Field and laboratory investigations of *Echyridella menziesii* (Unionida: Hyriidae) interactions with host fishes

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Abstract

Globally, the study of freshwater mussels has increased markedly in recent years, spurred on by recognition that many mussel populations are declining or are already extinct. The New Zealand freshwater mussel *Echyridella menziesii* (Unionida: Hyriidae) has a current threat classification of 'At Risk, Declining', in part reflecting disruption of recruitment on host fish. The life cycle of *E. menziesii* includes a parasitic stage in which the larvae (glochidia) must attach to a host fish to transform into the juvenile stage. However, no research has been conducted to understand whether fish develop immunity that limits infestation rates, and little is known about the spatial and temporal variations of mussel-host interactions in natural environments in New Zealand. This study therefore aimed to: i) determine whether multiple infestations of *E. menziesii* glochidia influenced the suitability of common bully (*Gobiomorphus cotidianus*) as a host; and ii) quantify fish host associations over the glochidial release period in three contrasting Waikato streams.

Common bully considered naïve to *E. menziesii* were infested with glochidia in the laboratory, and placed in individual, flow-through tanks (22^{o}C) which were flushed every second day to measure glochidia detachment and juvenile excystment. Fishes were infested either one, two or three times, with 'control' fish infested for the first time in the second and third rounds. There were no differences in cumulative detachment rates of glochidia and no major reductions in metamorphosis success across multiple infestations. Metamorphosis success rate across all infestation rounds was ≤30%, and was highest when initial glochidia viability was also high. The lack of detectable immunity in common bully, at least after three sequential infestations, is promising for the future conservation of *E. menziesii* as host-fish are likely to be repetitively infested with glochidia over the mussel spawning season in a natural setting.

Infestation by glochidia was determined on the associated fish community, caught using a combination of electrofishing and minnow trapping. In parallel with these evaluations, mussel brood pouch development was assessed fortnightly from October 2018 to February 2019 at three Waikato stream sites. Peak glochidia release occurred in February when average monthly water temperatures were >18.8^{o}C. A field method was developed to quantify glochidial attachment on

external surfaces of fish, while internal attachment (gills, mouth) was quantified by laboratory dissection. While a range of fish hosts were identified, 86% of recorded glochidia were attached to *Gobiomorphus* species, including redfin bully (*G. huttoni*) and common/Crans bully (*G. cotidianus*/*G. basalis*). Most externally-attached glochidia (73%) occurred on caudal, pelvic and pectoral fins. Host associations appeared to favour benthic rather than pelagic species, and did not change throughout the mussel reproductive season despite seasonal change in fish communities.

This research demonstrates that common bully, and more broadly *Gobiomorphus* species, are important hosts for glochidia and do not develop immunity to repeated infestations. *Gobiomorphus* species have the potential to be very useful 'tools' to assist in restoring *E. menziesii* populations, and therefore conservation management must consider these host fish populations as well as other habitat and water quality factors affecting *E. menziesii*.

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Chapter One

General Introduction

1.1 Significance of freshwater mussels

Freshwater mussels (Order: Unionida) are one of the most globally diverse freshwater invertebrates (Bogan, 2008). The Unionida is divided among six families: Unionidae, Margaritiferidae, Hyriidae, Etheriidae, Mycetopodidae and Iridinidae (Nowak & Kozlowski, 2013). A review of the Unionida by Graf and Cummings (2007) estimated there are 840 species in total worldwide, with the largest family Unionidae having 674 species. There are an estimated 33 species in Australasia, with the majority belonging to the family Hyriidae (Graf & Cummings, 2007). Freshwater mussels are found in a wide range of habitats, ranging from soft sediment beds of lakes and ponds, to cobble and rock substrates in fast-moving rivers, although the majority of species are found in clear, highly oxygenated streams and rivers with sand, gravel, or cobble bottoms (Nobles & Zhang, 2011; Nowak & Kozlowski, 2013).

New Zealand is home to three native freshwater mussel species which belong to the unionid family Hyriidae (Graf & Cummings, 2011). All three freshwater mussel species have differing conservation status reported by Grainger et al. (2018) as follows: *Echyridella menziesii*, 'At Risk, Declining', *Echyridella aucklandica*, 'Threatened, Nationally Vulnerable', and *Echyridella onekaka* 'Data Deficient'. Previously, there has been considerable confusion regarding the number of freshwater mussel species in New Zealand. It was found that, historically, there had been overestimates in the number of species due to differences in shell morphologies later found to be a result of environmental conditions (Fenwick & Marshall, 2006; Phillips et al., 2007; Marshall et al., 2014). However, DNA barcoding technology was able to confirm that there are only three extant species of freshwater mussels known at present in New Zealand (Marshall et al., 2014). The widespread *E. menziesii* is found in a wide range of habitats from small fast-flowing streams to lakes (Walker et al., 2001). The distribution of *E. aucklandica* is restricted to the northern North Island, with outlier populations in Whanganui, Lake Wairarapa near Wellington and Lake Hauroko in Fiordland (Walker et al., 2001; Marshall et al., 2014), while the less common *E. onekaka* is restricted to the north-

western South Island (Fenwick & Marshall, 2006) (Figure 1.1). This study focusses on the widespread and sometimes abundant *E. menziesii*.

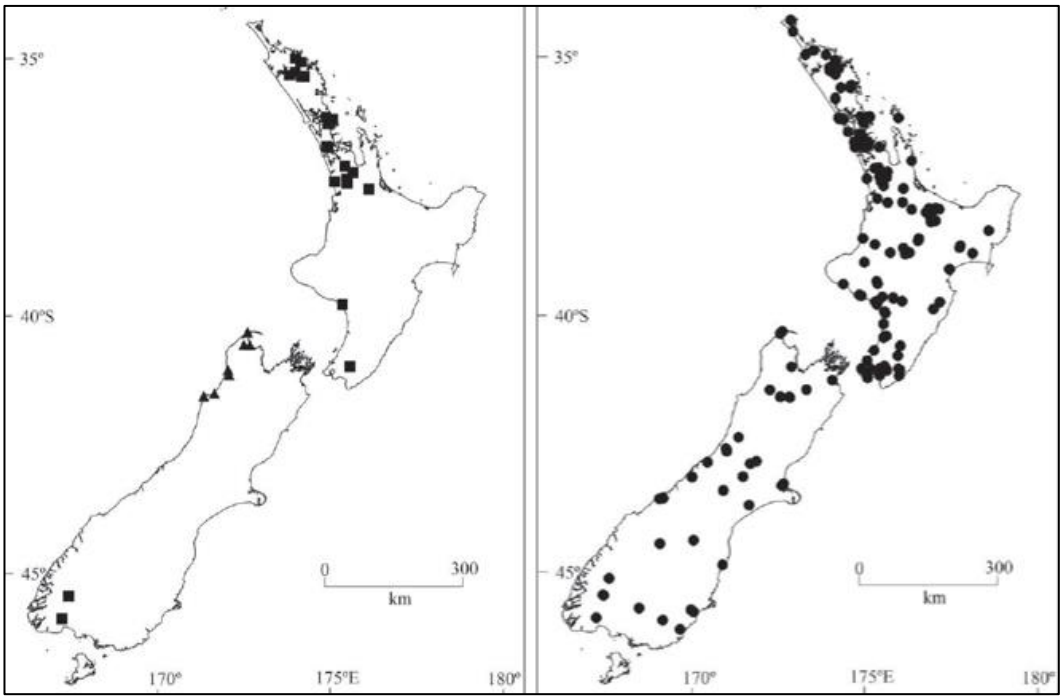


Figure 1.1. Figures from Marshall et al. (2014) showing recorded distribution of mussels on an outline map of New Zealand. Left: Distribution of Echyridella aucklandica (Gray, 1843) (■) and E. onekaka (Fenwick & Marshall, 2006) (▲). Right: Distribution of the widespread E. menziesii (Gray, 1843) (●).

All three native mussel species are likely long-lived, although longevity has only been measured for *E. menziesii*. Individuals as old as 33 years (84 mm) were reported in Lake Waipapa and the Waikato River (Roper & Hickey, 1994), compared to 13 years old (61 mm) in Lake Taupō (James, 1985). In Lake Waipori, Otago, Grimmond (1968) estimated the most frequent age ranged from 20-25 years, with the majority of specimens between 15-35 years, indicating a geriatric population. There are records of larger overseas species living longer still, such as freshwater pearl mussel (*Margaritifera margaritifera*) populations which frequently have mussels aged over 100 years, with the youngest individuals collected primarily 30-50 years old (Geist, 2010). The longevity and sessile lifestyle of unionid mussels such as *E. menziesii* makes conservation efforts particularly difficult, and solutions to increasing their abundance are not simple (Hare et al., 2019).

1.1.1 **Ecosystem services of freshwater mussels**

Freshwater mussels play a disproportionately important role in freshwater ecosystems when present in high numbers and are commonly referred to as ecosystem engineers or keystone species (Nowak & Kozlowski, 2013; Chowdhury et al., 2016; Vaughn, 2017). The presence of mussels creates habitat suitable for other species, and their loss would have complex consequences on the entire ecosystem (Chowdhury et al., 2016). Vaughn (2017) divided freshwater mussel functional roles into three main ecosystem service classes: regulating; supporting; and provisioning and cultural (Figure 1.2).

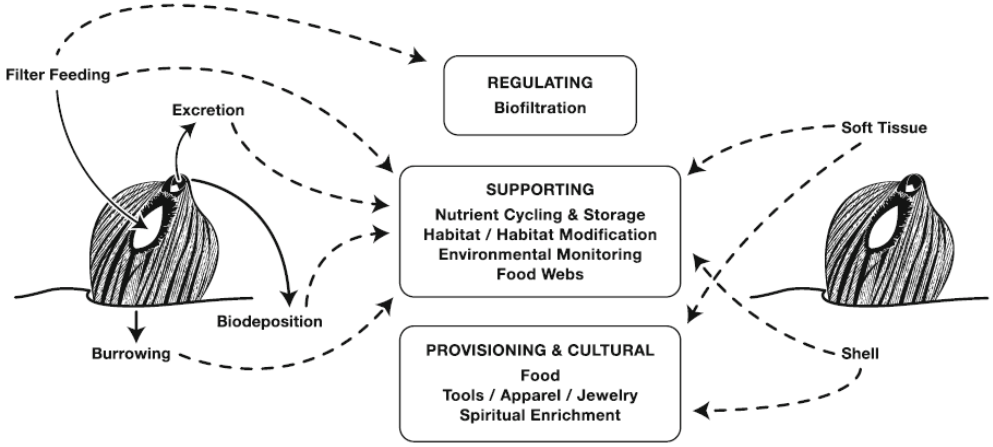


Figure 1.2. Ecosystem services that mussels provide in freshwater environments (Vaughn, 2017).

Regulating services: mussels as water purifiers

Freshwater mussels are efficient filter feeders removing particles from the water column and interstitial sediments (Vaughn, 2017). Research has shown them to be omnivores whose diet changes with habitat and food availability, feeding across trophic levels on bacteria, algae, detritus, zooplankton, and perhaps even dissolved organic matter (Vaughn et al., 2008; Vaughn, 2017). Though temperature-dependent filtration rates vary (Spooner & Vaughn, 2008), adult kākahi (*E. menziesii*) can filter approximately one litre of water per hour (Ogilvie & Mitchell, 1995; Roper & Hickey, 1995; Walker et al., 2001; Clearwater et al., 2014), therefore dense mussel beds can greatly influence water quality. These bivalves have the potential to deplete phytoplankton and other suspended particles in lotic systems, and influence nutrient budgets in lentic systems (Ogilvie & Mitchell, 1995; Cyr et al., 2017).

Supporting services: mussels recycle nutrients and modify habitat

As a result of their large size and sheer numbers, mussels can significantly influence both the biotic and abiotic conditions around them in some environments (Nobles & Zhang, 2011; Nowak & Kozlowski, 2013). Mussels convert food resources into biomass (soft tissue and shell), biodeposits (faeces and pseudofaeces), and dissolved nutrients (Strayer, 2014). Therefore, large mussel beds can play an important role in nutrient cycling, translocation, and storage, potentially reducing bioavailable nutrients in enriched aquatic environments (Vaughn, 2017).

Freshwater mussel beds have patchy distributions because they are limited to areas with stable sediments and low shear stresses. Consequently, dense patches of mussels can create local hotspots of biodiversity due to the bottom-up provisioning of nutrients, provision of habitat through their shells adding physical structures, and stabilisation of stream substrates, all factors that facilitate colonisation by other species (Vaughn, 2017). Accordingly, dense mussel beds can support more abundant and diverse macroinvertebrate communities than similar habitat without mussels (Beckett et al., 1996; Vaughn & Spooner, 2006; Aldridge et al., 2007; Vaughn, 2017). Additionally, crevices on mussel shells can provide protection from flow and predation for small invertebrates, and algae growing on mussel shells may attract grazing invertebrates (Allen et al., 2012).

Freshwater mussels have the potential to serve as bio-indicators of past and future environmental change, through changes in geochemistry of mussel shells linked to historical changes in physical and chemical conditions over large spatial and temporal scales (Brown et al., 2005). Since they are widespread, long-lived and are sessile suspension feeders, bio-accumulation of contaminants by mussels can be useful for monitoring purposes (Vaughn, 2017).

Provisioning and cultural services

Historically, New Zealand freshwater mussels, also referred to as kākahi or kāeo by some Māori (there are many other names in use), are of great cultural importance and significance (McDowall, 2002). Kākahi are considered a taonga (treasured) species due to their multi-functionality as food resources, tools and medicines (Hiroa, 1921; McDowall, 2002). Kākahi were harvested year round for mahinga kai (a traditional naturally-sourced food eaten either raw or cooked, used particularly

for orphaned children or the sick), or as a tool to cut hair or the umbilical cord of new-born babies, and for preparing flax to construct nets and baskets (Hiroa, 1921). Today, kākahi remain of importance to Māori and are referred to as a cultural keystone species, however, they are less frequently utilized as a food source.

1.2 Life cycle

Unionida mussels have a unique life cycle that includes an obligate parasitic phase where mussel larvae called glochidia attach onto a host fish (Figure 1.3) (Barnhart et al., 2008; Graf & Cummings, 2011). During the spawning season, female mussels are fertilized by gametes released by the male into the water column. Females draw these gametes in through inhalant siphons, and the eggs are fertilized in brood chambers within the female's gill where larvae develop (Barnhart et al., 2008). Once developed, these microscopic glochidia, are expelled into the water column, with release thought to be triggered by environmental cues including flooding and changes in water temperature (Walker et al., 2001; Lima et al., 2012). Once released and attached to host fish, glochidia become encysted in host epithelial cells on the gills or fins (or other body surfaces) where they complete metamorphosis to the juvenile stage (Fritts et al., 2012). Juvenile mussels then detach and settle on the stream or lake bed (Benson et al., 2017) where they are thought to live interstitially in sediments, feeding mostly via their ciliated foot, in contrast to adult mussels which feed by filtering overlying water (Yeager et al., 1994; Walker et al., 2001).

Metamorphosis from the mussel larval stage to free-living juveniles is dependent on obtaining nutrients from a suitable host fish (Barnhart et al., 2008; Haag & Stoeckel, 2015). The encystment phase of the life cycle is critical, as more than 99.99% of glochidia fail to reach a suitable fish host (Young & Williams, 1984; Ćmiel et al., 2018), requiring mussels to release a large number of glochidia as a survival strategy to compensate for failed attachment (Walker et al., 2001). Mussel-fish relationships vary in their degree of host-specificity, ranging from generalists where multiple fish are capable of producing viable juvenile mussels, to specialists that infest a single species of fish (Haag & Stoeckel, 2015). Freshwater mussel species have differing parasitic durations, for example, the freshwater pearl mussel, *M. margaritifera* has a parasitic phase reaching almost one year (Salonen & Taskinen, 2016), and *Margaritifera laevis* takes 40 to 50 days to develop into

juvenile mussels (Terui et al., 2014). In comparison, New Zealand's *E. menziesii* parasitizes fish for up to 22 days (Clearwater et al., 2014; Moore et al., 2019).

This unique life cycle makes mussels vulnerable to decline in streams and lakes because: i) they have a high risk, parasitic larval stage which is dependent on the presence of a suitable fish host; ii) the juveniles are susceptible to predation, drift, siltation and sediment toxicity; iii) adult mussels are exposed to water-borne contaminants as well as sediment-borne contaminants due to their filter-feeding and deposit-feeding, respectively; and iv) the long-lived adults require stable substrates and are therefore prone to disturbance from floods (Nowak & Kozlowski, 2013).

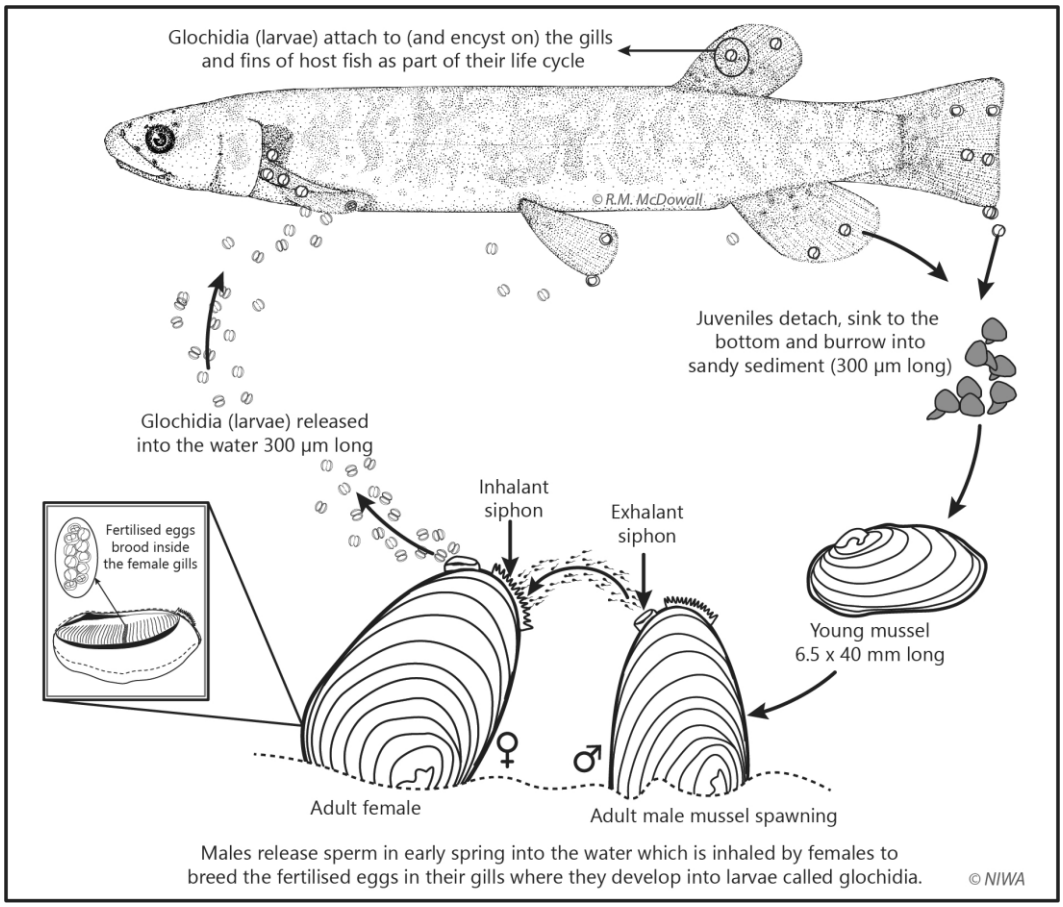


Figure 1.3. Summary of a New Zealand native freshwater mussel, *Echyridella menziesii*, life cycle. Diagram provided by NIWA, Hamilton (2018).

1.3 Causes of mussel decline

Freshwater mussels are considered one of the most endangered animal taxa globally (Lydeard et al., 2004; Lopes-Lima et al., 2014), exhibiting declines due to a variety of anthropogenic factors (Blakeslee et al., 2018) leading to widespread conservation

concern (Dudgeon et al., 2006; Nobles & Zhang, 2011). Since freshwater mussels are considered a keystone species, their decline in diversity and abundance may have multiple negative impacts on whole ecosystems (Nobles & Zhang, 2011).

A review of the causes of mussel decline by Downing et al. (2010) found that, out of the 124 articles considered, there were seventeen different factors posed as causes of extirpation of mussels. These factors were pooled into six broad categories: energy and food availability; exotic/invasive species; global climate change; exploitation by humans or others (e.g., introduced mammalian predators); population phenomena (e.g., recruitment failures, host availability, small population effects, genetic change, and small ranges); and mussel habitat alteration or destruction. Specific anthropogenic drivers of freshwater mussel decline include increased inputs of nutrients and other pollutants, alterations to water regimes, habitat degradation and fragmentation, and introductions of invasive species which all interact to stress mussel populations (Bogan, 2008; Strayer & Dudgeon, 2010; Slapansky et al., 2016). These factors impact not only freshwater mussels but also the fish they rely on for the critical parasitic larval stage. As elsewhere in the world, drivers of freshwater mussel decline in New Zealand are complex but are thought to include pollution, invasive species and habitat fragmentation, although there is limited information on habitat requirements and biology of the three native species (Hare et al., 2019).

1.3.1 **Pollution**

Contaminants in waterways as a result of anthropogenic activities are key drivers of freshwater mussel decline globally (Lydeard et al., 2004; Strayer et al., 2004; Blakeslee et al., 2018). Blakeslee et al. (2018) assessed mussel populations on the Delaware River, in the United States, which has experienced decades of water-quality degradation from both industrial and municipal sources. This decline is primarily a result of historical pollution of one of its major tributaries, the Lehigh River. The tributary was affected by acid-mine drainage as well as domestic and industrial waste, and even today it still has elevated nutrients, metals and sulphate levels. Blakeslee et al. (2018) found significant declines in the dominant species eastern elliptio (*Elliptio complanata*) immediately downstream of the confluence, indicating that water quality was having an impact on adult mussel populations.

Freshwater mussels can also bioaccumulate pollutants where toxicity thresholds will vary between metals, and according to factors such as species, life stage, water quality and sediment composition (Cope et al., 2008; Jing et al., 2019). An Australian study found six freshwater mussel species to be most sensitive to copper out of a range of key metals known to exceed their national freshwater guidelines, with the species being least sensitive to zinc (Markich, 2017).

In the Waikato River, New Zealand, decreases in mussel densities have been linked to increases in agricultural inputs and accumulations of geothermal contaminants (Roper & Hickey, 1994). Nutrient enrichment and/or eutrophication in waterways from surrounding land uses can result in deoxygenation, or even hypoxic conditions when oxygen concentrations are <2 mg L^{-1} (Yusseppone et al., 2018). This oxygen depletion can provoke behavioural responses in aquatic invertebrates (e.g., movement or metabolic reorganisation), particularly in lentic environments where stratification occurs (Yusseppone et al., 2018). Recent modelling studies indicated that 71% of river length located in pastoral farming areas in New Zealand had nitrogen levels that may affect the growth of sensitive aquatic species (Ministry for the Environment and Stats NZ, 2019). Nitrogen is one of several dissolved contaminants which, along with sediment-bound contaminants, are widespread throughout New Zealand's lowland waterways and are implicated as key factors contributing to mussel declines (Clearwater et al., 2014).

1.3.2 **Habitat quality and fragmentation**

The transport of fine particles into streams, caused by activities such as deforestation and agriculture, has a negative impact on benthic filter-feeding organisms (Wood & Armitage, 1997; Österling et al., 2010; Österling, 2015). Increased benthic sedimentation can smother beds of mussels, which may reduce foraging activity and growth of mussels, and clog interstitial spaces in stream substrates that juveniles feed in. Österling et al. (2010) found that juvenile mussel density was negatively correlated with turbidity and sedimentation, demonstrating that declining habitat quality impacts freshwater mussels, particularly at the juvenile stage, hindering recruitment (Österling et al., 2008). Additionally, excessive growth of macrophytes (typically non-native species) can clog water channels and alter habitat conditions at the sediment-water-interface, resulting in

adult mussels dispersing away from macrophyte beds to avoid adverse physicochemical conditions (Moore et al., 2019).

Fish with a diadromous life cycle are highly susceptible to habitat modification and disruption to connectivity throughout freshwater and marine environments (Franklin & Gee, 2019). Given that, without host fish, mussels are unable to complete their life cycles and disperse to suitable habitats, managing waterway connectivity is fundamental to mussel conservation (Franklin & Gee, 2019). The construction of barriers to upstream fish passage (e.g., weirs, culverts, dams) contributes to the decline of native diadromous fish in New Zealand (Leathwick et al., 2008), not only through restricted fish movement, but also through changes to natural flow regimes which can provide environmental cues for fish spawning. Factors limiting fish recruitment and numbers ultimately also impact long-term recruitment of mussel populations which need an annual influx of migrating fish hosts. Juveniles would also obtain a selective advantage by the upstream dispersal of migrating fish into habitat that is likely to have higher water quality and less sedimentation (Watters, 2001; Barnhart et al., 2008).

1.3.3 **Invasive species**

In New Zealand, a shift towards non-native fish communities is concerning for freshwater mussels (Collier et al., 2016a; Collier et al., 2016b). When high in biomass, non-native fish can have negative impacts on water quality, for example common carp (*Cyprinus carpio*) which excrete nutrients into waterways and disturb sediments (Collier & Grainger, 2015). Other non-native fish such as goldfish (*Carassius auratus*) and brown bullhead catfish (*Ameiurus nebulosus*) also feed by bioturbating bottom sediments which may result in the accidental consumption of juvenile mussels, therefore reducing recruitment of mussel populations in invaded waterbodies (Moore et al., 2019).

The introduction of non-native brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) to Lake Taupō, was proposed by McDowall (2002) as a cause for the decline of kōaro (*Galaxias brevipinnis*), a known host of *E. menziesii* glochidia. This proposition raised questions of the compatibility of other non-native freshwater fish as suitable hosts, including trout and catfish (McDowall, 2002). For

a fish to be considered a host of freshwater mussels, both glochidial infection and metamorphosis must occur (Tremblay et al., 2016). However, some fish (typically non-native species) may act as sinks for glochidia, where glochidia attach to a fish but do not metamorphose into juvenile mussels, thereby reducing mussel recruitment. A mathematical model used by Tremblay et al. (2016) suggests that round goby (*Neogobius melanostomus*, an invader of biodiversity hotspots in the Laurentian Great Lakes region), served more as a sink for glochidia than a host for mussels. Similarly, research by Moore and Clearwater (in press) supports the general assumption that non-indigenous fish (at least catfish, rudd (*Scardinius erythrophthalmus*) and goldfish) are unsuitable hosts of native freshwater mussels after comparing their development of juvenile mussels with native common bully (*Gobiomorphus cotidianus*) hosts.

In addition to fish, other groups of invasive species can directly or indirectly affect native mussel populations. As noted above, invasive macrophytes potentially act as habitat modifiers in lakes and reservoirs, affecting the suitability of bed sediments and the physicochemistry of the overlying water (Moore et al., 2019). Additionally, non-native mammalian predators such as rats (*Rattus* spp.) pose a direct threat to freshwater mussel populations through predation (Beveridge & Daniel, 1965; Theobald & Coad, 2002; O'Donnell et al., 2017; Moore et al., 2019). These opportunistic feeders dive to collect mussels and devour them on shore or in rat dens (Moore et al., 2019). *Rattus* spp. predation marks have been found on *E. menziesii* shells (Moore et al., 2019), and further, the diet of brown of Norway rats (*Rattus norvegicus*) on Mokoia Island, Lake Rotorua, has been found to include freshwater mussels as well as various seeds and other invertebrates (Beveridge & Daniel, 1965).

1.4 Why is mussel recruitment being disrupted?

Sampling of remaining *Echyridella* populations often shows size distributions that are heavily skewed towards large adults (James, 1985; Rainforth, 2008; Catlin et al., 2018). This size distribution suggests either: i) young mussels are simply too small to be identified during conventional sampling; ii) juvenile mussels occupy different microhabitats to those surveyed; iii) larvae and juveniles are more sensitive than adults to environmental contaminants; and/or iv) life cycle disruption

at the host-glochidia stage means juvenile production is low. If mussel recruitment is declining due to iii) and iv), then extinction debt is likely whereby population loss is delayed until older individuals die out (Kuussaari et al., 2009).

Phillips et al. (2007) reported that juvenile *E. menziesii* buried in the substrate were seldom found until they are at least 5 mm in length. To establish whether the abundance of juvenile mussels is being underestimated, sampling should target the habitat of juvenile mussels (e.g., sediment sampling in flow refugia) as they probably have differing requirements and feeding ecology compared to larger mussels, as noted by James (1985) and Catlin et al. (2018). Nevertheless, several studies report that the majority of remaining mussel populations lack juvenile recruitment despite intensive sampling, indicating that lack of juvenile recruitment is a real occurrence leading to ageing geriatric population structures (Vaughn & Taylor, 2000; Stöckl et al., 2014).

Larval and juvenile mussels (and host fish) are less tolerant of contaminants such as ammonia compared to adults, and this may be a factor contributing to the apparent lack of juveniles (Walker et al., 2001; Clearwater et al., 2014). Clearwater et al. (2014) conducted sensitivity tests where *E. menziesii* glochidia were exposed to dissolved copper, zinc and ammonia-nitrogen, and found glochidia were most sensitive to copper exposure. This research concluded that, under current Australian and New Zealand Guidelines for Fresh and Marine Water Quality (ANZECC), water quality guidelines for ammonia-nitrogen or copper, native juvenile mussels are not protected (Clearwater et al., 2014). Following the detachment of juvenile *M. margaritifera* from their host, mussels bury themselves into the sediment for a period of up to five years, a critical stage of the life cycle (Geist, 2010). During this phase they depend on a stable substrate of suitable composition and with adequate exchange between free-flowing water and interstitial water (Geist & Auerswald, 2007), suggesting fine sediment deposition and colmation (i.e., clogging of interstitial spaces), along with increased bed movement due to altered flow regimes, could also be factors affecting juvenile survival. Thus, sensitivity of larval mussels to contaminants and vulnerability of juvenile stages to sedimentation may contribute to reduced recruitment.

