Advancing the nursery culture of juvenile green-lipped mussel, *Perna canaliculus*

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This thesis is dedicated to my beloved parents who passed away, Susanto and Endang Purwati who inspired me with their courage to pursue my dreams.

To my beloved husband and son, Amron and Aufa for their understanding and unconditional love.

Verily, with every hardship comes ease (Quran 94:6)

Abstract

The green-lipped mussel, *Perna canaliculus*, has become one of the most valuable seafood exports from New Zealand with current annual export earnings of US$308 million based on the production of around 200,000 t each year. However, the green-lipped mussel aquaculture industry in New Zealand relies heavily on juvenile mussels, or spat, harvested from the wild. Consequently, with the rapid growth of the industry, shortages of spat have become an increasingly major constraint for the industry. One of the solutions is to optimise the quantity and quality of juvenile mussels produced from a hatchery. However, the production of juvenile mussels in a hatchery is costly compared to harvesting wild mussel spat, with the production of microalgal food for hatchery spat contributing around 30 – 50 % of total hatchery production costs. There is still limited information on the feed requirements of juvenile mussels and the optimum environmental conditions required to obtain the best performance from spat in the hatchery situation. Therefore, the aim of the research presented in this thesis was to investigate the feeding abilities of juvenile *P. canaliculus* of a range of sizes when exposed to microalgal food particles of various sizes, and to observe some of the potential key environmental conditions that may affect the performance of the mussels in a nursery situation (i.e., growth, retention and survival). Flow cytometry (FCM) was used to count microalgae to determine the filtering abilities of juvenile mussels of a range of sizes when exposed to a variety of microalgae species of different sizes. Potential key environmental conditions (i.e., light exposure, water motion and oxygen levels) were experimentally manipulated and the subsequent performance of juvenile mussels was measured to determine the optimal culture conditions. The results showed that during a short observation period (1 h) the smallest size class of juvenile mussels used in this study (0.5 – 0.9 mm) was more efficient at filtering larger sized microalgae species (i.e., > 6 µm cell diameter) compared to microalgae species of smaller size. This suggests that the current commercial practice of feeding juvenile mussels with small microalgae species (i.e., 3 – 5 µm cell diameter) is highly inefficient. Over a longer observation period (24 h), when a range of sizes of *P. canaliculus* spat were provided with a high concentration (40 cells μl^{-1}) of four microalgae species with different cell sizes (i.e., *Diacronema lutheri*, *Tisochrysis lutea*, *Chaetoceros muelleri*, and *Tetraselmis suecica*), the particle capture ability was found to increase in proportion to the size of the juvenile mussels. The largest microalgae species *T. suecica* tended to be captured at the lowest rate compared to a smaller microalgae species *D. lutheri*. Using the longer observation period (i.e., 24 h) with FCM measures found that juvenile mussels in the size class of 0.5 – 0.9 mm in shell length were highly efficient at capturing all four of the tested microalgae species at ~$2.4 – 2.9 \times 10^5$ cells $mussel^{-1}$ 24 h^{-1}. Results from the experiments testing the effects of environmental conditions on the performance of juvenile

mussels under culture conditions indicated that a combination of light exposure (i.e., some period of light exposure within 24 h) produced better growth, while high water motion (i.e., > 33 ml s^{-1} of aeration or > 5 cm^3 s^{-1} water flow) and high oxygen levels (i.e., > 80 %) increased the growth, retention and survival of juvenile *P. canaliculus*. Overall, the results of the research presented in this thesis provide valuable information that has the potential to greatly increase the efficiency of commercial nursery culture of juvenile *P. canaliculus* in New Zealand.

Keywords: juvenile green-lipped mussel, Perna canaliculus*, particle capture, environmental conditions, flow cytometer*

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Contents

List of Figures

List of Tables

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Extent of contribution by PhD candidate (%)	90

CO-AUTHORS

Name	Nature of Contribution
Andrew Jeffs	Experimental design, Preparation of manuscript, problem solving

The undersigned hereby certify that:

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- the above statement correctly reflects the nature and extent of the PhD candidate's contribution to this work, and the nature of the contribution of each of the co-authors; and
- that the candidate wrote all or the majority of thetext.

Name	Signature	Date
Andrew Jeffs		12 May 2019

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Chapter 1. General Introduction

1.1. The biology of bivalve feeding

The class of Bivalvia includes about 7,500 species from around 50,000 species within the Phylum Mollusca. Bivalves includes those molluscs with two shell valves such as, mussel, oysters, scallops and clams (Gosling, 2004). The bivalves have the greatest diversity of evolutionary modification of all the molluscs. This includes marked changes in their morphology, such as for the food gathering organs, which developed from a mouth in ancestral molluscs into two sets of complex filter-feeding gills in mussels (Willmer, 1990). Indeed, filter feeding has become highly evolved among the bivalves, with species, such as mussels, possessing some of the largest and most highly efficient ciliary feeding systems among aquatic organisms. The evolution of the gill filaments has included lengthening and folding the gill filaments, while also increasing the number of filaments which extend as far forward as the labial palps (Gosling, 2004; Gui et al., 2016a).

There are four types of gill structures found in bivalves. Typical examples of these four gill structures are found in the following four species; the blue mussel, *Mytilus edulis* (homorhabdic filibranch), the giant scallop, *Placopecten magellanicus* (plicate heterorhabdic filibranch), the Atlantic surf clam, *Spisula solidissima*, (eulamelibranch) and the soft-shell clam, *Mya arenaria* (plicate pseudo-lamellibranch) (Gosling, 2004).

The cilia covering the gill filaments in bivalves play the primary role in generating water currents for filtering and subsequent transport of captured particles. The lateral cilia on the gill filaments generate the water flow through the mantle cavity and the gills. The water is filtered by the latero-frontal cilia which also direct the water flow through the inter-filament spaces of the gills (lamellibranch) and leading to the ostia (eulamellibranch). The latero-frontal cilia play an important role in capturing particles from the water and direct them to the frontal surface of the gill filaments (Gosling, 2004; Gui et al., 2016). The abfrontal surface cilia which face the exhalent water flow on the gills are thought to not participate in the water pumping activity, because they are associated with high mucocyte densities and are small in size and are at low density (Dufour & Beninger, 2001). Once the particles suspended in the water are captured they are passed to the frontal cilia, where they are trapped in a fine mucus layer and transported towards the ventral ciliated particle grooves (Beninger & St-Jean, 1997) and then passed to the labial palps for further sorting before the food particles are ingested. This mechanism of particle

processing is common in the vast majority of bivalve species which have homorhabdic filibranch and eulamellibranch gill structures (Ward et al., 1993). Whereas, in bivalves with heterorhabdic filibranch gills there is a different mechanism for processing food particles, since their gills do not possess a ventral food groove. Rather, the food particles are passed along the pallial cavity to the dorsal region of the gills and into the plical troughs. Each plical trough consists of a principal filament, which process the particles into a mucus slurry which is discharged via the dorsal ciliary tracts and the food particles are then transported to the labial palps for final sorting. Unwanted food particles are embedded in viscous mucus and ejected from the principal filaments and onto the plicae of the ordinary filaments through the ventral ciliated tracts. Hence, there are separate functions of the different types of gill filaments in heterorhabdic bivalves, with the principal filaments associated with filtering and the ordinary filaments responsible for clearing (Beninger et al., 1993; Beninger et al., 1992). The gill filaments play a different role for processing food particles in pseudo-lamellibranch bivalves. The particles are captured on the frontal surface of the ordinary filaments, then passed to the ventral grooves to be transported directly to the labial palps for final sorting (Ward et al., 1994). These gills can control their rate of filtering particles from the water by altering the angle of the ciliary beat or by expansion of the muscular control of the ostia.

In general, the filtering efficiency of bivalves is a complex mechanism that relates to the interplay of the ciliary system and the nature of the suspended particles being processed. The particle selection capabilities of bivalves that facilitate the maximal energy and nutrient uptake have been elucidated in several studies. Various characteristics of the food particles, such as particle size (Gui et al., 2016a), shape, motility (Beninger et al., 2008; Manahan et al.,1982; Ward et al., 1993), density (Raby et al., 1997), and chemical cues (Espinosa et al., 2010; Rosa et al., 2017; Rosa et al., 2013) have been shown to contribute to particle capture and retention. Most studies have found that filter feeding bivalves have a size selection which is targeted toward capturing microalgal particles. Consequently, most bivalves are able to retain particles of 3–4 μm diameter with an efficiency of 100%, while only around 50% particles of 1 μm diameter are retained (Cucci et al., 1985). Motile flagellates as small as 1–2 μm in diameter as well as bacteria as small as 0.3–1.0 μm in diameter can be removed from suspension by adult mussel species at around 86% efficiency (Wright et al., 1982). The brown mussel, *Perna perna*, and the ribbed mussel, *Guekensia demissa*, are able to capture particles as small as 0.4–0.6 μm with low efficiency. The ability for capturing small particles is related to the narrow space (~0.5 μm) between the latero-frontal cilia (Manahan et al., 1982) and the complexity of the latero-frontal tracts, which varies among bivalves species (Ward et al., 1998). While most bivalves target particles of around 5-7 μm in diameter, some species target particles of larger sizes. For example, the giant scallops is inefficient at capturing particles of 5-7 μm in diameter, compared

to relatively large particles of 10-350 µm in diameter (Shumway et al., 1987), while the yesso scallop, *Patinopecten yessoensis*, captures particles up to 950 µm in diameter (Mikulich & Tsikhon-Lukanina, 1981). The green-lipped mussel, *Perna canaliculus*, from juveniles to adults is highly efficient at retaining particles of~5 µm diameter (Hatton et al., 2005), while adults have been reported to be able to capture and ingest large zooplankton of up to 620 µm and cannibalize its own spat of up to 2.4 mm (Zeldis et al., 2004).

Bivalves are exposed to a wide range of suspended particulate matter that includes both non-nutritious and nutritious particles (Newell & Jorda, 1983). Some bivalves have been shown to preferentially select and ingest particles not only based upon size and shape but also nutrient content (Baker et al., 1998; Kiørboe & Møhlenberg, 1981; Newell & Jordan, 1983; Ward et al., 1998). Generally, bivalves tend to be able to capture and consume highly nutritious food particles, while the less than nutritious particles, if captured, are usually sorted out and rejected as pseudofaeces (Defossez & Hawkins, 1997; Riisgård et al., 2011). In addition, there is good evidence that some bivalves also sort particles for their suitability as food at the surface of the gill. For example, adult eastern oysters, *Crassostrea virginica*, select suspended particles based upon the specific chemical interaction between lectins in the mucus of pallial organs and carbohydrates present on the surfaces of cells and particles in suspension (Espinosa et al., 2010a, 2010b). Likewise, the gill surfaces in adults of the golden mussel, *Limnoperna fortunei*, are able to reject microalgae of the genus *Trachelomonas* which are characterised by a tough and indigestible coating, or lorica (Wołowski & Walne, 2014). In contrast, there is positive selection and high capture of microalgae species from the Class Chrysophyceae, which are more readily digestible and an important source of EPA (Lang et al., 2011). Mussels, such as the blue mussel, are also known to utilise particle selectivity on at the gill surface using a combination of surface chemical compounds and electrostatic charges on the particles (Espinosa et al., 2010a; 2010b; Rosa et al., 2017; 2013)

1.2. Transition of feeding from larvae to plantigers in bivalves

The veliger larvae of bivalves rely on a band of cilia along the margin of the velum for capturing food particles. In benthic bivalves, the veliger larvae subsequently settles, metamorphoses and undergoes a rapid transition to feeding using newly formed ctenidial filaments. In blue mussels, recently settled plantigers develop only a few short ctenidial filaments that are not yet connected with ciliary disc (Dral, 1967). However, these gill filaments have similar dimensions, spacing and ciliature of the gill filaments found in adult mussels. Two-day-old plantigrades of blue mussel retain the velum, which can still play an important role in swimming and feeding. The velum lies on one half of the anterior of the mantle cavity, which

lies side–by-side with the mouth and oral palp. The remnant velum retains a clear separation by a thick margin of long cilia on the inner band and short cilia on the peripheral band. These cilia work together for maintaining feeding in a manner consistent with the preceding veliger larvae. The beating of the longer cilia generates a feeding current which is directed toward the peripheral cilia. The periperal cilia beat towards the mouth facilitating the transfer of particles into the mouth (Bayne, 1971). At a shell length of 240-260 µm the first ctenidial filaments are formed in the pediveliger of the blue mussel in preparation for settlement and metamorphosis. The first three gills filaments are formed simultaneously from the ridge of presumptive gill tissue which subsequently become ciliated and grow to extend into the mantle cavity (Bayne, 1971; Cannuel et al., 2009). At this stage, the ctenidial filaments do not act as food collectors and move sporadically and in an uncoordinated manner. Late pediveliger larvae at ~390 µm of shell length, typically have two rows of gill filaments that each consist of 4 – 5 filaments. The gill filaments start to function once the velum disintegrates, and the labial palps are developed in the post-settled plantiger (Bayne, 1971).

In contrast to the blue mussel, in the green-lipped mussel the gill filaments develop immediately after settlement. The velum is the only feeding organ present in the pediveliger stage of *P. canaliculus,* (Cannuel et al., 2009; Gosling, 2003; Gui et al., 2016c) which occurs at around 30 days post-hatch in the wild, and has a prodissoconch length of around 247 µm (Gui et al., 2016c; Jeffs et al., 1999; Redfearn et al., 1986). After settlement, the pediveliger metamorphoses into a plantiger, which develops three to four tiny ctenidial filaments on each side of the mantle cavity. The ctenidia of plantigers of 260 µm in shell length have eulateral frontal cilia (ELF) that are 12 – 16 µm in length (Gui et al., 2016c) and play an important role in capturing suspended particles at the frontal surface of the ctenidial filaments (Bayne, 1971; Cannuel et al., 2009). The ctenidial filaments increase in number and length as the juvenile mussels grow in size and this is thought to greatly improve their particle capture efficiency (Gui, 2012). By the time the juvenile mussels reach 400 – 500 µm in shell length, the number of ctenidial filaments has increased to around six to seven and they begin to take on the characteristic form of the homorhabdic ctenidial structure with a well-developed ventral food groove, which is used for more efficiently conveying filtered particles toward the mouth. The detail of diagonally arranged parallel strips of inter-lamellae connections is formed in the ventral half of ctenidial lamellae by the time the juvenile mussels reach approximately 10 mm in shell length (Gui et al., 2016c). Initial research indicates that the overall capacity to capture suspended particles, as well as the filtering efficiency and size range of particles that can be captured, increases dramatically as the size and complexity of the ctenidial morphology develops in juvenile *P. canaliculus* of increasing size (Gui et al., 2016c; Sim-Smith et al., 2005). For example, *P. canaliculus* of 6 mm in shell length can capture microalgae particles of 5 µm in

diameter with nearly 100 % efficiency based on a theoretical particle capture threshold (PCT) value, whereas smaller-sized juvenile mussels have much lower efficiency of capturing particles of 5 µm in diameter due to their more rudimentary ctenidial structure (Gui, 2012).

1.3. Biology and ecology of *Perna canaliculus*

The green-lipped mussel, *Perna canaliculus*, is endemic to New Zealand waters and is distributed throughout the North and South Islands of the country. It grows up to 140 mm in shell length and can form dense aggregations, known as mussel beds, of up to 100 individuals m^{-2} (Hickman, 1991). The predominant habitat of *P. canaliculus* is from the mid-littoral intertidal down to a depth of over 50 m (Buchanan, 1994). It prefers to inhabit rocky reefs, soft muddy sand seafloor, and among algal holdfasts in the intertidal, as well as on wharf piles (Morton & Miller, 1973). This mussel species has a wide tolerance of water temperatures (Hickman, 1991). In the South Island, green-lipped mussels can survive at seawater temperatures as low as 5.3 $^{\circ}$C, whereas in the North Island they can survive at temperatures as high as 27 $^{\circ}$C. In addition, this species has optimum growth in a narrow range of water salinity of 30 – 35 psu, although in laboratory experiments *P. canaliculus* can survive in water with salinity at 25 psu (Flaws, 1975).

The green-lipped mussel is dioecious and has high fecundity, with adult female mussels spawning up to 100 million eggs (Rusk, 2012). The reproductive cycle of this species is highly influenced by temperature. They usually spawn in early autumn or late spring (Alfaro et al., 2011). In northern New Zealand, mussels spawn in June to December, whereas in southern populations in the Marlborough Sounds spawning occurs in early summer and again in autumn-spring (Alfaro et al., 2011; Buchanan, 1994; Flaws, 1975). After fertilisation, the larvae are planktonic for up to six weeks (Alfaro et al., 2001; Jeffs et al., 1999). The embryos develop into trochophore larvae then, after 24 – 48 h, they metamorphose into veliger larvae (Young, 1985). Larval mussels have limited swimming abilities, being able to swim vertically in the water column. Larval dispersal is thought to occur passively through transport by water currents (Buchanan, 1994). By the time the larvae reach around 220-350 µm of prodissoconch length, the pediveligers (larvae with foot 'pedi') will settle on benthic substrate and metamorphose to juveniles or plantigers, which are more commonly known as spat (Jeffs et al., 1999).

The plantigers initially attach to the substrate by producing an adhesive mucoid exudate and subsequently during settlement they anchor themselves by producing strong proteinaceous threads known as byssus threads (Ganesan, 2012). After settling, early juvenile mussels are capable of moving by detaching their byssus threads from their primary settlement location and either crawling along surfaces with their muscular foot or by exuding a long thread of buoyant

mucous which facilitates passive drifting of the mussel on water currents to a new potential location (Buchanan & Babcock, 1997; Buchanan, 1994; Lane et al., 1985). If a suitable location is found the juvenile mussels will attach to the substrate by anchoring with new byssus threads. The movement and attachment of juvenile mussels into new locations is known as secondary settlement behaviour and is thought to be a major contributor to the large losses of seed mussels in aquaculture operations (Alfaro & Jeffs, 2003; Buchanan, 1994). Secondary settlement behaviour in mussels appears to be lost once they reach around 6 mm in shell length, since at this size they are unable to use mucous thread drifting behaviour to change location (Alfaro et al., 2011). The early juveniles from settlement to around 6 mm in shell length appear to be particularly vulnerable to the availability of suitable suspended food particles (Gui, 2012). Losses of large quantities of juvenile mussels within this size range appear to often be associated with environmental conditions and nutritional limitations that are normal in aquaculture operations and represent a major weakness in the production cycle (Carton et al., 2007).

1.4. Green-lipped mussel aquaculture in New Zealand

The aquaculture production of green-lipped mussels is of economic importance in New Zealand. The aquaculture of green-lipped mussels started nearly 40 years ago following the overharvesting of wild mussel populations in the Hauraki Gulf, as well as in the Marlborough Sounds and Tasman Bay (Hickman, 1991). Sold under the tradename, Greenshell™, the production of this mussel by the aquaculture industry has grown rapidly. It is currently the single most valuable seafood export from New Zealand with more than US$308 million of exports in 2019 (New Zealand Sustainable Aquaculture, 2019). The industry uses the Japanese longline method for culturing this mussel, a system consisting of a continuous dropper rope to which mussels attach with byssus threads, and the ropes are deployed in a series of loops, each of 5 – 15 m long that are suspended beneath a backbone line kept afloat at the sea surface by large plastic floats (Jeffs et al., 1999).

The majority of the juvenile mussels or spat used by the New Zealand mussel aquaculture industry is currently collected from the wild, with only a tiny proportion (< 5 % of all spat supply) being produced from a commercial shellfish hatchery (Aquaculture New Zealand, 2020; Jeffs et al., 1999). Around 80 % of the mussel spat used by the industry comes from Ninety Mile Beach where it washes ashore in large numbers attached to drifting debris, which is mostly composed of seaweed, hydroids and plant material (Jeffs et al., 2018). The beach cast material with attached spat is harvested and transported to mussel farms all over New Zealand in temperature-controlled vehicles. Upon arrival, the harvested material is placed alongside a polypropylene dropper rope and held in place by covering with a cotton mesh stocking, known

as mussock (Hayden & Woods, 1997). Subsequently, the mussock and the beach cast debris decomposes, leaving the mussels to either attach to the remaining dropper rope with byssus threads or leave altogether. Once the mussels become established on the dropper rope by reaching a larger size (> 10 mm in shell length), the mussels are stripped from the line and reseeded to another dropper rope in order to lower their density and to provide more uniform growth rates (Buchanan & Babcock, 1997). The mussels are usually then on-grown for 12 - 18 months at which time they are harvested at a size of around 90 - 110 mm in shell length (Aquaculture New Zealand, 2018).

Relying on wild spat supplies to seed mussel farms is unreliable in terms of the uncertain arrival time, quality and quantity of the spat (Alfaro & Jeffs, 2003; Alfaro et al., 2010). Ongoing shortages in the supply of spat has been a constraint for the development of the Greenshell™ aquaculture industry for many years (Carton et al., 2007; Foote, 2003). More recently this situation has motivated the aquaculture industry to develop land-based hatcheries to produce green-lipped mussel spat (Capson & Guinote, 2014; Ragg et al., 2010). However, hatchery production of spat tends to be more expensive than for supplies of wild spat, as it requires the culture of large quantities of good quality microalgae to feed the larvae, and even larger quantities for feeding the juvenile mussels in nursery culture (Gui, 2012). The culture of microalgae to feed larvae and spat is a major cost of hatchery and nursery production, usually estimated to be around 30 – 50 % of the total costs of this production phase (Laxmilatha et al., 2011; Ragg et al., 2010).

The spat of green-lipped mussels (< 6 mm in SL) also have secondary settlement behaviour which allows them to readily depart from growing structures and move to other locations using mucous bysso-pelagic drifting (Ganesan, 2012). The secondary settlement behaviour of juvenile *P. canaliculus* contributes to a sizable loss in mussel spat in the New Zealand aquaculture industry with 50 – 90 % of spat typically departing from growing structures (Hayden, 1995; South, 2018). The secondary settlement behaviour of juvenile *P. canaliculus* appears to be lost by the time the mussel spat reach 6 mm in SL as they become too heavy to be buoyed in the water column by an extruded mucous thread (Buchanan & Babcock, 1997). There is also some evidence that mussels of this size lose the glands associated with the production of the mucous used for drifting, i.e., *P. canaliculus* at size 5 – 6 mm in SL (Buchanan & Babcock, 1997) and *M. edulis* at size 1 – 2.5 mm in SL (Lane et al., 1982). The cause of secondary settlement behaviour in green-lipped mussel spat is unclear, however, variations in environmental conditions (Carton et al., 2007; Eyster & Pechenik, 1988; Hayden & Woods, 2011) and starvation (Carton et al., 2007) have been shown to affect the retention in early stage of mussels. This situation creates a dilemma for a mussel hatchery because they can either deploy the spat

at a smaller size (< 2 mm in SL) to minimise production costs but face losing a large proportion from secondary settlement behaviour, or they can on-grow the mussel spat until they reach a larger size (> 3 – 6 mm in SL) to reduce the losses but incur significant additional production costs, especially due to a massive increase in the requirement for cultured microalgal food.

Studies have shown that when spat are raised in nursery culture conditions to a larger size, then they are much less likely to leave the farm structures (Buchanan & Babcock, 1997; Carton et al., 2007; Sim-Smith & Jeffs, 2011), and thereby can be deployed more efficiently. The current hatchery nursery practice for *P. canaliculus* involves feeding the larvae and spat with several microalgae species that are commonly used in commercial shellfish hatcheries for raising larvae (i.e., *Tisochrysis lutea*, *Diacronema lutheri*, and *Chaetoceros muelleri*) until the mussel reach < 1.2 mm in SL (Gui, 2012) (S. Cumming, SpatNZ Ltd, pers. comm.). These microalgae species all have relatively small cell sizes. The spat of green-lipped mussels with a shell length of < 6 mm have previously been found to have difficulties in filtering small particles (< 5 µm in cell diameter) (Gui et al., 2016b; Jacobs et al., 2015). Therefore, finding microalgal species of an appropriate size for juvenile *P. canaliculus* of a range of sizes could greatly enhance feed delivery in nursery culture. Despite the importance of nutritional condition to the retention and survival of green-lipped mussel spat in aquaculture, there is relatively little known about the feeding biology and optimum environmental conditions required by mussels at this stage in their lifecycle. This lack of knowledge of the feeding biology and environmental conditions is not confined to the green-lipped mussel, but is common to all cultured mussel species. Thus, understanding the feeding abilities of mussel spat and their preferred environmental conditions could be used to greatly improve their performance in nursery culture.

1.5. The importance of understanding feeding biology of juvenile mussels

Understanding the feeding biology and filtering capabilities of mussels are important for helping to improve the efficiency of aquaculture practices. Many studies have focused on identifying the fractions of suspended particles that are filtered by mussels, the amount they filter, and the filtering mechanisms they use (Gui et al., 2016c; Rajesh et al., 2001; Riisgård et al., 1980; Tang & Riisgård, 2016; Tantanasarit et al., 2013; Tremblay et al., 2007; Wong & Cheung, 1999). However, recent studies have been more concerned with improving the understanding of the feeding biology of juvenile mussels (Gui et al., 2016a; Gui et al., 2016c; Jacobs et al., 2015; Sim-Smith et al., 2005) because such an understanding can lead to improvements in aquaculture practices. For example, providing particulate feed that better matches the specific filter feeding requirements of mussel spat in nursery culture may ultimately

lead to reductions in the subsequent loss of these mussels from farms once seeded out, and ultimately result in higher aquaculture productivity.

Several feeding studies have been conducted on juvenile mussels of different species, such as the zebra mussel (*Dreissena polymorpha*) (Dionisio Pires et al., 2004), blue mussel (*Mytilus edulis*) (Jacobs et al., 2015), and the green-lipped mussel (*P. canaliculus*) (Gui et al., 2016a; Gui et al., 2016b; Sim-Smith et al., 2005) to develop a better understanding of their fundamental feeding biology, including defining their capabilities for particle capture and selection. However, the ability of early juvenile mussels of different sizes to capture suspended particles of various sizes and different microalgal taxa remains to be characterised and is an important step toward delivering effective feeding for juvenile mussels in the nursery situation.

1.6. The importance of environmental conditions for juvenile mussel performance

The effect of various environmental factors on juvenile mussel performance, such as growth, retention, and survival, have been studied for several mussel species. However, those environmental factors which promote successful recruitment in a wild habitat may often be absent in aquaculture systems. Several environmental conditions which may greatly influence the performance of juvenile mussels have been identified and include; water motion (Alfaro, 2005; Pernet et al., 2003), water velocity and aeration (Alfaro, 2006; Hayden & Woods, 2011) and light exposure (photoperiod) (Carl et al., 2011; Lurman et al., 2013). Of particular interest for addressing poor retention of spat seeded onto mussel farms is that variation in environmental conditions has been shown to modify the secondary settlement behaviour of juvenile mussels (Carton et al., 2007; Eyster & Pechenik, 1988; Hayden & Woods, 2011). High water motion can promote the production of more byssal threads used for attachment to the substratum in juvenile mussels, including *P. canaliculus* (Alfaro, 2006) and *M. edulis* (Eyster & Pechenik, 1988). This would help to explain why a high water velocity of 40 cm s^{-1} resulted in significantly increased retention as well as growth of juvenile green-lipped mussels when cultured in experimental tanks (Hayden & Woods, 2011).

One of the strategies for improving water motion and oxygen concentration in a nursery situation where juvenile mussels are raised under controlled conditions in tanks, is to manipulate water flow and aeration, which can also generate turbulent water flow in tanks. Aeration is also considered to be important for dispersing and maintaining the food particles in suspension in nursery tanks so that the particles remain available for filter feeding by juvenile mussels (Alfaro, 2006). For example, low levels of dissolved oxygen and low water flow in experimental nursery culture conditions have been shown to greatly influence the settlement and mortality rate of *P.*

canaliculus larvae (Alfaro, 2005), however, the relationship of these individual environmental factors to juvenile mussel growth has not been confirmed.

Another environmental factor which may influence juvenile mussel retention and growth is light exposure or photoperiod. Photoperiod has been shown to influence feeding behaviour in the blue mussel (Nielsen & Strömgren, 1985) and the great scallop, *Pecten maximus* (Duinker, 1996), and growth in the eastern oyster (Medcof & Kerswill, 1965). Blue mussels with a shell size of 14 – 22 mm that were cultured in continuous darkness in experimental tanks for 18 days had greater shell growth (9 – 25 % higher) than those cultured under natural daylight conditions (Nielsen & Strömgren, 1985). A study in adult *P. canaliculus* (± 18 months old) showed a wider valve gape of mussels at night consistent with active nocturnal feeding compared to periods of exposure to more intense light (Lurman et al., 2013). Other studies also suggest that light exposure may influence byssal thread production in mussels. For example, byssus attachment behaviour in a respirometry chamber housing adult *P. canaliculus* was minimal during daylight hours, while the majority of byssal threads were produced during the night (Lurman et al., 2013). This behaviour was thought to be due to the mussels avoiding extending the foot during daylight and thereby making it vulnerable to attack from predators.

Determining the role of key environmental conditions in influencing the growth, survival and retention of juvenile mussels has the potential to greatly improve juvenile mussel performance in nursery aquaculture systems and to also improve their subsequent performance once seeded onto farms. Given this potential, there is value in assessing this opportunity to optimise the performance of nursery culture systems.

1.7. Aims of study

The overall aim of this thesis is to better understand the feeding biology of juvenile *P. canaliculus* and to observe some of the potential key environmental conditions that may enhance the mussel performance in a nursery culture situation prior to seeding out to coastal mussel farms. Information on the feeding ability of juvenile green-lipped mussel and the environmental conditions that can promote their performance are priorities for optimising green lipped mussel nursery production.

1.8. Thesis structure

Chapter One – *General Introduction.*

This chapter provides a general introduction to the current study by introducing the study organism, feeding biology in bivalves generally and outlining

previous studies on juvenile mussel feeding behaviour and identifying the specific research aims for the thesis.

Chapter Two – *Ability of juvenile green-lipped mussel* Perna canaliculus *to filter feed on microalgae of different sizes.*

The research presented in this chapter tests the hypothesis that the filtering ability of early juvenile *P. canaliculus* of a range of sizes does not change when exposed to suspensions of nine species of microalga of various sizes and shapes. Flow cytometry (FCM) techniques are used to determine the particle capture abilities of the early juvenile mussels by measuring changes in the concentration of microalgal cells over time and relative to a control solution of microalgae.

Chapter Three – *Continuous particle capture of juvenile green-lipped mussel (*Perna canaliculus*).*

The research presented in this chapter tests the hypothesis that the particle capture of juvenile mussels of a range of sizes is consistent over a 24-hour period when provided with suspensions of four species of microalgae. In testing this hypothesis, the research determines the continuous maximum capture and consumption of four species of microalgae by seed mussels when held under nursery conditions in the laboratory. Flow cytometry (FCM) techniques are again used to determine the particle capture abilities of the early juvenile mussels by measuring changes in the concentration of microalgal cells over time and relative to a control solution of microalgae.

Chapter Four – *Effect of different environmental conditions on growth, survival and retention of green-lipped mussel spat,* Perna canaliculus.

The research presented in this chapter tests the hypothesis that that growth, survival and retention of juvenile *P. canaliculus* is unaffected by differences in the environmental conditions (i.e., photoperiod, aeration, water flow, and oxygen) on the growth, survival and retention of juvenile mussels held under nursery conditions in the laboratory. This research was conducted as four separate experiments using an experimental mussel spat culture unit (conical tanks), with each experiment testing one of the four environmental factors (i.e., photoperiod, aeration, water flow, and oxygen concentration) with similar methods for each experiment.

Chapter Five – *General Discussion.*

The general discussion provides an integrated discussion of the findings from each of the research chapters and draws conclusions from these combined results especially in relation to how they could be used to improve the nursery production of juvenile mussels. Areas of potential future research arising from the current study are also identified.

The thesis has been constructed with a view to publishing the three central research chapters, and as a consequence there is a degree of repetition in introductory sections that could not be avoided by adopting this approach to the thesis structure.

Chapter 2. Ability of juvenile green-lipped mussels *Perna canaliculus* to filter feed on microalgae of different sizes

2.1. Introduction

A major constraint for the production of bivalves in aquaculture is providing suitable diets to maintain the rapid growth of early juveniles during the nursery phase (Carton et al., 2007; Foote, 2003). Following metamorphosis from larvae, the early juveniles usually have rudimentary filtering apparatus that develops rapidly as they grow in size (Cannuel et al., 2009; Gui, 2012). Associated with this development is a dramatic advance in their filtering abilities, especially their efficiency and ability to selectively capture suspended particles based on size, shape, surface properties and biochemical composition (Cognie et al., 2003; Gui et al., 2016c; Mafra et al., 2009). An improved understanding of these ontogenetic changes in filtering capabilities can greatly assist in the efficiency of feeding regimes during the nursery production of juvenile bivalves in aquaculture.

Mussels of various species and of various sizes have been shown to be able to select food particles while filter feeding on the basis of characteristics such as size, particle shape, morphology, density, motility, toxicity of the microalgae (Martínez-Pita, Sánchez-Lazo, & García, 2016; Raby et al., 1997; Viherluoto & Viitasalo, 2001; Von Elert & Stampfl, 2000), nutritional value, and cell concentration of microalgae (Galley et al., 2010; Mamat & Alfaro, 2014; Martínez-Pita et al., 2016). Different species of microalgae vary greatly in their nutritional composition, size, and shape, so that the quantification of the number of cells consumed by bivalves does not adequately represent their overall nutrient intake (Chitari & Anil, 2017) as a large number of small microalgae can be equivalent to small number of larger microalgae cells in terms of carbon biomass (Harrison et al., 2015; Safi & Hayden, 2010). Therefore, providing information on the total cell volume of microalgal particles that are captured by filtering bivalve can help to determine the total amount of microalgal cell biomass consumed.

For the green-lipped mussel, *Perna canaliculus*, the nursery production phase is particularly challenging because of a rapid increase in the demand for live microalgal feeds with the growing size of juveniles. Currently, strains of axenically cultured microalgae used for larval rearing of

this species (i.e., *Tisochrysis lutea*, *Chaetoceros muelleri*, *Diacronema lutheri*) are also used for feeding juveniles, requiring massive quantities of microalgal culture to maintain the growth of the juvenile mussels, which is commercially unviable (S. Cumming, SpatNZ Ltd, pers. comm.). However, raising the juvenile green-lipped mussels to a larger size and to a high nutritional condition before seeding onto long-lines in coastal waters is thought to greatly increase their subsequent survival and retention on the nursery lines (Carton et al., 2007; Foote, 2003). Previous studies have indicated that juvenile green-lipped mussels appear to have an ability to filter feed on particles of a wide range of sizes, providing a potential opportunity to deliver a feed regime that is used more efficiently by the mussels (Sim-Smith et al., 2005). One of the methods used to determine the selection of suspended particles by filter feeding bivalves is flow cytometry (Cucci et al., 1985; Shumway et al., 1985). Flow cytometry (FCM) examination of the filtering abilities of juvenile mussels of 1 - 6 mm in shell length (SL) found that the mussels were be capable of filtering axenic cultures of microalgal species over a wide range of sizes (3 - 30 µm cell diameter) with the filtering efficiency increasing rapidly as the size of the juvenile mussels increased (Sim-Smith et al., 2005). However, the filtration rates of the juvenile mussels measured with the FCM were inconsistent with the feeding rates commonly used in commercial juvenile production, suggesting experimental bias, possibly due to a proportion of the experimental mussels not actively filtering during FCM measurements (Sim-Smith et al., 2005; Jacobs et al., 2015).

Therefore, the research presented in this chapter tests the hypothesis that the filtering ability of early juvenile *P. canaliculus* of a range of sizes does not change when exposed to separate suspensions of nine species of microalga of various sizes and shapes. Experimental testing of the hypothesis used a combination of visual observations of the filtering activity of juvenile mussels and FCM measures of changes in the abundance of suspended microalgae cells. The results of this study may assist in identifying a suitable range of sizes for suspended particles to maximise the feed delivery to juvenile *P. canaliculus* in a commercial nursery culture setting.

2.2. Materials and methods

2.2.1. Juvenile mussel preparation

Hatchery-raised plantigers of *Perna canaliculus* (0.3 – 0.8 mm shell length - SL) were supplied by Shellfish Production and Technology New Zealand Ltd (SpatNZ Ltd) located in Nelson. Larger sizes of juvenile mussels (2 – 8 mm of SL) were collected from their natural habitat at Muriwai Beach in north-western New Zealand (36º 83.871' S, 174º 42.649' E) on 10 March 2017. All of the mussels were transported to the laboratory where they were separated from the material they were attached to and placed in a container filled with 1.5 l of filtered (0.2

µm) and UV treated seawater (FSW) held at 18 °C. The juvenile mussels were fed with axenically cultured microalgae (*Tisochrysis lutea*) at approximately 2×10^5 cells mussel^{-1} day^{-1} for three days while they acclimatised to the holding conditions. All the juveniles of *P. canaliculus* were starved for 24 h prior to commencing the experiment.

2.2.2. Juvenile mussel size classes

Juvenile mussels were sorted by size into five size classes using a series of graduated sieves (i.e., M_1, M_2, M_3, M_4 and M_5) and their shell length (SL) was measured using a stereo microscope (Meiji Techno, EMZ-13TR) with a graduated reticule. Mussels in size class M_1 were post-settlement plantigers sourced from the hatchery with a mean SL of 0.65 ± 0.01 mm (±SE, n = 90); M_2 were post-settlement juveniles sourced from the hatchery with a mean SL of 1.21 ± 0.02 mm (±SE, n = 90); M_3 were early juveniles sourced from Muriwai Beach with a mean SL of 2.41 ± 0.03 mm (±SE, n= 90); M_4 were juvenile mussels with a mean SL of 4.39 ± 0.04 mm (±SE, n=90); while M_5 were larger juvenile mussels sourced from Muriwai Beach with a mean SL of 6.34 ± 0.03 mm (±SE, n=90).

2.2.3. Preparation of phytoplankton culture

Nine species of single-celled microalgae that represented a wide range different cell sizes and that were available as axenic cultures were accessed and transferred to the seawater laboratory at the University of Auckland where they were cultured axenically to the exponential phase of growth for use in experimental feeding to juvenile mussels of a range of sizes (Table 2.1 and Fig. 2.1). The cell dimensions of the nine axenic microalgae species were measured with a fluorescence microscope (Leica DM2500) by examining at least 15 randomly selected cells and measuring their diameter (a perpendicular diameter of the cells was also measured where the cells were not spherical), cell height and cell length. A mean for each measure was calculated from the replicate measurements. Aliquots of axenic cultured microalgae were diluted with filtered and sterilized seawater to a concentration of $5.7 – 6.2 \times 10^3$ cells ml^{-1} ready to be used for filtering experiments. This microalgae concentration was selected based on pilot experiments that assessed the accuracy of the FCM counting for a range of concentrations of microalgae supplied to juvenile mussels for 60 min. A 2 ml aliquot of this solution was then measured with a micropipette and used to fill the 3 ml wells of a BD Falcon 24 well plate. The well plate was used as a convenient platform for observing replicate mussels under a binocular microscope to closely observe their feeding behaviour.

