Evaluating the implications of recent filter-feeding *Daphnia* invasions for kākahi (*Echyridella menziesii*)

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Abstract

Freshwater mussels function as key ecosystem engineers due to their highly efficient filter feeding and bioturbating abilities, which enhance water clarity and promote nutrient cycling. Although they play a crucial role in facilitating these ecosystem services, freshwater mussels globally are in decline. New Zealand's native freshwater mussels, traditionally referred to as kākahi or kāeo, are no exception. On a global scale, freshwater mussels are impacted by a suite of anthropogenic stressors, including pollution, habitat loss and the establishment of non-indigenous species. However, factors contributing to mussel declines in New Zealand are less understood. Recent studies have suggested that non-native species may be a key contributing factor. At present, one-third of non-native invertebrate species that have established in New Zealand's freshwater lakes are zooplankton; two of these are highly efficient filter-feeding *Daphnia* species. Various studies have demonstrated that *Daphnia* invasions can have serious ecological impacts in their receiving environments, as these organisms are capable of modifying zooplankton communities and limiting the availability of algal food resources for other organisms. New Zealand studies are yet to explore the possibility of non-native *Daphnia* competing with freshwater mussels for algal food resources.

In response to this hypothesis, my research aimed to; i) examine the effects freshwater mussels have on *Daphnia* via predation and/ or interference competition, and ii) investigate the effect of non-native *Daphnia* on kākahi, through exploitative competition for algal food resources. Controlled tank experiments were used to investigate whether New Zealand's most widespread kākahi species (*Echyridella menziesii*) could prey on zooplankton. Two-hour predation trials were undertaken on two small native zooplankton species (*Brachionus calyciflorus* and *Bosmina meridionalis*), and two non-native cladoceran species (*Daphnia pulex* and *Daphnia galeata*). Kākahi were only found to remove statistically significant numbers of the small rotifer species *B. calyciflorus* (30.2%) and the large cladoceran *D. pulex* (1.7%). These findings indicate that kākahi are unable to remove ecologically significant numbers of either non-native *Daphnia*. Thus, *Echyridella menziesii* is not suitable to be used as a biomanipulation tool to remove non-indigenous *Daphnia* species from shallow lakes and ponds. Kākahi could remove moderate

quantities of the small native zooplankton species *B. calyciflorus* (30.2%) over the two-hour period. This suggested that small zooplankton, particularly rotifer species, may function as an important food source for kākahi. Additional laboratory experiments were undertaken to test the algal removal capabilities of *D. pulex* and of adult and juvenile *E. menziesii* over a three-hour period. Both kākahi life stages were found to consume a broad range of algal taxa, including diatoms, green algae, and filamentous algae, which ranged in size (between 33.6 and 348.0 μm). Comparatively, *D. pulex* was unable to consume statistically significant numbers of the same algal taxa. Instead, *D. pulex* consumed smaller algal species and microbes. As such, my findings suggest a limited niche overlap between the two grazers and, therefore, *D. pulex* is unlikely to reduce algal food availability for kākahi. Due to these differences in algal removal, it is possible that these grazers could be used together as a biomanipulation tool to remove a wide size range of algal biomass in shallow lake systems.

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Chapter 1

General Introduction

1.1 Background

Although regularly overlooked, invertebrate species make up 99% of the globe's animal diversity and many of these species play key roles in ecosystem function (Lydeard et al. 2004). In freshwater environments mussels (Mollusca: Bivalvia) play an important role in nutrient recycling. These bivalves act as ecosystem engineers as they can modify habitats physically, chemically and biologically (Zaiko and Daunys 2015; Vaughn and Hakenkamp 2001). For example, filter-feeding mussels can enhance benthic-pelagic coupling as they filter seston from the water column and produce biodeposits of faeces and pseudofeces at the sediment-water interface; these activities help to both enrich the sediments and enhance water clarity in the system (Zaiko et al. 2010). The spent shells of mussels can also modify freshwater habitats by providing physical structure that helps to stabilize sediments (Vaughn et al. 2008). Freshwater mussels can effectively modify entire freshwater ecosystems from the bottom up, making them key players in the freshwater aquatic environment. In recent years, bivalves have become one of the world's most vulnerable and threatened taxonomic groups (Lydeard et al. 2004). Mussel declines have been linked to multiple stressors, such as the invasion of non-native species, anthropogenic pollution, and habitat modification (Naimo 1995; Bauer 1988; Bauer 1986; Brainwood et al. 2006). One of the key factors that appears to be impacting freshwater mussels globally is the establishment of non-native species such as invasive fish, macrophytes, and mammals in their environments (Lopes-Lima et al. 2016; Moore et al. in press).

Considering these issues, this chapter will address causes of freshwater mussel declines followed by a specific focus on the impacts of invasive species. It will also discuss freshwater mussels in New Zealand and their significance to local people, followed by potential causes for their declines in relation to non-native taxa and the

potential effects of two non-indigenous zooplankton (*Daphnia pulex* and *Daphnia galeata*). Additionally, this chapter will describe previously documented interactions between freshwater mussels and zooplankton on both a local and global scale, with the expectation of identifying the types of relationships that occur between these groups, before outlining the aims of this research.

1.2 Global decline of freshwater mussels

Although it is well known that freshwater mussels are impacted by anthropogenic factors and invasive species on a global scale, limited quantitative data is available to confirm the global decline, and much of the research is focused around European, Australasian, and American species (Ricciardi et al. 1998; Anthony and Downing 2001; Lydeard 2004). This makes it extremely difficult to ascertain the severity of Unionoida decline. However, it is clear from the few published studies available that freshwater mussels are at serious risk. For example, the freshwater European pearl mussel (*Margaritifera margaritifera*) has declined by more than 90% over the last century and is now extinct throughout broad areas of Europe due to illegal pearl fishing, water pollution, and habitat destruction (Bauer 1986). Bauer (1988) has suggested that pollution in the form of nitrate, phosphate, and calcium from farming and sewage outputs has been particularly detrimental to the adult stage of this species. Similarly, in North America, over 70% of freshwater mussel species are endangered or are at risk of extinction. In this case, research has suggested that high concentrations of ammonia, which are often associated with wastewater treatment plants, agricultural runoff, and industrial wastes, are a key factor limiting mussel survival (Augspurger 2003). The invasion of the zebra mussel (*Dreissena polymorpha*) has also played a key role in the decline of the native North American mussels through its biofouling capabilities. These highly effective invaders, native to Ukraine and Russia, have spread throughout waterways in the United States, smothering and outcompeting a multitude of native species for phytoplankton food resources due to their highly effective filter feeding capabilities (Ricciardi et al. 1998).

1.2.1 Impacts of non-indigenous species on freshwater mussels

Various studies have examined the possible impacts of non-native species on freshwater mussels, with three taxa primarily identified as contributors to freshwater mussel decline: non-native macrophytes, fish, and mammalian pests (Lopes-Lima et al. 2016). For example, in the lakes of Portugal, swan mussels (*Anodonta cygnea*) have been adversely affected by the non-native macrophyte species *Eichhornia crassipes* (water hyacinth). At the end of the growing season, macrophytes die off producing masses of organic matter; this causes a reduction in the oxygen availability that, in turn, causes high mortalities of *A. cygnea* on an annual basis (Lopes-Lima et al. 2016). Earlier research conducted by Burlakova and Karatayev (2007) on the giant floater freshwater mussel (*Pyganodon grandis*) and the paper pondshell mussel (*Utterbackia imbecillis*) in Texan (USA) lakes highlighted that the introduction of macrophytes is strongly associated with mussel declines. The presence of non-native macrophytes, such as the Eurasian watermilfoil (*Myriophyllum spicatum*) and the American lotus (*Nelumbo lutea*), were associated with significant reductions in unionids across several lakes. Although no single factor causing mussel declines could be determined, Burlakova and Karatayev (2007) suggested that the non-native macrophytes played a key role in modifying the mussel's habitat. The addition of these macrophytes were associated with increases in shade, changes in water temperatures, depleted oxygen supplies, trapped fine sediments, and fluctuations in water levels. Burlakova and Karatayev (2007) also concluded that fluctuations in water levels likely facilitated predation by Muskrats (*Ondatra zibethicus*), which accounted for 19% of unionid losses. Similarly, more recent research in Shoal Creek, Alabama, USA, found Muskrats were preying on six different native species of freshwater mussel (Edelman et al. 2015). Comparatively, research in Western Australia has recently discovered that non-native fish may be contributing to the decline of their native freshwater mussel (*Westralunio carteri*), as laboratory studies have concluded that some non-native fish species are unsuitable hosts for the unionids glochidial larval stage (Klunzinger et al. 2012).

1.3 Freshwater mussels of New Zealand

New Zealand's native freshwater mussels, commonly referred to by their traditional Māori names kākahi or kāeo, belong to the large order Unionoida, which are distributed through all biogeographical eco-zones except for the Antarctic and oceanic regions (Bogan 2008). Bivalves within this order are unique in that they have an obligate parasitic larval stage in which glochidia attaches to a host fish species early in their lifecycle. To date, a diverse range of strategies for mussels to attract host fishes have been observed, yet very little is understood about the evolutionary origins behind these interesting parasitic mechanisms (Barnhart et al. 2008).

Until recently, there has been a considerable amount of confusion in the literature regarding the number of kākahi species in New Zealand. Overestimates of the number of species were made due to slight differences in shell morphologies among populations, which were later found to be related to variations in environmental conditions (Marshall et al. 2014; Phillips 2007; Fenwick et al. 2006). Recent advancements in DNA barcoding technology have allowed for simple sequencing of the mitochondrial gene, cytochrome c oxidase I (COI), which has confirmed that there are only three living species of freshwater mussel in New Zealand (*Echyridella menziesii*, *Echyridella aucklandica* and *Echyridella onekaka)* (Marshall et al. 2014); *E. menziesii* is both the most abundant and widely distributed species with populations distributed throughout New Zealand's North and South Islands (Figure 1.1) (Marshall et al., 2014).

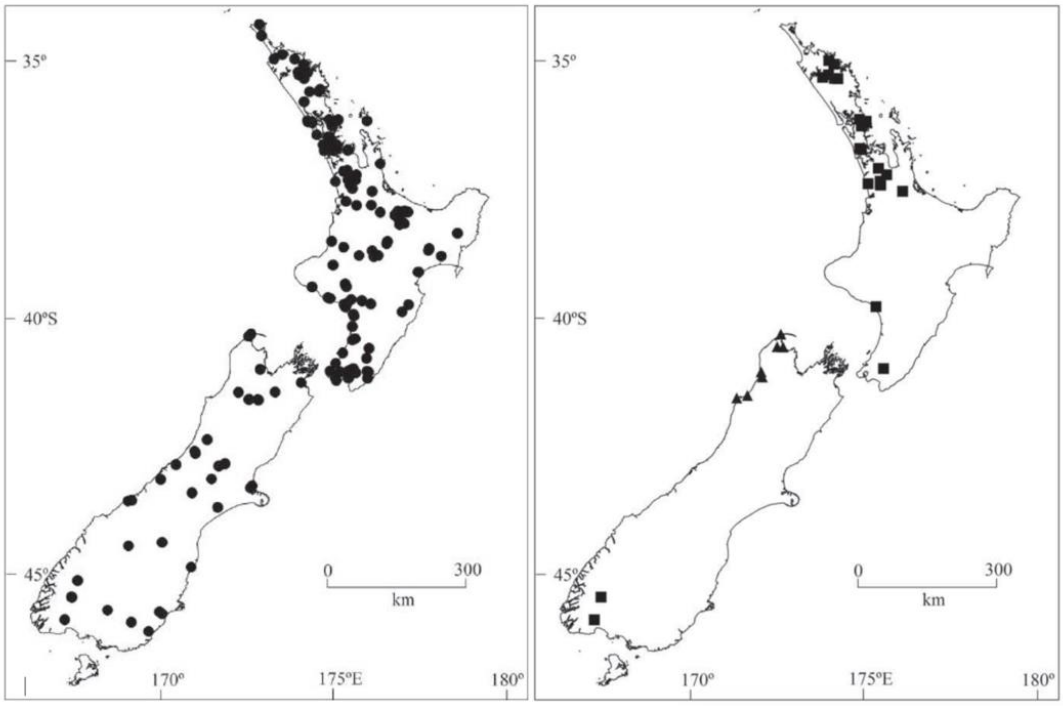

Figure 1.1. Distribution of New Zealand's three freshwater mussel species, *E. menziesii* (dots) is located on the left panel and *E. onekaka* (triangles) and *E. aucklandica* (squares) are located on the right panel (Marshall et al. 2014).

1.3.1 Significance to Māori

Historically, kākahi have played an important role as a food source, cutting tool, and medical treatments in Māori culture. Although freshwater mussels were mostly thought to be bland in taste, they were often utilised to feed orphaned children who would suckle on the liquid produced by their cooked visceral mass. Due to their important role in nourishing motherless children, kākahi were often referred to by Maori as "the mother of the child" (ko te kākahi te whaea o te tamaiti) (McDowall 2011; Hiroa 1921). Interestingly, the juice from cooked kākahi was also utilised to help those with medical issues such as whitening of the eye. The shell of kākahi was also widely sought after; it was deemed particularly useful for its sharp edge that could be used to cut through human hair, flax, and the umbilical cords of newborns. The patterns and spirals carved by kākahi moving through the sediments were also considered significant for their likeness to the Māori patterns of toa, warrior tattoos and carvings (Hiroa 1921) (Figure 1.2). In recent years, these patterns have earnt mussels the name of "carvers of the lake bed" in the Te Arawa Lakes (Pers. Comm., I. Kusabs, Ian Kusabs and Associates Ltd, 2018).

Figure 1.1. Bioturbation patterns generated by kākahi moving through silica sand.