The other potential cause of geriatric population structures in mussel populations, life cycle disruption at the host-glochidia stage, is the focus of this thesis. Many native fish are diadromous and migrate between freshwater and saltwater at various stages of their life cycle (Smith, 2014), and declines in their abundance may have implications for mussel recruitment. *Echyridella menziesii* is thought to be a host generalist (Clearwater et al., 2011) parasitizing a range of native fish (Brown et al., 2017), compared to many overseas species which vary in host specificity from generalists to specialists (Haag & Stoeckel, 2015).

Several New Zealand native fish species, including common bully, banded kōkopu (*Galaxias fasciatus*), shortfin eel elvers (*Anguilla australis*), and Canterbury galaxias (*Galaxias vulgaris*), have been identified as hosts after artificial infestation in a laboratory (Brown et al., 2017). Other known hosts based on previous observations include kōaro, longfin eel (*Anguilla dieffenbachii*) and rainbow trout (*Oncorhynchus mykiss*) (Hine, 1978; Clearwater et al., 2014; Brown et al., 2017). In addition, recent laboratory trials with upland bully (*Gobiomorphus breviceps*) have shown that they can act as hosts to glochidia, though īnanga (*Galaxias maculatus*) seem to be poor hosts (Bob Brown, Manaaki Whenua Landcare Research, pers. comm. 2016; Catlin et al., 2018).

The presence of host fish, behavioural patterns, migratory movements, local distributions and abundance will all determine the actual exposure of fish to *E. menziesii* glochidia. Therefore, it is critical that there are fish present at key times to enable mussel life cycle completion (Atkins, 1979). Otherwise, without the host fish necessary for the continued reproduction of a given species, all other conservation efforts will be in vain as recruitment will be disrupted (Bogan, 1993).

1.5 Objectives and outline of thesis

There is limited knowledge of how the immune status of fish hosts contributes to the regulation of mussel abundance (Haag & Stoeckel, 2015). Many studies have conducted artificial infestations for various combinations of mussel and fish species, demonstrating that specificity of the glochidia-host association varies with the species of mussel (Kelly & Watters, 2010). In New Zealand, native common bullies have been confirmed as suitable hosts after artificial infestation, however, the

effects of multiple infestations have not been determined. Once glochidia attach to fish, survival is not guaranteed as glochidia may have difficulty encysting properly or transforming upon the host, which infers that an immune response by the fish may be occurring (Strayer, 2008; Slapansky et al., 2016). Therefore, the first aim of this thesis was to elucidate whether common bully remain suitable hosts (can produce viable juveniles) after multiple infestations of glochidia in a controlled laboratory experiment. An immune response to multiple infestations might suggest that regular supply of naïve fish from annual migrations was important for sustaining mussel population recruitment, thereby underscoring the critical role of upstream passage for diadromous species.

Ferreira-Rodríguez et al. (2019) recently summarized the top 20 research priorities for assessing freshwater mussel conservation status at a global scale. One key research priority is identifying primary mussel-host relationships and host histological compatibility (metamorphosis success). Despite the large amount of literature on host-parasite relationships, few studies have examined these interactions in natural situations (Haag & Stoeckel, 2015), and in New Zealand little is known about kākahi glochidia and juvenile stages generally (Phillips et al., 2007). Therefore, the second aim of this thesis was to quantify mussel-host relationships in the field through regular sampling of three Waikato streams during the *E. menziesii* glochidial release period.

Accordingly, the objectives for this thesis were to:

1. Determine whether common bully develop an immunity to repeated glochidia attachment in a laboratory setting;
2. Quantify glochidia attachment in the field on different structures (e.g., fins, mouth, gills) for a range of fish species;
3. Determine whether there is a temporal shift in host fish use due to migratory fish movement patterns and seasonal habitat use;
4. Determine whether fish habitat use in a stream influences the glochidia infestation rate (i.e., are benthic fish like bullies likely to have a higher infestation rates compared to pelagic fish which may encounter mussels less frequently?);

5. Investigate whether field infestation rates are higher on young migratory fish exposed to glochidia for the first time compared to older fish that may develop resistance later on in the season.

It is hoped that this fundamental research will help to improve the understanding of biological processes influencing kākahi populations and factors affecting juvenile survival, which will in turn allow more focussed conservation action for the species. Few studies document the presence of glochidia on fish in the field, and even then, these studies are one-off collections or opportunistic events (Kelly & Watters, 2010). My work involved developing a repeatable method to quantify glochidia attachment in the field and applying this systematically in three streams at fortnightly intervals over the mussel spawning period (summer). This work added a temporal element to glochidia attachment data, something which, to my knowledge, has not been studied before in New Zealand. Additionally, quantifying whether host fish develop an immunity to *E. menziesii* glochidia attachment will guide future conservation efforts, particularly in terms of mussel propagation methods.

This research was a part of the Cultural Keystone Species (CKS2020) project funded by the Ministry of Business, Innovation and Employment (MBIE), and led by the National Institute of Water and Atmospheric Research (NIWA). Māori communities in New Zealand have acknowledged declines in populations of mahinga kai (naturally-sourced food) species including tuna (eel), kōura (freshwater crayfish) and kākahi (freshwater mussel). The CKS2020 project aims to co-develop research methods, tools and products with whānau, hapū and iwi that inform new and innovative management approaches for the protection, restoration and economic development of keystone species.

This thesis comprises five chapters, with the two main results chapters set out in the general style of manuscripts to enable ease of editing for later submission to scientific journals. As a result, there is some repetition throughout the thesis. This chapter summarized relevant literature on freshwater mussels globally and within New Zealand, and set out the overall objectives of the thesis. Chapter two provides detailed descriptions of all study areas including catchment characteristics and site descriptions. Chapter three examines the response of common bully to multiple

exposures of *E. menziesii* glochidia in the laboratory. Chapter four investigates the temporal patterns of glochidia infestation on host fish in three Waikato streams. Chapter five is an overall discussion chapter which summarises the main findings from chapters three and four and includes some management ideas that may help improve the conservation status of freshwater mussels in New Zealand over the long term.

Chapter Two

Study Area

2.1 Physiography

The study was conducted in the central Waikato region of the North Island, New Zealand. Topography is generally flat within the Hamilton and Hauraki basins, while upland areas and ranges occur along both western and eastern coasts (Lowe, 2010). The study sites were within the Western and Central Hill Country and Waikato Lowland areas defined by Kettle (2013), where topography is rolling to hilly at elevations ranging from 100-200 m a.s.l. The volcanic cones of Pirongia, Karioi and Kakepuku rise above the hills surrounding the Hamilton basin, with extensive coastal dunes north of Marokopa, particularly around the entrances to Kāwhia, Aotea and Whāingaroa (Raglan) harbours (Schofield, 1967).

The Hamilton lowland area is bordered by dissected hills and ranges where Mesozoic rocks out-crop at the surface in ranges along its western and eastern margins over Tertiary sedimentary rocks (Schofield, 1967). Surface rocks include areas of limestone that form karst landscapes near Waitomo and further north towards Port-Waikato. Soils in the Waikato are a mixture of volcanic loams/loamy clays, gley and organic soils and hill country brown clays (Lowe, 2010). Climate in the region is warm-temperate and humid, with mean annual rainfall ranging from 960-1840 mm, although higher rainfall occurs on the Central Plateau (2759 mm) (Chappell, 2013). Mean daily maximum summer air temperatures range from 20-25^{o}C, while mean daily minimum air temperatures in the winter range from 0-8^{o}C (Chappell, 2013).

The majority of the region's lowland and hill-country land use is upland conservation estate in native forest, plantation forestry made up of primarily *Pinus radiata,* or pastoral agriculture supporting large dairy farms with some cattle and sheep farming (Chappell, 2013). Western areas are characterized by short and often steep coastal streams draining partially forested catchments, whereas the lowlands are characterized by low-gradient streams and rivers draining predominantly agricultural landscapes.

The Piako, Waikato, Waihou, Waipā and Waitoa rivers are the main water bodies transporting flows to coastal areas. Average specific discharges in the Waikato region vary, from 13 L s^{-1} km^{-2} in smaller tributaries draining the central hill country north of Hamilton and the spring fed streams in the Hauraki and Hamilton lowlands, to 60 L s^{-1} km^{-2} in the Coromandel, Taupō and western ranges (Waikato Regional Council, 2019). The largest river is the Waikato River, running 442 km north from its headwaters at Lake Taupō to its mouth at Port Waikato (Collier et al., 2010). The river drains 13% of the North Island, with an average discharge of 422 m^3 s^{-1} to the sea (Brown, 2010). The river is fed by over 17,000 km of tributaries draining a total catchment area of 11,013 km^2 (Collier et al., 2010). Eight hydro-electric dams occur in the upper reaches of the river (Taupō Gates to Karāpiro Dam), creating a series of hydro-lakes to generate electricity (Brown, 2010). Water quality data from measurements along the Waikato River from 2013-2017 were summarized by the Waikato Regional Council (2019). Ecological health (a measure of seven key water quality variables such as dissolved oxygen, temperature and turbidity) decreases in a downstream direction from 96% at the Taupō Gates to 59% at Tūākau Bridge. Phosphorus, nitrogen and turbidity increase downstream from 0.003 g P m^{-3}, 0.10 g N m^{-3}, and 0.4 NTU at the Taupō Gates, to 0.05 g P m^{-3}, 1.02 g N m^{-3}, and 10.5 NTU, at the Tūākau Bridge, respectively.

Vant (2018) reports trends in river water quality in the Waikato region generally from 1993 to 2017. Overall, there was an important deterioration in total nitrogen at nine of the ten sites on the Waikato River, and in 40% of other monitored rivers and streams in the region, most likely due to runoff and leaching of nitrogen from pastoral farming areas (Vant, 2018).

2.2 Mussel and fish communities

Two of the three species of freshwater mussels occurring in New Zealand are found in the Waikato region, *Echyridella menziesii* and *E. aucklandica* (Figure 2.1). Freshwater mussels are reportedly widespread in the upper Waikato River, compared to the lower reaches where the species is now rarely encountered in the river mainstem below Karāpiro Dam (Collier & Hogg, 2010). Reports of *Echyridella* densities in Lake Whakamaru of up to 30 m^{-2} have previously been recorded in stable areas of sandy mud between macrophyte beds (Coffey, 1997). In

Lake Arapuni, further downstream, mussel densities of 3-5 m^{-2} have been reported in muddy surface sediments (Coffey et al., 1998). The most downstream impoundment, Lake Karāpiro, has been reported to support large densities of mussels (Roper & Hickey, 1994; Walker et al., 2001). More broadly around the Waikato region, freshwater mussels in wadeable streams, including some of sites used in this study (see below), are monitored by Waikato Regional Council, one of only three councils in New Zealand monitoring mussel populations.

Figure 2.1. The two freshwater mussel species located in the Waikato region: *Echyridella aucklandica* (left) and *E. menziesii* (right).

Within the Waikato region, there are 22 native fish species and 14 introduced fish species (Table 2.1). At least 18 of these native fish are diadromous species that move between freshwater and marine environments during different stages of their life cycle (Speirs, 2001). Common bully and common smelt are the only two species found in every river system/catchment in the Waikato (Speirs, 2001). Every other fish species varies in distribution around the catchments of the region. Longfin and shortfin eels are found widely throughout rivers and streams, and torrentfish is found in riffle sections of large open gravel and boulder streams (Speirs, 2001). The largest threats to Waikato's freshwater fish populations are: i) impediments to fish passage; ii) reductions in water quality; iii) flow regime modifications; and iv) habitat loss (Speirs, 2001).

Because of the diadromous life history of many Waikato freshwater fish species, the Waikato Regional Council, in association with other regional councils, has incorporated freshwater fish into their state of the environment monitoring programme (David et al., 2016). Fish species densities and diversity can be used to understand the degree of connectivity of the sampled reach within the river system, which can be an important component of riverine health.

Table 2.1. Freshwater fish of the Waikato region. Adapted from Speirs (2001).

Common name	Latin name
Native fish	
Yellow-eye mullet	*Aldrichetta forsteri*
Shortfin eel	*Anguilla australis*
Longfin eel	*Anguilla dieffenbachii*
Australian eel	*Anguilla reinhardtii*
Torrentfish	*Cheimarrichthys fosteri*
Giant kōkopu	*Galaxias argenteus*
Kōaro	*Galaxias brevipinnis*
Dwarf galaxias	*Galaxias divergens*
Banded kōkopu	*Galaxias fasciatus*
Īnanga	*Galaxias maculatus*
Shortjaw kōkopu	*Galaxias postvectis*
Lamprey	*Geotria australis*
Black mudfish	*Neochanna diversus*
Crans bully	*Gobiomorphus basalis*
Upland bully	*Gobiomorphus breviceps*
Common bully	*Gobiomorphus cotidianus*
Giant bully	*Gobiomorphus gobioides*
Bluegill bully	*Gobiomorphus hubbsi*
Redfin bully	*Gobiomorphus huttoni*
Grey mullet	*Mugil cephalus*
Common smelt	*Retropinna retropinna*
Black flounder	*Rhombosolea retiaria*
Introduced fish	
Catfish	*Ameiurus nebulosus*
Goldfish	*Carassius auratus*
Grass carp	*Ctenopharyngodon idella*
Koi carp	*Cyprinus carpio*
Mosquito fish	*Gambusia affinis*
Rainbow trout	*Oncorhynchus mykiss*
Brown trout	*Salmo trutta*
Perch	*Perca fluviatilis*
Sailfin molly	*Poecilia latipinna*
Guppy	*Poecilia reticulata*
Brook Char	*Salvelinus fontinalis*
Rudd	*Scardinius erythrophthalmus*
Tench	*Tinca tinca*
Swordtail	*Xiphophorus helleri*

2.3 Field study sites

Three sites known to have large populations of *E. menziesii* were sampled between October 2018 and February 2019 to examine glochidia-fish host associations (Chapter 4). These sites were on Pakoka River, Ohautira Stream and Mangapapa Stream (Figure 2.2; see Figure 2.4 for site photos).

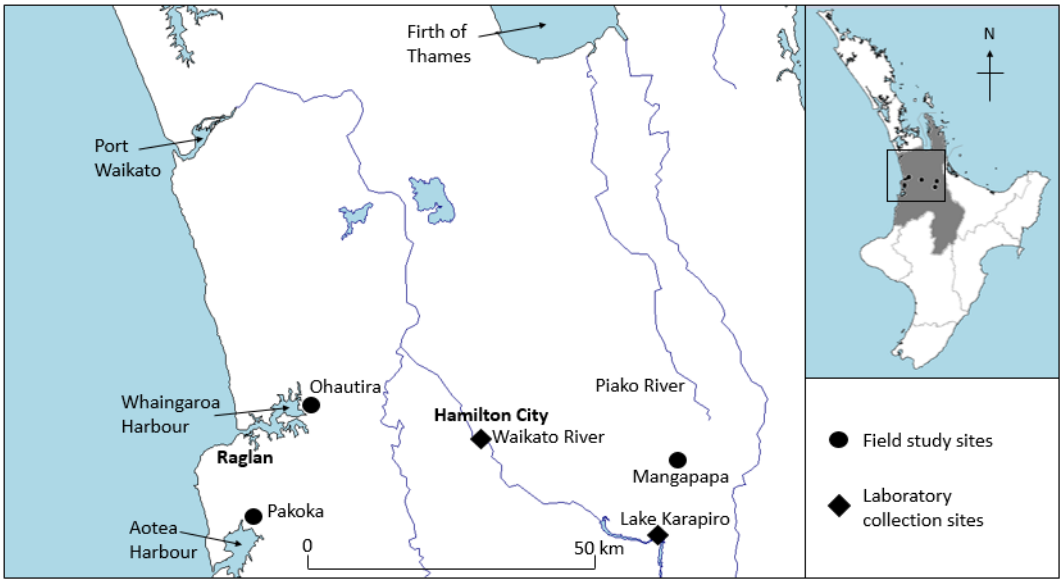


Figure 2.2. Locations of the field study sites and laboratory collection sites visited for the purposes of this research in the Waikato region, North Island (inset).

Figure 2.3. Site access points (◆) for Pakoka, Ohautira and Mangapapa sampling sites (left to right). Ohautira site is accessed off Ohautira Road.

2.3.1 Site descriptions

Pakoka River

Pakoka River (-37.92889, 174.869619, Figure 2.4A) flows south-west from the Wharauroa Plateau to its mouth in the Aotea Harbour. The river has a Strahler order of four with an upstream catchment area of 25.2 km^2, and downstream distance of 2.2 km to the sea from the point of sampling (NZFFD version 2.06.434, accessed

28 March 2019). At the time of the study, this site's surrounding land use was predominantly sheep and beef farming, with no fencing along the river. Partial shade was provided by a combination of native (notably, whekī or *Dicksonia squarrosa*) and exotic vegetation. Well-drained allophanic soils (Kauroa soil series) surround the river on moderately steep land posing a high erosion risk (Waikato Regional Council, 2018). Previous Waikato Regional Council sampling of Pakoka River in 2015 indicated *E. menziesii* densities of 1.58 m^{-2} and *E. aucklandica* densities of 0.08 m^{-2} (Table 2.2). The Waikato Regional Council does not have any fish data from the Pakoka River (J. Smith, pers. comm. 2018, Waikato Regional Council), nor was there any previous sampling recorded on the New Zealand Freshwater Fish Database (NZFFD).

Table 2.2. Previous data on mussel populations within a 50-m reach at Pakoka, Ohautira and Mangapapa sampling sites collected by the Waikato Regional Council from 2015 – 2017. Data supplied by A. Catlin, Waikato Regional Council.

Sampling details		**Pakoka**	**Ohautira**	**Mangapapa**
Survey date		16/04/2015	27/04/2017	21/04/2017
	Area sampled (m^2)	320.5	339.5	227.1
E. menziesii	Total no.	506	98	367
	Density (no. m^{-2})	1.58	0.29	1.62
E. aucklandica	Total no.	25	263	0[1]
	Density (no. m^{-2})	0.08	0.77	0

[1], one *E. aucklandica* found during the present study

Ohautira Stream

Ohautira Stream (-37.761688, 174.979656, Figure 2.4B) begins west of Ngāruawāhia and flows into the Whāingaroa Harbour. Livestock farming is the dominant land use within the catchment, with indigenous forest the main vegetation further upstream. The channel is incised with bank erosion evident, as shown in Figure 2.4B, and elevated sediment levels were observed during high flow events. At the point of sampling, the upstream catchment area was 44.9 km^2 and downstream distance to the sea was 2.1 km (NZFFD version 2.06.434, accessed 28 March 2019). Ohautira Stream is significantly shaded by riparian cover, and is fenced off from livestock. Imperfectly-drained recent soils surround the stream

(Mangapiko soil series), with wetness the main limitation for arable farming and development (Waikato Regional Council, 2018).

Waikato Regional Council's most recent mussel survey (April 2017) found *E. menziesii* densities of 0.3 m^{-2} and *E. aucklandica* densities of 0.8 m^{-2} within a 50-m reach (Table 2.2). Eight native fish species have been previously recorded in Maunganui Stream which flows into the Ohautira Stream (Table 2.3). The dominant species was redfin bully, with on average 123 caught over eight sampling dates (Waikato Regional Council, unpublished data). The next most common species present was īnanga averaging 14 per visit followed by longfin eels at 13.

Table 2.3. Average number of each fish species caught in an upstream tributary of Ohautira Stream (Maunganui Stream) over eight different sampling dates (2009 – 2018) and over four sampling dates at Mangapapa Stream (2014 – 2017). No previous fish data existed for Pakoka River.

	Ohautira trib. (n = 8)	**Mangapapa (n = 4)**
Banded kōkopu	5.3 ± 1.4	8.0
Brown trout	1.5 ± 0.5	-
Common bully	-	68.5 ± 10.5
Crans bully	-	146.0 ± 52.0
Common smelt	1.0	-
Giant kōkopu	2.0	-
Īnanga	14.4 ± 5.3	2.0
Kōura	-	20.3 ± 7.2
Longfin eel	13.3 ± 1.6	7.0 ± 2.0
Redfin bully	122.8 ± 23.8	-
Shortfin eel	2.1 ± 0.5	83.5 ± 44.7

Mangapapa Stream

Mangapapa Stream (-37.828458, 175.690303, Figure 2.4C) has a Strahler order of three, an upstream catchment area of 25.2 km^2 and a downstream distance to the sea of 110.6 km from the sampling site (NZFFD version 2.06.434, accessed 28 March 2019). The stream runs through a rural catchment with dairy farming the most common land use (Land and Water Aotearoa, 2017). The Mangapapa Stream drains into the Waitoa River, before converging with the Piako River, ultimately ending up in the Firth of Thames. The stream has minimal overhead cover, with

pockets of shade provided by flaxes. The dominant riparian vegetation at the sampling site consisted of pastoral grasses. Well-draining allophanic soil (Peria soil series) surrounds the stream where erosion is the main limitation for arable land (Waikato Regional Council, 2018).

A previous mussel survey in 2015 by the Waikato Regional Council found only *E. menziesii* at a density of 1.62 m^{-2} (Table 2.2). Additionally, previous Waikato Regional Council fish surveys found longfin and shortfin eels, īnanga, banded kōkopu, common bully/Crans bully and kōura (Table 2.3).

Figure 2.4. Site photos, facing upstream (left column) and downstream (right column) at all three study sites. Pakoka River (A), Ohautira Stream (B) and Mangapapa Stream (C).

2.3.2 **Physicochemical and habitat characterisation**

Spot samples of water temperatures, dissolved oxygen and conductivity taken fortnightly (usually before midday) over October 2018 to February 2019 with a YSI Pro2030 meter (Yellow Springs Instruments, Ohio, USA) showed that Pakoka River and Ohautira Stream had similar physicochemical conditions (Table 2.4). Mangapapa Stream differed, however, having the highest average temperature (17.6 ^{o}C), and the lowest average dissolved oxygen levels (9.5 mg L^{-1} and 99.9%) and specific conductivity (95 μs_{25} cm^{-1}) of all three sites.

Water temperatures increased from October to February, ranging from 11.7 – 18.4^{o}C, 12.7 – 18.2 ^{o}C and 14.8 – 21.6 ^{o}C at Pakoka, Ohautira and Mangapapa streams, respectively (Table 2.4). All measured dissolved oxygen concentrations were ≥8.0 mg L^{-1} and >89% saturation. Dissolved oxygen generally increased or plateaued from October to December/January, but values were lowest at each site during February. Specific conductivities increased over the summer period, peaking in February. Values ranged from 143.8 – 166.3, 145.5 – 153.8 and 88.6 – 105.2 μs_{25} cm^{-1} at Pakoka, Ohautira and Mangapapa streams, respectively.

Stream width, thalweg depth and overhead shade were measured once at 10 equally-spaced transects over a 50-m length of each stream in October. Pakoka River was the widest and deepest stream sampled (5.58 m and 0.33 m, respectively), whereas Mangapapa Stream was the narrowest at 4.59 m and shallowest at 0.17 m depth. Stream shade was measured using a spherical convex densiometer (Model A Wildco, Yulee, FL, USA) in the middle of each transect facing upstream, downstream, true left and true right. The total number of squares (out of a total of 96) filled by overhead canopy and banks on a convex mirror was recorded for each orientation, multiplied by 1.04, and then averaged to give % shade at every sub-reach (Harding et al., 2009). Canopy shade was lowest at Pakoka (7%) followed by Mangapapa (15%), compared to 68% shade at Ohautira (Table 2.5). The latter stream also had the largest amount of wood cover (4%), likely due to having the most intact riparian vegetation. No streams had significant patches of macrophytes at the study sites.

Table 2.4. Monthly physicochemical measurements (means presented where two measurements were taken in a month) and overall site means (n = 7-8) over October 2018 – February 2019 for Pakoka River, Ohautira Stream and Mangapapa Stream. Highest parameters for each stream are in bold.

	Dissolved oxygen (mg L^{-1})	**Dissolved oxygen (%)**	**Water temperature (ºC)**	**Specific conductivity (μs_{25} cm^{-1})**
Pakoka (n = 7)				
October (n = 1)	**11.5**	104.8	11.7	143.8
November (n = 2)	11.1	105.6	13.4	129.8
December (n = 1)	10.8	113.2	**18.4**	130.7
January (n = 2)	11.2	**117.2**	17.8	144.3
February (n = 1)	9.3	99.0	18.1	**166.3**
Mean ± SE	10.9 ± 0.3	108.9 ± 2.7	15.8 ± 1.2	141.3 ± 5.6
Ohautira (n = 7)				
October (n = 1)	10.6	98.6	12.7	145.5
November (n = 2)	10.5	99.7	13.2	142.3
December (n = 1)	**10.9**	**110.7**	16.4	129.0
January (n = 2)	10.1	105.5	17.8	142.5
February (n = 1)	8.8	93.2	**18.2**	**153.8**
Mean ± SE	10.2 ± 0.3	101.8 ± 2.5	15.6 ± 1.0	142.5 ± 3.4
Mangapapa (n = 8)				
October (n = 2)	9.8	96.7	14.7	88.6
November (n = 2)	**10.2**	98.7	13.9	97.1
December (n = 1)	9.7	**108.1**	20.1	92.0
January (n = 2)	9.3	105.6	**21.2**	95.7
February (n = 1)	8.0	89.4	21.0	**105.2**
Mean ± SE	9.5 ± 0.3	99.9 ± 2.6	17.6 ± 1.3	95.0 ± 2.2

Surficial substrate size was assessed across each transect by allocating ten discrete particles to one of the following size classes: <0.004 mm, clay; 0.004-0.06 mm, silt; 0.06-2 mm, sand; 2-8 mm, small gravel; 8-16 mm, medium gravel; 16-64 mm, large gravel; 64-128 mm, small cobble; 128-256 mm, large cobble; and >256 mm, boulder. Boulders and large cobbles accounted for 53% of Pakoka River substrates, while small cobbles and large gravels accounted for 66% of Ohautira Stream substrates. The bed of Mangapapa Stream was mainly (79%) bedrock (Table 2.5).

Table 2.5. Summary of stream characteristics and benthic substrate composition measured at Pakoka, Ohautira and Mangapapa sampling sites in October 2018; values are averages (± SE for width and depth). Substrates ≥20% are in bold.

	Pakoka	**Ohautira**	**Mangapapa**
Average width (m)	5.58 ± 0.20	5.12 ± 0.18	4.59 ± 0.20
Average depth (m)	0.33 ± 0.01	0.28 ± 0.04	0.17 ± 0.02
Stream shade (%)	6.6	68.7	14.9
Large wood cover (%)	0.1	3.5	0
Substrate			
Clay (%)	0	0	0
Silt (%)	4	1	1
Sand (%)	7	3	0
Small gravel (%)	6	5	9
Medium gravel (%)	7	13	2
Large gravel (%)	7	**32**	3
Small cobble (%)	16	**34**	1
Large cobble (%)	**33**	11	5
Boulder (%)	**20**	1	0
Bedrock (%)	0	0	**79**

2.4 Laboratory study collection sites

Gravid *E. menziesii* and common bully (*Gobiomorphus cotidianus*) were collected from two sites on the Waikato River for the laboratory experiments described in Chapter 3. Mussels were collected from Lake Karāpiro and common bully were collected from the Waikato River (Figure 2.2).

Lake Karāpiro is a riverine hydro-lake on the Waikato River south-east of Hamilton city (-37.947789, 175.651129). It is a multipurpose lake used for a variety of recreational, cultural and economic activities, including as a world class venue for rowing regattas (Matheson et al., 2010). However, the growth of a nuisance weed, hornwort (*Ceratophyllum demersum*), can become problematic for rowing and other activities as it can grow to a depth 8-10 m or more anchoring itself to the lake bottom. While this moderately-deep lake has reportedly high nutrient loads, dissolved oxygen levels exceed the ecological habitat standard of 80% saturation at all sites that supported nuisance weed beds prior to weed spraying (Matheson et al.,

2010), possibly because it is a riverine impoundment managed for the production of electricity (i.e., there is always significant water flow through most of the lake). As mentioned earlier, Lake Karāpiro supports a large population of *E. menziesii* which were collected from Bob's Landing (Figure 2.5A) for use in the laboratory experiment.

The collection site for common bully was on the lower Waikato River accessed via the Hamilton Gardens (-37.806242, 175.307638) (Figure 2.5B). Living mussels have not recently been collected in the lower Waikato River, including adjacent to the Hamilton Gardens site, despite extensive air-lift sampling in the area (Collier & Hogg, 2010; Collier et al., 2014). Fish caught at this site were therefore considered naïve in terms of previous exposure to *E. menziesii* glochidia.

One of the water quality monitoring sites along the Waikato River is at Narrows boat ramp, which is just upstream of Hamilton Gardens and downstream of Lake Karāpiro. Water quality data collected there from 2012-2016 (Table 2.6) showed five year median values of: 1.8 NTU for turbidity; total phosphorus 0.029 g P m^{-3}; total nitrogen 0.43 g N m^{-3}; and *Escherichia coli* forming 38 colony units 100 mL^{-1} (Tulagi, 2017). This is similar to Lake Karāpiro boat ramp where *E. coli* colony forming units (CFU) over five seasons from 2006 to 2017 were 30 CFU 100 mL^{-1} (Tulagi, 2017).

Figure 2.5. Bob's landing where large numbers of mussels are found (A) and Hamilton gardens (B) where *Gobiomorphus cotidianus* were collected but mussels are considered absent.