Table 2.1. Nine species of axenically cultured microalgae and their corresponding cell size as used in the study. d = diameter (a perpendicular diameter of the cell also measured where cells were not spherical), h = cell height and l = cell length.

Species	Taxa	Size (µm)
Nannochloropsis oceanica	Eustigmatophyceae	2 – 3
Nannochloropsis atomus	Chlorophyceae	3 – 6
Tisochrysis lutea	Prymnesiophyceae	3 – 8
Diacronema lutheri	Pavlovophyceae	4 – 6
Chaetoceros muelleri	Mediophyceae	7 (d) × 9 (h)
Rhodomonas salina	Cryptophyceae	6 (d) × 12 (h)
Tetraselmis suecica	Chlorophyceae	9 – 10 (d) × 12 – 14 (h)
Karenia papilionacea	Dinophyceae	28 (l) × 25 (d_1) × 19 (d_2)
Akashiwo sanguinea	Dinophyceae	80 (l) × 40 (d_1) × 26.8 (d_2)

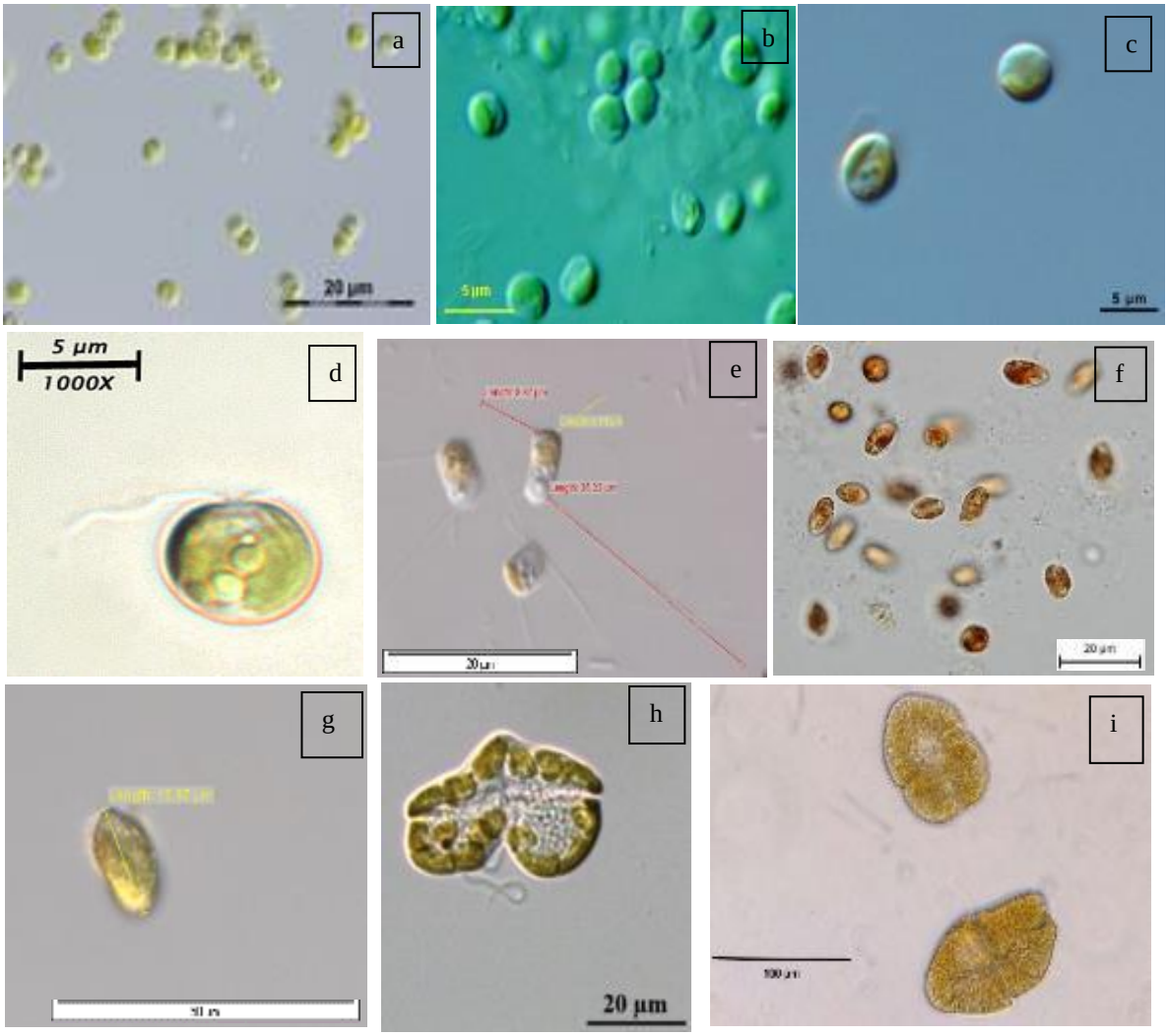

Figure 2.1. Microalgae species used in the study for determining filtration rates in juvenile green-lipped mussels; (a) *Nannochloropsis oceanica* (Baker, 2012), (b) *Nannochloropsis atomus* (Baker, 2012), (c) *Tisochrysis lutea* (Baker, 2012), (d) *Diacronema lutheri* (Baker, 2012), (e) C*haetoceros muelleri*, School of Biological Science, Sanjayasari. 12 September 2017, (f) *Rhodomonas salina* (Hill & Wetherbee, 1989), (g) *Testraselmis suecica*, School of Biological Sciences, Sanjayasari, 12 September 2017, (h) *Karenia papilionacea* (Fowler et al., 2015), (i) *Akashiwo sanguinea* (Munir et al., 2015).

2.2.4. Laboratory Experiment

Observations using video recordings from the camera of a stereo microscope during pilot experiments showed that juvenile green-lipped mussels filter intermittently at times. The pilot experiments consisted of placing individual juvenile mussels in the well of a BD Falcon 24 well plate that was filled with filtered seawater containing various concentrations of axenically cultured microalgae. Movement of the suspended microalgae in relation to the mussel could be observed with 40 - 100× magnification under the microscope. Typically after placing the juvenile mussel in the well plate with the microalgae suspension it would begin to open its valves

and the filtering activity would commence, which was evident from the movement of microalgae particles generated by the feeding current of the mussel. The presence of intermittent feeding in some juveniles during the pilot study, indicated that to reliably measure filtering rates it is necessary to concurrently observe their feeding behaviour to determine the period for which they were actively filter feeding, so that measurements of the reduction in suspended particles (microalgae) removed by filtering activity can be standardised by the duration of active filtering. Therefore, to undertake this experiment individual juvenile mussels of a known size were placed in a 2 ml well of a BD Falcon 24 well plate containing 2 ml of microalgal solution. For each well containing an experimental mussel, two control wells were also run, containing 2 ml of microalgal solution but no mussel. The filter feeding activity of each juvenile mussel was recorded for 60 min using a digital video (Samsung Galaxy A5) connected to a binocular microscope (Leica Wild M3C) and under continuous lighting (microscope light). Recorded video was subsequently replayed and the total time within the 60 min experimental period that the juvenile mussels spent actively feeding, as determined by the presence of opened valves and feeding currents (Fig. 2.2), were measured using a stopwatch.

2.2.4.1. Particle selection and counting

A flow cytometer (FCM) (BD Accuri C6) was used to enumerate suspended microalgal cells at t_0 (0 minute) immediately prior to the juvenile mussels being placed in the wells and for the control wells (without mussels), and then again after a 1 h period during which the juvenile mussels were intermittently filter feeding t_1 (60 min). FCM measurements were taken by sampling 260 µl of seawater from each well using a micropipette and transferred directly to the FCM for processing using a fast reading setting of 66 µl s^{-1}.

2.2.4.2. Rates of microalgal capture

The rate of capture of the microalgal cells by the juvenile *P. canaliculus* was measured by using the difference in suspended particles between t_0 and t_1, adjusted for any changes in particle numbers measured in the two controls without a mussel present, and by the amount of time during the 60 min experimental period the juvenile mussel was observed to be actively filtering. Hence, particle capture adjustment could be estimated as follows:

$$\text{Particle capture rate (cells min}^{-1}) = \frac{(t_0 - t_1)\ \text{treatment} - (t_0 - t_1)\ \text{control (cell)}}{\sum \text{Duration of active filtering (min)}}$$

2.2.4.3. Microalgae volume intake

The cell volume of each microalgae species was calculated by using appropriate volume equations from the literature that were based on the shape of the cells of the microalgae (e.g.,

spherical, elliptical, etc) (Table 2.2). The total cell volume intake for each microalgae species was calculated by multiplying the total number of microalgal cells captured by a juvenile mussel as measured by flow cytometry with the estimated volume of the individual cells for each microalgae species.

Table 2.2. The shape of microalgae species and their volume equation V (volume), r (radius), l (length), d (diameter), h (height).

Microalgae	Shape	Volume equation	Estimated cell volume (μm^3)	Source
Nannochloropsis oceanica	spherical	$4/3 \times 3.14 \times r^3$	8.12	(Hillebrand et al., 1999)
Nannochloropsis atomus	spherical	$4/3 \times 3.14 \times r^3$	47.69	(Hillebrand et al., 1999)
Tisochrysis lutea	sub-spherical	$4/3 \times 3.14 \times r^3$	87.07	(Hillebrand et al., 1999)
Diacronema lutheri	sub-spherical	$4/3 \times 3.14 \times r^3$	65.42	(Hillebrand et al., 1999)
Chaetoceros muelleri	oval cylinder	$(3.14/4) \times d_1 \times d_2 \times (h)$	296.73	(Olenina et al., 2006)
Rhodomonas salina	cone+half sphere	$(3.14/12) \times d^2 \times (h+d/2)$	141.3	(Olenina et al., 2006)
Tetraselmis suecica	spherical	$4/3 \times 3.14 \times r^3$	440.245	(Olenina et al., 2006)
Karenia papilionacea	flattened ellipsoid	$(3.14/6) \times l \times d_1 \times d_2$	6960.33	(Munir et al., 2015)
Akashiwo sanguinea	flattened ellipsoid	$(3.14/6) \times l\ d_1 \times d_2$	44881.07	(Munir et al., 2015)

2.2.4.4. Biomass of *Perna canaliculus*

To determine how the filter feeding of juvenile mussels changed in relation to their actual body size (i.e., tissue only), the dry biomass of tissues (BM) of juvenile mussels covering the range of sizes used in the study were determined. Ninety mussels within each of the five size classes (M_1 up to M_5, i.e., $n = 450$) that had been used in the feeding experiment were carefully rinsed with fresh water to remove salt, and then dried carefully with a paper towel. Each mussel was then dissected under a dissecting microscope (Leica Wild M3C) to separate the body tissues from the attached shell. The tissues were carefully dabbed with Whatman filter paper for approximately 5 min to remove water on the outside. The wet mass of the tissue was then measured on a microbalance (Mettler Toledo, Global Science). The tissue was then placed in a pre-weighed Eppendorf tube and freeze dried for 24 h and re-weighed to determine the dry mass. The shell length of each mussel was measured prior to the feeding experiment under a dissecting microscope (section 2.2.2).

2.2.5. Statistical analyses

A two-way analysis of variance (ANOVA) was used to compare the mean percent of the 60 min period that mussels spent actively filtering for the five mussel size classes and nine microalgae species. The percentage data were firstly transformed using an arcsine transformation and then assessed for both normality using a Shapiro–Wilk's test, and for homogeneity of variances using a Levene's test. Significant overall differences among means detected by the ANOVA were followed by pairwise Tukey HSD post-hoc tests to identify the differences between pairs of means, while also using a Bonferroni correction for inflated familywise error due to multiple testing of the same data set.

The mean particle capture rates and the mean cell volume capture rates of juvenile mussels of the five size classes and for the nine species of microalgae were both compared using a two-way ANOVA. The mean cell volume capture rate of juvenile mussel was log transformed prior to the analyses to ensure data conformed to the underlying parametric assumptions. Normality of the data and homogeneity of variances were confirmed a priori. Significant differences among means detected by the ANOVA were followed by Tukey HSD post-hoc tests to identify the differences between pairs of means with correction for error inflation.

Juvenile mussel shell length and body mass data were plotted as a relationship curve, and in the same way, the relationship between body mass of a range of sizes of juvenile mussels, their shell length and particle capture rate for nine species of microalgae were plotted and then curves of best fit determined in Sigma Plot version 14.0.

2.3. Results

2.3.1. Juvenile mussel actively filtering

Most of the experimental mussels opened their valves and began feeding almost immediately upon placement in the microalgal solution (Fig. 2.2). Most juvenile mussels subsequently spent the full 60 min experimental period actively feeding. The shortest total period that any individual mussel fed was for 33 min out of the possible 60 min and this was for one mussel from M_3 size class when fed the largest species of microalgae (*A. sanguinea*). Overall, the juvenile green-lipped mussel started to filter the microalgae as soon as they were placed in the well plate regardless of microalgae species. Hesitant filtering activity was shown by a small number of the juvenile mussels in M_3 when introduced to the largest species of microalgae (*A. sanguinea*). The mussels would hesitate feeding by stopping their active filtering, withdrawing their siphons and closing their valves, and then reversing the process to recommence filtering shortly afterwards. All microalgae species, including the largest species

of microalgae (*A. sanguinea*), were captured and ingested by all sizes of juvenile mussels as could be observed by the accumulation of the microalgae in the gut of the mussel, which was visible through the opaque shells of mussels in M_1 and M_2. It was also confirmed through the dissection of the fed juvenile mussels in M_3, M_4 and M_5 at the end of the experimental period (60 min). There were no pseudofaeces observed during any of the experiments. The time to the first appearance of faeces varied among all mussel size classes.

The largest size class of juvenile mussel category (M_5) was the fastest to produce faeces at~ 10 - 15 min after commencing feeding on microalgae. In contrast, juvenile mussels in M_1 began to produce faeces towards the end of the experimental period. These observations suggest marked differences in digestive capacity and processing times between different stages of development of juvenile mussels.

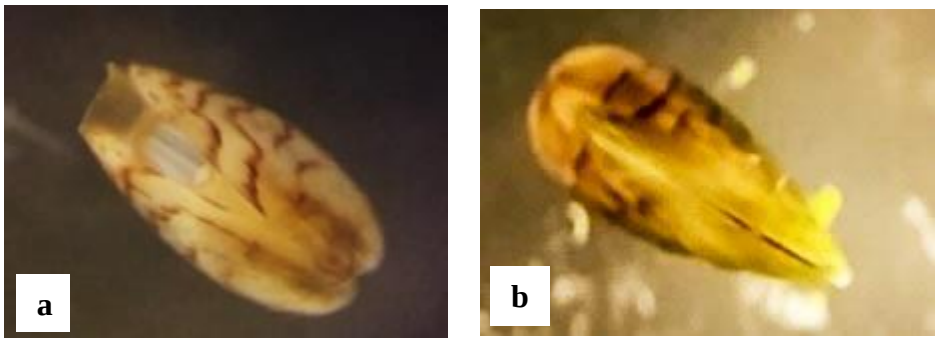

Figure 2.2. a) Juvenile mussel of M_3 size class actively filtering *Chaetoceros muelleri* showing valves widely separated and mantle extension, b) Juvenile mussel of M_3 size class not actively filtering when in a suspension of *Akashiwo sanguinea* as indicated by valves barely separated for juvenile mussel M_3 = size (2.0 – 3.0 mm).

The overall time spent filtering by the juvenile mussels of different size classes provided with nine different species of microalgae was significantly different ($F_{(8, 405)} = 12.93$, $P < 0.0001$). However, there was no significant interaction between mussel size class and the species of microalgae that was provided in terms of the time that mussels spent actively filtering ($F_{(32, 405)} = 1.13$, $P = 0.29$). Also, there was no difference in the mean amount of time mussels spent actively filtering among mussels of the five different size classes regardless of microalgae species ($F_{(4, 405)} = 0.67$, $P = 0.62$). Post-hoc analyses showed that mussels supplied with *A. sanguinea* had a significant reduction in filtering activity compared to all other microalgae species tested. The mean amount of time juvenile mussels spent actively filtering when exposed to *A. sanguinea* over the course of the experiment was 95.46 ± 0.02 (S.E.) % (i.e., 57.28 min out of 60 min period) (Tukey HSD, $P < 0.05$) (Fig. 2.3). Juvenile mussels provided with *A. sanguinea* were observed to often be slower to commence feeding and had more intermittent

filtering activity compared to juvenile mussels provided with other microalgal species in this study.

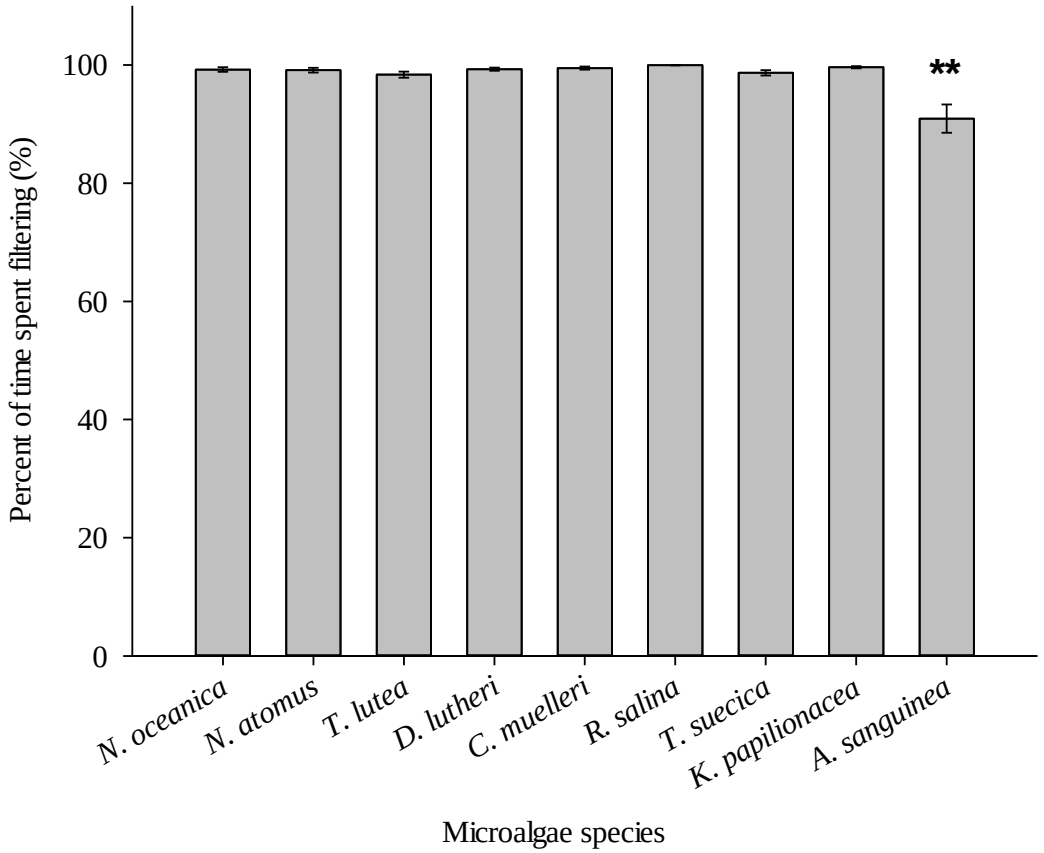

Figure 2.3. Mean percent of 60 min period of observation that juvenile mussels were actively filtering (± SE, n = 10) for five classes of juvenile mussel *Perna canaliculus* supplied with axenic cultures of nine microalgae species. Differences among the means for different microalgae species are marked with an asterisk (Tukey HSD, $P < 0.05$).

2.3.2. Particle capture ability

The overall ability of juvenile mussels to filter microalgae particles increased markedly with mussel size class ($F_{(4, 405)} = 819.69$, $P < 0.0001$) and varied significantly among microalgae species ($F_{(8, 405)} = 48.20$, $P < 0.0001$), however, the ability to filter different microalgal species was not consistent among mussel size classes for the different microalgae species (Size class*Microalgae species – $F_{(32, 405)} = 8.29$, $P < 0.0001$) (Fig. 2.4, Table 2.3).

Among mussels in the M_1 size class the highest mean particle capture rate was when they were provided with *R. salina* (25.40 cells mussel^{-1} min^{-1} ± 3.32) (Tukey HSD, $P < 0.05$), but this was not different to the rates for *T. suecica* or *C. muelleri*, but was higher than for the remaining six microalgae species (Tukey HSD, $P < 0.05$) (Fig. 2.4, Table. 2.3). The only other significant

differences in particle capture rates for M_1 mussels were for *T. suecica*, which was higher than for *A. sanguinea*, *K. papilionacea* and *D. lutheri* (Tukey HSD, $P < 0.05$).

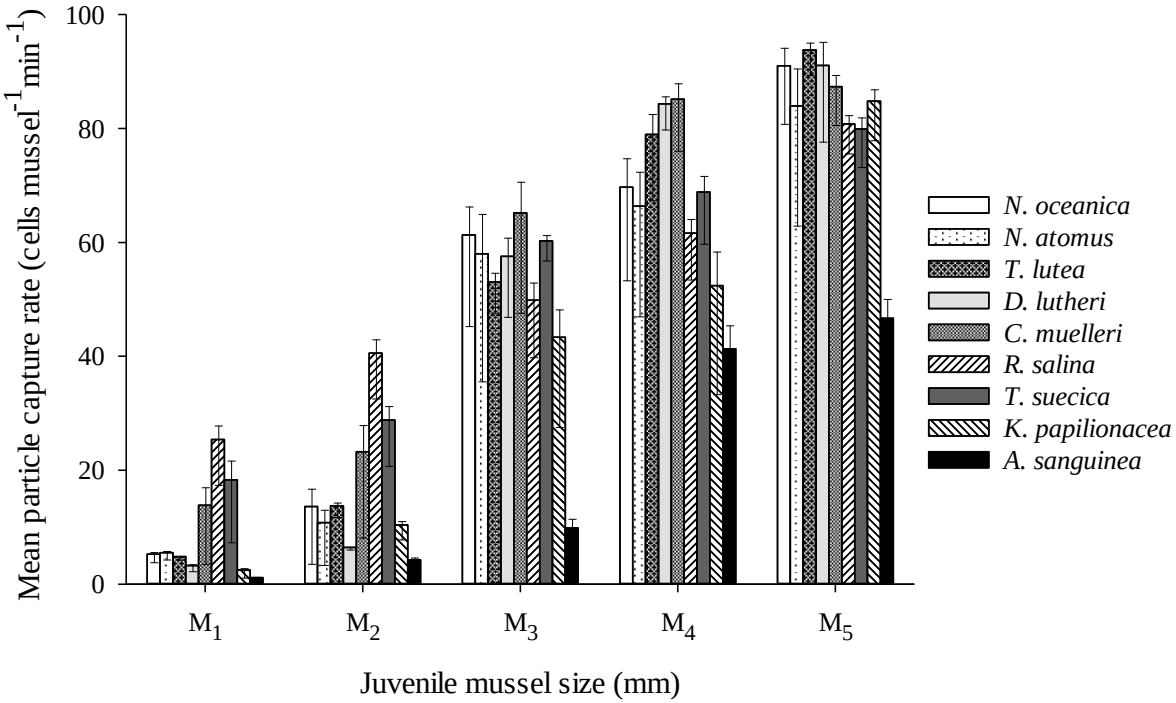

Figure 2.4. Mean particle capture rate for five size classes of juvenile mussels (*Perna canaliculus*) filtering axenic cultures of nine different species of microalgae of different sizes and supplied at the same cell density. Juvenile mussel shell length size classes; M_1 = 0.5 – 0.9 mm, M_2 = 1.0 – 1.9 mm, M_3 = 2.0 – 3.0 mm, M_4 = 4.0 – 5.0 mm and M_5 = 6.0 – 7.0 mm.

Among the mussels in the M_2 size class the highest mean particle capture rate was when they were provided with *R. salina* (40.54 cells mussel^{-1} min^{-1} ± 3.32) (Tukey HSD, $P < 0.05$), but this was not different to the rate for *T. suecica*, but was higher than for the remaining seven microalgae species (Tukey HSD, $P < 0.05$) (Fig. 2.4). The only other significant differences in mean particle capture rates for M_2 mussels was for *T. suecica*, which was higher than for *A. sanguinea*, *K. papilionacea* and *D. lutheri* (Tukey HSD, $P < 0.05$), but not for *C. muelleri*.

Among the mussels in the M_3 size class the lowest mean particle capture rate was when they were provided with *A. sanguinea* (9.85 cells mussel^{-1} min^{-1} ± 3.32) which was significantly lower than for all other microalgae species (Tukey HSD, $P < 0.05$) (Fig. 2.4). The mean particle capture rate for M_3 mussels when filtering *K. papilionacea* was significantly lower than for *N. atomis*, *T. suecica*, *N. oceanica*, and *C. muelleri* (Tukey HSD, $P < 0.05$). The mean particle capture rate for *C. muelleri* was also higher than for *R. salina* (Tukey HSD, $P < 0.05$).

Table 2.3. The mean particle capture rate (cells mussel^{-1} min^{-1}) of juvenile mussels in five size classes when placed in solutions of nine species of axenically cultured microalgae at the same cell concentration. Means without letters in common within each column are significantly different at $P < 0.05$ – Tukey HSD. M_1 = size (0.5 – 0.9 mm), M_2 = size (1.0 – 1.9 mm), M_3 = size (2.0 – 3.0 mm), M_4 = size (4.0 – 5.0 mm), and M_5 = size (6.0 – 7.0 mm).

Microalgae species	Juvenile mussel size class				
	M_1	M_2	M_3	M_4	M_5
N. oceanica	5.27 ± 0.45^{a}	13.63 ± 3.34^{a}	61.32 ± 5.32^{b}	69.69 ± 5.43^{ab}	91.10 ± 4.44^{b}
N. atomus	5.52 ± 0.38^{a}	10.78 ± 5.35^{a}	58.03 ± 7.51^{b}	66.38 ± 5.49^{ab}	83.96 ± 6.98^{b}
T. lutea	4.84 ± 0.16^{a}	13.77 ± 0.66^{a}	53.05 ± 1.77^{ab}	78.95 ± 3.84^{b}	93.77 ± 0.31^{b}
D. lutheri	3.26 ± 0.31^{a}	6.45 ± 0.10^{a}	57.57 ± 3.52^{ab}	84.32 ± 1.47^{b}	91.07 ± 4.44^{b}
C. muelleri	13.85 ± 3.40^{ab}	23.25 ± 5.01^{ab}	65.20 ± 5.82^{b}	85.15 ± 3.00^{b}	87.34 ± 2.22^{b}
R. salina	25.40 ± 2.64^{b}	40.54 ± 2.63^{b}	49.87 ± 3.33^{ab}	61.62 ± 2.69^{ab}	80.78 ± 1.70^{b}
T.s suecica	18.29 ± 3.63^{ab}	28.80 ± 2.66^{ab}	60.27 ± 1.13^{b}	68.86 ± 3.02^{ab}	79.91 ± 2.21^{b}
K. papilionacea	2.47 ± 0.43^{a}	10.37 ± 0.81^{a}	43.33 ± 5.23^{ab}	52.44 ± 6.34^{a}	84.81 ± 2.27^{b}
A. sanguinea	1.14 ± 0.19^{a}	4.26 ± 0.49^{a}	9.85 ± 1.76^{a}	41.30 ± 4.44^{a}	46.71 ± 3.63^{a}

Among the mussels in the M_4 size class the lowest mean particle capture rate was when they were provided with *A. sanguinea* (41.30 cells mussel^{-1} min^{-1} ± 3.32) which was significantly lower than for all other microalgae species (Tukey HSD, $P < 0.05$) (Fig. 2.4). The mean particle capture rate for M_4 mussels when filtering *K. papilionacea* was significantly lower than for *C. muelleri*, *D. lutheri*, *T. lutea*, and *N. oceanica* (Tukey HSD, $P < 0.05$). Likewise, the filtration rate on *R. salina* was lower than for *T. lutea*, *D. lutheri*, and *C. muelleri* (Tukey HSD, $P < 0.05$). The mean particle capture rate for *N. atomis*, *T. suecica* and *N. oceanica* were all lower for both *C. muelleri* and *D. lutheri* (Tukey HSD, $P < 0.05$).

Among the mussels in the M_5 size class the lowest mean particle capture rate was when they were provided with *A. sanguinea* (46.71 cells mussel^{-1} min^{-1} ± 3.32) which was significantly lower than for all other microalgae species (Tukey HSD, $P <0.05$) (Fig. 2.4). There were no other significant differences among the mean particle capture rates of the remaining eight microalgae species.

2.3.3. Microalgae volume intake

The rate of cell volume of microalgae that was filtered by juvenile mussels increased markedly with mussel size class ($F_{(4, 405)} = 1011.37$, $P < 0.05$) and among the nine species of microalgae ($F_{(8, 405)} = 2318.27$, $P < 0.05$) (Fig. 2.5, Table. 2.4), tending to increase with the corresponding increase in the cell size of the different species of microalgae. However, the rate

of cell volume that was filtered for the nine microalgal species was not consistent among the five size classes of juvenile mussels (Size class*Microalgae species – $F_{(32, 405)} = 16.11$, $P < 0.05$) (Fig. 2.5, Table. 2.4).

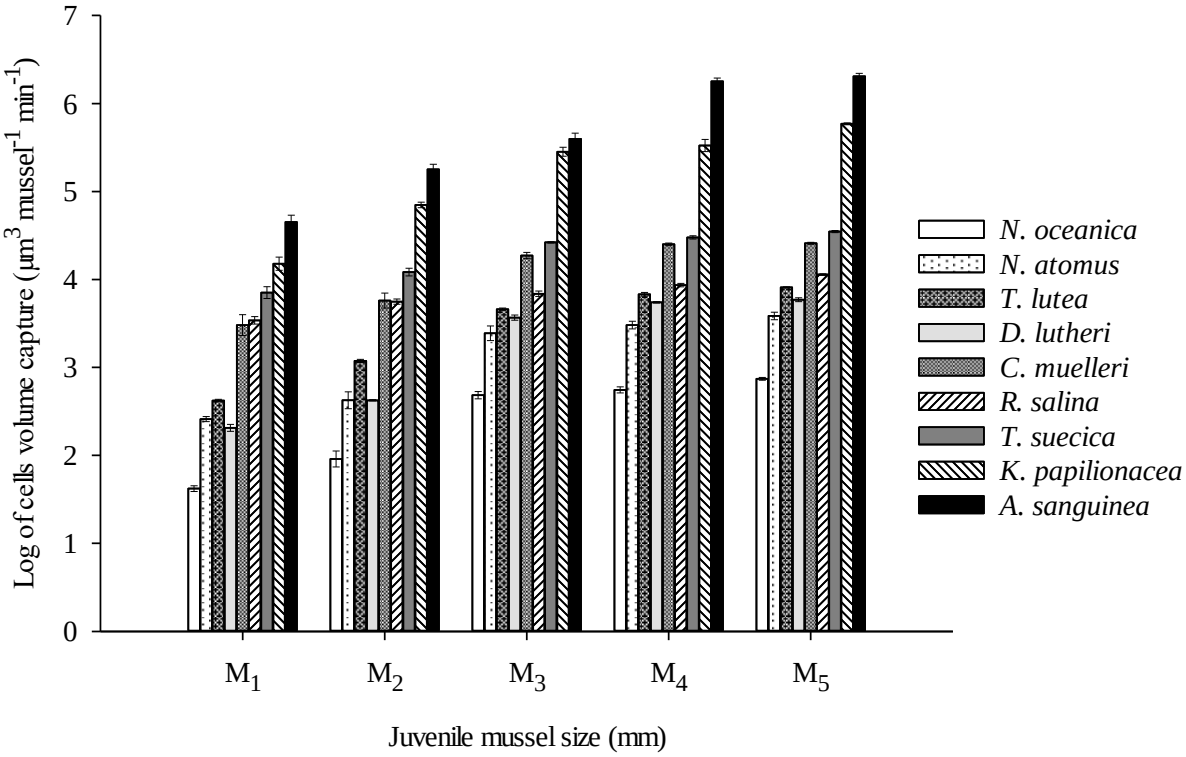

Figure 2.5. Mean rate of cell volume of microalgae filtered by five size classes of juvenile mussels (M_1-M_5) for nine species of microalgae provided at the same cell concentration (n = 90). Y axis is natural log of rate of cell volume to facilitate presentation of data.

Among all microalgae species provided, the highest mean cell volume intake was for *A. sanguinea* for four of the five size classes of juvenile mussels. The other species of large-celled dinoflagellate (i.e., *K. papilionacea*) was able to be captured at the same rate as *A. sanguinea* by juvenile mussel M_3, but this species was captured at a significantly lower rate than *A. sanguinea* for the other four size classes of mussels (Tukey HSD, $P < 0.05$).

The total cell volume of *R. salina* was captured at a similar rate as *C. muelleri* by juvenile mussels in size classes M_1 and M_2, but the rate of capture of these two species was lower than for *T. suecica* (Tukey HSD, $P < 0.05$). In contrast, the rate of cell volume capture of *C. muelleri* was higher than for *R. salina* in juvenile mussel size classes M_3, M_4 and M_5, and consistently reaching a similar rate of cell volume capture as the much larger *T. suecica* for these three size classes of mussels (Tukey HSD, $P < 0.05$). Most juvenile mussel size classes had a higher rate of cell volume capture for *T. lutea* than for *N. atomus* and *D. lutheri*, with only juvenile mussels

in size class M_4 having a similar capture rate for *T. lutea* and *D. lutheri*, despite the comparative difference in cell size (Tukey HSD, $P < 0.05$). Consistently the lowest rate of cell volume capture for the nine experimental microalgae species tested in the study was for *N. oceanica* for all five size classes of juvenile mussels (Tukey HSD, $P < 0.05$) (Fig. 2.5 and Table 2.4).

Table 2.4. Mean rate of cell volume of microalgae filtered by five size classes of juvenile mussels (M_1-M_5) for nine species of microalgae of different cell sizes provided at the same cell concentration. Means without subscript letters in common within each column are significantly different at $P < 0.05$ – Tukey HSD. Means without superscript letters in common within each row are significantly different at $P < 0.05$ – Tukey HSD. M_1 = size (0.5 – 0.9 mm), M_2 = size (1.0 – 1.9 mm), M_3 = size (2.0 – 3.0 mm), M_4 = size (4.0 – 5.0 mm), and M_5 = size (6.0 – 7.0 mm).

Microalgae	Rate of cell volume capture (µm^3 mussel^{-1} min^{-1})				
	M_1	M_2	M_3	M_4	M_5
N. oceanica	$4.31 \times 10^{1}{}_{a}{}^{a}$	$1.11 \times 10^{2}{}_{b}{}^{a}$	$5.01 \times 10^{2}{}_{c}{}^{a}$	$5.69 \times 10^{2}{}_{c}{}^{a}$	$7.44 \times 10^{2}{}_{d}{}^{a}$
N. atomus	$2.63 \times 10^{2}{}_{a}{}^{b}$	$5.14 \times 10^{2}{}_{b}{}^{b}$	$2.76 \times 10^{3}{}_{c}{}^{b}$	$3.17 \times 10^{3}{}_{c}{}^{b}$	$4.0 \times 10^{3}{}_{d}{}^{b}$
T. lutea	$4.21 \times 10^{2}{}_{a}{}^{c}$	$1.19 \times 10^{3}{}_{b}{}^{c}$	$4.62 \times 10^{3}{}_{c}{}^{c}$	$6.87 \times 10^{3}{}_{c}{}^{c}$	$8.16 \times 10^{3}{}_{d}{}^{c}$
D. lutheri	$2.13 \times 10^{2}{}_{a}{}^{b}$	$4.22 \times 10^{2}{}_{b}{}^{b}$	$3.77 \times 10^{3}{}_{c}{}^{b}$	$5.52 \times 10^{3}{}_{c}{}^{c}$	$5.96 \times 10^{3}{}_{cd}{}^{b}$
C. muelleri	$4.11 \times 10^{3}{}_{a}{}^{d}$	$6.89 \times 10^{3}{}_{b}{}^{d}$	$1.93 \times 10^{4}{}_{c}{}^{d}$	$2.53 \times 10^{4}{}_{c}{}^{d}$	$2.59 \times 10^{4}{}_{d}{}^{d}$
R. salina	$3.59 \times 10^{3}{}_{a}{}^{d}$	$5.73 \times 10^{3}{}_{b}{}^{d}$	$7.05 \times 10^{3}{}_{b}{}^{c}$	$8.71 \times 10^{3}{}_{bc}{}^{c}$	$1.14 \times 10^{4}{}_{c}{}^{c}$
T. suecica	$8.05 \times 10^{3}{}_{a}{}^{e}$	$1.27 \times 10^{4}{}_{b}{}^{e}$	$2.65 \times 10^{4}{}_{c}{}^{d}$	$3.03 \times 10^{4}{}_{c}{}^{d}$	$3.52 \times 10^{4}{}_{c}{}^{d}$
K. papilionacea	$1.72 \times 10^{4}{}_{a}{}^{f}$	$7.22 \times 10^{4}{}_{b}{}^{f}$	$3.02 \times 10^{5}{}_{c}{}^{e}$	$3.65 \times 10^{5}{}_{c}{}^{e}$	$5.90 \times 10^{5}{}_{d}{}^{e}$
A. sanguinea	$5.13 \times 10^{4}{}_{a}{}^{g}$	$1.91 \times 10^{5}{}_{b}{}^{g}$	$4.42 \times 10^{5}{}_{c}{}^{e}$	$1.85 \times 10^{6}{}_{d}{}^{f}$	$2.09 \times 10^{6}{}_{d}{}^{f}$

The scale of the differences in the rate of capture of microalgal cell volume among the different microalgae species of different sizes by juvenile mussels was very large. For example, among juvenile mussels in the M_1 size class the difference between the highest and lowest mean rates of capture of cell volume of microalgae was three orders of magnitude, i.e., *A. sanguinea* 5.13×10^4 µm^3 mussel^{-1} min^{-1} versus *N. oceanica* 4.31×10^1 µm^3 mussel^{-1} min^{-1}. This difference had extended to four orders of magnitude for mussels of the M_5 size class, i.e., *A. sanguinea* 2.09×10^6 µm^3 mussel^{-1} min^{-1} versus *N. oceanica* 7.44×10^2 µm^3 mussel^{-1} min^{-1}.

2.3.4. Particle capture versus mussel biomass and shell length

There was a close relationship between SL of juvenile mussels and their dry body mass (BM) with 87 % of the variation in the data described by a power curve function, $y = 0.01(x)^{2.1}$, ($R^2 = 0.87$, $P<0.05$). Initially BM of juvenile mussels exponentially increase in relation to SL

until they reached a size of about 2 mm in shell length at which point their BM began to increase more rapidly in relation to SL.

