depth. Differences between sites and depths within durations are indicated by 1 or 2 asterisks, respectively.

There were six significant correlations between the key biofouling taxa and *P. canaliculus* abundance in Experiment 1 out of a possible 26 (Table 2.4). In Experiment 2 there were no significant correlations from 16 tests (Table 2.4). The negative correlation between the numbers of *P. canaliculus* and Ischyroceridae (r (28) = -0.42, $p < 0.05$) accounted for 17.6 % of the variation (32.6 mussels) after 136 days in the field in Experiment 1. The numbers of *Paradexamine* spp. and *P. canaliculus* were negatively correlated at 136 (r (28) = -0.38, $p < 0.05$) and 176 (r (46) = -0.59, $p < 0.001$) days accounting for 14 % (25.9 mussels) and 15.2 % (20.1 mussels) of the total variation. The positive correlation between the numbers of *M. galloprovincialis* and *P. canaliculus* at day 84 in Experiment 1 (r (34) = 0.37, $p < 0.05$) explained 14 % of the variation accounting for 19.9 mussels. The biomass of sessile invertebrates was negatively correlated with the number of *P. canaliculus* after 136 (r (28) = -0.63, $p < 0.05$) and 178 (r (46) = -0. 39, $p < 0.01$) days explaining 40 % and 15.2 % of the variation or 73.3 and 20.1 mussels, respectively.

Table 2.3. Results of CAP modelling the number of juvenile *Perna canaliculus* as a function of the biofouling assemblage.

	Duration	Mobile inverts.			Algae			Sessile inverts.		
	(days)	r^2	p	m	r^2	p	m	r^2	p	m
Expt. 1	15	0.02	0.837	2						
	44	0.00	0.988	2						
	84	0.13	0.241	3	0.02	0.961	4	0.26	0.108	5
	136	**0.57**	**0.004**	**5**	0.42	0.154	8	0.15	0.24	3
	178	**0.37**	**0.005**	**5**	0.08	0.277	3	**0.43**	**0.003**	**9**
Expt. 2	8	**0.34**	**0.04**	**6**						
	77	0.03	0.73	2	0.01	0.887	2	**0.24**	**0.016**	**1**
	138	**0.39**	**0.015**	**4**	0.06	0.444	2	0.12	0.172	2

Bold text indicates a significant relationship at $p < 0.05$.

Table 2.4. Spearman's rank correlations between the number of juvenile *P. canaliculus* and key biofouling organisms.

	Duration	n	Isch	Para	Cap	Myt	Alg	Invert
Expt. 1	15	18	0.03	-0.14	0.09	-0.12		
	44	36	-0.04	-0.02	0.14	-0.06		
	84	36	0.27	-0.08	-0.02	**0.37***	-0.02	-0.23
	136	30	**-0.42***	**-0.38***	-0.09	-0.06	-0.04	**-0.63***
	178	48	-0.28	**-0.59*****	-0.14	-0.15	-0.06	**-0.39****
Expt. 2	8	36	-0.01	-0.31	0.29	-0.03		
	77	21	-0.22	0.22	-0.05	-0.04	0.1	-0.36
	138	36	-0.01	-0.31	0.29	-0.03	0.09	-0.07

Duration is in days. Isch = Ischyroceridae, Para = *Paradexamine* spp., Cap = Caprella spp., Myt = *Mytilus galloprovincialis*, Alg = Algae (DW), Invert = Sessile invertebrates (DW).
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

2.4. Discussion

This study indicates that the majority of juvenile *P. canaliculus* seeded onto commercial mussel farm ropes for aquaculture are lost during the early stages of farm production. Overall, 64.4 and 78.3 % of seeded mussels were lost in Experiments 1 and 2 over 176 and 136 days, respectively, with greatest losses (42.9 and 49.1 %, respectively) occurring during the first 1 to 2-weeks of the production cycle. Biofouling communities developed rapidly and changed over the duration of both field experiments and comprised a suite of invertebrate and algal taxa, sometimes in very high abundance. There were significant losses of juvenile *P. canaliculus* during periods of significant changes in the biofouling assemblage (e.g., between days 44 and 84 in Experiment 1, and between days 77 and 136 in Experiment 2). However, relationships between biofouling assemblages, or their dominant taxa, and the abundance of *P. canaliculus* were inconsistent, and generally small where they occurred, suggesting relatively weak effects of the biofouling assemblage during the first few months of aquaculture production.

The massive loss of seed mussels from nursery ropes during early production stages is highly inefficient, especially given that mussel aquaculture in New Zealand is constrained by seed resources (Alfaro et al. 2010, Jeffs et al. 2018). The percentages of mussels disappearing from the experimental ropes in the field after 15 and 8 days (42.9 and 49.1 %, respectively) is similar to those reported after 1 month (46 %) in another field study (South et al. 2017), indicating that initial losses of seed upon deployment to farms may be the major loss of mussels during the early stages of aquaculture. It is possible that such early losses are caused by the transport process from the hatchery to the farm site that is likely highly stressful for the juvenile mussels (Webb & Heasman

2006, Carton et al. 2007). This result provides considerable impetus for directing future research into transport and seeding methodologies and their effects on subsequent seed-retention.

The development of biofouling on culture ropes was highly variable exhibiting distinct shifts between sampling durations and often spatial variation among sites and depths. Generally, there was a succession from an amphipod dominated assemblage to one that was dominated by algae, and, finally, to a complex mixed assemblage in which the blue mussel, *M. galloprovincialis*, was a dominant species. Similar patterns of biofouling development have been recorded in the study region (Woods et al. 2012, South et al. 2017) and in other parts of the world (Greene & Grizzle 2007, Bloecher et al. 2013). Observation of the successional process was hampered in Experiment 2 by the reduced number of sampling occasions and losses of experimental ropes. Regardless, the observed temporal patterns of abundance in biofoulers were similar in that the amphipods, Ischyroceridae, and to a lesser extent *Caprella* spp., numerically dominated the assemblage during the middle of the experiment and then declined, while *M. galloprovincialis* and sessile invertebrates increased and persisted until the end of the experiment. Notable differences between the two experiments were the lower abundances of *Paradexamine* spp., *M. galloprovincialis*, algae and sessile invertebrates in Experiment 2 that likely reflect seasonality of the species. For example, *M. galloprovincialis* typically reproduces and recruits in spring (Kennedy 1977, Atalah et al. 2017). This could explain the increase in the abundance of this species throughout Experiment 1, which ran from mid-winter to mid-summer, and its reduced and finally more stable abundance in Experiment 2, which ran from mid-summer to late-autumn. The seasonality of many of the biofouling taxa found on the experimental ropes is not well understood,

but the results presented here suggest considerable temporal variability. Further studies of temporal patterns of recruitment of biofouling are required if aligning mussel seed husbandry practices with patterns of biofouling development that are more conducive to retaining seed mussels is a goal for reducing stock losses (Sievers et al. 2014).

The biofouling assemblages that developed over the shortest deployment durations in this study (15 & 8 days in Experiments 1 & 2, respectively), when the greatest losses (42.9 and 49.1 % or 2.9 & 6.1 % per day) across sites and depths in Experiments 1 & 2, respectively) of *P. canaliculus* occurred, were characterised by relatively low taxonomic richness and abundances of small crustaceans such as amphipods. For example, losses of *P. canaliculus* were greatest over the first 8 days of Experiment 2 during which period taxonomic richness was < 10 per experimental rope and the abundances of key taxa were at their lowest. Despite these low abundances after 8 days, the assemblage of mobile invertebrates predicted 34 % of the variation in the number of *P. canaliculus*, with greater abundances of biofoulers being associated with greater numbers of *P. canaliculus* suggesting that positive interactions among the crop species and biofoulers might be important during the early stages of mussel aquaculture. By contrast, the significant reduction in *P. canaliculus* abundance between 44 and 84 days in Experiment 1 (total = 22.5 %, per day = 0.3 %) and days 8 and 77 in Experiment 2 (total = 38.5 %, per day = 0.5 %) coincided with intense recruitment of caprellid amphipods, high abundance of gammarid amphipods (*Paradexamine* spp. and members of the Ischyroceridae), and the initial colonisation by algae. However, if the recruitment of these organisms did reduce the abundance of *P. canaliculus*, this impact was transient, because there was only one significant negative relationship (of 16 analyses) between

the abundance of *P. canaliculus* and the biofouling assemblages or their key taxa at the end of these periods. Furthermore, the one significant relationship between the assemblage of sessile invertebrates and the number of *P. canaliculus* at day 77 in Experiment 2 did not persist until the end of the experiment.

The data from this study indicates that the effects of biofouling on juvenile mussel retention are inconsistent over time and space and appear to reflect small-scale variations in its development. Significant relationships between the assemblage and abundance of biofoulers and *P. canaliculus*, when retention was spatially and temporally stable (days 136 and 178 in Experiment 1), suggest that variations in the impact of biofouling might occur at small scales (among experimental ropes). For example, there were negative correlations between the numbers of *Paradexamine* spp. and *P. canaliculus* at days 136 and 178 in Experiment 1 that accounted for 38 and 59 % of the variation in *P. canaliculus* abundance, respectively. These relationships may reflect differential resource availability and use, or direct and indirect negative interactions with the crop species. However, since *Paradexamine* spp. are mesograzers that are commonly associated with macroalgae (Amsler et al. 2013), it seems most likely that they use resource spaces that are unused by *P. canaliculus*, such as the thalli of macroalgae. By contrast, sessile invertebrates, such as ascidians and hydroids, compete for space and food with mussels in aquaculture (Fitridge & Keough 2013, Fletcher et al. 2013b), which could account for the negative correlations between sessile invertebrate biomass and *P. canaliculus* abundance at 136 ($r(28) = -0.63$, $p < 0.05$) and 178 ($r(46) = -0.59$, $p < 0.01$) days. Alternatively, *P. canaliculus* abundance might limit the recruitment and development of

sessile invertebrates through the pre-emption of settlement space. More detailed experimental work is required to test interactions among biofoulers and crop species.

Overall, the results from the present study suggest that biofouling is not the major cause of losses of *P. canaliculus* juveniles. Other possible causes of loss include predation by fish (Hayden 1995), mortality due to heat or desiccation stress (Webb & Heasman 2006), secondary settlement migrations due to transport induced stress (Carton et al. 2007), and underlying variations in fitness among individuals (Sim-Smith & Jeffs 2011). However, one important caveat is that there were no massive infestations of biofouling taxa that are considered to be problematic (e.g., *Ciona robusta*) during the experiments assessed here, and it is possible that extreme densities of certain biofouling species can have a greater impact on juvenile retention. Determining the occurrence of extreme biofouling events requires structured temporal monitoring, whereas manipulative experiments are required to determine their impacts.

The presence of sessile invertebrates such as *Diplosoma listerianum, Aplidium phortax Watersipora subtorquata* at the end of the experiments has important implications for mussel aquaculture in New Zealand. Such species can be transported among sites with the seed mussels, where they can negatively impact the crop species via competition for food or direct negative effects such as smothering (Lesser et al. 1992, Woods et al. 2012, Fletcher et al. 2013b, Sievers et al. 2013). Similarly, the blue mussel, *M. galloprovincialis*, which is a problematic fouling species in the region (Forrest & Atalah 2017), was more abundant than the crop species at the end of both early aquaculture production periods indicating that the structures used to grow *P. canaliculus* are

also beneficial for blue mussels and might be an important route into the grow-out phase of mussel aquaculture.

Conclusions

The results of this study show that most losses of juvenile mussels during early production in mussel aquaculture in New Zealand occur during the initial few weeks after deployment. This has tremendous relevance to the relay and deployment processes currently employed by the New Zealand aquaculture industry and suggests that they should be a focus of research. By contrast, biofouling accumulation had inconsistent relationships with the abundance of *P. canaliculus,* which was relatively stable during the period of intense and dynamic development of the biofouling assemblage. However, the biofouling assemblage was found to contain many taxa, some of which can be problematic for aquaculture, and therefore merit continued research.

Chapter 3.

Inefficient seeding of wild-sourced mussels in aquaculture: the role of seeding substrate on the timing and magnitude of seed-losses

3.1. Introduction

Most of the juveniles used as seed for much of the world's mussel aquaculture are sourced from the wild (Hickman 1976, Alfaro et al. 2004, Peteiro et al. 2007a, Capelle et al. 2014). However, the settlement and recruitment of juvenile mussels in the wild is highly variable in space and time, which can impose severe limits on the availability of seed mussels for aquaculture operations (Jeffs et al. 1999, Smaal 2002b). Juvenile mussels to be used as seed are either captured at settlement by placing artificial substrates into the water column or collected post-settlement from a variety of different structures, such as from macroalgae and wild mussel beds (Alfaro & Jeffs 2002, Filgueira et al. 2007, Capelle et al. 2016b). Soon after capture, juvenile mussels are relayed to a mussel farm and seeded into aquaculture production systems. However, the process of seeding juvenile mussels is highly inefficient with most juveniles being lost soon after they are deployed, further limiting aquaculture operations (Smaal 2002b, Capelle et al. 2016a, South et al. 2017). There are many potential causes of losses of mussels following relay that include predation (Hayden 1995, Peteiro et al. 2010), transport-induced stress (Webb & Heasman 2006, Carton et al. 2007), underlying variations in fitness of the juveniles (Sim-Smith & Jeffs 2011), density-dependent processes (Frechette & Lefaivre 1990), environmental stressors (Myrand & Gaudreault 1996), and secondary settlement migrations (Bayne 1964, Buchanan & Babcock 1997) that might have ontogenetic (von der Meden et al. 2010) or environmental triggers (Newell et al. 2010).

Losses of the green-lipped mussel, *Perna canaliculus*, in the early stages of aquaculture production in New Zealand, where this species is the most important export product from aquaculture (> 70 % of export revenue worth NZ$ 311 million per annum; Aquaculture New

Zealand 2016), are a substantial constraint on production. The New Zealand mussel industry is heavily reliant on wild mussel seed that are mostly (ca. 80 %) obtained from the shoreline at one site, Ninety Mile Beach, in the far north of the country (Hickman 1976, Alfaro et al. 2010, Jeffs et al. 2018). Juvenile mussels are delivered to the shore attached to a variety of substrates that mostly comprise various species of macroalgae and other material including hydroids, terrestrial plant matter, shells and sand (Jeffs et al. 2018). Juvenile mussels attached to such substrates are called 'Kaitaia spat' (named after a nearby township) and are shipped around the country to be seeded into aquaculture (Jeffs et al. 1999, Alfaro et al. 2010, Jeffs et al. 2018).

Mussel aquaculture in New Zealand uses long-line systems that consist of parallel backbone lines, suspended by buoys and below which are suspended lead-cored, fibrous plastic ropes to depths of ca. 5 – 10 m. Seeding involves deploying the Kaitaia spat into long-line aquaculture by holding the substrates (e.g., macroalgae, hydroids) to which the juvenile *P. canaliculus* are attached, in place alongside the ropes with a mesh sock. The spat are then grown in a nursery farm for a period of ca. 3 – 6 months until they attain a shell length of > 10 mm after which they are stripped from the lines and re-seeded into farms for on-growing. The process of seeding Kaitaia spat is highly inefficient with most seeded mussels being lost during the nursery period (Webb & Heasman 2006, Hayden & Woods 2011). The timing and magnitude of these losses are unknown, as is the magnitude of subsequent losses of juveniles during the grow-out phase. Determining when these losses occur, the number of mussels lost, and the potential underlying causes of loss are an important first-step towards addressing the issue of poor retention in mussel aquaculture, especially in the New Zealand context.

3. Inefficient seeding of wild-sourced mussels

The density of juvenile *P. canaliculus* in the Kaitaia spat can be as great as 2×10^6 kg^{-1}, although it can vary seasonally (Hickman 1976, Alfaro et al. 2004, Jeffs et al. 2018). Furthermore, the density and size of juvenile mussels attached to the macroalgae and other substrates in the Kaitaia spat can vary markedly, with typically fewer, larger mussels on coarsely-branched macroalgae, while higher numbers of smaller mussels are on finer, more complex macroalgae (Hickman 1976, Alfaro & Jeffs 2002, Alfaro et al. 2004). It is unknown whether variations in the macroalgae to which the juvenile *P. canaliculus* are attached affects their retention during aquaculture production. For example, it is possible that fine macroalgae degrade more quickly than coarse macroalgae, sooner becoming an unstable surface for attachment. It is also possible that as the macroalgae degrade they modify local environmental conditions, such as water pH or dissolved oxygen content, that might negatively affect retention by causing mortality or triggering secondary settlement.

Secondary settlement is a behavioural process employed by the juvenile mussels to migrate among substrates after the larvae have initially settled out of the plankton and metamorphosed to juveniles, and the secondary settlement behaviour is thought to allow the juveniles to optimise their final settlement position (Bayne 1964, Sigurdsson et al. 1976, Lane et al. 1985, Buchanan & Babcock 1997). The process of secondary settlement is a likely source of losses of juvenile *P. canaliculus* in mussel aquaculture, because it appears to be a pronounced feature of this species (Buchanan & Babcock 1997, Jeffs et al. 1999, South 2016). However, few field-based studies have assessed rates of secondary settlement and their relative importance compared to other mechanisms of loss, such as mortality of the juveniles.

This study used a field experiment to repeatedly assess the retention of Kaitaia spat over a three-month aquaculture nursery period. The effects of substrate characteristics, that have previously been shown to modify the abundance and size of juvenile *P. canaliculus*, were assessed to determine whether variations in the seed-substrate can affect retention. *In situ* retention and patterns of secondary settlement were compared among different component substrates of the Kaitaia spat. Finally, small-scale water quality conditions associated with the substrates and the mussels were assessed and considered in the context of the observed patterns of retention.

3.2. Materials and methods

3.2.1. Study site and source of juveniles

The field component of this study was undertaken at a mussel farm operated by Sanford Ltd in Clova Bay in the Marlborough Sounds, New Zealand (-41° 05' 40.7" S, 174° 00' 50.7" E). Clova Bay is a sheltered site in mid-Pelorus Sound that is used as a nursery site for juvenile *P. canaliculus*. Following harvest from Ninety Mile Beach the Kaitaia spat are packed in 10 kg lots into plastic bags and transported (1108 km) to the Marlborough farming region in refrigerated trucks over a period of c. 36 h. They are then seeded onto lead-cored polypropylene dropper ropes, with the Kaitaia spat being held in place with a mesh socking made of polyester and cotton. Dropper ropes are suspended beneath backbone lines until the juvenile mussels have grown to at least 10 mm in shell length (typically > 3 months of age), when they are removed from the water, mechanically stripped from the ropes and re-seeded at a reduced density for grow-out. This study concerns the period between initial seeding of Kaitaia spat and re-seeding of larger juveniles, which hereafter is referred to as the ‘nursery period’. The Kaitaia spat material used in this study

was obtained from a commercial consignment that arrived at Clova Bay on 16 September 2017. The laboratory component of the work was undertaken at the Cawthron Aquaculture Park in Nelson, c. 4 h travel by car and then vessel from the field site.

3.2.2. Retention and migration of juvenile Perna canaliculus

A field experiment was used to assess how the morphology of the various macroalgal substrates contained within the Kaitaia spat affected the retention and migration of the juvenile mussels in Kaitaia spat during the early phase of aquaculture production. The consignment of Kaitaia spat was separated into four substrate groups based on the morphology of the macroalgae to which they were attached. Since the macroalgal component of the Kaitaia spat was a relatively homogenous mix of many seaweeds, the substrate groups contained more than one species. The four morphological groups were: (1) fine macroalgae, (2) coarse macroalgae, (3) a mix of fine and coarse macroalgae, and (4) un-manipulated Kaitaia spat. The fine macroalgal group consisted of species such as *Halopteris congesta*, *Plocamium* spp., *Ballia calitricha* and *Pterocladia capillacea* that are typically small (< c. 10 cm), have thin thalli (< 0.5 cm) and are often highly branched and structurally complex. The coarse macroalgal group contained only fucoid macroalgae, mostly *Landsburgia quercifolia*, *Carpophyllum maschalocarpum* and *Xiphophora chondrophylla,* all of which had thick thalli (> c. 1 cm in blade width), a turgid texture and relatively low morphological complexity. The mix of fine and coarse macroalgae (hereafter mixed macroalgae) was created by combining the two groups (ratio = 1:1 by wet weight). The Kaitaia spat contained a mix of coarse and fine macroalgae and other algal, invertebrate (hydroids) and terrestrial taxa (e.g., pine needles), and represents the typical deployment situation. Fifteen × 20 g samples from each of the four

morphological groups were each seeded onto 18 cm sections (60 sections in total) located at the middle of an individual 40 cm long mussel dropper rope by enveloping the macroalgae and mussels in a fine-meshed sock made of a mix of polyester and cotton as used by industry, and holding them in place with cable-ties. This left 11 cm both above and below the seeded 18 cm section. In addition, a total of 15 sections of dropper rope that were 18 cm long were also enveloped in mesh sock, but no substrate or juvenile mussels were added. These sections provided an estimate of the arrival of secondary settling mussels *in situ*, as well as a baseline contrast for laboratory assays. Mussel abundance and shell-length (mm) at the start of the experiment was estimated from five × 20 g samples of each morphological group.

The 40 cm long experimental dropper ropes were secured (with cable-ties) onto six 80 × 100 cm frames (12 or 13 dropper ropes per frame) made from polyvinyl chloride (PVC) pipe (22 mm ⌀) that were further divided midway with a further 100 cm length of PVC pipe to form two 40 cm rectangular sections. These frames were deployed beneath two buoys (3 per buoy, 50 m apart due to space constraints at the mussel farm) at a depth of 5 m, on a mussel backbone line that had been seeded with Kaitaia spat material earlier in the day. Weights were added to the bottom of the frames to align the ropes in the water column, simulating typical deployments of Kaitaia spat. Five replicate experimental ropes for each of the four substrate groups and the controls were collected on the 5 October 2017 (19-day deployment), and 26 October 2017 (40-day deployment). There was a final collection on 14 December 2017 (89-day deployment), however, due to an error at seeding four and six experimental ropes with coarse and mixed macroalgae were retrieved, respectively. Once the experimental dropper ropes were recovered in the field they were bagged,

stored in an insulated box, and returned to the laboratory where the 18 cm experimental ropes were cut from each of the 40 cm ropes and placed into individual aquaria (see 3.2.3). Care was taken not to dislodge mussels during this process. Occasionally when mussels detached from the 18 cm sections of rope during handling, they were added to the aquarium along with the rope and the attached mussels. The number and size of mussels that were retained *in situ* (i.e., on the 18 cm sections) during the field experiment were assessed as the total number of *P. canaliculus* per aquarium, after the laboratory experiments (see section 3.2.4). The number of dead mussels as identified by empty and degraded valves was counted. Mussels were removed from the remaining 22 cm of rope on the experimental droppers and enumerated to quantify vertical migrations of the juveniles from the seeded sections. Finally, many mussels had migrated to the frames at the end of the experiment (day 89); therefore, these were also counted. In this study, retention was defined as the number of mussels remaining *in situ* on the 18 cm sections of experimental rope. It was assumed that mussels on the adjacent sections of rope, control ropes and frames, had migrated via pedal crawling or mucus drifting from the nearby seeded mussels. However, it is also possible that they could have arrived as drifters from other experimental ropes, or the mussel lines on the farm, and it is also possible that interchange among the unseeded and seeded sections of rope took place during the experiment.

3.2.3. Fine-scale mussel rope conditions

The 18 cm of sections of experimental rope including the outer socks and mussels were held separately in 1.8 L aquaria (11.3 × 11.3 × 28.2 cm), made from food grade plastic, to allow sampling of the small-scale chemical conditions around the ropes. Ropes were suspended from

plastic rods at the top of the aquaria using monofilament fishing line and 316 grade stainless weights to align them vertically in the water column. Filtered (1 μm), UV treated seawater at ambient temperature and salinity (35 ± 0.1 psu) was pumped through the aquaria using two 250 L h^{-1} aquarium pumps via two PVC manifolds in a flow-through system. Outlet pipes were capped with a 0.25 mm nylon mesh to prevent escapees and no food was provided during the experimental periods. The experimental ropes, the juvenile mussels and any fouling organisms that had recruited during the field deployments were held for a period of 3-days. One sample of water from each aquarium was taken on each consecutive day to describe the local environment in terms of the concentration of dissolved oxygen (DO), pH, and concentrations of Nitrate-N (NO_3N), total Nitrogen (Nitrite-n plus Nitrate-n), and total Ammoniacal-N (NH_4N). Samples for DO were carried out on the first day of incubation, pH was sampled on the second day, and nutrients were sampled on the third day. Water samples were taken from among the polypropylene fibres of the experimental dropper ropes at a depth of ca. 8 cm using syringes. The flow-through system was shut-off prior to sampling for all water quality variables. Similar samples were taken from the tanks containing the pumps to provide a baseline contrasts for the experimental ropes. Time elapsed since the flow-through was shut down was recorded for each sample and used as a covariate in the statistical analyses.

To quantify DO among substrate groups and the controls, one water sample (1 ml) per aquarium was injected into a flow cell containing a polarographic oxygen electrode (MI 730 Micro-Oxygen electrode, Microelectrodes Inc., Bedford, NH) connected to a Cameron Instruments OM 200 oxygen meter. The oxygen electrode was held within a water jacket

(Cameron Instruments, Guelph, Canada) maintained at 16 °C. DO values were determined using measured oxygen partial pressures, salinity and barometric pressure, and calculated using oxygen solubility and saturated vapour pressure values (Benson & Krause 1984). Total pH was quantified from a 10-ml sample of seawater from each aquarium using a pH meter (Mettler Toledo, Columbus, OH). A similar approach was used to quantify nutrients, although a 100-ml sample of water was taken. Nutrient analyses were sent to Hill Laboratories (Hamilton, NZ) on the day of collection in a cooled, insulated container. The samples were processed using industry standard flow injection analytical techniques. The wet-weights of the remaining macroalgae were also quantified to the nearest 0.01 g at the end of each period in the laboratory.

3.2.4. Percentage and size of retained juvenile **Perna canaliculus** *in the laboratory*

Many mussels moved from the ropes to the aquaria bases, rims and outlet pipes during laboratory experiments, therefore mussels that were retained on the ropes or had migrated to the aquaria (including weights and outlet pipes) were counted and measured for each aquarium. All samples were washed over a nested stack of sieves (0.25, 0.5, 1 and 2 mm). Fifty randomly selected mussels that remained on the ropes and 50 mussels attached to the aquaria were measured (to 0.01 mm) for each experimental rope using image analysis software (Image J) to determine if there were size differences between retained and migrating mussels. For the controls, all mussels were measured unless the total number of mussels was > 50. The number of days the experimental ropes were in the field was used to define ‘duration’ throughout this chapter although the data were quantified after the number of days in the field plus 3 days in the laboratory.

3.2.5 Statistical analyses

Most analyses in this study were performed using univariate permutational analysis of variance (PERMANOVA) based on similarity matrices of Euclidian distances using 9999 (count data) or 999 (size data) permutations to estimate p-values. Differences among levels of important factors were assessed with pairwise PERMANOVAs. Variance homogeneity was tested with permutational analysis of multivariate dispersions (PERMDISP) using distances to the centroid of the data cloud, a procedure that is equivalent to Levene's test (Anderson et al. 2008). Data were transformed (log (x + 1) or square-root) where necessary to meet the assumption of variance homogeneity. If homogeneity was not achieved the analyses proceeded but the results were interpreted cautiously at $p < 0.01$ and this was noted in presenting the results.

***Retention, migration and mortality of juvenile* Perna canaliculus**

Differences in the mean size of the mussels among Substrate Groups at the start of the experiment were analysed with a single factor PERMANOVA. The mean number of *P. canaliculus* juveniles that were retained *in situ* (i.e., on the 18 cm sections of experimental rope) were analysed with PERMANOVA testing for the crossed effects of Duration of deployment (random; 4 levels = 0, 19, 40, 89 days) and Substrate Group (fixed; 4 levels = fine macroalgae, coarse macroalgae, mixed macroalgae and Kaitaia spat material). A similar analysis was employed to assess the mean total number of mussels per experimental rope (i.e., including juveniles that had migrated onto the adjacent un-socked sections of rope). The mean abundance of mussels that were assumed to have migrated to adjacent un-socked sections of the experimental ropes, and the number of dead mussels remaining *in situ*, were assessed with the same model as above, except Duration had three levels

(19, 40 and 89 days), because these response variables were not enumerated at the start of the experiment. Finally, a one-way PERMANOVA was used to test the effects of Duration (random; 3 levels = 19, 40, 89 days) on the mean number of mussels settling on the control ropes (i.e., rope sections covered in mesh sock but with no juvenile mussels or substrate).

Fine-scale mussel rope conditions

To determine any differences in the degradation of the substrates on which the juvenile mussels were seeded, the wet weight of remaining macroalgae was assessed with a PERMANOVA testing among Durations (random 4 levels 0, 19, 40 and 89 days) and Substrate Groups (fixed 4-levels, fine, coarse, mixed and Kaitaia spat material). To examine patterns of change in the water quality conditions immediately surrounding the mussels, mean concentrations of DO, pH, Nitrate-N (NO_3N), total Nitrogen (Nitrite-N plus Nitrate-N) and total Ammoniacal-N (NH_4N) were analysed using a model that included Duration (random 3 levels 19, 40 and 89 days) and Treatment (fixed 6-levels, fine, coarse, mixed and Kaitaia spat material, control and tank water). Control experimental ropes were included to give a baseline for field conditions without seaweed, and the tank water acted as a baseline against which to assess biological functioning associated with deployment in the field. The time elapsed since the water-flow was ceased, and its interaction with Duration, were included as a covariate in all analyses.

Percentage and size of retained juvenile* Perna canaliculus *in the laboratory

To quantify patterns of mussel retention in the laboratory, the mean percentage of mussels retained on the experimental ropes during the experiments in the laboratory was assessed with a 2-way

PERMANOVA testing for the effects Duration (random; 3 levels = 19, 40, 89 days) and Substrate Group (fixed; fixed; 5 levels = controls, fine macroalgae, coarse macroalgae, mixed macroalgae, and Kaitaia spat material).

Variation in mean mussel size among substrate groups (fixed; 5 levels = controls, fine macroalgae, coarse macroalgae, mixed macroalgae, and Kaitaia spat material) and Retention Status (fixed; 2 levels, retained and migrated) was assessed for each duration (19, 40 and 89 days) separately.

Finally, Pearson product moment correlation analyses were carried out between the total number of mussels retained in the field (i.e., the total number of mussels per rope/aquarium), and the percentage retained on the experimental ropes after the laboratory period, to test whether retention in the laboratory varied with mussel abundance. Correlation analyses were also used to examine the relationship between the water quality variables, the total number of mussels retained in the field, and the percentage retained in the laboratory.

3.3. Results

3.3.1. Retention, migration and mortality of juvenile Perna canaliculus *in the field*

The mean shell length of the juvenile *P. canaliculus* used in this study was similar on coarse (2.14 ± 0.05 mm) and fine macroalgae (2.19 ± 0.05 mm) but were both larger than for mixed macroalgae (2.0 ± 0.05 mm) and Kaitaia spat (2.0 ± 0.05 mm) ($F_{3, 996}$ = 4.6, p = 0.002). There were fewer juvenile *P. canaliculus* per 20 g^{-1} macroalgae on coarse macroalgae (2724.4 ± 169.0, mean ± SE) at the beginning of the experiment compared to all other treatments (pairwise t = 2.5 – 3.9, p <

0.05), with fine macroalgae having the greatest abundance (4323.6 ± 415.0). There were large losses of mussels between consecutive durations throughout the experiment (Duration $F_{3, 64}$ = 300.16, p = 0.0001, Fig. 3.1, Table 3.1). After deployment at the mussel farm for 19 days, 52.8 – 72.9 % (64.8 % across all substrate groups), of the originally seeded mussels were lost from the 18 cm sections of rope. By 40 days, 69.6 – 76.7 % (72.7 % across all substrate groups) of the initial number of seed mussels had been lost. At the end of the experiment (89 days), 74.4 – 84.9 % (80.4 % across all substrate groups) of the original starting numbers of seed mussels had been lost. Retention during the experiment was greater for coarse macroalgae (25.6 % of the starting abundance during the entire experiment) compared to the other substrate groups (< 17.9 %), but at the end of the 3-month nursery period (89 days) there were no differences in the number of *P. canaliculus* juveniles between the coarse macroalgae and the other treatment groups (Fig. 3.1). The abundance of juvenile mussels associated with different substrates varied for individual Duration levels, but there were no consistent patterns (Duration × Substrate Group $F_{9, 64}$ = 3.06, p = 0.0039, Fig. 3.1, Table 3.1). At the end of the experiment there were fewer mussels on the mixed macroalgae (590.5 ± 27.2) compared to the fine macroalgae (725.6 ± 38.8, Fig. 3.1).

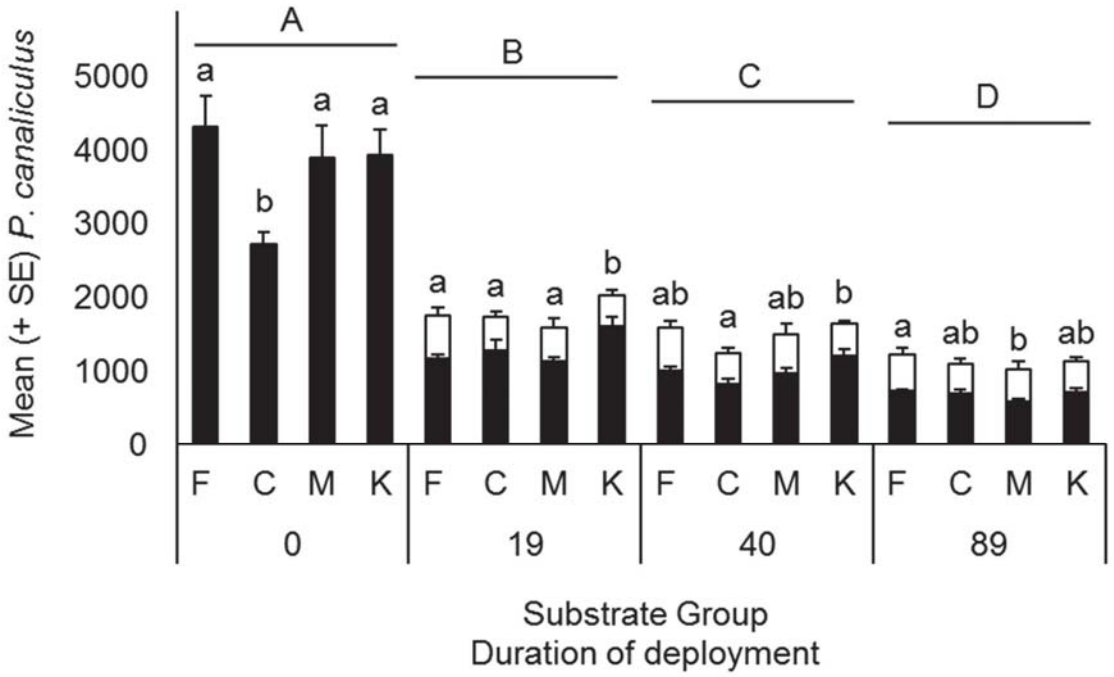

Figure 3.1. Retention (mean number + SE) of *Perna canaliculus* on four Substrate Groups at 0-, 19-, 40- and 89-days following seeding onto a mussel farm. F = fine macroalgae, C = coarse macroalgae, M = mixed macroalgae, and K = Kaitaia spat. Black bars show *in situ* mean retention of mussels on the 18 cm sections of rope that were seeded with 20 g of spat material at the beginning of the experiment. White bars show the mean number of mussels that migrated onto adjacent un-socked sections of rope (22 cm) stacked on top of the bars presenting mean retention. The sum of the black and white bars = Total mussels per experimental rope. Different black letters indicate pairwise differences among (capitals) and within (lower-case) Durations for *in situ* mussel retention. Grey letters indicate pairwise differences within Durations for the Total mussels per rope, where these differ from *in situ* retention.