1.3.2 Kākahi decline

In New Zealand, there is no formally published quantitative data available to determine the severity of kākahi decline (Pers. Comm., M. Hamner, Waikato Regional Council 2017). Nonetheless, it is clear from historic accounts generated by Māori, that kākahi are far less abundant than in pre-European times (Rainforth 2008; McDowall 2011). Iwi of Whanganui formally noted the decline of kākahi during their Planning Tribunal hearings in 1989 - 1990. During the tribunal, various scientists and iwi fisherman tested these claims and were unable to locate any kākahi within the Whanganui River (Rainforth 2008). Furthermore, Rainforth (2008) analysed historic records and suggested that kākahi numbers most likely began to decline in the 1950s, as reports from Māori elders (kaumātua) suggest

kākahi were still abundant throughout the 1940s. However, it is difficult to determine whether these verbal accounts are accurate as kākahi have a lifespan of over 50 years (Grimmond 1968). As such, declines may have gone unnoticed for many decades. At present, the conservation status of *Echyridella menziesii* is "at risk – declining", while *E. aucklandica* is considered "threatened – nationally vulnerable"; these statuses are given due to their current population size and their predicted continued declines. Comparatively, New Zealand's third kākahi species, *E. onekaka*, is classified as "data deficient" (Grainger et al. 2018).

1.3.3 Impact of non-indigenous species on New Zealand's freshwater mussels

Despite obvious causes for freshwater mussel declines overseas, mechanisms leading to kākahi decline in New Zealand are yet to be explored in depth. Nonetheless, the impact of non-indigenous species are thought to be one of the leading causes of kākahi losses. This includes impacts by both mammalian and freshwater taxa. Although there are no Muskrats in New Zealand, predation markings on spent kākahi shells made by *Rattus ratus* (ship rat) have been observed on stream banks throughout the Waikato (Pers. Obs., A. Pearson; Pers. Comm., K. King, University of Waikato 2017; Moore et al. in press). Further, the diet of brown or Norway rats (*Rattus norvegicus*) on Mokoia Island (Lake Rotorua) has been found to consist of various seeds and invertebrates, including freshwater mussels (Beveridge and Daniel 1965). This may indicate that non-indigenous mammals are also playing a role in impacting kākahi in New Zealand. It has also been suggested that the establishment of non-native fishes in freshwater systems may negatively impact kākahi reproduction (Phillips 2007; Moore et al. in press). Kākahi have an obligate parasitic stage in which glochidia are required to attach to a native fish host species to disperse (Barnhart et al. 2008). Therefore, the number and variety of non-native fish species, and associated declines in native fish in New Zealand's waterways, may be cause for concern (Phillips 2007). It is possible that non-indigenous fish (e.g. Koi carp, Rudd, and Perch) may be unsuitable hosts for glochidia (Collier and Grainger 2015), which may explain why poor recruitment success has been observed in kākahi populations in recent years (Moore et al. in press).

Along with the previously mentioned factors, it is also possible that other aquatic taxa are impacting kākahi. In recent years, numerous non-indigenous invertebrates have invaded New Zealand lakes, including several zooplankton species which make up one third of the known established invertebrates. Further, invasions of zooplankton appear to be on the rise, with 60% of non-native invertebrates identified in New Zealand lakes since 2000 being zooplankton (Duggan and Collier 2018). At present, three of the eight non-native zooplankton species established in New Zealand lakes belong to the genus *Daphnia* (Duggan and Collier 2018). In lakes, *Daphnia* have been recorded to have strong grazing capabilities, making these microscopic organisms highly effective herbivores, particularly in high densities (Beisner 2001; Balvert et al. 2009). Thus, *Daphnia* may have the ability to compete with kākahi.

1.3.4 Potential impacts of non-indigenous Daphnia *on kākahi*

Although it is probable that non-indigenous mammals and anthropogenic factors are likely impacting New Zealand's native freshwater mussels, it is possible other mechanisms may also be contributing to kākahi losses. A theory that is yet to be explored in New Zealand is competition between kākahi and filter-feeding zooplankton such as non-native *Daphnia*, which have recently widely invaded New Zealand lake ecosystems (Branford and Duggan 2017; Duggan and Collier 2018). Since 2000, two large non-native cladocerans have become widely established in New Zealand's lakes and ponds (Figure 1.3); the Holarctic *Daphnia galeata* and the North American *Daphnia pulex* (Balvert et al. 2009; Duggan et al. 2012; Burns et al. 2017; Branford and Duggan 2017).

Figure 1.2. Image of *Daphnia pulex* (left) and *Daphnia galeata* (right) at 40x magnification.

In lake ecosystems, *Daphnia* function as highly effective herbivores due to their efficient grazing capabilities and generalist feeding strategies (Beisner 2001; Balvert et al. 2009). This makes these grazers particularly effective at modifying water clarity in shallow waterbodies. For example, small scale experimental work undertaken by Vanni (1986) determined that the addition of *D. pulex* has negative implications for zooplankton. *D. pulex* was associated with a strong reduction in phytoplankton biomass and zooplankton abundance in enclosures located within North American ponds; these reductions were possibly the result of interference competition as *D. pulex* was likely able to outcompete the smaller zooplankton species (Vanni 1986). Similarly, the colonization of *Daphnia galeata* in a recently filled mine pit (Lake Puketirini) in New Zealand's North Island has had similar effects on the lake's biota; the establishment of *D. galeata* was associated with a strong increase in water clarity and changes in zooplankton composition. That lake switched from having a zooplankton community dominated by native rotifer species such as *Asplanchna, Brachionus, Keratella* and *Polyarthra*, to being solely dominated by *D. galeata*, with several rotifer species being extirpated from the

system (Balvert et al. 2009). The highly efficient uptake of phytoplankton by *D. galeata* observed at Lake Puketirini may indicate the potential for a direct competitive interaction between *Daphnia* invaders and filter-feeding kākahi in shared lake and pond ecosystems (Balvert et al. 2009; Marroni et al. 2017). Historically, New Zealand's lake ecosystems were predominantly associated with smaller, less efficient filter feeding cladoceran species such as *Bosmina* and *Ceriodaphnia* (Mourelatos and Lacroix 1990; Chapman et al. 2011). As these species have relatively low feeding efficiencies, they are unlikely to cause any significant resource competition for kākahi. Comparatively, the efficient non-native *Daphnia*'s filter feeding may be cause for concern (Mourelatos and Lacroix, 1990; Balvert et al. 2009). In light of this hypothesis, it must be considered whether the recent widespread *Daphnia* invasions pose a threat to New Zealand's native freshwater mussel species.

1.4 Global mussel and zooplankton interactions: Do mussels consume zooplankton?

In recent years, laboratory studies have found freshwater mussels to be capable of consuming various zooplankton species (Nichols and Garling 2000; Monlina et al. 2011). Previously, research into the feeding abilities of freshwater mussels had concluded that mussels function purely as primary consumers in freshwater systems, consuming only algae, suspended particles and bacteria (McMahon and Bogan 1991; Vanderploeg et al. 1995; Silverman et al. 1997). Although our understanding of freshwater mussel filtering and predation abilities has improved, few studies have explored whether mussels utilise zooplankton as a food source or whether they passively filter zooplankton from the water column as they filter (Nichols and Garling 2000). Nonetheless, whether mussels use zooplankton as a source of nutrition or unintentionally filter them while collecting food, their filtering abilities may still affect the composition of zooplankton communities. Furthermore, their feeding mode could also have additional effects on other grazing species in the food chain, or even larger predatory species such as fish that utilise larger zooplankton species as a primary food source.

In the international literature, few studies have considered trophic interactions between zooplankton and freshwater mussels (Wong et al. 2003; Hwang et al. 2004; Molina et al. 2012; Marroni et al. 2017). These studies have predominantly considered the effects and impacts associated with bivalve invasions for native zooplankton communities. For instance, the freshwater golden mussel (*Limnoperna fortunei*), native to Southeastern Asia, has been well studied as this species has established populations in numerous water bodies throughout South America. This particular bivalve impacts microcrustacean species of the floodplains (Cataldo et al. 2012; Molina et al. 2011). For example, in laboratory experiments the survival rate of local microcrustaceans exposed to *L. fortunei* was assessed using three copepod and five cladoceran species; despite the bivalves relatively small size (average 19.9 ± 1.6 mm), it was found that *L. fortunei* could remove zooplankton species up to a length of 1.1 mm (Molina et al. 2012). Interestingly, predation was the suggested interaction at play as no zooplankton were found within the pseudofeces which suggests that *L. fortunei* was digesting zooplankton rather than rejecting them during the filtration process (Molina et al. 2011). Similar results were also produced during a mesocosm experiment that investigated the impacts of *L. fortunei* on local Paraná floodplain zooplankton communities. These findings indicated that *L. fortunei* was also highly effective at rapidly depleting rotifer populations. Nevertheless, despite being consumed in smaller numbers, it was found that smaller cladoceran species represented the largest biomass in the golden mussel's diet (Molina et al. 2012). An earlier study on the invasion of *L. fortunei* in the Paraná River floodplains found a strong reduction in the chlorophyll *a* concentration and zooplankton species abundance (including the rotifer *Keratella*) due to the grazing activities of *L. fortunei*. However, it is not clear whether these effects were a result of the mussel's direct predation on the zooplankton or indirect competition for phytoplankton resources (Molina et al. 2008). Overall, these studies show that *L. fortunei* can prey on larger zooplankton species and suggests that zooplankton function as an important food resource for these freshwater mussels (Molina et al. 2011; Cataldo et al. 2012; Molina et al. 2012).

Other key bivalve and zooplankton research has been conducted by Kissman et al. (2010) on two *Dreissena* mussel invaders in the United States, both native to Eastern Europe; the quagga mussel (*Dreissena bugensis*) and the zebra mussel

(*Dreissena polymorpha*). Research on 50 Michigan lakes has highlighted that the invasion of these freshwater mussels has had significant consequences for both micro-zooplankton, such as ciliates and rotifers, and larger macro-zooplankton, such as cladocerans, in these stratified lake systems. Lakes containing the non-native mussel species were found to have significantly lower micro- (44% lower) and macro-zooplankton (40% lower) biomass, suggesting that freshwater mussels can also impact larger zooplankton species. This research concluded that the dreissenids were likely causing *Daphnia* declines through resource competition, as it has previously been suggested that the *Dreissena* spp. are unable to consume macro-zooplankton (Kissman et al. 2010).

Finally, recent research by Marroni et al. (2017) in Uruguay has also explored the idea of competitive interactions between zooplankton and freshwater mussels through a series of laboratory studies. Specifically, this study compared phytoplankton consumption between a natural zooplankton community collected in Laguna Blanca Lake and two fresh water mussel species; the native mussel species *Diplodon parallelopipedon* and the non-native species *Corbicula fluminea*. These experiments indicated that the natural zooplankton community produced a much lower phytoplankton grazing pressure than the two tested bivalves. The authors also looked at the native and non-native bivalve's ability to consume zooplankton, and found that both mussel species were capable of preying on small sized nauplii and rotifers but not on larger cladoceran and copepod species (Marroni et al. 2017).

Overall, it is unclear from the literature whether kākahi in New Zealand are likely able to prey on *Daphnia* species or whether kākahi can compete with non-indigenous *Daphnia* for algal food resources. However, it is plausible that non-native *Daphnia* may pose a significant threat to kākahi in New Zealand, as they have largely evolved in the absence of large, highly efficient filter feeding zooplankton (native *Daphnia* are uncommon). As such, research into the feeding behaviour of kākahi needs to be undertaken to determine if large zooplankton species are a viable food resource for these bivalves. Further, it will be important to ascertain whether kākahi can feed on and/ or filter feed on *Daphnia* from the water column, as this will indicate whether kākahi can be used as a biomanipulation tool to mitigate *Daphnia* invasions in the future.

1.5 Study objectives

This research aims to investigate the effect of non-indigenous *Daphnia* (*Daphnia pulex* and *Daphnia galeata*) on New Zealand's most common native fresh water mussel, *Echyridella menziesii*, in the form of two manuscripts for publication. As such, this thesis will have some repetition of key points and findings between chapters. The first paper (Chapter 2) will aim to determine whether kākahi prey on invasive *Daphnia* species, and determine whether kākahi could be used as a biomanipulation tool to remove non-native zooplankton from invaded waterbodies. The second paper (Chapter 3) will determine whether the widespread invader *Daphnia pulex* has the potential to compete with *E. menziesii* for algal resources.

1.5.1 Key objectives:

i) To determine whether kākahi can filter out non-native *Daphnia* species from the water column, by counting zooplankton before and after mussel exposure, to determine if a loss has occurred.

ii) To determine whether *Daphnia pulex* and kākahi compete for the same algal food resources, by counting the number of algal cells consumed by each species.

1.5.2 Research questions to be addressed:

i) Can kākahi filter out non-indigenous *Daphnia* species?

ii) Is it plausible for kākahi to be used as a biomanipulation tool to remove unwanted *Daphnia* spp. from invaded shallow lakes?

iii) In high densities, can *Daphnia pulex* limit algal food availability for kākahi?

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Chapter 2

Echyridella menziesii (Bivalvia: Hyriidae) as a predator on zooplankton of different sizes; are large non-indigenous *Daphnia* a potential food source? [1]

2.1 Abstract

Interactions between two recent invaders to New Zealand, the cladocerans *Daphnia galeata* and *D. pulex*, and native filter-feeding freshwater mussels, are unknown. We examined predation rates of the common native mussel *Echyridella menziesii* (kākahi, kāeo) on non-native *Daphnia* (comparatively large zooplankton in New Zealand), relative to two common native species, the smaller cladoceran *Bosmina meridionalis* and rotifer *Brachionus calyciflorus*. Controlled laboratory experiments were conducted in which each zooplankton species was exposed to bivalve predation for a two-hour period. Comparing treatments to non-mussel controls, removal rates of non-indigenous *D. pulex* and native *B. calyciflorus* were statistically significant. Nevertheless, kākahi removal rates may not be ecologically significant for daphnids (1.7% *D. galeata* and 7.4% *D. pulex*). Kākahi removed 8.8 % of *B. meridionalis* and 30.2% of *B. calyciflorus*, suggesting that small, feeble zooplankton species (particularly rotifers) are most susceptible to predation, and potentially function as an energy source for native freshwater mussels.