There was a close relationship between measured rates of particle capture versus the SL for individual mussels whilst filter feeding on nine different microalgae species with 75 % of the variation in the data described by a power function, $y = 20.95(x)^{0.77}$, ($R^2 = 0.75$, $P<0.01$). Likewise, there was a close relationship between measured rates of particle capture versus the BM (mg) of mussels for nine microalgae species with 78 % of the variation in the data described by a power function, $y = 105.08(x)^{0.28}$, ($R^2 = 0.78$, $P<0.01$) (Fig. 2.6). Initially, the particle capture rate (cells $mussel^{-1}$ min^{-1}) of mussels from the five size classes (M_1-M_5) increased exponentially in relation to their SL and BM. However, by the time they reached around 1 mm SL and approximately < 0.2 mg BM their rate of particle capture began to decrease proportionately in relation to the increases in both their SL and BM, as estimated from their individual capture rate derived from measures of filtering on nine different species of microalgae (Fig. 2.6).

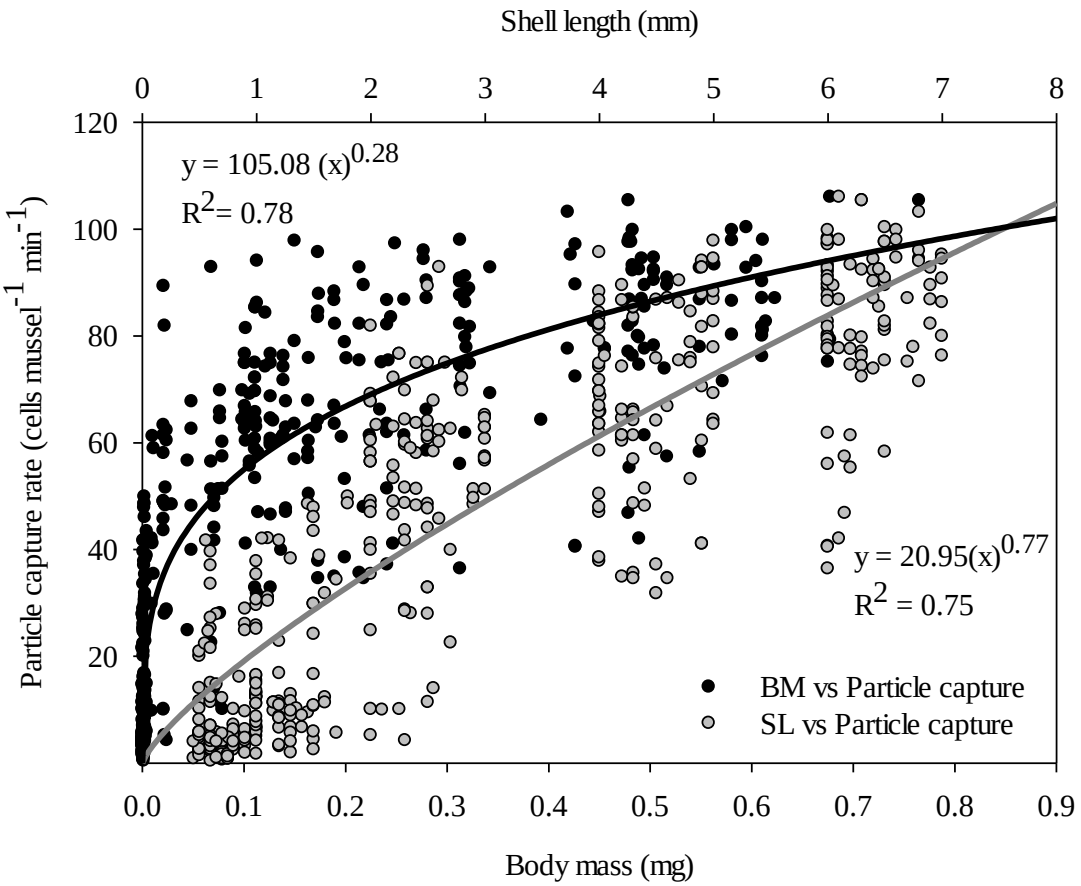

Figure 2.6. a) Juvenile mussel SL versus actual particle capture rate for mussels sampled over the five size classes (M_1-M_5) and for nine species of microalgae (n = 90), b) Dry body mass for mussels sampled over the five size classes (M_1-M_5) versus actual particle capture rate for individual juvenile mussels calculated from measures on nine microalgae species. BM = dry body mass, SL = shell length, Pc = mean particle capture rate.

2.4. Discussion

Determining the filtering capabilities of juvenile green-lipped mussels in relation to their size and for a range of microalgae species has the potential to lead to improvements in the efficiency of feeding in the nursery culture situation. Microscopic visual observations combined with FCM methods used in this study provided an effective approach for accurately determining the filtering capabilities of juvenile mussels over a short period of time when supplied with axenic cultures of nine species of unicellular microalgae of varying cell sizes.

2.4.1. Filtering behaviour of juvenile mussels

The ability of mussels to regulate filtering activity is a fundamental means by which mussels can control their intake of suspended particles, and is reported to vary in relation to the numbers and physical qualities of the ambient suspended particles or water quality conditions (Mamat & Alfaro, 2014; Rajagopal et al, 1991; Rajesh et al., 2001). In the current study the experimental juvenile green-lipped mussels in five size classes ranging from 0.5 - 7 mm in SL showed consistently high rates of filtering when provided with the same concentration of nine different microalgae species over a 60 min experimental period. The consistent rapid commencement and continuation of filtering by the mussels indicates the acceptability of all nine species of microalgae to the mussels (Tang & Riisgård, 2016), despite the marked differences in the size and taxa of these species. The 24 h period of starvation for the mussels prior to the experiment commencing may have also assisted in promoting this filtering behaviour. Therefore, the results may not represent longer term patterns of feeding activity where gut fullness and satiation may curtail continued feeding. Mussel feeding behaviour can change over time, with short experimental periods showing different results to those from longer experiments (Riisgard & Randløv, 1981). Furthermore, conducting experiments in small volumes of suspended microalgae in the well plates (i.e., 2 ml) would have limited the overall number of cells available for filtering, due to serial depletion of cell concentrations resulting from prior filter feeding. Consequently, the rates of particle capture could be expected to have declined in this study over the one hour experimental duration. Regardless, these results do provide an indication of the overall propensity and capacity for juvenile mussels of a range of sizes for filtering the various microalgal species. Only one species of microalgae, *A. sanguinea*, affected filtering activity with mussels of all size classes spending on average 4.2 % less time actively filtering than for mussels provided with the eight other species of microalgae, i.e., 95.5 % versus 99.7 % of 60 min spent actively filtering. Some of the juvenile mussels supplied with this large microalga (i.e., *A. sanguinea*) were observed to be hesitant to commence filtering, while others paused filtering

activity for short periods during the 60 min experimental period. However, for all other species of microalgae hesitation in feeding by the juvenile mussels were absent or rare.

The occurrence of hesitation in feeding in some juvenile mussels when exposed to *A. sanguinea* could be an indication of ingestion preferences in bivalves species (Defossez & Hawkins, 1997; Safi & Hayden, 2010; Widdowset al., 1979). Three species of juvenile mussels; *M. edulis*, *Ruditapes philippinarum* and *Tapes decussatus* showed high rejection of pseudofaeces contained particles size between 7.5 to 22.5 µm in diameter when fed with SiO_2 coated microsphere beads (Defossez & Hawkins, 1997). Current study did not observe the present of pseudofaeces regardless of the microalgae species provided, including. Several studies have reported that *A. sanguinea* is capable of producing toxins under certain circumstances, such as competition with other microalgae species (Xu et al., 2017; Yang et al., 2020). Adult *Pecten maximus* showed rejection of toxic dinoflagellate *Cerataulina pelagica*, although it did not affect their growth (Chauvaud et al., 2001). However, adult *P. canaliculus* showed higher ingestion of flagellated cells for both small and large cells, including dinoflagellate species *A. sanguinea*, *Gryrodinium spirale*, *Amphidinium*, *Dinophysis* spp., and *Karenia* spp,. but did not reject *Karenia* spp. which are commonly considered to be toxic (Safi & Hayden, 2010). The preferential size dependent rejection of larger particles could be a sign of adaptive behaviour in the natural environment, especially when they exposed to a large number of inorganic particles, or if the amount of available smaller particles with good organic content is higher (Defossez & Hawkins, 1997).

2.4.2. Particle capture capability of juvenile green-lipped mussel

Particle capture depends on the interaction of the particle size with the degree of development and complexity of the filtering apparatus (Dame, 2016; Gui, 2012; Newell & Shumway, 1993; Ward & Shumway, 2004). Prior work has documented that the development of gill structures play an important role in the ability of juvenile mussels to capture suspended particles. For example, a study of ontogenic change in the morphology of ctenidial filaments in the green-lipped mussels showed that the efficiency of the capture of suspended particles by the gills of juvenile mussels is dependent on the development of the laterofrontal surfaces of the ctenidial filaments, especially the eu-latero-frontal (ELF) cilia lining the ctenidial filaments (Gui et al., 2016c). Short ELF cause difficulties for mussels to capture and transport suspended particles (Gui et al., 2016a; Jørgensen et al., 1984). The rapid increase in the length and number of ELF as the juvenile mussels grow in size has been found to closely correspond to greater efficiency in particle capture and transport (Gui et al., 2016c).

The current study found that there is a substantial increase in the ability of juvenile mussels for capturing suspended particles as the juvenile mussels increase in size. The green-lipped mussels of less than 2 mm of SL (i.e., both M_1 and M_2) had higher rates of particle capture for the microalgae *R. salina, T. suecica, C. muelleri,* which all have cells that are 7 – 20 µm in diameter, compared to the smaller (i.e., < 5 µm in diameter, *N. oceanica*, *N. atomus*, *T. lutea*, and *D. lutheri*) and larger microalgae (i.e., > 20 µm cell in diameter, *A. sanguinea* and *K. papilionacea*). The ability to capture smaller microalgae improved markedly in mussels of 2 - 5 mm in SL (i.e., M_3 and M_4) with capture rates for *N. oceanica*, *N. atomus*, *T. lutea*, and *D. lutheri* matching or exceeding that of the larger *R. salina*, *T. suecica,* and *C. muelleri*. This indicates a development of the filtering capabilities, allowing for more efficient removal of smaller particles (i.e., < 5 µm). Mussels of 6.0 - 7.0 mm in SL (i.e., M_5) showed increased filtering rates for all sizes of microalgal species indicating an overall increasing filtering capacity.

The efficiency of particle capture varies among species of bivalves, which can be partly due to differences in feeding behaviour (Ward et al., 1998) and the development of the morphological structure of the ctenidia and labial palps (Ward et al. 1998, Ward & Shumway 2004). For example, pectinids, *Ostrea edulis* (Møhlenberg & Risgård, 1978) and *Monia squama* (Jørgensen et al., 1984) are less efficient at retaining suspended particles of < 2 µm in diameter, which is thought to be due their small or lack of laterofrontal cirri. Whereas, *Dreissena polymorpha*, which possess longer laterofrontal cirri, are able to retain smaller particles of 1.5 µm in diameter (Jørgensen et al., 1984).

Several researchers have provided evidence that high selectivity of smaller versus larger particles may possibly occur in juvenile bivalves which have well developed gill structure (Gui, 2012; Ward & Shumway, 2004). Juvenile *P. canaliculus* of > 6 mm in SL are considered to have a well developed gill structure that is similar to adult mussels (Gui, 2012). The current study found that the M_5 size class of mussels (i.e., 6 – 7 mm in SL) showed a high efficiency in filtering smaller particles at the same rate as the larger particles, except for the largest microalgae species (*A. sanguinea*). Furthermore, the mussels of this size class were able to filter at a 24 % higher rate than mussels of 4.0 – 5.0 mm SL for the smallest microalgae *N. oceanica* (2 – 3 µm cells in diameter). Similar results have been found for the juvenile stages of other commercially important bivalve species. For example, juveniles of the Pacific oyster, *Crassosstrea gigas,* also have poor filtration rate when provided with suspended particles of a small size, such as microalgae cells of 2 µm in diameter (i.e., *Nannochloropsis* sp.) (Brown et al., 1998). Juvenile blue mussels (*M. edulis*) of between 1.5 and 25.0 mm in SL have lower clearance rates on particles of < 2 µm cell in diameter (i.e., bacteria and picophytoplankton) compared to larger sized particles of > 6.6 µm cell diameter (i.e., nanophytoplankton) (Jacobs et al., 2015). This is

consistent with the proposition that the removal efficiency of suspended particles for most bivalves decreases when going below a size threshold, which is usually within the range of between 1 to 7 µm cell diameter depending on bivalve species (Ward & Shumway, 2004). Therefore, the current results show that smaller juvenile green-lipped mussels of < 2 mm in SL (i.e., M_1 and M_2) are inefficient at capturing smaller suspended particles < 5 µm cell in diameter, whereas larger juvenile mussels (i.e., size classes M_3, M_4 and M_5) are able to capture the smaller particle sizes (i.e., < 5 µm in cell diameter) much more efficiently, even matching the filtering rate for larger particles.

Pseudofaeces were not apparent in the feeding juvenile mussels in this study, but were not expected as the concentration of microalgae was well below the pseudofaeces threshold (Figueiras et al., 2002). In current study the microalgae cells concentration was relatively low at 11.4 – 12.4 cells μl^{-1}. This experimental concentration was chosen to accommodate the observation of filtering behaviour of a range of sizes of juvenile mussel under the microscope. Particle rejection as pseudofaeces will normally occur if it is embedded in mucus (Kiøbroe & Møhlenberg, 1981) or if the particle is of low organic content (Defossez & Hawkins, 1997; Safi & Hayden, 2010). However, at suspended particle concentrations below the pseudofaeces threshold, particles that may otherwise be rejected at pseudofaeces will most frequently be retained by the gills and ingested by most bivalves (Kiøbroe & Møhlenberg, 1981). Adult *P. canaliculus* have been found to prefer to ingest both small and large flagellated species including *A. sanguinea* and *Karenia* spp. over diatom species (Safi & Hayden, 2010). These preferences may reflect that dinoflagellates not only have higher carbon ratios (Menden-Deuer & Lessard, 2000), but also have higher percentage of assimilation in adult *P. canaliculus*, i.e., dinoflagellates (84.5%) compared with flagellates (77.9%) and diatoms (61.7%) (Ren et al., 2006). The high rejection of both large diatom speices (*Cosinodiscus* sp., *Rhizosolenia setigera*) and small diatom (*Pseudo-nitzschia* sp., *Navicula* sp.) into mucus-bound pseudofaeces, suggested that diatoms are not a preferred food source for *P. canaliculus* (Ren et al., 2006; Safi & Hayden, 2010) and other bivalves (Chauvaud et al., 2001; Menden-Deuer & Lessard, 2000). Although, diatoms are important food components for the bivalves, the physical properties of the cells (i.e., the siliceous frustule) and low carbon to silica ratios makes it more difficult for the bivalves to digest the cells of diatoms compared to the other microalgal taxa (Safi & Hayden, 2010; Tomas, 1997).

There were differences in the timing of the production of faeces, largely relating to the size of the juvenile mussels, and most likely representing differences in the digestive capacity of the juvenile mussels of different sizes. There has been little research describing the changes in

digestive structure and processing capabilities of early juvenile mussels, but it is likely to play a major role in the adsorption of food material, and warrants further research attention.

2.4.3. Cell volume intake of microalgae and feeding efficiency

While microalgae species vary greatly in size, shape and their nutritional composition, quantification of the number of cells consumed by bivalves does not adequately represent their biomass or nutrient intake (Chitari & Anil, 2017) since the capture and consumption of a large number of small microalgae cells may be equal to a few larger microalgae cells in terms of carbon biomass (Harrison et al., 2015). Regardless, converting the cell count to total cell volume captured during bivalve feeding provides a proxy for estimating biomass or nutrient intake in the absence of more detailed comparative information on the nutritional composition of the microalgae species.

Overall, juvenile mussels of a wide range of sizes (i.e., M_1 – M_5) showed an ability to capture greater total volumes of cells for increasingly larger microalgae species (Fig. 2.5, Table 2.4). For example, providing larger microalgae, such as *K. papilionacea* and *A. sanguinea*, consistently resulted in higher capture of total cell volume by juvenile mussels compared to smaller sized microalgae, such as *N. oceanica*, at approximately 600× and 2000× higher total cell volumes respectively. Spat of blue mussels of 401 µm in SL were able to capture cells of 15 – 25 µm in diameter 55 % more efficiently compared to particles of < 5 µm in diameter (Raby et al., 1997). The capability of juvenile blue mussels in ingesting larger sizes of particles is likely to be advantageous from an energetics point of view, as the volume of cells that are 20 µm in diameter are 300 times greater compared to cells of 3 µm in diameter (Raby et al., 1997). Poor capture efficiency of cells may lead to increased metabolic cost of feeding as filtration activity may need to be increased to obtain sufficient algal cells to fulfil nutritional requirements, and consequently this may cause growth reduction of mussels (Laing & Psimopoulous, 1998; Raby et al., 1997). The energy required for filtration activity, including food acquisition and movement of the captured particles through the gut, has been estimated at 3 to 7.6 % of total energy expenditure in juvenile blue mussels and Chilean mussels (*M. chilensis*) depending on filtering efficiency (Navarro & Winter, 1982; Widdows & Hawkins, 1989). Consequently, providing larger microalgae (i.e., 16 – 30 µm cell diameter) to juvenile Chilean mussels (i.e., 0.25 g dry tissue biomass) was the most efficient in terms of energy balance (i.e., 1.3 cal.day^{-1}) even when delivered at low cell concentrations (i.e., 10^6 cells l^{-1}) (Navarro & Winter, 1982). Hence, the results of the current study indicate that providing larger microalgae cells may greatly improve the efficiency of filter feeding in juvenile mussels, with a possible corresponding reduction in the energetic cost of particle processing. The efficiency of feeding larger microalgae

cells to juvenile green-lipped mussels needs to be further assessed, whilst also ensuring that possible differences in the nutritional composition of different microalgae is also taken into consideration.

2.4.4. Particle capture in relation to mussel shell length and weight

There is typically considerable variation in the relationship between filtration rate with both shell length and tissue dry weight, either within or between species of bivalve mussels (Gosling, 2003; Jacobs et al., 2015; Riisgård et al., 2014). This variation could be due to several factors, such as different sizes of individual bivalves or different experimental conditions. In general, weight-specific filtration rate declines as the size of juvenile mussels increases (Gosling, 2004). For example, juvenile blue mussels of 1.5 – 25 mm in SL living in natural habitats show a marked variability of weight-specific filtration rate throughout 3 years of experimental assessment (Jacobs et al., 2015; Riisgård et al., 1980).

The results of the current study clearly show that the smallest size class of juvenile mussels (i.e., M_1) consistently had the highest particle capture rate in relation to their dry body mass (BM) for all species of microalgae compared to all other size classes of juvenile mussels (Fig. 2.7). Post-metamorphic juvenile mussels are likely to have limited remaining tissue transferred from the larval phase, such that the capture of food particles at this time is critical for resourcing their recovery and subsequent rapid growth of their body mass. Consequently, the filtering ability of juvenile green-lipped mussels increases markedly in relation to their increasing body size as they grow.

2.4.5. Nursery feeding regimes for green-lipped mussels

Hatchery production of the green-lipped mussel in New Zealand relies on culturing spat in a nursery facility to a size of up to 1 mm (four weeks old) to 2 mm in SL prior to their deployment onto growout lines at sea (Hickman, 1976; South et al., 2017). There are several microalgae species provided for the mussel spat during this nursery phase, i.e., *T. lutea*, *D. lutheri*, *C. muelleri* (S. Cumming, pers. comm.), and *Chaetoceros calcitrans* (Brown et al., 1998). Most of these microalgae have relatively small cells (i.e., diameter of 3 – 5 µm) (Brown et al., 1998; Hillebrand et al., 1999). Other small microalgal species that are commonly used in bivalve larviculture, are also used in commercial nurseries raising juvenile green mussels (*Perna viridis*), i.e., *Isochrysis galbana* and *C. calcitrans* (Laxmilatha et al., 2011). However, in the current study juvenile mussels over a wide range of sizes consistently captured microalgae with larger cell sizes (i.e. > 6 – 20 µm) as well as, or more efficiently than, those used in current commercial nursery practice. For example, the M_1 size class of mussel was able to capture both *T. suecica* and *R. salina*, which have larger cells in diameter, at higher rates than the smaller *D.*

lutheri which is used in the commercial nursery for feeding plantigers of green-lipped mussel of this size. In addition, the improving particle capture ability of juvenile mussels once they reach 2 – 3 mm in shell length (M_3), enables mussels of this size to more efficiently filter the smallest size of microalgae. The highest efficiency in capturing the smallest microalgae cells was achieved by the time the juvenile mussel reached 6 – 7 mm in shell length (M_5). The results of the present study provide compelling evidence that providing microalgal cells of larger size has the potential to greatly improve feeding efficiency of the early stages of juvenile *P. canaliculus*.

The current study suggests that instead of the nursery culture of juvenile green-lipped mussels ending after 4 weeks as currently occurs, there may be advantages in extending the nursery stage until the mussels reach 4 – 5 mm in SL, by which time they would have the capacity to feed more efficiently on a wider size range of natural food particles. There is good evidence that early juvenile mussels with poor nutrition have poor retention in the farm situation (Carton et al., 2007; Sim-Smith & Jeffs, 2011; Supono et al., 2020). However, extending the nursery phase is likely to cause a significant increase in microalgae production cost as the particle capture rate increases dramatically in relation to increasing mussel size. Therefore, providing larger sized food particles which have good quality nutritional composition is one possible solution to improving feed delivery in nursery culture of the New Zealand green-lipped mussels. Future work should evaluate factors other than particle size, such as the biochemical composition of microalgae that are important in improving feed delivery to juvenile *P. canaliculus* during the nursery phase of production.

2.5. Conclusion

In conclusion, this study revealed that early juvenile green-lipped mussels (i.e., < 1 mm SL) are inefficient in capturing microalgae of a small cell size (< 6 µm in cell diameter) which are commonly provided as the principal feed in commercial nursery production. However, this size of juvenile mussel captures larger microalgal cells (> 6 - 20 µm in cell diameter) much more efficiently providing the opportunity to improve the efficiency of nursery feeding. Providing larger microalgae with high quality biochemical composition might be more effective for nursery feeding of juvenile *P. canaliculus* in the nursery situation. Further research is required to determine whether the nutritional requirements of juvenile green-lipped mussels can be met by larger cultured microalgae species, and whether the higher intake of larger microalgae is maintained over longer periods of feeding by the juvenile mussels.

Chapter 3. Continuous particle capture of juvenile green-lipped mussel (*Perna canaliculus*)

3.1. Introduction

The single most valuable aquaculture export from New Zealand is the green- lipped mussel, *Perna canaliculus*, with annual exports currently amounting to US$308 million based on the production of around 200,000 t of mussels (New Zealand Sustainable Aquaculture, 2019). Increasing aquaculture production of green-lipped mussels has placed greater pressure on the supply of seed mussels, also known as spat, to this industry. In recent years, shortages in spat supply have become a major constraint to the further growth of this industry. Most of the spat used by the industry comes from gathering or catching wild spat, with around ~ 60 % of the spat harvested from Ninety Mile Beach at the northern tip of the North Island (Alfaro et al., 2010; Jeffs et al., 1999). Drift seaweed and other debris with large numbers of attached mussel spat washes ashore intermittently to this beach, whereupon it is harvested, sorted, and then sent by refrigerated trucks to mussel farms around much of New Zealand where it is seeded out onto mussel farms (Jeffs et al., 1999). One of the solutions to increase the quantity and certainty of supply of mussel spat to the mussel aquaculture industry is the development of artificial mussel spat production in a hatchery. A commercial hatchery for mussel spat was built near Nelson in 2015 and is now producing a small proportion (~ 5 %) of the total mussel spat supply for New Zealand (Capson & Guinote, 2014). Compared to wild mussel spat, the production of spat in a hatchery is costly, with a significant proportion of the cost due to the expense of producing a large quantity of high quality microalgae to support the nursery phase of hatchery production, which typically can contribute 30 – 50 % of production costs (Laxmilatha et al., 2011; Ragg et al., 2010). Therefore, providing juvenile mussels with an optimum quantity of microalgae is very important for controlling production costs. Inadequate nutritional condition of spat due to a poor feeding history prior to seeding onto mussel farms has been shown to greatly reduce their subsequent retention on the farms (Foote, 2003; Hatton et al., 2005; Hayden & Woods, 2011). The feed requirements of spat of green-lipped mussels are poorly defined and are likely to change markedly with increasing size of the spat, as well as other factors (Gui et al., 2016b; Supono et al., 2020; Ward & Shumway, 2004).

For example, the filter feeding abilities of juvenile *P. canaliculus* have been shown to be greatly influenced by ontogenetic changes in particle capture efficiency of their gill structures (Gui et al., 2016c), which in turn is influenced by particle size, shape (Raby et al., 1997; Sim-Smith et al., 2005; Ward & Shumway, 2004) and particle concentration (Gui et al., 2016b). However, most of these previous studies have relied on experiments undertaken over short periods of time, typically 1 h or less (Gui et al., 2016a; Sim-Smith et al., 2005). Spat in nursery systems are typically supplied continually with cultured microalgae to excess to ensure they are fed to satiation, which has the potential to be wasteful if this feed is inadequately utilised by the cultured spat. Quantifying the continuous particle capture abilities of spat held under ideal conditions has the potential to significantly improve the efficiency of feed delivery in nursery culture of spat. The research presented in this chapter tests the hypothesis that the particle capture of juvenile mussels of a range of sizes is consistent over a 24 hour period when provided with suspensions of four species of microalgae. In testing this hypothesis, the research determines the continuous maximum capture and consumption of four species of microalgae by seed mussels when held under nursery conditions in the laboratory.

3.2. Materials and methods

3.1.1. Experimental feeding test

Axenic stock culture of four microalgae species used in this study were *Diacronema lutheri* (~3 – 6 µm, volume ~65.42 μm^3 $cell^{-1}$), *Tisochrysis lutea* (~4 – 8 µm, volume ~87.07 μm^3 $cell^{-1}$), *Chaetoceros muelleri* (~7 – 9 µm, volume~296.73 μm^3 $cell^{-1}$), and *Tetraselmis suecica* (~9 – 14 µm, volume ~440.24 μm^3 $cell^{-1}$). These four microalgae species are used to feed green-lipped mussel spat in the nursery culture situation in New Zealand. All of the microalgae species were axenically cultured at 21 ℃ in 1 l flasks of sterile filtered seawater which were supplemented with Guillard's F/2 media containing silica (Sigma-Aldrich) under continuous full spectrum illumination (24 h). The microalgae species used for the experimental feeding of mussels were harvested during the exponential phase of population increase.

3.1.2. Juvenile mussel preparation

Juvenile green-lipped mussels were collected from Maori Bay, northwest of Auckland, New Zealand (36º 83.871' S and 174º 42.649' E). The mussels were then transported to the seawater laboratory in the School of Biological Sciences at the University of Auckland. Upon arrival, the mussels were detached from seaweed by washing them with high pressure freshwater over a fine mesh sieve. Approximately 1000 mussel spat of various sizes were held in 4-L glass beakers which were supplied with filtered seawater (UV and 1 µm) and continuous aeration for 3 days

and fed with a 1: 1: 1: 1 mixture of the four microalgae species based upon cell count ml^{-1} to make up a total concentration 4×10^4 cells ml^{-1} or equal to 1.6×10^6 cells $mussel^{-1}$ day^{-1}. The glass beakers were cleaned every day, and the seawater replaced and maintained at 18 ºC with sufficient continuous aeration to maintain the microalgae in suspension. The light cycle 12L:12D was maintained throughout the experimental period. After three days of acclimation, 50 mussel spat were randomly selected from a beaker and were graded by using a series of graduated sieves and the resulting size classes of mussels had their shell length measurement determined using digital images that were analysed with Image J software. The five size class categories of mussels based on their shell length were: M_1 = 0.5 – 0.9 mm, M_2 = 1.0 – 1.9 mm, M_3 = 2.0 – 3.0 mm, M_4 = 4.0 – 5.0 mm, and M_5 = 6.0 – 7.0 mm. Five individual mussels of each size class were randomly selected and transferred into individual experimental conical tanks containing 200 ml of filtered seawater (UV and 1 µm). The conical tanks were made from inverted 1500 ml plastic bottles with the base of the bottle cut off. A small round air-stone of 2.2 cm in diameter was sealed into the neck of the plastic bottle through which air was bubbled into the tank at a rate of 33 ml s^{-1}. From the top and centre of each tank a short length of attachment substrate for the seed mussels was suspended from a length of twine weighed down with a stainless-steel nut (316 grade stainless steel). The attachment substrate consisted of fine plastic mesh (1 mm square nylon mesh) measuring 5 × 18 cm. Each combination of four microalgae species versus five size classes of mussels had 10 replicate experimental tanks making a total of 200 experimental tanks.

After the five juvenile mussels were size graded and transferred into the conical tanks, they were acclimated to the conditions in the conical tanks for the next two days including feeding on an axenic microalgal species prior to undertaking the measurements of their particle capture abilities. Each day each experimental tank was emptied, rinsed with filtered seawater and then re-filled with 200 ml of filtered seawater to which 4×10^4 cells ml^{-1} of the selected microalga species (i.e., 8×10^6 cells $tank^{-1}$) was added. After 2 days of acclimation to feeding on a microalga species, the continuous capture of the microalga by the mussel spat in the tank was measured over the following 24 h in the following manner. Each experimental tank was cleaned and re-filled in the same manner as for previous days. In addition, three control tanks, which contained axenic microalgae species without mussels for each treatment combination were also set up. At 5 min after re-filling each tank, a 500 µl water sample was taken from each tank with a micropipette and used to determine the initial concentration of the microalga species in each tank (t_0 = 0 h). The mussels were then left to feed on the microalga for 24 h at which time a 500 µl water sample was taken from each of the experimental and control tanks to measure the final microalga concentration (t_{24} = 24 h). A flow cytometer (FCM) (BD Accuri C6) was used to

enumerate suspended microalgal cells in the 500 µl water samples at t_0 and t_{24} using fast reading setting of 66 µl s^{-1}.

A measure of the capture of microalgal particles by juvenile mussels was assessed by comparing changes in the measured concentration of microalgal cells over 24 h in each tank after adjusting for any changes in microalgal cell concentration in the control tanks. The measure of particle capture (cell $mussel^{-1}$ 24 h^{-1}) was calculated as:

$$\text{Particle capture (cells mussel}^{-1}\text{ 24 h}^{-1}) = ([t_0 - t_{24}] - a] \times 200)/5$$

Where t_0 and t_{24} are the measured microalgal cell concentrations (cells ml^{-1}) at the beginning and the end of feeding time t (i.e., 24 h), and a is the correction factor derived from any changes in cell density in the control tanks:

$$a = (Cont_0 - Cont_{24})$$

Where $Cont_0$ and $Cont_{24}$ are cell concentrations (cells ml^{-1}) in the control tanks at the beginning and the end of the experimental period (Coughlan, 1969).

3.1.3. Juvenile mussel faeces production

Observations of the production of faeces by juvenile mussels of a range of sizes (i.e., M_1 to M_5) which were supplied with four species of microalgae were conducted separately by placing individual mussels in a well of a 24 well plate (BD Falcon) with a solution of axenically cultured microalgae. The aim of the observation was to confirm the pattern of faecal production by the mussels over the experimental feeding period (i.e., 24 h). Each well was filled with 2 ml of seawater containing cultured microalgae at the same concentration used in the experimental tank, i.e., ~8×10^5 cells ml^{-1}. Each well contained a single juvenile mussel of each of the various size classes, which were distributed randomly across the well plate. There were five replicates for each combination of treatments of microalgae species and juvenile mussel size class, i.e., a total of 100 individual mussels being observed.

Each mussel was observed separately under a dissecting microscope at four-time intervals t_1 (1 h after microalgae were introduced to the juvenile mussels in the wells), t_6 (6 h), t_{12} (12 h) and t_{24} (24 h). The presence of faecal strings was observed under a stereo microscope (EMZ-8TR, Meiji Techno) at each observation time within each treatment combination. The faecal string was carefully removed from individual experimental wells by using a 1 ml plastic pipette and were transferred to a glass petri dish which was filled with 2 ml of filtered seawater (Berry & Schleyer, 1983). The faeces were then examined under a fluorescence microscope (inverted, Nikon Ti-E) (Liutkus et al., 2012) to determine whether there were any undigested microalgae

present to provide an indication of the ability of juvenile mussels to digest the four species of microalgae over the feeding period.

3.1.4. Particle capture in relation to mussel size

To determine how the continuous particle capture of juvenile mussels changed in relation to their biomass, the dry biomass of tissues (BM) of the mussels used in the study were determined. After the feeding experiment was completed, the shell length of individual juvenile mussels from the various size classes (M_1 up to M_5, i.e., n = 200 of each size class) were measured from calibrated images of the mussels captured with a digital camera (Samsung Galaxy A5) connected to a dissecting microscope (EMZ-8TR, Meiji Techno). Each mussel was carefully rinsed with freshwater to remove salt, dried carefully with a paper towel and then dissected under a dissecting microscope in order to separate the body tissues from the attached shell. The tissues were carefully dabbed with Whatman filter paper for approximately 5 min to remove water on the outside of the tissue. The wet mass of the tissue was then measured on a microbalance (Mettler Toledo, Global Science). The tissue was then placed in a pre-weighed Eppendorf® tube and freeze dried for 24 h and re-weighed to determine the dry mass. The SL and BM of a range of sizes of juvenile mussels from each experimental treatment were plotted and curves fitted to best describe their relationship with the corresponding measure of continuous particle capture by the juvenile mussels of the different size classes.

3.1.5. Statistical analyses

A two-way analysis of variance (ANOVA) was used to compare the mean continuous particle capture and total cell volume capture of juvenile mussels among the tanks for each of the five size classes and for the four species of microalgae for the 24 h feeding period. The data were log transformed prior the analysis. A priori the data were assessed for normality using a Shapiro–Wilk test, and for homogeneity of variances using a Levene's test. Significant ANOVA results were followed by pairwise Tukey's HSD post hoc tests to identify differences between pairs of means using a Bonferroni correction to control for inflated family-wise error due to multiple testing.

The long term (24 h) and short term (1 h) observations (sourced from Chapter 2) on the particle capture ability of a range of sizes of juvenile mussel which were supplied with four microalgae species were compared by extrapolating the short term data (to 24 h by multiplying with total min (i.e., within 24 h)). Student's *t*-tests were then used to compare the mean number of microalgal particles captured for the two sets of observations for each of the five size classes of mussels and four microalgae species.

All the data were analysed by using Minitab version 18 and Sigma Plot version 14.

3.2. Results

3.2.1. Continuous particle capture by juvenile *P. canaliculus*

There was a slight reduction in the number of microalgae particles in the control tanks (i.e., container without mussels) over the 24 h observation period, however, the reduction was consistently small (i.e., < 3 %) and most likely the result of a small proportion of microalgae cells settling out in the tank.

Continuous particle capture abilities over a 24 h period increased markedly over a range of five size classes of juvenile mussel ($F_{(4, 180)} = 115.43$, $P < 0.0001$) and among the four species of microalgae ($F_{(3, 180)} = 5.57$, $P < 0.0001$). There was no interactive effect between species of microalgae and the size classes of juvenile mussels (Microalgae species*Size class of mussels - $F_{(12, 180)} = 1.15$, $P = 0.32$) (Fig. 3.1 and Table 3.1).

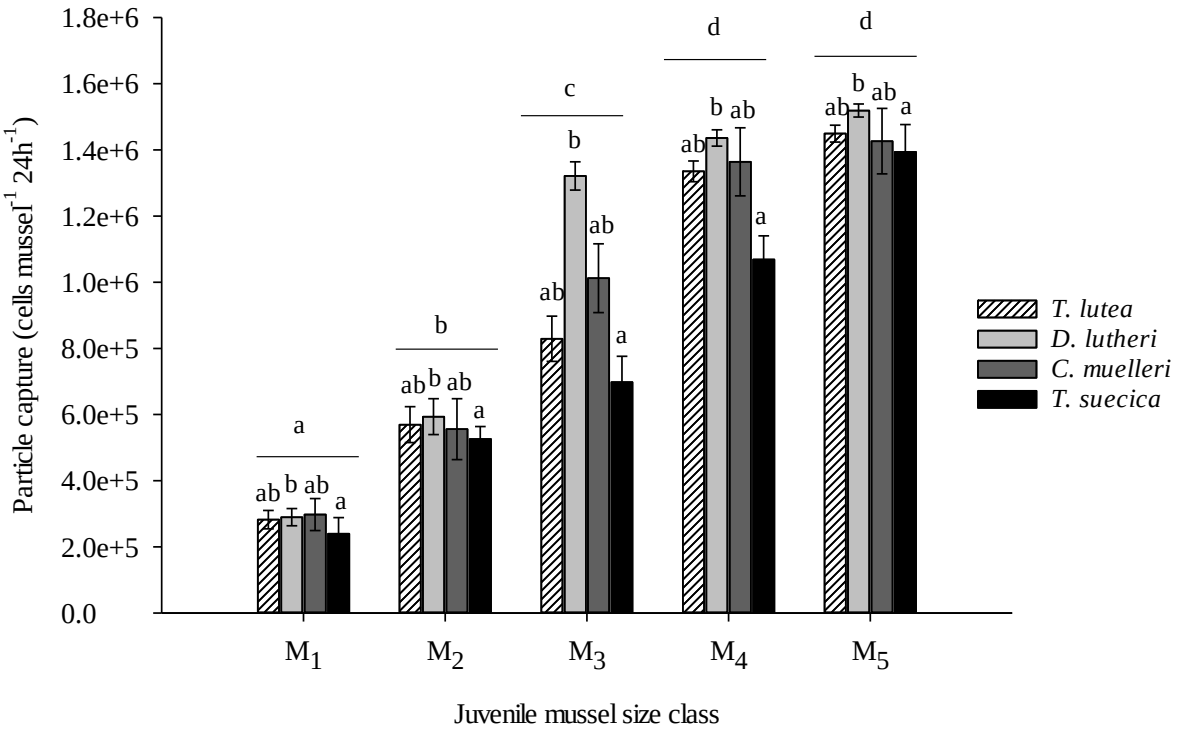

Figure 3.1. Mean particle capture for five size classes of juvenile mussels filtering axenic cultures of four different species of microalgae and supplied at the same cell density over a 24 h period. Size classes of juvenile mussels by shell length; M_1 = 0.5 – 0.9 mm, M_2 = 1.0 – 1.9 mm, M_3 = 2.0 – 3.0 mm, M_4 = 4.0 – 5.0 mm and M_5 = 6.0 – 7.0 mm. Different letters above the group of bars indicate significant differences in the main effect for mean values for the juvenile

mussel size. Different letters above the individual bars indicate significant differences between means for the main effect of microalgae. For details of differences among means see Table 3.1.