Table 3.1. Results from PERMANOVA analyses testing for the effects of Duration and Substrate Group on the retention and migration of juvenile *Perna canaliculus* after 19, 40 and 89 days in the field.

	Source	f/r	df	MS	*F*	p	perms
Retained	Duration	r	**3**	**4638.20**	**300.16**	**0.0001**	**9964**
in situ	Substrate (Su)	f	3	97.58	2.07	0.1743	9958
	Du x Su	r	**9**	**47.29**	**3.06**	**0.0039**	**9932**
	Residuals		64	15.45			
	PERMDISP				2.29	0.085	
Total	Duration	r	**3**	**2782.40**	**182.97**	**0.0001**	**9955**
mussels	Substrate (Su)	f	3	108.73	2.91	0.0903	9959
	Du x Su	r	**9**	**37.37**	**2.46**	**0.0164**	**9932**
	Residuals		64	15.21			
	PERMDISP				2.38	0.085	
Migrated	Duration	r	2	12609	0.67	0.5278	9947
	Substrate (Su)	f	**3**	**54888**	**12.83**	**0.0039**	**9961**
	Du x Su	r	6	4255	0.23	0.9681	9952
	Residuals		48	18900			
	PERMDISP				2.32	0.094	

F = *Pseudo F.* **Bold** = significant at α = 0.05.
Sqrt-transformed data used in Retained *in situ* and Migrated.

Including mussels that had migrated onto the un-socked sections of the experimental ropes (i.e., Total mussels) into the analyses showed a similar pattern of temporal decline to *in situ* retention, although losses were smaller overall, with overall mean reductions of 50.8, 58.4 and 66.8 % compared to the average starting density across all substrates at 19, 40 and 89 days, respectively (Duration $F_{3, 64} = 182.97$, p = 0.0001, Fig 3.1, Table 3.1). There were no effects of Duration when the mean number of mussels that had migrated onto the adjacent un-socked sections of experimental rope was analysed separately (Duration $F_{2, 48} = 0.67$, p = 0.053, Fig. 3.1, Table

3.1). However, more juvenile *P. canaliculus* had migrated onto un-socked sections of experimental rope adjacent to fine macroalgae (553.7 ± 33.1) compared to coarse macroalgae regardless of Duration (420.64 ± 27.9; Substrate Group $F_{3, 48} = 12.83$, p = 0.0039, Table 3.2).

The mean number of dead mussels across all Substrate Groups decreased from 311.7 ± 38 at 19 days, to 91.55 ± 10.4 at 89 days (Duration $F_{2, 48} = 17.47$, p = 0.0001, Fig. 3.2). Overall, there were fewer dead mussels in the coarse Substrate Group (93.64 ± 13.9) compared to the mixed macroalgae (216 ± 36) and Kaitaia spat (286 ± 43.98) groups ($F_{3, 48} = 7.23$, p = 0.0171, Fig. 3.2). All dead mussels passed through the 2-mm sieve at days 19 and 40, whereas 50 out of 1831 were captured by the 2-mm sieve at day 89.

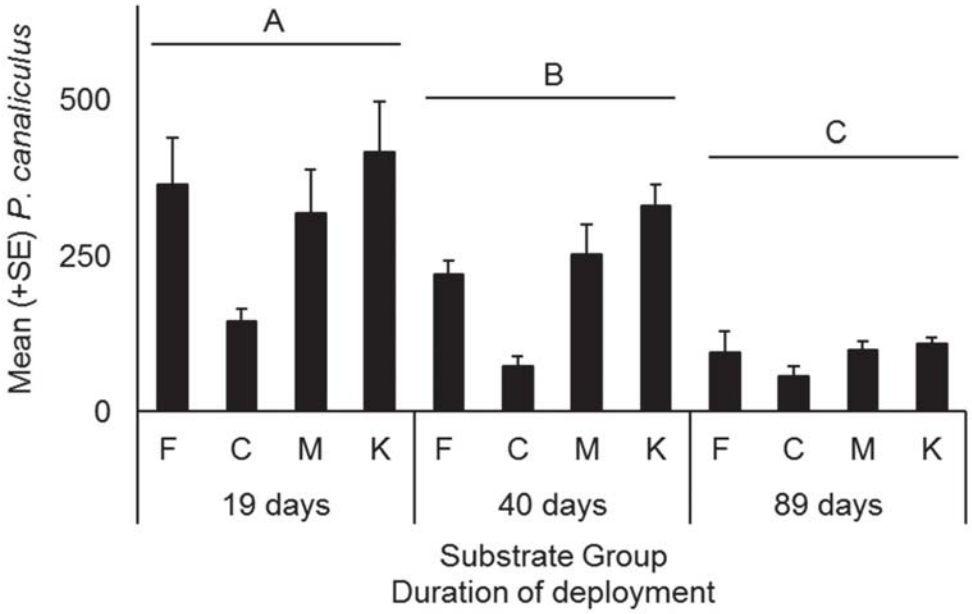

Figure 3.2. The mean number (+ SE) of dead *Perna canaliculus* per 18 cm dropper rope among Substrate Groups after 19, 40 and 89 days in the field. F = fine macroalgae, C = coarse macroalgae, M = mixed

macroalgae, and K = Kaitaia spat. Different capital letters depict overall differences among durations. Different lower-case letters indicate pairwise differences among Substrate Groups over time. There were no interactive effects.

The mean abundance of live juvenile *P. canaliculus* increased through time on the control ropes to 233.2 ± 34.76 at 19 days, and again from 221.4 ± 55.49 to 468.2 ± 93.94 between 40 and 89 days ($F_{2, 12} = 4.42$, p = 0.02, Fig. 3.3). At the end of the experiment, a total of 4448 mussels had migrated to the PVC frames.

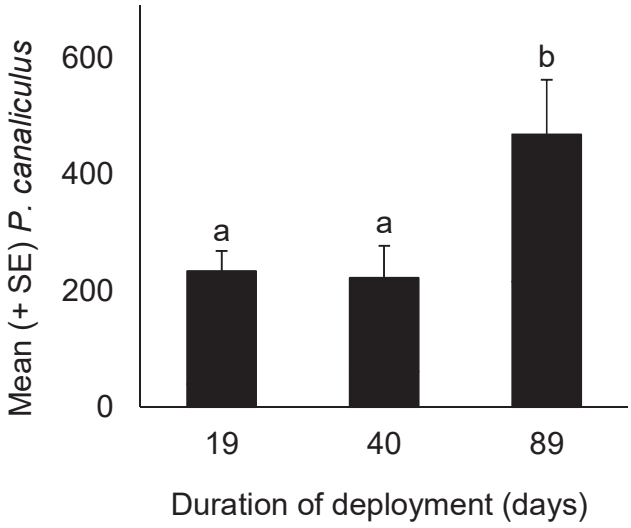

Figure 3.3. Numbers of *Perna canaliculus* (mean number + SE) on control ropes after 19, 40 and 89 days in the field. Different letters above bars indicate pairwise differences.

3.3.2. Fine-scale mussel rope conditions

The mean wet-weight of the macroalgae remaining on the ropes initially decreased from 6.4 ± 0.5 g to 3.8 ± 0.5 g over the first 19 days of the experiment and then again from 3.3 ± 0.4 g to 1.4 ± 0.2 g between days 40 and 89 (Duration $F_{3, 64} = 35.55$, p = 0.0001, Fig 3.4a). Across durations, coarse macroalgae (5.4 ± 0.9 g) weighed more than the mixed Substrate Group (3.5 ± 0.7g, Substrate Group $F_{3, 64} = 5.25$, p = 0.0227).

There were overall differences in the water quality of the mussel ropes among Durations in all analyses (Fig. 3.4), with fewer effects of Treatment, which were only detected for Dissolved Oxygen (DO) and NH_4N (Fig. 3.4b & f). Mean DO concentration increased by 0.03 mg L^{-1} across all Treatments from 7.86 ± 0.005 mg L^{-1} to 7.89 ± 0.008 mg L^{-1} between 19 and 89 days (Duration $F_{2, 54} = 8.53$, p = 0.0005) and there was an overall effect of Treatment ($F_{5, 54} = 5.06$, p = 0.0108) with coarse macroalgae having 0.007 mg L^{-1} less than the Kaitaia spat, and 0.004 mg L^{-1} more DO than on the control ropes. The Kaitaia spat and mixed macroalgal substrate groups had 0.051 mg L^{-1} and 0.044 mg L^{-1} more DO, respectively than the tank water. Water pH declined by 0.077 units from 7.926 ± 0.019 to 7.849 ± 0.014 between 19 and 40 days and was stable for the rest of the experiment ($F_{2, 54} = 6.9604$, p = 0.0023, Fig. 3.4c). There were no co-varying effects of time in the analyses of DO and pH.

3. Inefficient seeding of wild-sourced mussels

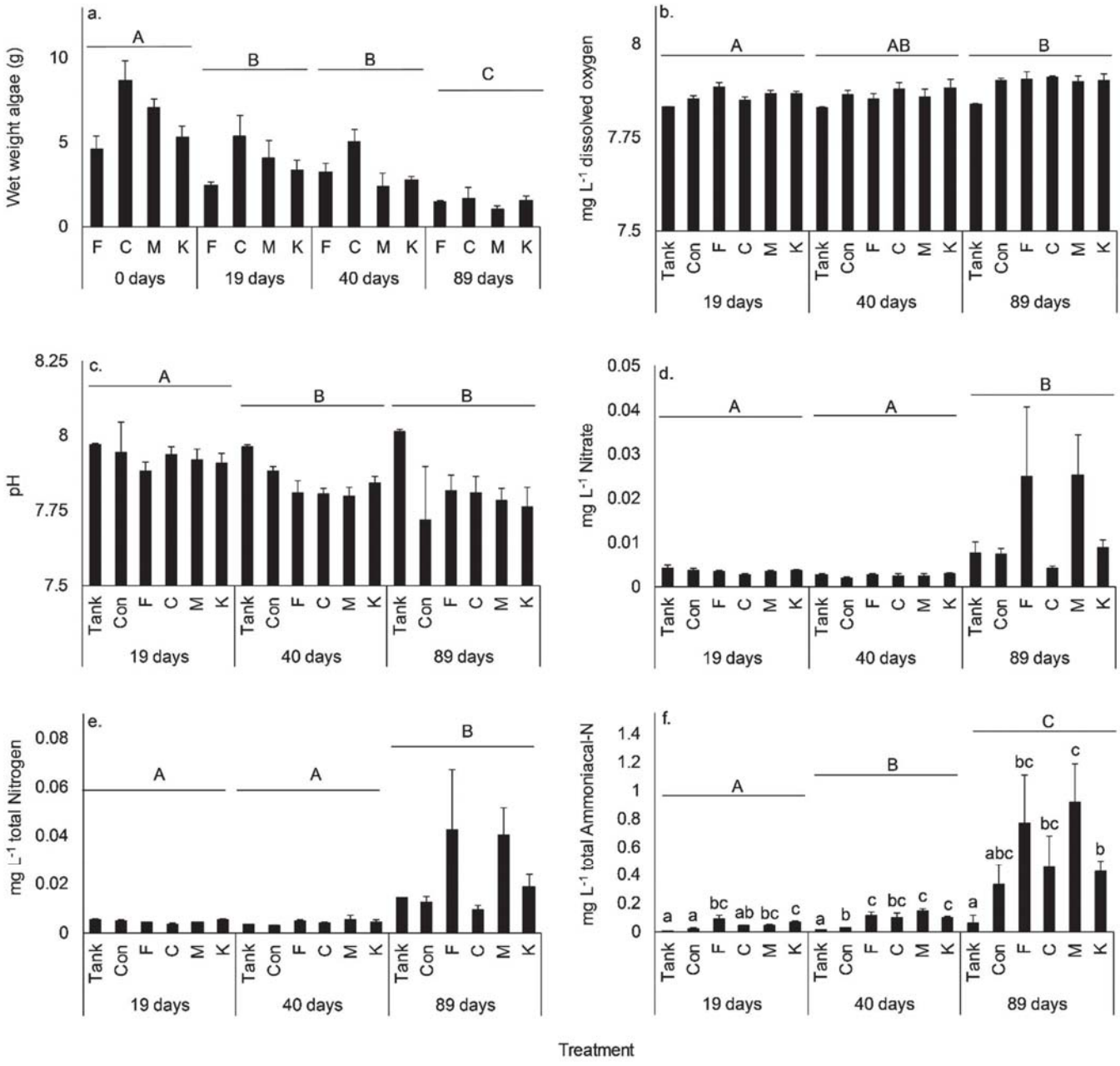

Figure 3.4. Mean (+ SE) substrate wet weight biomass (a), and water quality variables describing conditions around mussel ropes (b – f), among Durations and Treatments during laboratory assays lasting 3 days following period of either 19, 40 or 89 days in the field. Tank = water samples from the holding tank, Con = control ropes, F = fine macroalgae, C = coarse macroalgae, M = mixed macroalgae, and K = Kaitaia spat. Different capital letters indicate pairwise differences among Durations. Different lower-case letters indicate differences among Treatments within Durations following a Duration × Treatment interaction. In graph a, coarse macroalgae weighed less than mixed macroalgae across Durations ($t = 3.6$, $p = 0.038$). Note different scales on the x axis in graph a.

Mean Nitrate (NO_3N) and total Nitrogen (NO_xN) increased between days 40 and 89 by 0.011 mg L^{-1} and 0.018 mg L^{-1}, respectively (NO_3N $F_{2, 50} = 14.44$, $p = 0.0001$, NO_xN $F_{2, 50} = 9.68$, $p = 0.0003$, Fig 3.4d & e). Mean total Ammoniacal-N (NH_4N) increased over time and varied among treatments with all substrate groups (fine, coarse, mixed and Kaitaia spat) having elevated mean values of NH_4N compared to the tank water (Duration × Treatment $F_{2, 50} = 5.42$, $p = 0.0001$, Fig 3.4f). Typically, mean NH_4N among substrate groups was similar except that coarse macroalgae had less NH_4N than the mixed macroalgae and Kaitaia spat after 19 days, and the mixed macroalgae had more NH_4N than the Kaitaia spat after 89 days (Fig. 3.4f). The control ropes had more NH_4N compared to the tank water at 40 days. The amount of NH_4N also co-varied with time elapsed since the flow-through was shut down (Time elapsed × Duration $F_{2, 50} = 3.60$, $p = 0.0365$).

3.3.3. Percentage and size of migrating juvenile Perna canaliculus *in the laboratory*

Mean retention on the experimental ropes following the laboratory assays was 49.1 ± 4.4 % (19 days), 41.1 ± 2.9 % (40 days), and 28.7 ± 2.4 % (89 days) of the total number of mussels per experimental rope (i.e., in each aquarium) regardless of Substrate Group, with 19 and 40-day durations having greater retention than 89 days (Duration $F_{2, 60} = 17.9$, p = 0.0001, Fig. 3.5). Retention was greater on the control ropes (59.0 ± 5.3 %) compared to coarse (37.9 ± 2.1 %) and mixed (30.6 ± 2.7 %) macroalgae across durations (Substrate Group $F_{4, 60} = 18.1$, p = 0.0007, Fig. 3.5). By contrast, the size of the mussels varied among Substrate Groups and between Retention Status for each of the experimental periods (Fig. 3.6, Table 3.2). At 19 days, the juvenile *P. canaliculus* on control ropes (2.7 ± 0.1 mm) were on average > 0.62 mm smaller than in the other substrate groups, while the mussels on the coarse macroalgae (3.7 ± 0.1 mm) were on average largest by > 0.24 mm, and on the fine macroalgae, they were on average 0.2 mm larger than for the Kaitaia spat (Fig. 3.6a). After 40 days, the control ropes (4.2 ± 0.7 mm) had the smallest mussels by on average > 1.7 mm, followed by mussels on the Kaitaia spat (5.9 ± 0.7 mm) that were on average > 0.44 mm smaller than in the other Substrate Groups (Fig. 3.6b, Table 3.2). After 89 days, the controls and fine macroalgae had the smallest mussels by on average > 0.6 mm (Fig. 3.6c, Table 3.2). Across all Substrate Groups the mussels retained on the ropes were smaller, on average, than those that migrated by on average 0.45 mm at 19 days and 0.51 mm at 89 days but were bigger by on average 0.76 mm at 40 days (Fig 3.6, Table 3.2).

The number of *P. canaliculus* retained in the field (i.e., the total number of mussels per rope/aquarium) was negatively correlated with the percentage retained after the laboratory

experiments regardless of Duration (r = -0.66 to -0.75, $p < 0.02$). At 19 days, DO concentration was negatively (r = -0.62, $p = 0.02$), and pH was positively (r = 0.53, $p < 0.09$) correlated to the percentage of juveniles remaining in the laboratory. NH_4N concentration had a negative relationship with the percentage of juveniles retained at 19 days (r = -0.56, $p = 0.05$) and 40 days (r = -0.59, $p = 0.02$), and a positive correlation with the total number of mussels retained during the field experiment at 40 days (r = 0.65, $p = 0.001$). The wet weight of the macroalgae was negatively correlated to the number of juveniles retained in the field after all durations (r = 0.48 to 0.64, $p < 0.015$) while there was a negative correlation between the wet weight of the macroalgae and the percentage of juveniles retained in the laboratory at the end of the experiment (day 89, r = -0.42, $p = 0.036$).

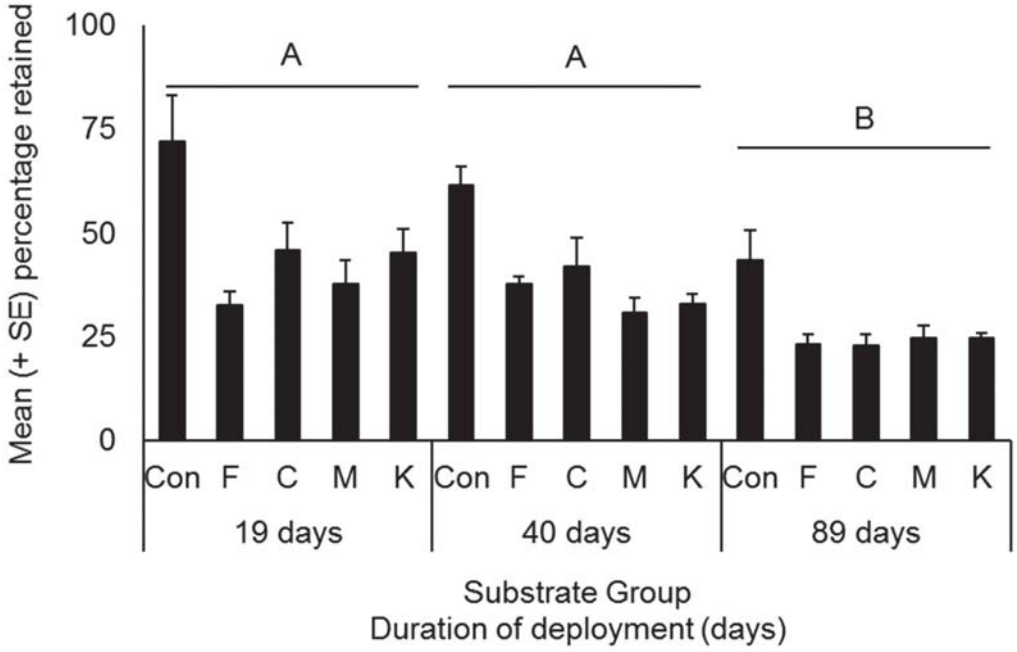

Figure 3.5. The mean (+ SE) percentage of retained *Perna canaliculus* among Substrate Groups after 19, 40 and 89 days in the field followed by 3 days in laboratory aquaria. Con = control (no macroalgae), F = fine macroalgae, C = coarse macroalgae, M = mixed macroalgae, and K = Kaitaia spat. Different capital letters indicate pairwise differences among Durations.

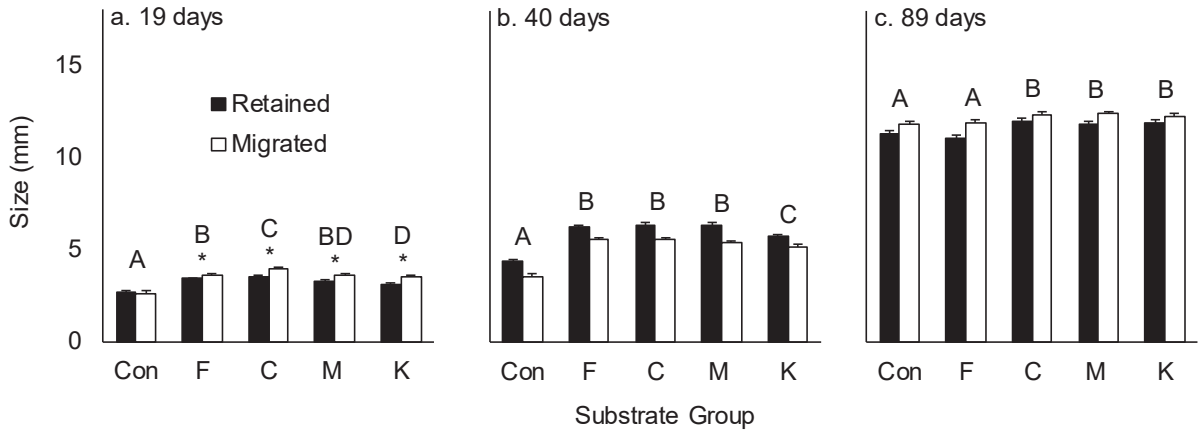

Figure 3.6. The mean (+ SE) size of retained (black bars) and migrated (white bars) *Perna canaliculus* in four Substrate Groups following seeding out in the field for 19, 40 or 89 days followed by 3 days in laboratory-aquaria. F = fine macroalgae, C = coarse macroalgae, M = mixed macroalgae, and K = Kaitaia spat. Different capital letters indicate pairwise differences among Substrate Groups within the same Duration pooling across retained and migrated groups. Asterisks indicate significant differences ($p < 0.05$) between the mean sizes of retained and migrated mussels within a Substrate Group following an overall significant Substrate Group × Retention Status interaction. The mean size of the retained mussels was greater than for migrated mussels regardless of Substrate Group at 40 days (graph b), ($p = 0.01$), whereas at 89 days the mussels were smaller (graph c, $p = 0.01$).

Table 3.2. Results from PERMANOVA analyses testing for the effects of Substrate Group and Retention Status (retained vs migrated) on the mean size of juvenile *Perna canaliculus* after 19, 40 and 89 days in the field followed by 3 days in laboratory aquaria.

Source	f/r	df	MS	*F*	P	perms
				19 days		
Substrate (Su)	f	**4**	**2.42**	**34.4**	**0.001**	**999**
Retention Status (RS)	f	**1**	**1.53**	**21.8**	**0.001**	**997**
Su × RS	f	**4**	**0.23**	**3.3**	**0.008**	**999**
Residuals		2202	0.07			
PERMDISP				14.0	0.001	
				40 days		
Substrate (Su)	f	**4**	**5.06**	**72.4**	**0.001**	**998**
Retention Status (RS)	f	**1**	**6.04**	**86.3**	**0.001**	**995**
Su × RS	f	4	0.07	1.0	0.425	998
Residuals		2190	0.07			
PERMDISP				3.6	0.002	
				89 days		
Substrate (Su)	f	**4**	**0.54**	**10.3**	**0.001**	**999**
Retention Status (RS)	f	**1**	**1.30**	**24.9**	**0.001**	**999**
Su × RS	f	4	0.04	0.8	0.509	999
Residuals		2490	0.05			
PERMDISP				6.1	0.001	

F = *Pseudo-F.* **Bold** text indicates significance at $p < 0.01$.
Data were log (x + 1)
transformed

3.4. Discussion

The results of this study reveal the complexity of the retention issue in mussel aquaculture, because losses of juvenile mussels were due to both secondary settlement and to a lesser extent, mortality. Enormous losses of juvenile *Perna canaliculus* were recorded (74.4 – 84.9 % of the starting density after 89 days), with most of the losses occurring during the first 19 days (64.8 %) but continuing throughout the nursery period. There were no clear effects of the type of substrate on the retention of juvenile mussels, although the number of mussels retained was correlated to the concentration of NH_4N early in the experiment, and the remaining biomass of the substrates on which they were seeded, indicating that the decomposition of the spat material may play a part in mussel losses. Importantly, the high percentage (> 70 %) of mussels that moved in the laboratory experiments showed that many juvenile *P. canaliculus* can migrate away from their point of attachment throughout the early period of aquaculture production. This is a phenomenon that is enormously problematic for efficient and sustainable aquaculture production.

This is the first study to assess losses of Kaitaia spat throughout the nursery period (from the time of seeding to re-seeding) and indicates that early losses can have the greatest impact on overall retention. The pattern of major losses of juveniles occurring early in production shown in this study was similar to field experiments that assessed the retention of hatchery-reared juveniles in the Marlborough Sounds, although the initial losses of Kaitaia spat in this study (64.8 % over 19 days from seeding) were greater than the losses of hatchery spat (46.4 %, South et al. 2017). The occurrence of the greatest losses of juvenile mussels very early in the nursery period implicates the handling, relay and seeding of juvenile mussels into aquaculture as a potential cause because

the greatest losses and numbers of dead mussels were observed soon after deployment. Juvenile *P. canaliculus* can experience temperature variations of > 24 °C, wind-chill and desiccation due to transport in the open and from vehicle refrigeration units and sustained emersion that can impact the fitness and retention of the juveniles during relay from the collection-site to the farm-site (Webb & Heasman 2006, Carton et al. 2007, Heasman 2013). Therefore, it is possible that procedural aspects of the relay and seeding processes impact the retention of seed mussels in the aquaculture situation and were responsible for the enormous early losses recorded in this current study. Further research is warranted to determine and mitigate the effects of transportation on retention of juvenile mussels in aquaculture.

The number of dead *P. canaliculus* juveniles found on the experimental ropes after 19 days (311 ± 38.0 across macroalgal groups, Fig. 3.2) accounted for 17.9 % of the total number of mussels that remained *in situ* during this experiment, supporting the notion that either transport induced stress or underlying variations in fitness could be responsible for the early losses of mussels recorded in this study (Carton et al. 2007, Sim-Smith & Jeffs 2011). The dead *P. canaliculus* juveniles found at 19 days were smaller (all passed through a 2-mm sieve) than the average size of the living juveniles (3.34 ± 0.02 mm shell length), indicating that smaller mussels may have had greater rates of mortality, or that mortality occurred soon after deployment, when the average size of the mussels was smaller (1.90 ± 0.02 mm). Decreases in the number of dead mussels over time (Fig. 3.2), and the lack of any substantial increase in the size of dead mussels, suggest that mortality occurred early in the nursery period and that the shells of the dead mussels were subsequently washed away by currents. Given that significant reductions in the number of

dead mussels were recorded during the later periods of this study (days 40 and 89), it is possible that the mortality data from day 19 are also affected by prior losses of dead individuals which would lead to an underestimate of the number of dead juvenile *P. canaliculus* at this time.

An important caveat in this study is that mussel-exchange among experimental ropes is likely to have occurred given the apparent mobility of the mussels and the deployment of replicate ropes on frames, a procedure that was adopted due to spatial constraints for experimental deployments on the commercial farm. Mussel exchanges between the experimental ropes could perhaps reduce the strength of the findings, especially if migrating mussels preferred one of the macroalgal groups, although this does not generally appear to be the case given the similar numbers of mussels among Substrate Groups and consistent temporal declines in *P. canaliculus* abundance.

The results of this study represent a significant step towards separating the relative effects of secondary settlement processes and mortality as causes of mussel losses in aquaculture. Many mussels appeared to migrate from the seeded sections of ropes to the adjacent, un-socked ropes and the control ropes, indicating that secondary settlement behaviour of the *P. canaliculus* juveniles can reduce retention in the field, as has been previously hypothesised (Jeffs et al. 1999, South et al. 2017). By the end of the experiment, secondary settlement had reduced the number of mussels deployed at the beginning of experiment (74,427 in total, 3721.4 ± 213.6 per experimental rope), by at least 19.6 % (14,598 in total across control ropes, adjacent un-socked sections of rope and the frames). At the same time (89 days), mortality accounted for at least 2.5 % (1831 in total, 91.6 ± 10.4 per experimental rope) of the total number of mussels deployed at the start of the experiment, whereas the number retained *in situ* (16,987 in total, 606.7 ± 42.7 per experimental

rope) was 22.8 % of the total number initially deployed. Therefore, around 44.9 % of the original number of mussels remained unaccounted for at the end of the experiment. It is impossible to determine the cause of these losses, but they likely include secondary settlement migrations away from the nursery ropes altogether, mortality at least at the greater rates observed earlier in the experiment (i.e., at 19 days, Fig. 3.2) and other factors not tested here such as predation by fish (Hayden 1995, Morrisey et al. 2006).

In this study, retention was defined as the number of mussels remaining *in situ* on 18 cm sections of experimental rope. In the field experiment, mussels that had made secondary settlement migrations to adjacent sections of rope were considered 'lost', in line with other studies of retention (Hayden & Woods 2011). Such movements would not be considered losses in the commercial aquaculture setting, because the mussels attached to the rope would still be available for re-seeding at the end of the nursery period (Total mussels = black + white bars in Fig 3.1). However, the numbers of mussels on the adjacent un-socked 22 cm sections of rope did not increase over time after day 19, despite significant *in situ* losses and increases in the number of mussels arriving at the control ropes (Fig. 3.3). This suggests that the scale of the migrations was possibly limited by the availability of space. Space limitation and other density-dependent processes could be important for retention, especially in the case of Kaitaia spat, which are commonly grossly over-seeded to buffer the losses of seed mussels occurring during the nursery period (Meder et al. 2005b). Juvenile *P. canaliculus* that had moved to the un-socked sections of rope maintained a consistent density over time (20.2 - 22.4 mussels cm^{-1} for days 19 to 89), whereas the density remaining *in situ* reduced from 72.2 – 37.5 mussels cm^{-1} between 19 and 89 days. Further support

for the possibility that retention might be a density-dependent process comes from the significant negative correlations between the total number of retained juvenile *P. canaliculus* and the percentage retained during the laboratory experiments that indicate that mussels at greater density are less likely to be retained during periods of stress.

It is possible that movements along the seeded rope would not result in increased overall retention in a typical commercial deployment of Kaitaia spat, when juveniles are deployed in extremely high density along the entire length of the aquaculture rope, rather than being deployed next to vacant space (i.e., the un-socked sections) as in this current experiment. This continuous high density of mussels along the ropes may result in an overall increase in departure of mussels from the seeded rope versus movements along the rope. Decreases in seed mussel retention with increasing seed density have been shown in cultured blue mussels (*Mytilus* spp.) in many parts of the world (Lauzon-Guay et al. 2005, Frantzen 2007, Lachance-Bernard et al. 2010), although the fate of lost mussels (i.e., whether they died or migrated) is rarely quantified. The effects of density at seeding on density-dependent growth, mortality and rates of secondary settlement have not been studied for juvenile *P. canaliculus* in New Zealand but are necessary if efficient use of the seed resource is to be achieved.

The number of *P. canaliculus* retained *in situ* declined by 22.4 % between days 19 and 40, and again by 28 % between days 40 and 89 (Fig. 3.1) showing that substantial losses can occur throughout the nursery period. The low percentages (28.7 - 49.1 %) of mussels retained on the experimental ropes once they had been placed into aquaria in the laboratory for only a further 3 days suggest that juvenile mussels can migrate at any point during the nursery period, and the

percentage of juveniles making these migrations can increase over time (Fig. 3.5). The juveniles probably experienced a variety of stressors in route to, and in the aquaria, that might have triggered their migrations. For example, the juveniles were emersed for ca. 6 hours prior to re-immersion in the aquaria and received no food during the experimental periods in the laboratory. While the laboratory conditions were possibly stressful for the juveniles, the low rates of retention indicate that mussel aquaculture is vulnerable to losses even towards the end of the nursery period when local conditions may become unfavourable despite the mussels being bigger.

At the start of the experiment there were fewer mussels on the coarse macroalgae compared to the other macroalgal groups, a situation that has been reported in other studies of Kaitaia spat (Alfaro & Jeffs 2002, Alfaro et al. 2004, Jeffs et al. 2018). Overall, the coarse Substrate Group had the greatest retention of juvenile mussels during this study (25.6 %), and fewer mussels migrated from it to the adjacent un-socked sections of rope. However, the mean number of juvenile *P. canaliculus* on the coarse macroalgae was similar to all other substrates at the end of the experiment suggesting that similar quantities of seeded spat material can yield similar numbers of retained mussels, despite different starting densities and differential patterns of retention among its constituent macroalgal components. Indeed, the abundance of *P. canaliculus* became similar among Substrate Groups after 19 days in the field due to high initial losses from the fine and mixed macroalgae and the Kaitaia spat groups. It is possible that an underlying density threshold or carrying capacity is in operation such that the migrations continue no matter what the starting density or substrate until the carrying capacity is reached.