Table 2.6. Routine sampling and bathing water quality monitoring summary data collected from Narrows boat ramp on the Waikato River upstream of Hamilton in 2016, and 5-year median values from 2012 – 2016 (Tulagi, 2017).

		Mean	**Median**	**Min**	**Max**	**5-year median**
Ecological health	Dissolved oxygen ($g\ m^{-3}$)	9.7	9.6	8.1	11.7	9.9
	Dissolved oxygen (%)	98.2	97.3	91.0	114.7	97.5
	pH	7.5	7.4	7.2	7.7	7.4
	NH_4-N ($g\ m^{-3}$)	0.015	0.013	0.005	0.028	0.015
	Temperature (^oC)	16.3	16.4	11.2	22.9	16.0
	Total Kjeldahl nitrogen ($g\ m^{-3}$)	0.18	0.17	0.11	0.27	0.16
	Total nitrogen ($g\ m^{-3}$)	0.49	0.52	0.25	0.83	0.43
	Total phosphorus ($g\ m^{-3}$)	0.029	0.029	0.022	0.038	0.029
	Turbidity (NTU)	2.2	1.6	1.2	4.2	1.8
	Specific conductivity ($mS\ m^{-1}$)	15.6	16.0	14.2	16.9	15.9
Recreation	Black disk (m)	2.10	2.20	1.20	2.50	1.80
	Escherichia coli (no. $100\ mL^{-1}$)	67	50	19	200	38
Water supply	Chlorophyll *a* ($g\ m^{-3}$)	0.008	0.006	0.002	0.026	0.005
Drinking water	Arsenic ($g\ m^{-3}$)	0.02	0.02	0.02	0.03	0.02
	Boron ($g\ m^{-3}$)	0.22	0.22	0.19	0.28	0.25

Chapter Three

Investigating the response of common bully (*Gobiomorphus cotidianus*) to multiple exposures of *Echyridella menziesii* glochidia

3.1 Introduction

The New Zealand freshwater mussel *Echyridella menziesii* has a life cycle unique to Unionida, involving an obligate ectoparasitic larval stage where glochidia must attach onto a fish host to transform into juveniles (Neves & Widlak, 1988; Walker et al., 2001). After embryonic development in the brood chambers of female gills, gravid mussels release glochidia into the water column, and these have 2–4 days to attach onto and parasitize a fish host (Clearwater et al., 2014). While attached, glochidia become encysted by host epithelial cells (Nezlin et al., 1994), and metamorphose into juveniles, relying on the fish host for nutrients and dispersal (Walker et al., 2001). After up to 22 days (Clearwater et al., 2014; Moore & Clearwater, in press), juveniles drop off their hosts (excyst), depending on environmental factors such as water temperature (Hastie & Young, 2003), and are thought to begin deposit-feeding in stream substrates until emerging to the surface as adults (Walker et al., 2001; Patterson et al., 2018)

Globally, unionids vary from generalists to specialists in their host specificity (Haag & Stoeckel, 2015). *Echyridella menziesii* is thought to be a host generalist as mussels are known to parasitize a range of native fish (Clearwater et al., 2011). Brown et al. (2017) found that *E. menziesii* can successfully attach and transform into viable juveniles (meaning the presence of an active, pedal-feeding foot) on the head, mouth and fins of common bully (*Gobiomorphus cotidianus*), banded kōkopu (*Galaxias fasciatus*), shortfin eel elvers *(Anguilla australis*), longfin eel elvers (*A. dieffenbachii*) and Canterbury galaxias (*Galaxias vulgaris*) after artificial infestation. In addition, confirmed hosts from previous field studies include kōaro (*Galaxias brevipinnis*) (Brown et al., 2017), and rainbow trout (*Oncorhynchus mykiss*) (Clearwater et al., 2014). Percival (1931) also recorded giant bully

(*Gobiomorphus gobioides*) as having glochidia attached, but so far there has been no evidence of transformation on that species.

Despite the number of confirmed hosts, there is still concern over the 'At Risk, Declining' conservation status of *E. menziesii* (Grainger et al., 2018). Known stressors and threats to kākahi include ecosystem changes, pollution, and invasive species (Hare et al., 2019). In addition, fragmentation or reduced connectivity of mussel populations and their hosts, clashes with economic or development priorities that adversely affect habitats, and limited knowledge of the biology and habitat requirements of mussels are also factors thought to be contributing to freshwater mussel decline in New Zealand (Hare et al., 2019).

One poorly understood component of the mussel-host relationship is whether hosts can develop resistance to glochidia attachment after repeated exposures, leading to unsuccessful transformation of juveniles. Once glochidia are attached, survival is not guaranteed as they may have difficulty encysting properly or transforming upon the host (Strayer, 2008; Slapansky et al., 2016). Evidence from North American mussel species suggests that some host fishes may develop acquired immunity to parasitic glochidia attachment after multiple exposures (Watters & O'Dee, 1996; Rogers & Dimock, 2003; Dodd et al., 2005; Ćmiel et al., 2018). In addition, some studies have found that fish age or size can influence parasitic infection (Klunzinger et al., 2010; Slapansky et al., 2016; Schneider et al., 2017).

This theory of acquired immunity to glochidial infection has not yet been explored for any of New Zealand's fish species, but it has potential to be a significant factor limiting mussel populations if annual recruitment of naïve, diadromous host fish is limited by downstream barriers. This adaptive immunity may involve gradual antibody production that determines whether a host fish becomes resistant to glochidia after multiple infections (Barnhart et al., 2008). Therefore, the aim of this study was to measure glochidia transformation rates after successive infestations of common bully by *E. menziesii* glochidia in the laboratory to determine if adaptive immunity occurs. Common bully is a benthic species endemic to New Zealand, maturing at one year with a generation time of two years (West et al., 2014). This diadromous species exhibits tolerance to highly eutrophic conditions, and is widespread and abundant throughout New Zealand (West et al., 2014). I

hypothesised that the number of juvenile mussels produced from common bully would decline over three infestation cycles due to acquired immunity, and that this effect would be greater for larger fish as they host higher numbers of glochidia. Specific objectives were to: i) compare cumulative detachment rates of untransformed glochidia over consecutive 20-day infestations to see how many glochidia unsuccessfully attached; ii) determine whether the number of viable juveniles excysted by common bullies decreased over repeated infestation cycles, and; iii) examine whether the size (mm) or surface area (cm^2) of fish used in the experiments influenced the number of viable juveniles produced.

3.2 Methods

Three consecutive laboratory trials were conducted between January and March 2019 in the eco-toxicology laboratory at the National Institute of Water and Atmospheric Research, Hamilton, New Zealand. Native common bully (*G. cotidianus*) were selected for artificial infestation of *E. menziesii* glochidia based on: i) their abundance and wide distribution that overlaps with freshwater mussel distribution (McDowall, 1990; Maceda-Veiga et al., 2016); ii) occupying benthic littoral habitat (Rowe, 1999) during the mussel glochidia release season leading to potentially high encounter rates with glochidia in the wild (Klunzinger et al., 2012); iii) previously determined suitability as hosts for freshwater mussels (Brown et al., 2017); and iv) common bullies not being a species of conservation concern (i.e., conservation status of 'Not Threatened'; (Dunn et al., 2018), as fish were overdosed with anaesthetic (>0.8 mg L^{-1} AQUI-S for 20 min) and disposed of at the end of the experiment. This research had approval from the National Institute of Water and Atmospheric Research (NIWA) Animal Ethics Committee (AEC222).

3.2.1 Glochidia and fish collection

Glochidia harvesting

Echyridella menziesii were collected by snorkeling in water 0.6-1.5 m deep in Lake Karāpiro, south of Hamilton (see Figure 2.2, Chapter 2 for more detail). Mussels were collected on two separate occasions for experiments one and two, but experiment three used the remaining mussels housed in the laboratory since experiment two. Mussels were kept immersed in lake water as much as possible during the process of collection and inspection to prevent early release of glochidia (S. Clearwater, NIWA, pers. comm. 2018). After collection in dive bags, mussels were transferred underwater into 20 L buckets filled with lake water. Mussels were assessed for sex and reproductive status by gently opening the mussel shell (~10 mm) to inspect the marsupium (or brood pouch). Females with mature glochidia were identified as those with an enlarged, orange/brownish brood pouch.

Approximately 40 females with mature glochidia were placed in buckets containing source water (kept cool with ice packs to reduce stress-induced glochidia release) and transported back to the laboratory. Mussels were then housed in a 100 L tank where there was a gradual transition from 100% lake water to dechlorinated tap

water. During this process, in which mussels were acclimated over a week, water was constantly aerated in a constant temperature room set to 15°C and a 16:8 h light:dark cycle. Ammonia concentrations (API® Ammonia Test Kit) were monitored every other day and water was exchanged if concentrations exceeded 0.5 mg L^{-1}. Water temperature was measured every other day with an YSI model 55 meter (Yellow Springs Instruments, Ohio USA).

To collect glochidia, mussels were removed from the 15°C constant temperature room, gently cleaned of material loosely adhered to the shells and placed individually in 300 mL cups filled with dechlorinated tap water (Figure 3.1A). Glochidial release was stimulated by allowing water temperature to gradually increase to ambient laboratory temperature (approximately 22°C). Released glochidia were assessed for percent viability by exposing a subsample of 90-200 glochidia to 1 mL of brine solution (98-100 ppt of concentrated oceanic seawater), and counting the number of open, half-closed and closed glochidia before and within 1 min of brine exposure (Taeubert et al., 2012a). Glochidia that closed when exposed to brine were considered viable (e.g., glochidia golden in colour Figure 3.1B). Glochidia released from mussels that had >85% viability were pooled, and viability was recalculated to determine the combined viability of the pooled glochidia. This viability was then used to calculate the volume of solution required to produce ~2000 viable glochidia L^{-1} for infestation baths (Dodd et al., 2005; Patterson et al., 2018).

Fish collection

To reduce the possibility of an acquired immune response from previous glochidia exposure, common bully were collected from the Waikato River near Hamilton Gardens (-37.806242, 175.307638) where *E. menziesii* populations are not thought to be present (S. Clearwater, NIWA, pers. comm. 2018). Although dead shells are common in parts of the lower Waikato River, living mussels have not recently been collected despite extensive air-lift sampling of benthic substrates, including adjacent to the site used for bully collection in the present study (Collier & Hogg, 2010; Collier et al., 2014).

Eight fine-mesh (2 mm) minnow traps were set overnight along the littoral zone until >60 suitably-sized fish (>30 mm length) were captured. The fish were kept in

a 100 L tank containing dechlorinated tap water adjusted to 3-5 ppt saline solution by addition of natural seawater, and acclimated to laboratory conditions (>1 week) in a constant-temperature room (20^{o}C, 16:8 h light:dark). Each tank had a recirculating pump with a biofilter (Fluval 206 canister filter) to remove excess food and fish waste products from the water. Ammonia was monitored daily and water exchanged if necessary (>0.5 mg L^{-1}), as for mussels. Once fish were sufficiently acclimated (as indicated by consuming 5-10 % body weight per day of frozen blood worms), and in suitable condition (i.e., no external evidence of fungal infection or fin damage), artificial infestation was performed following the protocol used in Moore and Clearwater (in press).

3.2.2 Artificial infestation procedure

Three artificial infestations were conducted on common bully in a constant temperature room set to 20^{o}C. For each infestation, the fish were exposed to a homogenous glochidial suspension in batches of four individuals (Dodd et al., 2005). Infestation baths were vigorously aerated to keep glochidia in suspension for each 15 min exposure. After infestation, fish were transferred to a water bath without glochidia for another 15 min to remove loosely attached or non-attached larvae. After the first infection, infested fish were randomly assigned to 10 L, self-cleaning tanks (Pentair Aquatic Eco-Systems; PC90 tanks, LID90I-4 lids, and BAF10.01-4 baffles) with filters (150 µm mesh) receiving the outflow of each tank to collect detached glochidia or excysted juveniles (Figure 3.1C). After subsequent infestations, the same procedure was used but repeat-infested fish were returned to the same tanks as in the previous experiment, with control fish (never previously exposed to glochidia) randomly assigned to the remaining individual tanks. The tanks were supplied with dechlorinated water recirculated via a pump after it had passed through biofiltration. A single rectangular shelter made from PVC piping (11 cm x 7 cm x 5.5 cm, length x width x height) was provided for the fish in each tank.

In addition to individual tank exposures, a bulk exposure of native fish was performed using the same methods to provide ancillary data on glochidia transformation progress. This was done by quantifying internal and external glochidia attachment on days two, four and eight post-infestation (four fish per

exposure). These fish were held in four 100 L tanks (i.e., naïve, and as required, fish infested once, twice or three times) with an assortment of rocks and PVC piping for shelter, and maintained throughout the experiments.

The first experiment (21 January-10 February 2019) was conducted on 28 fish, of which 12 were placed in individual tanks (Table 1, experiment one) and 16 were in a bulk exposure tank (Table 2, experiment one). Meanwhile, a separate 100 L tank of naïve fish was maintained for infestations in subsequent experiments. For the second experiment (21 February-11 March), the healthiest eight of the 12 fish from the first trial (selected based on feeding behaviour and absence of fungal infection) were re-infested, and four new common bully (control fish from the naïve tank) were infested for the first time and placed in individual tanks (Table 3.1, experiment two). Additionally, 12 fish were infested for the first time (from the naïve tank) in bulk exposures, and the remaining "infested once" bulk exposure fish (those not sacrificed, n = 12), were infested for a second time (i.e., 36 fish were infested in total in round 2). On the third and final experiment (11-31 March), the same eight of the original 12 fish were infested for the third time, and another four control fish (from the naïve tank) were infested for the first time and placed in individual tanks (Table 3.1). Additionally, the remaining bulk fish that were previously infested once and twice were infested again, resulting in fish infested twice (n = 5) and three times (n = 7), respectively (Table 2). Another four naïve fish were infested for the first time for monitoring (i.e., 28 fish were infested in total in experiment three). Between each infestation round, fish were given at least four days rest when no live juveniles were collected in the outflow sieve cups.

Table 3.1. Sequence of experiments and fish involved for the twelve individual tanks (total number of fish used in individual tanks; n = 20).

	Number of fish		
Experiment	**Infested once**	**Infested twice**	**Infested three times**
1	12	-	-
2	4	8	-
3	4	-	8

Table 3.2. Numbers of fish in each bulk exposure tank during each stage of the experiment (there were four naïve fish remaining at the end of the experiment). Total number of fish initially held in the bulk tanks was 60.

	Number of fish			
Experiment	***Tank 1*** **Naïve**	***Tank 2*** **Infested once**	***Tank 3*** **Infested twice**	***Tank 4*** **Infested three times**
1	44	16	-	-
2	28	12	12	-
3	4	4	5	7

3.2.3 Post-infestation procedures

Water flow through the fish tanks was maintained at a constant rate (~0.5 L min^{-1}) using a Hailea HX-6830 pump to promote self-cleaning and reduce potential fish predation of glochidia or juvenile mussels. Water temperature and ammonia concentrations were monitored every second day, as described previously, for the duration of each 20-day trial. Fish were fed frozen blood worms after tanks had been flushed with a high flow of water (i.e., >3 L min^{-1}) for >15 min to ensure glochidia or juveniles were removed from tanks into the outflow filters.

Glochidia examined in a Bogorov tray at 16x magnification were classified as either open, closed or juveniles, which were identified as alive if there was active pedal movement and/or active contraction of the adductor mussel (Taeubert et al., 2013). Any closed glochidia were held for at least a week after collection and observed every other day to positively confirm their status as transformed juveniles or detached glochidia. Laboratory trials were considered complete 20 days after each infestation when no more juvenile mussels were extracted from the tanks, indicating there were no juveniles remaining attached to the fish.

3.2.4 Location of glochidia attachment

Fish from the bulk exposure were euthanized (>0.8 mg L^{-1} AQUI-S for 20 min) and examined periodically from day two onwards to assess glochidia attachment on internal (mouth and gills) and external (dorsal, adipose, pectoral, pelvic, anal and caudal fins, lips and operculum cover) structures. All fish housed individually in tanks were also assessed using the same methods at the end of each experiment. Once no longer required for the trials, fish were euthanized, measured (body length to the nearest mm), weighed to the nearest 0.01 g, and body and fin surface areas were measured following methods described by O'Shea et al. (2006) where fins and tail were dissected from each fish and their surface areas traced on to paper. The remaining fish head and torso were carefully wrapped in paper, then the surface areas of the wrapper, and the fin and tail tracings were used to calculate the surface area of the fish. Fish surface area across experiments averaged 18.5 cm^2 and ranged from 10.8-35.6 cm^2. The average length of fish was 58.9 mm, while average weight was 2.4 g (Table 3.3). There was one mortality during the first experiment when the fish in one tank died, leaving 19 fish in total in the individual tanks.

Table 3.3. Mean (± SE) values for fish weight, length and surface area of fish held in the individual tanks, from all three experiments combined (n = 19).

	Mean ± SE	**Range**
Weight (g)	2.4 ± 0.3	1.1 - 5.9
Length (mm)	58.9 ± 2.1	48.0 – 81.0
Surface area (cm^2)	18.5 ± 1.6	10.8 - 35.6

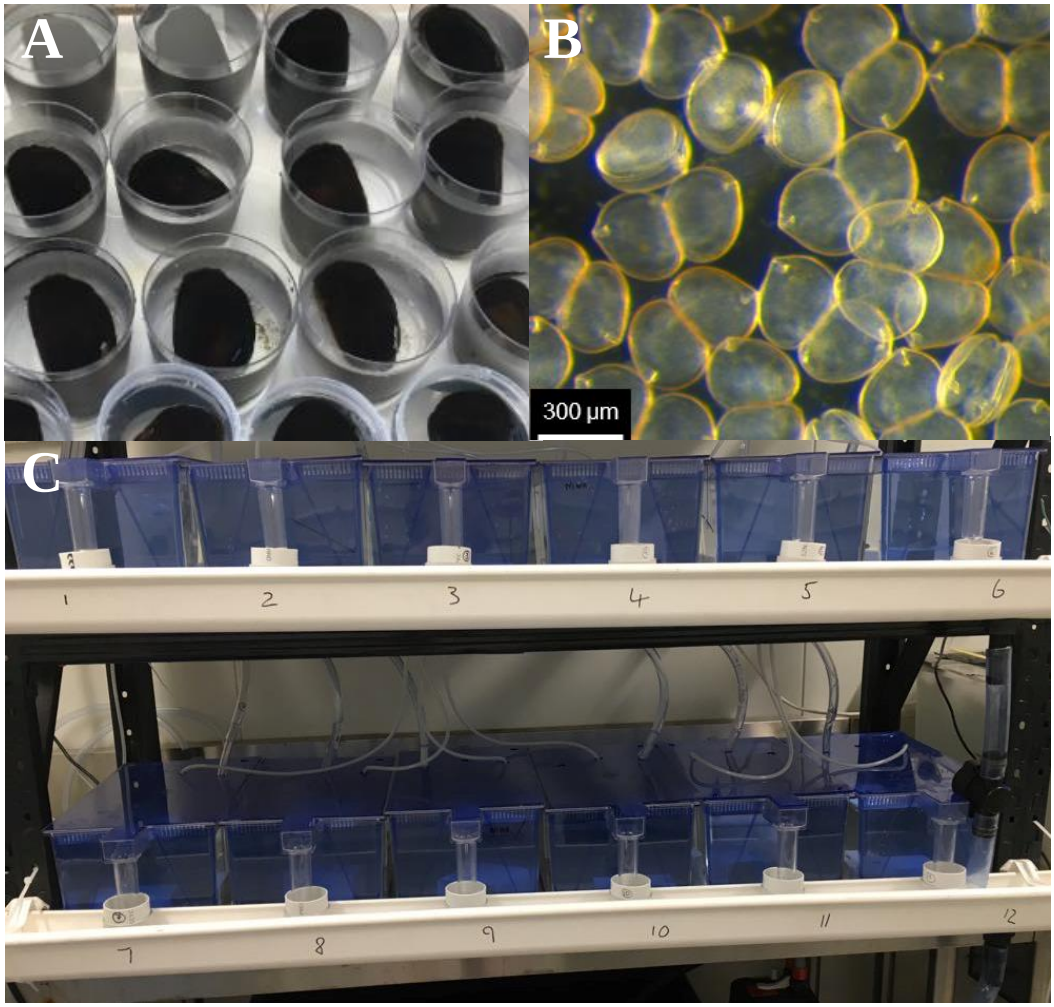


Figure 3.1. A – freshwater mussels placed at room temperature in individual cups to stimulate glochidia release; B – viable glochidia with a golden tinge; C – the 12 individual 10 L tanks set up with aeration tubing.

3.2.5 Statistical analysis

STATISTICA (v 13; StatSoft, Oklahoma, USA) was used for all statistical analyses. Data were tested for normality and homogeneity of variance using Shapiro-Wilk and Levene's tests (significance level: $P < 0.05$), respectively. Not all the data were found to be normal even after log (x+1) transformation, and therefore non-parametric tests were used.

The Kruskal-Wallis test, followed by a post-hoc multiple comparisons test where a significant main effect was detected, was used to test for: i) differences between water chemistry variables across experiments; and ii) differences in glochidia loss/detachment, and excystment as juvenile mussels, between controls across experiments. Mann-Whitney U test was used to determine whether there was a statistically significant difference (using adjusted Z and P values) between the number of detached glochidia and excysted juveniles from control and treatment fish within each experiment. Glochidial loss and juvenile excystment were analysed with and without standardisation by fish surface area (i.e., glochidia $fish^{-1}$, juvenile $fish^{-1}$, glochidia detached cm^{-2}, juveniles excysted cm^{-2}).

Kolmogorov-Smirnov (K-S) test was used to statistically compare accumulation curves of the average numbers of juveniles produced and detached glochidia. Metamorphosis success, measured as the percent of attached glochidia that metamorphosed to the juvenile stage and were recovered alive out of the total number of glochidia which attached to fish, was compared between infestations. Spearman rank correlation analysis was used to explore the relationships between total juveniles produced or detached glochidia and fish length, surface area and weight. Correlation analysis was performed for control or treatment fish in each experiment as well as across the three experiments combined.

Relationships between the number of glochidia or juvenile mussels produced vs length, and the number of juveniles $fish^{-1}$ cm^{-2} and length were explored but had low explanatory power (i.e., each variable explained <6% of the variance in number of juveniles) (Appendix 2).

3.3 Results

3.3.1 Viability and infestation of glochidia

Glochidia viability prior to the infestation of fish ranged on average (± SE) from 89.5 ± 5.1% to 96.1 ± 3.0% (Table 3.4). The lowest viability count of pooled glochidia was 80% and the highest viability was 100%. For the first, second and third infestations, the glochidia of two, four and three mussels were pooled, respectively, to achieve the desired concentration (Table 3.4). During the infestation process, common bullies settled on the tank bottom and would take cover behind the aerators where glochidia kept in suspension would settle on the tank bottom, increasing the likelihood of fish-glochidia contact.

Table 3.4. The number of mussels used in each experiment to extract viable glochidia. Individual mussel viability ranges for subsamples before the glochidia of individual mussels were pooled together, and subsample viability of the pooled glochidia are both shown.The mean viability is the average of the pooled glochidia viability subsamples, where n = number of subsamples.

	Infestation 1	**Infestation 2**	**Infestation 3**
No. mussels used	2	4	3
Individual mussel viability range (%)	85.9 - 95.1 (n = 6)	80.0 - 99.0 (n = 12)	90.3 – 100 (n = 6)
Pooled glochidia viability range (%)	85.9 - 95.1 (n = 4)	80.0 - 97.3 (n = 3)	90.3 - 100 (n = 3)
Mean viability (%) ± SE	90.9 ± 1.9	89.5 ± 5.1	96.1 ± 3.0

Water chemistry variables measured every other day ranged from 5.6 to 9.6 mg L^{-1} dissolved oxygen, 20.9 ^{o}C to 22.7 ^{o}C temperature, and 2 to 7 ppt salinity across all experiments (Table 3.5). The lowest and highest salinities and temperatures were recorded during experiment two. The highest dissolved oxygen occurred during experiment one, and the lowest was recorded in experiment two. However, there were no significant differences in salinity, dissolved oxygen, or temperature between experiments one, two, and three ($H = 0.86$, $P = 0.65$; $H = 1.49$, $P = 0.47$; $H = 3.94$, $P = 0.14$).

Table 3.5. Mean (± SE) and range for water chemistry variables measured in randomly selected tanks every other day.

		Experiment		
		One	**Two**	**Three**
Salinity (ppt) n=10	Average ± SE	3.9 ± 0.5	3.9 ± 0.2	3.9 ± 0.1
	Range	2 - 7.0	2 - 4.5	3.5 - 4.5
Dissolved oxygen (mg L^{-1}) n=10	Average ± SE	7.1 ± 0.3	6.6 ± 0.2	6.8 ± 0.1
	Range	6.3 - 9.6	5.6 - 7.2	6.4 - 7.5
Temperature (ºC) n=10	Average ± SE	22.1 ± 0.1	22.0 ± 0.2	22.3 ± 0.1
	Range	21.9 - 22.4	20.9 - 22.7	21.5 - 22.6

3.3.2 Glochidia detachment

Glochidia were shed over the entire course of each infestation. Between 26 and 54% of total glochidia that detached from fish (i.e., open and closed glochidia that did not transform into juveniles) dropped off within the first two days of each experiment for control fish, compared to 37-58% in treatment fish (Figure 3.2; Figure 3.3). The number of detached glochidia accumulated steadily over time for experiments one and two. During experiment three, glochidial detachment appeared more delayed with a large number of glochidia detaching between days 12 and 14 post-infestation in both the control and treatment fish. However, K-S tests suggested no statistically significant differences between the accumulation curves of control fish for each experiment ($P > 0.05$), with the differences between experiments two and three only marginally not statistically significant ($P = 0.06$). The accumulation curves of detached glochidia between experiment two control and treatment fish, and experiment three control and treatment fish were not statistically different ($P > 0.05$).

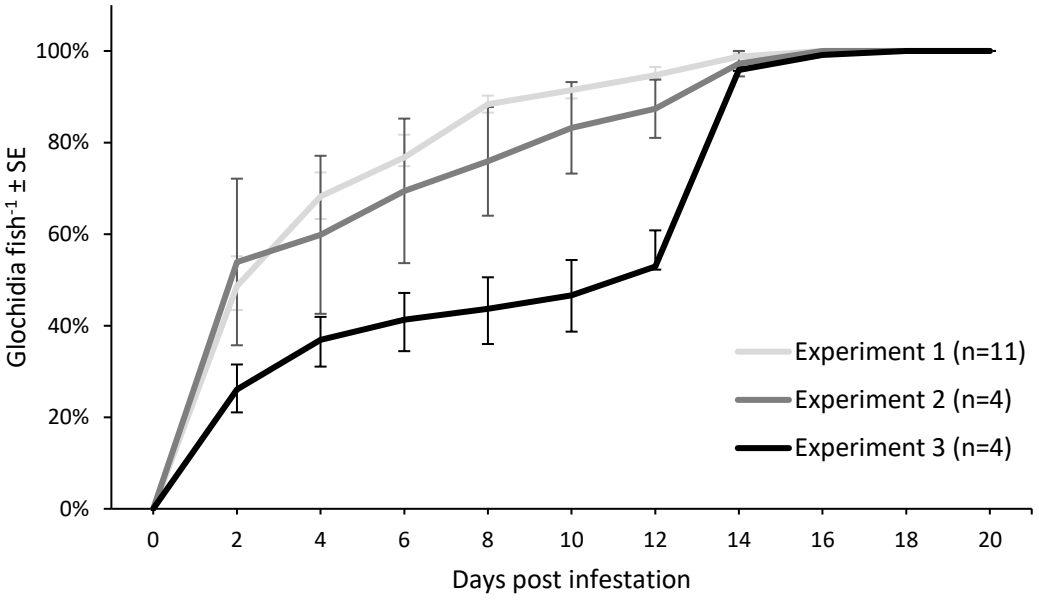


Figure 3.2. The cumulative mean (± SE) % of glochidia detached over 20 days for control fish in each experiment, i.e. fish infested once.

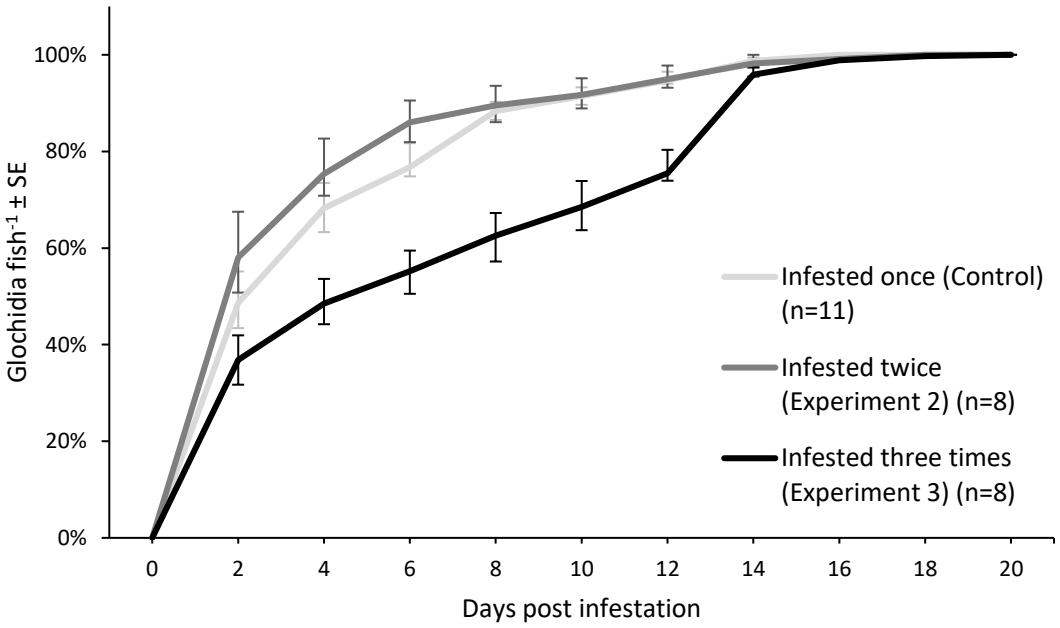


Figure 3.3. The cumulative mean (± SE) % of glochidia detached over 20 days for treatment fish in each experiment, i.e. fish infested once, twice or three times.