Regardless of mussel size class the only difference in mean particle capture ability was observed between the microalgae *T. suecica* 7.85×10^5 and *D. lutheri* at 1.03×10^6 cells mussel^{-1} 24 h^{-1} (Tukey's HSD, $P < 0.05$). There were no other differences among the measures of mean particle capture including for *T. lutea*, and *C. muelleri* with 8.93×10^5, and 9.31×10^5 cells mussel^{-1} 24 h^{-1} respectively.

Regardless of microalgal species, the lowest overall particle capture was observed for juvenile mussels of M_1 size class at 2.77×10^5 cells mussel^{-1} 24 h^{-1} (Tukey's HSD, $P < 0.05$). The next lowest was observed for juvenile mussels of M_2 size class at 5.61×10^5 cells mussel^{-1} 24 h^{-1} (Tukey's HSD, $P < 0.05$). This was lower than the particle capture measured for the M_3 size class of juvenile mussel which had an overall mean particle capture of 9.63×10^5 cells mussel^{-1} 24 h^{-1} (Tukey's HSD, $P < 0.05$). However, this was lower than the overall mean particle capture measured for both M_4 and M_5 which were 1.30×10^6 and 1.45×10^6 cells mussel^{-1} 24 h^{-1} respectively and were not different (Tukey's HSD, $P < 0.05$) (Fig. 3.1 and Table 3.1).

Table 3.1. Mean particle capture by five size classes of juvenile mussels (M_1-M_5) for four species of microalgae provided at the same cell concentration over 24 h. Different superscript letters after the treatment levels (i.e., microalgae species, and juvenile mussel size class) indicate significant differences within the treatment means (Tukey's HSD, $P < 0.05$). M_1 = size (0.5 – 0.9 mm), M_2 = size (1.0 – 1.9 mm), M_3 = size (2.0 – 3.0 mm), M_4 = size (4.0 – 5.0 mm), and M_5 = size (6.0 – 7.0 mm).

Microalgae Species	Mean particle capture (cells mussel^{-1} 24 h^{-1})				
	M_1 [a]	M_2 [b]	M_3 [c]	M_4 [d]	M_5 [d]
T. lutea [ab]	2.82×10^5	5.69×10^5	8.29×10^5	1.34×10^6	1.45×10^6
D. lutheri [b]	2.89×10^5	5.93×10^5	1.32×10^6	1.44×10^6	1.52×10^6
C. muelleri [ab]	2.98×10^5	5.56×10^5	1.01×10^6	1.36×10^6	1.43×10^6
T. suecica [a]	2.40×10^5	5.26×10^5	6.98×10^5	1.07×10^6	1.39×10^6

There were marked differences between the measures of particle capture from direct measurements taken over 24 h and extrapolation from 1 h (i.e., data from Chapter 2) for the same five size classes of juvenile mussel and four microalgae species tested (Tables 3.1, 3.2). Extrapolating short term (1 h) particle capture consistently underestimated the actual measures

over 24 h regardless of the size class of juvenile *P. canaliculus* or the four microalgae species provided. These underestimates ranged from 8 to 64 times (Table 3.2).

Table 3.2. Mean particle capture by five size classes of juvenile mussels (M_1-M_5) for four species of microalgae provided at the same cell concentration when extrapolated from 1 h measure to 24 h. The two means actual capture rate (24h) versus extrapolation capture rate were compared with a Students *t*-test and where significant indicated with *t*-value and $P < 0.01$, the size of the difference in the means is provided (Δ). M_1 = size (0.5 – 0.9 mm), M_2 = size (1.0 – 1.9 mm), M_3 = size (2.0 – 3.0 mm), M_4 = size (4.0 – 5.0 mm), and M_5 = size (6.0 – 7.0 mm).

Microalgae species	Extrapolation of mean particle capture (cells mussel^{-1} 24 h^{-1})				
	M_1	M_2	M_3	M_4	M_5
T. lutea	6.97×10^3	1.98×10^4	7.67×10^4	1.14×10^5	1.35×10^5
t- test	29.1, $P < 0.01$	31.3, $P < 0.01$	24.5, $P < 0.01$	47.0, $P < 0.01$	103.9, $P < 0.01$
Δ	40 ×	29 ×	11 ×	12 ×	11 ×
D. lutheri	4.69×10^3	9.29×10^4	8.29×10^4	1.21×10^5	1.31×10^5
t- test	30.5, $P < 0.01$	43.3, $P < 0.01$	36.8, $P < 0.01$	101.1, $P < 0.01$	47.1, $P < 0.01$
Δ	62 ×	64 ×	16 ×	12 ×	12 ×
C. muelleri	1.99×10^4	3.34×10^4	9.39×10^4	1.23×10^5	1.26×10^5
t- test	10.5, $P < 0.01$	6.8, $P < 0.01$	16.9, $P < 0.01$	23.1, $P < 0.01$	27.8, $P < 0.01$
Δ	15 ×	17 ×	11 ×	11 ×	11 ×
T. suecica	2.63×10^4	4.15×10^4	8.68×10^4	9.92×10^4	1.15×10^5
t- test	6.8, $P < 0.01$	21.9, $P < 0.01$	10.2, $P < 0.01$	29.6, $P < 0.01$	32.8, $P < 0.01$
Δ	9 ×	13 ×	8 ×	11 ×	12 ×

3.2.2. Continuous cell volume captured

Continuous cell volume captured over a 24 h period increased markedly over a range of five size classes of juvenile mussel ($F_{(4, 180)} = 115.43$, $P < 0.0001$) and among the four species of microalgae ($F_{(3, 180)} = 171.89$, $P < 0.0001$). There was no interactive effect between species of microalgae and the size classes of juvenile mussels (Microalgae species*Size class of mussels - $F_{(12, 180)} = 1.15$, $P = 0.32$) (Fig. 3.2 and Table 3.3).

Regardless of mussel size class, the lowest mean continuous cell volume capture was observed for both microalgae *D. lutheri* at 6.75×10^7 and *T. lutea* 7.78×10^7 µm^3 mussel^{-1} 24 h^{-1}, but there was no difference between these values. Higher mean cell volume capture was observed for both *C. muelleri* 2.76×10^8 and *T. suecica* 3.46×10^8 µm^3 mussel^{-1} 24 h^{-1}, but there was no difference between these values (Tukey's HSD, $P < 0.05$).

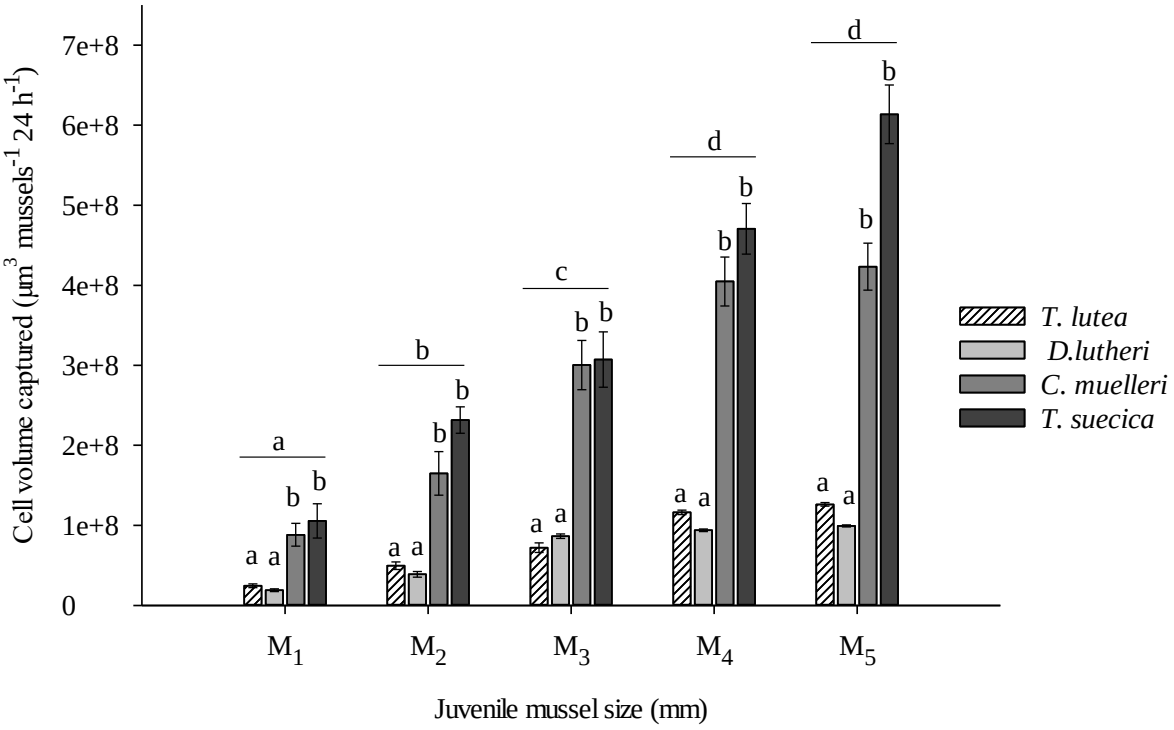

Figure 3.2. Mean continuous total microalga cell volume captured for five size classes of juvenile mussels filtering axenic cultures of four different species of microalgae and supplied at the same cell density over a 24 h period. Size classes of juvenile mussels by shell length; M_1 = 0.5 – 0.9 mm, M_2 = 1.0 – 1.9 mm, M_3 = 2.0 – 3.0 mm, M_4 = 4.0 – 5.0 mm and M_5 = 6.0 – 7.0 mm. Different letters above the group of bars indicate significant differences in the main effect for mean values for the juvenile mussel size. Different letters above the individual bars indicate significant differences between means for the main effect of microalgae. For details of differences among means see Table 3.3.

Regardless of microalgal species, the lowest overall continuous cell volume capture was observed for juvenile mussels of M_1 size class at 5.92×10^7 µm^3 mussel^{-1} 24 h^{-1} (Tukey's HSD, $P < 0.05$). The next lowest was observed for juvenile mussels of M_2 size class at 1.21×10^8 µm^3 mussel^{-1} 24 h^{-1} (Tukey's HSD, $P < 0.05$). This was lower than the cell volume capture measured for the M_3 size class of juvenile mussel which had an overall mean cell volume capture of 1.91×10^8 µm^3 mussel^{-1} 24 h^{-1} (Tukey's HSD, $P < 0.05$). However, this was lower than the overall mean cell volume capture measured for both M_4 and M_5 which were 2.71×10^8 and 3.16×10^8 µm^3 mussel^{-1} 24 h^{-1} respectively and were not different (Tukey's HSD, $P < 0.05$) (Fig. 3.2 and Table 3.3).

Table 3.3. Mean continuous total microalga cell volume captured by five size classes of juvenile mussels (M_1-M_5) for four species of microalgae provided at the same cell concentration over 24 h. Different superscript letters after the treatment levels (i.e., microalgae species, and juvenile mussel size class) indicate significant differences within the treatment means (Tukey's HSD, $P < 0.05$). M_1 = size (0.5 – 0.9 mm), M_2 = size (1.0 – 1.9 mm), M_3 = size (2.0 – 3.0 mm), M_4 = size (4.0 – 5.0 mm), and M_5 = size (6.0 – 7.0 mm).

Microalgae Species	Mean total cell volume captured (μm^3 $mussel^{-1}$ $24\ h^{-1}$)				
	M_1 [a]	M_2 [b]	M_3 [c]	M_4 [d]	M_5 [d]
T. lutea [a]	2.46×10^7	4.96×10^7	7.22×10^7	1.16×10^8	1.26×10^8
D. lutheri [a]	1.89×10^7	3.88×10^7	8.64×10^7	9.39×10^7	9.94×10^7
C. muelleri [b]	8.83×10^7	1.65×10^8	3.00×10^8	4.05×10^8	4.23×10^8
T. suecica [b]	1.05×10^8	2.31×10^8	3.07×10^8	4.71×10^8	6.14×10^8

3.2.3. Continuous particle capture in relation to mussel size

The relationship between SL of juvenile mussels and their dry body mass (BM) showed a close relationship with 89 % of the variation described by the power fitted curve, i.e., $y = 0.03(x)^{1.33}$, ($R^2 = 0.89$, $P<0.01$). The BM of juvenile mussels initially exponentially increases in relation to their SL until the mussels reached a SL of around 2 mm at which point their BM began to increase more rapidly in relation to SL.

Daily particle capture of juvenile green-lipped mussels provided with four different microalgae species increased with SL and BM which was well described with power functions respectively $y = 508045.39(x)^{0.63}$, ($R^2 = 0.84$, $P<0.01$) and $y = 1755303.45(x)^{0.22}$, ($R^2 = 0.78$, $P<0.01$), explaining 84 and 78 % of the variability in the data respectively (Fig. 3.3). Initially, the daily particle capture by mussels increased exponentially in relation to their SL and BM until they reached around 1 mm SL and at approximately < 0.1 mg BM at which point their rate of particle capture began to decrease gradually in relation to the increases in both their SL and BM (Fig. 3.3).

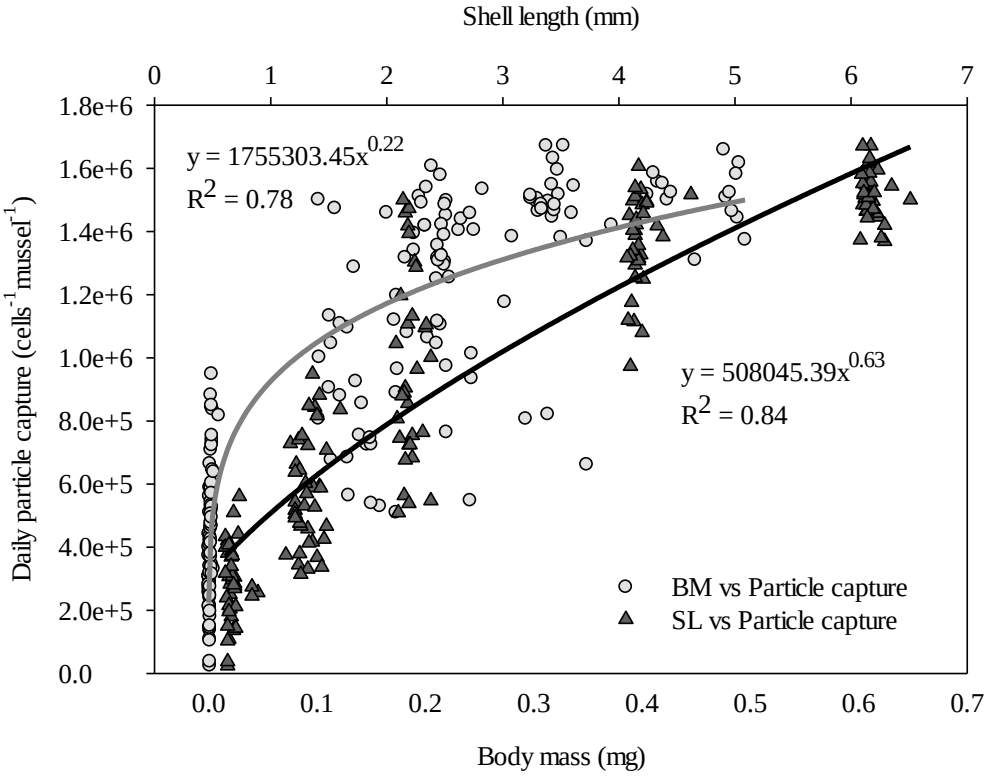

Figure 3.3. Daily (i.e., 24 h) particle capture for juvenile mussels in relation to SL and BM when supplied with four species of microalgae.

3.2.4. Faeces production over 24 h

The juvenile mussels were observed to begin filtering (i.e., shell gape and mantle extension to the shell margin and displacement of suspended particles adjacent to the exhalant siphon) almost immediately once placed in the solution of suspended microalgae regardless of the microalgae species provided and the size class of mussels. The first faeces were consistently produced within an hour of the experiment commencing regardless of microalgae species and mussel size. Mussels also consistently continued to produce faecal strings continuously over the 24 h of experimental period. Close examination of the faecal strings indicated that undigested cells were consistently present in all species of microalga regardless juvenile mussel size class.

3.3. Discussion

3.3.1. Continuous particle capture and cell volume capture for juvenile mussels

The filtering abilities of bivalves are dependent upon a variety of endogenous and exogenous factors, such as the state of development of filtering apparatus in growing juveniles (Cannuel et al., 2009; Gui et al., 2016c), and the concentration and physical qualities of suspended particles, such as microalgae (Brown et al., 1997; Gui et al., 2016a; Gui et al., 2016b;

Mamat & Alfaro, 2014; Newell et al., 2001; Pascoe et al., 2009; Rajesh et al., 2001; Ren et al., 2006; Tang & Riisgård, 2016; Ward & Shumway, 2004). These factors are likely to be highly important for ensuring the efficient delivery of live microalgal feeds to juvenile mussels whilst in nursery culture. For example, the feeding abilities of juvenile green-lipped mussels undergoing nursery culture are poorly defined, while studies of the morphology of the filter feeding apparatus (Gui et al., 2016b; Gui et al., 2016c) and short term feeding studies (Gui et al., 2016b; Sim-Smith et al., 2005) (Chapter 2) suggest their particle capture abilities are likely to change markedly with increasing size of the spat, as well as with different species of microalgae. However, short term measurements of particle capture (i.e., less than a few hours) in bivalves could be misleading if applied for longer term culture conditions (Sim-Smith et al., 2005), due to processes such as diurnal patterns of feeding behaviour (Lurman et al., 2013) or feeding satiation. Therefore, the aim of current study was to measure the continuous particle capture abilities of juvenile green-lipped mussels of a range of sizes (i.e., from 0.5 mm (M_1) to 7.0 mm (M_5) SL) when exposed to a high concentration of four microalgae species (i.e., 4×10^4 cells ml^{-1} or equal to 40 cells μl^{-1}).

The results indicated that the juvenile mussels were able to filter all microalgal species at a high rate for the 24 h period, and that particle capture abilities of the juvenile mussels increased markedly with their increasing size. Mean particle capture of juvenile mussels doubled from size class M_1 to M_2, and increased by 3.5 times from M_1 to M_3 mussels, and 5 times from M_1 to M_4 and M_5 size classes regardless of the microalgae species provided. Of the four microalgae species experimentally fed to the juvenile mussels, *T. suecica* tended to have the lowest particle capture rate for every size class of juvenile mussel while *D. lutheri* tended to have the highest. Taken over all size classes of juvenile mussels the mean particle capture of *D. lutheri* was on average 31.2 % higher than for *T. suecica*. There were no other differences in particle capture among the four species of microalgae that were revealed by the experiment.

The overall rapidly increasing particle capture abilities of juvenile mussels of increasing size when supplied with four different microalgae species is most likely due to the filtering capabilities of the mussels improving as the gill strucures develop and increase in size (Cannuel et al., 2009; Gui et al., 2016c). For example, juvenile green-lipped mussels of 0.3 mm SL which were fed with artificial feed (My Spat, Inve Technologies, Belgium) with a particle size range of 2 – 15 µm in diameter captured 47.5 % fewer suspended particles than mussels of 1.2 mm SL over a period of 1 h (Gui et al., 2016b). Likewise, the overall particle capture capabilities of juvenile green-lipped mussels increased by nearly an order of magnitude from mussels of 0.5 mm in SL to those of 6 – 7 mm (Chapter 2). The ontological development of the fine scale morphology of the gill structures of juvenile green-lipped mussels appears particularly

important to their improving filtering abilities (Gui et al., 2016c). Most importantly the length and number of the eulatero-frontal cilia (ELF) increase rapidly from early post-settlement juvenile (i.e., < 1 mm SL), greatly improving the particle processing abilities of the pallial organ. The short ELF in small juvenile mussels (i.e., < 1 mm SL) appear to cause difficulties for capturing and transporting suspended particles. In contrast, larger juvenile mussels (i.e., 6 – 7 mm SL) have more highly developed gill morphology, which are similar to the highly efficient filtering structures found in adults of this species (Gui et al., 2016c). It would seem that particle capture efficiency in juvenile mussels involves a complex interplay of the overall size and degree of development of the gill structures, as well as particle size and concentration (Dame, 2016; Gui, 2012; Newell & Shumway, 1993; Ren et al., 2006; Riisgård et al., 2014; Ward & Shumway, 2004).

The increasing particle capture ability could be also caused by the cell concentration provided in current study (40 cells μl^{-1}) which is likely to be under the threshold of optimum concentration for juvenile mussels. Likewise, it was confirmed that pseudofaeces production was absent in current study as supplied concentrations of microalgae were below the threshold for generating pseudofaeces, so that an increase in the experimental particle concentration may have increased the pseudofaeces production (Gosling, 2004). In general, at food particle concentrations below the pseudofaeces threshold, all particles retained by the gills will be directly ingested (Kiørboe & Møhlenberg, 1981). For example, in adult blue mussel, the pseudofaeces production will rise rapidly as the food particle concentration increases from 50 cell μl^{-1} to 100 cells μl^{-1} (Gosling, 2004). This indicates the cell concentration of 40 cells μl^{-1} used in this current study was likely to be close to the optimum concentration without producing pseudofaeces for a range of sizes of juvenile green-lipped mussels. However, the current study indicates that the experimental size range of juvenile mussels have the capacity to readily process higher concentrations of suspended particles.

Other factors that may influence filtering ability include the ambient environmental conditions. For example, it has previously been suggested that different experimental set ups may also cause variation in the measures of filtering abilities and subsequent absorption efficiency (Ren et al., 2006; Sim-Smith et al., 2005). This may have been the cause of the marked discrepancies in the measures of the filtering abilities of juvenile green-lipped mussels between 1 h and 24 h in this current study. Differences in the experimental set up, including differences in the initial microalgae concentration and the subsequent depleted concentration of microalgae, between short term (1 h) and long term (24 h) experiments may potentially explain this result. A similar discrepancy was found between short term FCM measures of particle capture in juvenile *P. canaliculus* (i.e., 1 mm in SL) and optimum feeding regimes in commercial

aquaculture practice, i.e., laboratory FCM = 4×10^3 cells mussel^{-1} day^{-1} (Sim-Smith et al., 2005) versus commercial culture conditions = 2×10^5 cells mussel^{-1} day^{-1} (Bruce, 2005; Gui et al., 2016b). The efficiency of mussels in selectively capturing particles is known to vary depending on the abundance and composition of suspended phytoplankton (Ren et al., 2006). For example, small blue mussels (i.e., 35 mm SL) ceased or reduced their filtering activity when exposed to low (i.e., 4 cells µl^{-1}) or high (> 30 cells µl^{-1}) concentrations of *Isochrysis galbana*, with the most efficient filtering occurring between those two concentrations (Pascoe et al., 2009). Changes in feeding behaviour could also explain the discrepancies between the short and long term measures of particle capture observed in juvenile mussels in this study. For example, adult *P. canaliculus* show a wider valve gape at night (i.e., continuous dark conditions) consistent with nocturnal filtering activity (Lurman et al., 2013). A similar result was found in the valve gape of adult *Mytilus galloprovincialis*, for which the valves opened wider (i.e., up to 6.2 ± 0.5 mm) at night time (Comeau et al., 2018). This is a possible explanation for the discrepancy in the results of the current study as the mussels in the 24 h experiment were subjected to 12L:12D cycle, whereas mussels in the 1 h experiment were held under continuous light.

Accurately determining whether bivalves are filter feeding is challenging and is considered to be a possible cause for the high variability among measures of bivalve filtering efficiency (Comeau et al., 2018; Maire et al., 2007; Newell et al., 2001). The valve gape behaviour is often used as a proxy for active filter feeding and valve gaping behaviour has been shown to correlate directly to the changes in suspended food particle abundance that is consistent with filter feeding in adult *Mytilus edulis*. Valve opening and closing behaviour is strongly influenced by the ambient feeding conditions. For example, mussels were observed to close their valves when exposed to a concentration of *Rhodomonas* sp. below 7×10^2 cells ml^{-1} or 0.9 µg chl-a L^{-1} (Riisgård et al., 2006). *Mytilus galloprovincialis* will open their valves when exposed to strong tidal flow and higher food concentrations of > 1 µg chl-a L^{-1} (Maire et al., 2007). However, other studies show that the valve gape of adult *M. galloprovinciallis* was less initially for in situ experiments only, whereas the valve gape remained high for the rest of the experimental period (10 days), regardless of fluctuations in the available food concentrations (Comeau et al., 2018). The current study confirmed that juvenile mussels were consistent in rapidly commencing filtering once placed in a suspension of cultured microalga based on the opening of the valves and the appearance of filtering water current. The filtering activity was observed to be continuous thereafter. However, the use of measures of valve gape in juvenile bivalves as a reliable indicator of feeding activity may require further investigation, as valve gape may be modulated by several physiological processes, such as oxygen consumption, photoperiod and

pumping rate, as well as filtering activity (Comeau, et al., 2018; Maire et al., 2007; Newell et al., 2001).

The feeding responses of bivalves to variations in the quality of food particles has been extensively studied (Brown et al., 1998; Mamat & Alfaro, 2014; Rajesh et al., 2001). One of the key variables that relate to the quality of potential food particles for filtering bivalves is their size. In the case of phytoplankton, selectivity for different sizes greatly influences the volume of the cells that are captured by bivalves, which will ultimately be important for their nutrient intake (Chitari & Anil, 2017; Harrison et al., 2015). In the current research, four species of microalgae (i.e. *D. lutheri*, *T. lutea*, *C. muelleri*, and *T. suecica*) were supplied at similar initial concentrations of 40 cells μl^{-1} across all juvenile mussel size classes. The results showed that estimates of the total volume of phytoplankton cells captured by juvenile mussels (0.5 – 7.0 mm SL) over 24 h varied and the cell volume capture increased as the juvenile mussel increased in size. Mean total cell volume of microalgae captured by juvenile mussels doubled from size class M_1 to M_2, and increased by three times from M_1 to M_3 mussels, and~5 times from M_1 to both M_4 and M_5 size classes regardless of the microalga species provided. Larger cell volume of both microalgae *T. suecica* and *C. muelleri* tended to be captured on average by 4 × more total cell volume compared to both microalgae *D. lutheri* and *T. lutea* (i.e., both smaller cell volumes) regardless of the juvenile mussel size classes (Table 3.3), despite the total number of both *T. suecica* and *C. muelleri* cells captured being lower (Table 3.2). There were no other differences in total cell volume captured among the four species of microalgae that were revealed by the experiment. In contrast, during a short period of experimental feeding (1 h) (Chapter 2), the total number of *T. suecica* cells that were captured was higher than for *D. lutheri* for juvenile mussels of two size classes M_1 (0.5 – 0.9 mm SL) and M_2 (1 -1.9 mm SL). The comparatively low capture of the larger cells of *T. suecica* over (24 h), could be of the result of gut satiation triggering reduced filtering, a common behaviour observed in other bivalves (Riisgård etal., 2011). Such feeding inhibition is unlikely to be observed in short term feeding experiments (i.e., 1 h), resulting in measurements during this time recording high initial capture of the larger and more accessible cells (Gui et al., 2016a). From an energetic point of few, ingesting larger cells would be more advantageous for a juvenile mussel versus ingesting smaller ones, particularly as the volume of a 20 µm diameter cell is 300 times that of a 3 µm diameter cell (Raby et al., 1997). This would assume that the nutritional content of cells is directly proportional to the cell size so that a large number of small cells are equivalent to few larger cells in term of carbon biomass, which in general appears to be the case (Harrison et al., 2015).

3.3.2. Continuous particle capture in relation to mussel size

The relationship of shell length to juvenile mussel body mass was best described by an exponential function, which is similar to previous studies on *P. canaliculus* and *M. edulis*. With increasing shell length there is a relative increase in shell content and the body mass able to be enclosed within the valves (Hickman & Illingworth, 1980; Jacobs et al., 2015; Walne, 1970). There was a corresponding non-linear relationship (i.e., a power function) for the total number of phytoplankton cells captured in 24 h in relation to both the mussel shell length (SL) and body mass (BM). Filtration abilities did not continue to increase in direct proportion to body size despite the rapid increase in the size and complexity of the gill structures that have been described for this size range of juvenile *P. canaliculus* (Gui et al., 2016c). The relatively high efficiency of the filtering ability of small juveniles of this species, despite their small and rudimentary gill structure most likely reflects the critical importance of securing nutritional resources to fuel the establishment and growth of this benthic settler.

3.3.3. Faeces production over 24 h

The results from the current study show that faeces started to be produced within the first hour and continued to be produced throughout the observation intervals (i.e., 6, 12 and 24 h) regardless of the size of the juvenile mussels or the species of microalga provided. When placed in high concentrations of food particles, bivalve molluscs can limit intake either by reducing their filtration activity or discharging excess captured particles as pseudofaeces (Iglesias et al., 1992). However, no pseudofaeces were observed in this study regardless of mussel size or phytoplankton species provided. Generally, bivalves will not initiate filter feeding in dilute suspensions of food particles (Gosling, 2004) with filtration commencing once ambient food concentration reaches a critical threshold. For example, to commence active filtering adult blue mussels require initial food concentration of about 50 cells μl^{-1} of the microalga *Phaeodactylum tricornotum* and filtering activity will remain high with microalga concentrations of up to 70 cells μl^{-1} (Foster-Smith, 1976). However, adult blue mussels will filter feed at maximum capacity when exposed to *Rhodomonas baltica* at a concentration of 3-10 cells μl^{-1}, but filtration activity will decrease when the concentration is increased to 15 cells μl^{-1} (Riisgård, 1991).

The current study provides relatively higher concentrations of suspensions of microalgae compared to previous studies (i.e., approximately 8× higher) (Bruce, 2005; Gui et al., 2016), however, the concentration provided in the research was 1.2 – 6 × lower than suppled to juvenile mussels during the commercial nursery culture *P. canaliculus* (S. Cumming, pers. comm). The food particle concentration used in the current study was selected as an optimum concentration to be delivered to the juvenile mussels based on previous work (Gui et al., 2016a; Sim-Smith et

al., 2005), as well as from an estimation of feed consumption in the commercial juvenile mussel nursery situation (S. Cumming, pers.comm.) and the reported optimum concentration for blue mussels (Gosling, 2004). Providing excessive concentrations of microalgae to juvenile mussels has the potential to be wasteful if it is inadequately utilised by the mussels, such as resulting in the diversion of filtered microalga cells into the production of pseudofaeces. Current commercial nursery practice has found that the juvenile mussels capture between 6 – 62% of the total food particles provided (S. Cumming, pers. comm.). This indicates that the relatively high microalgal concentration provided in the present study (i.e., 40 cells μl^{-1}) was below the pseudofaeces threshold and within the upper limit of filter feeding capabilities of juvenile green-lipped mussels. This suggests juvenile green-lipped mussels are capable of making good use of relatively high phytoplankton abundance when available. However, suspension feeding bivalves exposed to increasing seston concentrations may reduce the time spent filtering or reduce their clearance rate (Foster-Smith, 1976). Therefore, it is possible that given the lack of production of pseudofaeces in this current study, that juvenile mussels were reducing their filtering activity, which could explain the differences observed in the extent of filtering observed between *T. suecica* and *D. lutheri* for the juvenile mussels.

Despite the particle concentrations, other factors such as carbon content or nutritional content of food particles play an important role in particle capture preferences of bivalve feeding (Dupuy et al., 1999; Sieburth et al., 1978; Ward & Shumway, 2004). Under natural coastal conditions, different seasons will affect the composition of the abundance of suspended microalgae, and as a consequence it is likely to influence the particle selection by bivalves, such as mussels (Defossez & Hawkins, 1997; Safi & Hayden, 2010). For example, adult *P. canaliculus* were observed to consistently capture both large (i.e., *Conscinodiscus* sp, *Ditylum brightwellii*) and small (i.e., *Pseudo-nitzschia* sp., *Navicula* sp.) diatoms from coastal waters. However, these diatom species were subsequently mostly rejected through mucus-bound pseudofaeces. In contrast, adult *P. canaliculus* preferred to ingest both small and large flagellated species including *A. sanguinea* and *Karenia* spp. versus diatom species (Safi & Hayden, 2010). This feeding preference may be due to dinoflagellates and flagellates having higher carbon ratios compared to diatoms (Menden-Deuer & Lessard, 2000), as well as have higher assimilation efficiency in adult green-lipped mussels, i.e., dinoflagellates (84.5%) compared with flagellates (77.9%) and diatoms (61.7%) (Ren et al., 2006). Although, diatoms are often a major component of seston in coastal waters, the physical properties of the cells (i.e., the siliceous frustule) makes diatom more difficult for the bivalves to digest and assimilate the cell contents (Safi & Hayden, 2010; Tomas, 1997).

In the current study microscope observations of faeces from juvenile mussels identified closely packed undigested microalgal cells regardless of the microalgae species provided or the size of juvenile mussels. Undigested microalgal cells in faeces can be caused by an overloading of both filtering apparatus and digestive systems caused by excessive microalgae concentration in the water column (Riisgård et al., 2011). Therefore, in the current study the presence of the undigested material in the faeces could be an indication of saturation of the feeding and digestive systems resulting from exposing mussels to a high concentration of microalgae particles. Overall, this suggests that juvenile green-lipped mussels, whilst having a high capacity for filtering particles, may have limited abilities to selectively manage their particle intake. A similar conclusion was drawn in relation to the lack of particle selectivity in the filtering behaviour of juvenile green-lipped mussels in previous studies of their feeding behaviour (Gui et al. 2016a, 2016b, 2016c).

3.3.4. Implications for green-lipped mussel aquaculture

The commercial green-lipped mussel hatchery in New Zealand, currently feeds their juvenile mussels with four species of cultured microalgae that were also used in this study *T. lutea*, *D. lutheri*, *C. muelleri* and *T. suecica* (S. Cumming, pers. comm.). The continuous particle capture observed in the current study for the smallest juvenile mussel size class (M_1 and M_2) was higher (i.e.,~ > 2.4 - 2.9 × 10^5 cells mussel^{-1} day^{-1}) than the previous study which estimated at 2 ×10^5 cells mussel^{-1} day^{-1} (Bruce, 2005) and compared to an extrapolation from short term (1 h) feeding observations (i.e.,~1.45 × 10^4 cells mussel^{-1} day^{-1}) (Table 3.2). The result of this study indicated that providing microalgae cells of a larger size may potentially reduce the amount of microalgae required for rearing juvenile green-lipped mussels of < 1 mm SL, (i.e., using the larger *T. suecica* versus *D. lutheri*). The current experiment tested only one concentration of microalgae under controlled laboratory conditions over a 24 h feeding period, suggesting that 40 cells µl^{-1} was still an acceptable concentration to stimulate efficient filter feeding for juvenile green-lipped mussels over a range of sizes. Thus, the result of the current study suggests that providing large microalgae species (i.e., *T. suecica*) may potentially reduce the microalgal food requirements for rearing green-lipped mussel spat (i.e., 0.5 – 7 mm SL) and improve their feeding efficiency in a commercial nursery situation. Given the cell concentrations would have exponentially decreased over the course of the experimental period, maintaining the microalgal cell concentration over the entire duration of the experiment would more tightly define the particle capture capabilities for the juvenile mussels. Further research to examine the assimilation efficiency of microalgae supplied to juvenile mussels at different concentrations appears to be warranted in order to understand the significance of the consistent appearance of undigested microalgae cells in the faeces of juvenile mussels.

3.4. Conclusion

This study revealed that when observed over 24 h, juvenile mussels of a range of sizes were continuously capturing all four microalgae species provided. The largest microalgae (i.e., *T. suecica*) had a lower number of total cells captured compared to the smallest microalgae (i.e., *D. lutheri*), however, the situation was reversed when the intake of total cell volume was compared. The continuous production of faeces by the juvenile mussels of a range of sizes over the 24 h experimental period indicates that the mussels continued to actively filter all four microalgae species. However, the presence of undigested microalgal cells in these faeces, and the lower number of *T. suecica* cells captured compared with *D. lutheri* may indicate satiation of the digestive function in juvenile mussels. Thus, providing larger sizes of microalgae species may potentially reduce the microalgae food requirement and improve feeding delivery for *P. canaliculus* spat in the nursery situation. Further studies of assimilation and growth efficiencies of juvenile mussels fed with microalgae species of larger cell size and at different concentrations should allow precise definition of the most efficient or minimum ration of a given microalgae diet.

Chapter 4. Effect of different environmental conditions on growth, survival, and retention of green-lipped mussel spat, *Perna canaliculus*

4.1. Introduction

One of the challenges for developing green-lipped mussel, *Perna canaliculus*, aquaculture in New Zealand is the extensive reliance on the wild-caught juvenile mussels, known as spat, for initiating the production cycle. Most of the wild mussel spat supply (~60 %) comes from one location, Ninety Mile Beach in northern New Zealand (Alfaro et al., 2010; Jeffs et al., 1999). Intermittently, large quantities of seaweed and other debris covered in very high numbers of spat washes ashore at this beach, and this material is subsequently collected, before being transported around the country to be used for seeding out mussel farms. Upon arrival at a mussel farm site, the debris with the attached spat is placed alongside fibrous polypropylene growout rope and held in place with cotton socking. Then the socking-covered rope containing the spat material is suspended in the seawater beneath the backbone lines which are held near the water's surface by large plastic floats on the mussel farm (Dawber, 2004; Hickman, 1991). Most of the remaining green-lipped mussel spat (~ 35 %) used in the New Zealand mussel aquaculture industry are caught as settling larvae on fibrous spat collecting ropes, which are deployed in locations with naturally high larval settlement. Once the settled mussels have established as juvenile mussels on the spat collecting ropes they are transferred to mussel farms for further on-growing. A small proportion of the spat (< 5 %) used by the New Zealand mussel industry is supplied by a commercial hatchery and these mussels are settled in the hatchery onto a growing rope before the rope is deployed onto mussel farms (South, 2018). Regardless of the source of mussel spat, following their seeding onto mussel farms, there is typically a great loss of spat from the growing ropes, usually more than 50 % and sometimes greater than 95 % (Hayden, 1995). For example, 64.4 – 89.0 % of hatchery-reared spat were lost 20 - 25 weeks after their deployment onto a coastal mussel farm in the Marlborough Sounds, in the South Island of New Zealand (South, 2018). This sizeable loss of spat from ropes is a major inefficiency in production in this industry. The rearing of green-lipped mussel spat in a nursery situation up to a size of 4 – 6 mm in shell length (SL) may greatly reduce spat losses because mussels of this larger size

lose their secondary settlement behaviour which is thought to be the major underlying cause of the losses of spat of a smaller size (Buchanan & Babcock, 1997; Gui, et al., 2016c; Sim-Smith & Jeffs, 2011). Therefore, developing more efficient methods for nursery culture of mussel spat to reach this size has the potential to lead to a marked increase in the efficient use of mussel spat in this industry. However, the efficient nursery culture of mussel spat relies on optimising nursery holding conditions to maximise the growth and survival of the spat, so as to minimise the duration and cost of operation of the nursery culture systems.