Keywords: freshwater mussels, Hyriidae, zooplankton, *Daphnia*, predation, filtration

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2.2 Introduction

Mussels function as primary consumers in freshwater ecosystems removing algae, bacteria and other suspended particles from the water column (McMahon and Bogan 1991). Further, several laboratory studies have demonstrated that freshwater bivalves are capable of consuming zooplankton (Nichols and Garling, 2000; Molina et al. 2011). Mechanisms used by mussels to sort particles both prior to, and following ingestion, have raised questions regarding whether mussels utilise zooplankton as a food source, or simply passively filter them from the water column and subsequently deposit them as pseudofeces (Nichols and Garling 2000). Nevertheless, regardless of whether mussels function as intraguild predators that consume zooplankton for nutritional purposes, or simply act as incidental predators (Polis and Myers 1989), their filtering activity has the potential to influence both zooplankton densities and community structure.

To date, few studies have considered trophic interactions between zooplankton and freshwater mussels (Wong et al 2003a; Hwang et al. 2004; Molina et al. 2008, Molina et al. 2012; Marroni et al. 2017). Of those studies, most have focused on the impact of non-indigenous bivalves on native zooplankton communities, or the diets of propagated mussel species. For example, a number of studies have examined the impacts of the invasive golden mussel (*Limnoperna fortunei*), native to South-eastern Asia, on microcrustaceans of the Paraná floodplains in South America (Cataldo et al. 2012; Molina et al. 2011). Molina et al. (2011) determined that, despite their relatively small size (average 19.9 ± 1.6 mm), *L. fortunei* were capable of preying on zooplankton species up to 1.1 mm in length. Similarly, mesocosm studies investigating the impact of *L. fortunei* on Paraná floodplain zooplankton have indicated *L. fortunei* are highly effective at depleting rotifer populations, although cladoceran species represent the largest biomass in the golden mussels diet (Molina et al. 2012). Another well studied species is the non-indigenous zebra mussel (*Dreissena polymorpha*) in North America; this bivalve has had profound effects on native zooplankton communities in the Laurentian Great Lakes (MacIssac et al. 1991; Leach 1993; MacIsaac et al. 1995). While a number of papers exist examining the predatory effects of non-native mussel species on native

zooplankton, none to date have examined the effects of native mussels on non-native zooplankton.

In recent years, a number of non-indigenous zooplankton species have been identified in New Zealand waters, with zooplankton making up one-third of the total number of known non-native invertebrate species established in New Zealand lakes (Duggan and Collier 2018). Among these, two *Daphnia* species, the Holarctic *Daphnia galeata* and North American *D.* '*pulex*', were first recorded in 1993 and 2005, respectively, and are becoming increasingly widespread (Duggan et al. 2006; Duggan et al. 2012; Branford and Duggan 2017). Daphnids are known to function as highly effective herbivores due to their efficient grazing capabilities and generalist feeding strategies (Beisner 2001). As such, these grazers are particularly effective at modifying water clarity and zooplankton community composition, particularly in shallow water bodies (Balvert et al. 2009). Historically, New Zealand's zooplankton communities have primarily been dominated by smaller, less efficient filter-feeding cladoceran species, such as *Bosmina*, and rotifers (Chapman and Green 1987; Duggan et al. 2002). The potential interactions between New Zealand's freshwater mussels and zooplankton, including the highly efficient filter-feeding *Daphnia* invaders, have yet to be explored.

New Zealand has three species of freshwater mussel, of which *Echyridella menziesii* (commonly referred to as kākahi or kāeo) is the most widely distributed, with populations throughout New Zealand's North and South Islands (Marshall et al. 2014). *Echyridella* belong to the large order Unionoida (Marshall et al. 2014), which are unique in having an obligate parasitic larval stage, requiring them to attach to a host fish (Barnhart et al. 2008). On a global scale, freshwater mussels are in decline (Lydeard et al. 2004). At present, the severity of freshwater mussel decline in New Zealand is difficult to ascertain. However, historic records generated by Māori have indicated that kākahi are now far less abundant than in pre-European times (Rainforth, 2008; McDowall, 2011). Currently, the causes of kākahi decline in New Zealand are not fully understood.

At present, it is unclear whether kākahi prey on zooplankton, including non-indigenous *Daphnia* species. We aimed to determine whether *E. menziesii* are able

to prey on invasive *Daphnia* species in New Zealand. Further, we aimed to determine the relative susceptibilities of two common native zooplankton species, the small cladoceran *Bosmina meridionalis* and rotifer *Brachionus calyciflorus,* which are expected to be more susceptible to predation due to their more feeble swimming abilities.

2.3 Materials and methods

2.3.1 Animal collection

Fifty-three adult *Echyridella menziesii* (mean length, mm ± 1 SD: 60.98 ± 3.3 mm, range 52.9 - 67.4 mm) were collected on 27 March 2018 from Ngongotaha, Lake Rotorua (average <1 m depth; 38°04'25.8"S, 176°12'58.9"E). Kākahi were located by wading. A bathyscope was used to observe their siphons at the sediment-water interface; this method allowed individual bivalves to be collected by hand. Collected *E. menziesii* were transported to the laboratory in a 12 L bucket filled with lake water. Mussels were acclimatised to room temperature before being transferred to a large aquarium (length 92 cm, width 38 cm, height 92 cm) filled with 105 L of dechlorinated tap water, containing three oxygen bubblers and a layer of silica sand. Mussels were kept in the aquarium for a period of one week to acclimatise to the laboratory conditions (18°C and a 16 h light: 8 h dark cycle) before commencing experiments. Ten percent of the tank water was changed every second day to avoid ammonia build up and mussels were fed daily with 0.8 mL of Reid Mariculture Shellfish Diet 1800 and 0.4 mL of Reid Mariculture Nanno 3600 microalgae diluted in 2 L of dechlorinated tap water. On trial days, experiments were undertaken at the mussel's regular algal feeding time, and the mussels were not fed until the trial was complete (a period of 24-31 hours since their last algae feeding; e.g., Soto and Mena 1999).

Four zooplankton species were collected using a horizontal tow with a plankton throw net (mesh size 45 μm); the non-indigenous cladocerans *Daphnia galeata* and *D. pulex,* and the indigenous cladoceran *Bosmina meridionalis* and rotifer *Brachionus calyciflorus*. *Daphnia pulex* was collected from Triangle Pond, Flatbush, Auckland (36°57'23.2"S, 174°54'23.8"E) on 2 March 2018. On the same

day, *B. calyciflorus* was collected from Cameron Lake (Kareaotahi), Rukuhia (37°51'17.3"S, 175°18'07.5"E). *Daphnia galeata* and *B. meridionalis* were collected from Lake Te Kōutu, Cambridge (37°53'20.3"S, 175°28'18.4"E), on 5 March 2018. All zooplankton samples were kept in separate 10 L buckets, filled with their source water for return to the laboratory. Zooplankton were kept in the same temperature-controlled room as *E. menziesii*, and therefore exposed to the same day: night cycle and ambient temperature. A coarse bubbler (with no air stone attached) was used to ensure that oxygen levels within buckets did not deplete.

2.3.2 Tank preparation and experiment details

Eighteen rectangular aquaria (length 36 cm, width 18 cm, height 17.5 cm) were used for this experiment. Three small plastic cups (a.k.a., tumblers; 60 mL volume) were attached to the bottom of each tank, to hold mussels upright during the trials. Tumblers were pre-rinsed with tap water and soaked in dechlorinated water for a 48-hour period, during which, the water was renewed daily. Three cleaned tumblers were glued 9 cm apart along the centre of each rectangular tank using Nirox clear silicone sealant. The silicone was then left to dry for of 24 hours before being rinsed with dechlorinated tap water.

Aquaria were filled with 6 L of dechlorinated tap water and kept at 18°C for the duration of the experiment. In preparation for the trials, each of the three cups had a layer of Parafilm (101.6 x 101.6 mm) stretched over them to form a membrane; this layer was used to simulate the support of surrounding sediments in the absence of sand. The tanks were placed on top of towels to reduce vibrations disturbing filtering mussels. In total, 32 trials were randomly allocated to one of four treatments; 1) *D. galatea* and *E. menziesii*, 2) *D. pulex* and *E. menziesii*, 3) *B. meridionalis* and *E. menziesii*, and 4) *B. calyciflorus* and *E. menziesii*. Further, 32 tanks were randomly allocated as one of four control types (i.e. without bivalves); 1) *D. galatea*, 2) *D. pulex*, 3) *B. meridionalis*, and 4) *B. calyciflorus*. To begin the experiment, three *E. menziesii* were randomly selected from the main acclimatisation tank. Healthy bivalves were selected based on two criteria; (1) kākahi were seen to be filtering with both siphons open in the sediment, and (2) kākahi retracted their siphons and closed their shells in response to handling

(Molina et al. 2011). A slit was cut down the centre of the Parafilm membrane and a mussel was inserted into each of the cups with their siphons orientated upwards. To ensure *E. menziesii* could still open their shells to filter, each mussel was twisted a few degrees to the right and left in the Parafilm to loosen its grip. Then, *E. menziesii* were left for a 15-minute period to allow them to acclimatise to their new environment and begin filtering. Water samples were collected using a 40 μm mesh from randomly chosen zooplankton specie's buckets and transferred into a Petri dish. Fifty large individuals of each species were manually selected and counted into separate Petri dishes using a Pasteur pipette under a dissecting microscope (Olympus SZ60). The Petri dish containing the zooplankton was added to each of the tanks once the 15-minute mussel acclimatisation period was complete.

After a two-hour period the mussels and Parafilm were removed and placed into separate 1 L containers filled with dechlorinated water. The Parafilm and mussels were rinsed off into the tank water using a wash bottle. All water from the aquaria was then filtered through a 40 μm mesh; each side of the tank and the cups were subsequently rinsed twice into the mesh to collect any leftover plankton. The mesh contents were then washed into a Petri dish and the remaining zooplankton counted out live under a dissecting microscope using a Pasteur pipette. Dead zooplankton were included in the total zooplankton remaining counts as these may have been killed during handling, while small crustacean juveniles were not included, as these had likely fallen from the brood pouches of adults during or after experiments. This same protocol was undertaken for the control tanks, with no mussels included. In total, this resulted in 64 trials across a three-day period (5-7 March 2018), with each of the treatments and controls being repeated eight times in total. The body lengths (excluding spines) of 50 individuals of each of the zooplankton species were measured to estimate the average size of individuals used in experiments. Zooplankton were measured under a compound microscope using an eyepiece micrometer calibrated with a slide micrometer.

2.3.3 Data analysis

T-tests were undertaken to compare the predation rates of each zooplankton species when exposed to *E. menziesii*. Data were transformed using a natural logarithm (ln)

in Microsoft Excel to achieve normality of the dataset, assessed using the Shapiro-Wilks normality test in Statistica (version 13.2). Prey-based ingestion rates (IR, prey h^{-1} $mussel^{-1}$) and clearance rates (CR, mL h^{-1} $mussel^{-1}$) were calculated for each zooplankton species using the following equation (MacIsaac et al. 1991):

$$\text{Clearance Rate (CR)} = V(\ln[E_0/E_t] - \ln[C_0/C_t])\ /tn$$

$$\text{Ingestion Rate (IR)} = CR.\ C_0$$

Volume V is represented in mL, E_0 represents the mean initial treatment prey densities and E_t represents the mean final treatment prey densities; both after exposure to *E. menziesii*. C_0 represents the beginning control prey densities, C_t represents the final control prey densities, t represents time in hours, and n the number of predators present.

2.4 Results

The average numbers of zooplankton remaining after mussel exposure was lower relative to their controls for all zooplankton species tested (Figure 2.1). Average differences of 1.73% for *Daphnia galeata*, 7.40% for *D. pulex*, 8.80% for *Bosmina meridionalis* and 30.23% for *Brachionus calyciflorus* were observed between treatments and controls. On average, control trials had more zooplankton retrieved than were added to the aquaria at the start of the experiment, particularly for smaller species, likely due to a combination of zooplankton reproduction and difficulty in transferring exact numbers of small zooplankton from live concentrated samples. Indeed, selecting individuals of smaller size zooplankton (e.g., *Brachionus*) from concentrated samples will have resulted in the accidental suction of additional, unintended individuals; this problem was less apparent with larger species (*Daphnia*), as their greater size allowed individuals to be more easily picked individually and contaminants more easily excluded. Further, while we could confidently eliminate young produced by *Daphnia*, rotifers produce young of a similar size (and same number of cells) as their mothers, making it difficult to identify offspring from adults. Nevertheless, while we cannot be certain of the exact

numbers of zooplankton individuals added at the start of the experiment, due to random experimental order, no bias should exist between the numbers of zooplankton added to the treatments and controls.