There were no differences in the size of the mussels between the fine and coarse macroalgal substrates at the start of this experiment, in contrast to other studies of Kaitaia spat that have typically found larger mussels on coarse macroalgae (Alfaro & Jeffs 2002, Alfaro et al. 2004). This situation had changed after 19 days in the field, when the mean shell length of juveniles in the coarse macroalgae was greater than all other groups by at least 0.24 mm, suggesting that perhaps greater numbers of smaller mussels were lost from this group, or that they grew more. The small size of mussels that had migrated to the control ropes (Fig. 3.6a & b) and those that were found dead (Fig. 3.2) support the hypothesis that smaller mussels were lost. Indeed, this is in line with other studies of settlement that have shown mussels of this smaller size to be mobile (Alfaro 2006b, South 2016). By contrast, mussels migrating in the laboratory at 19 days, when the mussels were on average between 3.1 and 3.9 mm in shell length, were larger than those remaining attached to the experimental ropes for all other substrates (Fig. 3.6a). This pattern of larger migrating mussels was reversed at 40 days when the average size of mussels was between 5.2 and 6.4 mm (Fig 3.6b) and repeated at 89 days when mussels were sized between 11.1 and 12.4 mm (Fig. 3.6c). Together, the size data obtained in this study highlight the complexity of mussel losses throughout the nursery period and suggest that secondary settlement behaviour in juvenile *P. canaliculus* can vary markedly with ontogenetic changes, as has been shown in studies of wild mussels (Buchanan & Babcock 1997, Erlandsson et al. 2008, von der Meden et al. 2010).

The water quality data obtained in this study provide a preliminary description of how the Kaitaia spat material modifies the local environment of seeded mussel ropes. All the water quality variables varied significantly over time, with the greatest changes being found between 40 and 89

days (Fig. 4a - f), when there was a 28 % reduction in the number of juvenile *P. canaliculus* across treatments. Dissolved oxygen and nutrients were at their greatest concentrations, and pH at its lowest value at the end of the experiment when the wet weight of macroalgae was also at its lowest. This is surprising in the case of dissolved oxygen, because the mussels were bigger at the end of the experiment and were likely consuming more DO. It is possible that this is an artefact of sampling and reflects a gradient of DO from more depleted in the water column, to less depleted near the degrading algae and rope. Nutrients were highly variable at 89 days, possibly reflecting greater decomposition of the macroalgae or, in the case of NH_4N, excretion by the mussels and biofouling organisms. The positive correlations between the number of juveniles retained in the field and the wet weight of the macroalgae support the hypothesis that the breakdown of the seaweed would affect retention, especially because the retained mussels were not attached to the macroalgae at the end of the experiment, rather they were attached to each other and the outer sock (pers obs).

NH_4N concentration had a significant negative relationship with the percentage of mussels retained on ropes in the laboratory at 19 days (r (24) = -0.56) and 40 days (r (24) = -0.59), and a positive relationship with the total number of mussels retained at day 40 (r (24) = 0.65), suggesting a negative feedback between greater numbers of mussels that might increase NH_4N and then decrease their retention. The lack of any correlation at the end of the experiment, when NH_4N was at higher concentrations, and the mussels were bigger, suggests that smaller juveniles might be more sensitive to variations in NH_4N. Alternatively, the toxicity of NH_4N might have been reduced later in the experiment due to the reduced pH of the seawater (Tomasso 1994). Toxicity of NH_4N

is due to the concentration of unionised NH_3N that varies with pH, with only 1 % of NH_4N being NH_3N at a pH of 7 compare to 10 % at a pH of 8 (Downing & Merkens 1955, Epifanio & Srna 1975). The negative association between the percentage of juveniles retained and the concentration of DO is unlikely to be due to its negative effects on the mussels, because all of the concentrations (7.86 ± 0.005 mg L^{-1}) were well within the range of 6.4 ± 0.8 and 12.0 ± 0.7 mg L^{-1} that was found to have no effect on the settlement and mortality of juvenile *P. canaliculus* in a laboratory study (Alfaro 2005). Similarly, such small differences in pH as were found at day 19, have not been found to have adverse effects on juvenile molluscs in other studies (Range et al. 2011).

This study followed the retention of juvenile *P. canaliculus* associated with various components of Kaitaia spat, sampled from one consignment of Kaitaia spat, throughout the nursery period (89 days). It is possible that differences in retention among components of the spat material will vary within and among consignments, given that the composition of the Kaitaia spat material is temporally variable (Jeffs et al. 2018). Furthermore, the four substrates tested in this experiment were necessarily broad (e.g., coarse vs fine macroalgae), due to the spat material being comprised of lots of small pieces of many different species of macroalgae and other material. Whether retention varies among families or species of macroalgae within the Kaitaia spat material, or among consignments of Kaitaia spat remains untested. Similarly, the size of mussels attached to the spat material can be highly variable and might influence retention through variations in secondary settlement behaviour, fitness and competitive interactions in the cohorts of mussels.

Conclusions

The majority of juvenile *P. canaliculus* seeded into aquaculture in this study were lost, showing that the nursery process for Kaitaia spat can be highly inefficient, particularly in the first few weeks after seeding. Juveniles were shown to have made secondary settlement migrations and died, albeit in smaller numbers, thereby reducing retention throughout the nursery period. However, the fate of at least 49 % of the seeded mussels is unknown. The results presented here have important implications for long-line mussel aquaculture because they point to the seeding process and juvenile behaviour in enormous losses of mussels, highlighting the relative importance of early losses compared to those later in the nursery period (i.e., 64.8, 22.4 and 28.0 % at 19, 40 and 89 days, respectively). Aspects of the seeding process and their effects on retention warrant further research if the underlying causes of juvenile loss are to be understood and mitigated. The data presented here also suggest that mussel density can affect retention with fewer mussels migrating away from experimental ropes when they were in lower density in the aquaria, or once they had relocated onto the un-socked sections of rope, possibly due to elevated NH_4N concentrations. Further and more targeted studies into the effects of seed density are required to determine optimal levels of seeding and improve the efficiency of mussel aquaculture in New Zealand.

Chapter 4.

Differential effects of adult mussels on the retention and fine-scale distribution of juvenile seed mussels and biofouling organisms in long-line aquaculture

4.1. Introduction

Increases in the global human population and its pressure on wild stocks of fish and shellfish have resulted in the rapid emergence of aquaculture to meet our growing demand for seafood (Naylor et al. 2000, Pauly et al. 2002). Mussel aquaculture has grown to become a major global industry during the last 30 years (Smaal 2002b, Carrasco et al. 2014, FAO 2016). The majority of mussel aquaculture relies on wild sources of larval and juvenile mussels to seed aquaculture structures (e.g., ropes, rafts and benthic mussel beds) in coastal production facilities. However, natural settlement of mussels is variable in space and time, and this variability causes considerable uncertainty in the continuity of aquaculture production (Carrasco et al. 2014). Access to wild seed sources for aquaculture is also increasingly being constrained by regulations such as quota allocation and reduced or managed access to seed-catching areas and seasons (de Vooys 1999, Smaal 2002b). The early stages of mussel aquaculture production can be extremely inefficient, with massive quantities of juveniles frequently lost soon after capture, further compounding the vulnerability of aquaculture operations to natural population fluctuations (Peteiro et al. 2007b, Capelle et al. 2014). Retaining juvenile mussels within aquaculture production systems in the face of a natural tendency for losses is commonly referred to as ‘retention’. Increasing the retention of juvenile seed mussels in the early production cycle would greatly increase the overall efficiency of the mussel aquaculture industry and lessen its susceptibility to natural variations in larval supply and settlement.

The New Zealand mussel industry is based on the aquaculture of the endemic green-lipped mussel, *Perna canaliculus* (Gmelin, 1791), and this industry has grown to an annual production of

101,311 t since its development in the 1970s (Aquaculture New Zealand 2016). The issue of retention has received attention in New Zealand, where the mussel aquaculture industry is almost entirely reliant on a single ephemeral wild source of seed mussels (Alfaro & Jeffs 2003, Alfaro et al. 2010). In recent years, overall production has been severely impacted by intermittent periods of limited availability of wild mussel seed. Furthermore, the retention of juvenile *P. canaliculus* on aquaculture growing substrata, such as fibrous nursery ropes, can be very poor, with losses that range from 50 to 100 % (Jeffs et al. 1999, Webb & Heasman 2006, Hayden & Woods 2011). Consequently, a few studies have specifically addressed the causes of low retention, focusing on methods of determining the quality of juveniles captured from the wild (Webb & Heasman 2006, Sim-Smith & Jeffs 2011), impacts on fitness and behaviour that can occur during transport or due to poor handling (Webb & Heasman 2006, Carton et al. 2007) and environmental conditions during early production (Alfaro 2006a, Carton et al. 2007, Hayden & Woods 2011). However, the issue of retention is complex and far from understood, largely due to the small number of studies that have addressed this problem.

One of the most important factors governing the retention of *P. canaliculus* is likely to be the secondary settlement behaviour that is a pronounced feature of this species. Secondary settlement has been observed in *P. canaliculus* juveniles of 0.3 – ca 6 mm in length (Buchanan & Babcock 1997, Jeffs et al. 1999). Primary settlement occurs when mussel larvae of 240 – 300 μm transition from pelagic to benthic modes of life and undergo metamorphosis. Mussel larvae often settle initially on filamentous structures, such as algae, and this has been proposed as a mechanism to avoid consumption by, or competition with, conspecific adults, although there is supporting

(Bayne 1964, Alfaro 2006b) and refuting (Lasiak & Barnard 1995, Erlandsson et al. 2008) evidence for this being advantageous. Secondary settlement is the process by which juvenile mussels detach and migrate away from their initial larval settlement sites to explore, select and re-settle in alternative locations (Bayne 1964). Movement can occur via the juvenile initiating mucus drifting or pedal crawling behaviour that operate over medium (10s to 100s m) or small (cm) scales, respectively (Bayne 1964, Buchanan & Babcock 1997, Carton et al. 2007, Le Corre et al. 2013, South 2016). The ability of large numbers of juvenile mussels to migrate away from aquaculture growing structures is enormously problematic not only because these mussels are lost from commercial production, but also because it creates vacant space for colonisation by biofouling organisms which can compete with the remaining cultured mussels and create problems for subsequent aquaculture processing (Fitridge et al. 2012). Furthermore, small-scale movements of juvenile mussels are of interest in New Zealand because seed mussels are usually attached to degradable substrata (e.g., algae or, in the case of hatchery-reared juveniles, coir ropes) when they are deployed at farm sites for on-growing. Therefore, it is essential that seed mussels migrate to permanent substrata (i.e., suspended growing ropes) if they are to remain in production.

The triggers of secondary settlement behaviour in juvenile mussels are not well understood and currently cannot be managed. There appear to be many potential triggers of secondary settlement processes in mytilid mussels, including changes in the local environment (Carton et al. 2007, Hayden & Woods 2011) and changing habitat requirements or behaviour (Alfaro & Jeffs 2002, Alfaro et al. 2004, von der Meden et al. 2010). Furthermore, juvenile seed mussels on growing structures will likely experience intense pressure from the ongoing settlement of a wide

range of biofouling organisms competing for the same space (Hurlbut 1991, Holthuis et al. 2015). While biofouling organisms are known to impact the later stages of mussel aquaculture production, their effect on the retention of juveniles has not been examined in any detail.

One potential cause of secondary settlement migration is the proximity of the juvenile mussels to populations of conspecific adults. In natural habitats, mussels either settle as larvae directly among adults (Lasiak & Barnard 1995, Dobretsov & Wahl 2001) or arrive as secondary settlers following dispersal from primary settlement sites (Bayne 1964, Alfaro 2006b, Newell et al. 2010). Behavioural responses to physical and chemical properties of the substratum can be important determinants of secondary settlement location in mussels (Alfaro et al. 2004, von der Meden et al. 2010). For example, juvenile *Perna perna* actively seek out adult conspecifics over small (cm) scales (Cáceres-Martínez et al. 1994, Erlandsson et al. 2008, Porri et al. 2016). The mechanisms used by juveniles to remotely locate adult mussels is unknown, but is likely to be a waterborne chemical cue, as has been observed for the primary larval settlement in a range of invertebrates, including mussels (Anderson 1996, Steinberg et al. 2002, de Vooys 2003, Alfaro et al. 2006, Morello & Yund 2016). It is possible that as the juvenile mussels grow, their affinities for chemical cues or surficial features shift to those associated with adults and therefore trigger secondary settlement (Bayne 1964, von der Meden et al. 2010). Determining whether this is the case could be of tremendous relevance for the development of approaches for reducing the losses of mussel juveniles in aquaculture operations.

If juvenile mussels undergo secondary settlement to recruit among adults, then it is possible that contact or close-proximity to adult mussels might suppress secondary settlement behaviour.

For example, juvenile *P. canaliculus* > 2 mm have been shown to secondarily settle into adult beds and recruit to the adult population (Alfaro 2006b). In addition, adult mussels can provide a structural refuge from predators and abiotic stressors to reduce post-settlement mortality (Bertness & Grosholz 1985). If the presence of adult mussels suppresses secondary settlement of juveniles by providing chemical cues or physical refuge, then seeding adult individuals alongside conspecific juveniles for on-growing might offer an opportunity to reduce the losses of juveniles currently observed in many mussel farming operations.

This study used a field experiment to test whether deploying conspecific adults with juvenile seed mussels increases the retention of juveniles. In addition, small-scale movements of seed mussels were assessed to consider how the addition of adults might affect the distribution of seed mussels on nursery structures typically used in aquaculture production. The development of the biofouling assemblages was also described to gain a better understanding of which organisms might be problematic for mussel seed during the early stages of production.

4.2. Materials and methods

4.2.1. Study site and source of juvenile mussels

This study was undertaken on a long-line mussel farm operated by Sanford Ltd in outer Pelorus Sound in the Marlborough Sounds, New Zealand (40° 57' 18" S, 174° 3' 39" E, Fig. 4.1). The juvenile mussels used in this study were hatchery-reared by SpatNZ Ltd (Nelson, New Zealand). Hatchery-reared juveniles within a single cohort have consistently shared developmental histories, such as shared parentage, *ad libitum* access to food and managed densities. In the hatchery, larval

mussels were settled onto fibrous coir (coconut fibre ropes ca. 10 mm in diameter) ropes that are transported to the nursery site and deployed alongside a typical fibrous polypropylene nursery rope which is suspended in the water column from a buoyed surface line to form a nursery rope. The polypropylene rope and coir rope containing the juveniles are held together with a mesh sock placed over the two strands which collectively form a nursery rope. However, only the polypropylene rope is a permanent structure and the coir and the sock can degrade and are subsequently lost during production.

4.2.2. Experimental design

A field experiment was initiated on 13 October 2015 to test whether the presence of adult *Perna canaliculus* on nursery ropes would increase the retention of conspecific juvenile seed mussels. This experiment was deployed in October (i.e., early austral spring) because this is typically a period of low primary settlement of *P. canaliculus* at the study site and therefore our estimates of retention were less likely to be confounded by over-settling conspecifics early in the experimental period. At other times of the year, new cohorts are easy to determine using size and morphological characteristics (Redfearn et al. 1986, Atalah et al. 2016). Furthermore, the mussel farm used in this study was in 30 m deep water, with the experiment deployed at 4 m depth, while the secondary settlement by wild mussels typically occurs deeper in the water column (Alfaro & Jeffs 2003).

Fifty experimental nursery ropes (45 cm in length, hereafter ‘experimental ropes’) were assigned to 1 of 5 treatments that included two densities of live adult *P. canaliculus* (5 and 20 adults per experimental rope, hereafter PL (*P. canaliculus* Low) and PH (*P. canaliculus* High), respectively), two densities of empty adult shells that were included to examine the structural

effects of adult mussels without their biological functioning such as defecation, byssus production, filter feeding or chemical signalling (5 and 20 shells per experimental rope, hereafter SL (Shell Low) and SH (Shell High), respectively), and a control to which no adults or shells were added (hereafter, C). The high adult mussel and shell density of 20 rope^{-1} (i.e., 44 m^{-1}) was chosen to reflect typical densities of mussels of the size used in this experiment at cropping, in a commercial aquaculture setting. An additional 10 ropes were seeded and transported to the study site, but were not deployed, being retained for analyses to provide an estimate of the starting density on experimental ropes. The live adult mussels used in this experiment were obtained from a nearby mussel farm and were 93.1 ± 2.1 SE mm (n = 20) in shell length. Biofouling organisms were removed from the live adults, which were then deployed at even intervals along the experimental ropes to replicate a typical arrangement of adult mussels on a growing line. The rope and the mussels were then held together by covering with a mesh stocking. The adult mussel shells were 91.7 ± 1.0 mm (n = 20) in shell length and were glued together to represent the physical form of adult mussels and glued (Coral Glue Eco Tec Marine) to the polypropylene rope at regular intervals. Similar amounts of glue were added to all the other polypropylene ropes used in this experiment as a procedural control.

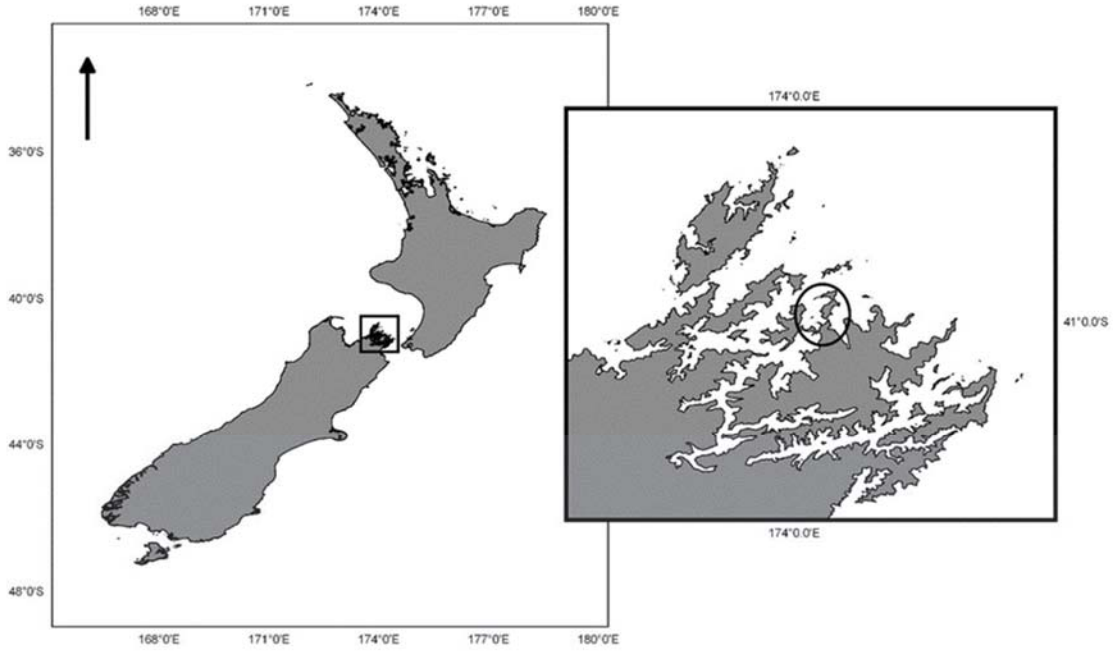

Figure 4.1. New Zealand, showing the Marlborough Sounds (inset) and the study site in outer Pelorus Sound (circled).

Perna canaliculus are grown in a longline culture system that comprises continuous looped ropes suspended from two buoyed longlines (Jeffs et al. 1999, Woods et al. 2012). Each buoy is 6 – 8 m apart, forming 'bays' between buoys from which the nursery and the later grow-out ropes are hung. The present experiment occupied one such bay made available on a commercial mussel farm and involved the deployment of five rectangular frames (100 × 90 cm) consisting of two wooden vertical rods (20 × 10 × 900 mm) intersected horizontally at the top, middle and bottom by three cylindrical nylon rods (10 × 1000 mm; Fig. 4.2). The frames were lashed at 1 m intervals

to the backbone lines and hung at a depth of 4 m. The frames were weighted to align them vertically in the water column. Two replicates of each experimental treatment were cable-tied to each of the frames (Fig. 4.2). These experimental ropes were positioned in a haphazard order, ~15 cm apart on the frame and sat vertically in the water column to reflect the typical position of a commercial mussel aquaculture nursery rope used for rearing juvenile mussels of this species. Each experimental rope was seeded with 45 cm length of coir rope coated in hatchery-raised juveniles that were seeded according to standard industry practices. The hatchery-raised juveniles were approximately 6 wk post-settlement, and their shell length ranged from 0.29 to 1.76 mm with a mean length of 1.01 mm ± 0.01 (n = 498). All of the experimental ropes were transported to the study site in a cool and damp environment to reduce the likelihood of mortality or modified secondary settlement behaviour as artefacts of factors occurring during transport (Carton et al. 2007).

After 30 d (hereafter, 1 mo), one experimental rope from each treatment from each frame was randomly collected by removing the frames from the water and cutting the cable ties. Sampling was repeated at the end of the experiment after 145 d (hereafter, 5 mo). At each sampling, five experimental ropes per treatment were collected. Experimental ropes were returned to the laboratory and separated into the three substrata (coir, polypropylene rope and socks). Large biofouling organisms were removed with tweezers, and each substratum was then washed over a 250 µm sieve to ensure any wild primary mussel settlers were also captured. Experimental substrata were checked after washing to ensure that all juvenile mussels were sampled. The numbers of juvenile *P. canaliculus* within each substratum were counted, and 50 individuals were

measured from each experimental rope using image analysis (to 0.01 mm precision) and Vernier calipers (to 0.5 mm precision) at 1 and 5 mo, respectively. The shell length of 20 randomly selected *Mytilus galloprovincialis* that settled on each experimental rope were measured for the experimental ropes that were sampled at 1 mo. All biofouling organisms (> 1 mm) were identified to the highest possible taxonomic resolution (usually to family level or higher) and counted. The dry weights (after 48 h at 50 °C) of biofouling algae and sessile invertebrates were quantified. All settlers of *P. canaliculus* (assessed by smaller size than hatchery juveniles), *M. galloprovincialis* and *Modiolarca impacta* > 250 µm on the experimental ropes were identified and counted.

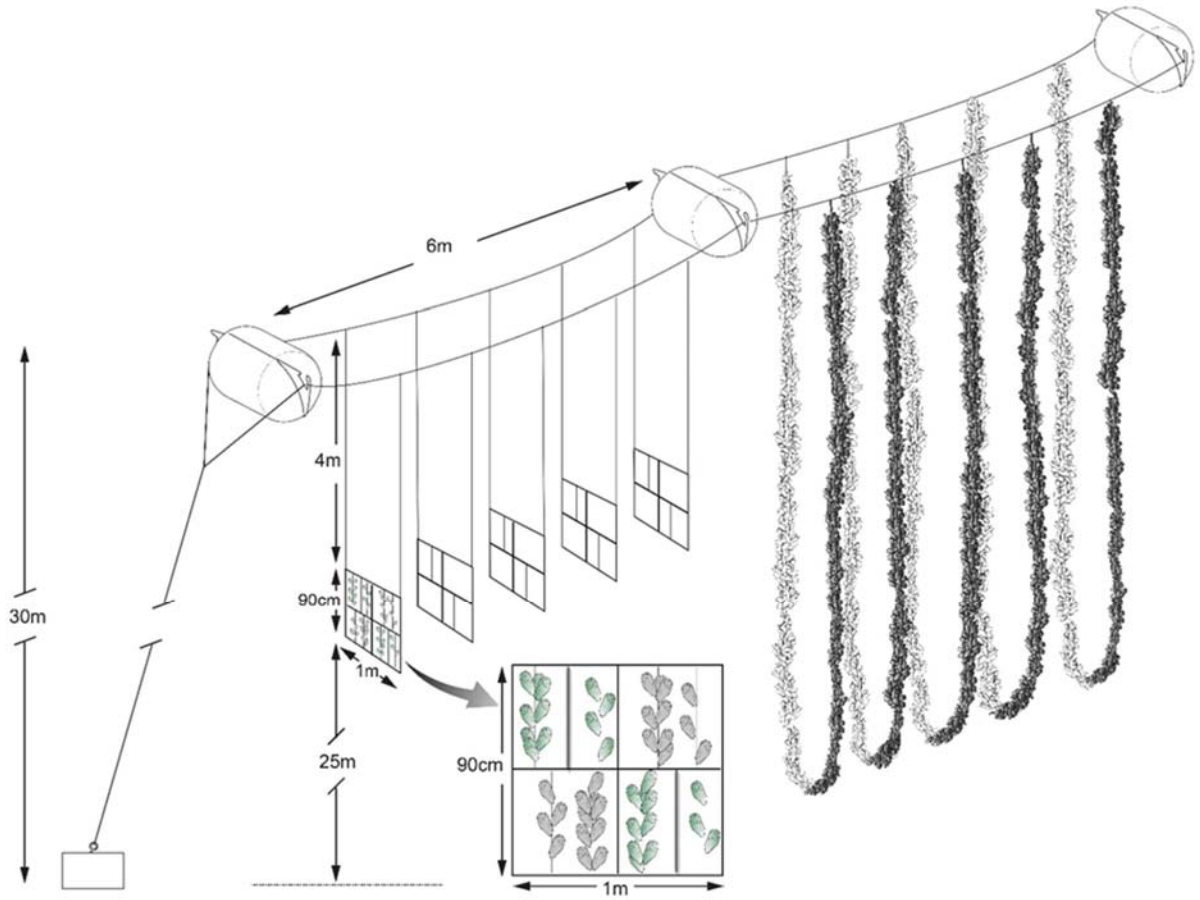

Figure 4.2. Experimental set-up on a long-line culture system at the study site in outer Pelorus Sound. Frames were 1 m apart. The inset depicts the spatial arrangement of replicates on the frames. The treatments were a control with no mussels added to experimental ropes (C; thick black line) and experimental ropes with low or high densities of either live adult *Perna canaliculus* (*Perna* Low [PL] and *Perna* High [PH], green mussels) or their shells (Shells Low [SL] and Shells High [SH], grey mussels). Experimental ropes were ~15 cm apart on the frames with 1 replicate treatment^{-1} frame^{-1} sampled after two deployment durations (1 and 5 mo).

4.2.3. Statistical analyses

Variation among experimental factors for all analyses in this current study was tested using permutational analysis of variance (PERMANOVA), which has no assumption of data normality, but assumes homogeneity of variances (Anderson et al. 2008). Cochran's *C*-test was used to assess the variance structure of data used for each univariate analysis. Where there was significant heterogeneity, data were transformed (square root, log (x + 1) or arcsine-square root) to stabilise variances. When transformation failed to homogenise the data, the results from analysed data were considered significant only at $p < 0.01$ to decrease the risk of Type 1 error. For multivariate tests, the PERMDISP routine was used to assess data dispersion. PERMANOVA was used to analyse univariate and multivariate data using distance matrices based on Euclidean distances and Bray Curtis similarities, respectively. Non-metric multidimensional scaling (nMDS) was used to visualise differences in biofouling assemblage composition prior to multivariate tests. Pairwise comparisons were used to determine between-treatment differences for significant effects in the full models. Tests were based on 999 (size data) or 9999 (all other data) permutations.

Retention of juvenile mussels

The effect of Duration (0, 1 and 5 mo) on the number of juvenile mussels was tested with a PERMANOVA using only the untreated control experimental ropes to determine patterns of retention on typical aquaculture substrata.

Effects of adult mussels on juvenile retention, distribution and size

The planned analysis included the factors Treatment (C, SL, SH, PL and PH), Substratum (coir, polypropylene rope and sock) and Duration (1 mo, 5 mo). However, separating the substrata turned out to be impossible after 5 mo due to the interwoven biofouling and mussel byssus. Therefore, the number of juveniles retained was summed across substrata for each experimental rope to test for the effects of Treatment and Duration on juvenile retention using a PERMANOVA model with the factors Treatment (C, SL, SH, PL and PH, fixed effect), Duration (1 and 5 mo, fixed) and Frame (1–5, random). Where Frame was used as a factor in the analysis, variation due to the highest-order interaction could not be calculated due to insufficient replication and was assumed to be a component of the residual variation (Anderson et al. 2008). Therefore, factors are tested for the presence of main effects over and above interactions involving frames (Quinn & Keough 2002), with the caveat that the significance of main effects might not be spatially consistent. A separate PERMANOVA was undertaken using only the 1 mo data to examine the distribution of juveniles on the three substrata, thereby assessing small-scale migrations (i.e., the number of juveniles that had moved from coir to the other substrata). The factors Treatment (C, SL, SH, PL and PH) and Substratum (coir, polypropylene rope and sock) were fixed while Frame (1–5) was a random factor. Additional separate analyses were performed for each individual substratum (coir,

polypropylene rope and sock) after 1 mo to test the effects of Treatment (fixed) and Frame (random) on the percentage of the total number of juveniles on each experimental rope. Finally, the size of the juvenile *P. canaliculus* was analysed separately at 1 and 5 mo for the effects of Treatment (fixed) and Frame (random). Size frequency distributions for each of the durations (0, 1 and 5 mo) were inspected to determine whether any natural settlement of *P. canaliculus* was present.

Effects of adult mussels on the recruitment of biofouling organisms

Data for the six most abundant taxa, the total biomass of sessile invertebrates and algae and the entire biofouling assemblage (multivariate data were fourth-root transformed) were pooled across substrata to test for the effects of Treatment (C, SL, SH, PL and PH, fixed), Duration (1 and 5 mo, fixed) and Frame (1–5, random). The similarity of percentages (SIMPER) routine was used to determine the proportional contribution of individual biofouling taxa to variation among treatments for multivariate data. To test whether the most abundant biofoulers after 1 mo were distributed differentially among substrata, the factors Treatment (C, SL, SH, PL and PH, fixed), Substrata (coir, polypropylene rope and sock, fixed) and Frame (1–5, random) were analysed with PERMANOVA.

After 5 mo, many of the adult mussels had been lost from the experimental ropes. Therefore, correlation analyses (Pearson's product moment) were used across live mussel treatments (i.e., PL and PH) to determine whether the number of remaining adults might be associated with the magnitude of biofouling development. The number or biomass of common biofouling taxa was correlated (Pearson's product moment on log (x + 1) transformed data) to the

number of juvenile *P. canaliculus* after 1 mo and 5 mo separately to determine whether biofouling may be implicated in juvenile losses. Correlation analyses were done across experimental mussel treatments.

4.3. Results

4.3.1. Retention of juvenile mussels

At the beginning of the experiment, 787.5 ± 20.4 (mean ± SE, n = 10) juvenile *Perna canaliculus* were attached to the experimental ropes. Considerable reductions in the abundance of juvenile mussels were observed on the untreated control ropes after 1 mo (decreasing to 422.4 ± 61.1 mussels, i.e., 46.4 % less) and then again after 5 mo (145.6 ± 10.2 mussels), resulting in a significant effect of Duration ($F_{2,12}$ = 65.7, $p < 0.001$, Table 4.1) in the analysis. On average, retention of juveniles after 5 mo was 18.5 % (i.e., an 81.5 % loss relative to initial abundance).

Table 4.1. PERMANOVA testing for the effects of deployment duration (0, 1 and 5 mo) on the number of juvenile *Perna canaliculus* retained on experimental ropes.

Source	f/r	df	MS	F/t	p	perms
			Main test			
Duration	f	2	**4.608**	**65.721**	**0.0001**	**9952**
Residual		17	7.011			
			Pairwise t-tests			
0 > 1				**3.567**	**0.002**	**2855**
0 > 5				**6.844**	**0.0005**	**2228**
1 > 5				**4.467**	**0.0072**	**91**

Bold text indicates significance at $p < 0.05$

4.3.2. Effects of adult mussels on juvenile retention, distribution and size

After 1 mo, all the live adult mussels added to the experimental treatments (i.e., PL, PH) had attached to the polypropylene rope, survived and remained. Despite being spread out along the polypropylene rope on deployment, the adult mussels moved along the ropes to form clumps, and generally all adults on individual experimental ropes were attached to one another. Several vacant patches of adult mussel byssus on the ropes suggested that adults had attached and subsequently moved on multiple occasions during this first month. After 5 mo, the number of adult mussels remaining ranged from 0 to 18 (0, 0, 8, 16, 18) in the PH and 2 to 5 (2, 3, 4, 5, 5) in PL treatments. However, there was no correlation between the number of remaining adult mussels and the number of juveniles on the experimental ropes (Pearson's r (9) = 0.31, p = 0.38). Additionally, two live adult mussels were found on one of both the C and SH experimental ropes, after presumably having byssus-walked around the frame. All mussel shells (SL and SH treatments) remained at the end of the experiment. It was not possible to separate the different substrata from the experimental ropes

after 5 mo in any of the treatments because the remaining coir and sock were tightly bound to the polypropylene rope with byssus from the juveniles and fouling by blue mussels *Mytilus galloprovincialis*.

The presence of live adult mussels or mussel shells, whether at high or low density on experimental ropes, had no effect on the retention of juvenile *P. canaliculus* (Treatment $F_{4, 16}$ = 1.8, p = 0.169; Fig. 4.3, Table 4.2). Among the five treatments, the number of juvenile mussels declined between 1 and 5 mo (Duration $F_{4, 16}$ = 98.83, p < 0.01; Fig. 4.3, Table 4.2). On average, all treatments had less than 25 % of the original starting abundance of juvenile mussels at the end of the experiment.

Table 4.2. PERMANOVA testing for the effects of seeding live adult mussels or their shells and deployment duration (1 and 5 mo) on the number of juvenile *Perna canaliculus* remaining on experimental ropes.

Source	f/r	df	MS	F	p	perms
Treatment	f	4	0.22	1.85	0.169	9948
Duration	f	1	**10.11**	**98.835**	**0.008**	**5264**
Frame	r	4	0.05	0. 70	0.602	9946
T × D	f	4	0.05	0.66	0.636	9956
T × F	r	16	0.12	1.68	0.151	9943
D × F	r	4	0.1	1.41	0.269	9953
Residual		16	0.07			

f/r = fixed/random. **Bold** text indicates significance at p < 0.05

One month following deployment of the experimental ropes, there were differences in small-scale migrations (i.e., juveniles that had moved from coir to ropes or outer socks) of juvenile

mussels among the three substrata for the five treatments (Treatment × Substratum, $F_{8, 56} = 3.1$, $p < 0.01$, Table 4.3), with greater numbers of juveniles found on the polypropylene rope in PH (64.6 ± 8.7) than in SH (25.2 ± 7.4), SL (25.0 ± 5.7) and PL (43.6 ± 6.8) treatments (pairwise *t*-tests $p < 0.05$).