The effect of various environmental factors on mussel attachment to the substrate, such as wave motion (Alfaro, 2005; Pernet et al., 2003), water flow and aeration (Alfaro, 2006), and light exposure, including photoperiod, (Carl et al., 2011; Lurman et al., 2013) whilst held under laboratory conditions are known to be of varying importance among several mussel species. High water motion appears to promote stronger attachment through the production of more byssus threads in juvenile *P. canaliculus* (Alfaro, 2006). High water velocity (40 cm s^{-1}) has also been associated with prevention of spat migration in *P. canaliculus* on mussel farms (Hayden & Woods, 2011) and faster growth in spat of *Perna viridis*, possibly as a result of assisting with the delivery of food particles to the juvenile mussels (Rajagopal et al., 1998). Aeration is one of the most effective methods for generating water motion in a tank culture situation, however, aeration also improves levels of dissolved oxygen, which may also have an influence on spat performance independently, or in concert with, any effect produced by the water motion resulting from the aeration (Alfaro, 2006). Low oxygen levels and low water flow have been associated with increased mortality and reduced settlement of larval *P. canaliculus* (Alfaro, 2005). Furthermore, low oxygen concentrations at below 2 mg l^{-1} have been shown to induce a rapid reduction in respiration (i.e., < 0.33 mg O_2 h^{-1}) and filtration activity (i.e., < 2.09 l h^{-1}) in adult blue mussels, in comparison to those exposed to higher oxygen concentrations (Tang & Riisgård, 2018).

Another environmental factor, which may influence mussel spat attachment and growth is light exposure and photoperiod. Exposure to longer periods of diurnal darkness has been found to result in higher growth as measured by shell length in juvenile blue mussel, *M. edulis* (Nielsen & Strömgren, 1985) and the eastern oyster, *Crassostrea virginica* (Medcof & Kerswill, 1965). For example, juvenile blue mussels of 14 – 22 mm SL that were cultured in tanks under continuous darkness for 18 days had 9 - 25 % greater shell growth than those cultured under natural daylight conditions (Nielsen & Strömgren, 1985). In addition, juvenile blue mussels appeared to have longer periods of feeding activity under 24 h of continuous darkness than when exposed to continuous dim daylight conditions (Nielsen & Strömgren, 1985). It appears likely that *P. canaliculus* also responds to photoperiod because adults (± 18 months old) show a wider

valve gape of mussels at night, consistent with active nocturnal feeding compared to periods of exposure to more intense light during the day (Lurman et al., 2013; Trottier & Jeffs, 2015). Furthermore, byssus attachment behaviour in adult *P. canaliculus* was observed to be minimal during daylight hours with the majority of byssal threads being attached during the night (Lurman et al., 2013).

Given the potential of light regime, oxygen concentration, and water motion to influence the growth, survival, and retention of mussel spat in nursery culture systems, there is value in assessing these environmental factors with a view to optimising the performance of nursery systems for *P. canaliculus*. Therefore, the aim of the present study was to identify the importance of these environmental parameters to the performance of mussel spat so that the effectiveness of the nursery culture for spat of green-lipped mussel can be maximised by applying optimum environmental conditions. This research tests the hypothesis that growth, survival and retention of juvenile *P. canaliculus* is unaffected by differences in the environmental conditions (i.e., photoperiod, aeration, water flow, and oxygen) on the growth, survival and retention of juvenile mussels held under nursery conditions in the laboratory.

4.2. Materials and methods

4.2.1. Preparation of spat

Juveniles of *Perna canaliculus* were supplied from a commercial shellfish hatchery located in Nelson, New Zealand; Shellfish Production and Technology New Zealand Ltd (SpatNZ Ltd). The size of mussel spat used in the study were typical of those entering the nursery phase of production, ranging in shell length from 0.5 to 1.5 mm (i.e., plantigers that were ~30 days post-settlement). All the mussels were held in 10 l aerated holding tanks for three days, which had been filled with UV treated and filtered (5 µm) seawater held at 18 °C in the seawater laboratory of the School of Biological Sciences, University of Auckland. The tank was cleaned every day, with the seawater changed by draining it through a 250 µm sieve to retain unattached mussels prior to being refilled with fresh filtered seawater. Immediately prior to experimentation, sets of 100 mussels were randomly selected from the holding tank and their shell length was determined using a digital camera (Samsung Galaxy A5) connected to a dissecting microscope (Leica Wild M3C). The shell length of the mussel spat was then measured from the digital images using Image J software (Fitzpatrick et al., 2013) and individual mussels were randomly allocated to one of the 20 identical tanks used for each of the experiments.

4.2.2. Preparing microalgae culture for spat feeding

Four species of microalgae that are commonly used in commercial shellfish hatchery and nursery systems were cultured using standard axenic culture methods until the exponential phase of culture, prior to feeding to the mussel spat (Sim-Smith et al., 2005): *Tisochrysis lutea*, *Nannochloropsis atomis*, *Chaetoceros muelleri*, and *Diacronema lutheri*. Initially, in the holding tank (first 3 days) and for the first 10 days of the experiment, mussels were fed the microalgae at a concentration of 200,000 (2×10^5) cells $spat^{-1}$ day^{-1} which is known to promote filtering activity in green-lipped mussel spat of this size regardless of the microalgal species (Bruce, 2005; Gui et al., 2016b). After 10 days the food concentration was increased to ~800,000 (8×10^5) cells $spat^{-1}$ day^{-1} to ensure the growing mussel spat were fed to satiation (Bruce, 2005). Across the experimental period the four microalgae species were fed to the juvenile mussels on a daily rotation (i.e., one species of microalga after another per 24 h) at identical concentrations. This rotation feeding was used due to the limited capacity of the laboratory to provide mass culture of microalgae species. The spat were transferred to conical experimental tanks after the initial three days in the holding tank.

4.2.3. Experimental design

The experimental mussel spat culture unit was made from 20 plastic conical drink bottles (1.5 l) which were placed upside down on a holding table with the base of each bottle cut off (Fig. 4.1) (Gui et al., 2016b). A small round air stone of 2.2 cm in diameter was sealed into the neck of each bottle through which air was bubbled into the tank at a rate of 1 l min^{-1} unless experimental conditions required otherwise as described below. A short length (5 × 18 cm) of attachment substrate made from fine plastic mesh (1 mm square nylon mesh) was suspended within each tank to facilitate the attachment of mussel spat. The attachment substrate was held in position by being tied to a cotton string, which was weighed down by tying a stainless-steel nut (316 grade stainless steel) at the end of the string (Fig. 4.1). The tanks were small enough in size and of a conical shape, which helped to ensure good mixing of the enclosed water, especially with the provision of aeration and water flow.

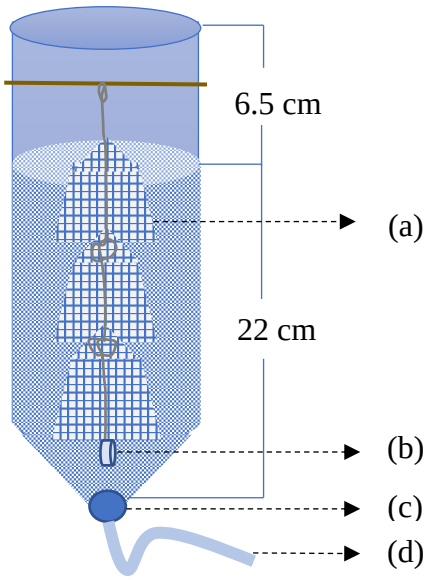

Figure 4.1. An experimental conical tank made from a 1.5 l plastic drink bottle; (a) plastic mesh for mussel spat attachment, (b) stainless steel nut for weighing down material, (c) air stone, and (d) air supply tube. A total of 20 conical tanks were used in the experimental mussel spat culture unit used for this research.

Four separate experiments were undertaken using the experimental mussel spat culture unit (i.e., conical tanks), with each experiment testing one of the four environmental factors (i.e., photoperiod, aeration, water flow, and oxygen concentration) with similar methods used for setting up and running the experimental culture unit for each experiment. Each of the four experiments had four treatments consisting of four different variations of the environmental factor being examined. Each treatment had five replicate conical tanks.

For each of the four experiments the performance of the 100 mussel spat (i.e., growth, survival, and retention) in each of the 20 conical tanks were observed for a 25 day experimental period which is sufficient time for experimental differences in the response variables to be observed (Alfaro, 2006). The apparatus was housed in a temperature-controlled room to help maintain constant temperature (18 °C) in each conical tank.

Each day during the experimental period each conical tank was cleaned by draining the seawater from each tank through a 250 µm sieve to catch any unattached mussels, and then rinsed with clean filtered seawater and the washings drained through a sieve, before refilling the tank with clean seawater. Shells of dead mussels caught on the sieve were counted (further explained in section 4.2.4.2). Any detached live mussels were counted and returned with the attachment substrate to the corresponding experimental tank after the tank had been refilled with freshly filtered seawater.

For the photoperiod, aeration and water flow experiments the water quality (i.e., dissolved oxygen and temperature) was measured with a YSI (Professional Plus) water quality instrument for each conical tank once a week immediately after the tank had been cleaned (t_0) and after a 24 h period immediately prior to cleaning and water replacement (t_{24}). For the oxygen concentration experiment, the water quality measurements were conducted for each tank every day immediately after the tank was cleaned and refilled (t_0) and again after 24 h immediately prior to water replacement (t_{24}).

Photoperiod

This experiment used four light regime treatments; (1) 24 h dark (24D), (2) 12 h light and 12 h dark (12L:12D), (3) 6 h light and 18 h dark (6L:18D), and (4) 24 h light exposure (24 L). All conical tanks were individually wrapped in black plastic to block any ambient light from the laboratory entering the tanks. The experimental tanks receiving light exposure (i.e., all except the 24D treatment) were equipped with lids holding two LED lamps (0.6 W) with an output intensity of 4 cd which emitted light down into the conical tank when operating. The lamps were connected to automatic timers that were set according to the required experimental light regime. The light intensity was measured by using a Sekonic light meter.

Aeration

This experiment had four aeration treatments; (1) without aeration (NA), (2) low aeration of 2 ml s^{-1} (LA), (3) medium aeration of 33 ml s^{-1} (MA), and (4) high aeration of 125 ml s^{-1} (HA), which were each supplied through the air stone at the base of the conical tanks. The aeration was provided from miniature gas diaphragm pumps (Koge Electronics Co. Ltd, China) for which the air output was individually controlled using a voltage regulator and was set at the outset of the study and checked at each tank cleaning event. The aeration treatments provided in the current study were based on a previous study, which indicated that there is a potential for aeration to influence the performance of juvenile mussels (Alfaro, 2006).

Water Flow

This experiment used four water flow treatments; (1) without water flow (NF), (2) low water flow of 1 – 2 cm^3 s^{-1} (LF), (3) medium water flow of 4 –5 cm^3 s^{-1} (MF), (4) high water flow of > 5 – 8 cm^3 s^{-1} (HF). The different water flows were provided by a 12 V micro-pump (Qingdao Ahead Electric Co., Ltd) attached to the lid of each conical tank to circulate water inside each conical tank via intake and outflow pipes placed into the tank made from aquarium air tubing (Fig. 4.2). Filter mesh (80 µm) was placed over the ends of both pipes to prevent mussels entering the tubing. The water flow produced by each micro-pump was adjusted through

varying the voltage supplied to the micro-pump. The water flow treatments in the current study were based on water flows identified in previous studies (Alfaro, 2005; Hayden & Woods, 2011).

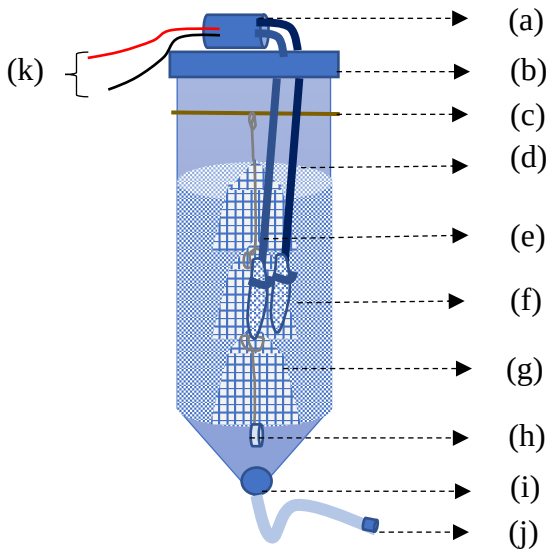

Figure 4.2. Experimental conical tank set up for different water flow regimes; (a) micro-pump, (b) tank lid, (c) stick holder, (d) inflow pipe, (e) outflow pipe, (f) filter mesh (80 µm), (g) plastic mesh substrate, (h) stainless steel nut, (i) air stone, (j) stopper, and (k) electrical wires connected to power supply.

Oxygen concentration

This experiment used four dissolved oxygen treatments; (1) 40 % (O_{40}), (2) 60 % (O_{60}), (3) 80 % (O_{80}), and (4) 100 % (O_{100}). The different dissolved oxygen concentrations in the experimental tanks were achieved by supplying individual tanks with adjusted gas mixtures at a constant flow of 33 ml s^{-1} through the air stone at the base of each conical tank. The O_{100} treatment used air that had been stripped of carbon dioxide by passing it over soda lime, while all other dissolved oxygen treatments were achieved with appropriately adjusted mixtures of air and pure nitrogen (N_2) gas. The DO treatments provided in the current study were based on a preliminary study on two separate subsets of experiment (i.e., aeration and water flow) which incidentally resulted in various oxygen concentrations among treatments. The DO treatments were selected to provide oxygen concentrations similar to those in these prior experiments after t_{24} (final) measurement of DO concentration with the aim of separating any effect of oxygen concentration on juvenile mussel performance (Alfaro, 2005).

4.2.4. Performance of spat

The performance of mussel spat in response to the experimental treatments was assessed by determining differences in the growth, survival, and retention of mussel spat measured for the 100 mussels in each conical tank as described below.

4.2.4.1. Growth

The starting size of *P. canaliculus* spat was determined by measuring the initial shell length (SL) of mussels (n = 100) at the outset of the experiment for each conical tank.

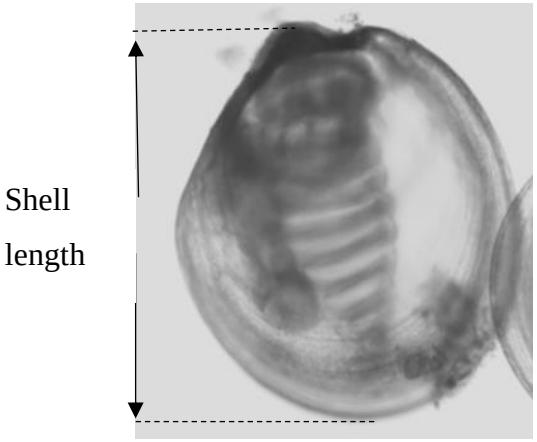

Figure 4.3. Spat of *Perna canaliculus* showing the locations for measuring shell length from apex of shell and greatest perpendicular distance to the shell margin.

All mussels were placed in a petri dish and a digital image recorded with a calibration scale within the frame. The shell length of each mussel was then determined from the image using Image J software (Fig. 4.3). This process was repeated again at the end of the experiment to determine the final size of the remaining mussels. An estimate of the growth of mussel spat for each tank was determined by comparing the mean final size of the mussels with their mean initial size, assuming there is no size selective mortality of mussels during the experiment.

4.2.4.2. Survival

On day 25 all mussels were removed from each tank and their status recorded (i.e., alive or dead, and whether they were attached or unattached to substrate, and if they were attached, what substrate they were attached to (i.e., mesh or bottle). The number of live mussels at the end of the experiment (day 25) was used to calculate the percentage of survival of the initial number of mussels stocked into each tank, (i.e., 100 mussel spat). The total number of dead juvenile mussels caught in the sieves each day over the course of the experiment was used to provide confirmation of the survival estimate.

$$\text{Survival (\%)} = \frac{\text{number of surviving mussels in tank after 25 days}}{\text{number of mussels initially placed into tank}} \times 100$$

4.2.4.3. Retention

To measure the percentage retention of seed mussels, the number of mussels remaining attached to the mesh substrate at the end of the 25 day experimental period was calculated as a proportion of the total number of mussels remaining alive in the tank at the end of the 25 day experiment (Carton et al., 2007).

$$\text{Retention (\%)} = \frac{\text{number of mussels attached to mesh substrate after 25 days}}{\text{number of live mussels at the end of the 25 days}} \times 100$$

4.2.5. Statistical analyses

A one-way nested ANOVA was used to confirm the initial shell length of the mussels was uniform among conical tanks and treatments for each experiment. With this requirement fulfilled, the final size of mussels was compared among conical tanks and treatments with the same statistical approach. When a significant difference amongst means was detected by the ANOVA, a pairwise comparison of means using Tukey's HSD test was used to identify differences the between mean final shell length.

One-way ANOVAs were used to compare mean survival and mean retention of mussels among treatments for each experiment. All percentage data (i.e., spat survival and retention) were firstly arcsine transformed prior to analysis. All data were assessed a priori for normality using a Shapiro-Wilk's test, and for homogeneity of variance using a Levene's test. Where an ANOVA was significant, pairwise Tukey's HSD post-hoc tests were then used to identify the differences between pairs of means whilst also applying a Bonferroni correction for inflated familywise error due to multiple testing of the same data set.

Water quality parameters (i.e., temperature (ºC) and DO (%)) were compared among treatments in each experiment (i.e., photoperiod, aeration, water flow, and oxygen concentration treatments) with a one-way nested ANOVA. This analysis was used to confirm the initial water quality (t_0) for each environmental condition was uniform among the conical tanks and treatments for each experiment. The final water quality (t_{24}) of each environmental condition was compared among conical tanks and treatments with the same statistical approach. Any significant differences detected among means was followed by pairwise comparisons of means using Tukey's HSD tests.

The statistical analyses were performed using SPSS version 24, Minitab version 18 and Sigma Plot version 14.0.

4.3. Results

4.3.1. Photoperiod

4.3.1.1. Temperature and dissolved oxygen

Temperature

The initial mean water temperature (t_0) among the four photoperiod treatments and for each conical tank within each photoperiod treatment were consistent at 19.14 ± 0.03 ºC ($F_{(3, 40)}$ = 0.84, P = 0.48 among treatments, and $F_{(16, 40)}$ = 1.29, P = 0.25 within treatments). However, after 24 h (t_{24}) the mean water temperature declined in all experimental tanks. There were temperature differences among the four photoperiod treatments ($F_{(3, 40)}$ = 12.53, P < 0.0001), and these differences were consistent among the individual tanks within each photoperiod treatment ($F_{(16, 40)}$ = 1.19, P = 0.32) (Fig. 4.4).

After 24 h (t_{24}) tanks in the 12L:12D and 24 D treatments had the lowest mean temperatures at 18.25 ± 0.01 ºC and 18.36 ± 0.01 ºC respectively. The tanks in the 6L:18D treatment had a higher mean temperature (18.43 ± 0.01 ºC) than for the 12L:12D, but this was not higher than the 24D treatment. The mean water temperature in the 24L treatment (18.56 ± 0.01 ºC) was higher than for both the 12L:12D and 24D treatments, but not the 6L:18D treatment (Tukey's HSD, P < 0.05) (Fig. 4.4).

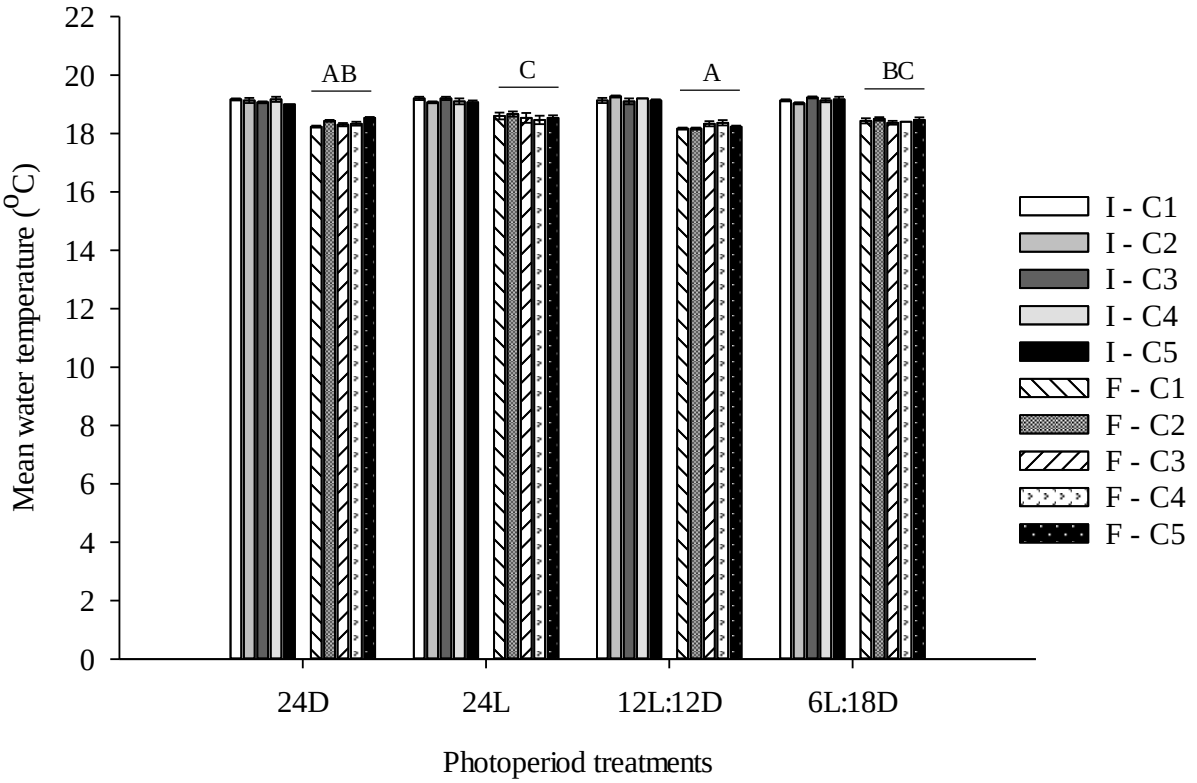

Figure 4.4. Mean water temperature for four photoperiod treatments and within five replicate tanks within each treatment measured at initial (I = t_0) and final (F = t_{24}) time points at weekly intervals over a 25 day experimental period, C (conical tank #), D (hours of dark), L (hours of light). Different letters above the group of bars indicate significant differences between the treatment means.

Dissolved oxygen

The initial (t_0) mean oxygen concentrations among the four photoperiod treatments and for each conical tank within each photoperiod treatment over the experimental period were consistent at 99.77 ± 0.15 % ($F_{(3, 40)} = 0.54$, $P = 0.66$ among treatments and among tanks within treatments $F_{(16, 40)} = 0.86$, $P = 0.62$).

There was a difference in the mean final (t_{24}) oxygen concentrations among the four photoperiod treatments ($F_{(3, 40)} = 3.49$, $P = 0.02$), however, it was not possible to subsequently distinguish any differences among the treatment means because they were very small in extent (Tukey's HSD, $P > 0.05$) (Fig. 4.5).

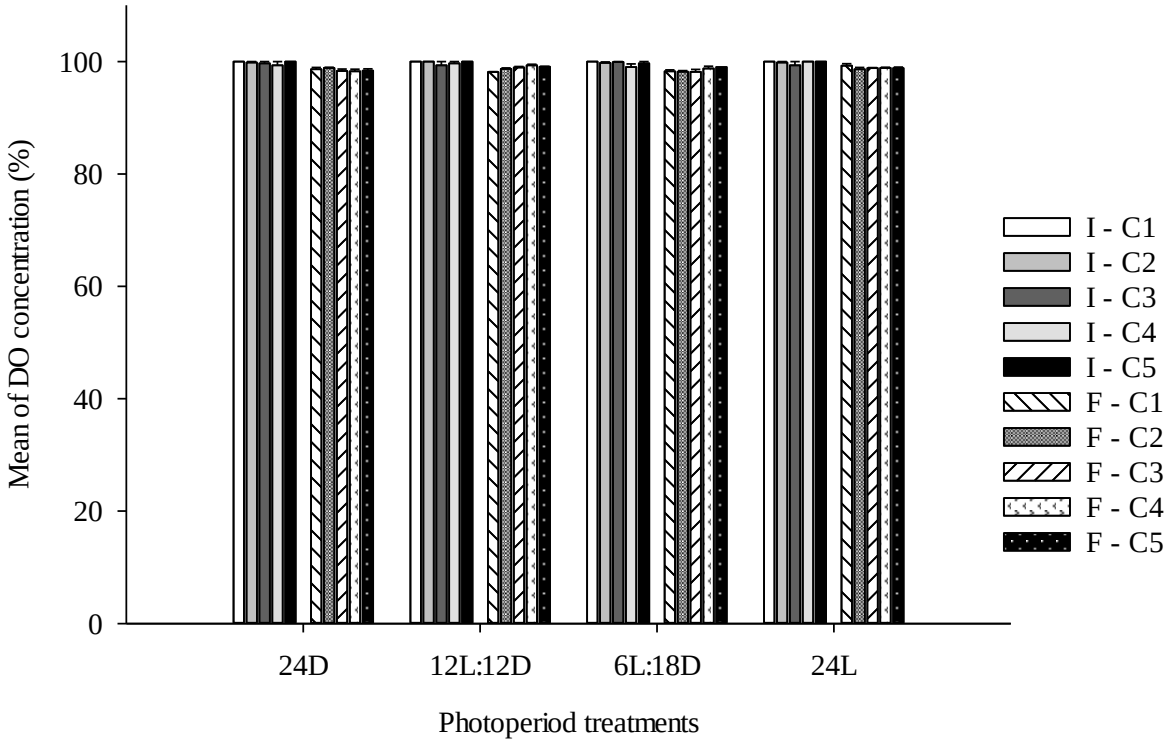

Figure 4.5. Mean oxygen concentrations for four photoperiod treatments and within five replicate tanks within each treatment measured initial (I = t_0) and final (F = t_{24}) at weekly intervals over a 25 day experimental period, C = (conical tank #), D (hours of dark), L (hours of light). There were no significant differences detected for mean DO for t_0. There were differences among treatments for t_{24}, but pairwise comparisons were unable to identify differences among the treatment means.

4.3.1.2. Growth

There was no significant difference in the initial mean shell length of mussels among the four photoperiod treatments ($F_{(3, 1980)} = 1.77$, $P = 0.15$), or for tanks within the photoperiod treatments ($F_{(16, 1980)} = 1.42$, $P = 0.12$). The overall initial mean shell length of the mussels was 0.99 ± 0.01 mm.

After the 25 day experimental period there were differences in the mean shell length of the mussels as a result of the different photoperiod treatments ($F_{(3, 1841)} = 15.66$, $P < 0.0001$), and there were also differences among tanks within the photoperiod treatments ($F_{(16, 1841)} = 9.76$, $P < 0.0001$).

Among the photoperiod treatments, the smallest final mean size of mussels was observed in the 24D treatment, i.e., 1.54 ± 0.18 mm. Greater mean shell lengths of mussels were identified in the three treatments which experienced some light exposure (i.e., 24L, 12L: 12D, 6L: 18D)

compared to 24D. However, there were no significant differences in mean shell length between any of these three treatments which had different durations of light exposure (Tukey's HSD, $P > 0.05$) (Fig. 4.6).

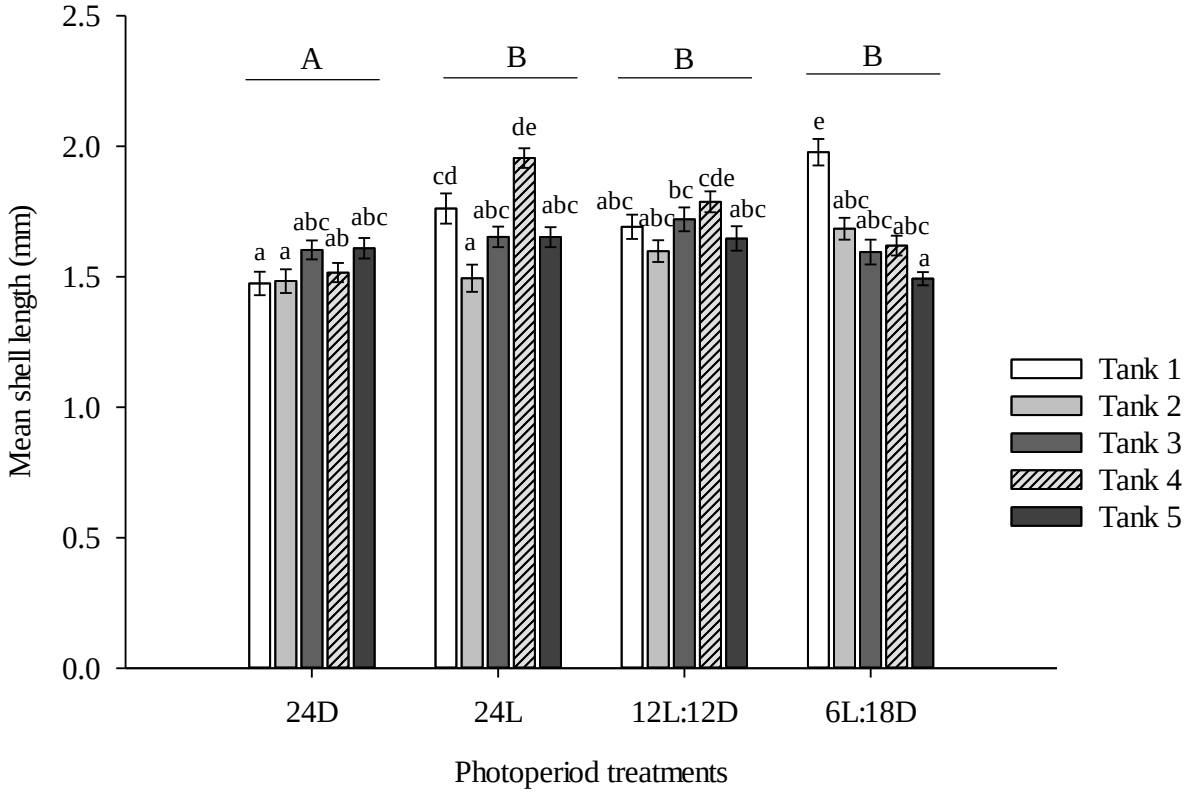

Figure 4.6 Final mean shell length of mussel spat raised for 25 days under four different photoperiod treatments, D (hours of dark), L (hours of light), conical tank #. Different letters above a group of bars indicate significant differences in the mean values for the treatments. Different letters above the individual bars indicate significant differences between means for individual tanks within treatments (Tukey HSD, $P < 0.05$).

There were differences in final mean shell length among individual tanks within all four photoperiod treatments (Tukey's HSD, $P < 0.05$) (Fig. 4.6). These differences among individual tanks were most pronounced for the 6L:18D and 24L treatments.

4.3.1.3. Survival

The overall mean survival of mussel spat was 90.3 ± 0.6 % after the 25 day experimental period regardless of the four photoperiod treatments ($F_{(3, 16)} = 0.87$, $P = 0.96$).

4.3.1.4. Retention

After the 25 day experimental period, there were no differences in the mean retention of the green-lipped mussel spat on plastic mesh substrate as a result of the different photoperiod

treatments ($F_{(3, 16)} = 1.70$, $P = 0.21$). Regardless of the photoperiod treatment, the overall mean proportion of mussel spat attached to the plastic mesh substrate was 79.7 ± 2.3 %, while 8.2 ± 1.2 % of mussels were attached to the bottle surfaces, and 12.1 ± 1.6 % of mussels were unattached on the bottom of the tank.

4.3.2. Aeration

4.3.2.1. Temperature and Dissolved Oxygen

Temperature

The initial (t_0) mean water temperature among the four aeration treatments and within treatments were consistent 19.15 ± 0.02 ºC (i.e., $F_{(3, 40)} = 1.33$, $P = 0.28$ among treatments, and among tanks within treatments $F_{(16, 40)} = 0.80$, $P = 0.67$). However, after 24 h (t_{24}) the mean water temperature declined in all experimental tanks, and there were temperature differences among the four aeration treatments at ($F_{(3, 40)} = 19.11$, $P < 0.0001$), and these differences were consistent among the individual tanks within each aeration treatment ($F_{(16, 40)} = 0.94$, $P = 0.53$).

The final mean water temperatures of the MA and HA treatments were both slightly lower (i.e., 18.25 ± 0.04 ºC and 18.35 ± 0.04 ºC respectively) than both the LA and NA treatments (i.e., 18.58 ± 0.04 ºC and 18.67 ± 0.04 ºC respectively) (Tukey's HSD, $P < 0.05$). There was no difference in final mean water temperatures between MA and HA treatments, and between LA and NA treatments.

Overall, there was a slight decline in mean water temperature after 24 h (t_{24}) for all of the four aeration treatments, i.e., decreasing from an overall mean of 19.15 ± 0.02 ºC at t_0 to 18.46 ± 0.03 ºC at t_{24}. However, the temperature drop between initial and final measurement was minor and still well within the temperature requirements of juvenile mussels.

Dissolved oxygen (DO)

The initial (t_0) dissolved oxygen concentration among the four aeration treatments and for each conical tank within each aeration treatment over the experimental period were relatively consistent at 99.99 ± 0.01 % ($F_{(3, 40)} = 0.79$, $P = 0.51$) among treatments and among tanks within aeration treatments ($F_{(16, 40)} = 0.67$, $P = 0.80$) (Fig. 4.7).

There was a difference in the mean oxygen concentrations among the four aeration treatments after 24 h (t_{24}) ($F_{(3, 40)} = 633.58$, $P < 0.0001$), however, there were no differences in the mean oxygen concentration among tanks within aeration treatments ($F_{(16, 40)} = 1.16$, $P = 0.34$).

Among the aeration treatments, the treatment without aeration (NA) had the lowest mean oxygen concentration after 24 h (t_{24}) of all four treatments (i.e., 56.19 ± 1.44 %). The experimental tanks in the LA treatment had an overall mean of 87.67 ± 1.24 % oxygen concentration after 24 h (t_{24}) which was significantly lower than for both MA and HA treatments (Tukey's HSD, $P < 0.05$). Experimental tanks in the MA treatment had a mean oxygen concentration of 99.29 ± 0.19 % after 24 h (t_{24}), which was slightly lower than for the HA treatment (i.e., 100.0 % ± 0.0) (Fig. 4.7).

Overall, the degree to which mean oxygen concentration had decreased after 24 h in the four aeration treatments was inversely proportional to the extent of aeration, i.e., dropping from an overall mean of 99.99 ± 0.01 % at t_0 to 56.19± 1.44 % at t_{24} in the NA treatment, whilst remaining high in the HA treatment 100.0 % ± 0.0 at t_{24}.

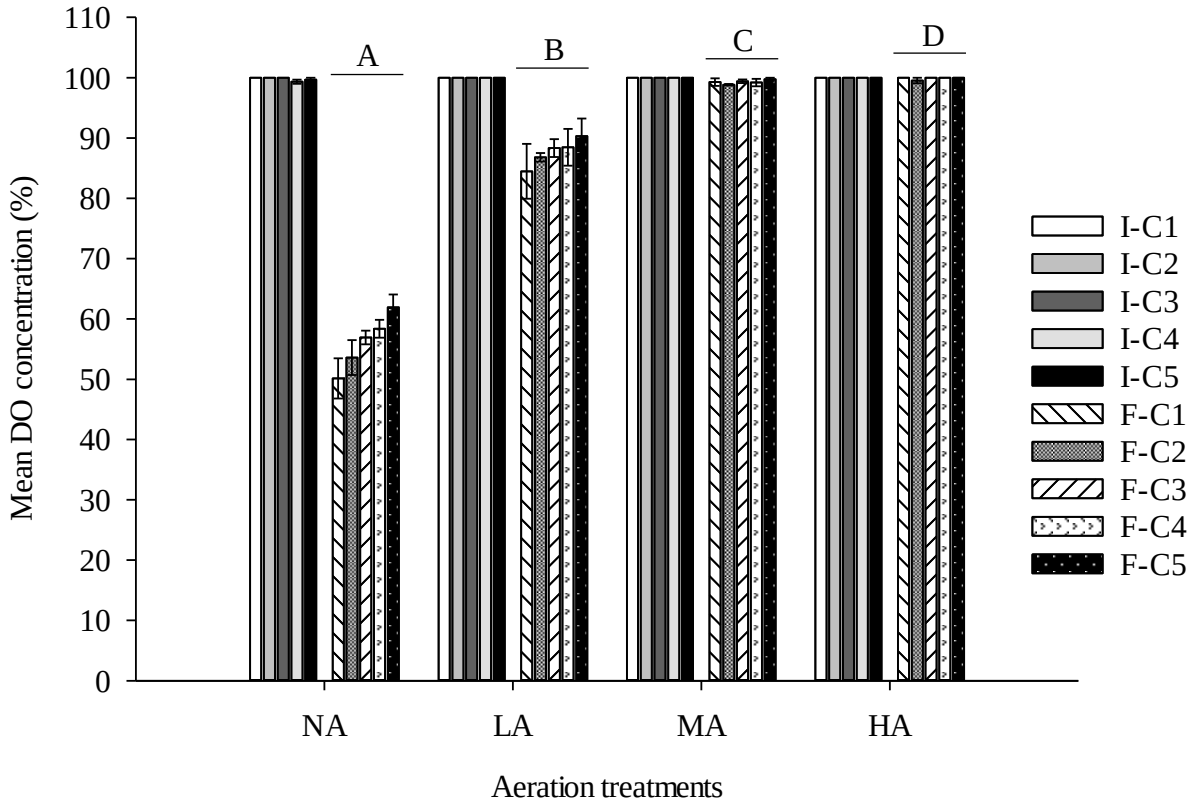

Figure 4.7. Mean oxygen concentration for four aeration treatments with five replicate tanks within each treatment measured initial (I = t_0) and final (F = t_{24}) at weekly intervals over a 25 day experimental period, C = (conical tank #). Without aeration (NA), low aeration of 2 ml s^{-1} (LA), medium aeration of 33 ml s^{-1} (MA), and high aeration of 125 ml s^{-1} (HA). Different letters above the group of bars indicate significant differences between the treatment means.

4.3.2.2. Growth

There was no significant difference in the initial shell length of mussels among the four aeration treatments ($F_{(3, 1980)} = 0.09$, $P = 0.97$), or for tanks within aeration treatments at ($F_{(16, 1980)} = 0.06$, $P = 1.00$) (Fig. 4.8). The overall initial mean shell length of mussel spat was 1.76 ± 0.01 mm.