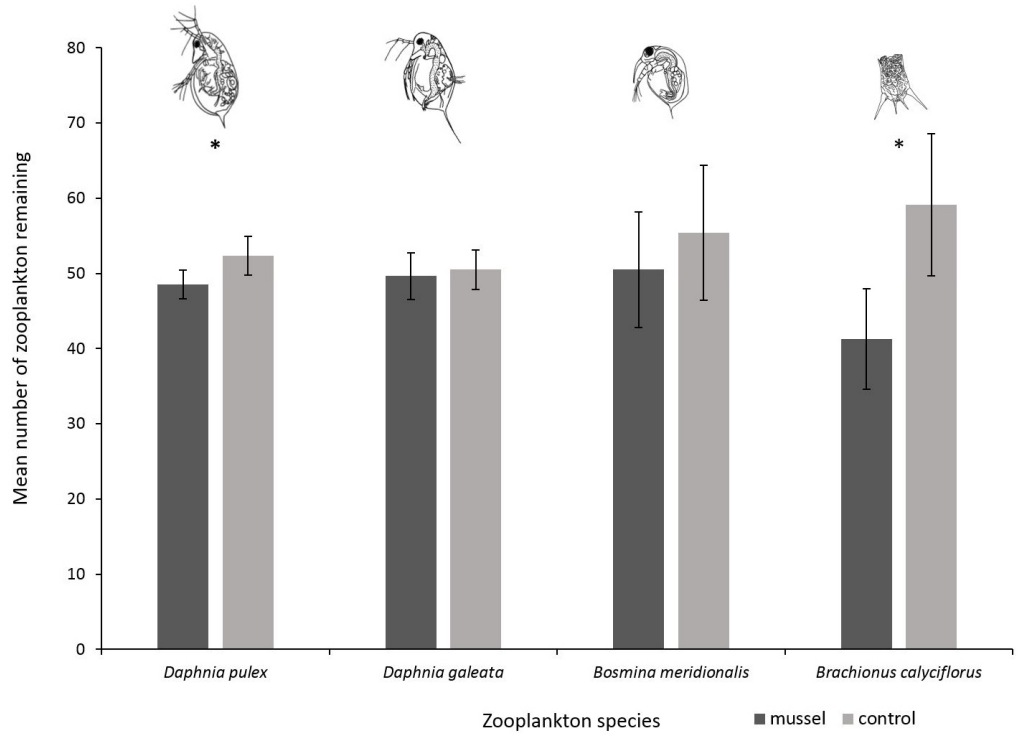

Figure 2.1. The mean number of zooplankton remaining after being exposed to mussel predation for a two-hour period and their associated controls (mean of eight replicates for each species control/treatment). Asterisks indicate a significant difference ($p < 0.05$) in the ln data between the average control and treatment result for each zooplankton species, while the error bars represent the standard deviation.

The recovery of two zooplankton species differed significantly between treatments and controls; *D. pulex* and *B. calyciflorus* (T-test P values 0.004 and <0.001, respectively). Of these, *B. calyciflorus* had the highest CR and IR overall when exposed to kākahi (Table 2.1). The next smallest zooplankton species, *B. meridionalis*, was found to have a similar CR and IR to the largest species tested, *D. pulex*. *Daphnia galeata* had the lowest CR and IR, indicating this species was the least susceptible to kākahi predation. No significant differences were found for *B. meridionalis* and *D. galeata* consumed between treatments and controls (T-test P values 0.240 and 0.538, respectively).

Table 2.1. Mean body lengths (±SD), excluding spines, of the four zooplankton species used in kākahi feeding laboratory trials; the mean clearance rate (CR) and ingestion rate (IR) of *Echyridella menziesii* for each species.

Species	Size (µm) (mean ± SD)	Mean Clearance Rate (CR) (mL h^{-1} $mussel^{-1}$)	Mean Ingestion Rate (IR) (prey h^{-1} $mussel^{-1}$)
Daphnia pulex	1581.95 ± 156.83	76.87	3.84
Daphnia galeata	1469.72 ± 171.66	17.48	0.87
Bosmina meridionalis	320.39 ± 55.47	92.16	4.61
Brachionus calyciflorus	285.24 ± 28.6	360	18

2.5 Discussion

2.5.1 Mussel predation

Kākahi had the highest clearance and ingestion rates for the rotifer *B. calyciflorus*, the smallest, most feeble zooplankton species tested; this was likely due to an inability of this rotifer to escape the suction generated by the mussel's siphon. Comparatively, exposure of *D. pulex* to *E. menziesii* produced very similar clearance and ingestion rates to *Bosmina meridionalis*, though of these only the *D. pulex* removal result was statistically significant; clearance and ingestion rates for both were far lower than that for *B. calyciflorus*. Similar results have been found in other studies focusing on zooplankton and bivalve interactions. For example, in South America, common crustacean zooplankton taxa (copepodites, *Ceriodaphnia dubia*, *Moina reticulata*, *M. micrura* and *Alona glabra*) from the Paraná floodplains were found to be significantly vulnerable to predation by adult (19.9 ± 1.6 mm) non-indigenous golden mussels (*Limnoperna fortunei*) (Molina et al. 2011). In this case the size, body shape and physiological behaviour were all suggested to play a key role in the zooplankton's susceptibility to predation (i.e., smaller, weaker species with poor evasive behaviours were more likely to be consumed). Subsequent mesocosm studies on *L. fortunei* and zooplankton of the Paraná floodplains have also found that these mussels are highly efficient at depleting rotifer populations, though cladocerans represented the largest biomass in the golden mussel's diet (Molina et al. 2012). Similarly, in our experiments, while we

found that *E. menziesii* was capable of removing statistically significant, but relatively small numbers of the largest crustacean *D. pulex* (1582.0 μm), due to their large size this equated to a larger consumed biomass (66.35 μg prey h^{-1} $mussel^{-1}$) than of *B. calyciflorus* (5.40 μg prey h^{-1} $mussel^{-1}$) (Dumont et al. 1975). Nonetheless, the low numbers of *D. pulex* consumed indicates that *E. menziesii* is unlikely to remove ecologically significant numbers of non-indigenous *Daphnia* from shallow lake systems, and are thus unlikely to lower population densities or alter crustacean community composition. Further, as we cannot be confident of the exact numbers of prey added at the initiation of the experiment, some caution might be placed on this narrow result. However, this does not exclude the possibility that *E. menziesii* can control daphnid populations through exploitative competition for phytoplankton resources.

In our study, kākahi were found to remove moderate numbers of the rotifer *Brachionus calyciflorus* (30.2%) compared to controls, which may suggest that rotifers function as an important food source for New Zealand's freshwater unionids. Previous research has illustrated that both marine and freshwater bivalves are capable of assimilating (i.e., incorporating carbon into their tissue) various micro- and meso-zooplankton species (Wong et al. 2003a; Wong et al. 2003b). In a laboratory study, which exposed two species of rotifers (*B. calyciflorus* and *Lepadella ovalis*) fed with a ^{14}C labelled microalgae suspension to invasive zebra mussels (*Dreissena polymorpha*), these small freshwater bivalves (11-14 mm) had assimilation efficiencies between ~37.4 and 54.0% (Wong et al. 2003a). Likewise, the invasion of *D. polymorpha* in the North American Great Lakes was found to have a strong impact on the composition of local rotifer populations; in particular, the abundances of *Keratella*, *Polyarthra* and *Synchaeta* species have been greatly reduced in Lake Erie (MacIssac et al 1991; Leach 1993; MacIsaac et al. 1995). Overall, these findings suggest that rotifers of various sizes can be important energy sources for many bivalve species. Further, these findings illustrate that bivalves can produce a significant top-down predation effect on small zooplankton in freshwater systems (Wong et al. 2003a; Wong et al. 2003b). Overall, it is probable that rotifer abundances may be negatively affected by predation pressure from kākahi in shallow New Zealand lakes.

2.5.2 Potential impacts of non-native Daphnia

If *B. calyciflorus*, as well as other rotifers, do contribute significantly to the diet of kākahi, it is possible that the invasion of large daphnids can negatively affect food availability of kākahi via reductions in rotifers through competition. For example, Gilbert (1985) found *Daphnia* caused rotifer reductions through competition for food resources, as *Daphnia* are much larger and capable of exploiting a broader range of food resources; this author found experimentally that *D. pulex* excludes *B. calyciflorus* and smaller rotifer species such as *Keratella cochlearis* from mixed cultures (Gilbert 1985). In addition to resource competition, mechanical damage by *Daphnia* has also been identified as a threat to small rotifers; for example, *Daphnia mendotae* has been found to trap *K. cochlearis* in their branchial chambers, causing damage, and in some cases mortality (Gilbert and Stemberger 1985). Nevertheless, it is possible that predation on small numbers of relatively high biomass non-native *Daphnia* may be able to offset this loss (and, indeed, these *Daphnia* may be consumed following their death). However, extrapolation of laboratory results to make generalisations across lakes is impossible; effects will be site-specific, being dependent on the relative numbers of rotifers and crustaceans in a waterbody at any given time (zooplankton assemblages differ among New Zealand lakes, many vary seasonally; e.g., Chapman et al 1985; Duggan et al. 2002), and the relative swimming abilities of each species present. Larger-scale mesocosm, or mussel transplant experiments, will be required to better assess mussel effects in-situ. In the New Zealand context, the invasion of *D. galeata* into a recently filled mine pit (Lake Puketirini) resulted in the disappearance of many native rotifers from the lake (e.g., *Asplanchna*, *Brachionus*, *Polyarthra* and *Keratella* spp.), resulting in *D. galeata* becoming the dominant zooplankton species in this community by number and biomass (Balvert et al. 2009). Elsewhere, Vanni (1986) found that North American *Daphnia pulex* greatly reduced the abundances of both phytoplankton and other zooplankton (particularly rotifers) when introduced to enclosures in a North American pond. These findings emphasize the possibility that non-indigenous *Daphnia* have the capability to negatively impact kākahi by eliminating smaller zooplankton as a potential food resource. Nevertheless, we did not determine in this study the mechanisms of removal of zooplankton by mussels; that

is, whether small zooplankton are consumed, providing an energy source for mussels, or whether they are deposited as pseudofeces without digestion.

As bivalves suspension feed from the water column, and will primarily consume phytoplankton, the loss of rotifers from their diet may not have a major impact on their energy intake. However, the highly efficient uptake of phytoplankton by *D. galeata* observed at Lake Puketirini may also indicate the potential for direct competitive interactions for algal resources (Balvert et al. 2009; Marroni et al. 2017). Thus, competition for algal resources between non-indigenous *Daphnia* species and kākahi requires exploration. Nevertheless, as both mussels and *Daphnia* consume phytoplankton, and the mussels are unable to easily consume *Daphnia*, these species may act synergistically to remove algae from the water column. As such, these species might be useful as biomanipulation tools in concert to improve water quality in New Zealand lakes (e.g., Burns et al. 2013).

2.6 Conclusion

Our research indicates that *E. menziesii* are unable to consume ecologically significant numbers of the tested non-indigenous *Daphnia* species (*D. galeata* and *D. pulex*). Thus, our findings suggest that kākahi would not be suitable as a biomanipulation tool in lake and pond systems to remove unwanted non-native daphnids through predation. Further, our research highlights that rotifers potentially function as a food source for kākahi, as significant numbers of *B. calyciflorus* were removed by the kākahi in our experiments. As such, the presence of non-native *Daphnia* in lake and pond systems may negatively impact kākahi as they can eliminate rotifers as a food source. It is clear that further research is needed to ascertain whether *Daphnia* pose a significant threat to kākahi through resource competition. As kākahi likely consume primarily smaller, more feeble species among the zooplankton, it is worth noting that the distribution of kākahi may significantly influence the zooplankton community composition in New Zealand. Further, mussel decline is likely having profound effects on predation rates on small zooplankton across New Zealand.

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Chapter 3

Dividing the algal soup: is there niche overlap between native bivalves (*Echyridella menziesii*) and non-native *Daphnia pulex*? [2]

3.1 Abstract

New Zealand's native freshwater mussels (commonly referred to as kākahi or kaeo) are considered to be in decline, yet the mechanisms driving these declines are unclear. The establishment of the highly efficient filter-feeding, North American, *Daphnia pulex* in New Zealand lakes may lead to competition for algal resources with kākahi. In response to this potential interaction, we conducted a controlled laboratory experiment to determine whether there is overlap in the algal resources utilised by each taxon. *Echyridella menziesii* (both adult and juvenile stages) and high densities of *Daphnia pulex* were exposed to mixed algal samples for a period of three hours to determine whether the same algal resources were being removed by the grazers. Relative to controls, our results indicated that both adult and juvenile *E. menziesii* could remove a broad range of algal taxa including diatoms, green algae and filamentous species ranging between 33.6 to 348.0 μm in size. *Daphnia pulex* exposed to the same algal taxa were unable to consume significant numbers of the same algae species relative to controls. These results suggest a limited niche overlap for the two grazers as *E. menziesii* could remove large particles and *D. pulex* is only capable of utilizing smaller particles. As such, these grazers could be used in combination as a biomanipulation tool to remove a broad range of algal taxa in eutrophic lakes.

Key words: bivalve, zooplankton, phytoplankton, filtration, juvenile, Hyriidae, competition, resource partitioning.

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3.2 Introduction

Freshwater equates to only 0.01% of the globe's water, yet these habitats house nearly 6% of the world's species, making them among the most biodiverse ecosystems in the world (Gleick 1996; Gatti 2016). Nevertheless, freshwater ecosystems are also considered to be among the most at risk in the world, with rates of biodiversity declines exceeding that of terrestrial habitats (Dudgeon et al. 2006). The introduction of non-indigenous species has been identified as a major driver in recent extinctions, and is considered a key threat to global biodiversity (Bellard et al. 2016; Gatti 2016).

Although much effort goes into the detection of potential new non-native species entering New Zealand's borders, invasions of small invertebrates and microscopic taxa are still occurring (Branford and Duggan 2017; Duggan & Collier, 2018). Invasions of aquatic invertebrates have predominantly comprised of zooplankton, which make up one-third of the known established invertebrate species in New Zealand lake ecosystems (Duggan and Collier 2018). The large Holarctic cladoceran *Daphnia galeata* has been established in New Zealand since at least 1993 (Duggan et al. 2006). This cladoceran has been found to have profound effects on native zooplankton communities. Their arrival in Lake Puketirini was associated with the elimination of several native rotifer taxa (*Brachionus*, *Polyarthra*, *Asplanchna*, and *Keratella* species) (Balvert et al. 2009), likely due to the large size and superior competitive abilities of this *Daphnia* (Gilbert 1985; Gilbert and Stemberger 1985). The switch to a system dominated by *Daphnia* in Lake Puketirini also resulted in an increase in water clarity, due to a loss of algal biomass caused by the *Daphnia*'s efficient filter feeding abilities (Balvert et al. 2009). Other *Daphnia* invasions have had similar effects across the globe. The invasion of *Daphnia lumholtzi* in Lake Springfield, Illinois, USA, has altered the natural crustacean community, with substantial declines in native cladoceran and copepod species (Kolar et al. 1997). Elsewhere, high densities of *Daphnia* in spring result in a 'clear water phase' in shallow lakes, due to an increase in phytoplankton grazing (Talling 2003).