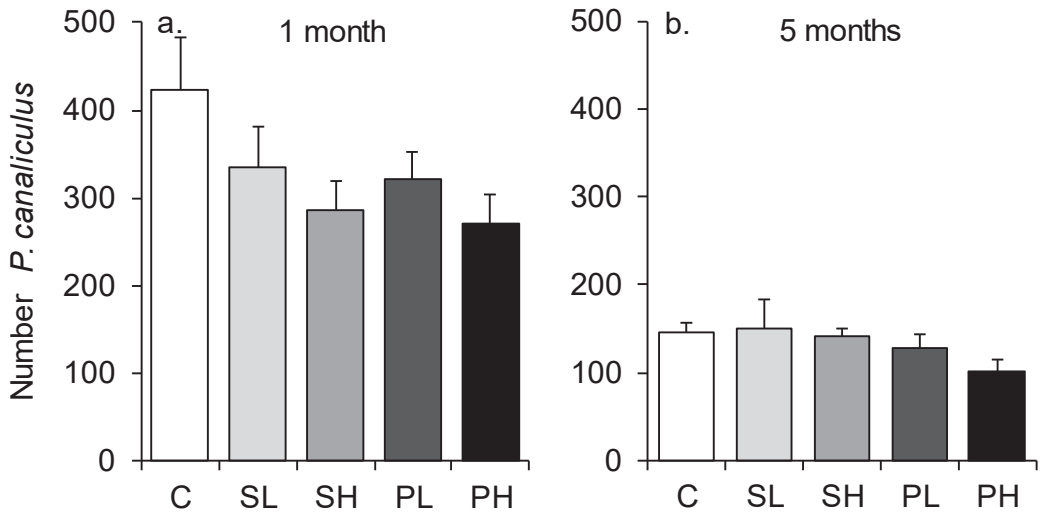

Figure 4.3. Effects of the factors Treatment and Duration of deployment on the mean (± SE) number of juvenile *Perna canaliculus* remaining on 45 cm experimental ropes at (a) 1 mo and (b) 5 mo. C: control (0 adult mussels or shells), SL: shells at low density (5 empty *P. canaliculus* shells), SH: shells at high density (20 empty shells), PL: *P. canaliculus* at low density (5 live adults), and PH: *P. canaliculus* at high density (20 live adults).

Table 4.3. PERMANOVA testing for the effects of seeding live adult mussels or their shells on the distribution of juvenile *Perna canaliculus* on aquaculture substrata after 1 mo.

Source	f/r	df	MS	F	p	perms
Treatment	f	4	0.32	0.72	0.5947	9962
Substratum	f	2	**31.54**	**415.41**	**0.0009**	**9914**
Frame	r	4	0.18	1.66	0.1784	9941
T × S	f	8	**0.62**	**5.81**	**0.0004**	**9955**
T × F	r	16	**0.44**	**4.11**	**0.0002**	**9924**
S × F	r	8	0.08	0.71	0.6828	9941
Residual		32	0.11			

Bold text indicates significance at $p < 0.05$

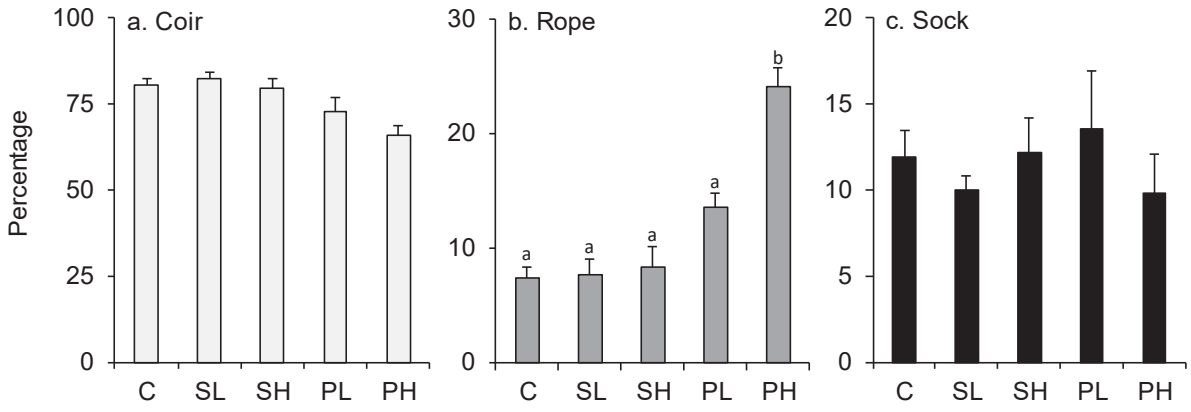

Figure 4.4. Mean percentage (± SE) of remaining juvenile *Perna canaliculus* on (a) coir, (b) polypropylene rope and (c) sock substrata among the five experimental treatments 1 mo after placement in the field. Treatment abbreviations as in Fig. 4.3. Different lower-case letters indicate significant differences between treatments. Note different scales on the y-axes.

4. Effects of adult mussels on juvenile retention

The majority (> 66 % in all treatments) of the juvenile seed mussels remaining at 1 mo were still attached to the coir, and there were no differences among treatments (Treatment $F_{4, 16} = 1.82$, $p = 0.17$, Fig. 4.4a, Table 4.4). A greater percentage (> 10 %) of juvenile mussels was found on the polypropylene rope in PH (24.1 ± 1.6 SE) compared to all other treatments (Treatment $F_{4, 16} = 23.01$, $p < 0.0001$, Fig. 4.4b, Table 4.4). There were no differences in the percentage of mussels attached to the socks among treatments (Treatment $F_{4, 16} = 0.49$, $p < 0.743$, Fig. 4.4c, Table 4). The number of *P. canaliculus* did not vary among experimental frames in any of the analyses

Table 4.4. PERMANOVA testing for the effects of seeding live adult mussels or their shells on the percentage of the remaining juvenile *Perna canaliculus* on aquaculture substrata after 1 mo.

Source	f/r	df	MS	F	p	perms
				Coir		
Treatment	f	4	12.74	0.49	0.7425	9952
Frame	r	4	16.68	0.65	0.6453	9967
Residual		16	25.51			
				Rope		
Treatment	f	4	**254.87**	**23.01**	**0.0002**	**9956**
Frame	r	4	7.95	0.71	0.5952	9959
Residual		16	11.07			
				Outer sock		
Treatment	f	4	12.74	0.49	0.7425	9964
Frame	r	4	16.68	0.65	0.6453	9955
Residual		16	25.51			

f/r: fixed/random. **Bold** text indicates significance at $p < 0.05$

One month following deployment, the juvenile *P. canaliculus* had attained an average size of 1.66 ± 0.01 mm in length (range = 0.69 – 2.92 mm), representing an average growth increment

of 0.65 mm. By 5 mo the juveniles had grown to 20 ± 0.004 mm (range = 6 – 36 mm). However, at both deployment periods, the size of juveniles varied among frames (1 mo; Treatment × Frame $F_{16, 1225} = 2.09$, $p < 0.01$, 5 mo $F_{16, 1225} = 2.2$, $p < 0.01$, Fig. 4.5, Table 4.5), but no clear patterns among treatments emerged from post hoc analyses (Fig. 4.5). Mussels smaller than experimental cohorts were not observed throughout the experiment, indicating that there was an absence of primary settlement of *P. canaliculus*.

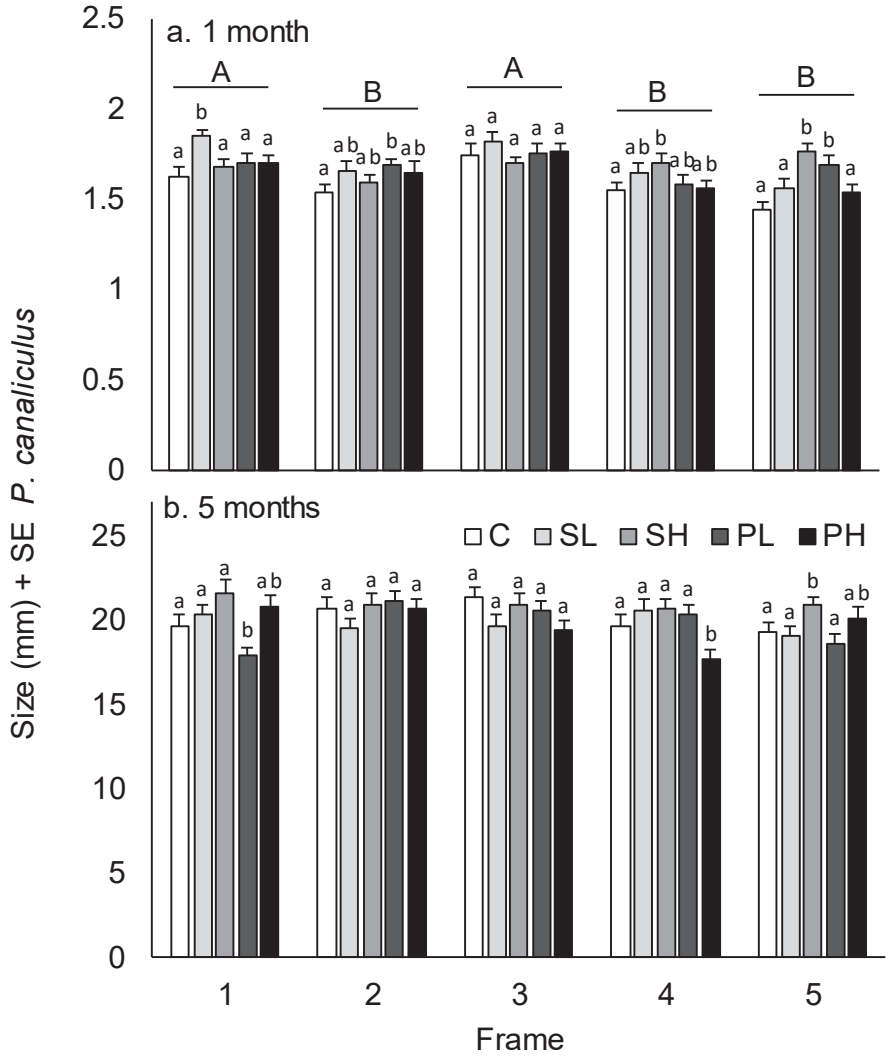

Figure 4.5. Effects of the factors Treatment and Frame on the size (mean length + SE in mm) of *Perna canaliculus* at (a) 1 and (b) 5 mo. Treatment abbreviations as in Fig. 4.3. Different lower-case letters indicate significant differences between treatments following a significant Treatment × Frame interaction. Different capital letters indicate significant differences among frames where a main effect of Frame was observed. Note different scales on the y-axes.

Table 4.5. PERMANOVAs testing for the effects of adding live mussels or their shells to aquaculture substrata on the size (length in mm) of juvenile *Perna canaliculus* after 1 and 5 mo.

Source	f/r	df	MS	F	p	perms	MS	F	p	perms
			1 month				5 months			
Treatment	f	4	0.018	2.738	0.074	999	0.029	1.277	0.315	999
Frame	r	4	**0.035**	**10.944**	**0.001**	**999**	0.016	1.783	0.129	999
T x F	r	16	**0.007**	**2.090**	**0.007**	**998**	**0.023**	**2.492**	**0.002**	**999**
Residual		1225	0.003				0.009			

f/r: fixed/random. **Bold** text indicates significance at $p < 0.05$

4.3.3. Effects of adult mussels on the recruitment of biofouling organisms

In this experiment, 68 biofouling taxa recruited to the experimental ropes, including amphipods, bivalves, ascidians, macroalgae and bryozoans. The six taxa that numerically dominated the biofouling assemblage were the mytilid mussels *M. galloprovincialis* and *Modiolarca impacta* and the amphipods Ischyroceridae, *Paradexamine* spp., *Caprella* spp. and *Parawaldeckia* sp. There were significant negative correlations among the number of *Caprella* spp. (r (24) = –0.500, $p < 0.05$), *M. galloprovincialis* (r (24) = –0.501, $p < 0.05$) and *P. canaliculus* juveniles at 1 mo, but not at 5 mo.

The factor Duration (1 and 5 mo) explained the greatest amount of variation in the abundance of biofouling taxa (Fig. 4.6, Table 4.6). The abundance of Ischyroceridae, *Paradexamine* spp. and *Caprella* spp. decreased between 1 and 5 mo, while *M. galloprovincialis*,

Parawaldeckia sp. and *M. impacta* increased in abundance. In addition, the biomass of sessile invertebrates and algae increased significantly between 1 and 5 mo.

Variation in the abundance of biofouling taxa among the treatments was more complex. The addition of shells (SH) or live adult mussels (PH) had significant positive effects on the number of *M. galloprovincialis* at 1 mo (Treatment × Duration, $F_{4, 16} = 6.41$, $p < 0.01$, Fig 4.6a, Table 4.6) with the abundance of *M. galloprovincialis* in the PH (371.8 ± 22.0) treatment being greater (30.4 – 58.8 %) than in any other treatment (Fig. 4.6). The SH (258.0 ± 14.8) treatment had significantly greater numbers of *M. galloprovincialis* than SL (199.8 ± 21.5) and C (153.2 ± 18.1, Fig. 4.6a) at 1 mo. These *M. galloprovincialis* were between 0.25 and 1.5 mm in shell length, with a mean length of 0.81 ± 0.01 mm. There were no differences in the number of *M. galloprovincialis* among treatments at 5 mo (Fig. 4.6a), although there was a significant positive correlation between the number of *M. galloprovincialis* and the number of adult *P. canaliculus* remaining on the experimental ropes (Pearson's r (24) = 0.78, $p = 0.008$).

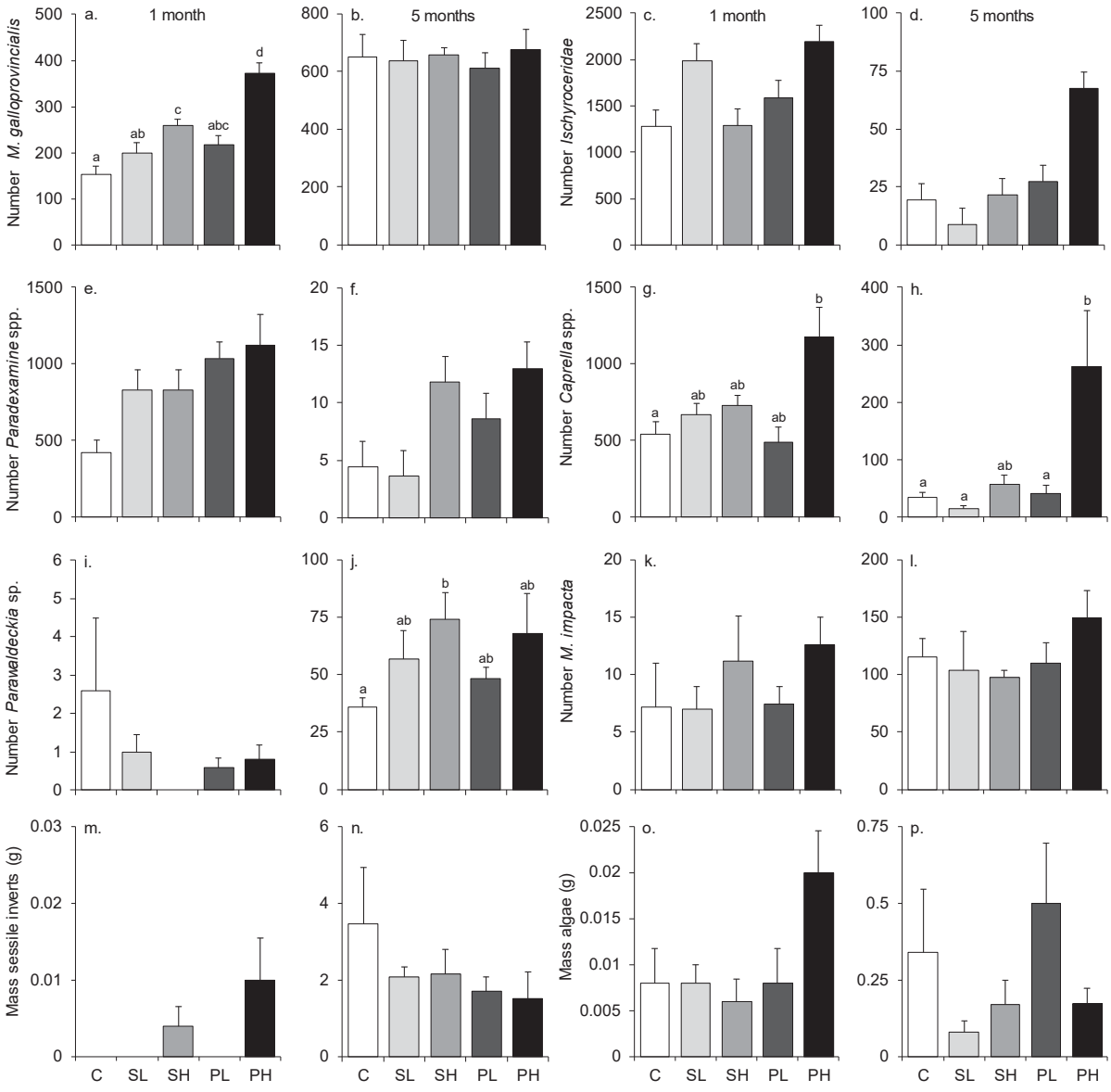

Figure 4.6. Effects of the factors Treatment and Duration (1 and 5 mo) on the mean (+ SE) abundance of the main biofouling organisms on the 45 cm experimental ropes. Treatment abbreviations as in Fig. 4.3. Different letters above bars indicate pairwise differences at $p < 0.05$ for significant Treatment × Duration interactions. Pairwise results for significant main effects of Treatment are presented in Table 4.6. Duration was significant for all taxa ($p < 0.01$). Note different scales on the y-axes.

Table 4.6. PERMANOVAs testing for the effects of seeding live adult *Perna canaliculus* or their shells and deployment duration on the abundance and assemblage of biofouling organisms after 1 and 5 mo.

Source	f/r	df	MS	F	p	perms	MS	F	p	perms	MS	F	p	perms
			Mytilus galloprovincialis				*Ischyroceridae*				*Paradexamine* spp.			
Treatment	f	4	**0.32**	**12.67**	**0.0002**	**9939**	**1.59**	**8.2**	**0.0008**	**9959**	**1.99**	**5.87**	**0.004**	**9961**
Duration	f	1	**13.18**	**123.97**	**0.0099**	**5262**	**234.9**	**4055**	**0.0088**	**5194**	**279.67**	**685.9**	**0.009**	**5304**
Frame	r	4	0.06	1.78	0.1846	9951	0.04	0.1	0.9777	9959	0.42	1.02	0.43	9958
T × D	f	4	**0.24**	**6.41**	**0.0024**	**9940**	1.23	3.25	0.0385	9949	0.49	1.16	0.368	9957
T × F	r	16	0.02	0.66	0.7865	9932	0.19	0.51	0.9068	9950	0.34	0.81	0.665	9944
D × F	r	4	0.1	2.77	0.0626	9948	0.05	0.15	0.961	9949	0.4	0.97	0.454	9962
Residual		16	0.03				0.37				0.41			
			Caprella spp.				*Parawaldeckia* sp.				*Modiolarca impacta*			
Treatment	f	4	**3.85**	**9.74**	**0.0004**	**9954**	0.05	0.17	0.9453	9957	1.03	1.66	0.188	9938
Duration	f	1	**97.99**	**225.76**	**0.0082**	**5311**	**152.3**	**683.4**	**0.0082**	**5261**	**75.21**	**92.79**	**0.011**	**5276**
Frame	r	4	0.73	2.01	0.1449	9950	0.39	2.22	0.1168	9956	0.83	0.97	0.453	9944
T × D	f	4	**1.44**	**3.96**	**0.0223**	**9951**	**0.73**	**4.19**	**0.0169**	**9959**	0.47	0.55	0.717	9957
T × F	r	16	0.39	1.08	0.4314	9937	0.3	1.74	0.1415	9940	0.61	0.71	0.762	9931
D × F	r	4	0.43	1.19	0.3602	9957	0.22	1.26	0.3247	9957	0.81	0.94	0.476	9970
Residual		16	0.36				0.17				0.86			
			Sessile invertebrates				Algae				Assemblage			
Treatment	f	4	0.06	1.29	0.3047	5263	0.06	1.29	0.3047	5269	368.31	1.43	0.058	9876
Duration	f	1	**0.72**	**47.22**	**0.0072**	**9958**	**0.72**	**47.22**	**0.0072**	**9941**	**23637**	**90.9**	**0.008**	**5303**
Frame	r	4	0.01	0.27	0.8962	9944	0.01	0.27	0.8962	9941	206.14	1.36	0.162	9925
T × D	f	4	0.07	1.28	0.3147	9952	0.07	1.28	0.3147	9939	**369.7**	**2.45**	**0.001**	**9900**
T × F	r	16	0.05	0.96	0.5415	9950	0.05	0.96	0.5415	9955	**256.96**	**1.7**	**0.004**	**9903**
D × F	r	4	0.01	0.28	0.8934	9937	0.01	0.28	0.8934	9935	**260.01**	**1.72**	**0.038**	**9842**
Residual		16	1.89				0.05				150.56			

Cochran's C; *Ischyroceridae* C = 0.23, $p < 0.05$; *Caprella* spp. C = 0.24, $p < 0.05$

f/r: fixed/random. **Bold** text indicates significance at $p < 0.05$ or 0.01 where data were heterogeneous.

See Fig. 6 for pairwise tests following significant Treatment × Duration interactions (note these were inconclusive for Ischyroceridae)

Pairwise *t*-tests for main effects (see Fig. 4.3 for treatment abbreviations); Ischyroceridae: PH > C, SL, SH, PL (t = 3–4.9, $p < 0.05$); *Paradexamine* spp.: C < SH (t = 3.9, $p < 0.01$), C < PL (t = 3.8, $p < 0.01$), C < PH (t = 3.9, $p < 0.05$), SL < SH (t = 2.9, $p < 0.05$); *Parawaldeckia* sp.: C < SH (t = 5.3, $p < 0.01$)

4. Effects of adult mussels on juvenile retention

There were greater numbers of the amphipods Ischyroceridae, *Paradexamine* spp. and *Caprella* spp. on PH compared to C treatments indicated by either significant main effects of Treatment or Treatment × Duration interactions (Fig. 4.6c-h, Table 4.6). For example, the tube-building amphipods Ischyroceridae were significantly more abundant on PH compared to C and SL treatments (Treatment, $F_{4, 16}$ 8.21, $p < 0.001$, Fig.4.6c-d, Table 4.6) overall. By contrast, the amphipod *Parawaldeckia* sp. was more abundant on SH (74 ± 11.24) compared to C (36 ± 3.8) experimental ropes at 5 mo (Treatment × Duration, $F_{4, 16} = 4.19$, $p < 0.05$; Fig. 4.6i - j, Table 4.6). There were no main or interactive effects of Treatment on the numbers of the bivalve *M. impacta* Fig. 4.6k, Table 4.6) or the biomass of sessile invertebrates or algae (Fig. 4.6m-p, Table 4.6). There were no effects of Frame in the analysis of count or biomass data for biofouling taxa.

4. Effects of adult mussels on juvenile retention

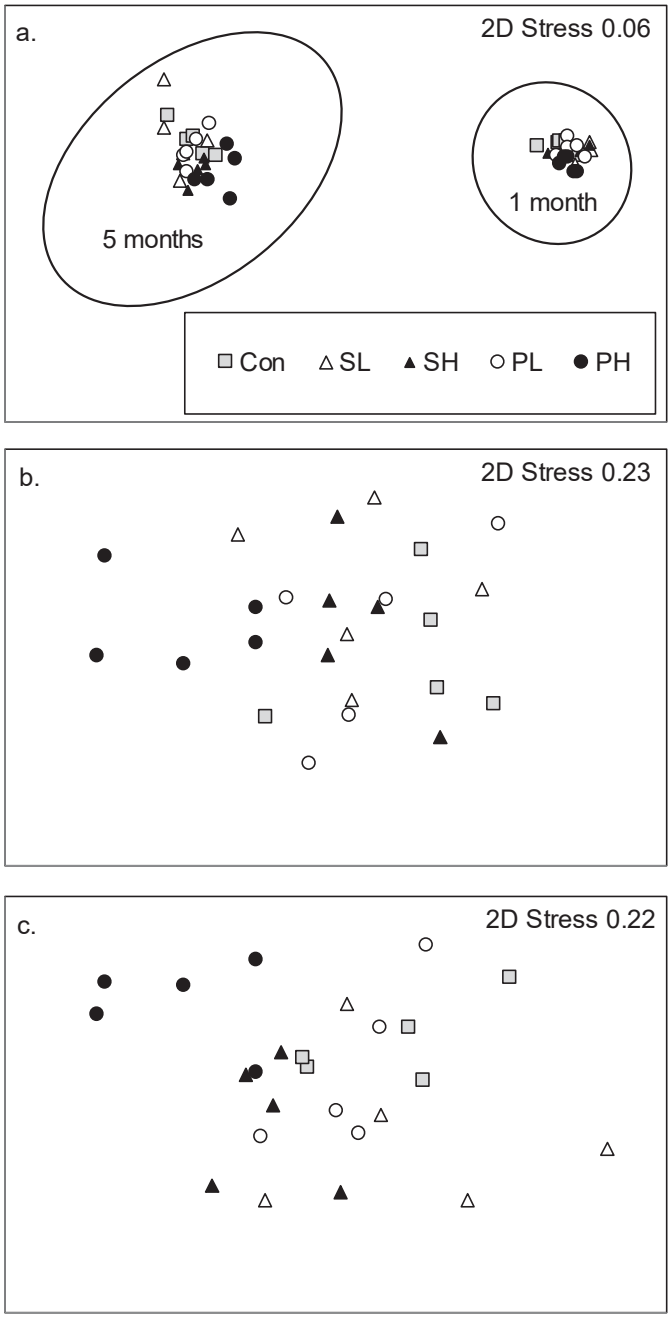

Figure 4.7. Non-metric multidimensional scaling plot showing data dispersion in five treatments (a) between, (b) within 1 mo and (c) within 5 mo deployment durations. Replicates for each of two deployment durations (1 and 5 mo) are encircled in (a). Treatment abbreviations as in Fig. 4.3.

There were distinct differences in the composition of the biofouling assemblages on the experimental ropes between 1 and 5 mo (Duration, $F_{1, 16} = 90.91$, $p < 0.01$, Fig. 4.7a, Table 4.6). The biofouling assemblage on the PH treatment at 1 mo varied from all other treatments except PL (Treatment × Duration, $F_{4, 16} = 2.45$, $p < 0.01$, pairwise *t*-tests: PH different from C, SL and SH, $t = 1.94 – 2.35$, $p < 0.05$; Fig 4.7b). There were no differences in biofouling composition among treatments after 5 mo of immersion (Fig. 4.7c). Differences in assemblage composition among treatment or Duration levels were not always consistent among replicate frames (Treatment × Frame, $F_{16, 16} = 1.70$, $p < 0.01$; Duration × Frame, $F_{4, 16} = 1.72$, $p < 0.01$, Table 4.6). Following the significant effect of Duration, SIMPER analysis indicated that there were greater numbers of Ischyroceridae, *Paradexamine* spp. and *Caprella* spp., and fewer *M. impacta*, Tanaidacea (data not presented due to low overall relative abundance) and *M. galloprovincialis* (accounting for > 50 % of the dissimilarity) after 1 mo compared to 5 mo. After 1 mo, the taxa that contributed > 50 % of the dissimilarity to among-treatment differences (Ischyroceridae, *Paradexamine* spp., *Caprella* spp. and the masking crab *Notomithrax minor*, which was in low relative abundance) were in greater abundance in the PH treatment.

4.3.4. Distribution of biofouling organisms on aquaculture substrata

At 1 mo, the most abundant biofouling organisms predominantly recruited to the sock and polypropylene rope (Fig. 4.8, Table 4.7). Greater numbers of *M. galloprovincialis* were found on

the polypropylene rope in PH compared to all other treatments, and on the sock in PH compared to SL and C (Treatment × Substratum, $F_{8, 32} = 5.3$, $p < 0.001$, Fig. 4.8a Table 4.7). Additionally, the C treatment had fewer *M. galloprovincialis* individuals than SH on the polypropylene rope, and SH and PL on the sock, however, there were no differences on the coir. There was also some spatial variability in the abundance of *M. galloprovincialis* (Treatment × Frame, $F_{16,32} = 2.6$, $p < 0.05$, Table 4.7). At 1 mo, the number of Ischyroceridae was greater on the polypropylene rope substratum in SL compared to C, and greater on the sock in PH compared to C treatments (Treatment × Substratum, $F_{8, 16} = 5.01$, $p < 0.001$, Fig 4.8b, Table 4.7). On the coir, there were significantly greater numbers of Ischyroceridae in C compared to SL, SH and PL treatments. *Paradexamine* spp. were more abundant on the polypropylene rope and sock than on the coir (Substratum, $F_{2, 16} = 33.0$, $p < 0.001$, Fig. 4.8c Table 4.7), but did not vary in abundance among treatment levels. The number of *Caprella* spp. varied among the treatments on each of the substrata (Treatment × Substratum, $F_{8, 16} = 6.7$, $p < 0.001$, Fig. 4.8d, Table 4.7), with more on the coir in C than all others, except PH, and fewer on the sock in C compared to PH treatments. There were greater numbers of *Caprella* spp. on the rope in PH compared to SL treatments.

Table 4.7. PERMANOVAs testing for the effects of adding live adult *Perna canaliculus* or their shells on the distribution of key biofouling organisms within experimental ropes after 1 mo in the field.

Source	f/r	df	MS	F	p	perms	MS	F	p	perms
			M. galloprovincialis				*Ischyroceridae* spp.			
Treatment	f	4	**1.167**	**6.546**	**0.0028**	**9956**	0.397	0.733	0.6043	9955
Substratum	f	2	**17.127**	**68.953**	**0.0001**	**9953**	**39.887**	**106.31**	**0.0003**	**9939**
Frame	r	4	0.029	0.414	0.7986	9953	0.228	0.912	0.4788	9949
T × S	f	8	**0.365**	**5.3**	**0.0004**	**9956**	**1.255**	**5.013**	**0.0003**	**9940**
T × F	r	16	**0.178**	**2.591**	**0.0106**	**9941**	0.542	2.164	0.0192	9918
S × F	r	8	**0.248**	**3.611**	**0.0039**	**9937**	0.375	1.499	0.1826	9956
Residual		32	0.069				0.25			
			Paradexamine spp.				*Caprella* spp.			
Treatment	f	4	1.116	1.818	0.1712	9958	**3.076**	**6.929**	**0.0017**	**9973**
Substratum	f	2	**28.921**	**33.009**	**0.0009**	**9950**	**34.246**	**90.572**	**0.0006**	**9951**
Frame	r	4	0.991	1.41	0.245	9954	0.21	0.609	0.6642	9949
T × S	f	8	0.889	1.264	0.2909	9948	**2.315**	**6.701**	**0.0001**	**9947**
T × F	r	16	0.614	0.873	0.6102	9930	0.444	1.285	0.2602	9915
S × F	r	8	0.876	1.246	0.3033	9944	0.378	1.095	0.3909	9938
Residual		32	0.703				0.345			

Cochran's C;*Ischyroceridae* C = 0.71, p < 0.001; *Caprella* spp. C = 0.35, p < 0.001

f/r: fixed/random. **Bold** text indicates significance at p < 0.05 or 0.01 where data were heterogeneous

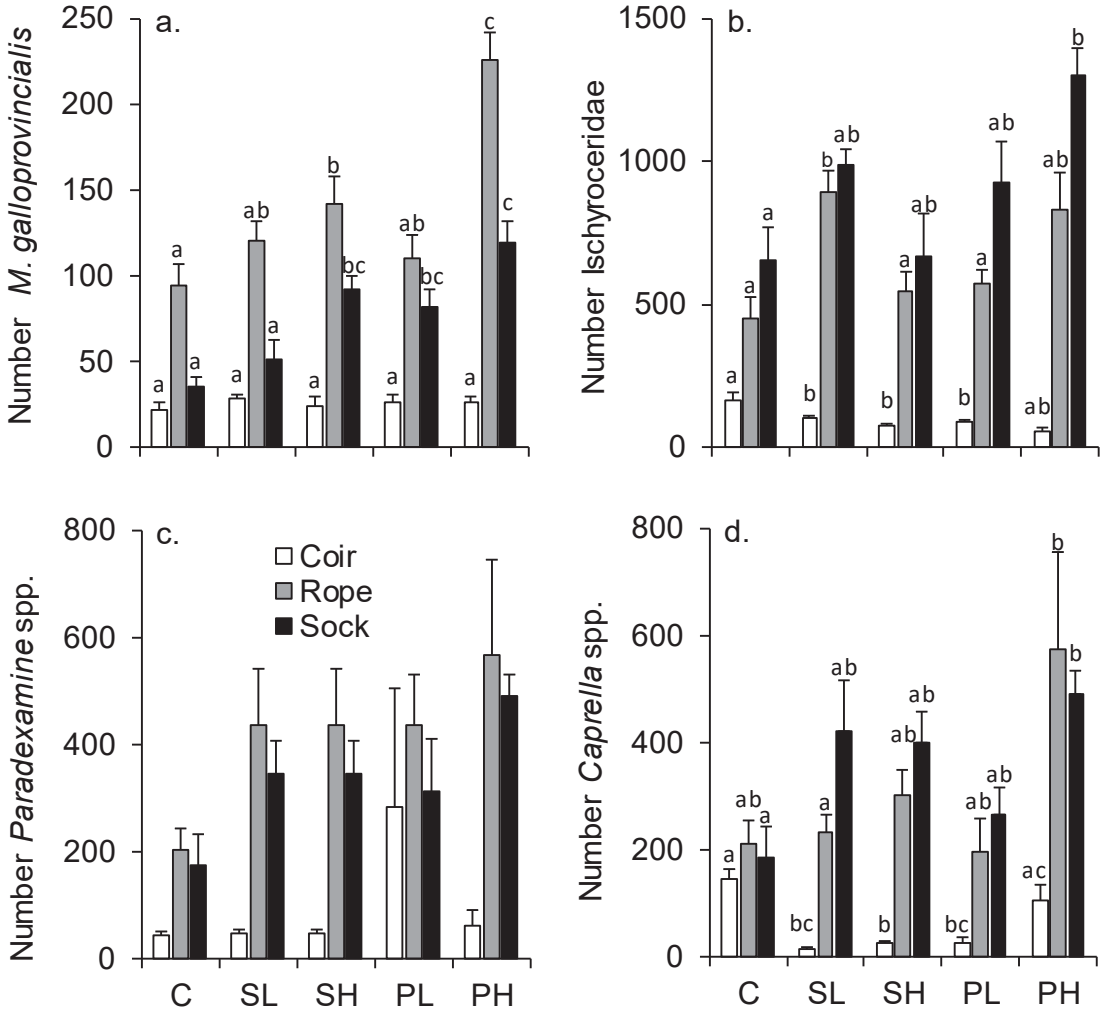

Figure 4.8. Mean (+ SE) number of (a) *Mytilus galloprovincialis*, (b) Ischyroceridae, (c) *Paradexamine* spp. and (d) *Caprella* spp. on the coir (white bars), polypropylene rope (grey bars) and sock (black bars) among treatments after 1 mo in the field. Treatment abbreviations as in Fig. 4.3; n = 5 ropes treatment^{-1}, all experimental ropes were 45 cm in length. Different lower-case letters above bars indicate significant differences among treatments within substrata following a significant Treatment × Substratum interaction. Note different scales on the y-axes.