The number of detached glochidia cm^{-2} of fish surface area that unsuccessfully transformed into juveniles decreased between experiments one and two, but increased between experiments two and three (Figure 3.4). Kruskal-Wallis tests showed there were also significant differences in the number of detached glochidia

from control fish between experiments (H = 11.42, P = 0.003). There were no significant pairwise differences between the number of glochidia detached cm^{-2} between experiments one and two controls (P = 0.36), however, there were significant differences between controls in experiments one and three, and two and three (P = 0.04, P = 0.003, respectively).

During experiment one, there were on average 1.8 ± 0.2 glochidia detached cm^{-2} of fish surface area. In experiment two, there was no significant difference between the number of glochidia detached cm^{-2} for control fish (0.7 ± 0.1) versus treatment fish (0.5 ± 0.1) (Z = 0.93, P = 0.35). During experiment three there was a marked difference between the mean number of glochidia detached cm^{-2} for control (8.86 ± 0.6 cm^{-2}) and treatment (6.12 ± 0.5 cm^{-2}) fish (Z = 1.95, P = 0.05) (Figure 3.4).

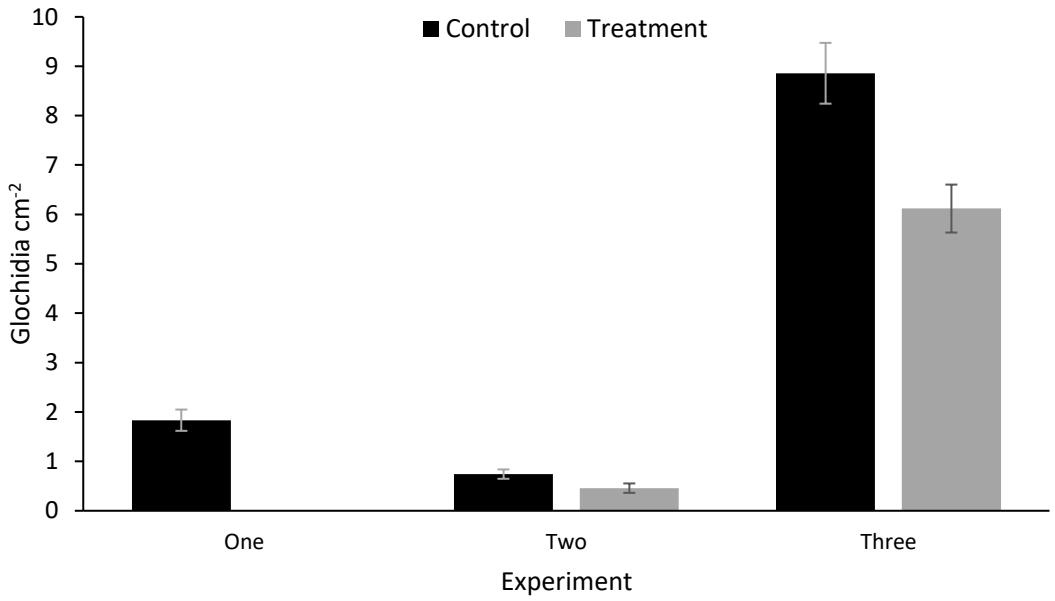


Figure 3.4. Mean (± SE) number of total glochidia detached cm^{-2} over 20 days for control and treatment fish across all three experiments.

3.3.3 Juvenile excystment

The largest number of juveniles produced over 20 days by any single fish were 25, 16 and 151 in experiments one, two and three, respectively. During the second experiment, there were five fish that did not produce any juveniles (one control fish and four treatment fish). These fish were removed from the cumulative analyses as there were not regarded as 'juvenile-producing fish' (see Appendix 3 for more detail). Juveniles were produced after four days post-infestation in experiments one and two, but not until six days in experiment three. Most juveniles were excysted

between days 6 and 14 before plateauing in all three experiments. There were no significant differences in accumulation curves of juvenile-producing control fish between experiments one and two, one and three, and two and three (K-S test, $P > 0.05$) (Figure 3.5).

The accumulation curves for the number of juveniles produced by treatment fish exposed for the second and third time is shown in Figure 3.6, and compared to fish from experiment one. As for the control fish infested once, the majority of juveniles from previously-infested fish in experiments two and three were produced between days 6 to 14. The accumulation curves of treatment fish between experiments did not statistically differ (K-S test, $P > 0.05$). Further, the accumulation curves between juvenile-producing control and treatment fish in experiments two and three were not statistically different ($P > 0.05$).

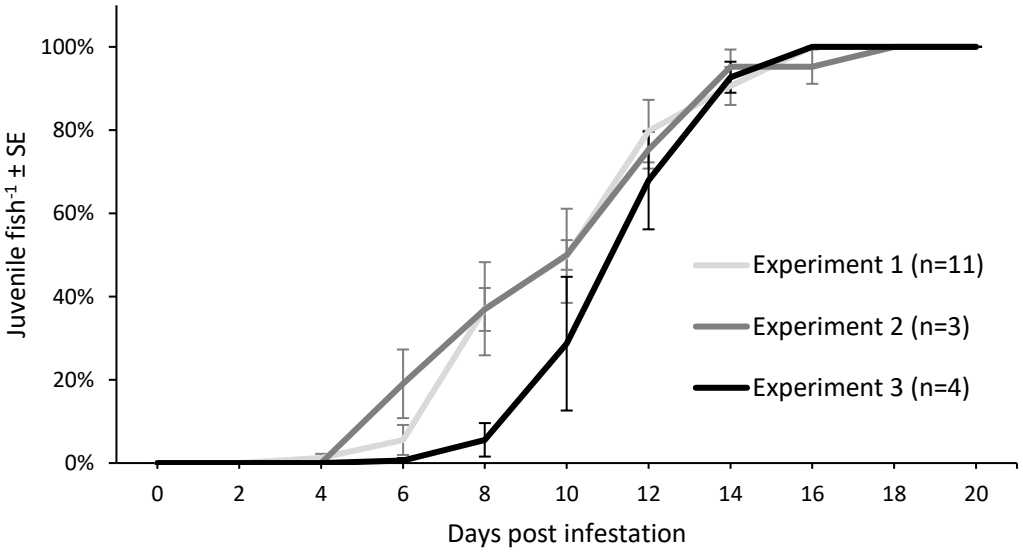


Figure 3.5. The cumulative mean (± SE) % of juveniles produced over 20 days for juvenile-producing control fish in each experiment (i.e., fish infested once).

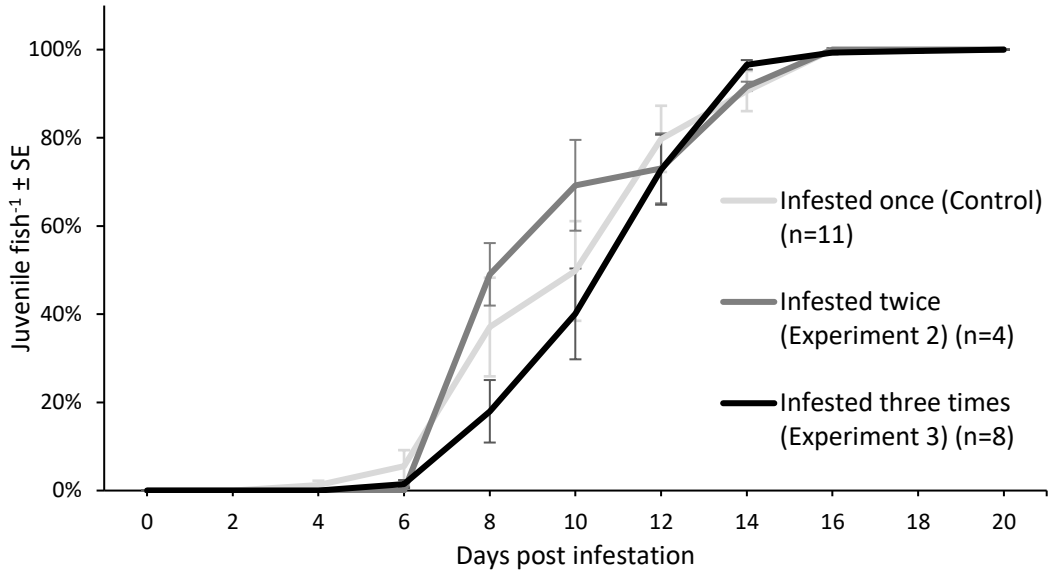


Figure 3.6. The cumulative mean (± SE) % of juveniles produced over 20 days for juvenile-producing treatment fish infested once, twice and three times.

The average number of total viable juveniles produced by individual fish infested once (control) ranged from: i) 9.3 ± 2.1 juveniles per fish or 0.5 ± 0.1 juveniles cm^{-2} in experiment one; ii) 7.5 ± 3.3 juveniles per fish or 0.4 ± 0.2 juveniles cm^{-2} in experiment two; and iii) 47.5 ± 14.6 juveniles per fish or 3.3 ± 1.0 juveniles cm^{-2} in experiment three (Figure 3.7, Figure 3.8). Kruskal-Wallis tests showed there were significant differences in the number of juveniles excysted from control fish between experiments ($H = 7.06$, $P = 0.03$). Between the control fish of experiments one and two, and two and three, there were no significant pairwise differences ($P > 0.05$), however, there was a significant difference between experiments one and three ($P = 0.03$). This was also evident in the standardized number of juveniles cm^{-2} between experiments one and three ($P = 0.01$) (Figure 3.8).

In experiment two, there was no significant difference between the number of juveniles produced by control (7.5 ± 3.33 juveniles) and treatment (2.5 ± 1.56 juveniles) fish (Mann-Whitney U (Z) = 1.33, P = 0.18). There was also no significant difference in the number of juveniles produced by each fish for control (47.5 ± 14.55 juveniles) and treatment (63.3 ± 16.11 juveniles) fish in experiment three ($Z = -0.43$, $P = 0.67$). Similar results were obtained after standardizing the number of juveniles produced by fish surface area. Thus, there was no significant difference ($Z = 1.50$, $P = 0.13$) between the number of juveniles produced by control

fish (0.4 ± 0.22 juveniles cm^{-2}) and treatment fish infested for the second time (0.1 ± 0.05 juveniles cm^{-2}), and no significant differences between the number of juveniles produced by control (3.2 ± 0.99 juveniles cm^{-2}) and treatment (2.8 ± 0.61 juveniles cm^{-2}) fishes infested three times (Z = 0.25, P = 0.80).

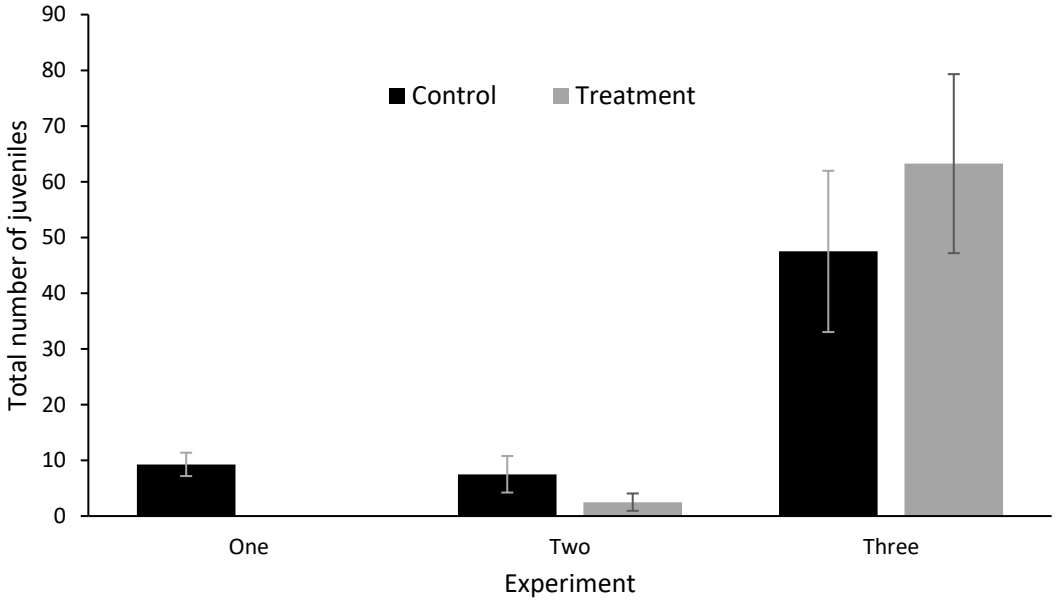


Figure 3.7. Mean (± SE) number of total juveniles produced per fish over 20 days for each experiment for fish infested once (controls) and two or three times (treatments in experiments two and three, respectively).

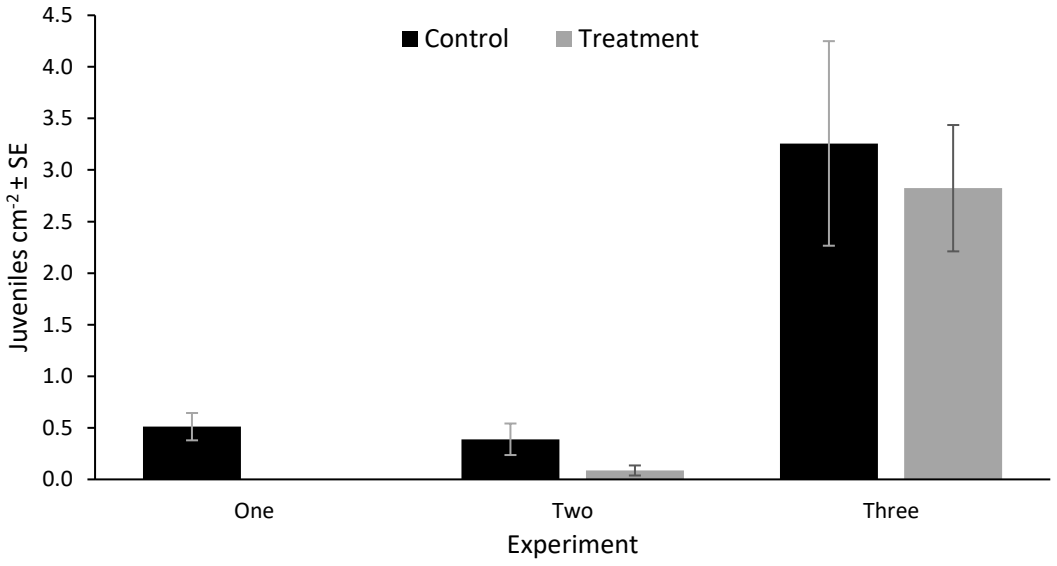


Figure 3.8. Mean (± SE) number of total juveniles cm^{-2} over 20 days for control and treatment fish across all three experiments.

3.3.4 Ratio of detached glochidia vs excysted juveniles

The ratio of the number of glochidia that detached to the number of juveniles produced for each individual fish was averaged across all fish per treatment. This ratio was compared for each experiment using standardized values, and was on average 3.8 times the number of glochidia:juveniles (Table 3.6). Thus, on average, 76.7% glochidia detached compared to 23.3% that encysted and transformed into juveniles, although there was some variability between experiments. Notably in experiment three, treatment fish had the highest percentage of juveniles produced (30%) compared to detached glochidia (70%), and experiment two treatment fish had the lowest percentage of juveniles produced (11.5%) compared to detached glochidia (88.5%).

3.3.5 Ratio of glochidia attached to internal vs external structures on the fish

Throughout all three experiments the fish from the bulk tanks were dissected on days two, four and eight. Of all the fish dissected, 15 had glochidia attached to them and six fish did not. Overall, out of the total number of glochidia attached, 43% of glochidia were attached internally (gills, mouth) (e.g., Figure 3.9A) compared to 57% attached externally to fish fins, lips and operculum (e.g., Figure 3.9B).

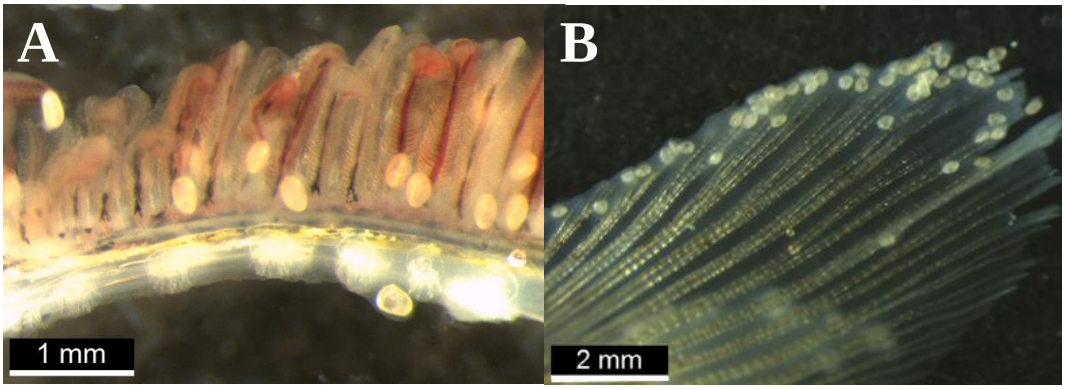


Figure 3.9. Examples of glochidia attachment to surfaces of fish dissected two days post-infestation: A – glochidia attached to the gills of a common bully; B – a pectoral fin of a common bully with glochidia attached and encysted.

Table 3.6. Ratio of detached glochidia and juveniles excysted (i.e., metamorphosis success) separated by experiment, based on average numbers of glochidia that detached and juveniles excysted per fish (cm^2). Overall means (± SE) are expressed as a percentage. Note: this includes fish that did not produce any juveniles, but had glochidia detach.

Experiment	Variable	No. fish	Detached glochidia (%)	Excysted juveniles (%)	Ratio
1	Control	11	78.8	21.2	3.7
2	Treatment	8	88.5	11.5	7.7
	Control	4	72.2	27.8	2.6
3	Treatment	8	70.0	30.0	2.3
	Control	4	74.0	26.0	2.9
Mean ± SE			76.7 ± 3.6	23.3 ± 3.6	3.8

3.3.6 Fish size relationships

Fish length explained 96% of variance in measured weight for the 19 fish used in this experiment (Figure 3.10). Additionally, there was a strong relationship between surface area and length (R^2 = 0.93). These results indicate that fish surface area calculations using the methods of O'Shea et al. (2006) strongly reflected fish size, and that length can be used to estimate surface area based on the equation for the derived surface area:length relationship (Figure 3.10).

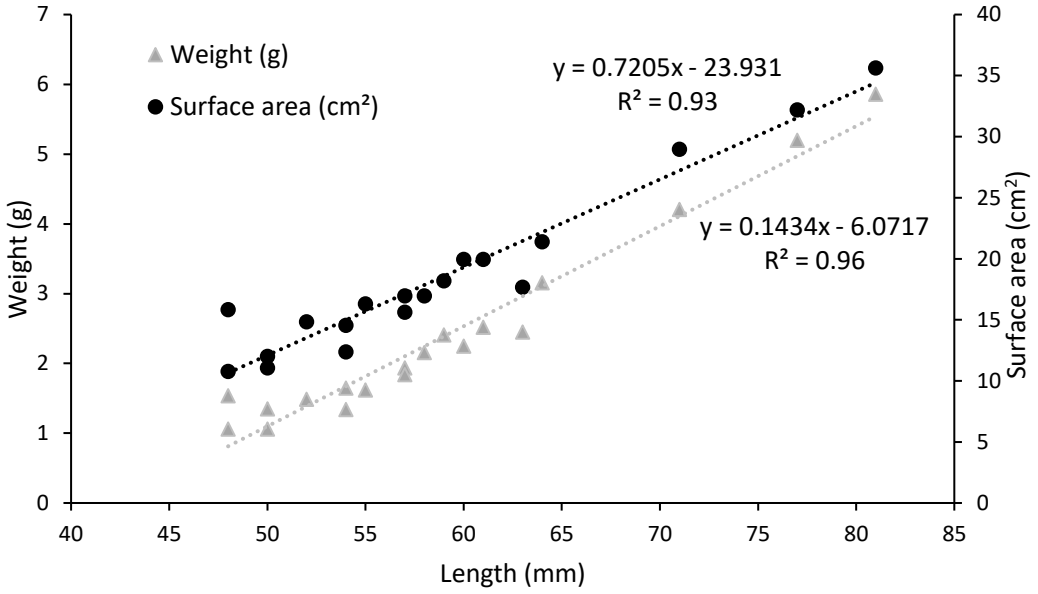


Figure 3.10. The relationship between fish length (mm) and surface area (cm^2), and fish length (mm) and weight (g), with R^2 values and equations.

Figure 3.11 explores the relationships between the total number of juveniles excysted per fish and fish surface area for each experiment. The strongest relationships were for treatment fish in experiments two and three, where 38% and 29% of variance in juvenile numbers was explained by fish surface area, respectively. The remaining R^2 values are all <0.15, indicating that fish surface area had low explanatory power for the number of juveniles produced. Similarly, the total number of juveniles or glochidia produced across all experiments was plotted against surface area and length (Figure 3.12, Figure 3.13). Surface area accounted for ≤10% of the variation in the number of glochidia detached from fish, and <5% for the numbers of juveniles excysted.

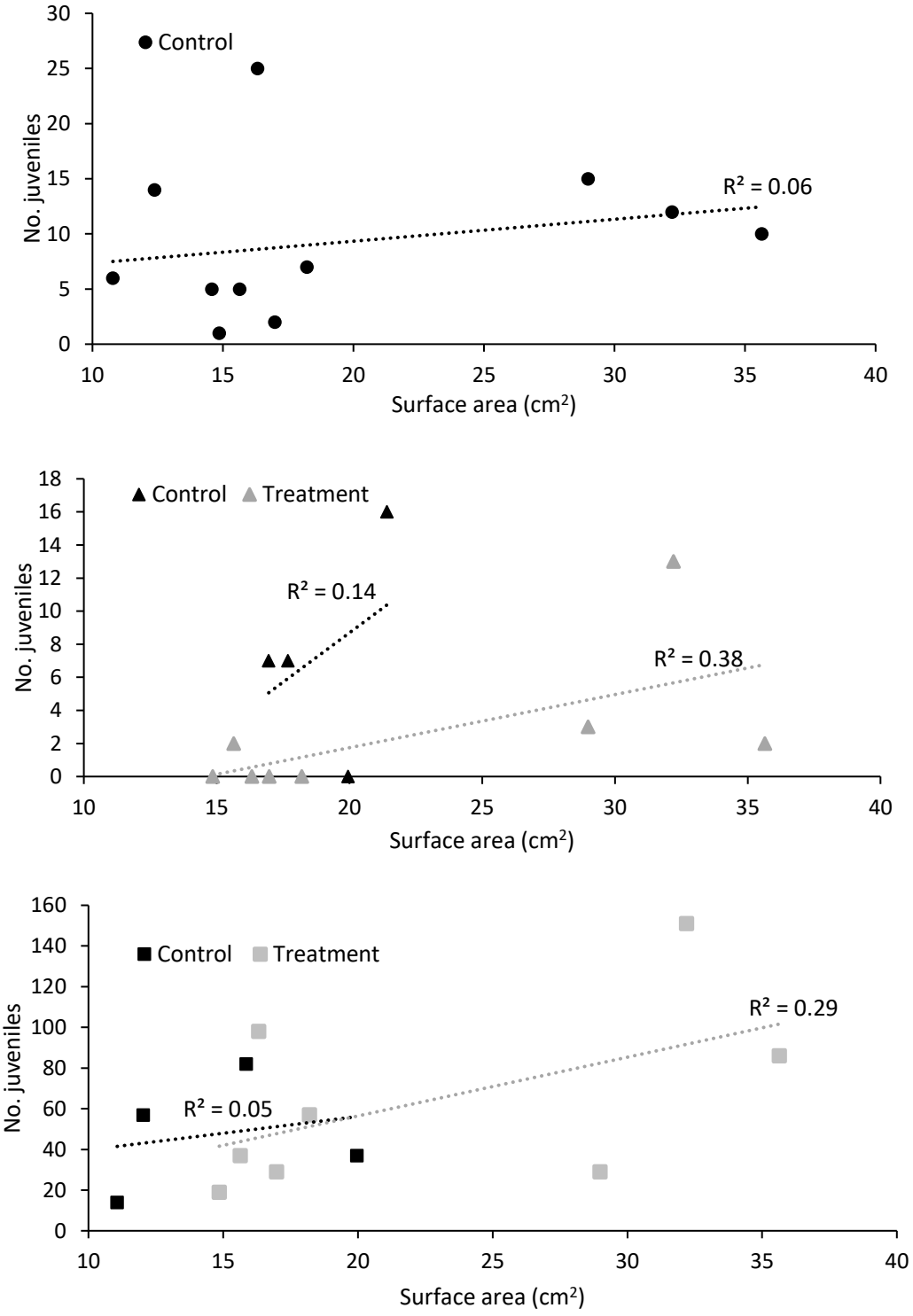


Figure 3.11. Total number of juveniles produced vs surface area (cm^2) for control and treatment fish for each experiment. From top to bottom, experiment one (control: n=11), experiment two (control: n=4, treatment: n=8) and three (control: n=4, treatment: n=8). Linear trend lines and R^2 values are included. Note: x axis begins at 10 cm^2.

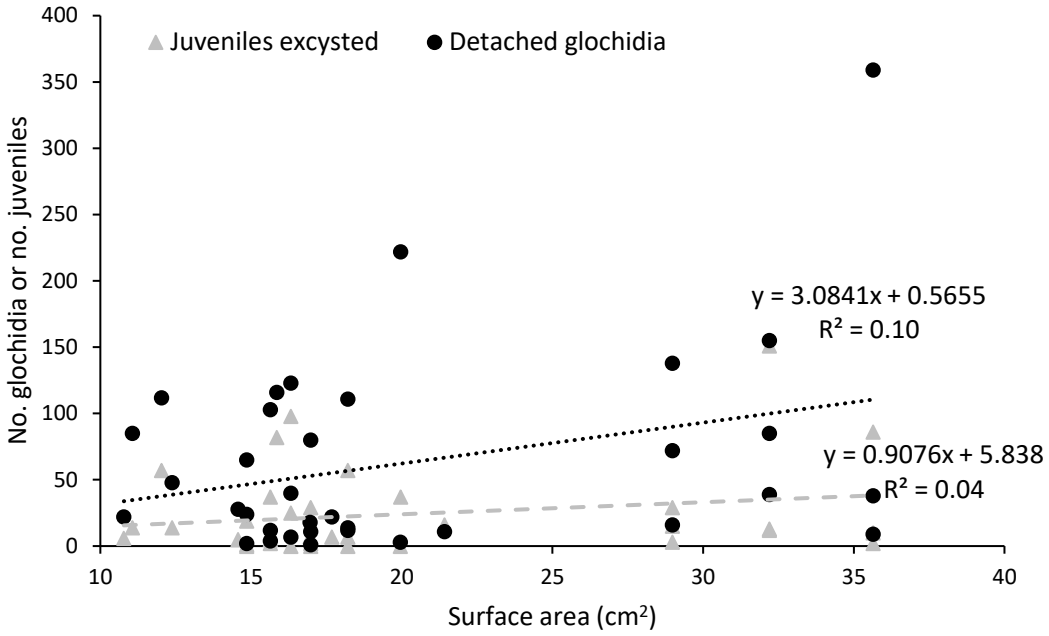


Figure 3.12. Total number of excysted juveniles or detached glochidia produced across all experiments vs fish surface area. Note: x-axis begins at 10 cm^2.

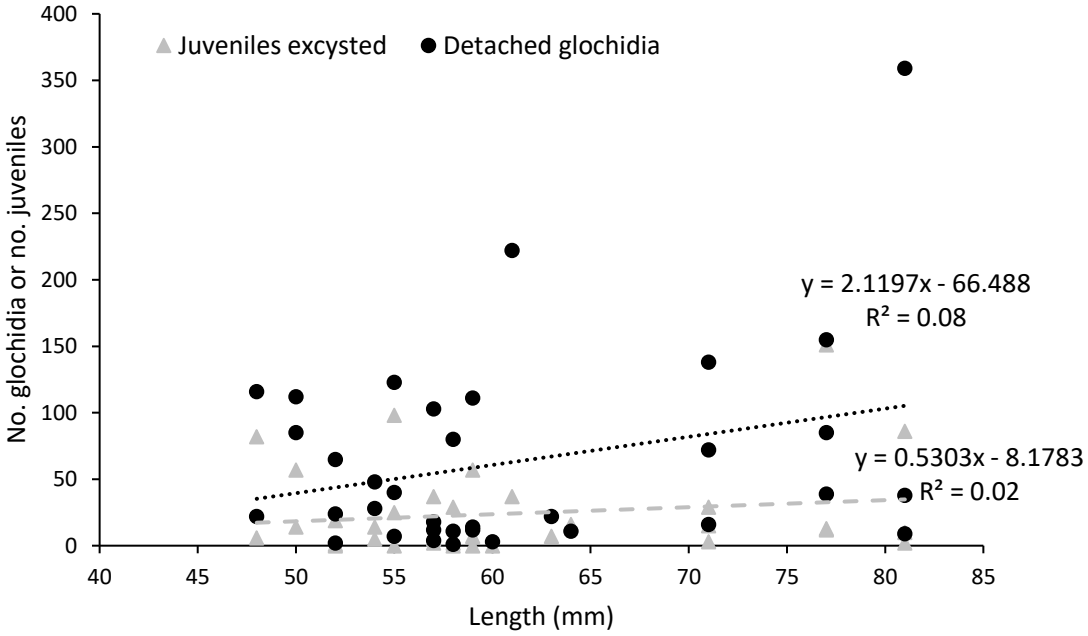


Figure 3.13. Total number of excysted juveniles and detached glochidia produced across all experiments vs fish length. Note: x-axis begins at 40 mm.