After the 25 day experimental period, there were differences in the mean of final shell length of the mussels as a result of the different aeration treatments ($F_{(3, 1704)} = 213.55$, $P < 0.0001$), and there were also differences among tanks within the aeration treatments ($F_{(16, 1704)} = 2.03$, $P = 0.01$).

Among the four aeration treatments, the smallest final mean shell length of mussels was observed in the NA treatment, i.e., 2.50 ± 0.05 mm. Mussels in the LA treatment had a greater mean shell length of 2.89 ± 0.05 mm. The greatest mean shell length was observed in both MA

and HA treatments at 3.83 mm ± 0.05 and 3.98 mm ± 0.05 respectively (Tukey's HSD, $P < 0.05$) (Fig. 4.8).

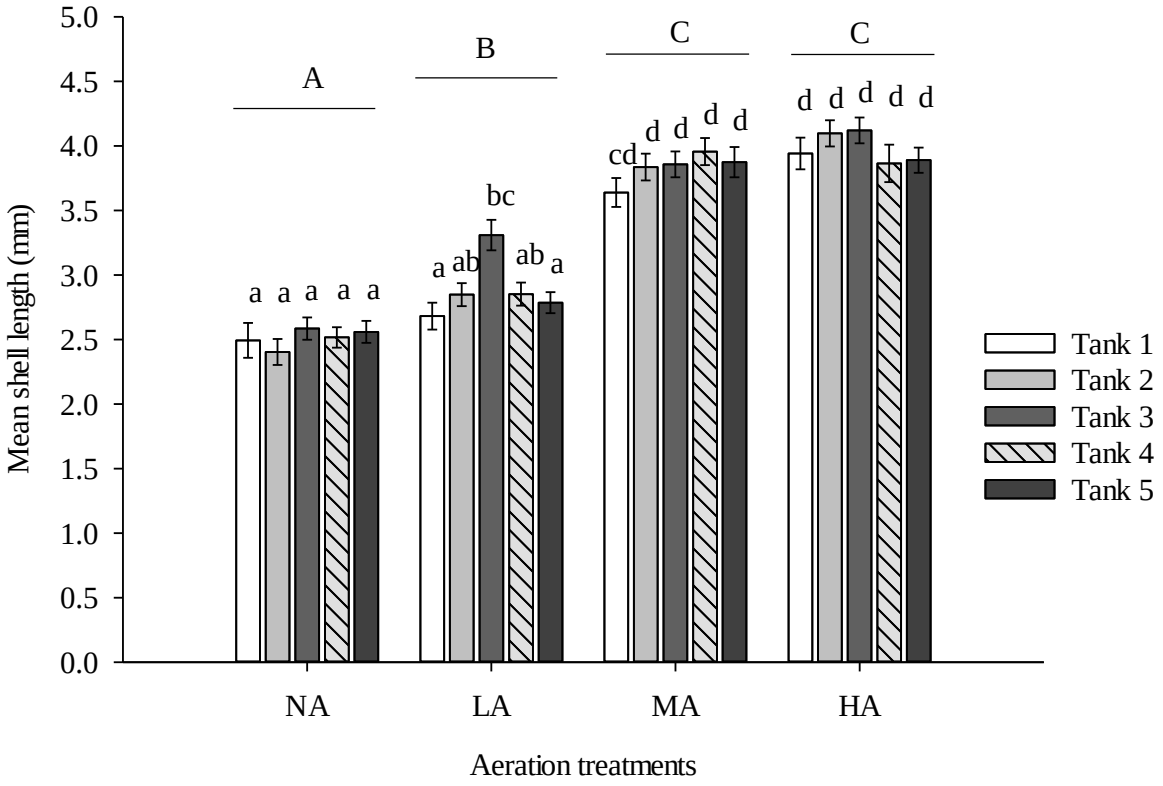

Figure 4.8. Final mean shell length of mussel spat raised for 25 days under four different aeration treatments, without aeration (NA, 0 ml s^{-1}), low aeration (LA, 2 ml s^{-1}), medium aeration (MA, 33 ml s^{-1}), and high aeration (HA, 125 ml s^{-1}). Different letters above the individual bars indicate significant differences between means for individual tanks within treatments. Different letters above a group of bars indicate significant differences in the treatment means.

There were differences in final mean shell length among individual tanks within all four aeration treatments (Tukey's HSD, $P < 0.05$) (Fig. 4.8). The differences among individual tanks were most pronounced for the LA treatment.

4.3.2.3. Survival

After the 25 day experimental period, there were differences in the mean survival of the mussel spat as a result of different aeration treatments ($F_{(3, 16)} = 10.12$, $P < 0.0001$).

The lowest mean survival among the aeration treatments was observed in the NA treatment, i.e., 68.9 ± 6.0 %. Greater mean survival of mussels were identified in the three treatments which were exposed to higher aeration (i.e., LA, MA, HA) with the treatment means ranging from 90.9

± 3.3 % to 92.5 ± 1.9 %. However, there were no significant differences in mean survival between any of these three treatments (Tukey's HSD, $P < 0.05$).

4.3.2.4. Retention

After the 25 day experimental period, there were differences in the mean retention of the green-lipped mussel spat on the plastic mesh substrate as a result of the different aeration treatments ($F_{(3, 16)} = 16.86$, $P < 0.0001$). Among the four aeration treatments, the lowest mean retention of mussels on the plastic mesh substrate was observed in the NA and LA treatments at 57.8 ± 5.9 % and 78.8 ± 4.3 % respectively. Higher mean retention of mussels on the plastic mesh was observed in MA treatments (i.e., 90.1 ± 2.8 %), this value was higher than NA treatment but not the LA treatment. The highest mean retention of mussels was observed in HA treatment at 97.7 ± 1.5 %, which was significantly higher than NA and LA treatments, but not the MA treatment (Tukey's HSD, $P < 0.05$). In addition, experimental tanks within the HA treatment had the lowest number of mussels attached to the tank walls and unattached on the bottom of the tank at 2.3 ± 1.7 % and 0.0 ± 0.0 % respectively. In contrast, the numbers of juvenile mussels that were attached on the walls and unattached on the bottom of the tanks tended to increase as aeration decreased. This resulted in the NA treatment having the highest number of mussels attached on the walls of the tank and unattached on the bottom of the tank at 24.3 ± 5.8 % and 18.0 ± 2.9 % respectively.

4.3.3. Water flow

4.3.3.1. Temperature and dissolved oxygen

Temperature

The initial (t_0) mean water temperature among the four water flow treatments and for tanks within water flow treatments were consistent at 19.06 ± 0.01 ºC ($F_{(3, 40)} = 0.2$, $P = 0.89$ among treatments and among tanks within treatments $F_{(16, 40)} = 1.31$, $P = 0.24$).

After 24 h (t_{24}) there was a difference in the mean water temperature among the four water flow treatments ($F_{(3, 40)} = 38.85$, $P < 0.0001$). However, these differences were consistent among individual tanks within water flow treatments ($F_{(16, 40)} = 1.63$, $P = 0.10$) (Fig. 4.9).

Among the water flow treatments, the lowest mean water temperature after 24 h (t_{24}) was observed in both HF and MF treatments, i.e., 18.31 ± 0.03 ºC and 18.4 ± 0.03 ºC respectively. The mean water temperature in the LF treatment (18.41 ± 0.03 ºC) was higher than the mean water temperature in HF treatment, but not the MF treatment. The highest mean water

temperature in all four water flow treatments was in the NF treatment (18.69 ± 0.03 ºC) (Tukey's HSD, $P < 0.05$) (Fig. 4.9).

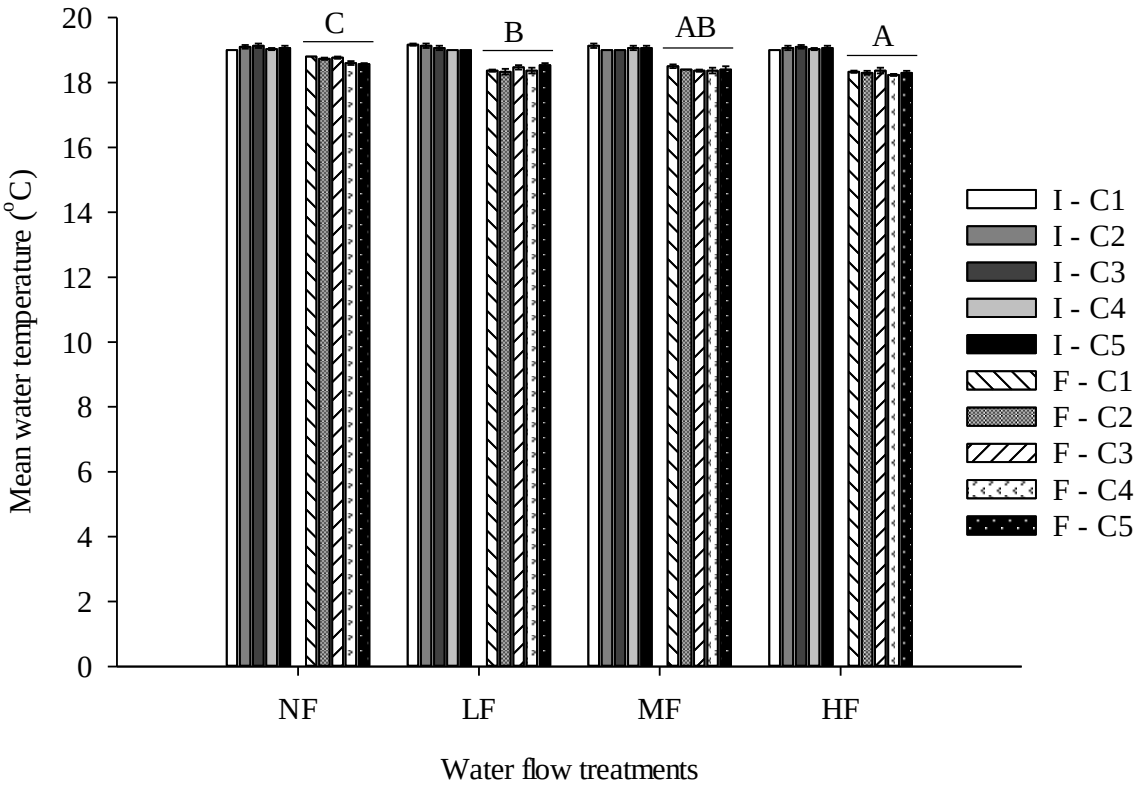

Figure 4.9. Mean water temperature for four water flow treatments with five replicate tanks within each treatment measured initial (I = t_0) and final (F = t_{24}) at weekly intervals over a 25 day experimental period, C = (conical tank #). Without water flow (NF, 0 cm^3 s^{-1}), low water flow (LF, 1 – 2 cm^3 s^{-1}), medium water flow (MF, 4 –5 cm^3 s^{-1}), and high water flow (HF, 5 – 8 cm^3 s^{-1}). Different letters above the group of bars indicate significant difference between the treatment means.

Overall, there was a trend for a small decrease in mean water temperatures after 24 h (t_{24}) for all four water flow treatments, i.e., declining slightly from 19.06 ± 0.01 ºC at t_0 to a mean temperature for each treatment ranging from 18.31 ± 0.03 ºC to 18.69 ± 0.03 ºC at t_{24}. However, these temperature variations between initial and final measurement were minor and well within the tolerance range of juvenile mussels.

Dissolved oxygen (DO)

The initial (t_0) mean oxygen concentrations among the four water flow treatments and for tanks within water flow treatment over the experimental period were consistent at 99.8 ± 0.03

% ($F_{(3, 40)}$ = 2.06, P = 0.12) among treatments and among tanks within treatments ($F_{(16, 40)}$ = 0.64, P = 0.83).

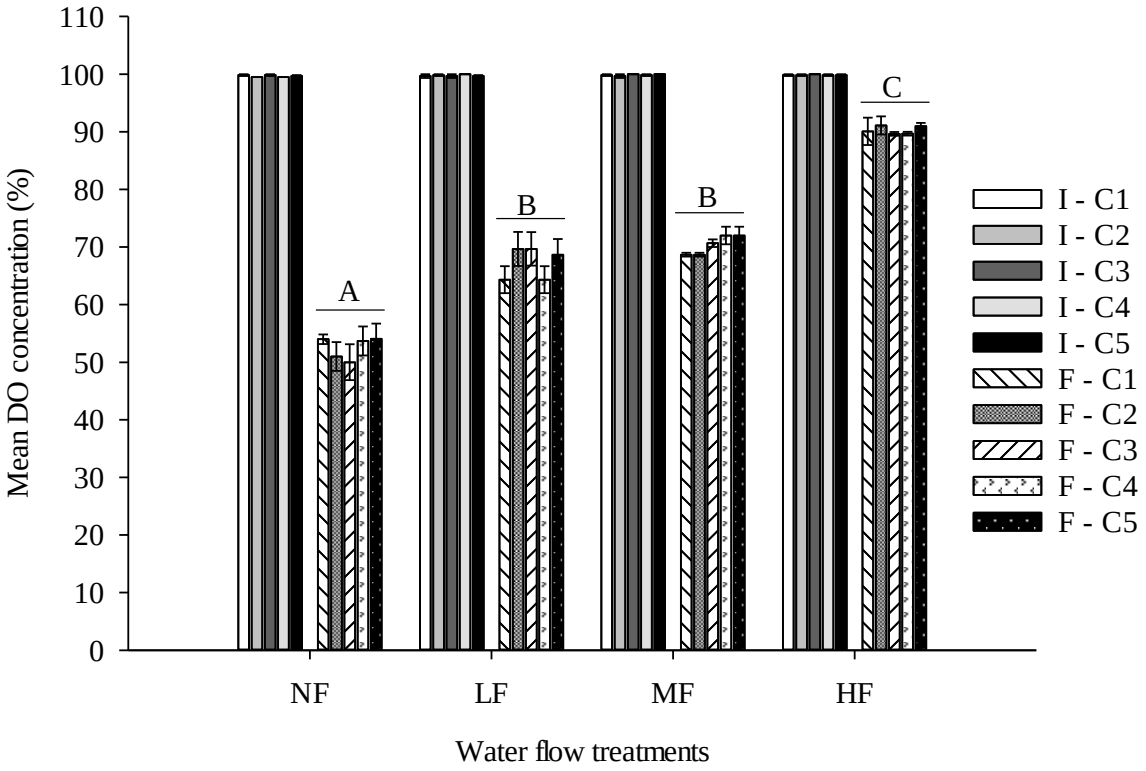

Figure 4.10. Mean oxygen concentration for four water flow treatments with five replicate tanks within each treatment measured initial (I = t_0) and final (F = t_{24}) at weekly intervals over a 25 day experimental period, C = (conical tank #). Without water flow (NF, 0 cm^3 s^{-1}), low water flow (LF, 1 – 2 cm^3 s^{-1}), medium water flow (MF, 4 –5 cm^3 s^{-1}), and high water flow (HF, 5 – 8 cm^3 s^{-1}). Different letters above the group of bars indicate significant differences between the treatment means.

After 24 h (t_{24}) there was a difference in the mean of final oxygen concentration among the four water flow treatments ($F_{(3, 40)}$ = 344.29, P < 0.0001), however, there were no differences in the mean final oxygen concentrations among tanks within water flow treatments ($F_{(16, 40)}$ = 0.82, P = 0.66).

Among the four water flow treatments, after 24 h (t_{24}) the lowest mean oxygen concentration was observed in the NF treatment, i.e., 52.55 ± 1.07 %. Higher mean oxygen concentrations were observed in both LF and MF treatments at 67.33 ± 1.23 % and 70.40 ± 0.57 % respectively. After 24 h (t_{24}) the highest final mean oxygen concentration was identified in the HF treatment at 90.29 ± 0.54 % (Tukey's HSD, P < 0.05) (Fig. 4.10).

Overall, there was a trend for a decrease in mean oxygen concentration after 24 h (t_{24}) for the water flow treatments, i.e., dropping from an initial 99.8 ± 0.03 % at t_0 to a mean oxygen concentration ranging among the four treatments from 52.55 ± 1.07 % to 90.29 ± 0.54 % at t_{24}.

4.3.3.2. Growth

There was no significant difference in the initial shell length of mussels among the four water flow treatments ($F_{(3, 1980)} = 0.17$, $P = 0.92$), or for tanks within the water flow treatments ($F_{(16, 1980)} = 0.05$, $P = 1.00$) (Fig. 4.12). The overall initial mean shell length of the mussel spat was 1.39 ± 0.01 mm.

After 25 days there were differences in the mean of final shell length of the mussels as a result of the different water flow treatments ($F_{(3, 1509)} = 27.8$, $P < 0.0001$), and there were also differences among tanks within the water flow treatments ($F_{(16, 1509)} = 2.99$, $P < 0.0001$).

Among the water flow treatments, the lowest final mean shell length was observed in the three lowest water flow treatments (i.e., NF, LF, MF) at 2.02 ± 0.0 mm, 2.10 ± 0.0 mm, and 2.10 ± 0.0 mm respectively. However, there were no significant differences among any of these three treatments. The greatest final mean shell length of mussel spat was in the HF treatment, i.e., 2.36 ± 0.02 mm (Tukey's, HSD, $P < 0.05$) (Fig. 4.11).

There were differences in final mean shell length among individual tanks within the four water flow treatments (Tukey's HSD, $P < 0.05$) (Fig. 4.11). These differences among individual tanks were most pronounced for the HF treatment.

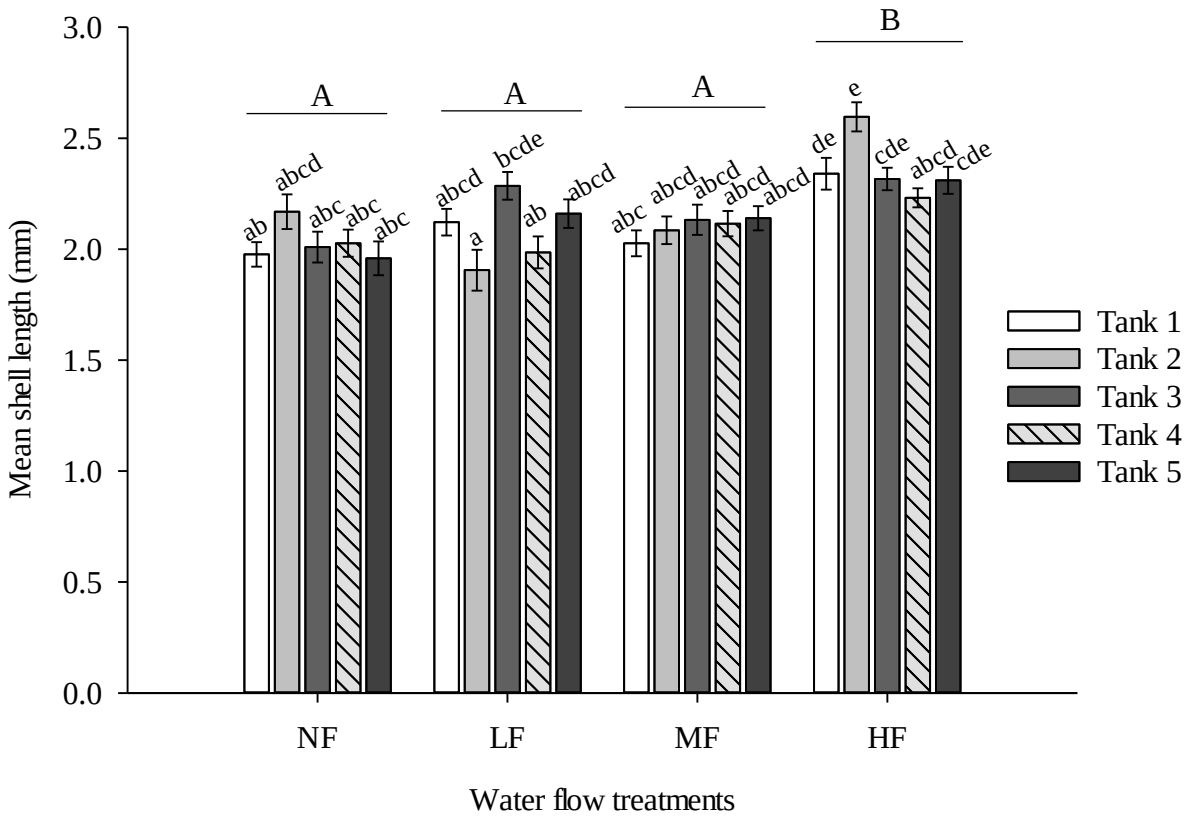

Figure 4.11. Final mean shell length of mussel spat raised for 25 days under four different water flow treatments, NF (0 cm^3 s^{-1}), LF (1 - 2 cm^3 s^{-1}), MF (4 - 5 cm^3 s^{-1}), and HF (5 – 8 cm^3 s^{-1}), tank #. Different letters above the group of bars indicate significant differences in the mean values for the treatments. Different letters above the individual bars indicate significant differences between means for individual tanks within treatments.

4.3.3.3. Survival

After 25 days there were differences in the mean survival of the mussel spat as a result of different water flow treatments ($F_{(3,16)} = 3.44$, $P = 0.04$).

Among the four water flow treatments, the lowest mean survival was observed in the three treatments with the lowest water flows (i.e., NF, LF, MF) at 67.0 ± 9.6 %, 70.0 ± 6.6 %, and 82.2 ± 6.7 % respectively. The highest mean survival was observed in the HF treatment at 90.0 ± 0.8 %, and this value was higher than NF and LF treatments, but not the MF treatment (Tukey's HSD, $P < 0.05$).

4.3.3.4. Retention

After 25 days there were differences in the mean retention of mussel spat on plastic mesh substrate as a result of different water flow treatments ($F_{(3, 16)} = 10.13$, $P < 0.0001$).

Among the water flow treatments, the lowest mean retention was observed in the three lowest water flow treatments, i.e., NF, LF, and MF treatments at 32.2 ± 2.9 %, 39.5 ± 2.5 %, and 47.5 ± 4.4 % respectively. The highest mean retention of mussels was observed in the HF treatment at 61.2 ± 4.8 %, this value was higher than retention in the NF and LF treatments, but not the MF treatment (Tukey HSD, $P < 0.05$). Correspondingly, in the three lower water flow treatments i.e., < 5 cm^3 s^{-1} (NF, LF, and MF) juvenile mussels tended to be located in other places within the tanks other than attached to the plastic mesh substratum. In contrast, the HF treatment (i.e., > 5 cm^3 s^{-1}) was characterised by a relatively smaller proportion of mussels attached to the surfaces of the tank (25.3 ± 3.5 %) and unattached on the floor of the tanks (13.5 ± 1.5 %).

4.3.4. Oxygen concentration

4.3.4.1. Temperature and dissolved oxygen

Temperature

The initial (t_0) mean water temperature among the dissolved oxygen treatments and for tanks within treatments over the experimental period were all consistent at 18.99 ± 0.15 ºC. ($F_{(3,\ 480)} = 0.32$, $P = 0.81$ among treatments and among tanks within the dissolved oxygen treatments $F_{(16,\ 480)} = 0.66$, $P = 0.83$).

After 24 h (t_{24}) there was a difference in the mean water temperature among the four dissolved oxygen treatments ($F_{(3,\ 480)} = 12.48$, $P < 0.0001$), however, these differences were inconsistent among tanks within dissolved oxygen treatments ($F_{(16,\ 480)} = 1.46$, $P = 0.11$) (Fig. 4.12).

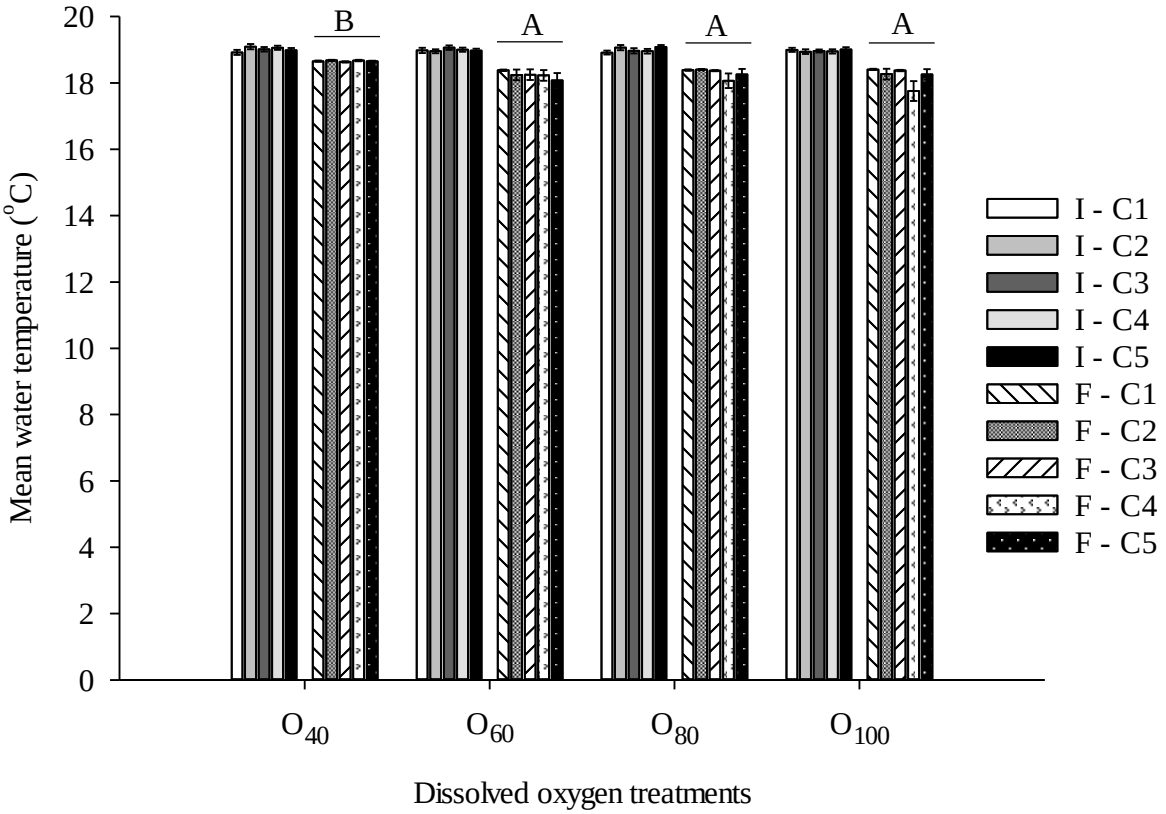

Figure 4.12. Mean water temperature for four oxygen concentration treatments with five replicate tanks within each treatment measured initial (I = t_0) and final (F = t_{24}) at weekly intervals over a 25 day experimental period, C = (conical tank #). Dissolved oxygen 40 % (O_{40}), dissolved oxygen 60 % (O_{60}), dissolved oxygen 80 % (O_{80}), dissolved oxygen 100 % (O_{100}). Different letters above the group of bars indicate significant differences between the treatment means.

Among the four dissolved oxygen treatments, the lowest mean water temperature after 24 h (t_{24}) was observed in three oxygen concentration treatments (i.e., O_{60}, O_{80}, O_{100}) at 18.24 ± 0.10 ºC, 18.29 ± 0.08 ºC, and 18.21 ± 0.11 ºC respectively. However, there were no differences among any of these three oxygen concentration treatments. The highest mean water temperature after 24 h (t_{24}) was observed in the O_{40} treatment at 18.66 ± 0.01 ºC (Tukey's HSD, $P < 0.05$) which was only slightly higher than for the other treatments (Fig. 4.12).

Overall, there was a trend for the mean water temperature after 24 h (t_{24}) for all dissolved oxygen treatments to decrease from the initial (t_0) mean temperature that was taken immediately following the water change, i.e., declining from 18.99 ± 0.15 ºC at t_0 to a temperature ranging among the four treatments from 18.21 ± 0.11 ºC to 18.66 ± 0.01 ºC at t_{24}. However, the decline

in temperature between initial and final measurements was less than 1 ºC and therefore trivial in terms of the biology of the mussels.

Dissolved oxygen

The initial (t_0) mean dissolved oxygen concentrations among the four dissolved oxygen treatments were significantly different, as well as for tanks within dissolved oxygen treatments ($F_{(3, 480)} = 117284.72$, $P < 0.0001$ among the oxygen concentration treatments and $F_{(16, 480)} = 15.36$, $P < 0.0001$ among the tanks within treatments).

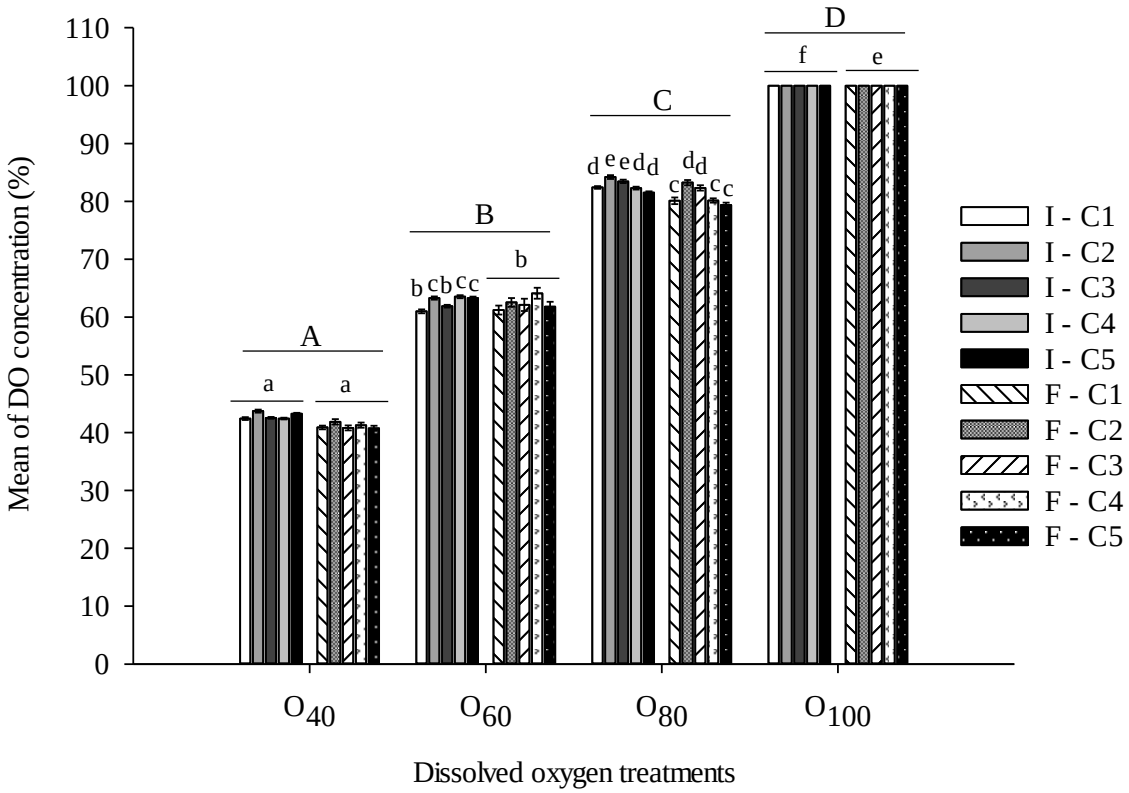

Figure 4.13. Mean oxygen concentration for four dissolved oxygen treatments with five replicate tanks within each treatment measured initial (I = t_0) and final (F = t_{24}) at daily intervals over a 25 day experimental period, C = (conical tank #). Dissolved oxygen 40 % (O_{40}), dissolved oxygen 60 % (O_{60}), dissolved oxygen 80 % (O_{80}), dissolved oxygen 100 % (O_{100}). Different letters above the individual bar indicate significant differences between means for individual tanks within treatments. Different letters above a group of bars indicates significant differences between the treatment means.

Among the dissolved oxygen treatments, the lowest mean initial oxygen concentration (t_0) was observed in the O_{40} treatment at 42.89 ± 0.14 %. A higher initial mean oxygen concentration was observed in the O_{60} treatment of 62.60 ± 0.21 %, but this value was lower than the initial mean oxygen concentration of the O_{80} treatment of 82.79 ± 0.21 %. The highest initial mean

oxygen concentration was in the O_{100} treatment at 100 ± 0.0 % (Tukey HSD, $P < 0.05$) (Fig. 4.13).

There were differences in the initial mean dissolved oxygen concentrations among individual tanks within all four oxygen concentration treatments (Tukey's HSD, $P < 0.05$). These differences were most pronounced in the O_{60} and O_{80} treatments.

After 24 h (t_{24}) there were differences in the mean oxygen concentrations among the four dissolved oxygen treatments ($F_{(3, 480)} = 15159.55$, $P < 0.0001$), and among tanks within treatments ($F_{(16, 480)} = 3.16$, $P < 0.0001$).

Among the dissolved oxygen treatments, the lowest mean oxygen concentration after 24 h (t_{24}) was observed in O_{40}, i.e., 41.05 ± 0.29 %. This value was lower than final mean oxygen concentration in the O_{60} treatment at 62.35 ± 0.58 %. In comparison the O_{80} treatment had a greater mean oxygen concentration of 81.04 ± 0.35 % and the highest mean oxygen concentration was in the O_{100} treatment at 100.0 ± 0.0 % (Tukey's HSD, $P < 0.05$).

After 24 h (t_{24}) there were differences in the mean dissolved oxygen concentrations among individual tanks within all four oxygen concentration treatments (Tukey's HSD, $P < 0.05$). These differences were more pronounced in the O_{80} treatment (Fig. 4.13).

4.3.4.2. Growth

There was no significant difference in the initial shell length of mussel spat among the four oxygen concentration treatments ($F_{(3, 1980)} = 0.04$, $P = 0.98$), or for individual tanks within the dissolved oxygen treatments ($F_{(16, 1980)} = 0.07$, $P = 1.00$). The overall initial mean shell length of the mussel spat was 1.15 ± 0.01 mm.

After 25 days there were differences in the final mean shell length of the mussels as a result of the dissolved oxygen treatments ($F_{(3,1700)} = 15.35$, $P < 0.0001$), and there were also differences among tanks within the dissolved oxygen treatments ($F_{(16, 1700)} = 1.97$, $P = 0.01$).

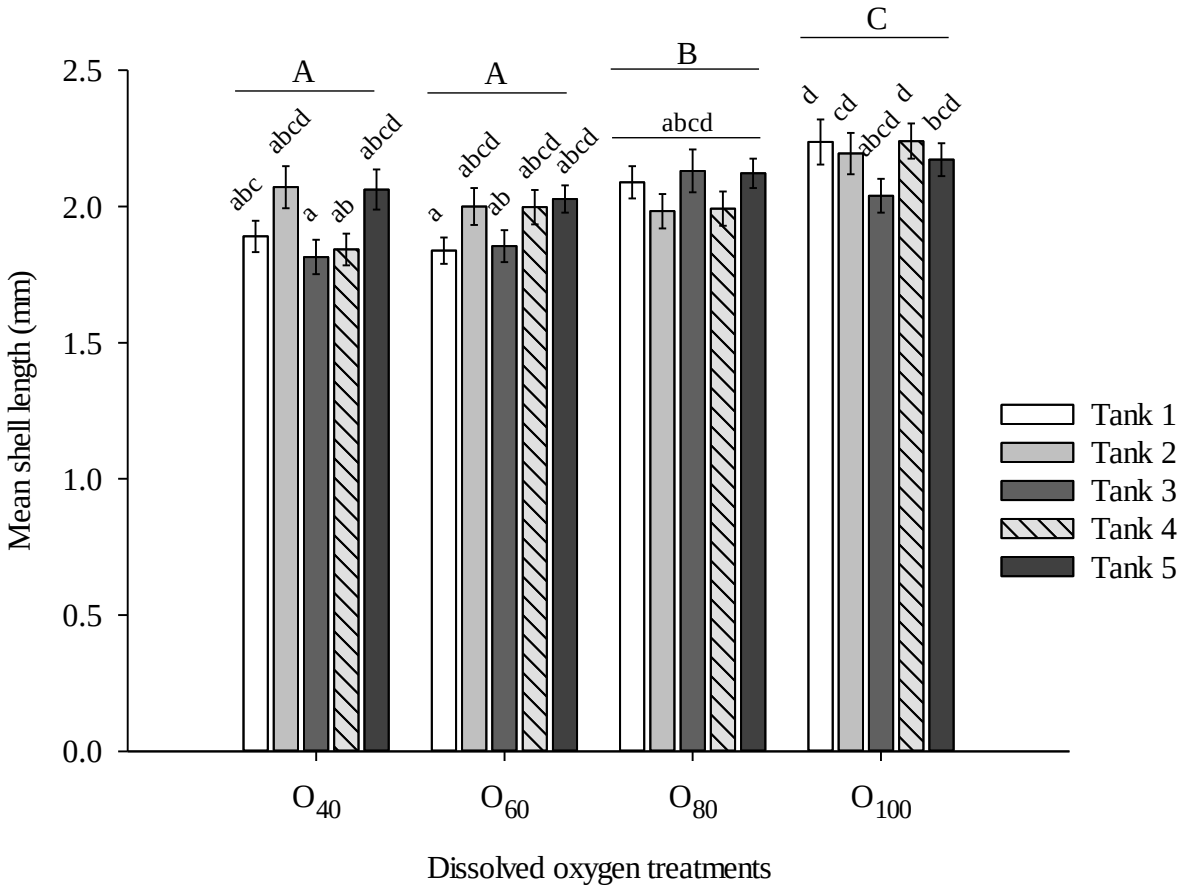

Figure 4.14. Final mean shell length of mussel spat raised for 25 days in four different dissolved oxygen treatments; dissolved oxygen 40 % (O_{40}), dissolved oxygen 60 % (O_{60}), dissolved oxygen 80 % (O_{80}), dissolved oxygen 100 % (O_{100}), tank #. Different letters above the individual bars indicate significant differences between means for individual tanks within treatments. Different letters above the group of bars indicate significant differences in the treatment means.

Among the four dissolved oxygen treatments, the smallest final mean shell length was observed in the O_{40} and O_{60} treatments at 1.94 ± 0.02 mm and 1.95 ± 0.03 mm respectively. In comparison, greater final mean shell length was observed in the O_{80} treatment 2.06 ± 0.03 mm, but this value was slightly lower than the final mean shell length of mussels in the O_{100} treatment at 2.18 mm ± 0.03 (Tukey's HSD, $P < 0.05$) (Fig. 4.14).

4.3.4.3. Survival

After 25 days there were differences in the mean survival of the mussel spat as a result of different dissolved oxygen treatments ($F_{(3, 16)} = 20.60$, $P < 0.0001$).