4.4. Discussion

The results of this study demonstrate that high losses of juvenile *Perna canaliculus* from nursery ropes can be experienced early in commercial aquaculture production. On average 46 % of the *P. canaliculus* juveniles were lost from experimental ropes after only 1 mo (average of 85.2 lost mussels rope^{-1} wk^{-1}) and fewer than 19 % of juvenile mussels remained after 5 mo (average of 13.4 lost mussels rope^{-1} wk^{-1}). These data support earlier estimates of losses and suggest that understanding the causes of these losses and mitigating against them would be beneficial to mussel production in New Zealand (Jeffs et al. 1999, Hayden & Woods 2011). Including live adult mussels, or their shells, at two different densities had no effect on the retention of juvenile mussels over 1 and 5 mo under conditions typical of the early production cycle. Therefore, adult *P. canaliculus* did not mitigate stressors or suppress secondary settlement behaviour in juvenile mussels. Furthermore, many of the adult mussels were themselves lost when they were deployed at high density, providing additional evidence against the usefulness of simultaneous deployments of adults and juveniles. However, the addition of live adult mussels or adult mussel shells did increase the abundance of some common biofouling organisms, in particular *Mytilus galloprovincialis*, which may have detrimental effects on mussel production (Fitridge et al. 2012, Sievers et al. 2013, Lacoste & Gaertner-Mazouni 2015, Forrest & Atalah 2017).

Secondary settlement behaviour is one likely cause of the observed losses of juvenile *P. canaliculus* (Jeffs et al. 1999, Carton et al. 2007). Juvenile *P. canaliculus* of the same size as the juveniles used in this study have been observed to settle into natural habitats, indicating that juveniles of this size range readily use secondary settlement behaviour to change location

(Buchanan & Babcock 1997, Alfaro & Jeffs 2003, Alfaro 2006b, South 2016). For example, pulses of settlement of *P. canaliculus* sized > 500 µm were observed on collectors deployed for periods of only 1 d at a time on a rocky shore in southern New Zealand (South 2016). In northern New Zealand, settlers arriving at an intertidal mussel bed were > 2 mm in length (Alfaro 2006b). Therefore, it appears that *P. canaliculus* juveniles of the size deployed in this study (mean = 1.01 ± 0.01 mm) are highly mobile and could have migrated away from the experimental ropes. While it might be possible that juveniles could have migrated among experimental ropes in this study, it is highly unlikely, as there was no significant variation in juvenile numbers among treatments that would be consistent with such movements (Fig. 4.3).

Secondary settlement migration from the coir or algae on which mussels are initially seeded is essential to mussel production in New Zealand, because the coir and algae degrade over time, while the polypropylene rope offers a stable, permanent substratum. After 1 mo, a greater percentage (~10 %) of juvenile mussels remaining on the experimental ropes had migrated from the coir to the polypropylene ropes when live adult mussels were added at the higher density. The underlying triggers of secondary settlement are not clear and could occur due to external factors such as chemical cues, changes in abiotic conditions or developmental changes in the juveniles. Alternatively, secondary settlement might be a response to negative changes in the immediate local environment, such as the recruitment of predatory species or organisms that modify food availability to the juveniles. It is possible that movements by the live adults along the nursery ropes could have displaced juveniles, causing them to re-locate onto the polypropylene rope. Juveniles might also have actively migrated away from adults, as a response to their biological functioning

(defecation, byssus production) or competition for food. Alternatively, juvenile mussels might have moved towards the adults that were mostly attached to the polypropylene rope (Porri et al. 2016). Movement of juveniles towards adults would provide support for the original hypothesis that adults might have some influence on the retention of juveniles, but the number of mussels undertaking such movements was insufficient to have any effect on the number of mussels retained during this experiment. The addition of live adult mussels or mussel shells to the experimental ropes led to an increase in the abundance of biofouling organisms, and this, or the presence of the adults, may have prompted the relocation of the juvenile mussels.

The ability of *P. canaliculus* juveniles to mucus-drift to facilitate secondary settlement ends at around 5 - 6 mm in shell length, and this might explain the reduced rate of loss of juvenile mussels from the experimental ropes observed after 1 mo (Buchanan & Babcock 1997). Losses of juvenile mussels from aquaculture substrata could also be due to other factors that include variations in genetics and fitness among individuals (Phillips 2002, Alcapán et al. 2007, Sim-Smith & Jeffs 2011), disease (Jones et al. 1996a), predation pressure (Hayden 1995, Peteiro et al. 2010, Capelle et al. 2016a), biofouling (Fitridge et al. 2012) and stressors associated with the relay of juveniles from the hatchery or wild collection sites to the nursery farm location that might increase mortality or trigger secondary settlement (Webb & Heasman 2006, Carton et al. 2007). Quantifying the relative importance of the causes of loss has not been satisfactorily achieved in this and other studies and remains an important research priority.

The addition of live adult mussels in high density to experimental ropes generally increased the abundance and composition of biofouling organisms after 1 mo in the field. Adding low

densities of live adults, or only the shells of adults, had weaker effects on biofouling development Fig. 4.6). Together this indicates that factors such as increased habitat area, refuge from predation and abiotic stress, as well as modification of food availability that are associated with adult mussels in high density, might have been important for many of the biofouling taxa recorded in this study (Commito & Rusignuolo 2000, Woods et al. 2012). Biofouling organisms can compete for food and space with cultured organisms, leading to reduced biomass and crop losses (Ramsay et al. 2008, De Nys & Guenther 2009, Sievers et al. 2013). The increased abundance of biofouling organisms associated with the presence of adult mussels (alive or shells) did not appear to affect the retention of juveniles. For example, the number of *P. canaliculus* juveniles was not significantly fewer where live adults were added in high density and where biofoulers such as *M. galloprovincialis* and *Caprella* spp. were in greater abundance. However, there were significant negative correlations between the number of remaining juveniles and the number of Ischyroceridae, *Caprella* spp. and *M. galloprovincialis*, at 1 mo, a trend that warrants further targeted research to determine possible effects of these species.

Mytilus galloprovincialis responded differentially to the presence of live adults versus shells after 1 mo (Fig. 4.6a). The number of *M. galloprovincialis* was more than double in the high live adult density treatment after 1 mo compared to the controls, clearly showing that experimental ropes bearing live mussels promote settlement or survival of *M. galloprovincialis*. Similar positive effects of adults on settlement have been reported in other studies of mytilid mussels (Nielsen & Franz 1995, Wahl 2001, Sardiña et al. 2009, Dolmer & Stenalt 2010). Adding mussel shells in high density also had a positive, albeit a smaller, effect on the number of *M. galloprovincialis*,

indicating that this species may be benefitting from the structural properties of *P. canaliculus* shells (Dolmer & Stenalt 2010). Given the small size (0.25 – 1.5 mm) of *M. galloprovincialis* settlers at 1 mo, it is likely that these arrived as primary settlers, although this experiment was not structured to test this. The greater number of *M. galloprovincialis* in treatments with live adult *P. canaliculus* could be the result of increased primary settlement due to chemical cues from the live adults (Alfaro et al. 2006) or the result of modified small-scale hydrodynamic patterns (Grizzle et al. 1996, Miron et al. 2000) and increased surface complexity associated with the adults (e.g., byssal threads) and shells (e.g., increased settlement area) (Cáceres-Martínez et al. 1994, Gribben et al. 2011). Alternatively, differences in the abundance of *M. galloprovincialis* among the treatments might have been due to variations in post-settlement processes such as mortality (Hunt & Scheibling 1997, von der Meden et al. 2012), secondary settlement (von der Meden et al. 2010, South 2016) or predation (Hayden 1995, Peteiro et al. 2010). The positive effects of live adults and shells of *P. canaliculus* on the abundance of its congener *M. galloprovincialis* contrasts with the absence of any effect on the conspecific juveniles deployed on the experimental ropes. It is possible that adult–juvenile interactions are only important early in the settlement process or that *M. galloprovincialis* and *P. canaliculus* have different underlying settlement-recruitment strategies. The lack of primary settlement of *P. canaliculus* during this study did not allow for any comparison among treatments or with the settlement of *M. galloprovincialis*.

The strong increase in the number of *M. galloprovincialis* on experimental ropes bearing high densities of adult *P. canaliculus* has important implications for the New Zealand mussel industry and the surrounding natural environment (Rius et al. 2011). The blue mussel *M.*

galloprovincialis has a wide global range with southern and northern lineages and is a successful invader of native ecosystems in many countries (McQuaid & Phillips 2000, Braby & Somero 2006, Branch et al. 2008, Gardner et al. 2016). In the Marlborough Sounds, *M. galloprovincialis* has increased in abundance over the last 25 yr and has become a problematic biofouling organism that competes for food and displaces crops of *P. canaliculus* on growing lines (Woods et al. 2012, Atalah et al. 2017, Forrest & Atalah 2017).

The most abundant biofouling taxa recruited differentially to the experimental substrata used to deploy seed mussels. For example, *M. galloprovincialis* settled heavily onto the polypropylene rope substratum (versus the socking or the coir), thereby occupying the space onto which *P. canaliculus* juveniles must migrate to remain in production. This result is in accordance with other studies that have shown *M. galloprovincialis* to have strong affinities for fibrous substrata such as the polypropylene ropes used in this study (Cáceres-Martínez et al. 1994, Carl et al. 2012b). The amphipods Ischyroceridae built tubes constructed from fine sediment on the sock and polypropylene rope, potentially modifying small-scale hydrodynamic processes and food delivery to juvenile mussels (Fitridge et al. 2012), for which feeding is known to be limited due to rudimentary feeding structures (Gui et al. 2016). Critically, this suggests that the substrata used for mussel culture in New Zealand can promote the settlement of nuisance species and that more research is warranted to understand their impact and methods to deter them.

Conclusions

This study shows how high losses of seed mussels can greatly affect the efficiency of mussel production in a typical aquaculture setting in New Zealand. The majority of juvenile losses

occurred within the first month, but continued over the following 5 mo. The addition of adult mussels to experimental ropes, while unsuccessful at increasing retention as originally hypothesised, provides considerable insight into how crops of mussels interact with their environment and the spatial and temporal dynamics of biofouling organisms in the early stages of aquaculture production. Overall, the presence of adult *Perna canaliculus* on experimental ropes increased the numbers of biofouling organisms subsequently arriving on these ropes, especially *Mytilus galloprovincialis*, most likely because of their provision of habitat and biological functioning. The polypropylene rope and outer sock substrata used by industry in nursery systems appear to promote the settlement of some biofouling organisms. Research into alternative systems is required to reduce the impacts of unwanted biofouling species on juvenile mussels in aquaculture.

Chapter 5.

Immersion at seeding triggers losses of juvenile mussels, reducing the efficiency of aquaculture operations

5. Immersion at seeding triggers losses of juvenile mussels

5.1. Introduction

Seeding juvenile mussels into aquaculture is highly inefficient with a large proportion of the seeded mussels typically being lost from aquaculture production (Webb & Heasman 2006, Capelle et al. 2016a, South et al. 2017). Losses of seed mussels create production problems in mussel aquaculture by compounding the high variability in the supply of wild seed, reducing the productivity and profitability of aquaculture operations, sometimes with significant societal impacts (de Vooys 1999, Jeffs et al. 1999, Imeson & van den Bergh 2006). However, the causes of losses of seed mussels in aquaculture are not understood and as a result they currently cannot be managed. The inability to manage large losses of juvenile seed mussels has become a major constraint to continuity and growth in the production from green-lipped mussel (*Perna canaliculus* Gmelin) aquaculture in New Zealand, where the industry is limited by its reliance on a single and highly variable natural source of seed mussels (Jeffs et al. 1999, Jeffs et al. 2018).

Recent studies have shown that losses of seed mussels can occur in the first few weeks after they are seeded into aquaculture production (Chapters 1 and 2 in this thesis, South et al. 2017). For example, losses of 49, 43 and 46 % of juvenile *P. canaliculus* have been recorded over deployment durations of 8, 15 and 30 days, respectively (Chapter 1 in this thesis, South et al. 2017). Although the underlying causes of the losses shown in these studies were not determined, they were shown to be independent of the development of biofouling (Chapter 1) or the presence of adult mussels on ropes (South et al. 2017). Other factors, such as fish predation and variations in water velocity, have been shown to affect the retention of seed mussels (Hayden 1995, Alfaro 2005, Hayden & Woods 2011). However, fish predation does not appear to be a major factor

affecting the mussels of the size that are typically seeded into aquaculture (1 – 2 mm) in the Marlborough Sounds, New Zealand, but it was found to be a major cause of loss when juveniles are greater than 5 mm in shell length (Hayden 1995). Similarly, while decreased water velocity can reduce retention via increased mortality, secondary settlement migrations, or decreased byssus production (Alfaro 2005, 2006a, Hayden & Woods 2011), its effects are likely to be site-specific and sustained, rather than occurring occasionally through time. However, the nature of the relationship between water velocity and other environmental variables, and the ontogeny of juvenile mussels is yet to be determined.

The relay of wild seed mussels from collection to farm sites is one of the most important processes in mussel aquaculture and is a likely source of spat losses early in aquaculture production (Carton et al. 2007, Heasman 2013, Calderwood et al. 2014, Capelle et al. 2014). In New Zealand, juveniles are collected in the far north of the country where they wash ashore attached to mixed natural substrates consisting of algal, invertebrate (e.g., hydroids) and terrestrial material, collectively known as ‘Kaitaia spat’, named after a nearby town (Hickman 1976, Jeffs et al. 1999). The Kaitaia spat are then sorted and packed in a process that can take more than a day and during which they experience temperature fluctuations and desiccation (Heasman 2013), before they are shipped to the growing regions in refrigerated trucks, a process that can take up to 78 hours (Jeffs et al. 1999). Finally, the Kaitaia spat are seeded out onto nursery mussel farms by placing the spat, and their substrates alongside a lead-cored polypropylene dropper rope, and held in place with a polyester and cotton mesh sock, and deployed into the sea (Jeffs et al. 1999).

5. Immersion at seeding triggers losses of juvenile mussels

The potential for aspects of the relay process to impact mussel retention has attracted some research attention in New Zealand. The effects of factors such as desiccation, temperature variations and starvation have been shown to affect the fitness (Webb & Heasman 2006) and retention (Foote 2003, Meder et al. 2005b, Carton et al. 2007) of juvenile *P. canaliculus*. For example, juvenile *P. canaliculus* that were emersed for 2 – 4 hours had reduced fitness (evidenced by mobility, gaping behaviour and vital stain uptake) compared to an immersed control group, suggesting that mussels experiencing desiccation might die or be less competitive during early production (Webb & Heasman 2006).

The timing and magnitude of losses of mussels from aquaculture structures in the first hours to weeks following seeding are unknown. It is possible that such losses occur either immediately upon seeding, or in the days (or weeks) following seeding. Determining the timing of losses of seed mussels early in aquaculture production, and understanding their causes, should be a major research goal if efficient and sustainable aquaculture practices are to be achieved. In this study, the possibility of immediate losses of seed mussels at the time of seeding was tested with three distinct laboratory experiments. The numbers, sizes and status (i.e., alive or dead) of mussels detaching from two different consignments of wild Kaitaia spat, and one batch of hatchery-reared juveniles was assessed to quantify losses and the immediate fate of juvenile *P. canaliculus* seeded into mussel aquaculture.

5.2. Materials and methods

5.2.1. Losses of Kaitaia spat

To test whether juvenile seed mussels can be lost immediately upon immersion at seeding, two separate experiments using different batches of Kaitaia spat were carried out. In Experiment 1, seven replicate sub-samples were collected haphazardly from each of two 10 kg bags of Kaitaia spat (n = 14 sub-samples in total, with an average weight of 1.08 g ± 0.14 SE). The Kaitaia spat had been collected from the surf zone at Ninety Mile Beach on 31 August 2017, sorted and packed as 10 kg lots in plastic bags and then transported by refrigerated truck over 1108 km (ca. 48 h) to Havelock in the South Island of New Zealand where they were held on the wharf in the truck overnight before being collected by the researcher on 2 September 2017. The balance of the consignment of Kaitaia spat was taken that same day and deployed onto mussel farms in the Marlborough Sounds. The sub-samples of Kaitaia spat were placed in individual small plastic bags that were stored upright and open in an insulated container containing a towel that had been wetted with seawater and wrung out to maintain a constant humidity and transported to the nearby Cawthron Institute (ca. 1 h) where the experiment was carried out. In the laboratory, the samples were immersed in 450 ml of 1 µm filtered seawater (FSW, 16 °C and 35 psu) contained in 500 ml glass aquaria, for a duration of 2-min. After this period, macroalgae and other substrates were removed with tweezers, bagged and frozen. The remaining FSW and any juvenile mussels that had dropped off were poured over a 250 µm sieve, and the glass aquaria were checked for any attached mussels. The content of the sieve was transferred to a petri dish and held in FSW for counting. Empty valves and mussels with gaping valves and that showed no movement (e.g., siphoning,

processing of particles in the gut, exploration with the foot, or closure upon agitation with tweezers) were considered dead. Those mussels in the aquaria that were attached to minute fragments (small epiphytes and broken pieces of algae ca < 5 mm that would likely pass through the mesh of the outer sock following seeding, Fig. 5.1) were counted separately. To remove and enumerate mussels retained on the natural substrates, the frozen samples were defrosted at room temperature and the mussels were washed from the natural substrats over a 250 µm mesh and counted. The natural substrates were checked under a dissecting microscope for any remaining mussels before being patted dry and weighed to a precision of 0.01 g. Shell length was measured for a sub-sample of 50 mussels (to 0.01 mm), using image analysis (Image J) to determine mussel-size at the start of the experiment, and for both those mussels that were retained on the natural substrates or became unattached in the aquarium after a 2-min immersion.

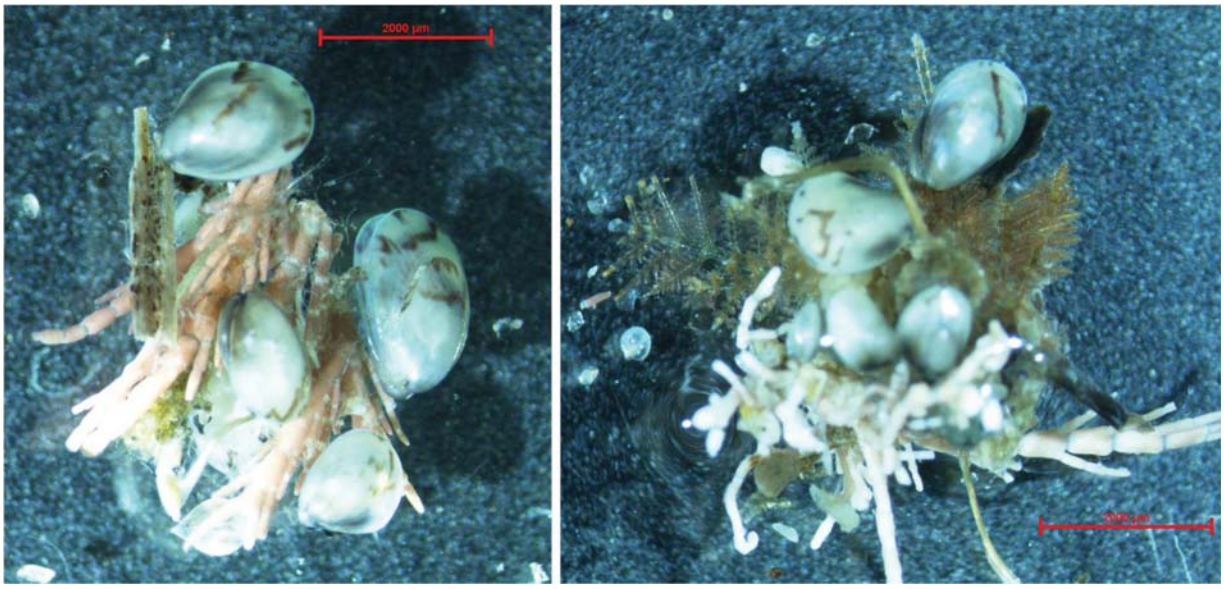

Figure 5.1. Juvenile *Perna canaliculus* attached to macroalgal fragments in Experiment 2.

5. Immersion at seeding triggers losses of juvenile mussels

Previous research suggests that the morphological complexity of substrates, especially macroalgae, that comprises the Kaitaia spat material can affect the number and size of the attached mussels (Alfaro et al. 2004, Jeffs et al. 2018). It follows that variations in macroalgal morphology and complexity might affect retention during early production. Therefore, in Experiment 2, the Kaitaia spat sample that was obtained on 16 September 2017 was separated into four groups based on their morphology (fine macroalgae, coarse macroalgae, a mixture of fine and coarse macroalgae, and un-manipulated Kaitaia spat) to assess their effects on mussel retention. The fine macroalgal group (hereafter, fine macroalgae) consisted of species such as *Halopteris congesta*, *Plocamium* spp., *Ballia callitricha* and *Pterocladia capillacea* that are typically small (< c. 10 cm), have thin thalli (< 0.5 cm) and are often highly branched and therefore of more complex morphology. The coarse macroalgal group (hereafter, coarse algae) contained only fucoid algae, mostly *Landsburgia quercifolia*, *Carpophyllum maschalocarpum* and *Xiphophora chondrophylla,* all of which had wide bladed thalli (> c. 1 cm), a turgid texture and are of comparatively low morphological complexity. The mix of fine and coarse macroalgae (hereafter mixed macroalgae) was created by combining the two groups (ratio = 1:1). The Kaitaia spat contained a mix of coarse and fine macroalgae and other macroalgal, invertebrate (hydroids) and terrestrial plant material, and represents the typical deployment situation.

To assess juvenile mussel retention among the macroalgal groups, 20 g of each of the substrate groups was placed into 950 ml (16 °C, 35 psu) of FSW in 1 L aquaria (17 cm height × 9 cm in diameter). The substrates and attached juveniles were removed from the containers after 2 minutes and placed in plastic bags and frozen for later analyses. Many of the detached mussels re-

settled on the container, therefore, the juvenile mussels were enumerated in two ways. Firstly, the seawater from each container was poured over a 250 μm sieve, capturing any unattached mussels or those that were attached to fragments of substrate. These were counted in freshwater with gaping (or empty) and closed valves being considered dead or alive, respectively (Webb & Heasman 2006). Secondly, the containers and the re-settled mussels were frozen at -20 °C, thawed and the mussels were removed from the walls of the aquarium with a jet of freshwater. The starting sizes of the mussels, and the size of the mussels that were still attached to the substrates, were unattached, or had re-settled on the containers following immersion, were measured using image analysis (n = 50 per group, per sample).

5.2.2. Losses of hatchery spat

A third experiment was performed to determine whether losses of hatchery-reared juveniles could occur at seeding. This was achieved by obtaining 3 m of coir rope with attached juvenile *P. canaliculus* from a mussel hatchery (SpatNZ Ltd, Nelson), on 24 October 2017. The coir rope was cut into 30 samples of 10 cm length that were haphazardly assigned to one of three emersion treatments; 0.25, 3 and 6 h, to test whether the time between emersion and re-immersion at seeding might influence retention. Samples were stored on open trays in insulated containers containing a wetted towel (with FSW) to maintain humidity. The container (and the samples of coir) were kept in a temperature-controlled room (16 °C) to reflect conditions typically encountered during industry transfers of spat. At the end of their assigned emersion duration the samples of coir with attached hatchery-reared spat were immersed in individual plastic aquaria (17 cm height × 9 cm in

diameter) containing 900 ml of filtered seawater (FSW). After 2-min of immersion, the 10 cm samples of coir were removed from the aquaria, bagged individually and frozen. Aeration via air-stones was added to each container to oxygenate the water and provide water motion. After 24 h the hosing attached to the air-stone was cut above the water line in the aquaria and carefully removed from the tank. The water from the tank and any loose juvenile mussels were poured over a 250 µm sieve. All loose mussels were counted immediately in fresh water and all gaping and empty valves were considered dead. Live mussels that washed out of containers were termed 'unattached'. The juvenile mussels were removed from the aquaria using the same methodology as in Experiment 2. Finally, the coir sections were defrosted, washed over a 250 µm sieve and the juvenile mussels enumerated as these were considered to have been retained during the experimental periods (0.25 – 6 h). The sum of the dead, drifting and secondary settlers was calculated to provide a total number of mussels per 10 cm of coir (i.e., the starting number of mussels on the coir). 30 mussels were measured to the nearest 0.01 mm for each 10-cm section of coir at the start of the experiment and for the retained and unattached mussels following the 2-min immersion.

5.2.3. Statistical analyses

All analyses in this study were done using permutational analyses of variance (PERMANOVA) based on dissimilarity matrices of Euclidean distances. P-values were constructed using 9999 or 999 permutations for count and size data respectively. Variance heterogeneity was checked with permutational analysis of multivariate dispersions (PERMDISP) using distances to the centroid of

the data cloud, a procedure that is equivalent to Levene's test (Anderson et al. 2008). Data were transformed log (x +1) where necessary to meet the assumption of variance homogeneity. If homogeneity was not achieved, results were interpreted cautiously at $p < 0.01$.

Losses of Kaitaia spat

In Experiment 1, single-factor PERMANOVAs were used to assess any differences between the two bags of Kaitaia spat for the mean number of juvenile *P. canaliculus* g^{-1} of Kaitaia spat at the start of the experiment, and the mean percentage of the number of seed mussels still attached to the Kaitaia spat substrate after immersion. Differences in the mean percentage of the number of mussels in each sample that were detached from the substrates, or attached to fragments of the substrates, were analysed with a PERMANOVA testing for the effects of Bag (random factor, Bags 1 and 2) and Distribution in the aquaria (fixed factor, detached vs attached to algal fragments). Finally, the number of dead mussels as a percentage of the total number of mussels was assessed between the two bags with a single factor analysis.

Single-factor PERMANOVAs were used in Experiment 2 to assess variation in the mean numbers of juvenile *P. canaliculus* among Macroalgal Groups (fine macroalgae, coarse macroalgae, mixed macroalgae and Kaitaia spat) for the total number of mussels per g on the substratum at the outset and the percentage of the total number of mussels per sample retained on the substratum. The mean abundances of juvenile *P. canaliculus* that were unattached, attached to fragments of substrate or had re-attached to the aquaria were assessed with a PERMANOVA model that tested for the effects of Substrate Group (fixed; fine macroalgae, coarse macroalgae,

mixed macroalgae and Kaitaia substrates) and their Distribution in the aquaria (Fixed factor, unattached, re-settled, and attached to substrate-fragments). Finally, the number of dead mussels as a percentage of the total number of mussels was compared among substrate groups with a single factor analysis.

In Experiment 1, differences in the mean size among individuals of *P. canaliculus* at the start of the experiment were compared to those that were retained on the Kaitaia spat substrate, or detached from it with a PERMANOVA testing for the effects of Bag (random factor, Bags 1 and 2) and Distribution (fixed factor, start and retained vs detached following immersion). Data transformation did not improve homogeneity of the variances; therefore, raw data were analysed and results interpreted cautiously ($p < 0.01$). In Experiment 2 the effects of Substrate group (Fixed factor, fine macroalgae, coarse macroalgae, mixed macroalgae and Kaitaia spat substrates) and Distribution (fixed factor, start and retained, unattached and re-settled following immersion) on the mean size of the juvenile *P. canaliculus* were assessed with PERMANOVA. Data transformation failed to stabilise variances for size data therefore results were interpreted cautiously ($p < 0.01$). To further assess possible size differences among the starting size and the retained, detached and re-settled mussels (Experiment 2 only), percentage-frequency plots were made for each bag in Experiment 1, and each substrate group in Experiment 2, using 0.25 mm bins. Finally, the percentage of the total number of mussels retained in each size-class was pooled across bags (Experiment 1) and substrate groups (Experiment 2) and analysed with linear regression.

Losses of hatchery spat

Differences in the mean number of total juvenile *P. canaliculus* on the 10 cm sections of coir rope (prior to the experiment), and the mean number of mussels attached to the coir rope after a 2-min immersion, among emersion durations were assessed with single factor PERMANOVAs. The mean abundance of juvenile *P. canaliculus* that detached from the coir rope and were either unattached in the water, or that had re-settled on the containers, were analysed with a PERMANOVA testing for the crossed effects of Emersion duration (fixed factor; 0.25, 3 and 6 h) and Distribution (fixed factor; unattached vs re-settled). Variation in the mean number of dead mussels among emersion durations (0.25, 3 and 6 h) was assessed with a single factor PERMANOVA. The mean size of hatchery-reared juveniles was assessed with a 2-factor analysis testing the effects of Emersion duration (fixed factor; 0.25, 3 and 6 h) and Duration (fixed factor, start and retained vs detached following immersion). Percentage-frequency plots were made for each bag in Experiment 1, each substrate group in Experiment 2 and the hatchery spat, using 0.25 mm size-classes. The percentage of the total number of mussels retained in each size-class was pooled across bags (Experiment 1), substrate groups (Experiment 2), or emersion durations (hatchery spat), and analysed with polynomial regressions.

5.3. Results

5.3.1. Losses of Kaitaia spat

On average, there were 3880.5 ± 778.5 (mean ± standard error) juvenile *P. canaliculus* g^{-1} of Kaitaia spat in Experiment 1, with no difference in the mean number of mussels between the two

bags of Kaitaia spat sampled ($F_{1, 13} = 0.44$, p = 0.55). However, after immersion in seawater there was a significant difference in the percentage of the total number of mussels retained between bags ($F_{1, 13} = 5.2$, p = 0.04, Fig 5.2a) with more mussels retained on samples from Bag 1 (65.6 ± 4.5 %) compared to Bag 2 (54.5 ± 1.9 %), equating to the retention of 2314.1 ± 999.8 mussels g^{-1} of Kaitaia material for samples from Bag 1, and 2420.9 ± 442.8 mussels g^{-1} for Bag 2.

The mussels that detached from the Kaitaia spat in Experiment 1 were either unattached, or still attached to small fragments of substrate that were released from the Kaitaia spat material and fell to the bottom of the aquaria (Fig. 5.2b). There was a greater percentage of unattached mussels for samples from Bag 2 (33.0 ± 1.4 %) compared to Bag 1 (21.4 ± 1.5 %), and the percentages attached to fragments of substrate were smaller than the unattached mussels for both Bags (Bag 1 = 13.0 ± 3.2 %, Bag 2 = 12.5 ± 1.5 %; Bag × Distribution $F_{1, 24} = 8.93$, p = 0.0052, Fig. 5.2b). Fewer than 15 mussels per sample were dead and there was no difference between the two bags in the percentage of the total mussels per sample that were dead at the end of the experiment (0.16 ± 0.04 %, Bag $F_{1, 13} = 0.5$, p = 0.4).

5. Immersion at seeding triggers losses of juvenile mussels

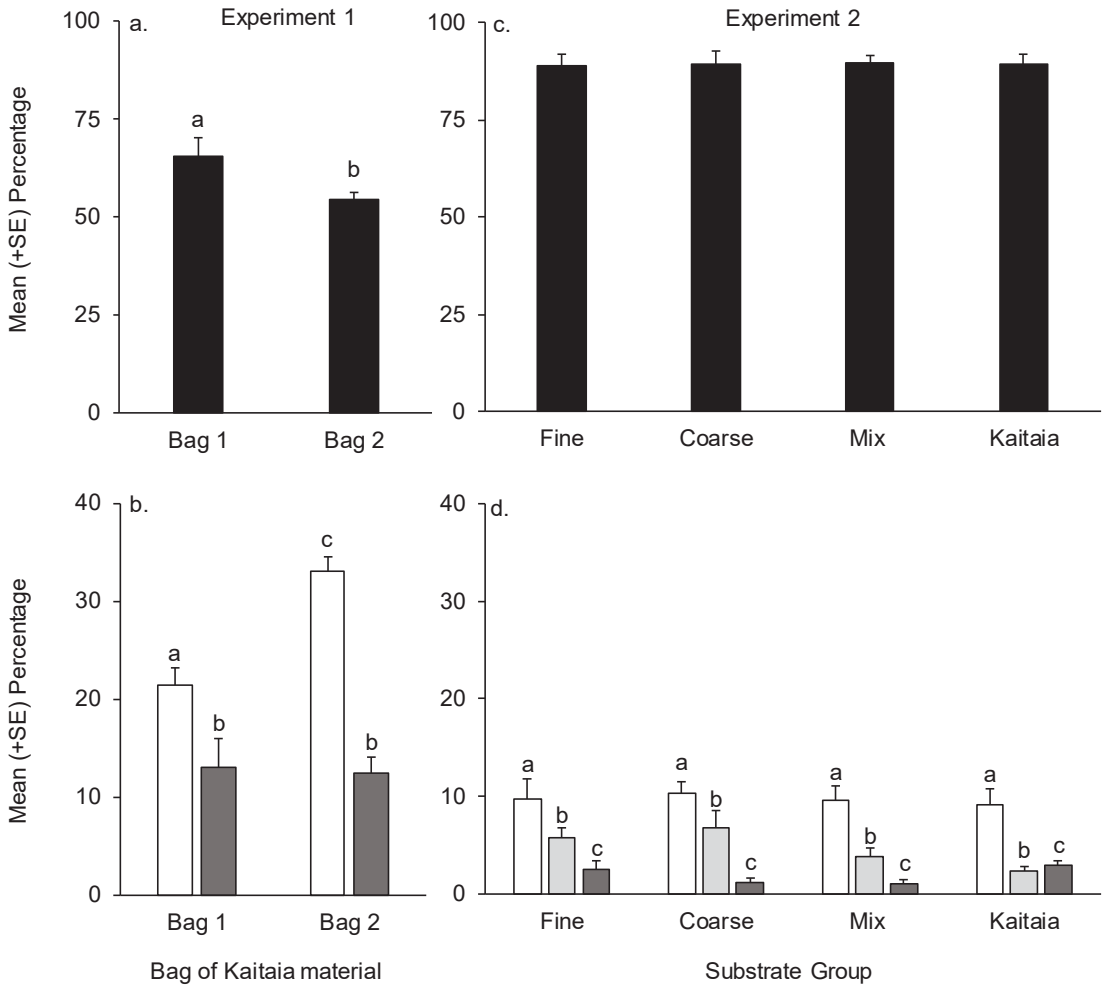

Figure 5.2. Mean (+ SE) percentage of the total number of *Perna canaliculus* g^{-1} Kaitaia spat retained after a 2-min immersion in Experiments 1 (a) and 2 (c) (black bars), and unattached (white bars), attached to small fragments of substrate (dark-grey bars) and re-settled on the aquaria (light-grey bars, Experiment 2 (d) only). Variation among bags (Experiment 1) and substrate groups (Experiment 2) are shown in a and b, and c and d, respectively. The same letter above bars indicate homogenous groups. In d, the same lower-case letters above bars indicate homogenous groups following a significant main effect of their Distribution in the aquaria.