3.4 Discussion

This study documents the first evaluation of multiple exposures of *Echyridella menziesii* glochidia to a New Zealand fish species, the native common bully (*Gobiomorphus cotidianus*). Comparison of glochidia detachment and juvenile excystment rates after three sequential infestations indicates that common bully did not acquire an immunity to *E. menziesii* glochidia, at least in a controlled laboratory setting. Therefore, individual fish appear likely to remain viable hosts throughout the mussel reproductive season and potentially over multiple years as common bullies reach 4-5 years of age (West et al., 2014). In fact, the excystment of juveniles significantly increased after three infestations over one season in this laboratory study, suggesting factors other than host compatibility can influence mussel reproductive success, as discussed below.

3.4.1 Differences in glochidia viability

Barnhart et al. (2008) considered that good glochidia hosts have >90% metamorphosis success whereas a lower proportion might succeed on marginal hosts. During these experiments, the most successful infestation round had 30% metamorphosis success, even though Brown et al. (2017) had confirmed common bully as a suitable host compared to other native fish in previous laboratory trials. Furthermore, there were many observations of glochidia encysted on common bully in my field study (Chapter 4). Haag and Staton (2003) state that such differences in metamorphosis success may be a result of experimental factors and/or changing fecundity of female mussels.

Viability tests were conducted on each batch of glochidia before creating a pooled batch for the host-fish exposures, as viability is a good indicator of mussel infectivity (Patterson et al., 2018). Fritts et al. (2014) state that this test is only helpful when viability is 90% or more, with viability <90% potentially resulting in low rates of infectivity, especially for glochidia harvested near the end of the brooding cycle. Due to a limited timeframe for this experiment, and a restricted number of captive mussels to choose from in the laboratory, mussels that had glochidia with viabilities >85% were pooled and the number of glochidia was increased in an attempt to compensate for the viability being <90%. Final glochidia

viability averages across all three infestations ranged from 89.5% ± 5.1 to 96.1% ± 3.0, close to the 90% threshold recommended by Fritts et al. (2014).

Treatment fish in the second exposure experiment had the lowest glochidia viability (89.5%) whereas fish in the third experiment were exposed to the highest glochidia viability (96.1%). Interestingly, experiment two treatment fish had the lowest metamorphosis success (11.5%) whereas experiment three treatment fish had the highest metamorphosis success producing the most live juveniles (30%), suggesting that even apparently small difference in glochidia viability may influence metamorphosis success. Similarly, control fish infested once in experiment two also had significantly lower glochidia detachment numbers and lower juvenile production rates compared to experiment three control fish, providing further evidence that glochidial performance was related to factors other than host immunity. Potential reasons for differences in glochidia viability, and therefore metamorphosis success, may include: i) glochidia viability is naturally variable between mussels, and for individual mussels over time; ii) a select number of female glochidia were pooled together per experiment; iii) females were fertilized by different males in the source location; and iv) the duration and temperature that mussels were acclimatized in the laboratory influenced glochidia viability.

Glochidia viability has been found to vary significantly between batches of glochidia from the same mussel and from different mussels (e.g., Fritts et al., 2014). In these experiments, mussels were collected on different dates (experiments one and two), or used different mussels collected at the same time (experiments two and three), which may explain the differences in glochidia viability. It is relatively easy to stimulate females to release their brood even when mussels are not ripe, for example during transport. Mussels that release glochidia prematurely can provide challenges if bringing mussels back to the laboratory for glochidial harvest as the yield may be low (American Society for Testing and Materials, 2006). Furthermore, in naturally-occurring dense mussel beds, many female mussels potentially at different stages of gonad development may serve as potential sources of glochidia, unlike the few female mussels selected to inoculate fish in these experiments which may not have reflected natural variation of glochidia supply.

Because females were collected from a natural population in Lake Karāpiro, it is unknown whether each female was fertilized by a single male or by multiple males, although the latter seems more likely. If so, variability in glochidia viability may result from differences in paternal fitness, as observed elsewhere (Christian et al., 2007; Ferguson et al., 2013; Wacker et al., 2018). Mussels used in experiments one and two were collected on separate occasions from Lake Karāpiro, while those used in experiment three were conditioned in the laboratory over one month (i.e., collected at the same time as the mussels used in experiment two), as I anticipated that finding viable glochidia in the field in March would be difficult given that the end of the *E. menziesii* glochidia release season occurs locally in February to March (Clearwater et al., 2014). In the laboratory, mussels were kept at ~15^{o}C, and then acclimated to room temperature (approximately 22^{o}C) when required for infestations. Research by Van Vreede et al. (1999) found that glochidial maturation can be stalled by holding paper pondshell mussels (*Utterbackia imbecillis*) in a laboratory setting at winter temperatures and increasing water temperatures when glochidial maturation is desired. Thus, holding mussels in a controlled temperature room for an extended period may lead to greater development time and explain why glochidia viability was higher in the third infestation.

Temperature is widely recognized as a major environmental cue affecting the release of glochidia by gravid mussels (Young & Williams, 1984; Watters & O'Dee, 2000; Hastie & Young, 2003; Österling, 2015), and gradual changes in spring temperatures can induce changes in species phenology (Stenseth & Mysterud, 2002; Schneider et al., 2018). Thus, early warming of water during the normal reproductive season (spring/summer) may stimulate glochidia release when the brood is not in the best condition, resulting in inefficient reproductive effort. Hastie and Young (2003) describe how variation in stream temperatures can delay reproduction in streams during cold years, while Roberts and Barnhart (1999) found that transformation success of flat floaters (*Anodonta suborbiculata*) on host golden shiners (*Notemigonus crysoleucas*) significantly decreased at higher temperatures. Schneider et al. (2018) suggested that temperature fluctuations cause mismatches between mussel reproduction and the availability of host-fish, and such fluctuations are predicted to become more frequent as climate change progresses.

3.4.2 **Glochidia detachment and excystment of juveniles**

While there was some initial suggestion of development of immunity or resistance to glochidia attachment between the first and second infestations, when there was almost a 50% reduction in the number of live juveniles produced, the percentage of glochidia that metamorphosed into juveniles compared to the number of shed glochidia remained relatively low throughout all three experiments. Although more glochidia were able to attach and transform during the third infestation, 70% of them did not transform successfully into juveniles, indicating low overall metamorphosis success for common bully. A transformation rate of 30% for the third experiment is not dissimilar to findings in the laboratory study of Rogers and Dimock (2003) who reported the fraction of total attached glochidia that successfully metamorphosed to paper pondshell mussel (*Utterbackia imbecillis*) juveniles decreased from >45% for the first two infestations to <26% for the third and fourth infections on bluegill sunfish (*Lepomis macrochirus*), although in that study metamorphosis success rates appeared to decrease with each infestation. The low metamorphosis success across the entire experiment in the present study may suggest that fish displayed some form of resistance to glochidia development unrelated to an acquired immune response. Across all three experimental infestations, 77% of glochidia detached from the bullies and 23% encysted and transformed, much lower than the >90% transformation rates described by Barnhart et al. (2008) suggesting different populations of common bully may vary in their host compatibility for *E. menziesii*.

In the present study, glochidia that dropped off within the first few days either did not attach successfully or failed to encyst on the fish. Glochidia continued to detach over the entire course of each infestation, as also found during the paper pondshell mussel infestation by Rogers and Dimock (2003). From an immunological perspective, the fish immune system would be expected to play an important role in protection from glochidia attachment or encystment through innate and/or adaptive responses (Lieschke & Trede, 2009). For example, fish may have an innate defensive response to glochidia attachment such that fish do not require previous exposure of the host individual to parasite antigens. Alternatively, fish may acquire immunity to glochidia via adaptive immune responses, as tested in the present study, for example through antibody production (Barnhart et al., 2008). Adaptive immunity may occur slowly in fish, therefore only affecting glochidia attachment

after multiple infections (Bauer & Vogel, 1987; Rogers & Dimock, 2003; Dodd et al., 2005).

The innate response of the fish immune system therefore determines whether the fish serves as a compatible host in the first instance, but adaptive immunity determines whether a host fish becomes resistant to glochidia after multiple infections (Barnhart et al., 2008). Some studies have reported that the production of antibodies in response to glochidia infestation increases with maturity of the host and rising water temperatures (O'Connell & Neves, 1999). In the present study, live juveniles excysted as early as four days post-infestation when water temperatures averaged 22^{o}C, compared to nine days during a study by Moore and Clearwater (in press) using the same experimental set-up and the same source of female mussels, but at average water temperatures of 21^{o}C. Although this temperature difference is small, it may become significant in terms of degree days over a period of time and may have limited the time available for antibody production.

This research also raises questions around whether there is a threshold for the number of times bullies may be infested with glochidia, and what may occur in the field within or between glochidia release seasons. Previous studies have found it may take at least two infestations for resistance to fully develop (Rogers & Dimock, 2003; Dodd et al., 2005), but that resistance can be lost quickly (Bauer & Wächtler, 2001; Dodd et al., 2006). It is therefore plausible that fish develop resistance to glochidia attachment within a glochidia release season, especially towards the end of summer, and then lose it over the winter in time for the next spawning season. In the Waikato, the glochidia release season extends October through to late March, with a peak thought to occur from December to January (Clearwater et al., 2011). This timing broadly coincides with spring and summer spawning of diadromous *Gobiomorphus* species, such as common bully. During spawning, males guard benthic nests until the eggs hatch within two to four weeks (McDowall, 1990), suggesting the potential for an increase in glochidia infestation for nest-guarding males.

Another key factor affecting recruitment, independent of transformation rate, could be the post-parasitic fitness of the juveniles which may affect their ability to survive in benthic sediments post-release. This was not monitored in the present study, and

nor were the juveniles from individual fish kept separate, so assessment of overall juvenile fitness and variability between fish was not possible. However, monitoring the post-attachment phase of juveniles to assess fitness would be advisable in future studies because, while host-fish may still produce live juvenile mussels, they may not necessarily survive for long. Marwaha et al. (2017) found that, although higher temperatures decreased the duration of the parasitic phase, there were strong positive relationships between the parasitic phase duration, size at excystment, and post-parasitic growth survival rates. Accordingly, juveniles that have longer parasitic phases appear to have greater fitness for a benthic lifestyle as they are larger and grow faster (Marwaha et al., 2017). Österling and Larsen (2013) also found evidence that the longer-term survival rate of juvenile mussels increases with greater size at excystment. These results suggest that warmer water temperatures may produce juvenile mussels faster at the expense of fitness, meaning that it is ecologically more beneficial for mussels to prolong the parasitic phase.

The above findings underscore the importance of water temperature regimes for the metamorphosis and fitness of juvenile mussels relative to host compatibility. The results also provide important insights into potential captive-rearing procedures for freshwater mussels to maximise juvenile recruitment for *E. menziesii* from common bully hosts specifically. Because host availability and compatibility are critical in the management of populations of endangered unionids (Schwalb et al., 2011; Douda et al., 2014; Haag & Stoeckel, 2015), a comprehensive understanding of factors affecting both fish immunology and juvenile fitness is important for conservation efforts.

3.4.3 **Fish size relationships**

The number of glochidia that attach to a fish at any one time will likely impact the success of metamorphosis, and potentially be more burdensome for smaller fish suggesting fish size may influence transformation. Österling et al. (2014) reported a decrease in foraging activity and/or dominance performance among brown trout (*Salmo trutta*) heavily infected by glochidia of freshwater pearl mussel (*Margaritifera margaritifera*), while Thomas et al. (2014) reported that glochidia attachment by the pearl mussel may impose a respiratory burden to brown trout. Similarly, Filipsson et al. (2018) reported that high glochidia loads may have

restricted oxygen uptake by the gills of brown trout, but that a low glochidia load did not influence host-fish performance in terms of competitive ability. However, Nezlin et al. (1994) reported that freshwater pearl mussel glochidia appeared to have a negligible effect on the gills of Atlantic salmon (*Salmo salar*).

During the present study, there did not appear to be any major changes in fish behaviour or activity compared to the acclimatisation period prior to infestation. For the size range of common bullies used in these experiments (>48 mm length), the number of glochidia attached to individual control fish was not strongly related to fish surface area (14% of variance explained). However, the number of juveniles produced by treatment fish was more strongly related to fish size (i.e., 38% and 29% of variance explained by size in second and third infestations, respectively). Although not statistically significant, this finding provides some support for the observation of Marwaha et al. (2019) who observed a shift in the host immune response, from resistance in younger fish to a higher tolerance in older fish, such that larger fish were more likely to be re-infested. However, this is not consistent with field observations by Roper and Hickey (1994) who noted that attachment rates for *E. menziesii* appeared much lower on adult native fish. While a rigorous assessment of fish size effects was not possible based on the laboratory experiments given the absence of small fish, my field study supports these findings as 33% of variance in glochidia numbers could be explained by fish length for commons/Crans bullies (Chapter 4).

Finally, although several studies report that glochidial infection is not harmful to the host, many do not consider delayed impacts of glochidial infection. Ooue et al. (2017) suggest there is a time lag in physiological and somatic responses to parasite infections such as glochidia. Therefore, while high densities of attached glochidia may limit some hosts, there may be additional impacts on host fitness following the excystment of juveniles. Monitoring of fish behaviour and development after excystment, particularly as fish grow through multiple seasons, would therefore be advisable to determine longer term effects of infestation on host fish.

3.5 Conclusion

Overall, while the degree of resistance to glochidial infestation may vary between mussel species and their hosts, resistance appears to never completely prevent glochidia transformation (Rogers & Dimock, 2003; Dodd et al., 2005; Strayer, 2008). The lack of detectable acquired immunity/resistance in common bully, at least over one reproductive season, is promising for the future conservation of *E. menziesii* as, in a natural setting, host-fish are likely to be repetitively infested with glochidia over the mussel spawning season. This laboratory study suggests that common bully will be able to continue to produce viable juvenile mussels even though metamorphosis success rates may be low (≤ 30%).

Chapter Four

Temporal patterns of glochidia infestation on host fish: A field investigation in three Waikato streams

4.1 Introduction

Unionid freshwater mussels have a complex life history involving a parasitic larval stage where the glochidia must find and attach to a fish host to complete development to the juvenile stage (Neves & Widlak, 1988; Walker et al., 2001). Female mussels are fertilized through gametes released by the male into the water column during the reproductive season. After drawing these gametes in through inhalant siphons, the eggs are fertilized in brood chambers within the female gill and larvae develop into glochidia (Barnhart et al., 2008). Glochidia are then released through the exhalant siphon of a female mussel into the water column (Neves & Widlak, 1988). These glochidia are small bivalves that parasitize internal gills, external fins or other tissue structures of a host fish (Rogers-Lowery & Dimock, 2006; Barnhart et al., 2008).

Many freshwater mussel species have evolved an array of strategies to transfer glochidia to a host fish. While some species broadcast glochidia that drift in stream currents (typically host generalists), others exhibit adaptations for host capture by particular fish species, including elaborate mimicry to lure hosts, or release of packages of glochidia called conglutinates that resemble fish prey (Barnhart et al., 2008). Once a glochidium has attached to fish tissue, it is encapsulated by epidermal or branchial epithelial cells, forming a cyst (Wächtler et al., 2001; Rogers-Lowery & Dimock, 2006). Although the attachment and subsequent encystment of glochidia occurs rapidly, it represents a short yet critical part of the life cycle of a freshwater mussel (Rogers-Lowery & Dimock, 2006). Glochidia are parasitic on fish deriving nutrition from its host that probably increases their chance of subsequent survival (Fisher & Dimock, 2002). Encysted glochidia may also obtain a selective advantage while on fish via dispersal upstream, before becoming benthic-bound juveniles and adults with sedentary lifestyles (Watters, 2001;

Barnhart et al., 2008; Terui et al., 2014). Therefore naïve fish species migrating upstream at the time of glochidial release may be important both for dispersal and as hosts of glochidia (Barnhart et al., 2008).

Reproductive success of most generalist species is dependent on the broadcasting of remarkably large numbers of glochidia to increase the likelihood of an encounter with a fish host (Neves & Widlak, 1988). However, this larval release strategy can be greatly diminished if the numbers of available fish hosts are insufficient during the period that glochidia are in the water column (Neves & Widlak, 1988). Therefore, freshwater mussels are dependent on strategies such as seasonal release to increase the probability of contact between their larvae and the availability of viable hosts, some of which may be migratory.

The first observations that glochidia were parasites were made on wild-caught fishes by Houghton (1862). Since then, and until the present day, there has been a research focus on laboratory fish infestations using various mussel and fish species combinations (Kelly & Watters, 2010). Few studies have systematically documented the presence of glochidia on fish in the field, and even then these studies tend to be one-off collections or opportunistic events (e.g., Kelly & Watters, 2010).

There are three freshwater mussel species in New Zealand, also known locally as kākahi or kāeo (among other names), which can dominate benthic invertebrate biomass on lake and stream beds, namely *Echyridella menziesii, E. aucklandica*, and *E. onekaka*. *Echyridella menziesii* is widespread throughout New Zealand, whereas *E. aucklandica* is less common and restricted in its distribution which includes streams in the Waikato region. *Echyridella onekaka* is only present in the upper South Island (Figure 1.1). The glochidia of *E. menziesii* are about 300 μm wide when released by gravid females and they remain this size upon detachment from host fish (Melchior et al., in press). In comparison, the European freshwater pearl mussel, *Margaritifera margaritifera,* glochidia are smaller (c. 70 μm) at the beginning of the parasitic phase, but when fully developed they are much larger (c. 400–500 μm) indicating growth on fish hosts (Young & Williams, 1984).

Seasonal changes in water temperature and floods likely serve as environmental cues for reproduction of freshwater mussels (Walker et al., 2001; Hastie & Young, 2003). Previous research on the *E. menziesii* life cycle by Clearwater et al. (2011) suggests that the males begin spawning in late winter (August), and that glochidia are released in mid-summer, as they were first detected in January. This timing indicates that *E. menziesii* is a short-term brooder with a tachytictic classification (Watters & O'Dee, 2000), whereby the female releases glochidia shortly after maturity in contrast to a long term brooder that retains larvae over winter and releases glochidia over an extended period (Gascho Landis et al., 2012).

Echyridella menziesii are thought to be host generalists as they have been seen to parasitize a range of native fish (Clearwater et al., 2011; Brown et al., 2017), but no study to date has attempted to quantify or describe the parasitic relationship for an ecological assemblage of mussels and fishes. Recent laboratory research involving artificial infestation has found that *E. menziesii* can successfully attach and transform themselves on the head, mouth and fins of common bully (*Gobiomorphus cotidianus*), banded kōkopu (*Galaxias fasciatus*), shortfin and longfin eel elvers *(Anguilla australis* and *A. dieffenbachii*) and Canterbury galaxias (*Galaxias vulgaris*) (Brown et al., 2017). Kōaro (*Galaxias brevipinnis*) is also a known host (Brown et al., 2017), along with rainbow trout (*Oncorhynchus mykiss*) (Clearwater et al., 2014). Percival (1931) recorded giant bully (*Gobiomorphus gobioides*) as having glochidia attached, but so far there has been no evidence of transformation to juveniles on that host species.

Given the reliance on fish hosts, conservation of freshwater mussels requires an understanding of the status of host species and implementation of measures to conserve them (Ferreira-Rodríguez et al., 2019). Today, 76% (39 of 51 species) of indigenous freshwater fish are recognized as threatened with or at risk of extinction in New Zealand (Ministry for the Environment and Stats NZ, 2019). These statistics include some fish species which are confirmed freshwater mussel hosts. Host fish behaviour, migratory movements, local distributions and abundance all determine the actual exposure of fish to glochidia, hence it is vital that there are fish present in sufficient numbers at the right time to enable mussels to complete their life cycle and sustain mussel population recruitment (Atkins, 1979). Understanding this relationship and knowing which fish species are utilized by which mussel species

is essential before any attempts are made to preserve an endangered freshwater bivalve species. Without the host fish necessary for the continued reproduction of a given species, all conservation efforts targeting unionid mussels will be in vain (Bogan, 1993).

Therefore, this field study examines the distribution and abundance of glochidia on fishes caught from three contrasting streams over five months (October 2018-February 2019) spanning the glochidial release period. The aim was to quantify host utilization by *E. menziesii* by addressing five objectives: i) develop a field methodology for assessing glochidia attachment; ii) determine whether glochidia release is triggered by seasonal changes in stream temperature; iii) determine whether there is a temporal shift in host fish preference due to migratory fish movement patterns and seasonal habitat use; iv) explore whether fish behaviour and habitat use in a stream corresponds to glochidia infestation rates (i.e., are benthic fish such as bullies likely to have a higher infestation rate compared to pelagic fish which are less likely to encounter mussels); and v) investigate whether infestation rates are higher on naïve young-of-the-year fish exposed to glochidia for the first time compared to older fish that may develop resistance later on in the season (using size as a proxy for age; see also Chapter 3).

4.2 Methods

4.2.1 Study sites

Fish and mussel sampling occurred fortnightly at three streams in the Waikato region between 3 October 2018 and 15 February 2019. Sampling sites were on two coastal streams of different sizes draining directly into the Tasman Sea on the west coast – Pakoka River and Ohautira Stream, and on the inland, Mangapapa Stream which drains into the Waitoa River in the Matamata-Piako District (Figure 4.1, see Chapter 2 for study site details).

These three sites were selected based on the following criteria: i) abundant freshwater mussel populations (>300 mussels in a 50-m reach) and evidence of recruitment based on a non-skewed population size structure; ii) absence of downstream barriers to fish passage; and iii) suitable for electrofishing according to the criteria of David et al. (2010). Freshwater mussel survey data were provided by the Waikato Regional Council (see Chapter 2).

Physicochemical characteristics were recorded on each site visit (dissolved oxygen, temperature, conductivity). Tidbit temperatures loggers (TidbiT v2 UTBI-001, Onset Computer Corporation, USA) installed at the start of the study recorded water temperature every 15-minutes for the duration of the study at each site.

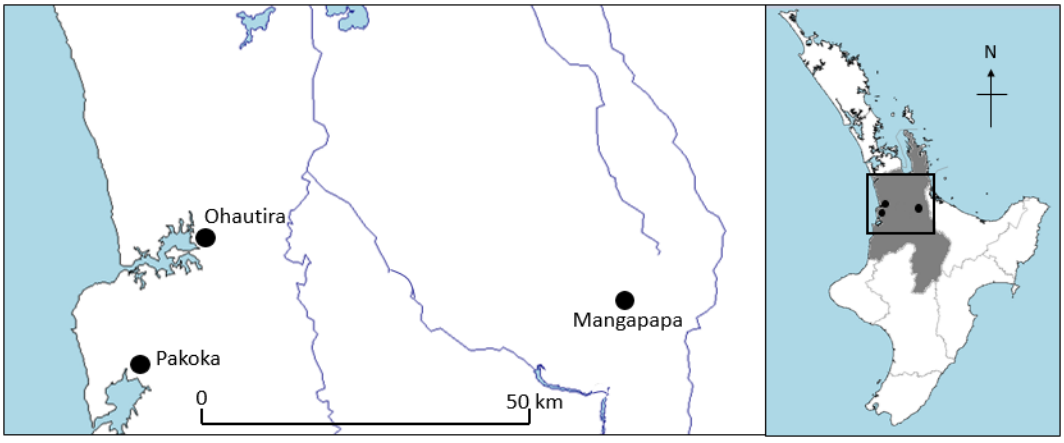


Figure 4.1. Locations of the three study sites in the Waikato Region, North Island (inset).

4.2.2 Glochidia development and release

Around 40 *E. menziesii* were randomly collected from each site monthly from October 2018 to February 2019 and held in 10 L buckets filled with source water for subsequent brood pouch assessment of females. Kakahi were gently opened (~5 mm) using a speculum to observe for swollen inner marsupial gill demibranchs (Aldridge, 1999) which were scored for brood pouch status (1-5) to assess female gravidity based on: i) volume (flat, small, fat, or very fat); ii) pigmentation (pale yellow, yellow or orange-yellow); and iii) presence or absence of eggs or glochidia within the inner demibranch (Melchior et al., in press). Otherwise, mussels were categorized as males or sexually immature females if the gill pigmentation was brown-orange, the gill volume was flat and no brood pouch was visible on the gills. Brood pouch status of 1-4 indicates the female is brooding, and 5 indicates the mussel has released her brood; scores indicating the pre-release or releasing of mature and viable glochidia (full or half-emptied brood pouch; i.e., stage 4) or post-release (emptied brood pouch; i.e., stage 5) (Melchior et al., in press) were of interest for this study as they indicated imminent or recent glochidial release. The length, width and height of each female mussel was measured to the nearest millimetre using Vernier callipers, and all mussel shells were visually scored for erosion, classed as a percentage of the mussel shell that was worn on a scale of 0-4: 0%, 1-25%, 25-50%, 50-75% or 75-100% worn, following McEwan (2015).

Six nested (250 µm and 50 µm mesh) stream drift nets were deployed at two cross sections along the Pakoka River and Ohautira Stream sites to survey for the presence of glochidia in stream drift. Mangapapa Stream was not included as the bedrock substrate was not suitable for securing drift nets. At Ohautira Stream, three drift nets were placed on the true left, middle and true right just downstream of a cluster of mussels, and another three drift nets were placed downstream of a large pool. At Pakoka River, the drift nets were placed downstream of a pool that contained >500 kakahi, and another three nets were placed 50 m further downstream. These same transects were sampled monthly (November 2018 to January 2019) to determine whether glochidia could be detected in the stream drift. Water velocity and depth at the net mouth were measured on each date, by taking an average of three measurements using a Marsh-McBirney Flo-Mate™ portable velocity flow meter and wading rod (Hach, 2009, U.S.A) so that numbers of glochidia collected could be expressed per m^3 of water filtered.

Nets were emptied at hourly intervals between times 09:00 and 13:00 by rinsing them with a wash bottle filled with source water and transferring the concentrated trapped material into 100 mL containers. Once back in the laboratory, 2-3 drops of Rose-Bengal were added to each sample and left for >24 hrs to stain any glochidia pink. Samples were then passed through a 250-µm and an 85-µm mesh sieve by rinsing with dechlorinated tap water to create two 40 mL samples of 85-250 µm and >250 µm fractions. For 250 µm samples a transfer pipette was used to extract 5 mL of sample and using a Bogorov counting tray to count the number of glochidia in the sample. A 3 mL subsample was extracted for enumeration from the 85-250 µm mesh samples which were a lot denser.

4.2.3 **Fish sampling**

Minnow traps and electrofishing were used to capture fish for observations of glochidia infestation, and to assess changes in fish community composition over time. Six fine-mesh (2 mm) minnow traps (not baited) were deployed at each site for several hours during the day in deep pool habitats outside the electrofishing reach. Two electrofishing approaches were used to characterise fish communities and obtain specimens for glochidial assessment: i) single pass fishing in the 50-m reach at monthly intervals to characterise abundance of dominant species, followed by spot-fishing if required, to augment single-pass catches for glochidial assessment; and ii) spot-fishing in between monthly reach-fishing, to target dominant species for glochidial assessment. Single-pass electrofishing is standard practice in New Zealand for estimating relative density in wadeable stream habitats and does not require fixed stop nets (David et al., 2010). Electrofishing has been reported to have little impact on fish and is harmless to European freshwater pearl mussels (*M. margaritifera*) when carried out correctly (Hastie & Boon, 2001).

Typically, the electro-fisher started on the edge of the bank sweeping side-to-side towards the stop netter positioned 2-4 m downstream. In general, a lane of approximately 6-8 m^2 was fished on each pass ensuring consistent fishing effort with no bias towards particular habitats. Once the width of the stream had been fished (moving across approximately 1.5 m each time), the fisher and stop netter moved upstream approximately 3 m and fishing continued upstream from bank-to-bank until a 50-m reach had been fished. If <10 fish of dominant species at a

particular site were caught, the fisher would continue to spot-fish upstream targeting likely habitats for these species. Upon capture, all fishes were held in 20 L buckets filled with source water until identified to species level and measured for total length (TL) to the nearest millimetre.

4.2.4 Analysis of glochidia infestation

Each fish was visually assessed in the field for *E. menziesii* glochidia infestation, initially by eye followed by confirmation using a 40x hand-held magnifier with a LED light (Magnifiers New Zealand Ltd., 12 mm lens). Glochidia were identifiable as whitish, bladder-like objects (c. 300 µm x 250 µm) with a rounded-triangular shape on the surface of the fish. Fish <80 mm were placed in a square glass viewer with stream water to keep fins upright, while fish >80 mm were placed in a viewing tray with water at a depth of 10 cm. Eels were anaesthetized in AQUI-S at 0.3 mL 10 L^{-1}, and fin clips were initially taken for laboratory confirmation of field observations. All potential external attachment structures (i.e., lips, dorsal, adipose, pectoral, pelvic, anal, caudal fins and operculum cover) on captured fish were examined, and the numbers of attached or encysted glochidia on each structure were recorded (Appendix 1). Encysted glochidia were recognisable as being fully enclosed by fish epidermal tissue (Figure 4.2A).