Among the four dissolved oxygen treatments, the lowest mean survival was observed for mussels in both O_{40} and O_{60} treatments at 58.0 ± 3.9 % and 67.8 ± 5.3 % respectively. These values were lower than the mean survival in the O_{80} and O_{100} treatments at 84.0 ± 1.7 % and

89.0 ± 2.6 % respectively (Tukey's HSD, $P < 0.05$), which were not significantly different from one another.

4.3.4.4. Retention

After 25 days there were differences in the mean retention of the mussel spat as a result of the four dissolved oxygen treatments ($F_{(3, 16)} = 31.76$, $P < 0.0001$).

The lowest mean retention was for three of the dissolved oxygen treatments (i.e., O_{40}, O_{60}, and O_{80}) and there were no significant differences among any of these treatments with an overall mean of 54.2 ± 1.8 %. The greatest mean retention of all four treatments was in the O_{100} treatment at 83.8 ± 2.7 % (Tukey's HSD, $P < 0.05$), which had a correspondingly small proportion of mussels attached on the surfaces of the tanks (8.5 ± 1.7 %) and unattached on the bottom of the tanks (7.6 ± 1.5 %). In contrast, in the three lower dissolved oxygen treatments larger proportions of juvenile mussels were attached to the surfaces of their tanks and unattached on the bottom of their tanks with overall means for O_{40}, O_{60}, and O_{80} treatments of 32.4 ± 1.3 % and 13.4 ± 1.7 % respectively.

4.4. Discussion

The aim of this study was to identify optimum photoperiod, aeration, water flow and dissolved oxygen conditions to maximize the performance (i.e., growth, survival and retention) of mussel spat being cultured in nursery conditions. The results indicate that the management of some of these key environmental variables have the potential to greatly improve the efficiency of nursery production of juvenile green-lipped mussels.

4.4.1. Photoperiod

Experimentally culturing mussel spat in continuous darkness (i.e., 24D treatment) for 25 days resulted in a 15 % smaller increase in mean shell length compared to all other photoperiod treatments where light was provided to some extent, i.e., 12L:12D, 6L:18D, 24L. This difference in growth could not be explained by the small differences in mean water temperature and dissolved oxygen levels detected among the treatments, and therefore are most likely due to the effect of the photoperiod treatment. However, despite the marked effect of the 24D treatment on the growth of the spat, there were no differences or trends in the growth of spat among the three treatments with varying periods of light exposure ranging from 6 to 24 h. This suggests that some period of light is required to elicit feeding in spat, or that the quality of the microalgae declined sufficiently in continuous darkness to affect the growth of the spat feeding upon it. There was considerable variability in the mean growth of mussels within replicate tanks within

photoperiod treatments, with the variability tending to increase in treatments with increasing light exposure. This may suggest that the presentation of light within individual tanks could have been variable due to possible shadowing effects of the downwelling experimental lighting arrangement that was used, which could have also influenced microalgae quality or spat feeding behaviour.

The experimental results indicate that some light exposure is required to promote growth in juvenile green-lipped mussels. Different species of mussels appear to respond in different ways to light exposure. Manipulating photoperiod in growing juvenile mussels (i.e., continuous darkness, dim day light exposure) can influence filtering behaviour in bivalves and their corresponding physiological condition which may in turn influence their survival and growth (Lurman et al., 2013; Nielsen & Strömgren, 1985; Strömgren, 1976). For example, juvenile blue mussels (*Mytilus edulis*) of 6.6 mm shell length grew 12.2 % and 8.61 % more over 60 days when reared in shaded natural light (50 % sunlight) compared with mussels grown under full sunlight and complete darkness respectively (Coulthard, 1929). A preliminary study in juvenile blue mussels of 14 – 22 mm SL showed increases in shell length of 20 % when reared under continuous darkness over 18 days compared to mussels grown under natural light (800 W m^{-2}) (control treatment) (Nielsen & Strömgren, 1985). The juvenile blue mussels produced more faeces when kept under continuous darkness which suggested they were feeding more vigorously or for longer periods (Nielsen & Strömgren, 1985). Juvenile eastern oysters, *Crassostrea virginica*, (20 – 21.3 mm shell length) and juvenile blue mussels (14 – 25 mm shell length) which were grown in shaded natural outdoor light produced 47.5 % and 15 % greater increases in shell length respectively compared to those grown without shade for 3 months (Medcof & Kerswill, 1965). Juvenile blue mussels (i.e., 38 – 47 mm shell length) which were raised at low irradiance (< 4 W m^2) showed higher growth than those raised in higher irradiance (i.e., 5.5 – 10 W m^{-2}) (Strömgren, 1976). The current experiment used two minature white LED lamps with an output of 4 cd luminous intensity which shone down into the conical tanks to provide a light irradiance of approximately 1.12 W m^{-2}. The lights provided in the current study can be categorised as relatively dim compared to natural daylight or bright artificial lighting, such as that used by Nielsen & Strömgren (1985). It is possible that providing more intense lighting in the current experiment may have elicited a response from the mussel spat in relation to the three lighted photoperiod treatments.

In the current study photoperiod treatments did not influence the survival of juvenile green-lipped mussels over 25 days of experimental culture with all treatments producing > 90 % survival. This indicates culture conditions were conducive to mussel spat survival regardless of photoperiod treatment.

The juvenile green-lipped mussels in the current study were retained on the plastic mesh substrate at similar levels (> 79 %) after experimental culture for 25 days regardless of the photoperiod treatment they were subjected to. This indicates that photoperiod did not significantly influence juvenile mussel retention. Previous studies have revealed that illumination commonly influences the movement and attachment behaviour of a number of species of mussels (Bayne, 1964; Kobak & Nowacki, 2007; Uryu et al., 1996), however, different developmental stages of mussels may respond to illumination differently. Some mussel species have been shown to attach to the substrate more readily when kept in the dark versus when exposed to light. For example, both small (i.e., < 10 mm) and large (i.e., > 10 mm) sizes of zebra mussel (*Dreissena polymorpha*) selected dark attachment sites versus illuminated sites regardless of various intensities of light exposure ranging from 0.1 – 100 lx provided for 24 h (Kobak & Nowacki, 2007). Pediveliger larvae of blue mussel did not show a response to 350 lx of illumination but demonstrated negative phototaxis for both 850 and 7200 lx (Bayne, 1964). Both small (< 15 mm shell length) and large (> 15 mm shell length) golden mussels (*Limnoperna fortunei*) showed negative phototaxis and positive geotaxis when exposed to light (Uryu et al., 1996). The lack of any differences in retention behaviour in response to the four photoperiod regimes in this current study could be due to either the relatively low light levels used in the current study (i.e., < 4 W m^{-2}), or that photoperiod does not influence attachment behaviour in this mussel species.

Further investigation related to various light intensity (i.e., high intensity or low intensity as in their natural habitats) over a range of sizes of juvenile mussels could be warranted to determine whether it may influence their retention and growth.

4.4.2. Aeration

Experimentally culturing mussel spat without aeration and with low aeration (i.e., NA & LA treatments) resulted in marked decreases in growth of mussel spat over the 25 day experimental period. These differences in growth could not be explained by the small differences in mean water temperature detected among treatments. However, it is highly likely that the observed differences in the growth of mussels are the result of the differences in the mean dissolved oxygen concentrations recorded in the tanks for these aeration treatments after 24 h of a water change. The oxygen concentration in both the NA and LA treatments were lower (i.e., 56.19 ± 1.44 % and 87.67 ± 1.24 % respectively) than for both the MA and HA treatments which both had mean oxygen concentrations at above 99 %. This reduction of growth associated with diminished dissolved oxygen conditions is consistent with the experimental results specifically testing the influence of this environmental factor in isolation in this current study.

Studies have shown similar results for other mussel species. For example, adult blue mussels of 63 – 66 mm shell length rapidly decrease their filtration activity when exposed to very low level of oxygen (i.e., 2 mg O_2 L^{-1}) for 30 min by showing valve closure (Tang & Riisgård, 2018). Despite the likely role of low dissolved oxygen in depressing juvenile mussel growth in the current study, it is possible that mussels in those treatments with higher aeration may also have benefitted from the increased circulation and suspension of microalgae food particles making them more readily available for capture by filter feeding. The growth in shell length of mussels in the HF treatment was substantially less than for the HA treatment (i.e., 69.8 versus 126.1 %) in the parallel experiments suggesting that where dissolved oxygen is maintained, water movement alone does not explain the increase in growth observed in juvenile mussels under high aeration conditions. It is perhaps the unique water turbulence or sound produced by aeration that is responsible for the greater observed growth in the HA treatment (Stanley et al., 2014; Wilkens et al., 2012).

There was some small variation in the mean growth of mussels within the replicate tanks within aeration treatments, especially in the higher aeration treatments. This may be due to smaller variations in dissolved oxygen conditions or the distribution of suspended food particles.

Previous studies have investigated the effect of water motion for a variety of mussel species (Alfaro, 2005, 2006; Pernet et al., 2003, Hayden & Woods, 2011), however, there is limited information on the direct effect of aeration on bivalve growth. There is evidence that aeration can be used to assist in maintaining gas exchange, dispersing suspended food particles and to enhance settlement and attachment behaviour in mussel larvae and juveniles (Alfaro, 2006; Widdows, 1991; Widdows & Staffs, 2006). Combination treatments of high water flow (i.e., 10 cm s^{-1}) with aeration in experimental tanks have been shown to stimulate increased attachment of *P. canaliculus* spat (i.e., 3 – 5 mm in shell length) by up to 60 % compared to in low water flow conditions (i.e., 1 cm s^{-1}) with aeration. However, providing aeration alone did not increase spat attachment (Alfaro, 2006).

The experimental culture of mussels without aeration (i.e., NA treatment) resulted in a 22.8 % lower survival of spat compared to the three higher aeration treatments (i.e., LA, MA, and HA). However, there were no significant differences in survival of spat among these three aeration treatments. The lower survival in NA treatment is most likely the result of the lower dissolved oxygen concentration observed in this treatment. A combination treatment of low water motion (i.e., 1 cm s^{-1}) with a low oxygen concentration (i.e., 6 mg O_2 L^{-1}) resulted in ~23 % lower survival in juvenile green-lipped mussels over 24 h compared to the treatment with high water flow (i.e., 10 cm s^{-1}) with high oxygen concentration (i.e., 12 mg O_2 L^{-1}) (Alfaro,

2005). This indicates that adequate oxygen concentration is required to support juvenile mussel survival.

The retention of juvenile mussels in the current experiment increased in relation to the amount of aeration, i.e., 57.8 %, 78.8 %, 90.1 %, and 97.7 % retention for the NA, LA, MA, and HA treatments respectively. A marked difference in mussel retention was identified between aeration < 33 ml s^{-1} (i.e., NA, LA) treatments and > 33 ml s^{-1} (i.e., MA, HA) treatments which is likely to be due to differences in dissolved oxygen concentrations or water turbulence among the treatments, or the combination of these two environmental factors generated by the aeration.

Increased water motion is known to stimulate byssal production in mussels (Nishida et al., 2003; Price, 1982; Young, 1985). For example, lower numbers of byssus threads (i.e., < 12 byssus threads) were produced over 24 h in juvenile *P. canaliculus* of 3 – 5 mm shell length in experimental tanks without aeration (i.e., < 0 ml s^{-1}) and high water flow (i.e., 10 cm s^{-1}) versus those with aeration (i.e., > 1 ml s^{-1}) and high water flow for which the mussels produced more than 16 byssus threads per mussel (Alfaro, 2006). Attachment in some mussel species at the time of larval settlement also appears to be influenced by water movement. For example, the pediveliger of blue mussels had only 0 – 30 % settlement on filamentous substratum when placed in a static glass culture dish for 24 – 48 h, but this increased markedly when the glass culture dish was agitated to generate water movement (Eyster & Pechenik, 1988). Likewise, larvae of blue mussels had 17.2 % higher settlement when exposed to high water velocity (i.e., 50 l h^{-1}) for 26 days observation time, compared to when the larvae were exposed to low water velocity (i.e., 20 l h^{-1}) (Pernet et al., 2003). Overall, the results from this current experiment indicate that providing vigorous aeration is likely to increase the retention of mussel spat in nursery holding systems.

4.4.3. Water flow

Experimentally culturing mussel spat in tanks with high water flow (i.e., HF treatment) resulted in 20.6 % greater increase in their shell length compared to three other treatments with lower water flow, i.e., NF, LF, HF. These differences in growth cannot be explained by the small differences in mean water temperature among treatments, but may be due to marked differences in final dissolved oxygen concentrations among the different water flow treatments or the effect of the water flow treatments themselves, or a combination of these two factors. The decrease in the growth of juvenile mussel was similar (i.e., 20.6 versus 20.4 %) to that was observed in the reduced dissolved oxygen treatments O_{40} and O_{60} in this current study, suggesting that reduced oxygen conditions were very likely to have contributed to the decreased growth of the mussels in the NF treatment, but may not be wholly responsible.

Water flow has been shown to affect growth, attachment and survival in many bivalve species, including mussels (Alfaro, 2006; Wildish et al., 1987; Wildish & Miyares, 1990). For example, adult blue mussels (i.e., 45 – 50 mm SL) had ~ 31 % higher filtration on cultured microalgae when exposed to low water flow (i.e., 6.8 cm s^{-1}) for a 1 h experimental period versus higher water flow (i.e., > 25 cm s^{-1}) (Wildish & Miyares, 1990). Small green-lipped mussels (i.e., 3 mm shell length) which were cultured on a sea-based farm for 8 weeks with water velocity ranging from 15 – 40 cm s^{-1} grew more than those grown under low water velocity (i.e., 1 – 10 cm s^{-1}) on the same farm (Hayden & Woods, 2011). These findings suggest that a combination of water flow and dissolved oxygen levels are most likely responsible for the marked differences in spat growth between experimental water flow treatments in the current study.

Mussel spat survival in the current experiment was significantly lower in the lower water flow treatments (i.e., NF and LF versus MF and H treatments). Like the growth of spat observed in this experiment, survival in these low water flow treatments was likely to be affected by a combination of reduced dissolved oxygen and water flow. A similar response has been shown by juvenile green-lipped mussel (0.5 – 3.0 mm shell length) when exposed to a combination of low oxygen concentration (6 mg O_2 L^{-1}) and low water flow (1 cm s^{-1}) for 24 h which resulted in 20 % lower survival compared with those at high oxygen concentration (i.e., 12 mg O_2 L^{-1}) and high water flow (10 cm s^{-1}) (Alfaro, 2005).

Likewise, larvae of *P. canaliculus* are also impacted by a combination of reduced dissolved oxygen and water flow. Pediveligers exposed to low oxygen concentration (i.e., 6 mg O_2 L^{-1}) and low water flow (i.e., 1 cm s^{-1}) had ~ 23 % lower survival over 24 h compared to when exposed to high oxygen concentration (i.e., 12 mg O_2 L^{-1}) and high water flow (i.e., 10 cm s^{-1}) (Alfaro, 2005).

Spat retention on the plastic mesh substrate in the current study increased with higher water flows in the current experiment. This difference could be due to water motion stimulating byssal thread production in the juvenile mussels (Nishida et al., 2003; Price, 1982; Young, 1985), but it is also likely to be related to the differences in oxygen concentration among the water flow treatments.

Juvenile green-lipped mussels (3 – 5 mm shell length) have previously been shown to have stronger attachment and higher byssal thread production when exposed to higher water flows experimentally (Alfaro, 2006). However, given the experimental design used by Alfaro, (2006) which combined the water flow and aeration treatments, it was not possible to identify the relative importance of water flow versus aeration to the retention of mussel spat. However, the

results of the current study indicate that water flow is of primary importance in juvenile mussel spat retention, and by comparison of the results with the aeration experiment there is an indication that water movement (i.e., > 33 ml s^{-1}) may also make some contribution to promoting spat retention.

Similar responses shown by juvenile *P. canaliculus* (3 – 5 mm shell length) when exposed to different water flows (i.e., 1, 5, and 10 cm s ¯1) with turbulence (aeration/ no aeration), revealed that high water flow (10 cm s^{-1}) with aeration for 24 h resulted in ~60 % higher numbers of spat attaching compared with low water flow (i.e., 1 cm s^{-1}) without aeration (Alfaro, 2006). In addition, high water flow with aeration was able to stimulate the juvenile mussels to produce 12 more byssal threads per mussel compared with those in a low water flow with aeration (Alfaro, 2006). A combination treatment of high dissolved oxygen concentration (12 mg O_2 L^{-1}) with high water flow (10 cm s^{-1}) on juvenile green-lipped mussel of < 3 mm SL for 24 h resulted in ~ 40 % higher settlement compared to mussels which were kept in a high dissolved oxygen concentration with low water flow (1 cm s^{-1}) (Alfaro, 2005). Higher retention of juvenile *P. canaliculus* was observed on ropes deployed on mussel farms which were exposed to the highest water velocities (i.e., 40 cm s^{-1}) resulting in ~21.1 % more spat remaining attached after 8 weeks compared to mussels on ropes which were exposed to low water velocities (i.e., 1, 4, and 10 cm s^{-1}) (Hayden & Woods, 2011). The results of the current study indicate that higher water flow is required to promote higher retention in juvenile green-lipped mussels.

4.4.4. Oxygen concentration

The experimental culture of juvenile mussels with increasing dissolved oxygen concentrations resulted in a corresponding increase in mussel growth. These differences in growth are unlikely to be caused by the small differences in final mean water temperature observed among the dissolved oxygen treatments. There was some variability in the mean growth of mussels among replicate tanks within dissolved oxygen treatments, however, these were minor differences compared to the marked differences among the dissolved oxygen treatments.

Previous studies have reported the effect of oxygen concentration and water flow on survival and retention of larvae and juvenile green-lipped mussel (Alfaro, 2006). However, no previous studies have reported reduction of growth in mussels associated with dissolved oxygen limitation, although it has been reported in juvenile *C. virginica* (Baker & Mann, 1992). Adult and juvenile blue mussels are known to reduce their shell opening and filter feeding activity under limiting dissolved oxygen concentrations (Tang & Riisgård, 2018; Wang & Widdows, 1993) which would be likely to ultimately result in reduced growth.

Experimental culture of mussel spat in low dissolved oxygen concentrations < 60 % (i.e., O_{40}, O_{60} treatments) resulted in 23.6 % lower survival of spat compared to those mussels cultured at higher oxygen concentrations > 80 % (i.e., O_{80}, O_{100} treatments). Juvenile *P. canaliculus* from a subtidal habitat (i.e., < 3 mm shell length) which were exposed to low oxygen concentration (i.e., 6 mg O_2 L^{-1}) and low water flow (i.e., 1 cm s^{-1}) for 24 h had a14 % lower survival compared with juvenile mussels kept under high oxygen concentration (i.e., 12 mg O_2 L^{-1}) and high water flow (10 cm s^{-1}) (Alfaro, 2005). However, given the experimental design used by Alfaro, (2005) it was not possible to identify the relative importance of water flow versus oxygen concentration to the survival of the mussels. However, in the current study it is clear that dissolved oxygen is of primary importance in survival of mussel spat, and by comparison of the results with the water flow experiment there is an indication that higher concentrations of dissolved oxygen are also likely to contribute to improved spat survival.

Experimental culture of juvenile green-lipped mussels in 100 % dissolved oxygen concentration (i.e., O_{100}) resulted in 29.6 % higher retention compared to three lower dissolved oxygen concentration treatments (i.e., O_{40}, O_{60}, and O_{80}). Despite the marked difference of the O_{100} treatment on mussel retention, there was no apparent trend in the retention of mussels in the three other oxygen concentration treatments. Lower dissolved oxygen levels may promote detachment of juvenile mussels as a precursor for migration in an effort to avoid less than ideal conditions. Juvenile green-lipped mussels are frequently found in natural habitats with high oxygen and water motion, such as exposed surf beaches (Alfaro et al., 2010; Jeffs et al., 1999). Low dissolved oxygen conditions may cause lower retention because juvenile mussels may have higher metabolic energy and oxygen demands to produce byssal threads. The energy requirement to produce byssal threads in adult green-lipped mussel has been estimated to be around 8 % of standard metabolic rate (Lurman et al., 2013). A similar estimate has been made for metabolic cost of byssal thread production in adult *M. edulis*, i.e., 8 % of total energy expenditure (Hawkins et al., 1986). Therefore, juvenile *P. canaliculus* may attempt to conserve energy by limiting byssus thread production required for attachment and retention under conditions of low dissolved oxygen.

4.4.5. Environmental conditions in nursery practices

The present study showed that the growth, survival, and retention of juvenile green-lipped mussels can be optimized through maintaining various environmental conditions (i.e., photoperiod, water flow, aeration and oxygen concentration) during the nursery phase of production. Providing some period of lighting to spat in nursery culture should help to maintain

growth, although further research is needed to determine the potential effect of different light intensities on spat performance.

The current study showed that maintaining high water flow and dissolved oxygen at saturation will maintain high growth, retention and survival of green-lipped mussel spat in nursery culture. This can be achieved with high levels of aeration, which provides both water movement and oxygen dissolution. Comparisons of the results for the high water flow (HF), oxygen saturation (O_{100}) and high aeration (HA) treatments from the three comparable, but separate, experiments showed that spat growth (i.e., increase of shell length) was 69.8, 89.6 and 126.1 % respectively, and survival 90, 89 and 92.5 %, and retention 61.2, 83.8, and 97.7 %. These comparisons indicate that high aeration (125 ml s^{-1}) delivers benefits in spat growth above providing either bubbling oxygen (33 ml s-^{1}) or water flow (5-8 cm^3 s^{-1}) alone, suggesting both contribute to the greatly improved spat growth in the HA treatment or that aeration may provide other unique benefits to spat growth. Likewise, the retention of spat in high aeration treatments appeared to be markedly greater than for either high water flow or oxygen saturation alone, indicating a synergistic effect or a uniquely positive attribute associated with aeration. This combined effect may due to boundary layer effects of small filtering bivalves with weak feeding currents being unable to access surrounding water with high oxygen and food particle abundance, without the assistance of external sources of water movement (Alfaro, 2006; Gui, et al., 2016a; Navarro & Winter, 1982). Alternatively, vigorous aeration may be associated with acoustic cues that have been associated with higher rates of attachment and growth in some marine invertebrates, including green-lipped mussels (Stanley et al., 2014).

The role of the environmental factors identified here for laboratory conditions may also assist in the selection of superior sea farm sites for seeding juvenile green-lipped mussels. High losses of juvenile mussels shortly after seeding onto farms are typical, with losses as high as 64.4 – 89 % after 20 - 25 weeks being reported (South, 2018). Deploying seed mussels to selected farm sites with high water flow (i.e., >8 cm^3 s^{-1}) and continuously high dissolved oxygen (i.e., >100 % dissolved oxygen) may help to reduce these costly losses of juvenile mussels whilst also improving growth rates in *P. canaliculus* culture.

4.5. Conclusion

In conclusion, variations in the environmental conditions tested in the current study (i.e., photoperiod, aeration, water flow and oxygen concentration) made large differences in the performance of juvenile mussels in conditions that were consistent with nursery culture. Increases of growth of (69.78 – 126.1 %), survival of (89 – 92.5 %) and retention of (61.2 – 97.7 %) over 25 days were observed through experimental variations in these four environmental conditions. The greatest improvements in commercial nursery culture of juvenile green-lipped mussels can be expected through maintaining high water motion (i.e., aeration > 33 ml s^{-1}, water flow > 8 cm^3 s^{-1}) and oxygen concentration and providing some lighting.

Chapter 5. General Discussion

5.1. Summary

The research presented in this thesis demonstrates the possibility of advancing feed delivery and the performance of juvenile *P. canaliculus* in the nursery culture situation by understanding their feeding abilities and manipulating several key environmental variables. This research included providing microalgae of different sizes to understand the filtering abilities of juvenile mussels (Chapter 2), assessing the continuous particle capture abilities of juvenile mussels (Chapter 3), and testing the role of some key environmental variables that can affect juvenile mussel performance during nursery culture (Chapter 4). The overall results of the research indicate that manipulating food particle sizes and some key environmental conditions (i.e., light exposure, aeration, water motion and levels of dissolved oxygen) can be used to improve the feeding efficiency and performance (i.e., growth, survival and retention) of spat during the nursery culture phase. The current results have potential for immediate application in green-lipped mussel nursery culture.

5.2. Filtering ability of juvenile *P. canaliculus*

In this current study the filtering ability of a range of sizes of juvenile *P. canaliculus* (i.e., 0.5 – 7 mm SL) were measured over the short term (1 h) and long term (24 h). For the short term measures (1 h), generally the smallest juvenile mussel size class (0.5 – 0.9 mm of SL) showed greater efficiency in capturing larger sized microalgae (i.e., 6 to 20 µm cells in diameter), while larger size mussels were comparatively more efficient in capturing small microalgae species (i.e., cells < 6 µm in diameter) (Fig. 2.4, Table 2.3). However, for the long term measures (24 h) all sizes of juvenile *P. canaliculus*, including the smallest size (i.e., < 600 µm SL), filtered four different microalgae species at a similar rate, except for *T. suecica* which was captured less efficiently than *D. lutheri* (Table 3.1). Extrapolation of the particle capture ability for five size classes of juvenile mussel from short term measures (1 h) produced underestimates of daily microalgae consumption that ranged from 8 to 64 times lower on average than that from the actual long term measures (24 h) for the same four microalgae species (Tables 3.1, 3.2). A similar scale of discrepancy was observed for short term (1 h) FCM measures for the capture of cultured microalgae (3 – 30 µm cell diameter) for juvenile green-lipped mussels (i.e., 1 mm in

SL) when compared with the feeding ration of microalgae used in a commercial nursery, i.e., 4 × 10^3 cells mussel^{-1} day^{-1} versus 2 × 10^5 cell mussel^{-1} day^{-1} (Bruce, 2005; Sim-Smith et al., 2005). These discrepancies indicate that short term FCM measures of filtering abilities do not accurately represent the actual filtering capabilities of juvenile mussels, possibly as a result of the ability of juvenile mussels to modulate their filtering activity in relation to environmental factors such as changes in the light dark cycle and particle concentration, as well as increasing their efficiency through time by adjusting filtering processes in response to the particle concentration and particle size (Brown et al., 1997; Gui, 2012; Gui, et al., 2016a; Lurman et al., 2013; Newell et al., 2001; Pascoe et al., 2009; Rajesh et al., 2001; Tang & Riisgård, 2016; Ward & Shumway, 2004).

Flow cytometry has been previously successfully applied to examining the filtering capability of adult and juvenile bivalves in a small number of studies (Baker et al., 1998; Jacobs et al., 2015; Shumway & Cucci, 1987; Sim-Smith et al., 2005). The current study revealed that for longer term experimental observations (i.e., 24 h) using FCM, the smallest size class of mussel (i.e., 0.5 – 0.9 mm in SL) were found to have particle capture abilities consistent with feeding rations commonly used in commercial nursery production of spat of this size (i.e.,~2.4 – 2.9 × 10^5 cell mussel^{-1} day^{-1} and 2 × 10^5 cell mussel^{-1} day^{-1}, respectively). In contrast, where FCM was used to determine feeding rates in short term feeding experiments (1 h) there was a substantial discrepancy with the feeding rates commonly used in commercial juvenile production (Chapter 2) (Sim-Smith et al., 2005). The presence of this discrepancy indicates that measurements of particle capture abilities of filter feeding bivalves should only be made over longer periods, and preferably under conditions that are commensurate with actual culture conditions, such as the nursery situation for spat.

The commercial Greenshell™ hatchery in New Zealand currently feeds their spat of up to 1 mm in shell length with three species of microalgae *Tisochyrysis lutea*, *Diacronema lutheri* and *Chaetoceros muelleri* (S. Cumming, SPATnz Ltd, pers. comm.) which are all small microalga species (i.e., < 6 µm cell diameter) that are commonly used in bivalve larval culture. The results of the current study, for both short and long term observations (i.e., 1 and 24 h) indicate that the microalga species with larger cell sizes are captured more efficiently on a total cell volume basis (Tables 2.4, 3.3, Figs. 2.5, 3.2). Therefore, providing larger sized microalgae (e.g., *Tetraselmis suecica*, *Dunaliela torteilecta*, *Rhodomonas salina*) could be a more efficient means for feeding spat provided the nutritional qualities of these microalgae are sufficient. Culturing large quantities of microalgae for nursery feeding of juvenile bivalves is a major cost for production (Laxmilatha et al., 2011). For example, to grow juvenile green-lipped mussels from 0.5 to 6.0 mm in shell length will take approximately 2.5 to 3 months (New Zealand Sustainable

Aquaculture, 2019) and requires a large quantity of microalgae, i.e., approximately 3.36×10^{11} to 1.6×10^{14} cells mussel^{-1}. Thus, replacement of microalgae with artificial particulate feeds has the potential to greatly reduce the production costs. The substitution of microalgae with artificial feed (i.e., MySpat^{TM}) of up to 25 % on juvenile green-lipped mussel (i.e., 1.1 mm in shell length) showed a similar response in growth and survival performance to a control treatment of spat fed with mixed algae over 21 days (Gui et al., 2016b). The results of the current study suggest that designing artificial feeds with a particle size ranging from 6 – 20 µm in diameter has the potential to greatly improve feeding efficiency and costs for the nursery production of juvenile green-lipped mussel.

The food of filter feeding bivalves consists of a wide variety of suspended particles, such as bacteria, phytoplankton, microzooplankton, detritus, as well as dissolved organic material (DOM) such as amino acid and sugars (Gosling, 2004; Sieburth et al., 1978). Other factors which could influence the filtering ability of juvenile mussels have not been tested in this current research, and include the size, shape, and surface properties of potential food particles, and the concentration of suspended particles. For example, the nutritional composition of microalgae (Brown et al., 1997; Brown et al., 1998; Gosling, 2004; Menden-Deuer & Lessard, 2000; Volkman et al., 1989) and the presence of possible toxic compounds in the microalgae (Li et al., 2002; Wang et al., 2011; Xu et al., 2017). Such other factors may play a significant role in influencing the selection of suspended particles, and may have also been at play in the current study for the microalgae species used in the experimental feeding where it was assumed the size of the cells was a defining feature for determining their vulnerability to filtration by juvenile mussels.

While the current study provides a first step to toward identifying the most suitable sizes of microalgae to feed juvenile green-lipped mussels, future research will need to consider other characteristics of suspended particles that potentially affect their filtration by juvenile mussels, including particle concentration, nutritional composition of particles, and shape and surface properties of the particles.

5.3. Environmental conditions for enhancing performance of juvenile mussels

The results from the current study on the effect of four environmental conditions on juvenile mussel performance indicated that high water motion and oxygen concentration greatly enhanced the growth, survival and retention of green-lipped mussel spat. Previous studies have investigated a range of natural environmental factors that may influence the settlement, growth and survival of juvenile mussels, i.e., air exposure, light exposure, water temperature, salinity,

and hydrodynamic forces (Hayden & Woods, 2011; Tan, 1975; Widdows, 1991). Under controlled conditions, such as in the current study or in a commercial nursery situation, providing water motion (i.e., aeration > 33 ml s^{-1}, water flow > 5 – 8 cm^3 s^{-1}) may help to maintain particles in suspension and the dissolved oxygen concentrations at > 80 %, which are likely to produce better performance in mussel spat. However, where mussel spat is placed on a coastal mussel farm, unfavourable environmental conditions such as low water flow or extreme hydrodynamic forces conditions (e.g., extreme wave action or tidal currents) may have a detrimental effect on the performance of the spat (Spencer, 2002). Limited water motion may cause other problems for spat, such as the accumulation of organic matter around the spat, which may also reduce water quality, e.g., oxygen depletion. Likewise, where spat material from Ninety Mile Beach is deployed onto grow out ropes on coastal mussel farms, the seaweed material with the attached mussel spat is held in place with a cotton stocking (Jeffs et al., 1999) and the subsequent decomposition of the seaweed material and cotton stocking is likely to result in localised depletion of oxygen. Reduced oxygen conditions and limited water motion due to the surrounding cotton stocking, may help to explain the poor retention of spat which is typical on seeded grow out lines (South, 2018), as this current study has found these environmental factors result in poor spat survival, growth and retention.

Therefore, developing new approaches to seeding out this mussel spat material onto coastal mussel farms may help to limit the likely effects of restricted water flow and reduced oxygen conditions on the subsequent performance of the spat. For example, selecting farm sites for seeding mussel spat that have higher natural water flow regimes (i.e., water flow, turbulence) may help to improve spat retention on seeded grow out lines. Alternatives to cotton stocking that are less restrictive on water flow and may disintegrate slowly rather than decompose, may also help to improve environmental conditions that favour better performance in mussel spat seeded onto grow out lines.

5.4. The value of the research approach

The results of the research presented in this thesis indicates that providing suitable particulate diets and environmental conditions are potentially useful for enhancing spat production in the nursery situation. It is well established that many bivalves are unable to efficiently remove cells in the picophytoplankton size range (< 2 µm diameter) (Dupuy et al., 1999; Tomaru et al., 2002; Widdows et al., 1979). Current results indicates that smaller juvenile green-lipped mussels < 2 mm in SL (i.e., M_1 and M_2) are inefficient at capturing smaller suspended particles < 5 µm cell in diameter, whereas larger juvenile mussels (i.e., size classes

M_3, M_4 and M_5) are able to capture the smaller particle sizes (i.e., < 5 µm in cell diameter) much more efficiently, even matching the filtering rate for larger particles. This is consistent with a previous study that demonstrated that the removal efficiency of suspended particles for most bivalves decreases when going below a size range of between 1 to 7 µm cell diameter depending on the bivalve species (Ward & Shumway, 2004). The differences in the lower size threshold of filterable particles among various species of bivalves appears to be related to a variety of factors, including the complexity of the morphology of the gills (Gui et al., 2016; Jørgensen et al., 1984), the size and shape of the particles (Cognie et al., 2003; Gui eta al., 2016; Safi & Hayden, 2010), and the nutritional composition of the particles (Defossez & Hawkins, 1997; Safi & Hayden, 2010). Hence, providing larger sized particles, such as microalgae of large cell diameter and high nutritional content is likely to improve their efficiency of feeding and feed delivery in the nursery situation for juvenile green-lipped mussels of < 1 mm SL. Moreover, larger sized particles may also provide higher intake of total cell volume in early juvenile mussels (Chitari & Anil, 2017; Raby et al., 1997). Converting the cell count to total cell volume captured during bivalve feeding has the potential to be applied to other bivalve species to help improve estimates of biomass or nutrient intake. The improved understanding of the ability of juvenile green-lipped mussels to capture suspended particles also helps to provide some initial insight as to whether juvenile mussels can directly utilise micro-phytoplankton-sized particles (>20 µm diameter) as significant food sources (Safi & Hayden, 2010; Sieburth et al., 1978).

The results of the current study also demonstrate that longer observation periods (i.e., 24 h) are more likely to provide representative measures of particle capture capability in juvenile bivalves when compared to short term observations (e.g., 1 h). Furthermore, conducting these measures using FCM under conditions which are commensurate to a realistic situation (e.g., commercial shellfish nursery upweller) versus an artificial laboratory situation, is more likely to deliver results that are representative of the practical application.

Environmental conditions such as high water motion and oxygen concentration were found to have the most pronounced effect on the performance of juvenile *P. canaliculus*. These findings have practical implications for making future improvements to the survival and retention of spat in nursery culture systems and in coastal mussel farms.

5.5. Limitation of the experimental work

It is important to consider the limitations of this current research and how these limitations may affect the interpretation of the results. A major challenge for assessing filtration in bivalves is the accurate determination of changes in the numbers of suspended particles over

time. In this study the challenge was addressed by initially using small volume of seawater and static culture conditions in BD Falcon 24 well plates which have small experimental chambers (i.e., 3 ml) but provide a practical experimental apparatus to observe the filtering activity of a range of sizes of juvenile mussel in a replicated array under the microscope. The initial concentration of microalgae provided for the experiment presented in Chapter 2 was based on a pilot study, which tested several initial concentrations to ensure the range of cell depletion could be measured effectively by FCM. The selected microalgae concentration that was subsequently used for a short period of observation (1 h) was 1.24×10^4 cells mussels^{-1} h^{-1} provided for an overall reduction of total available microalga cells ranging from ~ 2.3 – 45% for M_1 up to M_5 respectively during the 1 h experimental period. The changing concentration of the cells due to filtration would have increasingly reduced overall efficiency of filtering. This depletion was due to the limitation of the 3 ml volume of the well plate that was used to enable concurrent visual observations of the feeding behaviour of the mussels. The 24 h starvation of juvenile mussels prior to being exposed to experimental concentrations of microalgae may have affected their readiness to capture microalgae particles regardless of their particle size and shape. The filtering activity of the mussels in this study remained high throughout the short observation period of 1 h. In this regard the prior 24 h starvation prior to the experimental exposure may have affected the feeding behaviour of the juvenile mussels in the experiment. This was partially addressed through the subsequent experiment (Chapter 3) which ran the feeding exposure over 24 h and was not predicated by a 24 h starvation period for the experimental juvenile mussels. Also, in their natural habitat, marine invertebrates, such as mussels, are likely to be exposed to marked fluctuations in food availability (Chang et al., 2003) and most filter feeding bivalves are known to be able to regulate their filtering activity in response to the ambient feeding conditions (Jørgensen, 1996).

A higher concentration of microalgae was provided for the longer experimental feeding study (i.e., 24 h) (Chapter 3), which was 8×10^6 cells tank^{-1}, which is the equivalent of 40 cells µl^{-1}. The depletion of the microalgae over the experiment was ~ 15 % cells mussels^{-1} 24 h^{-1} for juvenile mussels of the size classes M_1, M_2, while >50 % cells mussels^{-1} 24 h^{-1} were removed by juvenile mussels of size classes M_3, M_4, and M_5. This indicated that the concentration of 40 cells µl^{-1} is likely to be under the threshold of optimum concentration for juvenile mussel size 0.5 – 6.0 mm in shell length. In the practical nursery rearing of spat, high food concentrations are typically provided in a single feeding event by feeding bulk quantities of highly concentrated cultured microalgae (S. Cumming, SPATnz Ltd, pers. comm.). However, supplying excessive microalgal concentrations has the potential to be wasteful if this feed is inadequately utilised by the cultured juvenile mussels, as there is evidence that in such situations juvenile mussels will

continue filtering but divert captured microalgae to pseudofaeces (Gui , pers. obs., Gui, 2012). Also, a proportion of microalgae will not be removed by filter feeding and is lost once the tank is flushed. For example, the nursery holding system usually provides the microalgae concentration in a range of 2 – 10 × 10^6 cells ml^{-1} day^{-1} according to the cell size of the microalgae species (S. Cumming, pers. comm.) with higher concentration provided for microalgae species with smaller cell size. It is estimated that at this concentration the spat could only filter the particle at~6 – 62% cells day^{-1} depending on the cell size of the microalgae.