5. Immersion at seeding triggers losses of juvenile mussels

The average number of juvenile *P. canaliculus* g^{-1} Kaitaia spat in Experiment 2 varied among Substrate Groups, with ascending numbers of mussels from coarse macroalgae (357.2 ± 78.0), mixed macroalgae (579.9 ± 101.2), Kaitaia spat (763 ± 66.8) to fine macroalgae (1069.9 ± 217.9) ($F_{3, 16}$ = 6.8, p = 0.0043). However, the mean percentage of the total number of juvenile *P. canaliculus* per sample that were retained after immersion was not different among the Substrate Groups (overall 83.9 ± 1.3 %, $F_{3, 16}$ = 0.8, p = 0.52, Fig. 5.2c). A greater percentage of the mussels became unattached from all the Substrate Groups after 2-min of immersion (9.7 ± 0.7 %) compared to those that re-settled (4.8 ± 0.6 %) or were attached to small fragments of substrate (1.8 ± 0.4, Distribution $F_{2, 48}$ = 46.4, p = 0.0001, Fig. 5.2d). Re-settled mussels accounted for a greater percentage of the total number of mussels per sample than the mussels attached to small fragments of substrate regardless of substrate group (t = 4.4, p = 0.0002, Fig 5.2d). Fewer than 11 mussels per sample were dead and there were no differences among Substrate Groups in the percentage of total mussels per sample that were dead at the end of the experiment (0.2 ± 0.03 %, Substrate Group $F_{3, 16}$ = 0.4, p = 0.75).

At the start of Experiment 1 there were no differences in the mean size of the juveniles between the two bags of Kaitaia spat. Following emersion, the mean size of juvenile *P. canaliculus* that detached from the Kaitaia spat were smaller (0.56 ± 0.02 mm) than the mean size at the start (0.75 ± 0.01 mm) and of the retained mussels (0.94 ± 0.01 mm; Distribution $F_{1, 1396}$ = 387.23, p = 0.001, Fig. 5.3a).

In Experiment 2, there were significant differences in the size of juvenile mussels among the substrate groups with coarse (2.14 ± 0.05 mm) and fine (2.19 ± 0.05 mm) macroalgae having

a greater mean shell length than the mixed macroalgae (2.0 ± 0.05 mm) and Kaitaia spat (2.0 ± 0.05 mm) substrate groups ($F_{3, 996} = 4.7$, p = 0.001, Fig. 5.3b). There was no difference in the mean size of mussels between coarse and fine macroalgae and similarly between mixed macroalgae and Kaitaia spat (yellow bars, Fig. 5.3b). However, the juvenile *P. canaliculus* that were unattached (1.40 ± 0.04 - 1.59 ± 0.04 mm, depending on Substrate Group) or had re-settled (1.77 ± 0.05 - 1. 59 ± 0.05 mm) were smaller than those retained on the substrates (2.27 ± 0.06 – 2.49 ± 0.05 mm; Macroalgal Group × Distribution $F_{9, 3931} = 3.9$, p = 0.0002, Fig. 5.4). The mussels that detached from all five of the substrate groups, when averaged across unattached and re-settled mussels (1.64 ± 0.71 mm) were 0.76 mm smaller than those retained on the various natal substrate groups (2.40 ± 0.91 mm). The mussels that re-settled were 0.40 mm bigger (1.84 ± 0.03 mm) than the unattached mussels (1.45 ± 0.02 mm) regardless of substrate group. Inspection of the percentage frequency distributions supported the analyses of mean size for both Experiments 1 (Fig. 5.4) and 2 (Fig. 5.5) in that there was clear trend for smaller mussels (i.e., ≤ 1.75 mm in shell length) in both experiments to detach from the substrates while larger mussels have a much greater propensity to be retained. There were significant and strong positive relationships between mussel size-classes and retention for both Experiments 1 ($R^2 = 0.99$, $p < 0.001$, Fig 5.6a) and 2 ($R^2 = 0.98$, $p < 0.001$, Fig. 5.6b).

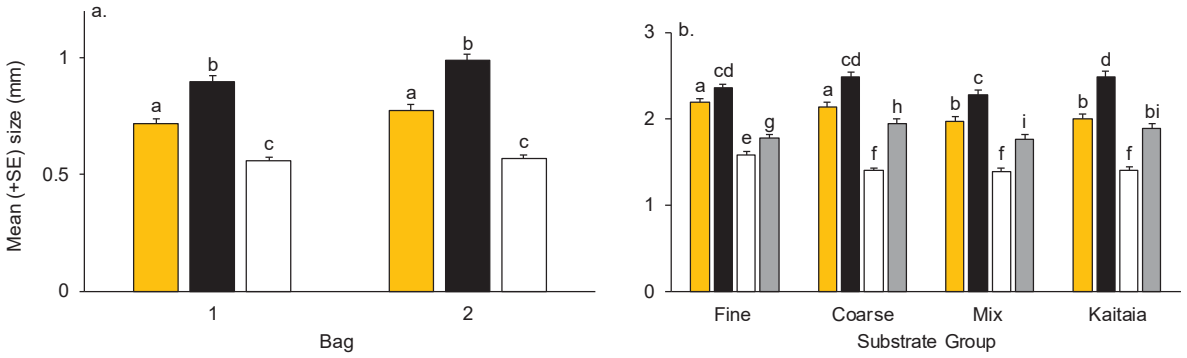

Figure 5.3. Mean (+ SE) size (mm) of juvenile *Perna canaliculus* in Experiments 1 (a) and 2 (b) at the start of the experiments (yellow bars), on the Kaitaia spat after a 2-min immersion (black bars), unattached in the aquaria (white bars) and re-settled on the aquaria (grey bars in b only). Variations between the two bags of Kaitaia spat and components (substrate groups) of the Kaitaia spat were assessed in Experiments 1 and 2, respectively. Note different scales on the y axes. The same lower-case letters indicate no differences between means following pairwise analyses.

5. Immersion at seeding triggers losses of juvenile mussels

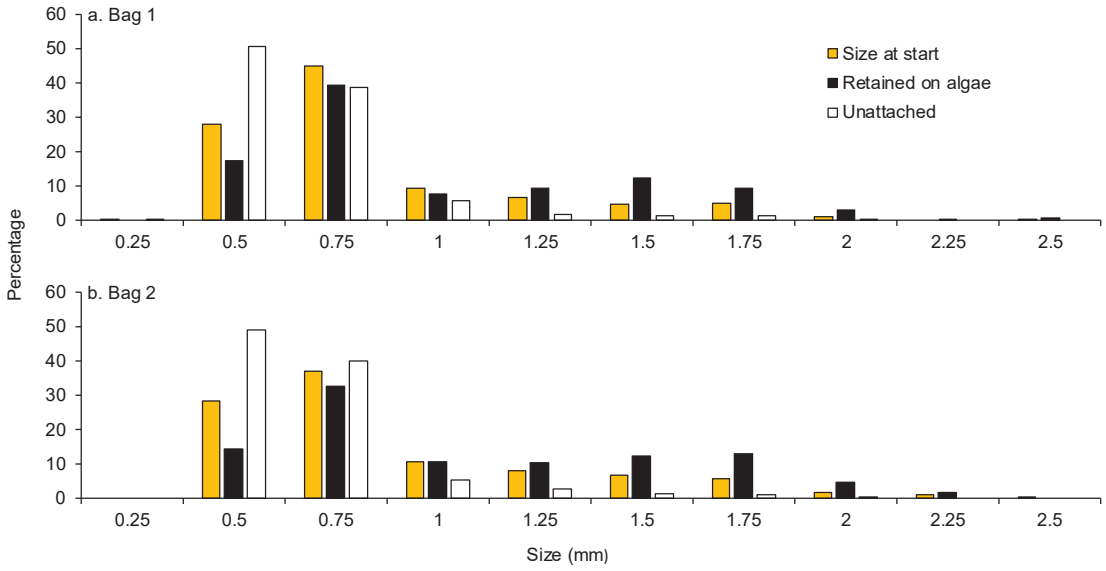

Figure 5.4. Percentage frequency (of total mussels sampled) for Bags 1 and 2 at the start of Experiment 1 (yellow bars) and retained on the substrate (black bars) and unattached (white bars) after a 2-min immersion in seawater. Numbers on the x axis denote maximum shell length for that size class.

5. Immersion at seeding triggers losses of juvenile mussels

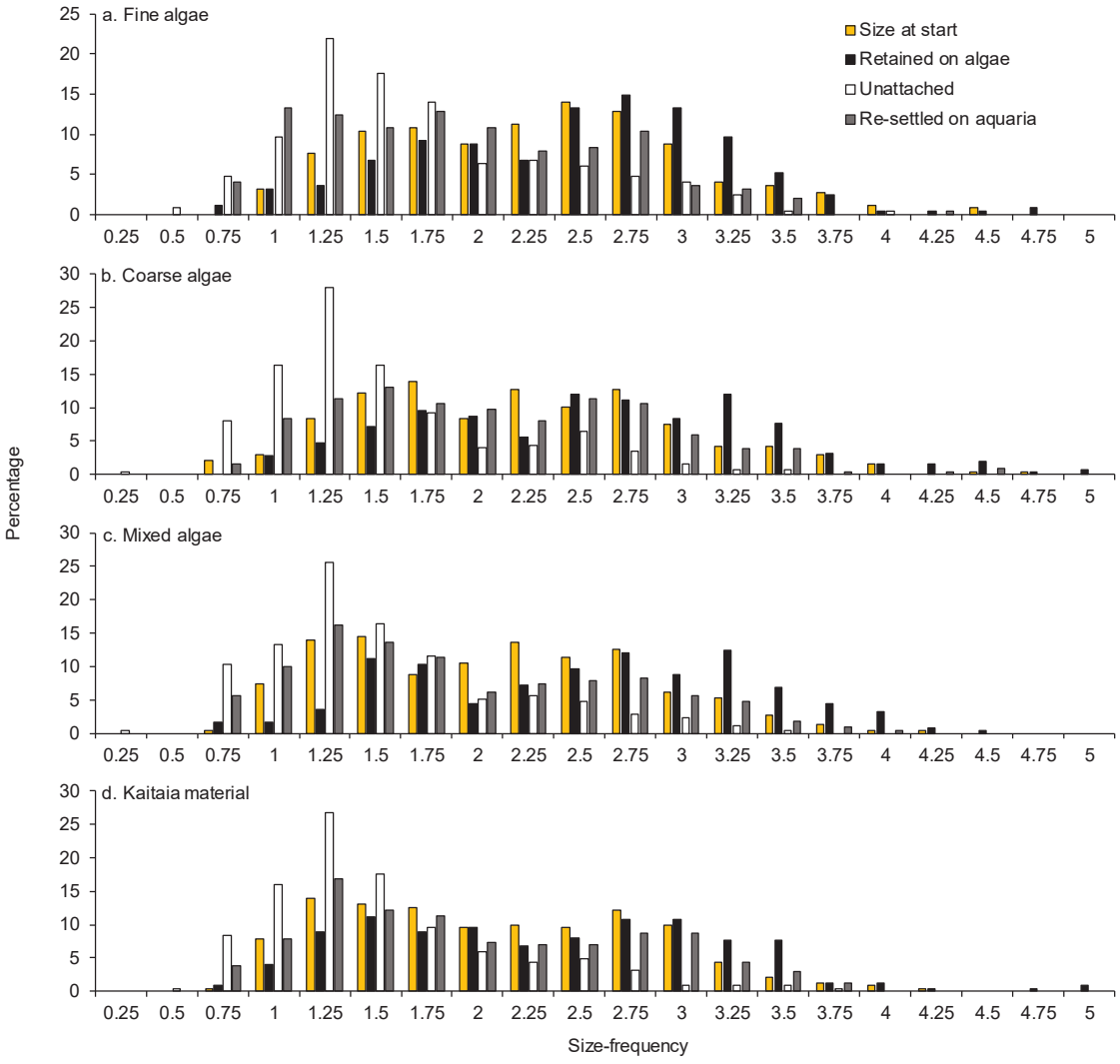

Figure 5.5. Percentage frequency (of total mussels sampled) for fine, coarse and mixed macroalgae, and Kaitaia spat at the start of Experiment 2 (yellow bars) and retained on the substrate (black bars), unattached (white bars) and re-settled (grey bars) on the aquaria after a 2-min immersion in seawater. Numbers on the x axis denote maximum shell length for that mussel size class.

5. Immersion at seeding triggers losses of juvenile mussels

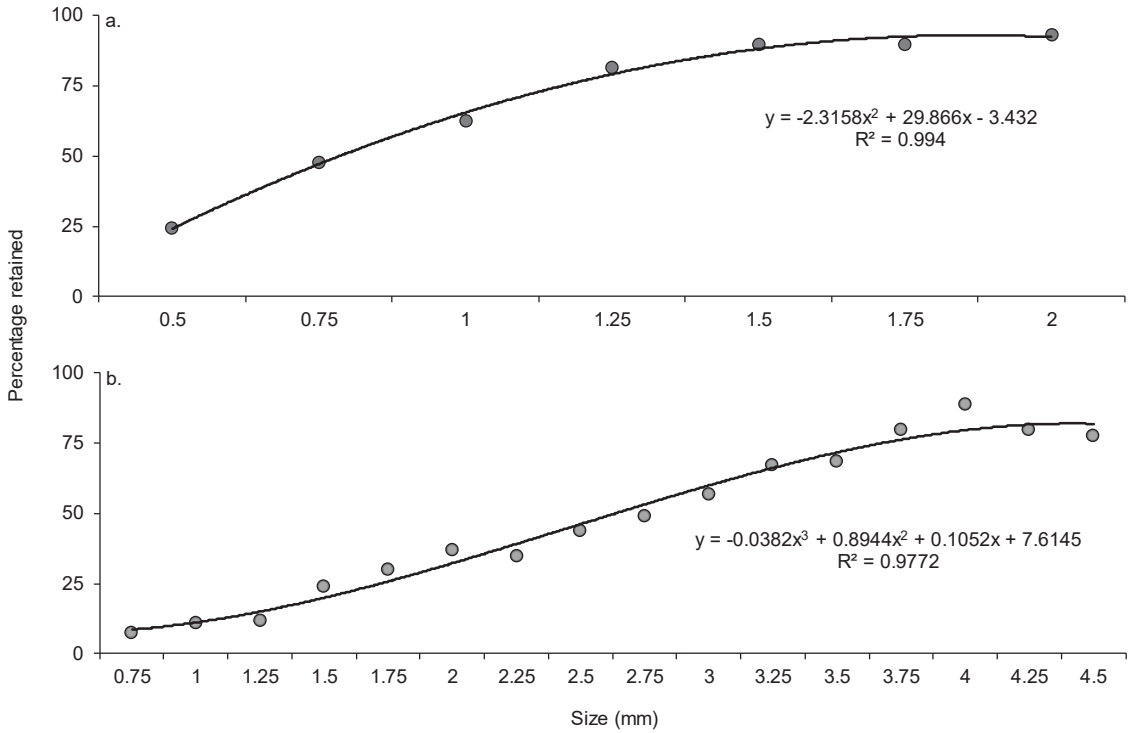

Figure 5.6. Percentage of the total number of mussels in each size-class retained after a 2-min immersion in seawater. In a, data are pooled across different bags of Kaitaia spat. In b, data are pooled across substrate groups. Numbers on the x axis denote maximum shell length for that mussel size class.

5.3.2. Losses of hatchery spat

The starting density of *P. canaliculus* juveniles on the sections of coir rope in Experiment 3 was 308. 2 ± 16.9 10 cm^{-1} and was consistent among the ropes used in the three emersion durations ($F_{2, 27}$ = 1.1, p = 0.35). However, the mean percentage of mussels retained on the coir rope was smaller after a 3 h (46.2 ± 2.8 %) emersion compared to 0.25 h (57.9 ± 1.9 %) and 6 h (58.8 ± 2.5 %) ($F_{2, 27}$ = 7.9, p = 0.0027, Fig. 5.7a). The mean percentage of the total number of mussels 10 cm^{-1} coir rope that remained unattached after immersion was significantly smaller (0.6 – 1.8 %) than for those that reattached to the aquaria and did not vary among emersion durations (Emersion × Distribution $F_{2, 54}$ = 5.5, p = 0.006, Fig. 5.7b). By contrast, the mean percentage of mussels that re-settled on the aquaria was greater after 3 h (52 ± 2.7 %) compared to after 0.25 h (41.8 ±1.9 %) and 6 h (38.8 ± 2.7 %) (Fig. 5.7b). On average, 93.0 ± 1.6 % of the mussels that left the coir rope would then settle onto the aquaria during the subsequent 24 h. The mean number of dead mussels was 3.1 ± 0.6 10 cm^{-1} that accounted for 1.1 ± 0.2 % of the total number of mussels per 10 cm of coir rope and there were no differences among three experimental emersion durations ($F_{2, 29}$ = 1.0, p = 0.4).

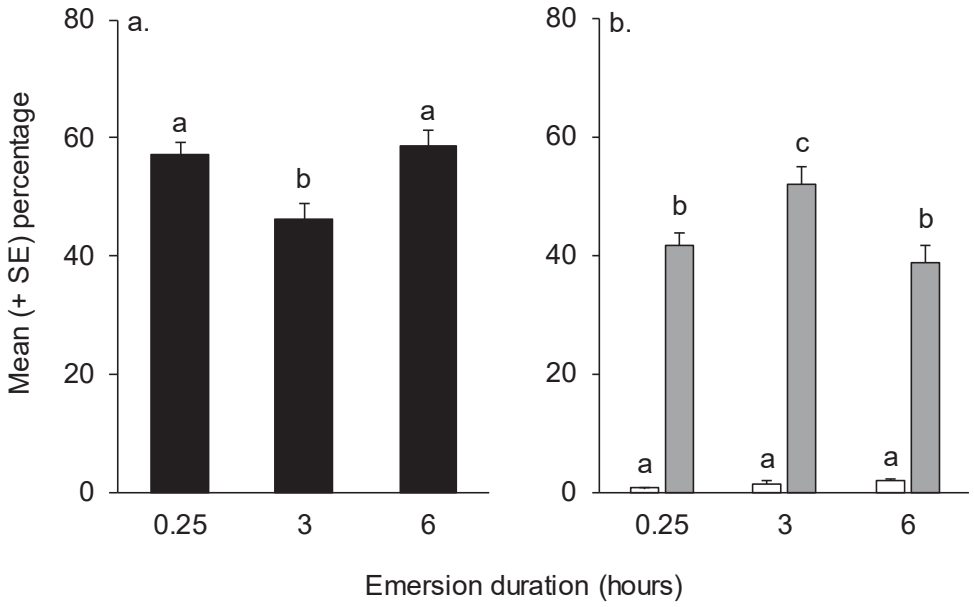

Figure 5.7. The effects of emersion duration on the mean (+ SE) percentage of hatchery-reared mussels retained on coir rope after a 2-min immersion in seawater (a), and the mean (+ SE) percentage of detached mussels during a 2-min immersion that remained unattached (white bars) or re-settled (grey-bars) after 24 h in aquaria (b).

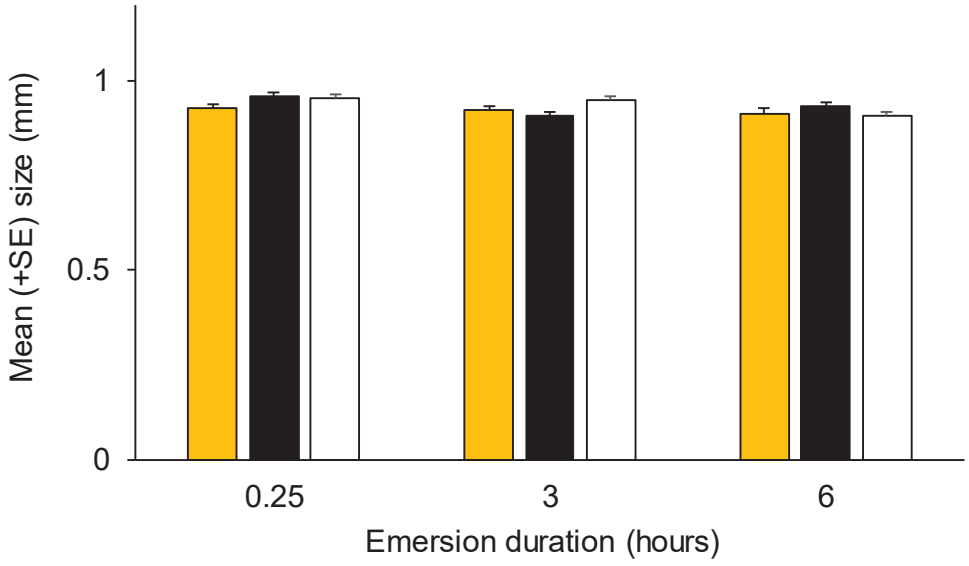

Figure 5.8. Mean (+ SE) size (mm) of juvenile *Perna canaliculus* in Experiment 3 at the start of the experiment (yellow bars), on the coir after a 2-min immersion (black bars) and detached from the coir (white bars). Variations among 0.25, 3 and 6 h emersion durations were assessed.

5. Immersion at seeding triggers losses of juvenile mussels

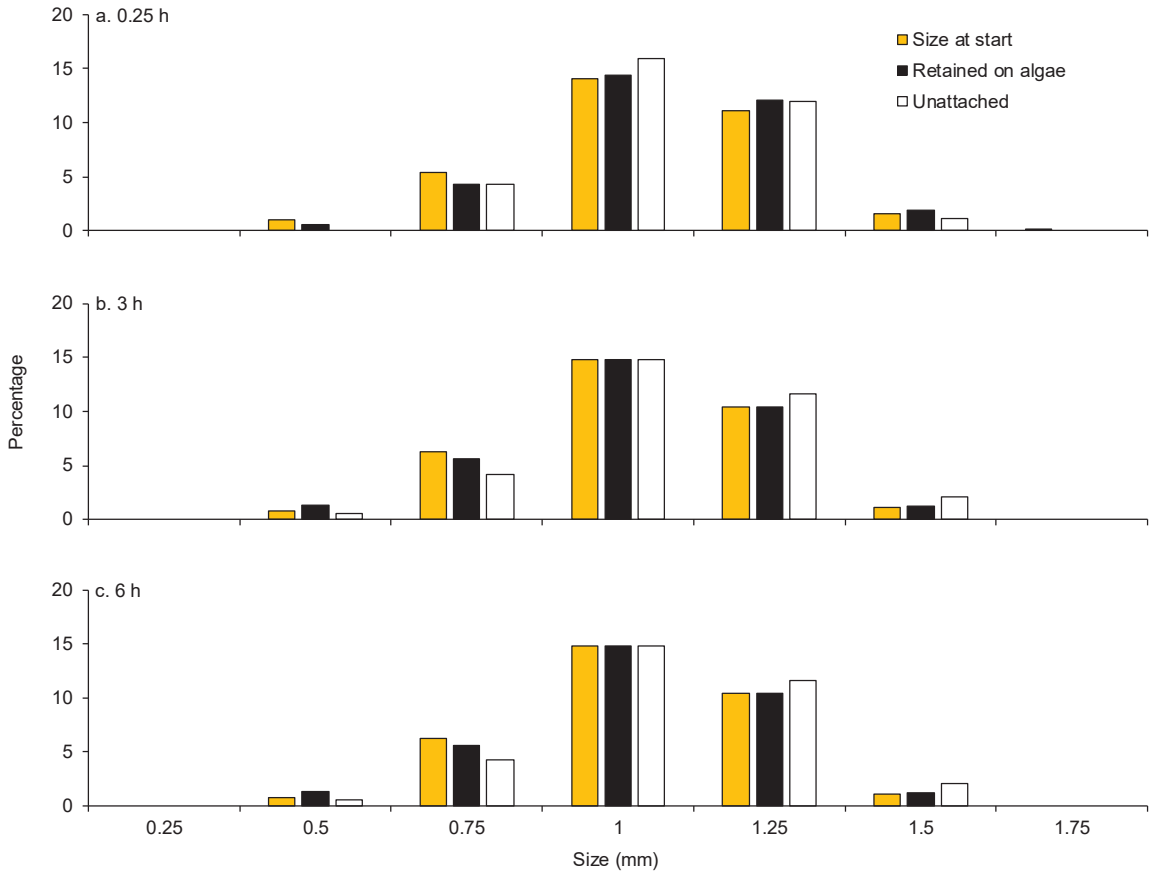

Figure 5.9. Percentage frequency (of total mussels sampled) for 0.25, 3 and 6 h emersion treatments at the start of Experiment 3 (yellow bars), retained on (black bars), or unattached (white bars) to the coir after a 2-min immersion in seawater. Numbers on the x axis denote maximum shell length for that mussel size class.

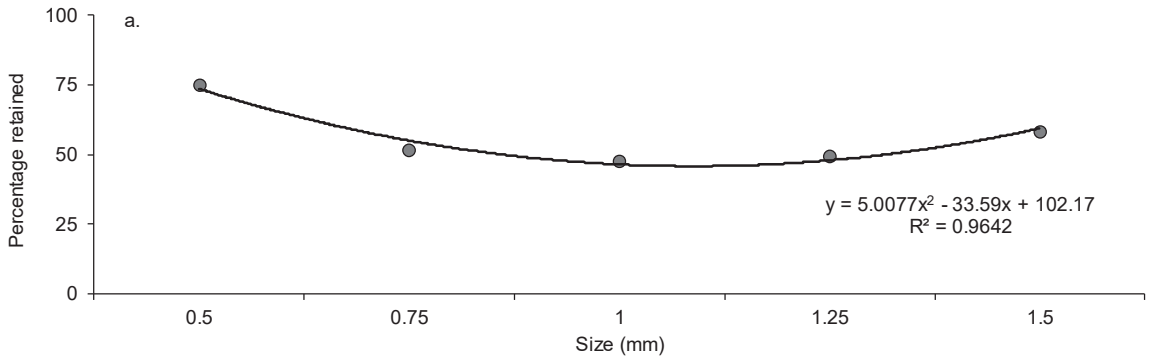

Figure 5.10. Percentage of the total number of mussels in each size-class retained after a 2-min immersion in seawater. Data are pooled across emersion durations. Numbers on the x-axis denote maximum shell length for that mussel size class.

There were no effects of Emersion treatment and Distribution on the mean size of mussels (0.93 ± 0.004 mm, Fig. 5.8). Inspection of percent-frequency data indicated similar percentages of the total number of mussels among size groups within size-classes (Fig. 5.9), while the percentage retained within size classes decreased from 0.5 mm to 1 mm and increased slightly thereafter (R^2 = 0.96, p = 0.03, Fig. 5.10).

4. Discussion

A large proportion of seed mussels, from both wild and hatchery sources, detached from their substrate immediately upon re-immersion in seawater, a situation typical of seeding operations in commercial aquaculture. This highlights the enormous inefficiency of the mussel aquaculture seeding process. While the scale of the experiments was small, the magnitude of the measured losses are equivalent to losses reported in the few field studies that have addressed the retention of juvenile *P. canaliculus* throughout the early stages of mussel aquaculture (Chapters 1 and 2 in this thesis, South et al. 2017). For example, up to 49.1 % of hatchery-raised seed mussels were found to have departed seeding ropes at 8 days after being seeded out (Experiment 2 in Chapter 2 of this thesis). In the current study most of the detaching mussels (> 42.6 %) were alive, and a high proportion (> 90 %) quickly re-settled when they were given the opportunity in Experiment 3 (Fig 5.7b). Therefore, it appears that early losses of mussels are not due to an inability of the detaching juveniles to attach to substrate, at least in the short-term, suggesting that many of the detached mussels might be fit for aquaculture production and constitute a significant lost resource.

There were clear differences in the number and size of the juvenile *P. canaliculus*, and their retention between the two consignments of Kaitaia spat used in Experiments 1 and 2 in this current study (Figs. 2 and 3). The mussels in the Kaitaia spat in Experiment 1 were, on average, 1.14 mm smaller in shell length, but more abundant (i.e., an additional 3188 g^{-1} of Kaitaia spat) than in Experiment 2. Mean retention of the Kaitaia spat in Experiment 1 was 60.1 ± 2.8 % compared to 83.8 ± 1.27 in Experiment 2 after immersion, suggesting that consignments of Kaitaia spat with larger juvenile mussels at lower densities may have better retention in the first few

minutes of seeding out. It is possible that larger mussels become more securely attached over time, or that the secondary settlement behaviour becomes less frequent with increased size (Lane et al. 1985, Buchanan & Babcock 1997, Fig. 5.6 in this study). Differences in the retention of juvenile *P. canaliculus* also varied between the two bags of Kaitaia spat that arrived as part of the same consignment (Fig. 5.2b). This variation in behaviour within the same consignment of mussels might be due to variations in conditions during collection and transport. For example, some Kaitaia spat is collected from shallow water on Ninety Mile Beach, while other Kaitaia spat is collected from beach-cast material, which could result in varying exposure to humidity and desiccation of Kaitaia spat used to fill individual bags (Webb & Heasman 2006, Alfaro et al. 2010). Bags of Kaitaia spat can also experience different temperature regimes during the extended period of handling and transport from Ninety Mile Beach until seeding out on a mussel farm (Heasman 2013). Further variability in retention might be due to variations among individual mussels or taxa within the Kaitaia spat. Such variations might arise through the coalescence of different accumulations of substrates that end up being included in Kaitaia spat, that may harbour different cohorts of mussels (Alfaro et al. 2010).

Since the number, size and fitness of the mussels within the Kaitaia spat, and the composition of the material itself, can be highly variable among consignments (Sim-Smith & Jeffs 2011, Jeffs et al. 2018), it is impossible to determine what processes led to the population characteristics of the juvenile *P. canaliculus* in this study. For example, there were fewer mussels attached to coarse macroalgae at the start of Experiment 2, and after 2-min of immersion, compared to the other macroalgal groups examined, suggesting that consignments with coarser macroalgae

might not yield as many mussels per gram of spat material. Furthermore, in contrast to previous studies of Kaitaia spat, the mussels found on the coarse macroalgae had a similar mean size and size-frequency distribution to those on fine macroalgae, which is typically considered to harbour smaller mussels (Alfaro & Jeffs 2002, Alfaro et al. 2004). More work is required to determine the factors that cause variation among cohorts, ages, attachment strengths, secondary settlement behaviour and densities, and that determine the size and distribution of juvenile *P. canaliculus* on the harvested Kaitaia spat and, ultimately, affect losses of seed mussels.

The sizes of the mussels detaching from the Kaitaia spat in Experiments 1 and 2 were smaller than that of the mussels that were retained on the Kaitaia spat. This is perhaps surprising, given that the overall mean size of the mussels was 1.14 mm smaller in Experiment 1 (0.75 ± 0.01 mm) than in Experiment 2 (1.89 ± 0.02 mm). It appears that smaller seed mussels within a cohort are more likely to detach compared to the larger mussels in that cohort. Smaller mussels might be more susceptible to the effects of desiccation, or other stressors arising during their relay from harvest to aquaculture site (Webb & Heasman 2006, Jenewein & Gosselin 2013). Across the experiments, mussels of ≤ 1.00 - 1.75 mm in shell length had a higher propensity to detach from their natal substrates, compared to mussels larger than this size (Figs. 5.4, 5.5, 5.9), suggesting a possible transition in the secondary settlement behaviour of mussels related to their size. It has previously been reported that secondary settlement behaviour in this species is not curtailed until the juvenile mussels exceed 5 - 6 mm in shell length (Buchanan & Babcock 1997). However, a decrease in the expression of this behaviour may provide opportunities to improve seed mussel retention by extending hatchery culture of seed mussels to a larger size. For example, the hatchery

spat used in Experiment 3 were, on average, 0.93 ± 0.004 mm in shell length and no individuals were > 1.75 mm (Fig. 5.9). Given that retention increased by 25.8 % (Experiment 1) and 30.6 % (Experiment 2) between 1 and 2-mm size classes (Fig 5.6), it is possible that on-growing in the hatchery to this larger size (2 mm) could possibly mitigate immediate losses of seed mussels. Furthermore, hatchery-culture to sizes greater than 2 mm would likely yield even better retention (Fig 5.6b).

Over 90 % of the 45.7 % of *P. canaliculus* juveniles that initially detached from the coir rope within 2-min of immersion in Experiment 3, re-settled during the subsequent 24 h (Fig. 5.7b), and less than 1.1 % died. The losses of hatchery-reared juvenile mussels observed in Experiment 3 immediately after immersion in seawater, and their subsequent re-settlement, suggests that secondary settlement processes are likely an important source of spat losses, as has been hypothesised in other studies (Jeffs et al. 1999, Carton et al. 2007, South et al. 2017). Secondary settlement is a suite of behaviours that allow juvenile mussels to relocate among substrates during their early life (Bayne 1964) and is considered to be a pronounced feature in *P. canaliculus* (Buchanan & Babcock 1997, Jeffs et al. 1999, Hayden & Woods 2011). However, it is difficult to compare the rates of secondary settlement, and patterns of re-settlement (i.e., attachment to other substrates) among the experiments in this study due to methodological differences. For example, differences in the number of unattached mussels that re-settled between Experiments 2 (28 .4 ± 2.7 %, Fig. 5.2d) and 3 (93 ± 1.6 %, Fig. 5.7b) were possibly due to the absence of water flow while the mussels were held in the aquaria in Experiment 2, which has been shown to reduce rates of settlement compared to more turbulent environments (Alfaro 2005, 2006a). However, the data

presented in this current study show that most mussels detaching from their substrate are alive at seeding, and the results of Experiments 2 and 3 suggest that many of these will re-settle or remain alive, at least over the following day, despite the possibly sub-optimal conditions in Experiment 2.

The high losses of hatchery spat that had *ad libitum* access to food throughout their development in the hatchery conflict with previous studies that have suggested a link between nutritional quality of the juveniles and their retention (Phillips 2002, 2004, Carton et al. 2007). For example, there was a negative effect of reduced food rations on the retention of juvenile *P. canaliculus* in a laboratory experiment, where a 6 day starvation period reduced retention by ca 30 % compared to a fed control (Carton et al. 2007). In this study, > 42 % of the nutritionally replete juvenile *P. canaliculus* detached from their coir substrate when they were immersed in seawater following a period of emersion. This indicates that the underlying nutrition of the juveniles might not affect retention through poor byssal attachment or by triggering secondary settlement as has been previously hypothesised. More detailed investigation into the role of nutrition in the retention of seed mussels is required. For example, it is possible that well-fed, fit mussels are more capable of making secondary settlement migrations than nutritionally deplete individuals.