With the exception of fish retained for laboratory examination (see below), fish were placed into a recovery bucket without anaesthetic (with battery powered aerators used as required), and returned to the stream unharmed after field glochidia assessment. A subsample (n = 5) of the bullies and īnanga, covering a range of lengths, was transported back to the laboratory to validate field observations, and inspect internal structures (i.e., mouth and gills) for glochidia. These species were euthanized by an overdose of AQUI-S (>0.8 mg L^{-1}) in individual containers, and later placed in the fridge until dissection (usually within 1-3 days of capture). During dissections, glochidia were occasionally observed floating in the AQUI-S-water solution that the fish was stored in. Therefore, the AQUI-S solution was sieved (60 µm mesh) to check for any detached glochidia or juveniles. These 'floating' glochidia were pooled together with external glochidia counts, as it was assumed they had dropped off external structures. Preliminary testing showed that glochidia stayed attached after one day in AQUI-S, however, the longer the fish

was left in AQUI-S the more glochidia detached (externally and internally attached glochidia on a fish, including those that had encysted, all detached and were in solution after 15 days).

Fins of euthanized fish were surgically removed and placed in a Petri dish of dechlorinated tap water for observation under a stereomicroscope (Wild M3B, Heerbrugg, Switzerland). Once the external surfaces of the fish had been checked to confirm field observations, the head and gills were examined using fine forceps to quantify internal glochidia load for each fish. After extracting the gill basket from the fish and counting any associated glochidia, the mouth cavity was flushed out using dechlorinated tap water to check for any remaining detached glochidia.

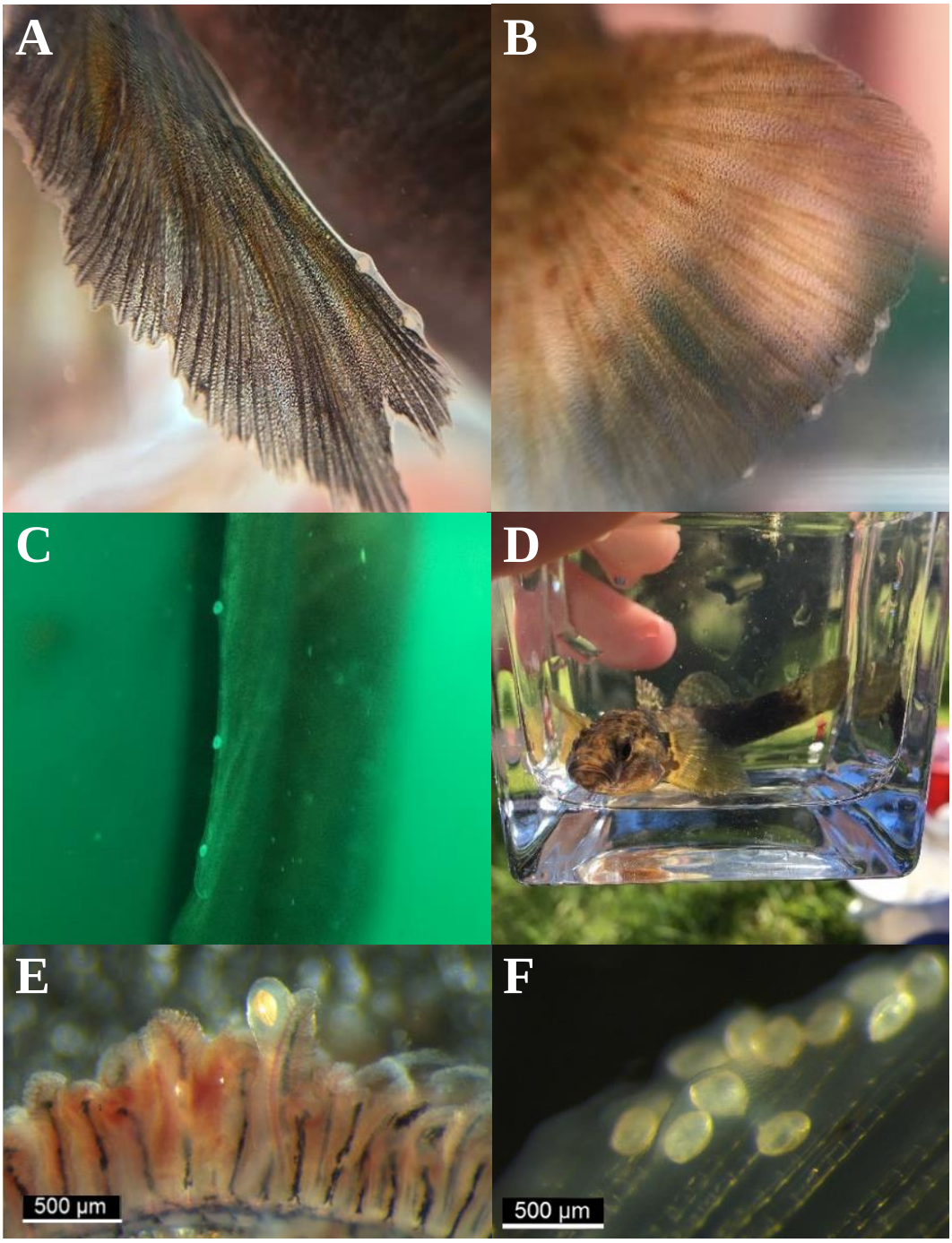


Figure 4.2. Glochidia attachment sites: A – right pelvic fin of a redfin bully showing encysted glochidia covered by fish tissue (~10x magnification); B – common bully tail (four glochidia); C – three glochidia attached but not encysted onto a longfin eel dorsal fin (in viewing tray for eels and larger fish); D – glass aquarium used to identify glochidia on smaller fish (<80 mm); E – a microscope image of a glochidium attached and encysted to the gills of a bully; F – a pectoral fin of a common bully with glochidia attached (some are encysted).

4.2.5 Statistical analysis

All statistical analyses were performed using Microsoft Excel (v 15.33), STATISTICA (v 13; StatSoft, Oklahoma, USA), and PRIMER 6 (v 6.1.15; Plymouth Marine Laboratory, Ivybridge, UK). All analyses were considered statistically significant at $P < 0.05$.

Analyses were performed on: i) 'reach-scale data,' collected from electrofishing the same 50-m reach each month; and, ii) 'pooled data' collected via the three sampling methods combined (50-m reach electrofishing, spot-fishing and minnow traps). The number of each fish species per m^2 and their average length was compared over time by site for reach data to determine the abundance of potential glochidia hosts and fish recruitment patterns.

Kruskal-Wallis (K-W) tests, followed by a post-hoc multiple comparisons test where a significant main effect was detected, were used to compare: i) mussel size (length, width, height) and shell erosion between sites; and ii) monthly brood pouch status between sites. The relationship between brood pouch pre-release (4) and post-release (5) stages and stream temperature was explored visually for all three sites to determine any consistent associations between the timing of glochidia release and water temperature.

Among-site differences in the taxonomic composition of the native fish assemblages were visualized using non-metric multidimensional scaling (nMDS) which creates two-dimensional plots of relationships among sites, where the distance between two points is proportional to their biological dissimilarity, as determined using a dissimilarity coefficient. Each nMDS was run on a Bray-Curtis dissimilarity matrix derived from log (x+1) transformed density data for all fish taxa. The log (x+1) transformation serves to downplay the influence of extremely abundant species while also accounting for rare species (Clarke et al., 2014). The nMDS plot was overlaid with fish species vectors using a correlation threshold of 0.2. Common and Crans (*Gobiomorphus basalis*) bullies were analysed together as the smaller fish of each species could not be differentiated. PERMANOVA was used to test for significant differences between fish community composition between sites. A Similarity Percentages (SIMPER) test was then used to determine which fish species were most responsible for differences in community composition

between clusters on the nMDS ordination, with significant clusters identified using similarity profile analysis (SIMPROF).

Glochidia attachment on fish from reach electrofishing was expressed as presence/absence to determine the frequency of infestation for different fish species. Spearman rank analysis was performed to explore fish-size relationships for redfin bullies and common/Crans bullies, using the number of glochidia attached to and length of each fish. The number of glochidia that attached to various external surfaces of bullies was expressed as average numbers of glochidia per structure and as a percentage of the total glochidia observed externally on field-collected fish. The non-parametric Wilcoxon test was used to compare internal vs external glochidia attachment data after laboratory dissections.

4.3 Results

4.3.1 Stream temperature

Maximum daily stream temperatures recorded at 15-min intervals between August 2018 and March 2019 were 25.3°C, 23.2°C, and 26.0°C at Pakoka, Ohautira, and Mangapapa, respectively (Table 4.1). Pakoka River had the lowest recorded temperature in September 2018 (9.4°C). Mangapapa Stream was the warmest, averaging 17.3°C from August to March. Monthly average stream temperatures across all three sites were lowest in August and increased over time to be highest in January-February (Figure 4.3).

Table 4.1. Summary of monthly temperature (°C) ranges (minimum-maximum) across all three sites, from August 2018 to March 2019, and overall average temperature for each site (n=14,873-18,818). The highest recorded temperature for each site is shown in bold. -, no data.

	Pakoka	**Ohautira**	**Mangapapa**
Mean ± SE (n = 14,873-18,818)	16.1 ± 0.02	17.0 ± 0.02	17.3 ± 0.03
Overall range	9.4 – 25.3	10.6 – 23.2	9.5 – 26.0
Monthly ranges			
August	10.5 –13.4	–	9.5 – 12.9
September	9.4 – 16.0	–	10.0 – 15.8
October	9.7 – 17.8	11.9 – 16.1	11.6 – 17.9
November	10.1 – 20.8	10.6 – 18.6	12.3 – 20.7
December	13.2 – 23.9	12.6 – 20.5	14.6 – 22.0
January	15.2 – **25.2**	15.2 – **23.2**	17.5 – **26.0**
February	13.4 – 24.1	14.0 – 22.0	16.0 – 24.7
March	13.6 – 19.4	14.2 – 19.2	16.6 – 20.4

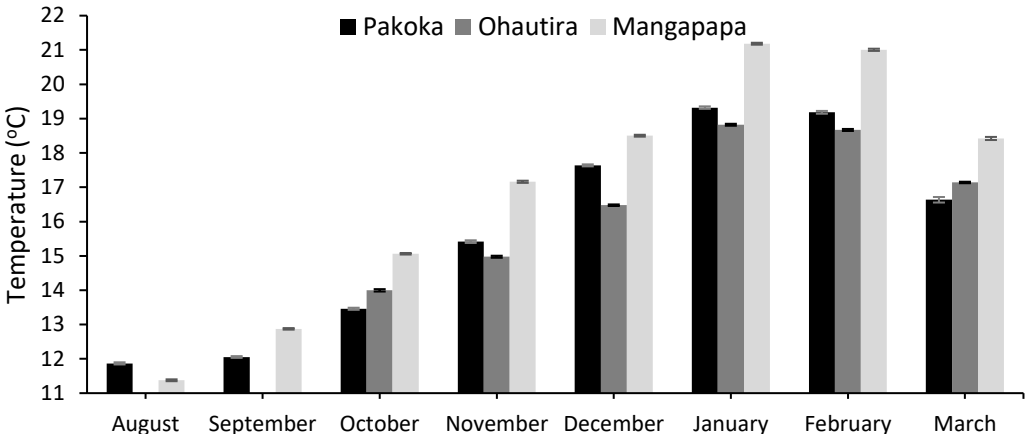


Figure 4.3. Summary of monthly temperature means (± SE) at each sampling site. Note: y-axis begins at 11°C; no data for Ohautira Stream in August and September.

4.3.2 **Characteristics of mussel populations**

Mussels collected during sexing and female brood pouch assessment were largest (length, width and height) in Pakoka River which also had the highest amount of shell erosion (Table 4.2). Mussels were on average smallest at Mangapapa Stream, followed by Ohautira and Pakoka. Females made up 51%, 46% and 55% of the sampled mussels at Pakoka, Ohautira and Mangapapa streams, respectively. Kruskal-Wallis tests showed there were significant differences ($P < 0.001$) between sites in the length, width, height, and shell erosion of mussels. The only instances where there were no significant pairwise differences was in shell height and shell erosion between mussels assessed at Ohautira and Mangapapa streams ($P > 0.05$).

Table 4.2. Overall averages (± SE) for shell erosion, sex, and size (females only) of *Echyridella menziesii* collected across all dates sampled (October – February) separated by site. Kruskal-Wallis H values are presented, where H values with $P < 0.001$ are in bold. -, not applicable.

		Pakoka	**Ohautira**	**Mangapapa**	**H**
Males and females	Total no. examined	176	182	201	-
	Shell erosion (0-4)	2.2 ± 0.07	1.5 ± 0.02	1.6 ± 0.06	**62.18**
Females	% females (n)	51 (90)	46 (83)	55 (110)	-
	Length (mm)	60.8 ± 0.76	56.0 ± 0.49	52.3 ± 0.56	**78.33**
	Width (mm)	19.8 ± 0.44	16.0 ± 0.23	14.8 ± 0.25	**83.16**
	Height (mm)	35.8 ± 0.50	30.7 ± 0.30	29.9 ± 0.44	**88.34**

4.3.3 **Brood pouch development**

Mean brood pouch stage over the sampling period was highest at Pakoka River (3.9) and lowest at Mangapapa (3.3). Kruskal-Wallis tests indicated significant differences in mean brood pouch status among sites across all dates ($H = 15.26$, $P < 0.001$) (Table 4.3). Pairwise comparisons indicated that overall brood pouch status in Mangapapa Stream was significantly lower to that in Pakoka and Ohautira streams ($P < 0.001$, $P = 0.015$, respectively). When separated by month, the statistically significant differences were between Ohautira and Mangapapa mussel brood pouch status during December ($P = 0.019$), and in February between Mangapapa compared to Pakoka and Ohautira streams ($P = 0.003$, $P = 0.009$, respectively).

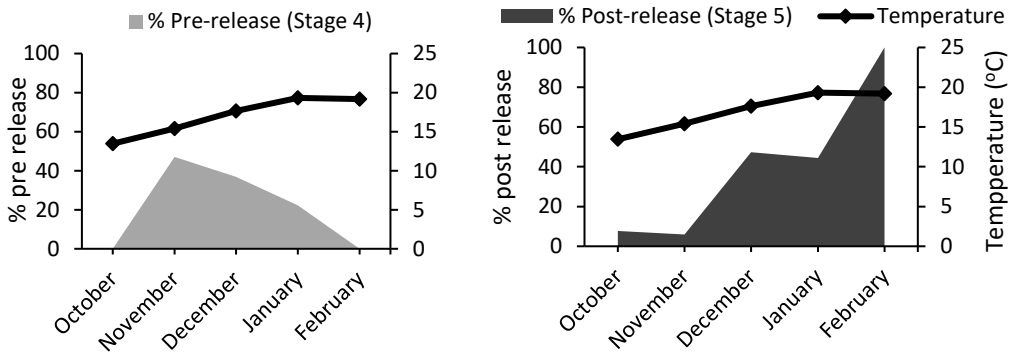
Table 4.3. Overall and monthly averages (± SE) of brood pouch status (October – February) at three sites. Bold H values indicate a significant ($P < 0.05$) difference detected using Kruskal-Wallis test and subsequent pairwise differences. n = 83-110 female mussels across all dates.

	Pakoka	**Ohautira**	**Mangapapa**	**H**
Mean brood pouch stage (1-5)	4.0 ± 0.12	3.8 ± 0.13	3.3 ± 0.12	**15.26**
Monthly averages				
October	2.5 ± 0.24	2.4 ± 0.36	2.7 ± 0.22	2.14
November	3.4 ± 0.21	3.1 ± 0.16	3.0 ± 0.28	2.88
December	4.1 ± 0.25	4.6 ± 0.16	3.8 ± 0.18	**9.37**
January	4.1 ± 0.20	4.4 ± 0.13	4.0 ± 0.19	1.42
February	4.9 ± 0.04	4.9 ± 0.05	3.1 ± 0.32	**19.13**

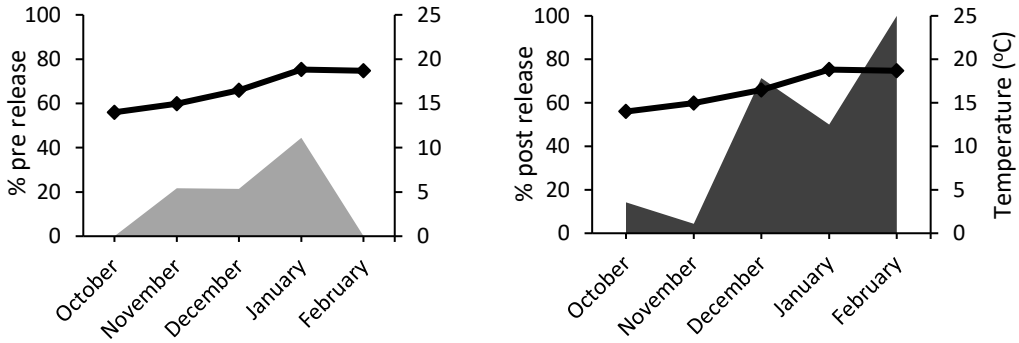
Brood pouches appeared to move from pre-release to post-release stages as mean monthly stream temperature increased over the summer period (Figure 4.4). Pre-release peaked in November at Pakoka River when stream temperatures averaged 15.4^{o}C, and post-release stages peaked in February when stream temperature was 19.2^{o}C. Mussel pre-release peaked at Ohautira Stream in January at average temperatures of 18.8^{o}C and post-release peaked in February when stream temperatures were averaging 18.7^{o}C. At Mangapapa Stream, pre-release peaked in December at a temperature of 18.5 ^{o}C and post-release in January when stream temperatures were 21.2^{o}C.

By January, most of the mussels sampled at each site had reached the pre-release stage and no mussels were at this stage in February. Mussels had released their brood or broods (i.e., post-release) by February with sharp peaks of 100% post-release for Pakoka and Ohautira, but at Mangapapa stream there was a more extended and lower peak at c. 40% post-release in January and February.

Pakoka



Ohautira



Mangapapa

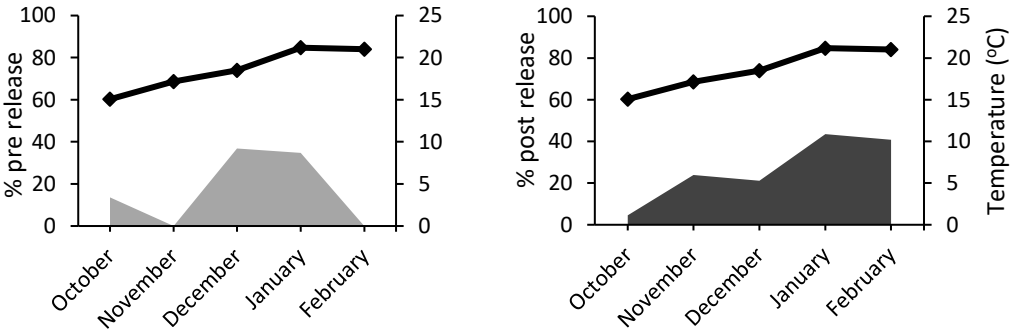


Figure 4.4. Percent of mussels at glochidia pre-release (left) and post-release (right) stages at three sites with monthly stream temperature averages shown as solid line. Note: there were no samples taken in early October or late February.

Stream drift sampling conducted monthly at Pakoka River and Ohautira Stream from November to January yielded few glochidia ($\leq$2.5 m^3 h^{-1}) with no apparent differences between true left, true right, and middle drift nets (data not shown).

4.3.4 **Fish community composition**

A total of 1073 native fishes were captured during the sampling across all three sites. Of those, 701 fish were obtained from electrofishing a 50-m reach, 308 from spot-fishing, and 64 from minnow traps. The most commonly occurring species in 50-m reach-scale sampling was shortfin eel followed by longfin eel and īnanga. Longfin eel dominated catches numerically across all sites (23.4%), followed by shortfin eel (22.8%) and īnanga (17.2%).

A two-dimensional non-metric multi dimensional scaling ordination (nMDS) plot had a stress value of 0.09 indicating that the ordination was a good representation of similarities between fish species assemblages among sites (Figure 4.5). A PERMANOVA, testing for differences in fish community composition between sites and over time showed significant differences (Pseudo-F = 11.29, P = 0.001). Pairwise testing between the sites but not taking time into account, revealed significant differences between all three sites ($P < 0.05$). SIMPROF analysis indicated two significant clusters at a similarity of 40%, shown as ellipses in Figure 4.5. One cluster (left) envelops the nearby coastal Pakoka and Ohautira sample dates, and the other cluster (right) contains Mangapapa Stream samples. Further, a SIMPER test revealed that the species most responsible for differences in community composition between clusters were īnanga (17.1% of dissimilarity) and longfin eels (15.2%) for the coastal stream cluster, and shortfin eels (30.4%) and common/Crans bully (17.5%) for the Mangapapa cluster. A vector overlay of fish species also highlighted the association of redfin bullies, common smelt (*Retropinna retropinna*) and lamprey (*Geotria australis*) to the western coastal streams, high densities of īnanga in Ohautira Stream during November, and the occurrence of torrentfish in Mangapapa Stream (Figure 4.5).

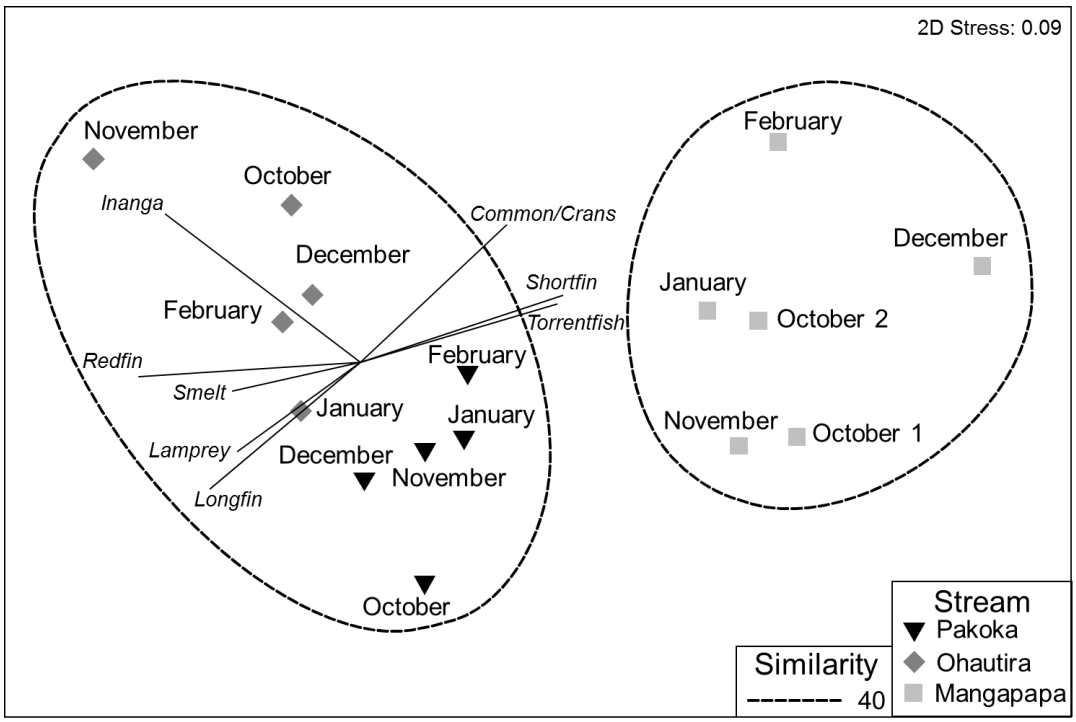
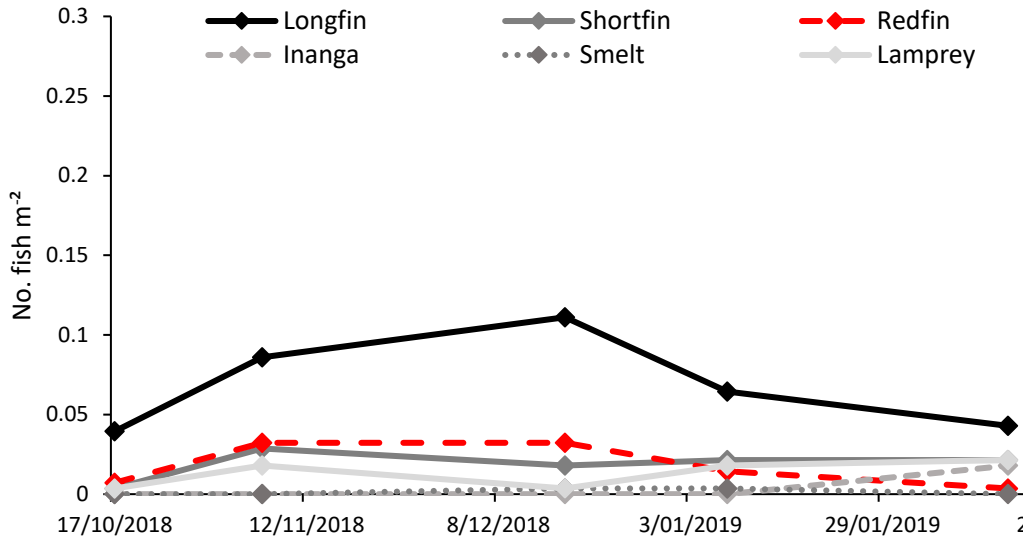


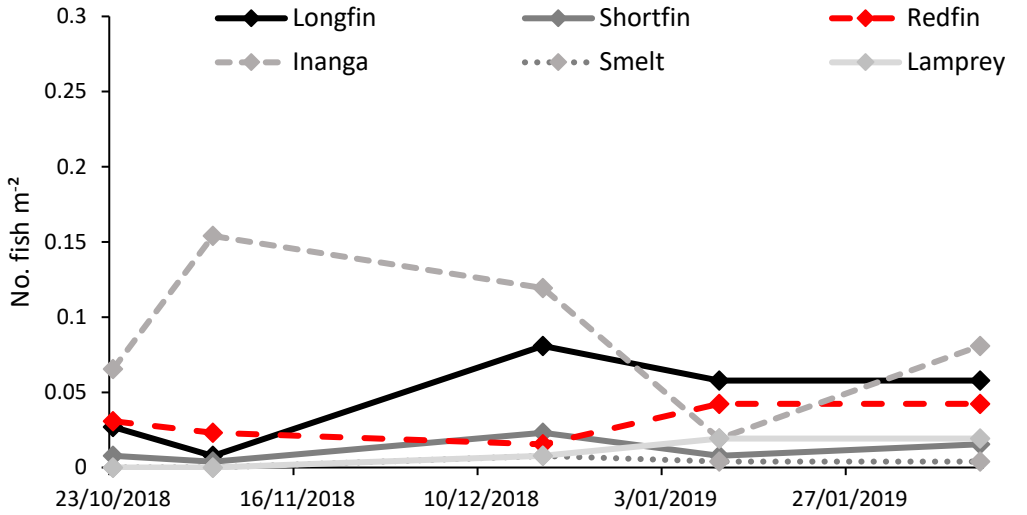
Figure 4.5. Non-metric multi-dimensional scaling (nMDS) ordination plot of log (x+1) transformed fish species abundance (fish m^{-2}) from reach electrofishing of Pakoka, Ohautira and Mangapapa streams on six occasions from October 2018 to February 2019 based on a Bray Curtis similarity distance matrix. Ellipses are based on a SIMPROF similarity of 40% showing statistically different clusters ($P < 0.05$); vector overlays of fish species show relationships with $r > 0.2$.

Changes in the abundance of individual species captured (per visit) over October 2018 to February 2019 are explored for all three sites in Figure 4.6. Longfin eels were the dominant species caught over all dates within a 50-m reach at Pakoka with highest densities in December (eels ranged from 87 to 650 mm). Redfin bully and shortfin eel were the next most abundant species at Pakoka, followed by īnanga, common smelt and lamprey (ammocoetes). Ohautira fish communities were dominated by īnanga which peaked in density (when schools of juveniles migrating upstream were intercepted) during October, followed by longfin eel and redfin bully which had higher numbers in later samples. Low densities of shortfin eel, lamprey and common smelt were also caught in Ohautira Stream. At Mangapapa Stream there was a marked December influx of shortfin eel elvers which dominated catches on most dates except in January/February when there was an increase in common/Crans bullies. Low densities of longfin eel and torrentfish were also caught in Mangapapa Stream.

Pakoka



Ohautira



Mangapapa

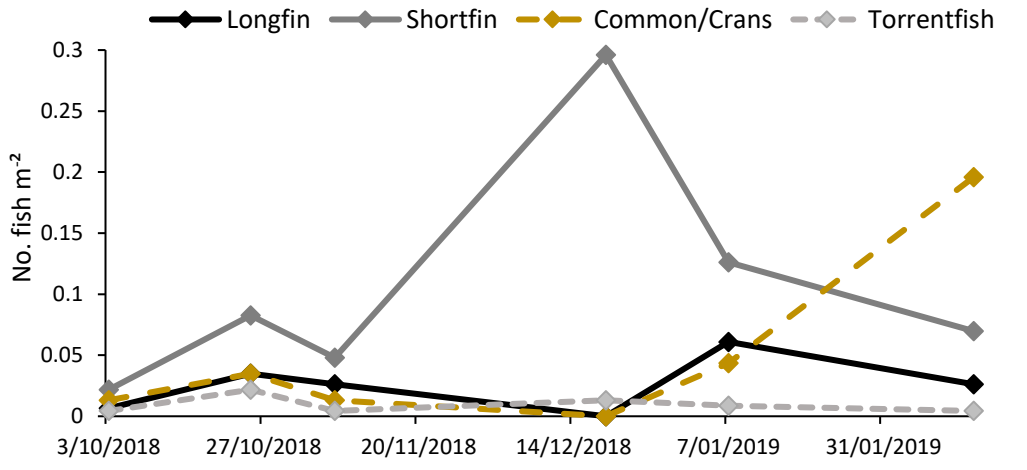


Figure 4.6. Density of fish species per m^2 over time for three sites based on reach-scale electrofishing. Note: differing x-axes scales between graphs.