One of the most recently detected zooplankton invaders in New Zealand's freshwaters is the North American cladoceran *Daphnia pulex*, which was first recognised in four South Island lakes in the Clutha river drainage system in 2005. *Daphnia pulex* has since become established in numerous lakes throughout the South Island, and has been identified in a number of ponds in Auckland, North Island (Branford and Duggan 2017). These invaded waterbodies have varied both in depth and trophic state, showing the tolerance of these non-native crustaceans to a broad range of environmental conditions (Burns 2013; Duggan et al. 2012). *Daphnia pulex* have been observed to reach high densities in shallow ponds in New Zealand, with maximum densities of 389 L^{-1} recorded in the littoral zone of a small Waikato farm pond (Pers. Obs., K. Le Quesne and A. Pearson, University of Waikato 2018). Similarly, *D. pulex* has been found to reach densities as high as 932 L^{-1} in Canadian sewage oxidation ponds (Daborn 1978). Due to the rapid spread of non-native daphnids across New Zealand, it must be considered what effect this efficient grazer is having on receiving environments and native fauna. Prior to the invasion of the two large daphnids (*D. galeata* and *D. pulex*), New Zealand was known to have few native cladoceran species, with lakes dominated by smaller, less efficient, filter feeding species such as *Bosmina meridionalis*, *Chydorus sphaericus* and *Ceriodaphnia* species (Chapman and Green 1987; Chapman et al. 2011). Two native *Daphnia* species can also be found in New Zealand, *D. thomsoni* and *D. tewaipounamu*; however, the former is uncommon in the North Island, while the distribution of the latter appears limited to alpine lakes in the South Island (Burns et al. 2017). Thus, it must be considered whether the establishment and spread of non-indigenous *Daphnia* are having an impact on other filter feeding lake taxa, such New Zealand's native freshwater mussels.

In aquatic systems, freshwater mussels (commonly named kākahi or kāeo in New Zealand) function as highly effective ecosystem engineers due to their bioturbation, biofiltering and nutrient recycling abilities (Vaughn 2018). Globally, freshwater mussels are in decline. They are impacted by a myriad of factors; the introduction of invasive species, pollution, freshwater pearl exploitation, and habitat loss have all been named as key factors influencing freshwater bivalve losses (Lydeard 2004; Anthony and Downing 2001; Ricciardi et al. 1998). There are three known species of freshwater mussel in New Zealand; *Echyridella aucklandica*, *E. onekaka*, and *E.*

menziesii, which all belong to the order Unionoida. *Echyridella menziesii* is the most common, with populations distributed throughout the two main islands (Marshall et al. 2014). Although kākahi provide invaluable ecosystem services, such as enhancing water clarity through filtration (Vaughn 2018), historic records by Māori suggest kākahi numbers have declined since pre-European times (Rainforth 2008; McDowall 2011). Yet, the mechanisms causing kākahi declines in New Zealand are poorly understood. Observations suggest that kākahi are likely experiencing poor recruitment success, with many populations skewed toward an adult dominated demographic with few smaller juveniles present.

To date, little research has considered potential freshwater mussel and zooplankton interactions, and their potential to compete for the same food resources (Marroni et al. 2017; Pearson and Duggan 2019). In a study by Marroni et al. (2017), the interactions between two freshwater mussels (the native *Diplodon parallelopipedon* and invasive *Corbicula fluminea*) and a native zooplankton community in Uruguay were compared. There, the two bivalves exhibited far higher filtration rates than the zooplankton community and caused significant decreases in phytoplankton densities. It is thought that the low zooplankton grazing rate is due to microbes being used as the primary food source instead of algae. Comparatively, *D. parallelopipedon* and *C. fluminea* were found to consume significant numbers of the micro-zooplankton community (i.e., rotifers and copepod nauplii). These findings indicate that bivalves affect algal food availability for zooplankton, but also impact small zooplankton directly through predation (Marroni et al. 2017). Pearson and Duggan (2019) have conducted the only laboratory trials on mussel-zooplankton interactions in New Zealand. These experiments found that small zooplankton (particularly rotifers) were highly susceptible to kākahi (*Echyridella menziesii*) predation, while the large non-indigenous *Daphnia pulex* and *Daphnia galeata* were not. However, it is currently unknown whether competition is occurring between non-indigenous *Daphnia* and kākahi for algal food resources. If both non-indigenous *Daphnia* and kākahi consume different algal material, it may be possible for these species to act as a biomanipulation tool to synergistically improve water quality, by reducing algal biomass in New Zealand lakes. In response to the knowledge gap surrounding these grazing interactions, our study aims to determine whether niche overlap exists between the non-indigenous *Daphnia pulex*

and *Echyridella menziesii*. We aim to determine the algal resources utilised by each species to evaluate whether competition for food resources is likely to occur. This research will also provide insights into differences in the algal clearance abilities of adult and juvenile life stages of kākahi, which are yet to be explored in the literature.

3.3 Methods

3.3.1 Mussel collection

Fifty-three adult *Echyridella menziesii* (kākahi) were collected from Lake Rotorua, Bay of Plenty (average <1 m depth; 38°04′25.8″S, 176°12′58.9″E) on 27 March 2018. Mussels were collected within 10 m of the shore with the aid of a bathyscope (Figure 3.1). From these specimens, ten healthy adults were selected based on two criteria; (1) kākahi appeared to be actively filtering with their inhalant and exhalent siphons out, and (2) kākahi retracted their siphons and closed their shells in response to touch (Molina et al. 2011). The ten adult bivalves selected had a mean length of 61.2 ± 3.4 (SD) mm with a range of 56 – 66 mm. In addition to the adult bivalves, ten juvenile mussels (mean length of 31.2 ± 3.6 (SD) mm; range of 23 – 35 mm) were collected by boat using the same method in Te Arero Bay, Lake Rotoiti, Bay of Plenty (average <0.5 m depth; 38°00′77.6″S, 176°.23′39.7″E) on 14 June 2018.

Figure 3.1. (A) Adult freshwater mussel moving through the sediments in Lake Rotorua. (B) A handful of smaller 'juvenile' freshwater mussels at Lake Rotoiti. (C) Shallow lake edge habitat where the juvenile freshwater mussels were found.

Mussels were transported to a temperature-controlled room (18°C, with a 16 h light: 8 h dark cycle) at the University of Waikato Aquatic Research Laboratory. Once acclimatised to the temperature, individuals were moved into two large tanks (length 92 cm, width 38 cm, height 92 cm) filled with 105 L of dechlorinated tap water. The tanks contained oxygen bubblers and a 2 cm layer of silica sand. Mussels were fed daily with 0.8 mL of Reid Mariculture Shellfish Diet 1800 and 0.4 mL of Reid Mariculture Nanno 3600 micro-algae diluted in 2 L of dechlorinated tap water. Additionally, ten percent of the tank water was replenished on alternate days to prevent ammonia build up.

3.3.2 Phytoplankton collection

Five waterbodies in the Waikato were sampled on 3 September 2018 to collect 60L of concentrated algae (an unnamed pond at Tamahere (37°50'14.3"S 175°23'37.7"E), Lake Areare (37°39'58.0"S 175°11'49.6"E), Lake Oranga (37°47'12.0"S 175°18'56.5"E), Waikato River at the Hamilton Gardens Ferry Terminal (37°48'24.6"S 175°18'18.3"E), and Lake Cameron (37°51'17.3"S 175°18'07.5"E)). These locations were selected based on the variety of algae found among these ponds during preliminary sampling the day prior to the experiment. Blue green algal species were avoided as preliminary experiments indicated that high concentrations of blue green algae may inhibit kākahi filtering. This was done by selecting waterbodies without these species or ones with very low concentrations of blue green algae. At each site, both 25 μm and 40 μm plankton throw nets were used to collect 15 L of algal concentrate. The algal concentrate was then returned to the laboratory and filtered through a 500 μm mesh to remove any large zooplankton species and detritus (Marroni et al. 2017), before being poured into a 125 L plastic PVC drum. An additional 40 L of dechlorinated tap water was added to the drum to reduce the overall algal concentrations.

3.3.3 **Daphnia pulex** *collection*

Daphnia pulex were sourced from Global Goldfish Fish Farm, Te Aroha (Waikato; 37°31'42.8"S 175°42'42.7"E), and purchased as three 2 L bags of concentrated individuals. The *Daphnia* were delivered on the day prior to the experiment and

were identified as *D. pulex* under a compound microscope (Olympus BH-2). The *Daphnia* were kept in the same temperature and light conditions as the kākahi, with the exception that the bubblers were used without air stones; this was done to prevent the entrapment of air bubbles under their carapaces.

3.3.4 Experimental setup

The experiment was undertaken over a 7.5-hour period inside a temperature-controlled room, set at 18^{o}C to simulate New Zealand's ambient lake summer temperatures (Green et al. 1987). The room was kept under dimly-lit red lighting conditions for the duration of the experiment to allow for experimenter visibility, but to discourage algal growth. Twenty square experimental aquaria (length 12.4 cm, width 12.4 cm, height 17.0 cm) were set up with two plastic cups (60 mL volume) attached 6 cm apart diagonally across the aquaria base using Nirox clear silicone sealant (full process described in Pearson and Duggan in press). A membrane was formed over each cup using Parafilm (101.6 x 101.6 mm), and the film was then slit in the center using a razor to create a pocket to hold the mussels upright during the experiment.

Fifteen aquaria were allocated one of three treatments; 1) 1000 *Daphnia pulex* (333.33 L^{-1}) + algal mix, 2) two adult *Echyridella menziesii* (equivalent to 13.33 mussels m^2) + algal mix, 3) two juvenile *E. menziesii* (equivalent to 13.33 mussels m^2) + algae; a further 5 aquaria were allocated as controls containing just the algal mix. This equated to five replicates per treatment/control type. The densities of *Daphnia* and mussels selected for the trial were based within numbers expected in natural lake and pond systems (Butterworth 2008; Pers. Obs. A. Pearson 2018). At the start of the experiment, the drum filled with lake water was stirred using a plastic garden stake in a figure of eight fashion to mix the algae and to counteract sinking. Three litres of the mixed algae solution (collected 1 L at a time) was added to each experimental bucket before commencement of the three-hour trial period.

For the adult kākahi treatment, two bivalves were randomly selected for each replicate. Each mussel was measured and weighed before being placed with their siphons orientated upward inside the slots of the Parafilm covered cups. The

Parafilm's grip was loosened by twisting the mussel to the right and left to ensure they could open their shells in the membrane. The same procedure was repeated for the juvenile mussel treatment, with the exception that two extra pieces of Parafilm (101.6 x 101.6 mm) were folded and placed in the bottom of the cups to prevent the mussels falling through the slots. Careful attention was taken to ensure that all mussels had their siphons out and were filtering during the three-hour experiment. In one case, an adult mussel treatment was re-conducted with new mussels as one adult mussel did not open its siphon after a 1-hour period. In the *Daphnia* treatment, 1000 adult *Daphnia pulex* were manually counted out into a Petri dish under a dissecting microscope (Olympus SZ60) using a Pasteur pipette before being added to the appropriate aquaria. Half way through the trial, all experimental aquaria were gently mixed using a new wooden paint stirrer to prevent algal particles from sinking out. A 100 mL container of water was also collected from the *Daphnia pulex* tank and was preserved with 90% ethanol; this sample was used to measure the body lengths (excluding spines) of 50 *Daphnia pulex* to estimate their average size. The *Daphnia* were measured using the eyepiece graticules of the compound microscope (Olympus BH-2) calibrated with stage micrometer.

3.3.5 Sample collection

Two 100 mL water samples were collected using a 50 mL syringe from each experimental aquaria at the start and end (0 and 180 minutes) of the trial period for algal count analysis. Each sample was preserved with 10 drops of Lugol's iodine solution. These samples were used to determine the initial and final algae concentrations and composition.

3.3.6 Algal cell concentrations

In preparation for the algal cell counts, settling tubes were attached to HYDRO-BIOS Utermöhl (counting) chambers using Glisseal silicon-free grease. 5 mL of reverse osmosis (RO) water was added to the Utermöhl chambers first to assist with even settling of the small but concentrated subsample volume. The algal samples were then gently mixed for 30 seconds in a figure of eight using an auto-pipette tip and a subsample of 3 mL (collected as 3 individual 1 mL aliquots) was added to a

Utermöhl chamber using the auto-pipette. A glass slide was placed over the settling tube to prevent dust settling in the algae sample and the Utermöhl tube was left for a minimum of twelve hours for algal settling due to the presence of small diatoms (Hötzel and Croome 1999). The following day, the slides were sealed with a glass coverslip in preparation of the algal counts.

Algal cells along the center transect of Utermöhl chamber were enumerated at 200x magnification using an inverted microscope (Olympus IX71) (Hötzel and Croome 1999). Only 'live' algal cells were counted (i.e. unbroken cells that contained chlorophyll); all others were rejected. The whole chamber was scanned from top to bottom and all algal cells, regardless of species, were counted to determine which were the most common. The dominant taxa (>65 cells per mL) were then selected, and an additional subsample was enumerated to ensure that at least 100 units of the dominant algal taxa were observed in the start samples (i.e. the samples taken prior to mussel/ *Daphnia* filtration) (Molina 2012). The greatest linear distance (GLD) was calculated for fifteen individuals /colonies /filaments for each of the dominant algal species using an Olympus (BH-2) compound microscope, calibrated using a stage micrometer (Naddafi et al. 2007).

3.3.7 Data analysis

Algal cell data was entered into Microsoft Excel (version 15.33) and used to calculate the average number of cells per mL using the following equation:

$$\text{Number of algal cells per mL } (N) = C\ f\ (A/baV)$$

The number of algal cells per mL in the original water sample is represented by N, while C represents the total number of algal cells counted in all chamber transects. A represents the area of the settling chamber, while a represents the area of the transect in mm^2. b indicates the number of transects counted and f indicates the concentration factor. Finally, V represents the volume of experiment water settled in mL.