The experiments used in this current study aimed to quantify how many mussels could be lost at the time of seeding, and to determine their short-term viability. However, the underlying causes of the initial losses recorded in this study were not examined. Many factors, such as emersion, desiccation and temperature stressors, occurring during the relay process could affect the health and retention of mussels in aquaculture (Webb & Heasman 2006, Carton et al. 2007,

Calderwood et al. 2014, Calderwood et al. 2015). Experiments 1 and 2 used Kaitaia spat that had been relayed from Ninety Mile Beach to the Marlborough Sounds as part of the standard commercial relay process for seed mussels. In Experiment 3, the longest emersion duration (6 h) represented a typical relay time between the hatchery and the nursery farm site. Emersion and desiccation are intrinsic to the relay of mussel spat in most aquaculture operations, given that juvenile mussels must be taken out of the water to move them among sites. For example, desiccation has been shown to reduce the retention of Kaitaia spat by 18 % compared to an immersed control over 10 d in a laboratory experiment (Carton et al. 2007). In the present study the percentage of mussels retained after a 3-h emersion was smaller than for 0.25 and 6 h. However, it seems unlikely that this difference had a biological cause given that the rate of retention did not continue to decrease over time. Regardless, even a short period of emersion was associated with substantial detachment from the coir and secondary settlement behaviour resulting in the re-attachment of over 90 % of the detached mussels.

It is possible to envisage how husbandry and transfer techniques might be altered to mediate losses of juvenile *P. canaliculus* at seeding, especially for mussels that are capable of re-settling. For example, brief periods of emersion followed by immersion might allow juveniles that are more likely to detach to be separated from those that are more strongly attached, or less likely to actively migrate away from their point of attachment. The detached mussels could then be allowed to re-settle, grown to a larger size (when secondary settlement is less likely) using hatchery technology, and deployed later. Indeed, some studies indicate that larger mussels (> 5 - 6 mm) are less likely to disperse via secondary settlement (Buchanan & Babcock 1997) and are more resilient

to desiccation stress, which is an intrinsic stressor in the relay process (Jenewein & Gosselin 2013). Therefore, culture to a larger size before deployment might be a useful route to increased retention. Cost-benefit analyses are required to assess the relative costs of the husbandry and feeding of spat in the hatchery, to those associated with losing large quantities of juveniles during early production. Alternative actions to increase retention that could be undertaken prior to seeding might be to vigorously agitate the seed mussels, via water motion, to increase the strength of their byssus attachment (Alfaro 2005, 2006a, Hayden & Woods 2011).

One important source of loss from the Kaitaia spat in this experiment, that has not been previously recorded, were of juvenile mussels attached to small fragments of substrate falling out from among the Kaitaia spat. Losses of mussels attached to fragments of substrate were 31.2 ± 2.5 % and 10.1 ± 1.9 % of the mean number of detached mussels in Experiments 1 and 2, respectively. These mussels were all small (< 1 mm) and sometimes in aggregations around small epiphytes or pieces of larger macroalgae (Fig. 5.1). It is possible that mussels settle to such structures while they are attached to larger macroalgae that are then broken up in the surf environment of Ninety Mile Beach (Newell et al. 1991, Alfaro et al. 2004), or settle to them as the mussels and macroalgae drift among the other material in the water column (Navarrete et al. 2015). Importantly, it is likely that mussels attached to minute pieces of substrate will not be trapped by the socking used to hold the Kaitaia spat against the mussel ropes at seeding and lost from the farm environment.

Conclusions

This study identifies the low rates of retention of seed mussels during the initial stages of aquaculture, where large quantities of mussels are lost immediately upon seeding. While the experiments presented here were done at small-scale and in the laboratory, they offer insight into the potential magnitude of the losses of seed mussels (16 - 47 %) after 2 minutes. Mortality of the juveniles was not important in the first few hours of aquaculture production. Rather, most mussels that detached (attached to substratum fragments or unattached) remained viable and were able to re-settle. More research is warranted to determine the causes of mussel loss and methods to mitigate those losses such as modified relay or seeding processes.

Chapter 6.

General discussion

6.1. Summary

The research presented in this thesis demonstrates profound inefficiencies in the early phase of the aquaculture of mussels in New Zealand, especially in the first few weeks after seeding, with overall retention of mussels reduced to less than 35 % of the starting density in all experiments (Table 6.1). The field-research assessed the retention of juvenile *P. canaliculus* in relation to different variables that conceivably have the potential to influence retention. These variables included the development of biofouling (Chapter 2), the type of material the mussels were seeded on (Chapter 3), and the presence of adult conspecifics (Chapter 4). However, the experimental factors tested in the field experiments generally had low levels or no statistical significance, except for the duration following seeding out which consistently accounted for the majority of the variation in all analyses (Table 6.1). Secondary settlement processes appeared to play an important role in immediate losses of seed mussels from grow-out structures (Chapters 3, 4, 5 and appendix 3). Therefore, future research should focus on developing a better understanding of the underlying ecological significance of secondary settlement behaviour in *P. canaliculus*, the triggers of secondary settlement, possible methods to arrest this behaviour, and aquaculture technologies to more efficiently manage seed mussels.

6.2. Retention of juvenile *Perna canaliculus* throughout the nursery period

The field experiments presented in this thesis (Chapters 2 – 4) are the first to assess the retention of juvenile *P. canaliculus* from the time of their seeding into aquaculture through to the subsequent recovery for re-seeding at lower densities for the final phase of grow-out. The results of these experiments demonstrated that the initial retention of the seed mussels is exceptionally poor. Previous accounts of such losses of seed mussels and their causes are anecdotal or lack conclusive or detailed data, as has been provided through this study (Jeffs et

al. 1999). Furthermore, the timing of losses of seed mussels during early production has previously not been determined, making it difficult to begin to understand and address the likely possible causes of poor retention. The results of the field experiments described in this thesis show that juvenile mussels are lost in very high numbers (42.9 - 64.8 % of the starting density within 8 – 30 days) following seeding, followed by smaller losses that can occur throughout the subsequent early production phase. This pattern of early loss was consistent for hatchery-reared juveniles (Chapters 2 and 4) and wild juveniles (Kaitaia spat; Chapter 3), although the greatest early losses of mussels following seeding were from the wild Kaitaia spat.

Previous studies of retention in juvenile *P. canaliculus* were carried out during discrete periods in early production and do not provide a complete picture of overall retention. For example, predation by the spotted wrasse, *Notolabrus celidotus*, had a major impact on retention when juvenile mussels were above 5 mm in shell length in experiments that lasted around 2 weeks (Hayden 1995). Likewise, experimental ropes seeded with Kaitaia spat were deployed to assess retention in the field and to provide a reference point for a laboratory assessment of the effects of water velocity (Hayden & Woods 2011). However, the juveniles used in the water velocity study by Hayden & Woods (2011) had been previously deployed for over 3 weeks to allow the spat to recover from the seeding process and would be unlikely to have behaved in a manner consistent with naive Kaitaia spat being seeding onto mussel farms. Similarly, juvenile mussels used in laboratory experiments are typically allowed to recover after immersion, a process that might serve to separate mussels that are more likely to migrate or die away from those that are used as experimental animals. For example, only mussels that remained attached to the substrate material of Kaitaia spat after an initial 3-day period of immersion were used in experimental work to test the effects of factors including desiccation and starvation on juvenile retention on sections of mussel rope (Foote 2003, Carton et al. 2007).

Using mussels that have already shown greater retention than their counterparts is likely to obscure patterns of retention at the scale of the individual batch of Kaitaia spat being assessed.

Understanding the timing of losses of juvenile mussels gives an important indication of where the major inefficiencies in early aquaculture lie and indicate the potential magnitude of benefits that might arise from any future interventions to manage and mitigate losses, depending on their timing during production. In chapter 3 for example, mitigation attempts targeting a production period of 40 - 89 days would only be able to address losses equating to 7.3 % of the overall number of mussels lost during the entire early production period. By contrast, mitigation attempted in the first 19 days of production would be targeting 85 % of the total number of lost mussels. However, the effectiveness of any interventions might also vary depending on the size of the mussels involved given the evidence from the current research which indicates that the size of juvenile mussels mediates their secondary settlement behaviour (chapter 5). This predicament is due to a limited understanding the processes causing the losses of mussels and a lack of research into possible mitigation measures.

Table 6.1. Summary of the research findings presented in this thesis.

Chapter	Factors tested	Response variables	Losses of *Perna canaliculus*	Other factors	Synthesis
Chapter 2	Effects of Duration of deployment, Site (× 2) and Depth (2 & 5-m). Two experiments were run at different times of the year to assess seasonality.	*P. canaliculus* retention and biofouling development.	Experiment 1: losses were 42.9 % (15 days), 60.2 % (89 days), and 64.4 % (178 days). Experiment 2: 49.1 % (8 days), 68.7 % (77 days), and 78.3 % (136 days). Small site-site variations among durations in Experiment 2. No effects of depth in either experiment.	Patterns of biofouling changed over time and varied among sites and depths. Generally, an amphipod assemblage developed early, followed by algae and then sessile invertebrates.	Biofouling development had occasional correlations with the number of mussels retained, but overall had little influence on *P. canaliculus* retention.
Chapter 3	Effects of substrata on which wild juveniles are seeded and duration of deployment on retention. Migrations of mussels onto adjacent and nearby ropes. Effects of the substrata and mussels on small-scale water conditions near the mussel ropes.	*P. canaliculus* retention from Kaitaia spat. Number of dead *P. canaliculus*. Wet weight of the substrata, DO, pH, nutrient concentrations.	52.8 – 72.9 % lost at 19 days, 69.6 – 76.7 % at 40 days, and 74.4 – 84.9 % lost at 89 days. Retention greatest for coarse algae (25.6 %) compared to all Substrate Groups (< 17.9 %).	Duration had most important effect on water conditions. Wet weight of the substrata decreased over-time, NH_4N was more variable among substrate groups and durations.	Losses of mussels generally appeared independent of the water conditions, except for a possible negative effect of NH_4N at days 19 and 40, that could have reflected variations in mussel abundance. Overall losses were due to secondary settlement (19.6 %), mortality (2.5 %) and unknown causes (44.9%).

Chapter 4	Effects of adding live adult mussels or their shells at different densities (5 and 20 mussels 45 cm^{-1}) as a route to increasing retention in hatchery-raised juveniles. Small-scale migrations of *P. canaliculus* and substrate preference of key biofoulers	*P. canaliculus* retention and biofouling development.	No effects of adding adults or their shells on retention. 46 % of mussels lost after 1 month, 81 % lost after 5 months.	Live individuals and shells of adult mussels generally increased biofouling development. Increased abundance of *Mytilus galloprovincialis* recruits at 1 month. *P. canaliculus* moved among aquaculture substrata. *M. galloprovincialis* settled mostly to the ropes, while Ischyroceridae settled on the outer socks.	No differences in retention among experimental mussel treatments despite differential patterns of biofouling, suggesting fouling had little impact on retention. Aquaculture substrata are complex and can control patterns of biofouling.
Chapter 5	Effects of a short (2-min) immersion on retention, replicating immersion at seeding, was tested in 3 Experiments. Expt. 1 tested for differences between bags of Kaitaia spat. Expt. 2 tested for differences among substrate components of the Kaitaia spat. Expt. 3 tested retention of hatchery-reared mussels after different emersion periods.	Percentage retained following immersion, mortality and size of the juvenile mussels. In Experiments 2 and 3, patterns of re-settlement were assessed.	Expt. 1: 34.4 % and 45.5 % lost from Bags 1 & 2. Expt. 2: 16.1 % lost across substrate groups. Expt. 3: 42.1 % (0.25-h emersion), 53.8 % (3-h), and 41.2 % (6-h).	In Expts.1 and 2, large numbers of mussels were attached to minute, drifting pieces of algae or other material that would not be retained by aquaculture substrata. > 90 % of detached mussels in Expt. 3 re-attached to the aquaria during the subsequent 24-h. The mean size of retained mussels was larger than those that detached in Expts. 1 and 2, but not in Expt. 3. Mussels less than 1.75 mm in shell length moved more than larger mussels.	Immersion can trigger secondary settlement in small mussels, even when they are nutritionally replete (i.e., the hatchery-reared juveniles in Expt. 3). Secondary settlement behaviour is a function of size.

The experimental chapters presented in this thesis assessed effects of factors with the potential to impact retention. Correlation analyses indicated occasional relationships between the biofouling assemblage and its major taxa in Chapter 2, and some indication that degradation of Kaitaia substrate material and associated concentrations of NH_4N might determine retention to a certain extent. However, none of the factors assessed experimentally strongly affected the numbers of *P. canaliculus* retained on the experimental ropes, especially when compared to the effects of deployment duration over the early phase of production. For instance, while the accumulation of biofouling organisms was particularly intense after the first few weeks of deployment in both experiments assessed in Chapter 2, the losses of juvenile mussels recorded over this period were small compared to those that had already occurred in the first 8 – 15 days (Experiments 1 and 2 in Chapter 2), and correlations between biofouling assemblages or dominant taxa were weak where they occurred. Similarly, the addition of live adult mussels or their shells to typical deployments of hatchery-reared spat (Chapter 4) had no effect on the numbers of mussels retained despite significant effects on the abundance of some biofouling taxa including the mytilid mussels *M. galloprovincialis*. Taken together, the results of the research presented in this thesis indicate that the factors that were examined, except for deployment duration, were only marginally responsible for influencing the observed large-scale losses of juvenile mussels. By contrast, insights from movements of juveniles during the experiments (Chapter 3, 4 and 5) suggest that secondary settlement of the juveniles at, and after seeding, can have a profound effect on overall retention.

6.3. Secondary settlement of juvenile *Perna canaliculus* in aquaculture

The findings from research presented in Chapters 3, 4 and 5 shows that many mussels move away from their initial position at seeding, clearly indicating that secondary settlement behaviour of the mussels is the predominant cause of early losses. The process of secondary settlement has been shown to be an important behaviour in many bivalve molluscs that increases the temporal and spatial scales over which recruitment can take place (deMontaudouin 1997, Lundquist et al. 2004). In mytilid mussels, secondary settlement is achieved via three distinct behaviours; (i) pedal crawling, (ii) mucus-drifting, and (iii) passive drifting (Nelson 1928, Bayne 1964, Sigurdsson et al. 1976, Board 1983, Lane et al. 1985, Buchanan & Babcock 1997). This multi-modal pattern of migration gives juvenile *P. canaliculus* great capacity to migrate over a range of spatial scales away from aquaculture substrata, especially after initial seeding when the juvenile mussels are small. Later in early aquaculture production, severance of the byssal attachment followed by pedal crawling is likely a more important behaviour, because mucus drifting in *P. canaliculus* is thought to have a maximal size of 5 – 6 mm (Board 1983, Buchanan & Babcock 1997).

The biological processes that drive secondary settlement in *P. canaliculus* are poorly understood and require further examination. Studies of *Mytilus edulis* in Europe and the United Kingdom showed that mussels that have undergone secondary settlement secrete byssal threads via glands in the pedal organ (Sigurdsson et al. 1976, Lane et al. 1985). These glands were capable of secreting both byssus (polyphenolic proteins) or mucus (mucopolysaccarides) threads, with long (100 mm) mucus threads being considered to be commonly employed for secondary settlement dispersal in the water column (Lane & Nott 1975, Sigurdsson et al. 1976, Lane et al. 1985,

Buchanan & Babcock 1997). In *M. edulis*, the production of mucus threads ends at around 1 – 2.5 mm in shell length along with the reduction of the glands involved in their secretion (Lane et al. 1982), whereas in *P. canaliculus* mucus-secretion appears to end at 5 – 6 mm in length (Buchanan & Babcock 1997). Primary larval settlers (ca. 0.25 mm) of *P. canaliculus* employ both proteins and polysaccharides for their attachment structures with 25 day-old primary settlers secreting more proteins (byssus) than 19-day old individuals (Petrone et al. 2008), although the composition of byssal threads in secondary settlement sized *P. canaliculus* has not been studied.

Juvenile mussels undergo important biological changes during the period when secondary settlement can occur and when the greatest losses were observed in the research for this thesis. For example, small (1 - 2 mm in shell length) *M. edulis* are undergoing significant physiological transition from an almost isomyarian (similar sized posterior and anterior abductor muscles) to a heteromyarian (reduced anterior abductor muscle) body plan (Baker & Mann 1997). While the timing of this transition has not been shown in *P. canaliculus*, the presence of an anterior abductor muscle in larvae (Rusk et al. 2017), and its absence in adults (Siddall 1980) suggest that such a transition might occur during early post-settlement stages. However, the underlying biological impetus for variations in the expression of secondary settlement behaviour, or other physiological processes occurring during the period when secondary settlement is possible, have not been assessed. Perhaps of greater importance is determining how biological processes interact with the environment to trigger secondary settlement migrations. It is also possible that there is a genetic impetus for secondary settlement behaviour which would have important implications for hatchery culture of spat and selective breeding programmes. Determining the drivers of biological and physiological changes occurring at secondary settlement would be of tremendous relevance to the

issue of retention in the mussel aquaculture industry and allow the development and evaluation of potential mitigation strategies.

Much of the evidence for the potential importance of secondary settlement in the retention of seed mussels comes from studies undertaken on wild populations in natural habitats. For example, the ability of *P. canaliculus* to disperse in the water column, as opposed to crawling along the seabed, was shown in an experimental study that presented isolated natural settlement substrata (i.e., macroalgae) in a natural situation (Buchanan & Babcock 1997). Furthermore, wild *P. canaliculus* > 0.5 mm in shell length have been shown to settle onto, and be lost from, artificial substrata over the course of 4 days (South 2016). Juveniles arriving into a natural adult bed of mussels in northern New Zealand were found to be larger than 2 mm in shell length suggesting that smaller mussels (< 2 mm) might initially avoid adult beds, or that they are consumed or out-competed by the adults (Bayne 1964, Alfaro 2006c, b, Porri et al. 2008). Together these studies of wild mussels suggest that juvenile *P. canaliculus* are highly mobile, especially when they are < 3 mm in shell length, when they move frequently among substrata. The hypothesis that the high mobility of *P. canaliculus* allows juveniles to recruit among adults, and that the presence of adults might encourage the juveniles to attach, provided the rationale for seeding juveniles alongside adults in Chapter 4 (Bayne 1964, Buchanan & Babcock 1997, Alfaro 2006b). However, experimental data suggest that this is not the case, because similar numbers of juveniles were lost regardless of whether adults were present. Still, it is possible that other environmental chemical cues could promote secondary settlement as has been shown for primary settlement of mytilids including *P. canaliculus* (Alfaro et al. 2006, Morello & Yund 2016). For example, chemical cues

exuded by predators (starfish and crabs) reduced the retention of *P. canaliculus* in a laboratory setting (Meder et al. 2005a).

One of the most problematic factors to manage in the aquaculture of *P. canaliculus* is the ability of small-sized mussels to migrate entirely away from their location at seeding via byssus or passive drifting (Chapters 3 and 5, Miron et al. 1995, Le Corre et al. 2013). Importantly, juvenile *P. canaliculus* are usually obtained and seeded into aquaculture at a point in their development when they are highly mobile, active and more likely to be lost (Buchanan & Babcock 1997, Alfaro 2005, South 2016). It is possible that juvenile mussels are forced to move due to the loss of their attachment substrata. In Chapter 3 for example, there was a strong negative relationship between the wet weight of the macroalgae to which mussels were initially attached and their retention in the field that is possibly due to the macroalgae becoming an unstable surface for attachment as they decompose. It is also possible that juveniles outgrow their attachment substrata and migrate to find a more suitable site. For example, it has been suggested that the fine-scale distribution of *P. canaliculus* on macroalgae is achieved via secondary settlement with juvenile mussels relocating among substrata of different morphology as they grow (Alfaro & Jeffs 2002, Alfaro et al. 2004).

In the aquaculture setting where juveniles are seeded in extremely high densities (2000 m^{-1} of dropper rope for hatchery spat and 50,000 m^{-1} for Kaitaia spat), it is possible that some of the mussels migrate away from the ropes to avoid competition with the other mussels. Migrations of mussels to unseeded sections of rope in Chapter 3 support this notion because the mussels moved away from a crowded situation and maintained a constant density on where they re-settled on

unseeded sections of rope throughout the experiment. Future studies should assess the importance of seed density by varying the abundance of mussels along the ropes. Importantly, it is unknown whether juvenile mussels will migrate into heavily seeded locations. Therefore, contrasts between retention of juvenile mussels on experimental ropes that are intermittently (i.e., leaving gaps of vacant rope) or continuously seeded with mussels could be a useful means of assessing the ability to retain a greater proportion of mussels seeded to dropper lines. Furthermore, understanding variations in density-dependent retention of different sized mussels is essential to planning for effective seeding practices to be achieved.

Small-scale secondary settlement migrations of the seed mussels are essential in mussel aquaculture in New Zealand because all the structures to which the mussels are attached at seeding (i.e., the Kaitaia spat material or coir) degrade over time (Foote 2003). Therefore, the juvenile *P. canaliculus* must move to the more stable plastic on-growing rope if they are to remain in aquaculture. This transition was documented in Chapter 4 with most of the retained juvenile mussels being attached to the coir after a 1-month deployment in the field, compared to at the end of the experiment when they were tightly attached to the on-growing rope and the remnants of the outer sock. A similar pattern of transition was observed in the experiments assessing retention and biofouling in Chapter 2 (data presented in Appendix 3). In this instance, intermediate samples along the time series (84 and 77 d in Experiments 1 and 2, respectively) showed that many mussels moved from the coir to the outer sock, prior to securing themselves, and the remaining outer sock, to the on-growing rope. Other species of mussels have been shown to have distributions that vary over time (Petersen 1984, Cáceres-Martínez et al. 1994, Pulfrich 1996, Newell et al. 2010). For example, *Mytilus edulis* settling on the blades of eel grass in Maine, USA, migrated down the

blades over time (Newell et al. 2010). In the Schleswig-Holstein Wadden Sea National Park (Germany), primary settlers of *M. edulis* were found in aggregations on the terminal axils of filamentous algae, whereas larger mussels migrated to the thicker branches (Pulfrich 1996). Similar distributions over small spatial scales have been for shown for *P. canaliculus* found on Kaitaia spat material with smaller mussels attached to the branch nodes of filamentous algae versus larger mussels that tended to be found on coarse structures such as the stipes and blades of fucoid algae (Alfaro et al. 2004).

The small-scale migrations of *P. canaliculus* juveniles could be due to a variety of factors. For example, the transition from the coir to the outer-sock could reflect either the degradation of the coir, which triggers re-location of the mussels, or the mussels could move to optimise food availability or attachment strength. For example, settlement into the axils of macroalgae or hydroids was found to increase the amount of substrate for attachment, allowing the mussel to attach its byssus threads at multiple points (Alfaro 2006a). It is possible that the mesh of the outer socks presents a more suitable attachment surface for juvenile mussels. However, it is also possible that the juveniles moved to improve their access to food. For example, the mussels attached to the coir in Experiments 1 and 2 of Chapter 2, or in Chapter 4, experienced the intense build-up of biofouling organisms on the outer sock that could have restricted water-flow to the mussels below (De Nys & Guenther 2009, Bloecher et al. 2013). Moving onto the outer sock would have allowed the juveniles better access to the water column and its food and reduced the potential for negative impacts of the local environment beneath the sock, such as elevated NH_4N, which was implicated in losses of Kaitaia spat in Chapter 3. Support for this hypothesis comes from studies of adult mussels that are less mobile when food is abundant, but move to less aggregated conditions when

food is depleted (van de Koppel et al. 2008). Further research into the small-scale migrations should assess substrate affinities of different sized juveniles, the dispersal modes employed to select them and the role of local environmental factors in triggering secondary settlement.

There are some potential routes to mitigating losses of juvenile seed mussels regarding their secondary settlement behaviour. Culturing juvenile mussels to a larger size, when secondary settlement behaviour appears less pronounced (Chapter 5, Fig. 5.6) might improve retention. For example, percent-frequency analysis of the retained mussels in Chapter 5 indicated that losses decrease with mussel size and that secondary settlement becomes supressed as the mussels grow. Poorly attached mussels could also be removed from aquaculture substrata with immersion, as has been shown in Chapter 5, and either re-settled onto growing structures via vigorous agitation (Alfaro 2005, 2006a) or cultured to a larger size before deployment into the field. These mitigation measures would require careful consideration in terms of their costs and benefits. For example, immersing Kaitaia spat to remove detaching mussels will have increased costs associated with the immersion process (paid work hours, infrastructure) and re-settling, on growing and feeding the juveniles. While this might improve production continuity, it might also increase production costs. Finally, and most relevant to hatchery culture, is the possibility of isolating genetic lines that are predisposed for retention and incorporating selection for those lines into selective breeding programmes, as has been done for fast-growing mussels (MacAvoy et al. 2008).

6.4. Estimating secondary settlement and mortality

Identifying the post-settlement processes (e.g., secondary settlement or mortality) that are involved in losses of juvenile mussels has been a significant challenge in ecology (Bownes & McQuaid

2009, von der Meden et al. 2012), in particular due to the small size of the juveniles, their dispersal capability, and the many factors that might cause their death (Hayden 1995, Jones et al. 1996a, Van de Ven 2007, Le Corre et al. 2013). Separating losses of juvenile mussels due to secondary settlement processes from losses via mortality was achieved in this study in a laboratory setting (Chapter 5) and to a lesser extent, in the field (Chapter 3). Still, the fate of ca 50 % of the mussels lost from experimental ropes in the field in Chapter 3 could not be determined. This inability to determine whether lost mussels are dead or alive has important ecological and industrial ramifications. In the wild, the live juvenile mussels migrating from macroalgae (Bayne 1964, Lawrie & McQuaid 2001) can replenish mussel beds (Newell et al. 2010), whereas their dead counterparts can potentially limit recruitment (Porri et al. 2016). In the aquaculture setting, determining whether seed mussels stay alive and migrate away from aquaculture structures, rather than dying, might allow for targeted loss-mitigation measures to be put in place. The results presented in Chapter 3 suggest that fewer mussels died than migrated during early production. However, these numbers are difficult to interpret in a field setting because dead mussels might be lost from the ropes and not counted. By contrast, live mussels could migrate away from, or among the ropes, as was shown by their secondary settlement on unseeded sections of experimental ropes and control ropes. Therefore, it is important that questions concerning the comparative status of retained versus migrated mussels, and the amount of them that die, are assessed in both field and laboratory settings.

6.5. Factors affecting the retention of juvenile *Perna canaliculus*

6.5.1. Possible effects of the relay process on the retention of juvenile mussels

Data from research presented in Chapter 3 suggest that substantial secondary settlement migration of the seed mussels onto nearby sections of rope, and to a lesser extent their mortality, can take place soon after relay and seeding. This implies that losses might result from the relay and seeding processes. The possibility of relay-associated effects on the retention of juveniles has provided the rationale for research in New Zealand (Webb & Heasman 2006, Carton et al. 2007) and elsewhere (Dare & Davies 1975, Calderwood et al. 2014, Calderwood et al. 2015). Desiccation and temperature stressors have been shown to reduce the retention (Carton et al. 2007) and fitness (Webb & Heasman 2006) of juvenile *P. canaliculus*, likely through impacts on mussel physiology and behaviour, as has been shown in studies of *M. edulis* seed in the United Kingdom (Calderwood et al. 2014). For example, in an experiment that assessed replicated transport on a mussel dredger vessel, juveniles that were held at greater depths (i.e., underneath other mussels) produced fewer byssus threads compared to those held at the surface of the mussel pile. It is possible that there are similar negative effects on Kaitaia spat that are bagged and piled into refrigerated trucks. Indeed, the temperature and humidity can vary significantly during collection, processing and transport of Kaitaia spat and among bags of the material during transport (Heasman 2013). However, no studies have assessed how variation during relay affects retention in the field. Such links between relay, spat-health, secondary settlement and death of the seed mussels are a possibility that should be addressed with further research. For example, experiments that manipulate relay conditions for seed mussels that are experimentally seeded out in the field and then followed over the subsequent

early period of aquaculture production are likely to yield important data. Ideally, future studies should aim to determine the fate of lost mussels and the comparative physiological state (e.g., bodily glycogen reserves, byssus production) or other biological characteristics (e.g., size, genetics) of retained versus lost mussels. Doing so will lead to a greater understanding of the characteristics of retained and lost mussels and will be essential in informing spat management practices.

6.5.2. Possible effects of juvenile fitness on **Perna canaliculus** *retention*

It has been hypothesised that losses of seed mussels from aquaculture are due to underlying variations in the fitness of the mussels (Foote 2003, Meder et al. 2005b, Carton et al. 2007, Sim-Smith & Jeffs 2011). For example, juvenile *P. canaliculus* that were starved for a period of 6 days had 30 % fewer mussels retained than un-starved mussels over a 10 d experimental (Carton et al. 2007) period. Similarly, juveniles that were either starved or received reduced rations had lower retention than well-fed individuals, after being out-planted in the field (Phillips 2002). To reduce the effects of variations in fitness among individual juvenile mussels that could be expected to occur among wild juvenile mussels of varying history, the field experiments in Chapters 2 and 4, and Experiment 3 in Chapter 6 used hatchery-reared juveniles. These are nutritionally replete, having been fed high quality food *ad libitum* throughout their development until the day of seeding. Therefore, hatchery-reared mussels were considered to provide better subjects for experimental work by reducing the confounding effects of wide variations in their health, size, genetics, and the substrate to which they were attached. However, these nutritionally replete mussels were also lost in enormous quantities, especially early in production when it could be expected, based on the

conclusions from previous studies, that their superior nutritional status would result in increased retention. It is still possible that these losses are due to underlying variations in fitness among individuals that arise from genetic differences (Strömgren & Nielsen 1989, Toro et al. 2004). For example, the larval size of mussels can be highly variable, even among siblings raised in the same environmental conditions (Bayne 1965, Toro et al. 2004). However, it is not known whether fitter mussels are more or less active, and likely to undergo secondary settlement than their less fit counterparts. Comparative determinations of the nutritional and physiological status of juvenile mussels that are retained versus those that express secondary settlement behaviour would test this possible explanation.

6.5.3. The effects of Biofouling in early **Perna canaliculus** *aquaculture*

Research presented in Chapters 2 and 4 of this thesis quantified the development of biofouling assemblages on the aquaculture substrata holding the seed mussels. Early communities are characterised by a succession from amphipods to macroalgae to an assemblage dominated by *Mytilus galloprovincialis* and other bivalves (e.g., *Modiolarca impacta*, Chapter 4), sessile invertebrates (arborescent bryozoans and colonial ascidians in particular), and the crop species. Few studies have assessed patterns of biofouling alongside recently seeded shellfish, despite the concern that biofouling is highly detrimental to aquaculture in nearly all cases (Fitridge et al. 2012). Rather, studies have typically assessed early patterns of biofouling on the netting of fish-cages (Greene & Grizzle 2007, Bloecher et al. 2013), on artificial settlement plates (Perkol-Finkel & Benayahu 2007, Sievers et al. 2014), on human structures such as wharf pilings (Cole et al. 2005) or on natural rocky reefs (Sousa 1979, Benedetti-Cecchi 2000). While such studies give

important insights into the potential timing, magnitude and effects of certain biofouling organisms, it is difficult to interpret such data in the context of an aquaculture system that is typically complex (e.g., fibrous plastic mussel ropes) and has been seeded with great quantities of a single species. Research presented in Chapters 2 and 3 of this thesis provide rare examples of studies that have assessed biofouling development alongside that of the crop species in the early stages of aquaculture. Furthermore, the results of the field experiments indicate that the major losses of juvenile *P. canaliculus* are likely to have occurred independently of biofouling with only occasional, generally weak and sometimes positive associations being made. This finding contrasts with the widely held perception that biofouling is detrimental to any stage of aquaculture production.

Many studies of settlement, recruitment and biofouling development in the marine environment use artificial (plastic or fibre-cement board) plates due to their uniformity and the ease with which they can be replicated. However, such surfaces are 2-dimensional, and it is possible that these do not well simulate the environments into which most biofouling organisms recruit. In this thesis the ropes, socks and material to which mussels are attached at seeding were used to quantify biofouling development, which provided a realistic settlement scenario and results that are directly relevant to the mussel industry (Hayden 1995). Separating the aquaculture substrata in this manner (Chapter 4) revealed that biofouling species can have differential patterns of settlement suggesting that their characteristics can influence biofouling development. For example, the tube-building amphipods from the family Ichscyroceridae, were more common on outer-socks, whereas the blue mussel *M. galloprovincialis* settled in greater abundance among the

fibres of the experimental ropes. Understanding substrate preferences of key biofouling taxa is important if their management and control are required.

The blue mussel was a ubiquitous fouler in all field experiments in this thesis (data for Chapter 3 presented in Appendix 4). The abundance of *M. galloprovincialis* has increased significantly in the Marlborough Sounds during the past 30 years to the extent that it is considered the most problematic fouling species in this region (Atalah et al. 2017, Forrest & Atalah 2017). The ecology of *M. galloprovincialis* as a fouling species is complex and varies from its ecology in wild settings where it is typically constrained to a narrow band in the mid-intertidal zone by competition with *P. canaliculus*. Typically, *P. canaliculus* is dominant in the low-intertidal and subtidal zones, where the two species co-occur (Menge et al. 2007, Petes et al. 2007). In the aquaculture situation, *M. galloprovincialis* can become the most abundant mussel species, especially in shallow water (< 5 m), reducing the efficiency of *P. canaliculus* aquaculture by competing for food or becoming entangled with the crop species and eventually falling off the lines when they become overcrowded and heavy, which often occurs as the mussel ropes are removed from the water for re-seeding (Forrest & Atalah 2017).

In the research presented in Chapters 2 and 4 in this thesis *M. galloprovincialis* was more abundant than the crop species at the end of the field experiments, its settlement was increased by the addition of live adult mussels and many individuals recruited among the fibres of the mussel ropes (Chapter 4). Therefore, it appears that the aquaculture system of shallow fibrous ropes and the *P. canaliculus* themselves are highly beneficial to the success of *M. galloprovincialis*. The finding that *M. galloprovincialis* can be more abundant than the crop species at the end of early

aquaculture production is important for husbandry techniques since this mussel is stripped alongside *P. canaliculus* at the nursery sites and re-seeded among it for the subsequent grow-out of *P. canaliculus* to a marketable size. It should also be noted that the majority of Kaitaia spat is seeded in late winter to spring, just prior to the major settlement peak of *M. galloprovincialis* in the Marlborough Sounds (Atalah et al. 2016, Atalah et al. 2017). The greater abundance of *M. galloprovincialis* in Experiment 1 compared to 2 (Chapter 2), carried out during winter-spring and summer-autumn, respectively, indicates that the timing of these deployments might favour settlement of this species. However, the primary settlement of *M. galloprovincialis* in the Marlborough Sounds and the arrival of beach cast Kaitaia spat in northern New Zealand occur at the same time of year making the over-settlement by *M. galloprovincialis* difficult to avoid. One mitigation measure that is currently being tested is the use of sunken aquaculture systems to avoid over-settlement by *M. galloprovincialis* that is typically greater in shallow water (Barrie Forrest pers com).