The relationships between the average length of fish caught and time were explored using regression analysis of reach data, and relationships with an $R^2 > 0.50$ have trend lines shown (Figure 4.7). At Pakoka River the average length of longfin eels and redfin bully increased over time ($R^2 = 0.53$ and 0.88, respectively) although the length range for both species was small at Pakoka, whereas there was no apparent change in mean shortfin length over time. There were declines in longfin and shortfin eel length over time at Ohautira Stream ($R^2 = 0.70$ and 0.58, respectively), increases in īnanga lengths ($R^2 = 0.84$), but no change in mean redfin bully length over time. In comparison, Mangapapa Stream appeared to have an influx of smaller (mm) fish (both eels and bullies) during late December as there was a marked decrease in average length for all species. There were no longfin eels caught at Mangapapa Stream during the December sampling.

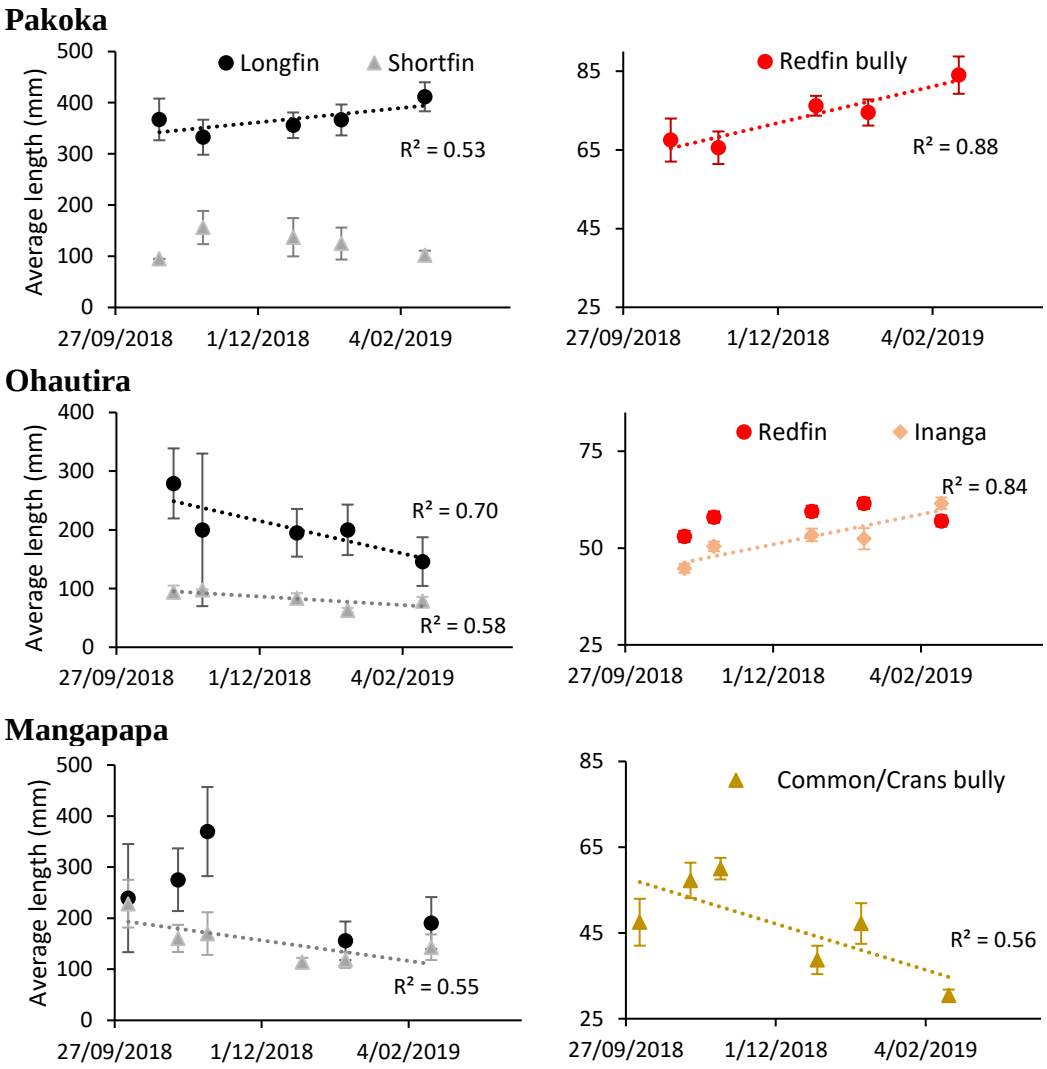


Figure 4.7. Average length (± SE) of eels and bullies over time at each site. Lines show regressions with an R^2 value >0.5.

4.3.5 **Quantifying glochidia attachment in fish communities**

Of the total fish collected across three sites during 50-m reach-scale electrofishing, 103 had visible glochidia (14.7% infested), with a total of 298 glochidia found. Of the 387 longfin and shortfin eels caught, 34 had one or more glochidia attached (i.e., 8.8% of eels caught were infested), while of 144 bullies (79 common/Crans, 65 redfin), 45% were infested with glochidia, comprised of 31 common/Crans bullies and 33 redfin bullies.

The percentage of fish caught within each 50-m reach that had glochidia attached externally was plotted over time (Figure 4.8), to examine temporal patterns of infestation. On the first site visit to Pakoka River in October 2018, the highest proportion of fish caught were infested with glochidia (40%), but from then onwards the proportion decreased over time. A similar trend was observed in Ohautira Stream where infestation rates declined from around 15% of fish in late October to 2-4% in January-February. The percentage of fish infested at Mangapapa Stream (on average 14 %) fluctuated more over time in comparison to Pakoka and Ohautira streams, with highest % infestation over late October/November and January/February (Figure 4.8).

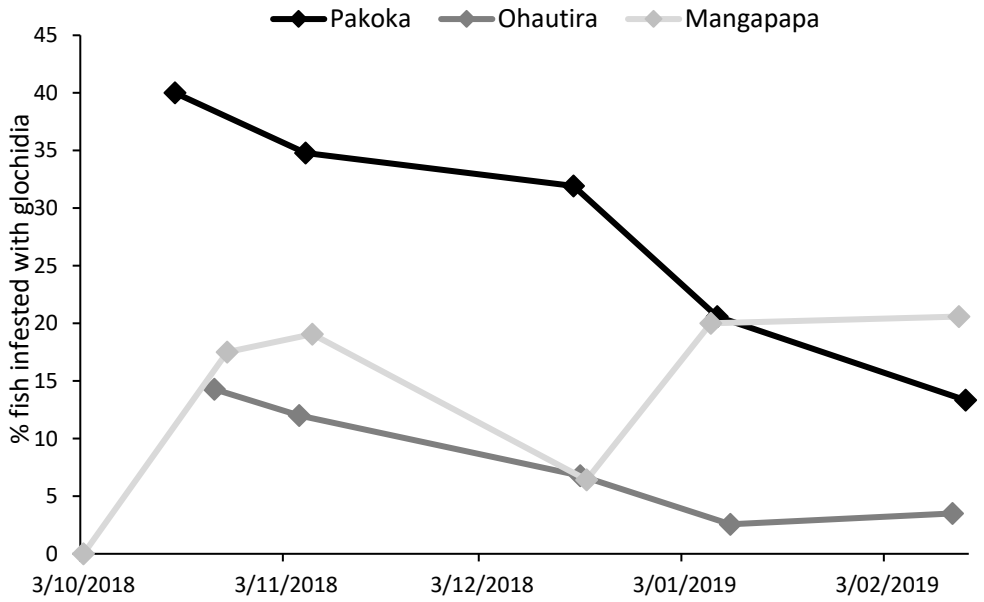
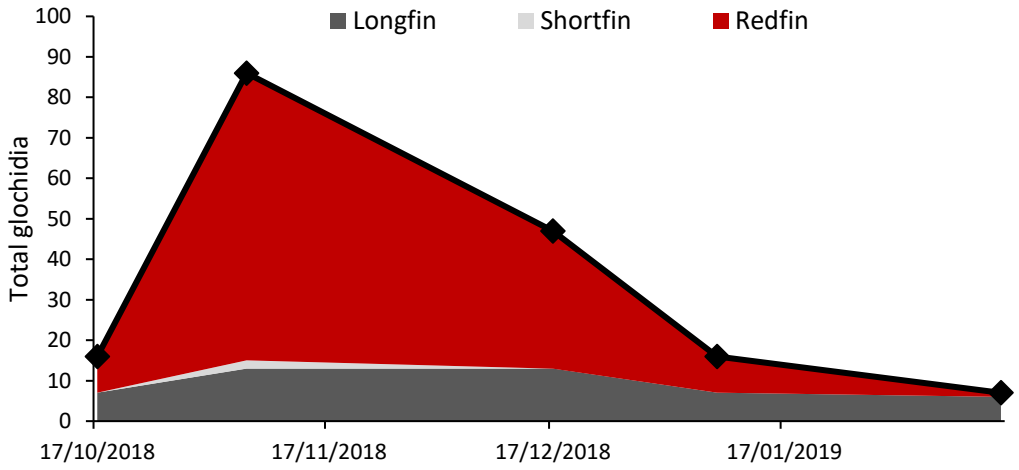


Figure 4.8. The percentage of fish infested with glochidia (presence/absence) out of the total number of fish caught across all sites from 50-m reach electrofishing.

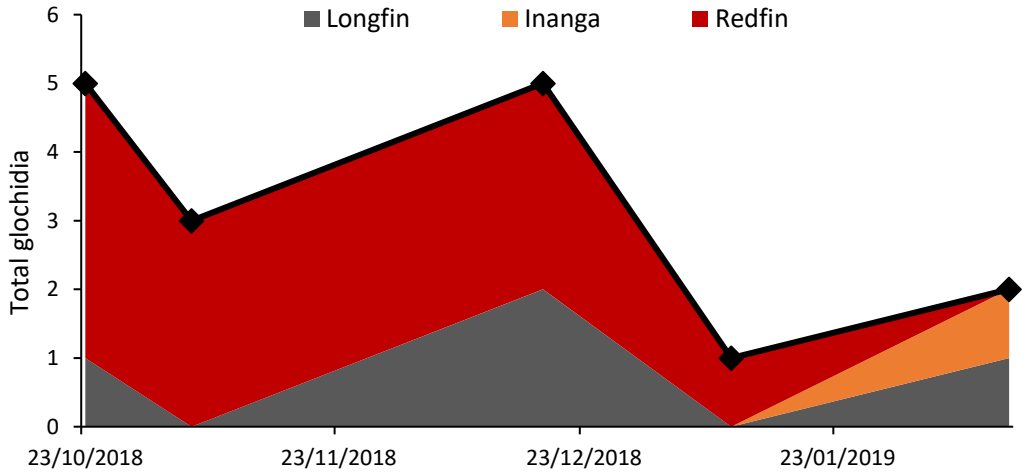
Using pooled data (reach-scale electrofishing, spot-fishing and minnow traps), 569 glochidia were observed on 187 fish, including 290 glochidia at Pakoka River, 29 at Ohautira Stream, and 250 at Mangapapa Stream. Redfin and common/Crans bullies collectively accounted for 86% (489) of total glochidia attached externally to fish (i.e., 237 at Pakoka River, 23 at Ohautira Stream, and 229 at Mangapapa Stream). The average number of glochidia attached to a redfin bully was highest at Pakoka River (4.6 ± 0.7) and lowest at Ohautira Stream (1.8 ± 0.4). At Mangapapa Stream, the average number of glochidia attached to common/Crans bully was 3.1 ± 0.4. The remaining 80 glochidia were attached to 47 fish which were a combination of īnanga (n = 1), torrentfish (n = 4), and longfin (n = 34) and shortfin eels (n = 8). At Pakoka River, one shortfin eel had two glochidia, and at Mangapapa seven shortfins had a total of 13 glochidia. For the 34 longfin eels collected across all three sites, 51 glochidia were found on 28 eels at Pakoka River, five glochidia on four eels at Ohautira Stream, and four glochidia on two eels at Mangapapa. No glochidia were observed externally on common smelt or lamprey.

Using reach-scale electrofishing only to explore temporal patterns, the maximum number of glochidia recorded on any visit to Pakoka Stream was 86 during early November, found on a combination of shortfin eel (n = 1 fish), longfin eel (n = 6) and redfin bully (n = 9) (Figure 4.9). The maximum number of glochidia recorded at any one visit at Ohautira Stream was five, for both October and December sampling, on longfin eel (n = 1) and redfin bully (n = 3). At Mangapapa Stream, the highest number of glochidia per visit was 35 recorded in October on common/Crans bully (n = 5), shortfin eel (n = 1) and torrentfish (n = 1).

Pakoka



Ohautira



Mangapapa

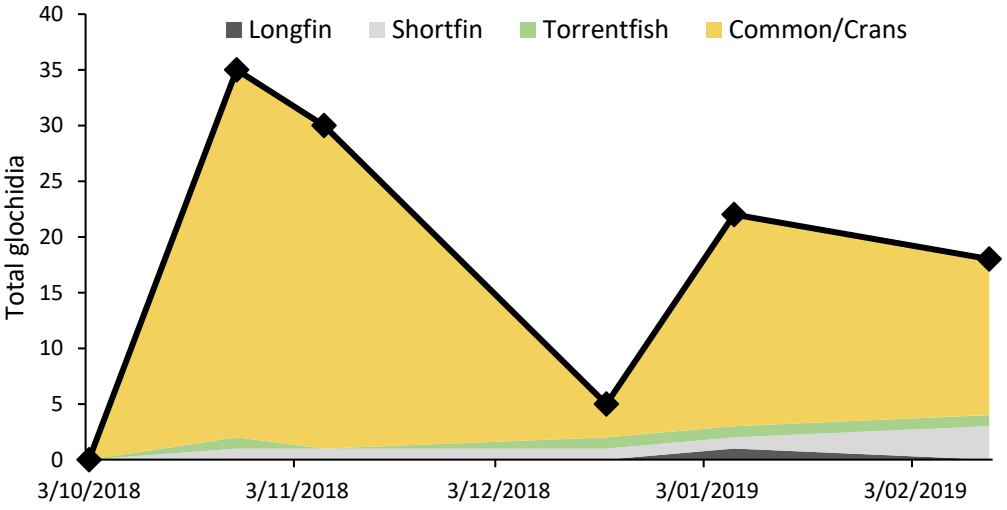


Figure 4.9. The total number of glochidia (◆) found externally on fish at each site per visit using reach-scale data separated by species. Note the different y-axes used for each graph.

4.3.6 **Fish size-glochidia relationships**

Relationships between total number of glochidia and infested bully length were explored for redfin and common/Crans bullies using pooled data (Figure 4.10). The R^2 value for common/Crans bullies indicated that 33% of variance in glochidia numbers could be explained by fish length, with large bullies generally supporting more glochidia (Figure 4.10). Redfin bullies did not show any trend between glochidia attachment and length ($R^2 = 0.01$). Likewise, R^2 values were low (<0.15) for relationships between total glochidia attachment and length for longfin and shortfin eels (Figure 4.11).

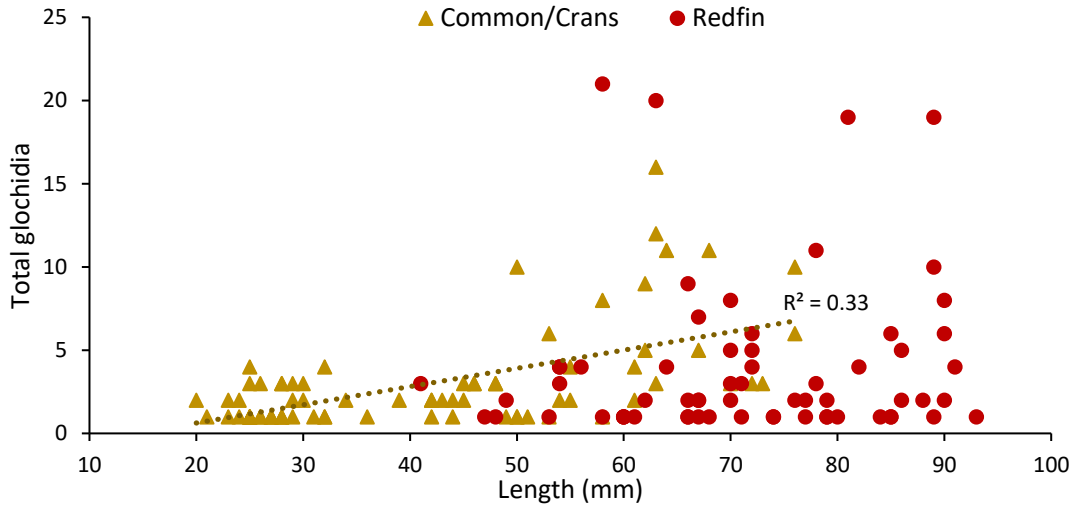


Figure 4.10. Total number of glochidia attached to all infested (≥1 glochidia) common/Crans and redfin bullies (i.e., pooled data) collected during the sampling season vs length (mm) of each fish. Lines show regressions with an R^2 value >0.3.

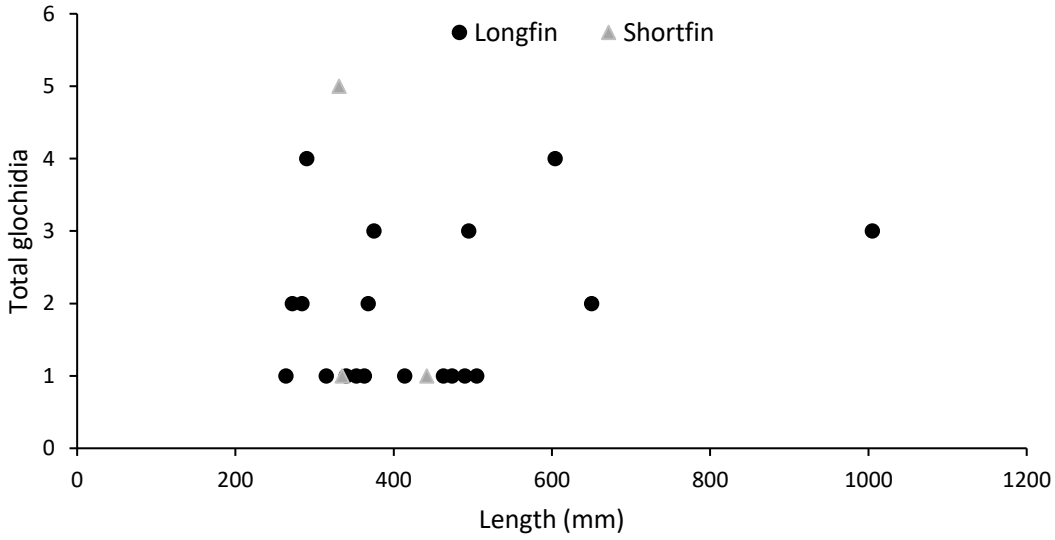


Figure 4.11. Total number of glochidia attached to all infested shortfin and longfin eels (i.e., pooled data) collected during the sampling season vs length (mm) of each fish.

The relationship between infested bully length and the number of glochidia attached was also explored by month to investigate temporal patterns (Figure 4.12; Figure 4.13). Early on in the season there appeared to be positive relationships with fish size (October to December for redfins, October to November for common/Crans). The regressions indicate that 34-56% of variance in glochidia attachment on redfin bullies at Pakoka and Ohautira streams could be explained by fish length during October to December. At Mangapapa Stream, R^2 values of 0.51 and 0.36 for October and November, respectively, suggests that the 51% and 36% of variance in glochidia attachment on common/Crans bullies could be explained by fish length for those months. However, in January/February, there appeared to be no relationship between fish length and glochidia attachment for redfin and common/Crans bullies across all three sites ($R^2 < 0.3$).

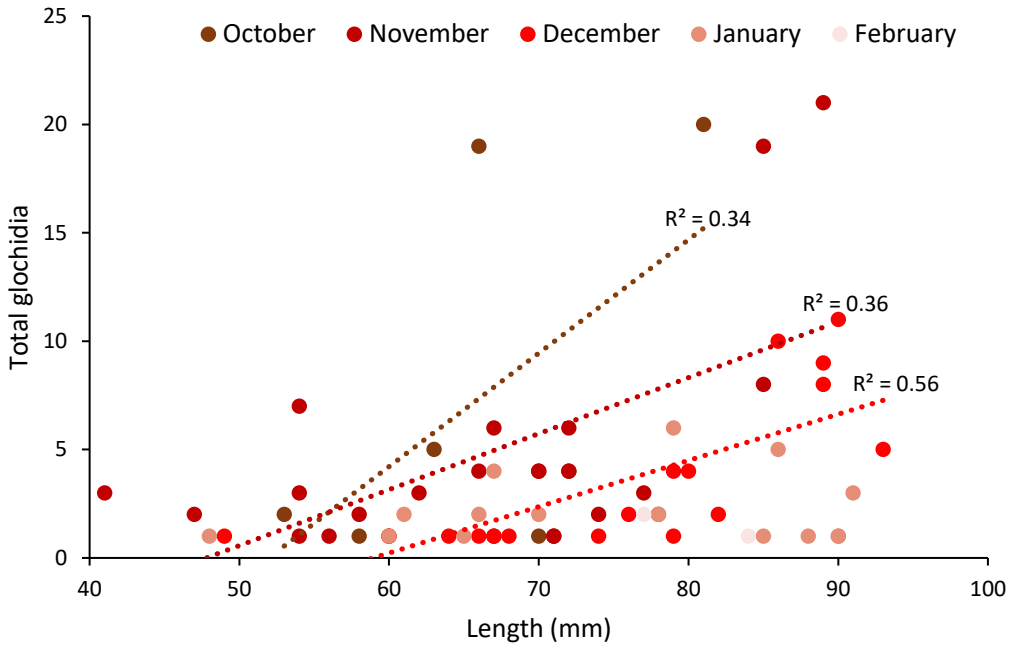


Figure 4.12. Total number of glochidia attached to infested redfin bullies collected each month from Pakoka and Ohautira streams vs length (mm) of each fish. Note: x-axis begins at 40 mm. Lines show monthly regressions with an R^2 value >0.3.

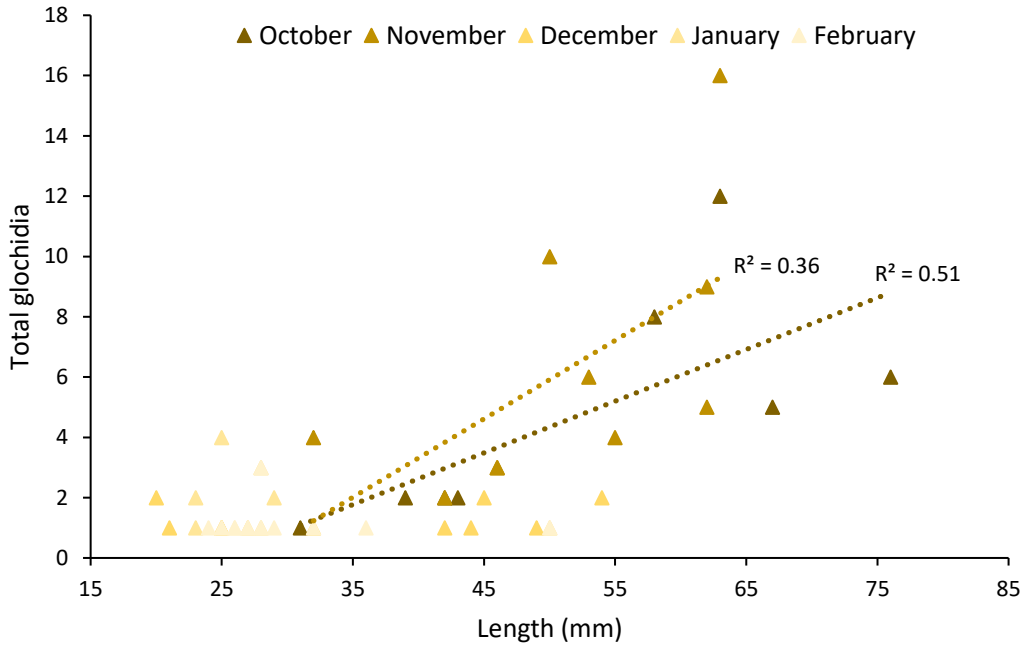


Figure 4.13. Total number of glochidia attached to infested common/Crans bullies collected from Mangapapa Stream each month vs length (mm) of each fish. Note: x-axis begins at 15 mm. Lines show monthly regressions with an R^2 value >0.3.

4.3.7 External glochidia attachment

For the 569 glochidia attached to fish (collected via reach-scale electrofishing, spot-fishing and minnow traps, i.e. pooled data), the most common attachment site for bullies was the caudal (tail) fin, most notably for redfin bully at Pakoka River (36%; Table 4.4). This compared to 26% and 16% of external glochidia on caudal fins at Ohautira and Mangapapa, respectively. Other common places of attachment were the pelvic fins (26%, 26%, 24% at Pakoka, Ohautira and Mangapapa streams, respectively) and pectoral fins (16%, 18%, 32%, respectively). The dorsal and adipose fins combined accounted for 11%, 13%, 13%, respectively. The least common attachment site on bullies from all three streams was the lips (≤1%). From field observations, glochidia appeared attached to torrentfish fins but not encysted (observed four times at Mangapapa Stream). The only observation of *E. menziesii* glochidia on īnanga was on the right pectoral fin of one fish from Ohautira Stream but it was not encysted. On eels, glochidia were found on the dorsal fin (n= 36), anal fin (n = 16), caudal fin (n = 8), and left and right pectoral fins (n = 7, 8, respectively) and were mostly encysted.

Table 4.4. The average number (± SE) of glochidia attached to external surfaces of infested bullies, and the percentage of glochidia attached to each external surface, separated by site. Redfin bullies were present at Pakoka (n = 52) and Ohautira (n = 13), and common/Crans bullies at Mangapapa (n = 75).

	Pakoka		**Ohautira**		**Mangapapa**	
	No.	%	No.	%	No.	%
Lips	0.04 ± 0.03	**1**	0	**0**	0.01 ± 0.01	**0**
Left operculum	0.12 ± 0.04	**3**	0.08 ± 0.08	**4**	0.11 ± 0.04	**3**
Right operculum	0.12 ± 0.07	**3**	0.08 ± 0.08	**4**	0.17 ± 0.05	**6**
Dorsal	0.17 ± 0.07	**4**	0.15 ± 0.10	**9**	0.19 ± 0.06	**6**
Adipose	0.31 ± 0.14	**7**	0.08 ± 0.08	**4**	0.21 ± 0.05	**7**
Left pectoral	0.35 ± 0.10	**8**	0.15 ± 0.10	**9**	0.45 ± 0.08	**15**
Right pectoral	0.37 ± 0.10	**8**	0.15 ± 0.10	**9**	0.52 ± 0.10	**17**
Left pelvic	0.56 ± 0.12	**12**	0.15 ± 0.10	**9**	0.32 ± 0.08	**10**
Right pelvic	0.63 ± 0.13	**14**	0.31 ± 0.13	**17**	0.43 ± 0.11	**14**
Anal	0.23 ± 0.07	**5**	0.15 ± 0.10	**9**	0.15 ± 0.05	**5**
Caudal (tail)	1.65 ± 0.32	**36**	0.46 ± 0.22	**26**	0.47 ± 0.10	**16**

4.3.8 **Internal:external ratio**

Nineteen percent of fish captured in the field (203 of 1073) were bought back to the laboratory for dissection to determine where there was internal glochidia attachment. Of these fishes, 76 were redfin bully, 56 īnanga, 39 common/Crans bully, 11 common smelt, 10 lamprey, four longfin eel, four shortfin eel, and three torrentfish. Only 104 of these fish (51%) had glochidia; 29 had only glochidia attached to the gills, 28 had glochidia only attached to fins, and 47 fish had glochidia attached both internally and externally. One common smelt and one redfin bully had glochidia of both *E. menziesii* and *E. aucklandica*, but in every other case, there was only one species of glochidia present. Common smelt had only *E. aucklandica* glochidia and īnanga had either *E. menziesii* or *E. aucklandica* glochidia.

A Wilcoxon matched pairs test on numbers of internal versus external glochidia on bullies found there was a significant difference in attachment locations for common/Crans bullies, with more *E. menziesii* glochidia attached to external surfaces (Table 4.5). However, there was no significant difference between redfin

bully glochidia attachment locations with both internal and external structures used to the same degree.

Table 4.5. Average number (± SE) of attached *Echyridella menziesii* glochidia for infested redfin and common/Crans bullies dissected in the laboratory. Significant differences are in bold.

	Internal glochidia	**External glochidia**	**Wilcoxon test**	
			Z	**P**
Common/Crans (n=39)	2.62 ± 0.63	3.78 ± 0.68	**1.98**	**0.047**
Redfin (n=76)	3.94 ± 0.90	3.69 ± 0.92	0.59	0.557

External glochidia attachment to redfin and common/Crans bullies was on average higher than internal attachment in October, although fish numbers for comparison were low (n = 11) (Figure 4.14). Internal attachment rates were slightly higher for redfins (n = 11-16 fish per month) for November and December, but external attachment was slightly higher in January on average. In February, only one glochidium was found on a redfin and it was attached internally (none were found externally attached in February). External attachment was usually slightly more than internal attachment for common/Crans bullies (n = 4-11 fish per month). Overall, there was a decrease over time in the number of glochidia attached internally and externally.