The cell concentration data was used to generate bar charts to visualize the algal cell depletions across treatments (Microsoft Excel; version 15.33). Non-metric multidimensional scaling (nMDS) was undertaken to create a 2-dimensional plot to visualize changes in algal community composition brought about by grazing. An Analysis of Similarities (ANOSIM) was performed to identify differences between algal communities across post grazing treatments relative to the starting samples (PRIMER; version 7.0.11). A Similarity Percentages (SIMPER) analysis was undertaken to determine the contribution of each algal species to the dissimilarity of the algal communities where ANOSIM inferred significant community differences. All data were transformed for multivariate analyses using a log(x + 1) transformation to down weigh highly abundant species, to prevent these having an undue influence on the analyses. Further, the multivariate analyses were based on Bray-Curtis similarities.

Clearance rates (CR) were calculated for each grazing taxon on each of the dominant phytoplankton species, using the following equation in Microsoft Excel (version 15.33) (MacIsaac et al. 1991):

$$\text{Clearance Rate (CR)} = V(\ln[E_0/E_t] - \ln[C_0/C_t])/tn$$

V represented the volume of water in the experimental aquaria (mL). The average initial and final algal (prey) densities are represented by E_0 and E_t respectively. C_0 represents the initial algal densities in the control while C_t represents the final control algal densities. Finally, t represents the time (hours) and n symbolises the number of grazers (i.e., 1000 *Daphnia pulex*/ two adult or juvenile *Echyridella menziesii*).

3.4 Results

3.4.1 Common algal taxa

Seven phytoplankton species were considered sufficiently abundant in the samples to be considered in analyses (Figure 3.2). Three species were filamentous diatoms (*Aulacoseira granulata*, *A. japonica*, and *Melosira varians*) and one was the

colonial diatom species *Asterionella formosa*. One unicellular green algae species was included in the analysis (*Closterium aciculare*), a single celled dinoflagellate (*Peridinium cinctum*) and a Synurophyceae species (*Mallomonas* sp.).

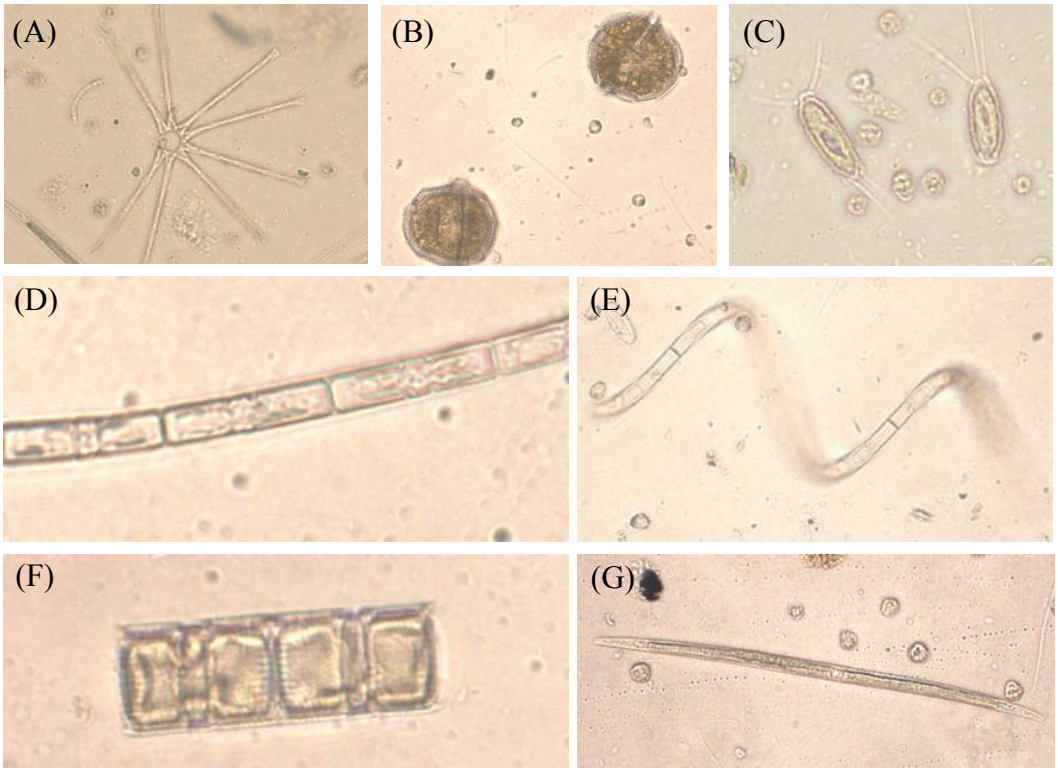

Figure 3.2. Images taken of dominant algal species at 200x magnification, captured using an Olympus IX71 inverted microscope and ASTRO IIDC Software (version 4.05.04). (A) *Asterionella formosa*, (B) *Peridinium cinctum*, (C) *Mallomonas* sp. (D) *Aulacoseira granulata*, (E) *Aulacoseira japonica*, (F) *Melosira varians* and (G) *Closterium aciculare*.

3.4.2 Differences in algal composition

Of the four-multicellular diatom species, *Asterionella formosa* had the largest average number of algal cells depleted across all treatments, with an average loss of 310 cells per mL for adult mussels, 218 cells per mL for juvenile mussels, and 77 cells per mL for *Daphnia pulex* (Figure 3.3). For the three-filamentous species (*Aulacoseira granulata*, *A. japonica* and *Melosira varians*), the average number of cells removed by the adult mussels and juvenile mussels were relatively similar, with adults ranging from 247 to 272 cells per mL, the juveniles ranging from 213 to 259 cells removed. The three filamentous algal species were found to have higher cell concentrations at the end relative to the start in the *Daphnia* treatment; these average cell numbers ranged from 2 to 49 cell per mL increases.

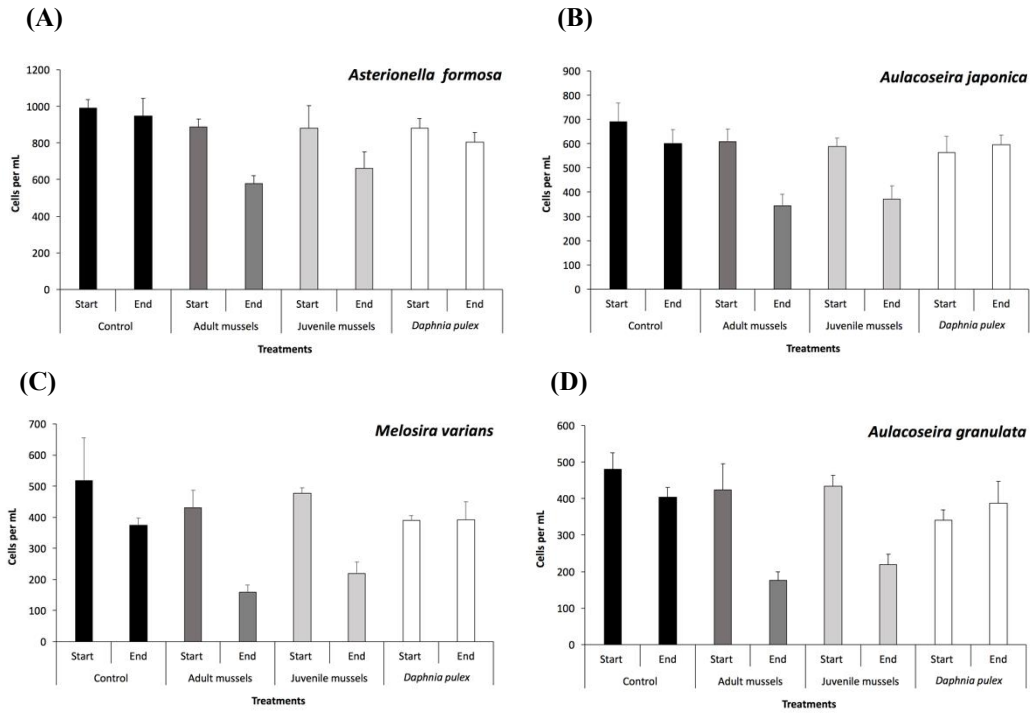

Figure 3.3. Average cells per mL (+SE) for each of the colonial phytoplankton species tested, at the start and end of each treatment as well as controls; (A) *Asterionella formosa*, (B) *Aulacoseira japonica*, (C) *Melosira varians* and (D) *Aulacoseira granulata.* The error bars represent the standard error.

Of the three unicellular algal taxa (Figure 3.4), the most common species, *Closterium aciculare*, had the highest average number of algal cells removed across all treatments, particularly in the mussel treatments, with over 500 cells per mL removed on average for both adult and juveniles. This was followed by *Mallomonas* sp., which had over 100 cells per mL removed by the adult and juvenile treatments (130 and 123 cells per mL respectively), while the *Daphnia* treatments had an average of 12 cell per mL less. Very few *Peridinium cinctum* were cells removed, with an average of less than 20 cells per mL for all treatments. Overall, both the unicellular and multicellular phytoplankton data showed a clear cell removal gradient, with the adult mussels removing the most, followed by the juvenile mussels, and then the *Daphnia pulex* with the least.

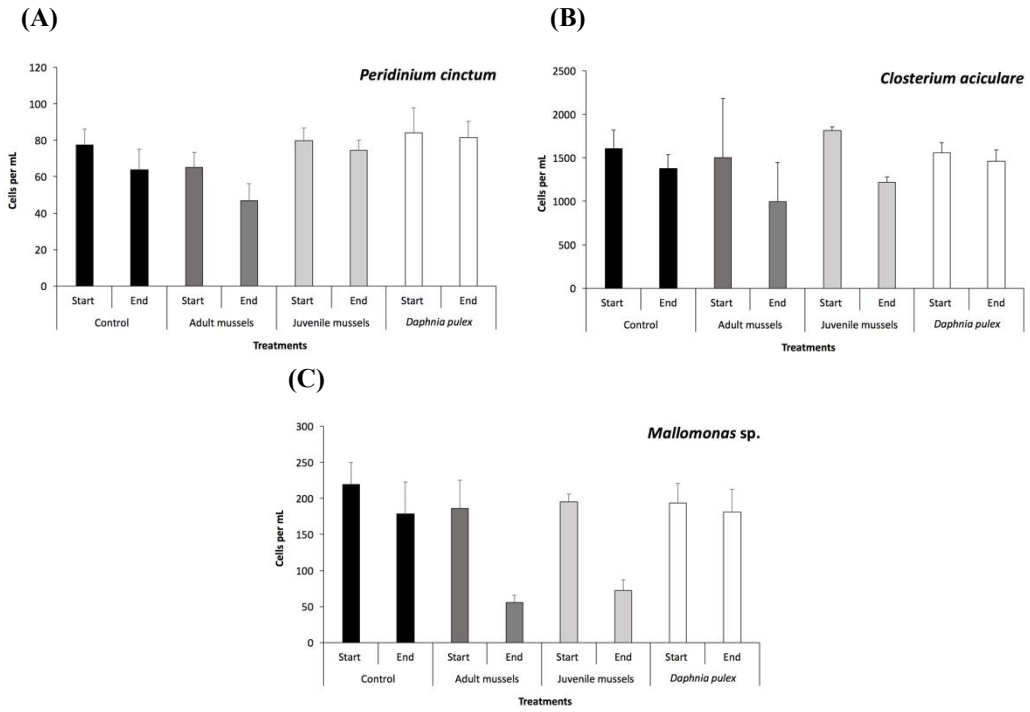

Figure 3.4. Average algal cells per mL (+SE) for each unicellular phytoplankton species tested in the trial at the start and end of each treatment; (A) *Peridinium cinctum*, (B) *Closterium aciculare*, and (C) *Mallomonas* sp. The error bars represent the standard error.

The nMDS plot (Figure 3.5) shows that the samples from the beginning of the experiment (the starting algal composition in each of the control and treatment replicates) were grouped to the left of the ordination, along with the control end and *Daphnia* end results (the final algal composition for both the *Daphnia* treatment and controls replicates). The juvenile mussel replicates from the end of the experiment were predominantly located in the centre of the plot, and the adult mussel samples are over to the right. The algal composition in the *Daphnia* samples at the end were relatively similar to those in treatment samples from the beginning of the experiment, as well as the controls from the beginning and end of the experiment. This is indicated by the overlap in samples in the upper, mid to left of the ordination. Comparatively, the algal composition in the juvenile and adult mussel replicates at the end of the experiment were not similar to the starting assemblages, controls at the beginning or end, or the *Daphnia* treatments, as the two mussel treatments were located further to the right on the ordination. The nMDS also indicates that there is a large difference between the cells per mL algal composition in the adult mussel and *Daphnia* treatments at the end of the experiment, as there is no overlap between the replicates of these treatments. A

stress value of 0.09 suggests the ordination provides no prospect of misinterpretation of the underlying similarity matrix (Clarke et al. 2008).