6.6. Limitations of the experimental work

It is important to consider the limitations of this work and how they might affect interpretation of the data. At the outset of this PhD research it became clear that the magnitude and timing of losses of juvenile mussels were unknown and detailed investigation into these was warranted. Each of the Chapters 2 – 4 has a descriptive element to them, describing spat-loss over time and space (Chapter 2) within the context of some wider research questions (e.g., the effects of co-seeding adult mussels as a route to improving retention – Chapter 4). Therefore, similar research was

undertaken in each of three Chapters, to allow their synthesis to provide some generality on the timing of losses during aquaculture production.

The major findings of the research presented in this thesis are largely the result of a series of field experiments that were spatially constrained due to access to, and working on, commercial mussel farms. Only one study could be replicated at the farm-scale and all studies would have benefited from replication at more sites to assess the generality of the findings (Underwood 1997). Spatial constraints also dictated the experimental design of the field studies, for example with the use of frames being adopted to deploy replicate experimental ropes. Ideally, ropes would have been hung individually from mussel farm backbone lines in a randomised array, which perhaps might have limited the losses of replicates experienced in Chapter 2 and increased their independence. However, this was impractical given the space, access and operational constraints on the mussel farms experienced in a commercial environment. Still, experimental ropes were always placed at generous distances from one another, proportionally more so than other recent studies using settlement panels (Wieczorek et al. 1996, Johnston & Keough 2000). Similarly, accessing the mussel farm was problematic in Experiment 2 of Chapter 2 and the reduced temporal resolution of the data set reduced the overall assessment of biofouling development in that experiment. This research would have benefited from the repetition of more of the experiments through time to assess the generality of the findings, especially the possible effects of major fouling episodes that did not occur during this study. Despite these limitations, the combined results of the field-based components of the study (Chapters 2 – 4) provide strong evidence of when losses of juveniles occur and their likely magnitudes and will perhaps provide some impetus to scale-up this experimental work to assess patterns of retention among multiple farms or at production scales.

6.7. Conclusion

The studies in this thesis have shed some light on the timing and magnitude of losses of seed mussels in early aquaculture production in New Zealand. The knowledge that most mussels are lost when they are small and soon after deployment into aquaculture has important implications for the management of mussel seed resources and the development of aquaculture practices to manage losses. The secondary settlement behaviour of *P. canaliculus* has been identified as the likely dominant cause of losses of juvenile mussels and should be a major focus of future research. Such research has the potential to reduce the large scale and costly losses of seed mussels, leading to more efficient and sustainable management of New Zealand's most important aquaculture resource.

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Appendices

Appendix 1. List of taxa and their maximum, minimum and frequency of occurrence in Experiments 1 and 2 of Chapter 2. Data are pooled across sites and depths. Phylum = phyla and sub-phyla. Freq. = frequency of occurrence in percent. **Perna canaliculus* are the crop species and were seeded into these assemblages. Experiment 1 total = 86 taxa. Experiment 2 total = 61 taxa.

			Experiment 1			Experiment 2		
Phylum	Family	Genus/species/complex	Min	Max	Freq (%)	Min	Max	Freq (%)
Annelida	Eunicidae	Eunicidae	0	1	0.7	0	0	0.0
	Onuphidae	Onuphidae	0	1	0.7	0	0	0.0
	Hesionidae	Hesionidae	0	1	0.7	0	1	1.4
	Nereididae	Nereididae	0	9	38.7	0	2	11.1
	Phyllodocidae	*Eulalia* sp.	0	1	2.0	0	1	1.4
	Syllidae	Syllidae	0	5	18.0	0	11	12.5
	Sabellidae	Sabellidae	0	9	7.3	0	0	0.0
	Serpulidae	Serpulidae	0	113	73.3	0	12	41.7
	Capitellidae	*Heteromastus filiformis*	0	1	1.3	0	0	0.0
	Opheliidae	*Armandia maculata*	0	0	0.0	0	5	12.5
	Magelonidae	Magelonidae	0	3	1.3	0	0	0.0
	Cirratulidae	Cirratulidae	0	0	0.0	0	2	1.4
	Terebellidae	Terebellidae	0	8	10.0	0	2	4.2
		Polychaete larvae	0	1	2.7	0	4	2.8
Bryozoa	Watersiporidae	*Watersipora subquarta*	0	0.02	12.0	0	0.01	26.4
	Bugulidae	*Bugulina stolonifera*	0	1.35	59.3	0	0.03	12.5
		Bugulina flabellata	0	4.62	48.0	0	1	43.1
Chlorophyta	Bryopsidaceae	*Bryopsis* sp.	0	0.01	7.0	0	0	0.0
	Cladophoraceae	*Cladophora* spp.	0	1	6.7	0	0.08	9.7
	Ulvaceae	*Ulva intestinalis*	0	0.06	0.7	0	0	0.0
		Ulva sp.	0	10.16	64.0	0	0.01	2.8

Chordata	Tripterygiidae	*Forsterygion varium*	0	2	4.7	0	1	1.4
	Didemnidae	*Didemnum* sp.	0	0.01	2.7	0	0.01	1.4
		Diplosoma listerianum	0	0.57	24.0	0	0.06	19.4
	Polyclinidae	*Aplidium phortax*	0	1.72	20.0	0	2.02	22.2
	Cionidae	*Ciona robusta.*	0	0.41 (12)	38.0	0	1.06 (11)	31.9
	Corellidae	*Corella eumyota*	0	0.99 (11)	31.3	0	0.01 (1)	8.3
	Pyuridae	*Pyura pachydermatina*	0	0.49 (2)	4.0	0	0	0.0
	Styelidae	*Asterocarpa humilis*	0	0.57 (13)	31.3	0	0.01 (1)	4.2
		Cnemidocarpa bicornuta	0	0.01 (1)	1.3	0	0	0.0
Cnidaria	Sertulariidae	*Amphisbetia bispinosa*	0	0.78	23.3	0	0.36	25.0
		Sertularella crassiuscula	0	0.02	2.0	0	0	0.0
Crustacea	Balanidae	*Balanus* sp.	0	1	1.3	0	0	0.0
		Cyprid	0	1	0.7	0	0	0.0
	Ampilescidae	*Ampilesca* spp.	0	140	59.3	0	72	65.3
	Caprellidae	*Caprella* spp.	9	7759	98.0	0	4924	98.6
	Corophiidae	*Apocorophium acutum*	0	258	69.3	0	53	51.4
	Dexamiidae	*Paradexamine* spp.	77	4813	98.6	0	496	98.6
	Ischyroceridae	Ischyroceridae	66	16926	100.0	1	25616	100.0
	Lyssanasidae	*Parawaldeckia* sp.	0	944	84.0	0	398	97.2
	Photidae	*Gammaropsis typica*	0	429	58.7	0	94	77.8
		Ampithoe sp.	0	8	9.3	0	2	11.1
		Aora sp.	0	267	33.3	0	10	25.0
		UID amphipod (1)	0	14	4.7	0	0	0.0
		UID amphipod (2)	0	2	1.4	0	0	0.0
		Copepoda	0	1753	87.3	0	22	87.5
	Hymenosomatidae	*Halicarcinus* spp.	0	7	39.3	0	4	25.0
	Majidae	*Notomithrax minor*	0	3	6.7	0	1	6.9
	Palaemonidae	*Palaemon affinis*	0	7	16.7	0	1	1.4
	Pinotheridae	*Nepinnotheres novaezelandiae*	0	6	26.0	0	7	27.8
	Porcellanidae	*Petrolisthes*	0	3	13.3	0	2	6.9
		UID Zoea	0	37	4.7	0	0	0.0

	Munnidae	Munnidae	0	2	1.3	0	0	0.0
	Idoteidae	Idoteidae	0	6	18.7	0	1	1.4
	Mysidae	Mysidae	0	4	2.0	0	1	1.4
		Ostracoda	0	20	47.3	0	11	48.6
	Tanaidacea	Tanaidacea	0	2197	73.3	0	304	77.8
Mollusca	Mytilidae	*Aulacomya maoriana*	0	2	6.0	0	0	0.0
		Modiolarca impacta	0	37	76.7	0	212	72.2
		Mytilus galloprovincialis	1	842	100.0	6	398	100.0
		*Perna canaliculus**	51	968	100.0	83	937	100.0
		Xenostrobus pulex	0	6	45.3	0	1	2.8
	Limidae	*Limaria orientalis*	0	3	9.3	0	8	16.7
	Hyatellidae	*Hiatella arctica*	0	30	67.3	0	12	50.0
	Pectinidae	Pectinidae	0	3	18.7	0	27	50.0
		Bivalve (UID < 500 µm)	0	5	31.3	0	12	40.3
	Trochidae	*Cantharidus* sp.	0	1	2.0	0	2	5.6
		Gastropoda spp. (UID)	0	31	86.7	0	19	47.2
	Ischnochitonidae	*Ischnochiton elongatus*	0	1	0.7	0	0	0.0
	Mopaliidae	*Plaxiphora caelatus*	0	1	0.7	0	0	0.0
Nematoda		Nematoda	0	336	56.7	0	44	55.6
Nemertea		Nemertea	0	11	4.0	0	1	16.7
Ochrophyta	Scytosiphonaceae	*Colpomenia* sp.	0	1.97	43.3	0	1.03	33.3
		Endarachne binghamiae	0	0.01	1.3	0	0	0.0
		Petalonia fascia	0	0.08	3.3	0	0	0.0
		Scytosiphon lomentaria	0	0.03	4.7	0	0	0.0
	Alariaceae	*Undaria pinnatifida*	0	0.68	3.3	0	0	0.0
		Uid filamentous brown	0	0.01	2.0	0	0	0.0
Porifera	Sycettidae	*Sycon ciliatum*	0	1.14	32.0	0	0.2	29.2
Rhodophyta	Ceramiaceae	*Ceramium apiculatum*	0	1.78	52.0	0	0.43	23.6
		Ceramium spp.	0	0.26	10.7	0	0.01	5.6
	Dasyaceae	*Heterosiphonia* sp.	0	0.11	1.3	0	0	0.0
	Delesseriaceae	*Myriogramme* spp.	0	0.25	15.3	0	0	0.0

	Rhodomelaceae	*Brongiartella australis*	0	0.21	6.7	0	0	0.0
		Laurencia distichophylla	0	0.01	9.3	0	0.29	8.3
		Polysiphoina abscissoides	0	8.65	63.3	0	1.86	43.1
	Lomentariaceae	*Lomentaria caespitosa*	0	0.87	36.0	0	0.01	8.3
		Lomentaria umbiculata	0	0.17	16.0	0	0.01	1.4
		Rhodophyta spp. (UID blades)	0	0.17	6.7	0	0	0.0

Appendix 2. PERMANOVA analyses used in Chapter 2 testing for differences in (2.2.1) the retention of *Perna canaliculus*, (2.2.2) taxonomic richness in Experiment 1, (2.2.3) taxonomic richness in Experiment 2, (2.2.4) structure of the biofouling assemblage, (2.2.5) the abundance of key biofouling organisms in Experiment 1, and (2.2.6) the abundance of key biofouling organisms in Experiment 2. Relevant results sections are given (in parentheses) in the titles.

2.2.1. PERMANOVAs testing for the effects of Duration of deployment (days), Site and Depth on the abundance of juvenile *P. canaliculus* in Experiments 1 & 2 (section 3.2.3.)

	Source	f/r	df	MS	*F*	p	Perms
				Experiment 1			
Sites 1 & 2	Duration	r	**3**	**110650**	**4.82**	**0.0029**	**9949**
	Site	f	1	18038	3.85	0.1405	9860
	Depth	f	1	16887	3.51	0.1512	9859
	Du×Si	r	3	4382	0.19	0.906	9951
	Du×De	r	3	4509	0.20	0.9038	9940
	Si×De	f	1	33684	2.07	0.2392	9848
	Du×Si×De	r	3	16154	0.70	0.5488	9955
	Residuals		134	22959			
Site 1	Duration	r	**4**	**118280**	**6.14**	**0.0001**	**9956**
	Depth	f	1	14497	0.60	0.4921	9866
	Du×De	r	4	24338	1.26	0.2845	9947
	Residuals		92	19270			
				Experiment 2			
Sites 1 & 2	Duration	r	**2**	**1.15**	**51.65**	**0.0001**	**9944**
	Site	f	1	0.01	0.08	0.8177	9873
	Depth	f	1	0.00	0.07	0.8274	9874
	Du×Si	r	**2**	**0.15**	**6.85**	**0.0021**	**9950**
	Du×De	r	2	0.04	1.59	0.2095	9939
	Si×De	f	1	0.07	4.78	0.142	9867
	Du×Si×De	r	2	0.01	0.65	0.5267	9947
	Residuals		75	0.02			

f/r: fixed/random, *F* = *Pseudo F*, **Bold** text indicates significance at $p < 0.05$,
Perms: number of unique permutations for each factor in the analysis,
Log (x + 1) transformed data were used in Experiment 2.

2.2.2. PERMANOVAs testing for the effects of Duration of deployment (days), Site and Depth on taxonomic richness in Experiment 1 (section 3.3.1.)

	Source	f/r	df	MS	*F*	p	perms	MS	*F*	p	perms
					All taxa				Mobile invertebrae taxa		
Sites 1 & 2	Duration	r	3	**39.01**	**326.40**	**0.0001**	**9938**	**899.95**	**128.29**	**0.0001**	**9965**
	Site	f	1	0.04	0.17	0.7024	9867	5.00	0.56	0.5017	9846
	Depth	f	1	0.01	0.02	0.8944	9856	3.02	0.20	0.6958	9859
	Du×Si	r	3	0.21	1.80	0.1529	9942	8.93	1.27	0.2887	9946
	Du×De	r	3	**0.46**	**3.84**	**0.0107**	**9964**	15.58	2.22	0.0894	9961
	Si×De	f	1	0.04	0.45	0.5449	9854	4.29	35.18	0.0056	9832
	Du×Si×De	r	3	0.09	0.76	0.5211	9954	0.01	0.00	0.9999	9959
	Residuals		134	0.12				7.01			
	PERMDISP				1.28	0.32			1.73	0.119	
Site 1	Duration	r	4	**8.48**	**466.55**	**0.0001**	**9954**	**16.70**	**169.44**	**0.0001**	**9944**
	Depth	f	1	0.01	0.38	0.5467	9841	0.01	0.10	0.7667	9850
	Du×De	r	4	0.02	1.17	0.3313	9938	0.09	0.95	0.44	9947
	Residuals		92	0.02				0.10			
	PERMDISP				0.93	0.595			1.91	0.113	
					Algal taxa				Sessile invertebrate taxa		
Sites 1 & 2	Duration	r	3	**12.02**	**87.14**	**0.0001**	**9962**	**29.74**	**414.07**	**0.0001**	**9948**
	Site	f	1	1.41	7.97	0.0577	9863	0.10	4.13	0.1296	9868
	Depth	f	1	0.23	1.10	0.3672	9817	0.01	0.05	0.8402	9868
	Du×Si	r	3	0.18	1.28	0.2872	9961	0.02	0.33	0.8084	9961
	Du×De	r	3	0.21	1.51	0.2213	9955	0.12	1.74	0.1577	9958
	Si×De	f	1	0.00	0.03	0.8719	9843	0.01	0.05	0.8399	9868
	Du×Si×De	r	3	0.07	0.49	0.6874	9950	0.12	1.64	0.1878	9953
	Residuals		134	0.14				0.07			
	PERMDISP				4.59	0.001			2.50	0.015	
Site 1	Duration	r	4	**13.36**	**95.68**	**0.0001**	**9954**	**20.16**	**359.77**	**0.0001**	**9957**
	Depth	f	1	0.08	0.46	0.5318	9840	0.01	0.44	0.5493	9864
	Du×De	r	4	0.18	1.26	0.2835	9964	0.02	0.40	0.8166	9944
	Residuals		92	0.14				0.06			
	PERMDISP				9.43	0.001			6.41	0.001	

f/r: fixed/random. *F* = *Pseudo-F* statistic.

Bold text indicates significant effects at p < 0.05 or 0.01 where data were heterogeneous

2.2.3. PERMANOVAs testing for the effects of Duration of deployment (days), Site and Depth on taxonomic richness in Experiment 2 (section 3.3.1.)

	Source	f/r	df	MS	*F*	p	perms	MS	*F*	p	perms
					All taxa			Mobile invertebrate taxa			
Sites 1 & 2	Duration	r	**2**	**2962.80**	**443.97**	**0.0001**	**9952**	**1090**	**231.03**	**0.0001**	**9944**
	Site	f	1	39.33	0.67	0.4953	9881	4.66	0.19	0.713	9868
	Depth	f	1	17.25	0.80	0.4632	9861	0.13	0.03	0.8826	9838
	Du×Si	r	2	**60.53**	**9.07**	**0.0004**	**9950**	**25.26**	**5.35**	**0.0063**	**9951**
	Du×De	r	2	**22.12**	**3.32**	**0.0437**	**9947**	5.37	1.14	0.331	9951
	Si×De	f	1	1.70	0.17	0.7216	9875	7.44	1.71	0.3096	9865
	Du×Si×De	r	2	9.96	1.49	0.2325	9945	4.34	0.92	0.4113	9939
	Residuals		75	6.67				4.72			
	PERMDISP				1.65	0.189			1.66	0.184	
					Algal taxa			Sessile invertebrate taxa			
Sites 1 & 2	Duration	r	**2**	**12.77**	**108.96**	**0.0001**	**9954**	**199.00**	**216.30**	**0.0001**	**9960**
	Site	f	1	1.60	3.27	0.1911	9870	0.00	0.00	0.9901	9869
	Depth	f	1	0.56	2.73	0.216	9860	1.97	0.67	0.4945	9875
	Du×Si	r	2	0.50	4.28	0.0177	9954	1.98	2.15	0.1327	9943
	Du×De	r	2	0.21	1.77	0.1826	9938	**2.99**	**3.25**	**0.0447**	**9949**
	Si×De	f	1	0.01	0.22	0.6831	9856	0.63	1.41	0.3471	9876
	Du×Si×De	r	2	0.04	0.31	0.7378	9967	0.43	0.47	0.6273	9942
	Residuals		75	0.12				0.92			
	PERMDISP				7.81	0.001			1.97	0.287	

f/r: fixed/random. *F* = *Pseudo-F* statistic.

Bold text indicates significant effects at p < 0.05 or 0.01 where data were heterogeneous

2.2.4. PERMANOVAs testing for the effects of Duration of deployment (days), Site and Depth on assemblage structure in Experiments 1 & 2 (section 3.3.1)

			Experiment 1					Experiment 2			
	Source	f/r	df	MS	*F*	p	Perms	df	MS	*F*	p	Perms
				Mobile invertebrates					Mobile invertebrates			
Sites 1 & 2	Duration	r	**3**	**29331**	**145.27**	**0.0001**	**9941**	**2**	**49985.00**	**243.81**	**0.0001**	**9941**
	Site	f	1	2972.8	2.3672	0.0743	9941	**1**	**3592.30**	**2.80**	**0.035**	**9947**
	Depth	f	1	481.73	1.2877	0.3206	9959	1	79.90	0.20	0.9644	9947
	Du×Si	r	**3**	**1273.4**	**6.3067**	**0.0001**	**9940**	**2**	**1319.80**	**6.44**	**0.0001**	**9915**
	Du×De	r	**3**	**376.97**	**1.867**	**0.0204**	**9913**	**2**	**412.07**	**2.01**	**0.0086**	**9926**
	Si×De	f	1	346.4	1.4635	0.2553	9952	1	197.13	0.67	0.7272	9950
	Du×Si×De	r	3	237.28	1.1752	0.288	9913	2	297.29	1.45	0.1099	9926
	Residuals		134	201.91				75	205.02			
Site 1	Duration	r	**4**	**26526**	**130.12**	**0.0001**	**9934**					
	Depth	f	1	258.96	0.7199	0.6623	9956					
	Du×De	r	**4**	**364.14**	**1.7863**	**0.0129**	**9926**					
	Residuals		92	203.85								
				Algae					Algae			
	Duration	r	**2**	**43401.00**	**31.73**	**0.0001**	**9938**	**1**	**32529.00**	**13.27**	**0.0001**	**9929**
Sites 1 & 2	Site	f	1	7254.00	2.13	0.1016	9947	1	4250.90	1.16	0.4353	9957
	Depth	f	1	7039.70	2.04	0.1123	9950	1	2773.90	1.08	0.4655	9961
	Du×Si	r	**2**	**3432.50**	**2.51**	**0.0021**	**9927**	**1**	3662.00	1.49	0.1735	9921
	Du×De	r	**2**	**3492.80**	**2.55**	**0.0022**	**9919**	1	2565.30	1.05	0.3917	9944
	Si×De	f	**1**	**2388.50**	**2.74**	**0.0437**	**9953**	1	1360.00	0.71	0.6649	9969
	Du×Si×De	r	2	862.93	0.63	0.8418	9922	1	1915.60	0.78	0.5675	9953
	Residuals		102	1367.70				43	2451.50			
				Sessile invertebrates					Sessile invertebrates			
Sites 1 & 2	Duration	r	**2**	**60408.00**	**32.74**	**0.0001**	**9909**	**1**	**17061.00**	**7.28**	**0.0001**	**9930**
	Site	f	1	13160.00	2.07	0.0728	9948	1	10255.00	1.39	0.3456	9967
	Depth	f	1	2454.60	1.41	0.2405	9941	1	2270.30	0.82	0.6019	**9957**
	Du×Si	r	**2**	**6417.70**	**3.48**	**0.0001**	**9906**	**1**	**7398.90**	**3.16**	**0.004**	9938
	Du×De	r	2	1737.90	0.94	0.5368	9911	1	2766.30	1.18	0.3056	9938
	Si×De	f	1	2445.30	1.32	0.2766	9947	1	1702.30	1.41	0.3332	9956
	Du×Si×De	r	2	1851.00	1.00	0.459	9910	1	1209.60	0.52	0.8132	9943
	Residuals		102	1845.30				43	2343.30			

f/r = fixed/random, Perms = number of unique permutations for each factor in the analysis.
Bold text indicates significance at $p < 0.05$.

2.2.5. PERMANOVAs testing for the effects of Duration of deployment (days), Site and Depth on the abundance of key biofouling taxa in Experiment 1 (section 3.3.2.)

	Source	f/r	df	MS	*F*	p	perms	MS	*F*	p	perms	MS	*F*	p	perms
					Ischerioceridae			*Paradexamine* spp.				*Caprella* spp.			
Sites 1 & 2	Duration	r	3	**116.09**	**367.88**	**0.0001**	**9953**	**17.33**	**55.98**	**0.0001**	**9966**	**38.03**	**62.40**	**0.0001**	**9946**
	Site	f	1	**3.88**	**47.07**	**0.0032**	**9867**	2.58	0.56	0.5045	9844	12.37	2.53	0.2037	9859
	Depth	f	1	0.09	1.14	0.3708	9852	0.22	0.18	0.7065	9845	0.13	0.06	0.8113	9834
	Du×Si	r	3	0.08	0.25	0.8661	9945	**4.68**	**15.13**	**0.0001**	**9969**	**4.95**	**8.13**	**0.0001**	**9947**
	Du×De	r	3	0.07	0.23	0.8709	9961	1.21	3.92	0.0126	9951	**2.07**	**3.40**	**0.0205**	**9963**
	Si×De	f	1	0.45	4.67	0.1134	9878	0.90	4.61	0.1152	9860	1.74	1.77	0.2664	9838
	Du×Si×De	r	3	0.09	0.29	0.8301	9949	0.19	0.63	0.5978	9945	0.99	1.62	0.1885	9946
	Residuals		134	0.32				0.31				0.61			
	PERMDISP				6.64	0.001			3.43	0.001			1.94	0.101	
Site 1	Duration	r	4	**52.54**	**184.78**	**0.0001**	**9948**	**59.10**	**90.44**	**0.0001**	**9966**	**107.74**	**167.05**	**0.0001**	**9945**
	Depth	f	1	0.39	3.83	0.1109	9845	0.59	0.51	0.5141	9880	0.58	0.33	0.594	9851
	Du×De	r	4	0.10	0.34	0.8473	9946	1.19	1.81	0.1323	9955	**1.80**	**2.79**	**0.03**	**9950**
	Residuals		92	0.28				0.65				0.64			
	PERMDISP				6.09	0.001			6.38	0.001			2.29	0.079	
					Mytilus galloprovincialis			Algae (g)				Sessile invertebrates (g)			
Sites 1 & 2	Duration	r	3	**50.53**	**337.65**	**0.0001**	**9946**	**17.94**	**170.23**	**0.0001**	**9946**	**13.32**	**158.84**	**0.0001**	**9949**
	Site	f	1	2.21	5.59	0.0953	9865	1.17	2.75	0.1924	9853	0.79	3.46	0.1565	9856
	Depth	f	1	1.47	3.46	0.1488	9833	0.04	0.15	0.7246	9861	0.19	3.95	0.1403	9863
	Du×Si	r	3	0.40	2.67	0.0511	9958	**0.43**	**4.09**	**0.0093**	**9955**	0.23	2.75	0.044	9956
	Du×De	r	3	0.43	2.87	0.0375	9954	0.24	2.31	0.0773	9956	0.05	0.56	0.6422	9949
	Si×De	f	1	1.62	25.52	0.0118	9865	0.57	6.95	0.0742	9875	0.00	0.42	0.5549	9888
	Du×Si×De	r	3	0.06	0.41	0.7422	9953	0.08	0.77	0.5139	9942	0.00	0.05	0.9848	9960
	Residuals		134	0.15				0.11				0.08			
	PERMDISP				2.28	0.021			4.66	0.001			6.83	0.001	
Site 1	Duration	r	4	**61.42**	**328.28**	**0.0001**	**9950**	**13.35**	**126.06**	**0.0001**	**9957**	**5.41**	**102.76**	**0.0001**	**9957**
	Depth	f	1	0.00	0.01	0.9235	9871	0.39	3.71	0.0605	9857	0.07	1.25	0.2671	9838
	Du×De	r	4	0.27	1.44	0.2356	9958	0.13	1.23	0.2972	9948	0.03	0.65	0.6321	9951
	Residuals		92	0.19				0.11				0.05			
	PERMDISP				1.92	0.089			7.62	0.001			6.93	0.02	

f/r = fixed/random. *F* = *Pseudo-F* statistic.

Bold text indicates significant effects at p < 0.05, or < 0.01 where data were heterogenous.

2.2.6. PERMANOVAs testing for the effects of Duration of deployment (days), Site and Depth on the abundance of key biofouling taxa in Experiment 2 (section 3.3.2.)

Source	f/r	df	MS	*F*	p	perms	MS	*F*	p	perms	MS	*F*	p	perms
			Ischerioceridae				*Paradexamine* spp.				*Caprella* spp.			
Duration	r	**2**	**299.48**	**864.99**	**0.0001**	**9951**	**37.86**	**84.81**	**0.0001**	**9951**	**159.90**	**220.21**	**0.0001**	**9944**
Site	f	1	3.52	2.20	0.2562	9879	2.03	0.11	0.7726	9871	9.33	1.38	0.3472	9861
Depth	f	1	2.07	2.63	0.2241	9872	1.02	2.53	0.2364	9877	1.08	0.21	0.6913	9863
Du×Si	r	2	1.65	4.75	0.0107	9952	**18.69**	**41.86**	**0.0001**	**9951**	**6.97**	**9.60**	**0.0002**	**9943**
Du×De	r	2	0.80	2.31	0.1055	9931	0.40	0.89	0.4168	9951	**5.22**	**7.19**	**0.0018**	**9952**
Si×De	f	1	1.52	2.86	0.2086	9880	0.62	1.34	0.362	9871	0.68	0.52	0.5401	9887
Du×Si×De	r	2	0.54	1.56	0.2185	9958	0.46	1.04	0.3574	9960	1.32	1.82	0.1693	9952
Residuals		75	0.35								0.73			
PERMDISP				5.25	0.001			4.29	0.004			4.83	0.001	
			Mytilus galloprovincialis				Algae (g)				Sessile invertebrates (g)			
Duration	r	**2**	**36.75**	**167.92**	**0.0001**	**9951**	**2.05**	**37.29**	**0.0001**	**9955**	**5.02**	**31.02**	**0.0001**	**9942**
Site	f	1	0.36	2.04	0.2736	9846	0.05	0.28	0.6437	9876	0.86	1.51	0.328	9883
Depth	f	1	0.07	4.90	0.1366	9867	0.02	1.50	0.3248	9879	0.05	0.50	0.5601	9871
Du×Si	r	2	0.18	0.80	0.4574	9956	0.17	3.05	0.0509	9934	0.58	3.58	0.0343	9951
Du×De	r	2	0.01	0.03	0.9676	9938	0.01	0.24	0.7901	9953	0.09	0.55	0.5816	9960
Si×De	f	1	0.04	3.19	0.1881	9862	0.01	0.21	0.6939	9857	0.02	0.83	0.4505	9864
Du×Si×De	r	2	0.00	0.02	0.9783	9946	0.02	0.43	0.6571	9949	0.02	0.10	0.907	9947
Residuals		75					0.05				0.16			
PERMDISP				4.3338	0.003			6.40	0.001			11.28	0.001	

f/r: fixed/random. *F*; *Pseudo-F* statistic. Bold text indicates significant effects at p < 0.01 where data were heterogeneous.

Appendix 3. Supplementary data on the small-scale distribution of *Perna canaliculus* juveniles from Chapter 2

This appendix presents a sub-set of data not included in the main text of Chapter 2. Experimental ropes deployed to assess the retention of juvenile *Perna canaliculus,* and the development of biofouling were separated into their three composite substrata in the laboratory and prior to processing using the methods outlined in section 2.2 of Chapter 2. The number of *P. canaliculus* on each of these substrata was counted for each deployment duration. At days 136 and 178 in Experiment 1, and day 138 in Experiment 2 it had become impossible to separate the remaining outer sock from the rope, because they had become tightly bound with byssus from the *P. canaliculus* and the over-settling *Mytilus galloprovincialis*, therefore, the rope and remaining sock are considered together on these days. The retained juveniles remained attached to the coir early in the experiments. Data from Experiment 1 suggest that the coir can be an important attachment substrate for retained juveniles for at least 44 days. There was a clear transition of juveniles between coir and the outer sock at days 84 and 77 in Experiments 1 and 2, respectively, before the remaining outer socks became bound to the rope by byssus (Fig. 1).

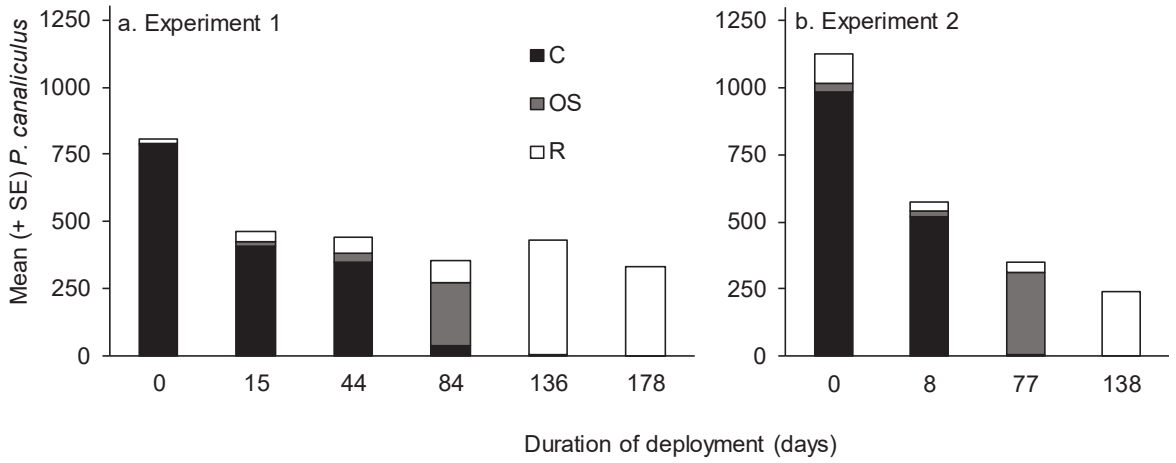

Figure 1. Stacked bar chart showing mean number of juvenile *Perna canaliculus* on coir (black bars), the outer sock (grey bars) and the rope (white bars). Note from day 136 in Experiment 1 and 138 in Experiment 2 rope and outer sock are considered together. Standard error not presented for presentation purposes.

Appendix 4. Additional data on *Mytilus galloprovincialis* abundance from experimental work in Chapter 3

This appendix presents mean abundance data for *Mytilus galloprovincialis* that settled on experimental ropes seeded with fine, coarse, mixed macroalgae and Kaitaia spat in Chapter 3. These mussels were enumerated at the same time and using the same procedures described in section 3.2.3 of Chapter 3. Data were analysed with analysis of variance (ANOVA) and there were no differences among substrate groups within durations (Fig. 1). By contrast, the mean number of *M. galloprovincialis* increased between durations ($F_{2, 58}$, p = 0.0001).

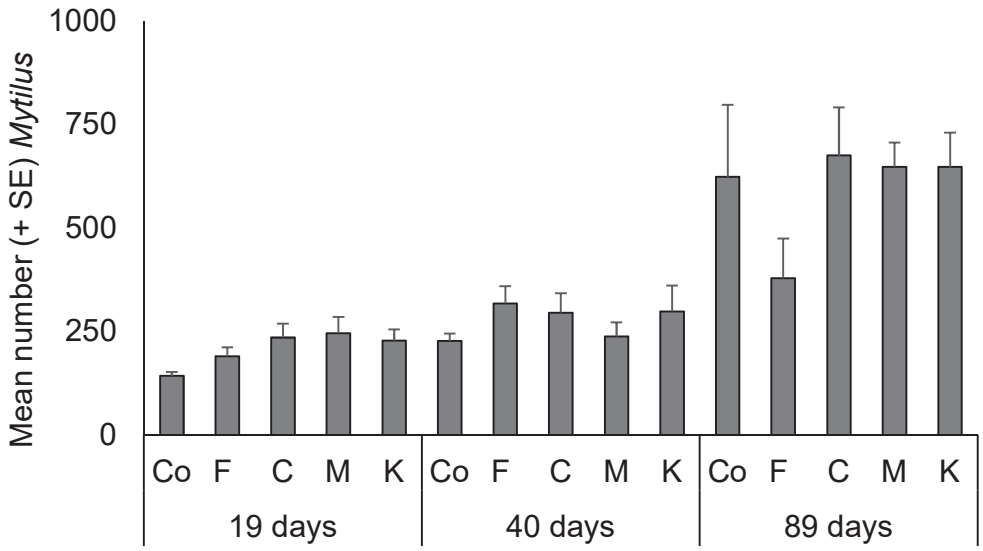

Figure 1. Mean (+ SE) number of *Mytilus galloprovincialis* among durations and substrate groups in Chapter 3. Co = control ropes with no seeded material or mussels, F = fine macroalgae, C = Coarse macroalgae, M = mixed macroalgae, and K = unmanipulated Kaitaia spat.