5.6. Future studies

It is anticipated that extending the culture of green-lipped mussel spat in the nursery situation up to a size where secondary settlement migration decreases (i.e., 4 – 6 mm shell length) may potentially mitigate the extensive losses of spat following seeding onto mussel farms. However, the economic feasibility of operating large scale Greenshell™ mussel nursery systems for an extended period to achieve this size of juvenile mussels may be excessively expensive (Laxmilatha et al., 2011, Ragg et al., 2010). Therefore, improving the efficiency of nursery protocols could have significant value in implementing this approach to managing mussel spat losses in this industry.

The findings of this current study indicate that there is the potential to improve the efficiency of commercial nursery culture of spat by providing them with larger microalgae cells, provided they can supply suitable nutrition. A logical next step for research would be to screen the potential of culturable large microalgae species for their potential to act as an effective feed for juvenile green-lipped mussels. In addition, the basis for particle selection in juvenile green-lipped mussels also require additional research, with studies that control for differences in density and surface properties of the particles. The results of the current research suggest that particle selection in juvenile *P. canaliculus* is limited, with particle selection based largely on the size range that can physically be captured by the gill structures, rather than any particle selection based on other properties, such as surface or chemical characteristics, as have been found in other bivalves (Rosa et al., 2017; Shumway & Cucci, 1987). A similar conclusion about the indiscriminate filter feeding behaviour of juvenile green-lipped mussels has been reached in previous studies (Gui et al., 2016a; 2016b). Such indiscriminate filtering behaviour has distinct advantages for designing and delivering artificial food particles (e.g., microcapsules) to substitute the relatively expensive mass production of microalgae species. If the capture of particles by juveniles mussels is a straightforward affair, then examining the digestibility and

assimilation of food particles becomes a priority in order to find the most efficient and suitable diets for juvenile *P. canaliculus* to improve their performance.

The results of the current study indicate that the current practices for seeding mussel spat material onto coastal mussel farms is suboptimal, with the lowered oxygen and water flow conditions created as a consequence of the seeding arrangement, likely to be contributing to the poor performance of spat routinely reported from commercial mussel farming operations. A number of alternative approaches that may overcome some of the unfavourable environmental conditions for seeding spat warrant further examination. The merits of seeding spat onto coastal farm sites with high water flow regimes or turbulence may help to promote spat retention. Likewise, alternative methods for deploying spat material onto grow out ropes that does not involve cotton socking that restricts water flow around the juvenile mussels, may also help to improve oxygen conditions and water flow regimes sufficiently, to promote better survival and retention of spat.

Together, the results of the research into the feeding and environmental requirements of juvenile green-lipped mussels presented in this thesis provide valuable insights into the biology at this stage of development of this mussel species. These results point toward a number of potential routes for further research with good prospects for overcoming some of the current difficulties with achieving a higher degree of performance from juvenile green-lipped mussels in the commercial production situation.

Reference

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

Alfaro, A. C. (2006). Byssal attachment of juvenile mussels, *Perna canaliculus*, affected by water motion and air bubbles. *Aquaculture*, *255*(1–4), 357–361.

Alfaro, A. C., & Jeffs, A. G. (2003). Variability in mussel settlement on suspended ropes placed at Ahipara Bay, Northland, New Zealand. *Aquaculture*, *216*(1–4), 115–126.

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

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

Alfaro, A., Jeffs, A., Gardner, J., Breen, B. B., & Wilkin, J. (2011). *Green-lipped mussels in GLM 9. (New Zealand Fisheries Assessment Report No. 48)*. Wellington.

Aquaculture New Zealand. (2018). Greenshell™ Mussels. Retrieved November 15, 2018, from https://www.aquaculture.org.nz/industry/greenshell-mussels/

Aquaculture New Zealand. (2020). *New Zealand Greenshell™ mussel spat strategy*. Nelson.

Baker, A. (2012). Phycokey -- an image based key to Algae (PS Protista), Cyanobacteria, and other aquatic objects. Retrieved March 28, 2017, from http://cfb.unh.edu/phycokey/phycokey.htm

Baker, S. M., Levinton, J. S., Kurdziel, J. P., & Shumway, S. E. (1998). Selective feeding and biodeposition by zebra mussels and their relation to changes in phytoplankton composition and seston load. *Journal of Shellfish Research*, *17*, 1207–1213.

Baker, S. M., & Mann, R. (1992). Effects of hypoxia and anoxia on larval settlement, juvenile growth, and juvenile survival of the oyster *Crassostrea virginica*. *Biological Bulletin*, *182*(2), 265–269.

Bayne, B. L. (1964). Primary and secondary settlement in *Mytilus edulis* (Mollusca). *Journal of*

Animal Ecology, *33*(3), 513–523.

Bayne, B. L. (1971). Some morphological changes that occur at the metamorphosis of the larvae of *Mytilus eduli*s. In *Proceedings of the 4th European Marine Biology Symposium* (pp. 259–280). Cambridge University Press.

Beninger, P. G., & St-Jean, S. D. (1997). The role of mucus in particle processing by suspension-feeding marine bivalves: Unifying principles. *Marine Biology*.

Beninger, P., St-Jean, S., Poussart, Y., & Ward, J. (1993). Gill function and mucocyte distribution in *Placopecten magellanicus* and *Mytilus edulis* (Mollusca: Bivalvia): the role of mucus in particle transport. *Marine Ecology Progress Series*, *98*, 275-282.

Beninger, Peter G., Valdizan, A., Decottignies, P., & Cognie, B. (2008). Impact of seston characteristics on qualitative particle selection sites and efficiencies in the pseudolamellibranch bivalve *Crassostrea gigas*. *Journal of Experimental Marine Biology and Ecology*, *360*, 9–14.

Beninger, Peter G., Ward, J. E., MacDonald, B. A., & Thompson, R. J. (1992). Gill function and particle transport in *Placopecten magellanicus* (Mollusca: Bivalvia) as revealed using video endoscopy. *Marine Biology*, *114(2)*, 281-288.

Berry, P. F., & Schleyer, M. H. (1983). The brown mussel *Perna perna* on the Natal coast, South Africa: utilization of available food and energy budget. *Marine Ecology Progress Series*, *13*(2), 201–210.

Brown, M. R., Jeffrey, S. W., Volkman, J. K., & Dunstan, G. A. (1997). Nutritional properties of microalgae for mariculture. *Aquaculture*, *151*(1–4), 315–331.

Brown, Malcolm R., McCausland, M. A., & Kowalski, K. (1998). The nutritional value of four Australian microalgal strains fed to Pacific oyster *Crassostrea gigas* spat. *Aquaculture*, *165*(3–4), 281–293.

Bruce, M. (2005). *Alternative and supplemental feeds for hatchery-reared Greenshell mussel spat - Part 1*. (Report#AKL 2005-041). New Zealand: NIWA.

Buchanan, S., & Babcock, R. (1997). Primary and secondary settlement by the Greenshell mussel *Perna canaliculus*. *Journal of Shellfish Research*, *16*(1), 71–76.

Buchanan, S. J. (1994). *The settement behaviour and recruitment of* Perna canaliculus. (MSc thesis). University of Auckland.

Cannuel, R., Beninger, P. G., McCombie, H., & Boudry, P. (2009). Gill development and its

functional and evolutionary implications in the blue mussel *Mytilus edulis* (Bivalvia: Mytilidae). *Biological Bulletin*, *217*(2), 173–188.

Capson, T. L., & Guinote, J. (2014). *Future proofing New Zealand's shellfish aquaculture: monitoring and adaptation to ocean acidification*. (Report# No. 136). Wellington: Ministry for Primary Industries.

Carl, C., Poole, A. J., Vucko, M. J., Williams, M. R., Whalan, S., & de Nys, R. (2011). Optimising settlement assays of pediveligers and plantigrades of *Mytilus galloprovincialis*. *Biofouling*, *27*(8), 859–868.

Carton, A. G., Jeffs, A. G., Foote, G., Palmer, H., & Bilton, J. (2007). Evaluation of methods for assessing the retention of seed mussels (*Perna canaliculus*) prior to seeding for grow-out. *Aquaculture*, *262*(2–4), 521–527.

Chang, F. H., Zeldis, J., Gall, M., & Hall, J. (2003). Seasonal and spatial variation of phytoplankton assemblages, biomass and cell size from spring to summer across the north-eastern New Zealand continental shelf. *Journal of Plankton Research*, *25(7)*, 737-758.

Chauvaud L, Donval A, Thouzeau G, Paulet YM, & Nézan. E. (2001). Variations in food intake of *Pecten maximus* (L.) from the Bay of Brest (France): influence of environmental factors and phytoplankton species composition. *Comptes Rendus de l'Académie Des Sciences - Series III - Sciences de La Vie*, *324*, 743–755.

Chitari, R. R., & Anil, A. C. (2017). Estimation of diatom and dinoflagellate cell volumes from surface waters of the Northern Indian Ocean. *Oceanologia*, *59*(3), 389–395.

Cognie, B., Barillé, L., Massé, G., & Beninger, P. G. (2003). Selection and processing of large suspended algae in the oyster *Crassostrea gigas*. *Marine Ecology Progress Series*, *250*, 145–152.

Comeau, L. A., Babarro, J. M., Longa, A., Padin, X. A. (2018). Valve-gaping behavior of raft-cultivated mussels in the Ría de Arousa, Spain. *Aquaculture*, *9*, 68–73.

Coughlan, J. (1969). The estimation of filtering rate from the clearance of suspensions. *Marine Biology*, *2*(4), 356–358.

Coulthard, H. S. (1929). Growth of the sea mussel. *Contributions to Canadian Biology and Fisheries*, *4*(1), 121–136.

Cucci, T., Shumway, S., Newell, R., Selvin, R., Guillard, R., & Yentsch, C. (1985). Flow cytometry: a new method for characterization of differential ingestion, digestion and

egestion by suspension feeders. *Marine Ecology Progress Series*. *24*, 201-204.

Dame, R. F. (2016). *Ecology of marine bivalves: an ecosystem approach*. (M. J. Kennish, Ed.) (2nd ed.). CRC press, Taylor and Francis Group.

Dawber, C. (2004). Lines in the water: a history of Greenshell mussel farming in New Zealand. Picton: River Press for NZ Marine Farming Association.

Defossez, J. M., Hawkins, A. J. S. (1997). Selective feeding in shellfish: size-dependent rejection of large particles within pseudofeces from *Mytilus edulis*, *Ruditapes philippinarum* and *Tapes decussatus. Marine Biology*, *129*, 139–147.

Dionisio Pires, L. M., Jonker, R. R., Van Donk, E., & Laanbroek, H. J. (2004). Selective grazing by adults and larvae of the zebra mussel (*Dreissena polymorpha*): application of flow cytometry to natural seston. *Freshwater Biology*, *49*(1), 116–126.

Dral, A. D. (1967). The movements of the latero-frontal cilia and the mechanism of particle retention in the mussel (*Mytilus edulis*). *Journal of Sea Research*, *3*, 391–422.

Dufour, S. C., & Beninger, P. G. (2001). A functional interpretation of cilia and mucocyte distributions on the abfrontal surface of bivalve gills. *Marine Biology, 138(2)*, 295-309.

Duinker, A. (1996). *Effect of photoperiod on growth and feeding rate of scallop spat (*Pecten maximus *L.)*. (Sci. Degree thesis). University of Bergen.

Dupuy, C., Le Gall, S., Hartmann, H. J., & Bréret, M. (1999). Retention of ciliates and flagellates by the oyster *Crassostrea gigas* in French Atlantic coastal ponds: Protists as a trophic link between bacterioplankton and benthic suspension-feeders. *Marine Ecology Progress Series*, *177*, 165–175.

Espinosa, E. P., Perrigault, M., Ward, J. E., Shumway, S. E., & Allam, B. (2010). Microalgal cell surface carbohydrates as recognition sites for particle sorting in suspension-feeding bivalves. *Biological Bulletin*, *218*, 75–86.

Eyster, L. S., & Pechenik, J. A. (1988). Attachment of *Mytilus edulis* L. larvae on algal and byssal filaments is enhanced by water agitation. *Journal of Experimental Marine Biology and Ecology*, *1124*, 99–110.

Figueiras, F.G., Labarta, U., & Fernández-Reiriz, M. J. (2002). Coastal upwelling, primary production and mussel growth in the Rías Baixas of Galicia. *Hydrobiologia*, *484*, 121–131.

Fitzpatrick, M. P., Jeffs, A. G., & Dunphy, B. J. (2013). Efficacy of calcein as a chemical marker of green-lipped mussel (*Perna canaliculus*) larvae and its potential use for tracking larval

dispersal. *Aquaculture Research*, *44*(3), 345–353.

Flaws, D. (1975). *Aspects of the biology of mussels in the Cook Strait area*. (PhD thesis). Victoria University of Wellington.

Foote, G. (2003). *Characterization of the viability of greenshell mussel spat,* Perna canaliculus *with respect to hatchery production and growth retention*. (MSc thesis). University of Auckland.

Foster-Smith, R. L. (1976). Some mechanism for the control of pumping activity in bivalves. *Marine & Freshwater Behaviour & Physiology*, *4*(1), 41–59.

Fowler, N., Tomas, C., Baden, D., Campbell, L., & Bourdelais, A. (2015). Chemical analysis of *Karenia papilionacea*. *Toxicon*, *101*, 85–91.

Galley, T. H., Batista, F. M., Braithwaite, R., King, J., & Beaumont, A. R. (2010). Optimisation of larval culture of the mussel *Mytilus edulis* (L.). *Aquaculture International*. *18*, 315–325.

Ganesan, A. M. (2012). *The role of bacterial biofilms on settlement and nutrition of mussel (*Perna canaliculus*) larvae and juveniles*. (PhD thesis). Auckland University of Technology.

Gosling, E. (2004). *Bivalve molluscs: biology, ecology and culture*. *Blackwell Publishing*. London: Blackwell Publishing.

Gui, Y. (2012). *Particle retention and selection by the spat of the green-lipped mussel,* Perna canaliculus. (PhD thesis). University of Auckland.

Gui, Y., Kaspar, H. F., Zamora, L. N., Dunphy, B. J., & Jeffs, A. G. (2016a). Capture efficiency of artificial food particles of post-settlement juveniles of the Greenshell™ mussel, *Perna canaliculus*. *Aquaculture*, *464*, 1–7.

Gui, Y., Zamora, L. N., Dunphy, B., & Jeffs, A. G. (2016b). Understanding the ontogenetic changes in particle processing of the Greenshell™ mussel, *Perna canaliculus*, in order to improve hatchery feeding practices. *Aquaculture*, *452*, 120–127.

Gui, Yue, Zamora, L., Dunphy, B. J., & Jeffs, A. G. (2016c). Evaluation of the formulated diet MySpat for feeding hatchery-reared spat of the green-lipped mussel, *Perna canaliculus* (Gmelin, 1791). *Aquaculture Research*, *47*(12), 3907–3912.

Harrison, P. J., Zingone, A., Mickelson, M. J., Lehtinen, S., Ramaiah, N., Kraberg, A. C., Sun, J., McQuatters-Gollop, A., & Jakobsen, H. H. (2015). Cell volumes of marine phytoplankton from globally distributed coastal data sets. *Estuarine, Coastal and Shelf*

Science, *162*, 130–142.

Hatton, S., Hayden, B. J., & James, M. R. (2005). The effects of food concentration and quality on the feeding rates of three size classes of the Greenshell™ mussel, *Perna canaliculus*. *Hydrobiologia*, *548*(1), 23–32.

Hawkins, A. J. S., Bayne, B. L., & Day, A. (1986). Protein turnover, physiological energetics and heterozygosity in the blue mussel, *Mytilus edulis* : The basis of variable age-specific growth. *Proceedings of the Royal Society of London. Series B. Biological Sciences*, *229*(1255), 161–176.

Hayden, B. J. (1995). *Factor affecting recruitment of farmed greenshell mussels,* Perna canaliculus *(Gmelin) 1971, in Marlborough Sounds.* (PhD thesis). University of Otago.

Hayden, B. J., & Woods, C. M. C. (2011). Effect of water velocity on growth and retention of cultured Greenshell™ mussel spat, *Perna canaliculus* (Gmelin, 1791). *Aquaculture International*, *19*, 957–971.

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

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

Hickman, R. W. (1991). *Perna canaliculus* (Gmelin) in New Zealand. *Estuarine and Marine Bivalve Mollusk Culture*, *21*, 325–334.

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

Hill, D. R. A., & Wetherbee, R. (1989). A reappraisal of the genus *Rhodomonas* (Cryptophyceae). *Phycologia*, *28*(2), 143–158.

Hillebrand, H., Dürselen, C. D., Kirschtel, D., Pollingher, U., & Zohary, T. (1999). Biovolume calculation for pelagic and benthic microalgae. *Journal of Phycology*, *35*(2), 403–424.

Iglesias, J. I. P., Navarro, E., Alvarez Jorna, P., & Armentina, I. (1992). Feeding, particle selection and absorption in cockles *Cerastoderma edule* (L.) exposed to variable conditions of food concentration and quality. *Journal of Experimental Marine Biology and Ecology*, *162*(2), 177–198.

Jacobs, P., Troost, K., Riegman, R., & van der Meer, J. (2015). Length- and weight-dependent clearance rates of juvenile mussels (*Mytilus edulis*) on various planktonic prey items.

Helgoland Marine Research, *69*(1), 101–112.

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

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

Jørgensen, C. (1996). Bivalve filter feeding revisited. *Marine Ecology Progress Series*, *142*, 287–302.

Jørgensen, C., Kørboe, T., Møhlenberg, F., & Riisgård, H. (1984). Ciliary and mucus-net filter feeding, with special reference to fluid mechanical Characteristics. *Marine Ecology Progress Series*, *15*, 283-292

Kiøbroe, T., & Møhlenberg, F. (1981). Particle selection in suspension-feeding bivalves. *Marine Ecology Progress Series*, *5*(3), 291–296.

Kobak, J., & Nowacki, P. (2007). Light-related behaviour of the zebra mussel (*Dreissena polymorpha*, Bivalvia). *Fundamental and Applied Limnology / Archiv Für Hydrobiologie*, *169*(4), 341–352.

Laing, I., & Psimopoulous, A. (1998). Hatchery cultivation of king scallop (*Pecten maximus*) spat with cultured and bloomed algal diets. *Aquaculture*, *169*(1–2), 55–68.

Lane, D.J.W., Beaumont, A.R.,Hunter, J. R. (1985). Byssus drifting and the drifting threads of the young post-larval mussel *Mytilus edulis*. *Marine Biology*, *84*, 301–308.

Lane, D. J. W., Nott, J. A., & Crisp, D. T. (1982). Enlarged stem glands in the foot of the post-larval mussel, *Mytilus edulis*: adaptation for bysso-pelagic migration. *Journal of the Marine Biological Association of the United Kingdom*, *84*, 301–308.

Lang, I., Hodac, L., Friedl, T., & Feussner, I. (2011). Fatty acid profiles and their distribution patterns in microalgae: a comprehensive analysis of more than 2000 strains from the SAG culture collection. *Plant Biology*, *11*, 124–140.

Laxmilatha, P., Rao, G. S., Patnaik, P., Rao, T. N., Rao, M. P., & Dash, B. (2011). Potential for the hatchery production of spat of the green mussel *Perna viridis* Linnaeus (1758). *Aquaculture*, *312*(1–4), 88–94.

Li, S. C., Wang, W. X., & Hsieh, D. P. H. (2002). Effects of toxic dinoflagellate *Alexandrium*

tamarense on the energy budgets and growth of two marine bivalves. *Marine Environmental Research, 53(2),* 145-160.

Liutkus, M., Robinson, S., MacDonald, B., & Reid, G. (2012). Quantifying the effects of diet and mussel size on the biophysical properties of the blue mussel, *Mytilus* spp., feces egested under simulated IMTA conditions. *Journal of Shellfish Research*, *31*(1), 69–77.

Lurman, G. J., Hilton, Z., & Ragg, N. L. C. (2013). Energetics of byssus attachment and feeding in the green-lipped mussel *Perna canaliculus*. *Biological Bulletin*, *224*(2), 79–88.

Mafra, L. L., Bricelj, V. M., Ouellette, C., Léger, C., & Bates, S. S. (2009). Mechanisms contributing to low domoic acid uptake by oysters feeding on *Pseudo-nitzschia* cells. I. Filtration and pseudofeces production. *Aquatic Biology*, *6*, 1–11.

Maire, O., Amouroux, J. M., Duchêne, J. C., & Grémare, A. (2007). Relationship between filtration activity and food availability in the Mediterranean mussel *Mytilus galloprovincialis*. *Marine Biology, 152(6),* 1293-1307.

Mamat, N. Z., & Alfaro, A. C. (2014). Evaluation of microalgal and formulated diets for the culture of the New Zealand pipi clam *Paphies australis*. *International Aquatic Research*, *6*, 1–16.

Manahan, D.T., Wright, S. H., Stephens, G. C., Rice, M. A. (1982). Transport of dissolved amino acids by the mussel, *Mytilus edulis*: Demonstration of net uptake from natural seawater. *Science*, *215*(4537), 1253–1255.

Martínez-Pita, I., Sánchez-Lazo, C., & García, F. J. (2016). Influence of microalga lipid composition on the sexual maturation of *Mytilus galloprovincialis*: A hatchery study. *Aquaculture Nutrition, 22(1),* 202-216.

Medcof, J. A., & Kerswill, C. (1965). Effect of light on growth of oysters, mussels, and quahogs. *Journal of Fisheries Board of Canada*, *22*, 281–288.

Menden-Deuer, S., & Lessard, E. J. (2000). Carbon to volume relationships for dinoflagellates, diatoms and other protist plankton. *Limnology and Oceanography*, *45*, 569–579.

Mikulich, L.V., & Tsikhon-Lukanina, E. (1981). Food composition of the yesso scallop. *Oceanology*, *21*, 633–635.

Møhlenberg, F., & Risgård, H. U. (1978). Efficiency of particle retention in 13 species of suspension feeding bivalves. *Ophelia*, *17*, 239–26.

Morton, J. E., & Miller, M. C. (1973). *The New Zealand Sea Shore*. *Collins*. London: Collins &

Sons. New Zealand.

Munir, S., Naz, T., Morton, S. L., & Siddiqui, P. J. A. (2015). Morphometric forms, biovolume and cellular carbon content of dinoflagellates from polluted waters on the Karachi coast, Pakistan. *Indian Journal of Geo-Marine Science*, *44*(1), 19–25.

Navarro, J. M., & Winter, J. E. (1982). Ingestion rate, assimilation efficiency and energy balance in *Mytilus chilensis* in relation to body size and different algal concentrations. *Marine Biology*, *67*(3), 255–266.

New Zealand Sustainable Aquaculture. (2019). *New Zealand aquaculture: A sector overview with key facts and statistics*. Nelson.

Newell , R. I. E. & Jordan, S. J. (1983). Preferential ingestion of organic material by the American oyster *Crassostrea virginica*. *Marine Ecology Progress Series*, *13*, 47–53.

Newell, C. R., Wildish, D. J., & MacDonald, B. A. (2001). The effects of velocity and seston concentration on the exhalant siphon area, valve gape and filtration rate of the mussel *Mytilus edulis*. *Journal of Experimental Marine Biology and Ecology*, *262*, 91–111.

Newell, Carter R, & Shumway, S. E. (1993). Grazing of natural particulates by bivalve molluscs: a spatial and temporal perspective. In *Estuarine and Coastal Ecosystem Processes* (pp. 85–148). Springer.

Nielsen, M. V, & Strömgren, T. (1985). The effect of light on the shell length growth and defaecation rate of *Mytilus edulis* (L.). *Aquaculture*, *47*(2–3), 205–211.

Nishida, A., Ohkawa, K., Ueda, I., & Yamamoto, H. (2003). Green mussel *Perna viridis* L.: Attachment behaviour and preparation of antifouling surfaces. *Biomolecular Engineering*, *20*, 381–387.

Olenina, I., Hajdu, S., Edler, L., Wasmund, N., Busch, S., Göbel, J., Gromisz, S., Huseby, S., Huttunen, M., Jaanus, A., Kokkonen, P., Ledaine, I., & Niemkiewicz, E. (2006). Biovolumes and size-classes of phytoplankton in the Baltic Sea. In *Baltic Sea Environment Proceedings* (Vol. 106, p. 144pp). Helsinki Commission.

Pales-Espinosa, E., P, Perrigault, M., & Allam, B. (2010). Identification and molecular characterization of a mucosal lectin (MeML) from the blue mussel *Mytilus edulis* and its potential role in particle capture. *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology*, *156(4)*, 495-501.

Pascoe, P. L., Parry, H. E., & Hawkins, A. J. S. (2009). Observations on the measurement and

interpretation of clearance rate variations in suspension-feeding bivalve shellfish. *Aquatic Biology*, *6*, 181–190.

Pernet, F., Tremblay, R., & Bourget, E. (2003). Settlement success, spatial pattern and behavior of mussel larvae *Mytilus* spp. in experimental "downwelling" systems of varying velocity and turbulence. *Marine Ecology Progress Series*, *260*(125–140).

Price, H. A. (1982). An analysis of factors determining seasonal variation in the byssal attachment strength of *Mytilus edulis*. *Journal of the Marine Biological Association of the United Kingdom*, *62*(1), 147–155.

Raby, D., Mingelbier, M., Dodson, J. J., Klein, B., Lagadeuc, Y., & Legendre, L. (1997). Food-particle size and selection by bivalve larvae in a temperate embayment. *Marine Biology*, *127*(4), 665–672.

Ragg, N. L. C., King, N., Watts, E., & Morrish, J. (2010). Optimising the delivery of the key dietary diatom *Chaetoceros calcitrans* to intensively cultured Greenshell™ mussel larvae, *Perna canaliculus*. *Aquaculture*, *306*(1–4), 270–280.

Rajagopal, S., Sasikumar, N., Azariah, J., & Nair, K. V. K. (1991). Some observations on biofouling in the cooling water conduits of a coastal power plant. *Biofouling*, *3*(4), 311–324.

Rajagopal, S., Venugopalan, V. P., Nair, K. V. K., Van Der Velde, G., Jenner, H. A., & Den Hartog, C. (1998). Reproduction, growth rate and culture potential of the green mussel, *Perna viridis* (L.) in Edaiyur backwaters, east coast of India. *Aquaculture*, *32*(4), 313–322.

Rajesh, K. V., Mohamed, K. S., & Kripa, V. (2001). Influence of algal cell concentration, salinity and body size on the filtration and ingestion rates of cultivable Indian bivalves. *Indian Journal of Marine Sciences*, *30*(2), 87–92.

Redfearn, P., Chanley, P., & Chanley, M. (1986). Larval shell development of four species of New Zealand mussels: (Bivalvia, *Mytilacea*). *New Zealand Journal of Marine and Freshwater Research*, *20*, 157–172.

Ren, J.S., Ross, A.H., Hayden, B. J. (2006). Comparison of assimilation efficiency on diets of nine phytoplankton species of the greenshell mussel *Perna canaliculus*. *Journal of Shellfish Research*, *25*, 887–892.

Riisgard, H. U., & Randløv, A. (1981). Energy budgest, growth and filtration rates in *Mytilus edulis* at different algal concentrations. *Marine Biology*, *61*(2–3), 227–234.

Riisgård, H. U. (1991). Filtration rate and growth in the blue mussel, *Mytilus edulis* Linaeus. 1758: Dependence on algal concentration. *Journal of Shellfish Research*, *10*(1), 29–35.

Riisgård, H.U., Egede, P. P., & Saavedra, B. I. (2011). Feeding behaviour of the mussel, *Mytilus edulis* : New observations, with a mini review of current knowledge. *Journal of Marine Biology*, *2011*, 1–13.

Riisgård, Hans Ulrik, Larsen, P. S., & Pleissner, D. (2014). Allometric equations for maximum filtration rate in blue mussels *Mytilus edulis* and importance of condition index. *Helgoland Marine Research*, *68*(1), 193–198.

Riisgård, Hans Ulrik, Randløv, A., & Kristensen, P. S. (1980). Rates of water processing, oxygen consumption and efficiency of particle retention in veligers and young post-metamorphic *Mytilus edulis*. *Ophelia*, *19*(1), 37–46.

Rosa, M, Ward, J. E., Holohan, B. A., Shumway, S. E., & Wikfors, G. H. (2017). Physicochemical surface properties of microalgae and their combined effects on particle selection by suspension-feeding bivalve molluscs. *Journal of Experimental Marine Biology and Ecology*, *486*, 59–68.

Rosa, Maria, Ward, J. E., Shumway, S. E., Wikfors, G. H., Pales-Espinosa, E., & Allam, B. (2013). Effects of particle surface properties on feeding selectivity in the eastern oyster *Crassostrea virginica* and the blue mussel *Mytilus edulis*. *Journal of Experimental Marine Biology and Ecology*, *486*, 59-68.

Rusk, A. B. (2012). *Larval development of the New Zealand mussel* Perna canaliculus *and effect of cryoperservation*. (PhD thesis). Auckland University of Technology.

Safi, K. A., & Hayden, B. (2010). Differential grazing on natural planktonic populations by the mussel *Perna canaliculus*. *Aquatic Biology*, *11(2)*, 113-125.

Shumway, S.E., Cucci, T.L., Newell, R.C., & Yentsch, C. (1985). Particle selection, ingestion, and absorption in filter-feeding bivalves. *Journal of Experimental Biological Ecology*, *91*, 77–92.

Shumway, S.E., Selvin, R. & Schick, D. (1987). Food resources related to habitat in the scallop *Placopecten magellanicus* (Gmelin, 1791): a qualitative study. *Journal of Shellfish Research*, *6*, 89–95.

Shumway, S. E., & Cucci, T. L. (1987). The effects of the toxic dinoflagellate *Protogonyaulax tamarensis* on the feeding and behaviour of bivalve molluscs. *Aquatic Toxicology*, *10*, 9–27.

Sieburth, J.M., Smetacek, V., Lenz, J. (1978). Pelagic ecosystem structure: heterotrophic compartments of the plankton and their relationship to plankton size fractions. *Limnology and Oceanography*, *23*, 1256–1263.

Sim-Smith, C. J., & Jeffs, A. G. (2011). A novel method for determining the nutritional condition of seed green-lipped mussels, *Perna canaliculus*. *Journal of Shellfish Research*, *30*(1), 7–11.

Sim-Smith, C., Mc. Cluskie, K., & Hall, J. (2005). *Measurement of clearance rate of* Perna canaliculus *spat using a flow cytometer*. (Report# NRAB051). New Zealand: NIWA.

South, P. M. (2018). *The retention of juvenile* Perna canaliculus *in aquaculture*. (PhD thesis) University of Auckland.

South, P. M., Floerl, O., & Jeffs, A. G. (2017). Differential effects of adult mussels on the retention and fine-scale distribution of juvenile seed mussels and biofouling organisms in long-line aquaculture. *Aquaculture Environment Interactions*, *9*, 239–256.

Spencer, B. E. (2002). *Molluscan shellfish farming*. Retrieved from https://leseprobe.buch.de/images-adb/00/88/00885744-5ffd-4819-8c9d-d2b0d05f3055.pdf

Stanley, J. A., Wilkens, S. L., & Jeffs, A. G. (2014). Fouling in your own nest: Vessel noise increases biofouling. *Biofouling*, *30*(7), 837–844.

Strömgren, T. (1976). Length growth of *Mytilus edulis* (Bivalvia) in relation to photoperiod, irradiance, and spectral distribution of light. *Sarsia*, *61*(1), 31–40.

Supono, S., Dunphy, B., & Jeffs, A. (2020). Retention of green-lipped mussel spat: The roles of body size and nutritional condition. *Aquaculture*, *520*.

Tan, W. H. (1975). Egg and larval development in the green mussel, *Mytilus viridis*. *Veliger*, *18*(2), 151–155.

Tang, B., & Riisgård, H. U. (2016). Physiological regulation of valve-opening degree enables mussels *Mytilus edulis* to overcome starvation periods by reducing the oxygen uptake. *Open Journal of Marine Science*, *6*(3), 341–352.

Tang, B., & Riisgård, H. U. (2018). Relationship between oxygen concentration, respiration and filtration rate in blue mussel *Mytilus edulis*. *Journal of Oceanology and Limnology*, *36*(2), 395–404.

Tantanasarit, C., Babel, S., Englande, A. J., & Meksumpun, S. (2013). Influence of size and

density on filtration rate modeling and nutrient uptake by green mussel (*Perna viridis*). *Marine Pollution Bulletin*, *68*(1–2), 38–45.

Tomaru, Y., Udaka, N., Kawabata, Z. I., Nakano, S. I. (2002). Seasonal change of seston size distribution and phytoplankton composition in bivalve pearl oyster *Pinctada fucata martensii* culture farm. *Hydrobiologia*, *48*(1), 181–185.

Tomas, C. R. (1997). *Identifying marine phytoplankton*. San Diego, CA: Academic Press.

Tremblay, R., Cartier, S., Miner, P., Pernet, F., Quéré, C., Moal, J., Muzellec, M. L., Mazuret, M., & Samain, J. F. (2007). Effect of *Rhodomonas salina* addition to a standard hatchery diet during the early ontogeny of the scallop *Pecten maximus*. *Aquaculture*, *262*(2–4), 410–418.

Trottier, O., & Jeffs, A. G. (2015). Mate locating and access behaviour of the parasitic pea crab, *Nepinnotheres novaezelandiae*, an important parasite of the mussel *Perna canaliculu*s. *Parasite*, *22*(13), 1–13.

Uryu, Y., Iwasaki, K., & Hinoue, M. (1996). Laboratory experiments on behaviour and movement of a freshwater mussel, *Limnoperna fortunei* (Dunker). *Journal of Molluscan Studies*, *62*(3), 327–341.

Viherluoto, M., & Viitasalo, M. (2001). Temporal variability in functional responses and prey selectivity of the pelagic mysid, *Mysis mixta*, in natural prey assemblages. *Marine Biology*, *138(3)*, 575-583.

Volkman, J. K., Jeffrey, S. W., Nichols, P. D., Rogers, G. I., & Garland, C. D. (1989). Fatty acid and lipid composition of 10 species of microalgae used in mariculture. *Journal of Experimental Marine Biology and Ecology*, *128(3)*, 219-240.

Von Elert, E., & Stampfl, P. (2000). Food quality for *Eudiaptomus gracilis*: The importance of particular highly unsaturated fatty acids. *Freshwater Biology*, *45(2)*, 189-200.

Walne, P. R. (1970). Studies on the food value of nineteen genera of algae to juvenile bivalve of the genera *Ostrea*, *Crassostrea*, *Mercenaria* and *Mytilus*. *Fish Investigation Series 2*, *26*(5), 62pp.

Wang, W. X., & Widdows, J. (1993). Metabolic responses of the common mussel *Mytilus edulis* to hypoxia and anoxia. *Marine Ecology Progress Series*, *95*, 205–214.

Wang, Y., Hu, M., Wong, W. H., Shin, P. K. S., & Cheung, S. G. (2011). The combined effects of oxygen availability and salinity on physiological responses and scope for growth in the green-lipped mussel *Perna viridis*. *Marine Pollution Bulletin*, *65*(5–12), 255–261.

Ward, J.E., Sanford, L.P., Newell, R.I.E., & MacDonald, B. A. (1998). A new explanation of particle capture in suspension-feeding bivalve molluscs. *Limnology and Oceanography Oceanogr*, *43*, 741–752.

Ward, J. E., MacDonald, B. A., Thompson, R. J., & Beninger, P. G. (1993). Mechanisms of suspension feeding in bivalves: Resolution of current controversies by means of endoscopy. *Limnology and Oceanography*, *38(2)*, 265-272.

Ward, J. E., Newell, R. I. E., Thompson, R. J., & MacDonald, B. A. (1994). In vivo studies of suspension-feeding processes in the eastern oyster, *Crassostrea virginica* (Gmelin). *Biological Bulletin*, *186*(2), 221-240.

Ward, J. Evan, & Shumway, S. E. (2004). Separating the grain from the chaff: Particle selection in suspension- and deposit-feeding bivalves. *Journal of Experimental Marine Biology and Ecology*, *300*(1–2), 83–130. h

Widdows, J., Fieth, P., & Worrall, C. M. (1979). Relationships between seston, available food and feeding activity in the common mussel *Mytilus edulis. Marine Biology*, *50*(3), 195–207.

Widdows, J. (1991). Physiological ecology of mussel larvae. *Aquaculture*, *94*(2–3), 147–163.

Widdows, J., & Hawkins, A. J. S. (1989). Partitioning of rate of heat dissipation by *Mytilus edulis* into maintenance, feeding, and growth components. *Physiological Zoology*, *62*(3), 764–784.

Widdows, J., & Staffs, F. (2006). *Biological effect of contaminants: measurement of scope for growth in mussels* (40th ed.). Copenhagen, Denmark: International Council for the Exploration of the Sea.

Wildish, D. J., Kristmanson, D. D., Hoar, R. L., DeCoste, A. M., McCormick, S. D., & White, A. W. (1987). Giant scallop feeding and growth responses to flow. *Journal of Experimental Marine Biology and Ecology*, *113*(3), 207–220.

Wildish, D. J., & Miyares, M. P. (1990). Filtration rate of blue mussels as a function of flow velocity: preliminary experiments. *Journal of Experimental Marine Biology and Ecology*, *142*(3), 213–219.

Wilkens, S. L., Stanley, J. A., & Jeffs, A. G. (2012). Induction of settlement in mussel (*Perna canaliculus*) larvae by vessel noise. *Biofouling*, *28*, 65–72.

Willmer, P. (1990). *Invertebrate relationship: Patterns in animal evolution*. Cambridge:

Cambridge University Press.

Wołowski, K., & Walne, R. L. (2014). *Strombomonas* and *Trachelomonas* species (Euglenophyta) from south-eastern USA. *Europian Jornal of Phycology*, *42*, 409–431.

Wong, W. H., & Cheung, S. G. (1999). Feeding behaviour of the green mussel, *Perna viridis* (L.): Responses to variation in seston quantity and quality. *Journal of Experimental Marine Biology and Ecology*, *236*(2), 191–207.

Xu, N., Wang, M., Tang, Y., Zhang, Q., Duan, S., & Gobler, C. J. (2017). Acute toxicity of the cosmopolitan bloom-forming dinoflagellate *Akashiwo sanguinea* to finfish, shellfish, and zooplankton. *Aquatic Microbial Ecology*, *80(3)*, 209-222.

Yang, Y., Huang, B., Tang, Y., & Xu, N. (2020). Allelopathic effects of mixotrophic dinoflagellate *Akashiwo sanguinea* on co-occurring phytoplankton: the significance of nutritional ecology. *Journal of Oceanology and Limnology*, 1–15.

Young, G. (1985). Byssus-thread formation by the mussel *Mytilus edulis*: effects of environmental factors. *Marine Ecology Progress Series*, *24*(3), 261–271.

Zeldis J., Robinson K., Ross A., & Hayden. B. (2004). First observations of predation by New Zealand greenshell mussels (*Perna canaliculus*) on zooplankton. *Journal of Experimental Marine Biology and Ecology*, *311*, 287–299.