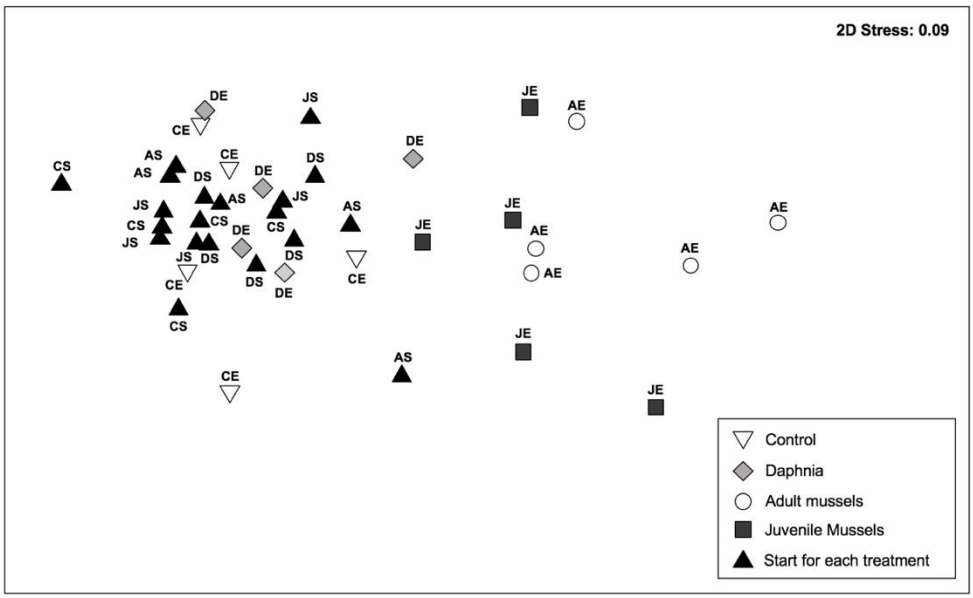

Figure 3.5. Non-metric Multi-dimensional scaling (nMDS) plot illustrating similarities and differences between algal counts (cells per mL) for each treatment at the end of the three-hour experimental period; *Daphnia pulex* (grey diamonds), adult mussels (white circles), juvenile mussels (grey squares). The starting algal counts for all treatments are also represented on the plot by black triangles and the controls are represented by white downward triangles. The 2D stress value was 0.09.

ANOSIM indicated that both the adult and the juvenile treatments were significantly different to the controls at the end of the experiment (Table 3.1) (Global *R*-statistic = 0.892 and 0.648 respectively, *P* = 0.008 (both)) and the start samples (Global *R*-statistic = 0.956 and 0.863 respectively, *P* = 0.001 (both)). This was also the case for the cell counts in the adult mussel treatment and *Daphnia pulex* treatment, which were seen to be statistically different to one another (Global *R*-statistic = 0.88, *P* = 0.008). Comparatively, the end *Daphnia pulex* composition was not different to that of the start samples (Global *R*-statistic = 0.13, *P* = 0.208).

Table 3.1. ANOSIM matrix p-value results showing where differences (significant p-values in bold) have occurred in the algal composition between the end of each treatment (*Daphina pulex*, adult and juvenile kākahi) and the control ending cell counts, as well as the controls and the starting composition for all treatments.

	Treatment and control starts	Adult mussel	Juvenile mussel	*Daphnia pulex*
Control end	0.115	**0.008**	**0.008**	0.817
Treatment and control starts		**0.001**	**0.001**	0.208
Adult mussels			0.333	**0.008**
Juvenile mussels				**0.008**

SIMPER analysis indicated that *Mallomonas* sp. contributed most to the dissimilarity between algal communities in adult mussel treatments compared to the control end (24.33%), followed by *Peridinium cinctum* (21.14%). The remaining algal species contributed 14.50% (*Aulacoseira granulata*) or less (*Aulacoseira japonica*, *Melosira varians*, and *Asterionella formosa*) to the dissimilarity of algal cells per mL between the adult mussel treatment ends and control end results. Similarly, for the juvenile mussels, *Mallomonas* sp. and *P. cinctum* contributed to the largest percentage of the dissimilarity (18.01% and 20.92% respectively) between the juvenile mussel treatments and control samples, while the remaining species contributed dissimilarities of 16.47% (*A. granulata*) and below (*Melosira varians*, *Aulacoseira japonica*, *Asterionella formosa*).

Adult *Echyridella menziesii* had the greatest clearance rate (CR) for all algal species, with the highest CR for *Mallomonas* sp. (498.69 mL h^{-1} adult $mussel^{-1}$), followed most closely by the *Aulacoseira granulata* and *Melosira varians* (351.10 and 335.84 mL h^{-1} adult $mussel^{-1}$, respectively) (Table 3.2). The next highest clearance rates were produced by the juvenile mussels, which had clearance rates slightly lower than the adult *E. menziesii*. The juveniles also cleared high numbers of *Mallamonas* sp. and *Melosira varians*. Both adult and juvenile mussels had the lowest clearance rates for *Peridinium cinctum*; adults produced a clearance rate of 64.57 mL h^{-1} $mussel^{-1}$, while the juvenile treatments were calculated to have a negative clearance rate, suggesting that grazing by the juvenile *E. menziesii* was unable to deplete the *P. cinctum* population. Unlike the clearance abilities of the freshwater mussels, *Daphnia pulex* was calculated to have a negative CR for all

algal species except *Asterionella formosa* (0.05 mL h^{-1} *Daphnia*$^{-1}$). This indicates that *Daphnia* were unable to deplete algal cells sufficiently during the three hour time period to result in a positive filtration rate.

Table 3.2. The average size of the algal unit measured as the greatest linear dimension (GLD) of the total algal unit (i.e. filament, colony, or cell) for each species, the (*) indicates species that comprised of many broken algal filaments. The mean clearance rate (CR) of each algal species in mL per hour for each individual grazer tested (adult *Echyridella menziesii*, juvenile *E. menziesii* and *Daphnia pulex*).

Species	Unit size (μm)	Structure	Adult mussel (mL h^{-1})	Juvenile mussel (mL h^{-1})	*Daphnia pulex* (mL h^{-1})
Asterionella formosa	140 ± 17.33	Colonial	192.87	120.08	0.05
Aulacoseira granulata	348 ± 112.89	Filament *	351.10	251.38	-0.30
Aulacoseira japonica	153.89 ± 38.71	Filament *	215.48	160.55	-0.19
Closterium aciculare	211.12 ± 28.23	Single cell	127.50	119.96	-0.09
Mallamonas sp.	33.64 ± 5.88	Single cell	498.69	396.02	-0.14
Melosira varians	150.41 ± 50.78	Filament *	335.84	227.77	-0.33
Peridinium cinctum	47.17 ± 5.31	Single cell	64.57	-65.62	-0.17

3.5 Discussion

3.5.1 Resource partitioning and selection

Kākahi were able to deplete a wide variety of algal taxa provided to them, including single celled, colonial, and filamentous species, while *Daphnia pulex* could not. At the end of the trial, the remaining algal community in the adult mussel treatment differed significantly to that of the *Daphnia pulex* treatment ($P = 0.008$). Declines in algal cell counts coupled with the high clearance rates for adult *Echyridella menziesii* indicate that adult kākahi could filter moderate quantities of all of the seven major algal species used in treatments, which had cells or colonies ranging in size from 33.6 ± 5.9 μm to 348.0 ± 112.9 μm. Comparatively, the remaining algal composition in the *D. pulex* treatment at the end of the experiment was found to be similar to that of the control (both beginning and end) and of the starting algal composition for each of the treatments. These findings suggest that even at the high *Daphnia* concentrations used in our experiment (333.33 L^{-1}), *D. pulex* is unable to efficiently remove algae within the size ranges provided, relative to the growth in the algal population.

Of the seven algal taxa tested in our study, clearance rates for *Daphnia pulex* indicated that *Asterionella formosa* (maximum colony length 140.0 ± 17.3 μm) was the only taxon reduced in the daphnid's presence (0.05 mL h^{-1} *Daphnia*$^{-1}$). In the literature, *Daphnia* have been described as highly efficient phytoplankton consumers in freshwater systems due their non-selective grazing behavior which result in 'clear water phases' in many temperate lakes (Horn 1981; Lampert 1986; Kasprzak et al. 1999). Similar to our experiments, the colonial diatom *A. formosa*, with cell sizes of 63.6 μm, was found to be a palatable food source for *Daphnia pulicaria* and *Daphnia thorata* sourced from Lake Washington, Seattle, USA; these *Daphnia* were observed to have clearance rates of 0.22 mL ind^{-1} hr^{-1} and 0.23 mL ind^{-1} hr^{-1} for *A. formosa*, respectively (Infante and Litt 1985). These findings are also in line with Ebert (2005), who suggested that *Daphnia* typically consume particles ranging from 1 μm up to 50 μm; however, larger particles of up to 70 μm have been found in the gut of large daphnids (Ebert 2005). Similarly, Burns (1968b) examined the relationship between the body size and maximum particle size ingestion of Cladocera, and found that *D. pulex* over 1.5 mm were able to ingest a maximum bead size of around ~40 μm in diameter. These findings suggest that the majority of phytoplankton found in our experiment was unpalatable due to being too large for the filtering apparatus of *D. pulex*. Thus, it is possible that the low clearance rate of *A. formosa* in our study may be due to the daphnids being only able to consume smaller broken fragments, or individual cells, of this colonial diatom.

Another trend seen in the feeding behaviour of *Daphnia* is the rejection of filamentous algal species. In our study, three of the clearance rates calculated as negative for *Daphnia pulex* were associated with the three largest filamentous algal species; *Melosira varians* (-0.33 mL h^{-1}), *Aulacoseira granulata* (-0.30 mL h^{-1}), and *Aulacoseira japonica* (-0.19 mL h^{-1}). Other cladocerans have also been recorded to have difficulty consuming filamentous algae. Long algal filaments were found to be a hindrance to *Daphnia rosea* as the filaments interrupted the daphnids filtering mechanism; for example, *D. rosea* was observed to vigorously reject filaments of blue-green algae that had collected in their thoracic chambers (Burns 1968a). These observations suggest that *Aulacoseira* and *Melosira* filaments were likely too long, and of an unsuitable shape, for the filtering apparatus of *D. pulex* in our experiment.

Surprisingly, *D. pulex* had negative calculated clearance rates even for the two smallest algal taxa; *Mallamonas* sp. (-0.14 mL h^{-1}) and *Peridinium cinctum* (-0.17 mL h^{-1}). To generate negative clearance rates, algae needed to be depleted more in the controls relative to the treatments. In our *Daphnia* trials, negative clearance rates may be explained by the nutrient excretion and recycling abilities of the *Daphnia* themselves (Vanni 2002). Zooplankton play a key role in supporting primary production through their excretory products, which provides soluble nitrogen and phosphorus supporting phytoplankton growth (Rivier et al. 1986; Attayde and Hansson 1999; Vanni 2002). Experimental work on the effect of *Daphnia* grazing by Rivier et al. (1986) found that natural densities of *D. pulex* (19 ind. L^{-1}) were sufficient to increase phytoplankton reproduction, particularly for pennate diatoms, which are typically associated with nutrient limited environments (Rivier et al. 1986). Therefore, the presence of 333.33 ind· L^{-1} of grazing *D. pulex* in the *Daphnia* treatment is expected to have led to higher rates of nutrient regeneration, allowing for higher algae reproduction when compared to the controls, which only contained small residual zooplankton grazers (e.g., rotifers and *Bosmina*) from the original algal sources. In addition to nutrient recycling, it is also possible that nutrient translocation has occurred in the *Daphnia* treatment, resulting in promoted algal growth (Vanni 2002; Rivier et al. 1986). *Daphnia pulex* had the ability to feed on bacteria and algae present in the water sourced from their original pond prior to entering the experiment. Due to the large densities of *Daphnia* in the trial, defecation may have resulted in higher fertilization rates occurring in the *D. pulex* treatments through nutrient translocation, supporting the primary production in this treatment relative to controls.

Recent work by Marroni et al. (2017) has found algae removal by zooplankton to be substantially lower than that of freshwater bivalves. A native subtropical zooplankton community consisting of rotifers, cladocerans and copepods from Laguna Blanca, Uruguay, were unable to effectively reduce the natural phytoplankton biomass, which was made up of "middle-sized" species (e.g. *Chlamydomonas* sp., *Euglena* sp., and *Monoraphidium* sp.). Like *Echyridella menziesii* in the current study, the bivalves *Diplodon parallelopipedon* and *Corbicula fluminea* were also found to produce a significantly higher grazing pressure than the natural zooplankton community under experimental conditions.

These authors have suggested that these findings may have been due to the lack of large cladocerans (such as *Daphnia*) in their experiments, and the potential for preferential feeding on microbes by the extant zooplankton community (Marroni et al. 2017).

In our study, both adult and juvenile kākahi were able to remove a broad range of algal taxa, including colonial (*Asterionella formosa*) and filamentous (*Aulacoseira* spp.) species, which had cells or colonies ranging in size from 33.64 ± 5.88 μm to 348 ± 112.89 μm. Freshwater mussels have also been identified elsewhere to remove a variety of particles from the water column, from small pieces of detritus to living zooplankton and algae (McMahon and Bogan 1991); particles larger than 20 μm are considered a potential food source (Vaugn et al. 2008). The largest clearance rates for *Echyridella menziesii* in our experiments were associated with the unicellular species *Mallomonas* sp., one of the smallest algal species we tested (33.64 ± 5.88 μm). *Mallomonas* sp. also contributed to the largest proportion of the dissimilarity (24.33%) seen between the end adult mussel treatment and controls, and was the second largest contributor in the juvenile mussel treatments compared to controls (18.01%). This suggests that small algal species observed in our experiment are particularly susceptible to mussel filtration. These findings are similar to that of early work by Nobes (1980), who examined retention efficiency of plastic beads. Laboratory experiments found that adult *E. menziesii* could remove from suspension 100% of plastic spheres that ranged in size between 30.1 – 80 μm. However, the retention ability was seen to drop to 66% for spheres between the sizes of 15.1 – 30 μm, and very small beads (5.1 – 15.0 μm) were unable to be retained (Nobes 1980).

Three-filamentous diatom species were associated with relatively high clearance rates for both juvenile and adult kākahi treatments in our experiment; the largest species, *Aulacoseira granulata* (average colony length 348 ± 112.89 μm), had the highest cell clearance rate measured (251.38 - 351.10 mL h^{-1}), followed by *Melosira varians* (150.41 ± 50.78 μm; 227.77 - 335.84 mL h^{-1}), and finally *A. japonica* (153.89 ± 38.71 μm; 160.55 - 215.48 mL h^{-1}). Like *E. menziesii*, many freshwater bivalves in the USA have also been found to consume a broad range of particle sizes (Bisbee 1984). Digestive gland analyses on two unionid bivalves, the

sandshell mussel (*Ligumia recta*) and the threeridge mussel (*Amblema plicata*), retrieved from a Wisconsin River fed selectively on phytoplankton species (Bisbee 1984); filamentous diatoms such as *Melosira italica* and *Melosira granulata* were found to be the most commonly consumed algal taxa for the larger sandshell mussel (118.8 mm: average of males and females). In contrast, threeridge mussel (98.3 mm) was found to contain mostly smaller green algal species (*Chlorella* sp., *Chlamydomonas* sp., and *Scenedesmus* sp.). Of the twenty algal species found during the bivalve gut analysis by Bisbee (1984), there was an overlap between the consumption of 16 algal species across the two mussels. However, it was noted that the algae ingested by the threeridge mussel (which are not much larger than adult kākahi in New Zealand), were often smaller in size, or consisted of smaller filamentous algae fragments (Bisbee 1984).

Overall, our findings suggest that the presence of non-indigenous *Daphnia pulex* in New Zealand lakes is unlikely to impact the availability of phytoplankton as a food source for kākahi, as the native bivalves can remove much larger algal species than *D. pulex*. Our research suggests that both adult and juvenile *Echyridella menziesii* can remove algae ranging between at least 33.64 to 348.00 μm, with the potential to consume larger particles. In contrast, *D. pulex* was unable to remove the large algae species used in this experiment, with literature suggesting algae ranging between 1 μm up to 50 μm are an appropriate size (Ebert 2005). Due to this limited niche overlap, it may be possible for *D. pulex* and *E. menziesii* to be used in concert as biomanipulation tools to removed unwanted phytoplankton in eutrophic systems. As kākahi are unlikely to prey on significant numbers of *D. pulex*, both grazers will be able to consume different algal size ranges which will hopefully lead to a decrease in algal biomass and longer 'clear water' phases in eutrophic systems (Pearson and Duggan in press). Further experimental and field work is required to determine whether *D. pulex* and *E. menziesii* can work together in concert to reduce algal densities from shallow eutrophic ponds and lakes.

3.5.2 Mussel clearance abilities

In New Zealand, several studies have focused on the effect of adult kākahi filtration through the measurement of chlorophyll *a* declines, particularly in relation to their

ability to remove phytoplankton biomass in eutrophic systems (Roper and Hickey 1995; Phillips 2007, Cyr et al. 2017). A simple filtration model generated by Ogilvie and Mitchell (1995) found that the *E. menziesii* population of Lake Tuakitoto, South Otago (12.3 g biomass/m^2) has the potential to filter a water volume equivalent to that of that lake every 32 hours. This filtration rate (9 L hr^{-1} g^{-1} at 19-21 °C) was generated by calculating the chlorophyll *a* decline for a single algal monoculture consisting of the small (4-6 μm diameter) green algae *Choricystis coccoides* (Ogilvie and Mitchell 1995). Although calculations by Ogilvie and Mitchell (1995) suggest *E. menziesii* at natural densities have the potential to regulate phytoplankton growth, less attention has been given to clearance abilities of kākahi in relation to specific algal taxa (Ogilvie and Mitchell 1995; White 2000). In our experiment, grazing rates varied among algal species, indicating that simple estimates of grazing rates based on single palatable species, are not an accurate method for calculating the overarching filtration abilities of kākahi. The highest algal clearance rates in our experiments were for the small *Mallomonas* sp., for both the adult (498.69 mL h^{-1} $mussel^{-1}$) and juvenile mussels (369.02 mL h^{-1} $mussel^{-1}$). Overall, the larger adult kākahi were able to filter higher numbers of algal cells relative to juveniles. On average, our clearance rates suggest adult kākahi removed 25% more algal cells than the juvenile mussels, when excluding the species *Peridinium cinctum* (which juveniles were unable to effectively remove). These differences in filtration abilities based on size are comparable to results generated on *E. menziesii* by others in the literature. Cyr et al. (2018) calculated clearance rates for *E. menziesii* based on declines in chlorophyll *a* for mussels from six Waikato and Rotorua lakes. As in our study, clearance rates for *E. menziesii* were seen to increase with mussel size. However, their filtration ability was found to range significantly (20 to 1300 mL $mussel^{-1}$ h^{-1}), suggesting that filtration abilities can be highly variable across individuals (Cyr et al. 2018). Evidence from our experiment suggests that some of these differences may also be explained by differences in algal availability across the six lakes tested.

The maximum algal clearance rate in our experiment of 498.69 mL h^{-1} $mussel^{-1}$ was generated by adult kākahi with an average shell length of 61.2 mm (± 3.4 SD). This is comparable to the clearance rates of *E. menziesii* found in Lake Taupo and Lake Tarawera (both, 480 mL ± 30mL h^{-1} $mussel^{-1}$), which had average shell lengths of

50.9 (± 5.6) mm and 51.1 (± 6.2), respectively. In terms of shell length, Lake Karapiro in the Waikato was found to have the closest mean shell lengths (65.7 mm ± 5.0) to the kākahi tested in our experiment (61.2 mm ± 3.4 SD). Although, these kākahi had clearance rates slightly higher than ours (620 mL ± 50 mL h^{-1} $mussel^{-1}$), this may have been due to the availability of a more palatable or chlorophyll rich algal species in Lake Karapiro (Cyr et al. 2018). To our knowledge, there is no previous published information on the individual clearance rates for juvenile *E. menziesii*. Interestingly, in our study, juvenile kākahi were seen to filter out substantial numbers of all species except *Peridinium cinctum*. The negative clearance rate calculated for *P. cinctum* (-65.62 mL h^{-1} juvenile $mussel^{-1}$) suggests that juvenile kākahi were unable to control this algae species. As juvenile kākahi likely have a lower siphoning ability relative to adult kākahi, and *P. cinctum* are motile, this may explain why they were unable to remove these small dinoflagellates.

3.6 Conclusion

Our laboratory experiments have indicated that kākahi and the non-indigenous zooplankton *Daphnia pulex* likely have a very limited niche overlap. Kākahi were found to remove a broad range of algal sizes, while *D. pulex* could not. Instead, it is thought that *D. pulex* is only capable of removing smaller algal taxa. As such, the feeding behaviour of *D. pulex* at natural (or even high) densities in lake ecosystems will not impact the overall algal food availability for kākahi. Further work is required to ascertain the full algal filtration abilities of *Echyridella menziesii*, particularly at the juvenile and earlier life stages. An improved understanding of the algal species grazed upon by *E. menziesii* will help others determine how kākahi can be used as a biomanipulation tool to combat algal blooms associated with eutrophication in the future.

3.7 Acknowledgements

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Chapter 4

Summary and Implications

4.1 Introduction

Globally, freshwater mussels are being impacted by a multitude of factors, such as habitat loss, pollution, exploitation and the introduction of invasive species (Ricciardi et al. 1998; Anthony and Downing 2001; Lydeard 2004). Historic Māori records coupled with observations of kākahi populations skewed toward adult dominated demographics suggest that freshwater mussels in New Zealand are in decline (Rainforth 2008; McDowall 2011). Nevertheless, factors influencing kākahi declines are less well understood.

One third of established invertebrates in New Zealand lakes have been zooplankton species (Duggan and Collier 2018); of these established taxa, two large daphnids (*Daphnia pulex* and *Daphnia galeata*) stand out as being potential competitors for kākahi in lake ecosystems, due to their highly efficient filter feeding abilities. In response to this hypothesis, the overarching aim of this research was, firstly, to assess whether kākahi can remove non-indigenous *Daphnia* from lake ecosystems. This question was used to determine whether kākahi function as zooplankton predators in lake ecosystems. Secondly, this research aimed to determine whether niche overlap exists between *Daphnia* and freshwater mussels, by assessing which algal resources each of the grazers can utilise. The findings from this question was used to determine whether *Daphnia* invasions negatively impact kākahi through resource competition.

4.2 Key findings

4.2.1 Echyridella menziesii *as a zooplankton predator*

Research conducted overseas has indicated that some bivalves can remove zooplankton from the water column through their filtering activities (Wong et al. 2003a and 2003b; Molina et al. 2011 and 2012; Marroni et al. 2017). Yet, prior to

my research, it was unknown whether New Zealand's native freshwater mussel *Echyridella menziesii* had the ability to impact zooplankton communities through their biofiltration activity. As such, it was unknown whether *E. menziesii* could be used as a biomanipulation tool to remove invasive *Daphnia* from shallow lake systems. To address these questions, laboratory experiments were undertaken to test whether *E. menziesii* could filter out the two non-indigenous zooplankton (*Daphnia galeata* and *Daphnia pulex*), as well as two smaller native zooplankton species (*Bosmina meridionalis* and *Brachionus calyciflorus*).

Kākahi could remove statistically significant numbers of the large cladoceran *Daphnia pulex* (7.4%) and the small rotifer *Brachionus calyciflorus* (30.2%) under shallow water experimental conditions. This suggests that *Echyridella menziesii* can function as a zooplankton predator in lake systems, particularly for small feeble species such as *B. calyciflorus* that cannot avoid the suction generated by the mussel's inhalant siphon. Nonetheless, *Echyridella menziesii* was unable to remove ecologically significant numbers of the two non-indigenous daphnids (1.7% *D. galeata* and 7.4% *D. pulex*) during the two-hour experimental period; therefore, kākahi is unlikely to function as a successful biomanipulation tool in shallow lakes to remove unwanted *Daphnia*. Nonetheless, these findings still indicate that the presence of kākahi in lake systems may play a key role in influencing zooplankton community composition, by preferentially removing smaller species. Additionally, the high removal rate of *Brachionus calyciflorus* suggests that small zooplankton such as rotifers may function as an important food source for kākahi. In this respect, *Daphnia* invasions may impact food availability for kākahi as some native rotifer species have been found to be removed from a New Zealand lake following *Daphnia galatea* establishment (Balvert et al. 2009). To evaluate this potential issue, further research is required to determine whether the bivalves can assimilate (i.e., incorporating carbon into their tissue) rotifers or whether they are just filtered out of water column and rejected as pseudofeces instead.

4.2.2 Algal resource competition

In shallow lake ecosystems, zooplankton and freshwater mussels function as the main consumers of phytoplankton biomass. Yet, few studies to date have

investigated the potential for competitive interactions between these invertebrates (Marroni et al. 2017). In New Zealand, potential competitive interactions between the now widespread non-indigenous *Daphnia* and native freshwater bivalves was yet to be considered. In response to this potential niche overlap, controlled laboratory experiments were undertaken to determine which algal taxa *Daphnia pulex* and adult and juvenile *Echyridella menziesii* could remove, to identify potential resource overlaps.

Algal counts indicated that *E. menziesii* can filter a broad range of algal taxa including large filamentous algae, diatoms, and green algae, with cells and colonies ranging in size between 33.6 to 348.0 μm in size. In contrast, *D. pulex* was unable to consume statistically significant numbers of the same algal species. Instead, *D. pulex* are likely to consume only smaller particles, and the size range of the natural algal species tested in our experiment were mostly unsuitable. Thus, it is clear from these findings that even when *Daphnia* are in high densities, there is only a limited niche overlap between the two grazers. Therefore, the spread of non-native *D. pulex* into lake ecosystems is unlikely to affect availability of algae for kākahi. Interestingly, juvenile kākahi (mean length of 31.2 ± 3.6 (SD) mm) filtered out the same algal taxa as adult kākahi, with the exception of the small dinoflagellate *Peridinium cinctum*. They also produced clearance rates only 25% lower than adult kākahi (61.2 ± 3.4 (SD) mm) on average for all algal species tested except for *P. cinctum*

These findings may have implications for the effect of kākahi in lake systems, especially in relation to nutrient recycling and their ability to function as a biomanipulation tool to remove algal biomass. In terms of phytoplankton removal, the lack of niche overlap between *D. pulex* and *E. menziesii* indicates there is potential for *Daphnia* and kākahi to be used in synchrony as a biomanipulation tool to mitigate excess algal biomass in eutrophic systems. My research suggests that kākahi can effectively remove a broad range of large algal taxa while in high densities the *Daphina* may be used to remove broken colonies and small algal taxa.

4.2.3 Implications and future research

Overall, my research has concluded that the invasion of non-indigenous *Daphnia* spp. is unlikely to be a factor influencing kākahi declines. Controlled laboratory experiments indicated that kākahi were unable to remove ecologically significant numbers of *Daphnia*. This suggested that there was potential for a niche overlap to occur between these two grazers in lake ecosystems. Nonetheless, additional algal filtering experimentation showed that mussels and *Daphnia* are unlikely to utilise the same algal food. Kākahi were found to remove larger sized algal particles, while *Daphnia pulex* could not. This indicates that daphnids are unable to reduce algal food availability for kākahi. Instead, my findings suggest that *D. pulex* is only able to remove small particles, or potentially only broken colonies and filaments. Due to differences in particle sizes filtered by *Daphnia* and kākahi, this may suggest that these taxa could be used in concert to clear algal biomass in eutrophic lakes. Nonetheless, the co-existence of these species need to be examined in longer term experimental trials, to test my predictions. Further research is also required to determine the filtering ability of both grazers across a broader range of algal species, including toxin producing algal blooms. It will also be important to ascertain how biomanipulations involving both *Daphnia* and kākahi will alter nutrient recycling and affect algal growth in lake systems.

My research has also highlighted that kākahi prey on small zooplankton, meaning the presence of these bivalves likely helps to shape zooplankton communities in New Zealand lakes. Further research is needed in this space to determine how the presence of kākahi alters zooplankton communities, and the consequences of kākahi decline for zooplankton community compositions. Additional research is also needed to analyse whether kākahi consume zooplankton for nutritional purposes, or simply passively filter them while utilizing other food resources. Understanding the dietary needs of kākahi may be useful in the future if freshwater mussels are ever propagated in captivity for conservation purposes to re-seed lake populations.

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