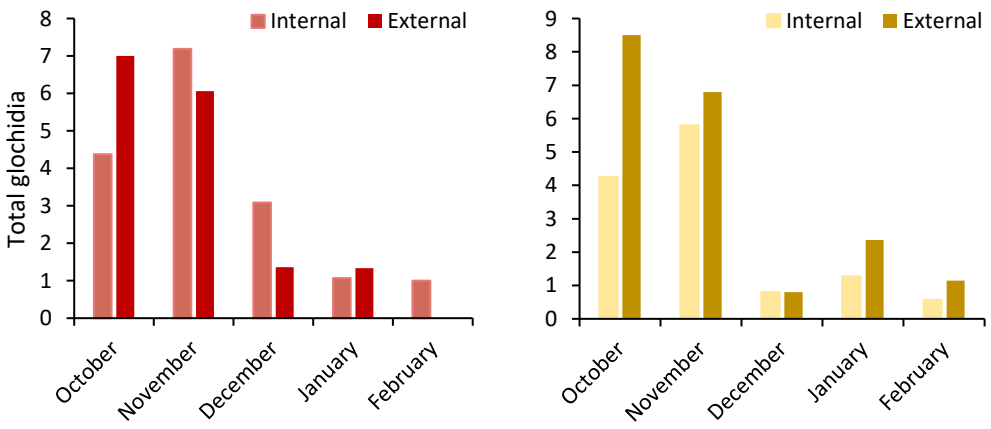


Figure 4.14. Average number of glochidia attached internally and externally per month for redfin (left – Pakoka and Ohautira combined) and common/Crans (right – Mangapapa) bullies.

4.4 Discussion

4.4.1 Method for stream-side glochidia assessments

Several studies have highlighted the need for a quantitative in-situ method of glochidia infestation of host fish surfaces to reduce the number of sacrificial fish required for freshwater mussel research (Geist, 2010; Taeubert et al., 2012b; Larsson, 2015; Ćmiel et al., 2018). The stream-side approach developed in this study to quantify external glochidia attachment by *E. menziesii* has general application for quantifying host suitability of a range of fishes with minimum mortality. The combination of a large tray for fish >80 mm and a miniature glass aquarium for smaller fish, coupled with the use of a field light source and magnifier, enabled glochidial infestation of different external surfaces of fish (e.g., lips, dorsal, adipose, pectoral, pelvic, anal, caudal fins and operculum cover) to be quantified. In addition, I was able to determine whether *E. menziesii* were encysted using this assessment method, although it did not include the inspection of internal structures such as the gills. Ćmiel et al. (2018) considered glochidia attachment on fins to be a good indicator of mussel presence in water bodies, so for this purpose there is no need to harm the fish by inspecting gills unless a particular mussel species has an obligate internal attachment strategy, as appeared to be the case for *E. aucklandica* in the present study.

Other investigators have recommended similar methods. Salonen and Taskinen (2016) state that electrofishing followed by a quick naked-eye check of fish provides a non-destructive way of detecting unrecorded *M. margaritifera* populations, that does not disturb the sensitive mussel bed itself or deplete the host fish population. However, this approach is likely only to be applicable during the glochidia release period which can be brief for some species (e.g., *Alathyria jacksoni* is a short-term brooder). *Margaritifera margaritifera* has been found to grow on a host during the parasitic phase (Young & Williams, 1984; Bauer & Vogel, 1987), making the identification of glochidia when freshly attached to host fish difficult. At the beginning of the parasitic phase *M. margaritifera* glochidia are small (c. 70 µm) and can only be observed under a microscopic compared to mature glochidia which are 400 to 500 µm in diameter (Salonen & Taskinen, 2016). In comparison, the glochidia of *E. menziesii* do not grow on their host, remaining about 300 µm in diameter for their infestation period (Melchior et al., in press). This large

and constant size makes it relatively easy to quantify glochidia attachment in the field during the October to February glochidia release period using the new method developed in this study.

Although the reproductive success of *E. menziesii* depends on the broadcasting of prodigious numbers of glochidia into the water column to increase the likelihood of encounter with compatible hosts (Neves & Widlak, 1988), the use of nets specifically designed in this study to detect glochidia in the drift was not successful. Few glochidia were detected, and analysis of drift samples was time consuming and required sub-sampling due to the volume of particulate material collected. The lack of glochidia in drift samples could be due to: i) the method not being efficient at or in suitable habitats for capturing suspended glochidia; ii) glochidia not suspending readily in the water column or becoming highly diluted; iii) release occurring mainly at night; or iv) glochidia rapidly attaching to fish after release. If the latter was true, this is ecologically beneficial as glochidia viability declines rapidly after release from the female, especially with increasing water temperatures (Bauer & Wächtler, 2001). In the laboratory I have observed that newly-released glochidia are negatively buoyant but easily resuspended which may explain their absence in drift net samples. Some mussel species release their glochidial load primarily at night (Haag & Warren, 2000; Culp et al., 2011), therefore daylight drift sampling may have underestimated the abundance of *E. menziesii* glochidia. Though, if glochidia remain in the drift for extended periods, diurnal differences in release may be largely obscured (Culp et al., 2011). Clearwater et al. (2014) suggests that *E. menziesii* glochidia have 2-4 days to attach to and parasitize a host, though this is temperature dependent (Melchior, 2017). The nets used were 20 x 30 cm and only covered <16% of the stream width at Pakoka and Ohautira sampling sites (drift nets were not used at Mangapapa due to bedrock substrate). In comparison, Neves and Widlak (1988) had 30 x 30 cm nets spaced 5 m apart (river width c. 30 m) and found that the greatest number of glochidia coincided with the highest water velocities. Future drift sampling to detect the presence of *E. menziesii* glochidia could include covering a larger portion of stream flow by having larger nets, more nets equally spaced across the river transect, targeting higher velocity areas, or possibly nocturnal drift netting.

4.4.2 **Mussel population characteristics**

Female mussels were largest at Pakoka River in terms of length, width and height followed by Ohautira and Mangapapa. Wu et al. (2018) found that fecundity was positively correlated with shell length in five mussel species in China. Similarly, Haag (2013) states that body size is a strong predictor of fecundity in North American freshwater mussels. Therefore, all other factors being equal, fish at Pakoka River appear to have the highest probability of glochidial infection because of the presence of high densities of large *E. menziesii* (see Chapter 2). Österling (2015) observed a positive relationship between adult mussel density and the number of gravid mussels. Similarly, Downing et al. (1993) found reproductive success declined with decreasing mussel density with very little fertilization occurring at densities <10 individuals m^{-1}. Few studies have linked mussel population densities with fish infestation rates, but an enlightening study by Schneider et al. (2019) suggests that relatively low mussel densities (possibly a critical population density threshold) can have a high reproduction potential, likely due to host fish availability and elevated encystment rates on fish.

In the present study, high mussel abundance and large mussel size at Pakoka River was mirrored by high fish infestation rates with a total of 290 glochidia recorded (c.f. 29 and 250 at Ohautira and Mangapapa, respectively), with an average of 4.6 ± 0.7 glochidia attached to each redfin bully compared to 1.8 ± 0.4 at Ohautira. Therefore, it is reasonable to suggest that larger female mussels coupled with higher mussel density led to high infestation rates on compatible fish hosts at Pakoka River. In contrast, Schneider et al. (2019) found that the level of glochidia infestation on fish was not dependent of the size of mussel individuals at a particular site.

During this temporal study, the numbers of gravid mussels (stages 4-5) increased as water temperatures increased, peaking when mean water temperatures were 18.8 - 21.2^{o}C. These findings are consistent with multiple studies which have found that the annual reproductive cycle of freshwater mussels is thermally driven (Hastie & Young, 2003; Taeubert et al., 2014). Warmer water temperatures initiate glochidial release by either: i) a summation effect; ii) a minimum threshold temperature; or iii) thermal shock (Hastie & Young, 2003). Hastie and Young (2003) found that glochidia release in a natural environment coincides with a thermal/hydrological event such as a sudden change in water temperature (>2^{o}C) and/or river level

(>0.1 m). In a laboratory environment, Taeubert et al. (2014) used four different temperature regimes to assess the suitability of *Phoxinus phoxinus* as hosts for *Unio crassus*, and found that a water temperature of 17°C had the highest metamorphosis success and the lowest host mortality, compared to 12°C, 20°C and 23°C.

Gravid mussels had released their brood (100% females) from Pakoka and Ohautira streams by February. In Mangapapa Stream, glochidial release appeared more delayed, with only 40% of females having fully released their brood at this time, and overall brood pouch status across October to February significantly lower or delayed compared to Pakoka and Ohautira sampling sites. Monthly temperature ranges were similar across all sites suggesting some other environmental factor in Mangapapa Stream may have been influencing glochidia maturation and release. In terms of location, Pakoka River and Ohautira Stream are both located on the west coast of the North Island, discharging directly into the sea within 50 km of each other, whereas Mangapapa Stream is located much further inland and discharges to the east coast (Figure 2.2). Marwaha et al. (2017) state that in rivers within the same geographical area and similar temperature regimes, glochidial release occurs around the same time, which was evident in *E. menziesii* brood pouch development. Additionally, the fish communities at Pakoka and Ohautira sampling sites were similar compared to Mangapapa Stream communities, perhaps indicating that distance inland and fish recruitment patterns may play a role in glochidia release timing in Mangapapa Stream. Unfortunately, the tail end of the female release period at Mangapapa Stream was possibly not captured, warranting further research to study year-long brood pouch status at a range of contrasting study sites.

4.4.3 **Host fish preference over a glochidia release season**

A range of fish species were assessed to see if glochidia were attached, yielding 569 glochidia on 187 fish. Species identified with *E. menziesii* glochidia included redfin and common/Crans bullies, longfin and shortfin eels, torrentfish and īnanga. *Echyridella menziesii* are thought to have a broadcast release strategy with generalist host requirements (Clearwater et al., 2014). This strategy is consistent with my field observations as seven different fish species had *E. menziesii* attached, although some hosts were clearly more infested than others. *Gobiomorphus* species accounted for 86% of total glochidia, while *Anguilla* had 13% of glochidia attached

despite being larger and more abundant than bullies in reach-scale sampling. Both torrentfish and īnanga accounted for <1% of glochidia attached and were apparently not favoured hosts. While there were glochidia attached to torrentfish, they never appeared encysted, therefore it is not certain that the species can produce juveniles (i.e., be a suitable host). To illustrate, Lellis et al. (2013) found *Elliptio complanata* glochidia attached to 16 fish species for up to 17 days without ever achieving metamorphosis during laboratory experiments. Future research should incorporate post-attachment phase monitoring to confirm whether the species actually produces viable juvenile mussels, as the broadcast release strategy means unsuitable fishes can be infested to the same degree as compatible fish hosts (Taeubert et al., 2012b), potentially leading to reduced recruitment. Of concern is that introduced invasive fish, including catfish (*Ameiurus nebulosus*), rudd (*Scardinius erythrophthalmus*) and goldfish (*Carassius auratus*), have recently been shown to be poor hosts of *E. menziesii* glochidia, with low glochidia transformation rates compared to common bullies (Moore & Clearwater, in press).

The host fish preference of glochidia did not appear to change over the glochidial release season with *Gobiomorphus* species (redfin and common/Crans bullies) serving as dominant hosts throughout the sampling period. An influx of smaller (20-30 mm) common/Crans bullies was observed at Mangapapa Stream during January to February (larger fish were not present December to February), but there was no evidence of smaller redfin bullies migrating into the west coast sampling sites. Several of the smaller common/Crans bullies had at least one glochidia attached to them, but no more so than larger bullies, suggesting that there is no host preference in terms of naïvety or size. *Anguilla* species (longfin and shortfin eels) were the next common hosts, and there was a seasonal influx of elvers at Ohautira and Mangapapa streams during December, but not at Pakoka River. Despite this influx, there were no obvious increases in the number of glochidia attached to the elvers, which may not have been naïve at Mangapapa Stream given the likelihood of encountering mussel beds during their long upstream migration. At Ohautira Stream, schools of īnanga whitebait were seen swimming upstream during sampling in early summer, however, only one īnanga was found to have *E. menziesii* glochidia attached to external surfaces on the right pectoral, and it did not appear to be encysted.

Although there were no strong correlations between fish size (bullies and eels) and the number of glochidia which attached to fish, when data was separated by month there was evidence of a trend for larger redfins and common/Crans to be more heavily infested early in the sampling season (October to December). This finding suggests that glochidia attachment is greater on larger fish early on in the season, likely due to more surface area/fin edge length for attachment and high glochidial release, but these may be displaced or outnumbered by juveniles migrating from sea from November onwards (Smith, 2014). Schneider et al. (2017) stated that in the wild, older hosts may become less suitable owing to acquired immunity from previous infestations. Similarly, Klunzinger et al. (2010) found a greater prevalence of glochidia on smaller fish suggesting that larger, older fish species develop partial or temporary resistance as they have had more opportunities to be infected. However, not all fish species develop an immunity to glochidia as large-bodied hosts have shown prevalent glochidia attachment, presumably due to a greater surface area for infection (Klunzinger et al., 2010). A laboratory-based experiment for the present study indicated that an immune response does not occur in repeatedly-infested common bullies (see Chapter 3).

While electrofishing can be highly efficient for catching larger fish as their size makes them more vulnerable to electric current and more visible to the fisher, it can be ineffective for bully fry <20 mm and shoaling species (Graynoth et al., 2012). The fish sampling did not detect an expected influx of redfin bullies <20 mm in size at the Pakoka or Ohautira sites, therefore it is possible that the upstream migration of redfins was missed, so their role as potential *E. menziesii* hosts cannot be evaluated here. Data collection for this research occurred fortnightly alternating between electrofishing a 50-m reach (single-pass fishing only) and spot-fishing to avoid targeting the same fish. However, fish densities were low which may suggest that fish actively avoid the electrical current, particularly at Pakoka where the substrate was cobbly with many interstitial refugia. Many studies have reported that previously shocked fish may actively avoid the current by burrowing further into the substrate, particularly elvers (Mahon, 1980; Peterson et al., 2004; Graynoth et al., 2012). This may partly explain why no recruiting elvers were found at Pakoka compared to the other two sites where the substrates provided little interstitial habitat.

4.4.4 **Benthic vs pelagic fish infestation rates**

My hypothesis, that benthic fish such as bullies might be more likely to have higher infestation rates compared to pelagic fish which are less likely to encounter mussels, was supported in this study. *Gobiomorphus* species (common/Crans or redfin) were the dominant *E. menziesii* glochidia hosts accounting for 86% of glochidia observed in the field. These three species, along with juvenile eels and torrentfish, are benthic dwellers living between coarse substrates in riffles and runs (McDowall, 1990). In particular, male common and Crans bullies are territorial and guard nesting sites during annual spawning (McDowall, 1990; West et al., 2014). Freshwater mussel beds can increase the heterogeneity of otherwise soft sediments (Vaughn, 2017) and *Gobiomorphus* species likely co-occur in these benthic areas.

Eel elvers (75 -120 mm) are abundant in riffles and runs living amongst the gravels, sometimes occupying the same habitat as torrentfish, while larger eels seek cover beneath logs and overhanging banks sometimes moving substantial distances from resting in pool habitats during daytime to nocturnally feed in riffles and runs (McDowall, 1990; Graynoth et al., 2012). It is plausible to assume that eels resting on stream bottoms and elvers moving through interstitial substrates will encounter gravid female mussels releasing glochidia into the water column, particularly in pool habitats when mussels can reach high densities. Eels are known to be compatible hosts for mussel species (Lellis et al., 2013), including *E. menziesii* but provided lower juvenile transformation rates than common bullies in a laboratory-setting (Brown et al., 2017); the present field-based study also suggests they are less suitable than bullies.

In comparison, pelagic, pool-dwelling fish such as īnanga and common smelt occur in shoals moving through open, gently flowing or still water (McDowall, 1990). Only one *E. menziesii* glochidia was found on īnanga and none on common smelt, while the rest were detected on benthic fish, suggesting that *E. menziesii* favour fish with benthic lifestyles making it easier for glochidia to attach to a host fish.

4.4.5 **Glochidia attachment sites**

The most important glochidia attachment sites for common/Crans bullies appeared to be the pectoral fins followed by the pelvic and caudal fins, in comparison to

redfins where the caudal fin was the most important attachment site followed by pectoral and pelvic fins. These fins are closer to the stream bed than the dorsal and adipose fins suggesting that bullies could be picking up glochidia which have settled to the bottom, as observed during laboratory exposures (Chapter 3), or they are encountering glochidia exiting exhalant siphons of buried mussels. The latter scenario may be more likely given that Jansen et al. (2001) stated that glochidia failing to attach to a host-fish eventually sink to the substrate where the likelihood of attachment is minimal and glochidia will perish. Some glochidia were also observed attached to dorsal and adipose fins of bullies, presumably after being broadcast into the water column by the female mussel. In contrast the majority of glochidia were attached to the dorsal fins of eels which may relate to their tendency (especially as elvers) to burrow into substrates. Clements et al. (2017) found that the closer the *M. magaritifera* beds were to good fish habitat, the higher the levels of excystment as juveniles. In other words, if a suitable host is in the right place at the right time, they will have glochidia attaching to them.

My results show that there was no difference in glochidia attachment between internal and external glochidia attachment for redfin bullies. The highest number of glochidia found attached internally and externally to any redfin (c. 89 mm length) was 61, which is not a very high glochidial load as Barnhart et al. (2008) considers a heavy infection to be hundreds of glochidia, although this would presumably also be relative to fish size. In comparison, the highest number of glochidia attached internally and externally to a single common bully (c. 68 mm length) during artificial infestation in the laboratory was 445 (Chapter 3), although some laboratory fish had <10 glochidia even after exposure to 2000 glochidia L^{-1} in artificial infestations. Common/Crans bullies had more glochidia attached to external surfaces than internal structures in the field. This is similar to common nase (*Chondrostoma nasus*) which had four times the number of *U. crassus* glochidia attached to fins than to gills (Ćmiel et al., 2018). The results of the present study demonstrate that the location and level of glochidia attachment is variable among fish species, possibly driven by habitat differences (i.e., fast/slower flowing water and/or burrowing by elvers).

4.5 Conclusion

The results from the field study clearly demonstrate that redfin and common/Crans bullies are the dominant hosts for *E. menziesii* throughout the glochidia release season. Glochidia attachment was not affected by fish size or whether they were naïve; rather if suitable benthic-dwelling host fish are in the right place at the right time, they will likely be infested. Based on New Zealand Freshwater Fish Database (NZFFD) data records, it is reasonable to suspect that redfin bully has experienced population declines of at least 20-25% over the past 20 years (Ling et al., 2014). Similarly, common bullies have been found to be in decline in both pasture and natural cover environments (Joy et al., 2019). This research has identified the fish species important for the reproductive success of *E. menziesii* in contrasting Waikato streams. Like *E. menziesii*, these fish species appear to be at risk of widespread decline, highlighting that integrated conservation efforts are required to protect not only mussel species but also suitable fish hosts.

Chapter Five

General Discussion

The overall aim of this thesis was to provide information on interactions between *Echyridella menziesii* glochidia and host fish in terms of the range of fish species that can serve as compatible hosts and whether they are capable of supporting multiple infestations. To address this aim, I conducted a laboratory experiment to determine whether common bully (*Gobiomorphus cotidianus*) would develop immunity to multiple infestations of glochidia, and undertook regular field sampling over summer to establish the dominant fish hosts for glochidia at three contrasting sites. Previous studies (e.g., Rogers & Dimock, 2003; Dodd et al., 2005; Ćmiel et al., 2018) have investigated the metamorphosis success of juvenile mussels on various fish hosts after multiple infestations, but this had not been explored for *E. menziesii* glochidia. Brown et al. (2017) identified suitable hosts for *E. menziesii* under controlled laboratory conditions, but I wanted to establish whether this was reflected in natural environments, and also to understand the temporal dynamics of this interaction with a diadromous fish community.

Almost all (99.99%) of glochidia broadcast by mussels fail to find a suitable host in the field, making the parasitic phase of the freshwater mussel life cycle the most critical (Young & Williams, 1984; Jansen & Hanson, 1991). This statistic emphasizes the need for mussels to have a huge reproductive output to compensate for the many glochidia which may not even reach a host or successfully transform into juveniles, and underscores the importance of understanding which fish species can serve as hosts for the few glochidia that attach. Thus, documentation of glochidia attachment in the field, coupled with an understanding of host immunity, provides important information about potential fish hosts and metamorphosis success, both of which are essential in determining whether fish can actually produce juvenile mussels (Lellis et al., 2013).

The research chapters presented in this thesis complement each other by exploring the obligate physiological part of the mussel-host relationship in the laboratory (Chapter 3), and confirming host compatibility for a wider range of species in the field (Chapter 4). Phillips et al. (2007) highlighted that the glochidial and juvenile

stages of the *E. menziesii* life cycle were critical knowledge gaps, recommending further experiments to improve understanding of factors limiting the survival of the critical early life-stage. This research contributes new information to the existing pool of knowledge by determining whether developing immunity to glochidia or failure to find and attach to a suitable fish host could contribute to declining *E. menziesii* populations in New Zealand.

5.1 Common bully response to multiple glochidia infestations

To address the first aspect of this study, I exposed naïve common bullies to *E. menziesii* glochidia and quantified their detachment and excystment as juvenile mussels. Previous studies have suggested that host fish may become resistant to glochidia upon re-infestation (Bauer & Vogel, 1987), compromising their ability to contribute to mussel recruitment. Rogers and Dimock (2003) carried out four consecutive infestations and found that bluegill sunfish (*Lepomis macrochirus*) developed an acquired immunity to glochidia of the paper pondshell mussel (*Utterbackia imbecillis*). They reported a reduction in metamorphosis success by the third and fourth infestations, but considered that a fifth infestation may be beneficial. Bauer and Vogel (1987) found that, after a second infestation, the number of glochidia of the freshwater pearl mussel (*Margaritifera margaritifera*) that remained attached to brown trout (*Salmo trutta*) decreased across a range of host fish sizes and glochidia mortality was higher in re-infested individuals compared to naïve fish.

In the present study, there appeared to be some decline in juvenile excystment after two infestations, but this was not carried over to the third infestation when active juveniles were produced in larger numbers than before. This finding reinforces the importance of conducting more than two infestations when assessing immune responses as other factors can clearly have an over-riding influence on metamorphosis success on some host fish. The three sequential infestations tested in the present study showed that common bullies used in this experiment did not develop an immunity to glochidia, but it is unclear whether this would persist for a fourth or even fifth infestation. During the course of the experiment, it became apparent that the viability of the glochidia used for infestation can have a substantial impact on the number that initially attach to host fish, accounting for the higher

transformation rate on the third infestation. Future experiments should examine whether glochidia viability of ≥90% is high enough because variation in viability seemed to explain variations in excystment rates in my laboratory experiment. Perhaps it is more important that viability remains constant between experiments, although this is difficult to control.

Barnhart et al. (2008) considered a "good" fish host to support the transformation of >90% glochidia into juvenile mussels. However, in my experiments only 12-30% of attached glochidia transformed into juveniles on common bully. The reasons for this are unknown, but these transformation rates are typical of those observed by Moore and Clearwater (in press) who reported a range of 15 to 62%. These transformation rates are also similar to the range observed for the paper pondshell mussel during multiple infestations on bluegill sunfish where the success of metamorphosis reduced from >45% in the first two infestations to <26% for the third and fourth infestations (Rogers & Dimock, 2003). Low metamorphosis success despite the apparent absence of an acquired immune response in common bully suggests there were underlying reasons causing glochidia detachment. For example, glochidia may not have attached sufficiently in the first two days for encystment by host tissue, or they may have attached to unsuitable parts of the fish such as scales, as observed elsewhere by Rogers and Dimock (2003). Finally, innate immunity (not requiring prior exposure) of the fishes may also contribute to the glochidia detachment that occurred throughout the duration of each infestation (O'Connell & Neves, 1999; Roberts & Barnhart, 1999; Rogers & Dimock, 2003), although its role in my experiments remains unclear.

Another aspect of this research that warrants further investigation is the monitoring of the fitness of juveniles after detachment. There was variation in the duration of the glochidia attachment phase, and research by Marwaha et al. (2017) found that juveniles attached to a host for longer benefitted from a size, growth rate and survival advantage compared to those detaching earlier. Thus, a further avenue of research would be to assess the health of the juveniles excysted during each infestation. In particular, information on post-detachment burial time, activity, growth, and feeding rates may reveal more subtle effects of immune responses to multiple glochidia attachments that compromise juvenile fitness.

5.2 Determining fish hosts in a natural setting

This study shed light on the temporal dynamics of fish host interactions with glochidia in three contrasting streams, and included the development of a quantitative method for assessing external infestation in the field supported by laboratory analysis of internal attachment structures. Host fish confirmation in a natural setting is necessary as such information will help establish which diadromous species can serve as hosts at different stages of the migration period, and whether declining mussel populations are linked to declining fish populations, as suggested for kōaro (*Galaxias brevipinnis*) in Lake Taupō (McDowall, 2002).

Eight native fish species were examined for the presence of external glochidia attachment. The only species caught which did not have any glochidia whatsoever (internal or external) was lamprey (*Geotria australis*), which is consistent with Neves and Widlak (1988) who reported an absence of glochidia on lamprey. There were only two cases of fish with both *E. menziesii* and *E. aucklandica* glochidia attached, one common smelt (Pakoka Stream) and one redfin bully (Ohautira Stream). Both fish had no glochidia attached externally underscoring the importance of also examining internal gill structures to confirm host compatibility.

This study encompassed one glochidia release season (October to February) at three sites so the generality of these findings must be viewed with some caution. The Mangapapa Stream was an inland, east coast draining stream and part of a larger river system, whereas Pakoka and Ohautira streams were short coastal streams draining directly to the sea but had contrasting abundances of *E. menziesii*. The differences in fish communities among sites enabled a range of host species to be evaluated from contrasting habitats. I was able to document the apparent conclusion of seasonal glochidia release at the Pakoka and Ohautira sampling sites because no brooding females were found in February at these locations. At Mangapapa Stream however, the early release period but possibly not the tail end of glochidial release was documented because early stage brooding females (i.e., stages 2 and 3) were present compared to 40% post-release females. In future, it would be beneficial to carry out a 12-month sampling survey, or at least extend sampling to longer than five months, to fully describe the entire seasonal reproductive cycle.

Of the eight fish species caught across the sites, īnanga (*Galaxias maculatus*), was the only *Galaxias* (whitebait) species caught. Future research into glochidia hosts would benefit by including sites with resident populations of banded kōkopu (*G. fasciatus*), shortjaw kōkopu (*G. postvectis*), giant kōkopu (*G. brevipinnis*) and kōaro. The latter species in particular was historically thought to be the main fish host for *E. menziesii* glochidia (McDowall, 2002). Additionally, sampling longer reaches and a wider range of habitats (i.e., 150 m instead of 50 m and pools by overnight netting or snorkeling), might increase the range of species caught. In a nationwide study, David et al. (2010) found that, on average, single-pass electrofishing a 150-m reach was required to detect most species present at a site, although additional spot-fishing as used in the present study may be required to obtain sufficient numbers of less common species for assessment of glochidia infestation.

One difficulty encountered in this research was being unable to identify in the field whether the bully species at Mangapapa Stream was common bully, Crans bully (*Gobiomorphus basalis*), or both. These highly cryptic species are difficult to identify and there are questions regarding the accuracy of existing keys for distinguishing between them (Graham et al., 2016). Some doubt therefore remains regarding the true identification of the bullies at Mangapapa, as noted by Graham et al. (2016) who observed differences in species identification there between years. This uncertainty likely will only be resolved with genetic analyses, and if Crans bully presence is confirmed in Mangapapa Stream another host species will be added to the list of native fish which *E. menziesii* glochidia can attach to. Given that this species is non-diadromous while common bully is diadromous, their temporal role as potential hosts of glochidia might be quite different. I am confident that fish collected from the Waikato River for the experiments (Chapter 3) were common bully as the New Zealand Freshwater Fish Database (NZFFD) does not have any records of Crans bully as far upstream as Hamilton Gardens, and previous expert species verification identified only common bully at that location.

Quantifying the suitability of hosts for glochidia attachment, encystment and metamorphosis success in the field is difficult as *E. menziesii* glochidia are only attached for a short period (i.e., up to 22 days (Clearwater et al., 2014; Moore & Clearwater, in press) or less as found in Chapter 3) and do not grow while on the

host. Thus, it is possible that glochidia may be lost soon after attachment on some fish, and even following encystment if adverse conditions are encountered, as observed in initial trials of anaesthetized fish in the present study (Hanrahan 2019, unpublished data). Furthermore, there is no way of knowing from field surveys whether viable juveniles are produced. When juveniles detach from a host they are seldom found as they are very small (<500 µm) and probably bury themselves into stream sediments (Geist, 2010). The duration that glochidia are attached to a host probably influences the survival of juveniles in the benthic life-stage, with larger juveniles thought to be a result of a longer parasitic phase, probably having more resources to sustain them during the initial stages of life in the benthic sediments (Österling & Larsen, 2013).

Monitoring of mussel population size-structure coupled with glochidia-host infestation rates will provide some insights into the longer-term recruitment processes and potential mussel population bottlenecks occurring at a site. For example, high rates of glochidia infestation over multiple years but no evidence of mussel recruitment might indicate either glochidia fail to transform on fish if juvenile habitat is not limited.

5.3 Management implications

Many factors likely contribute to declining mussel populations, including reproductive constraints involving glochidia viability, high glochidia mortality, low metamorphosis success, and limited host availability. In concert with other factors such as habitat and water quality degradation, any combination of these factors could reduce juvenile mussel recruitment and may explain why geriatric, senescing populations have been observed some in New Zealand streams and rivers (Roper & Hickey, 1994; Catlin et al., 2018).

Given their obligate association with fish hosts, freshwater unionid mussels are not only vulnerable to their own frailties, but also to those of their hosts (Barnhart et al., 2008). Geist et al. (2006) demonstrated that an inadequate host fish population is, alone, sufficient to explain a lack of juvenile recruitment. In New Zealand, it is evident that native freshwater fish populations, including confirmed glochidia hosts (particularly redfin and common/Crans bully), are declining (e.g., McDowall, 1990;

Franklin et al., 2014; Ling et al., 2014; West et al., 2014; Dunn et al., 2018; Joy et al., 2019). Reasons for their decline are largely due to habitat degradation (e.g., due to water abstraction and land use changes), fish passage constraints and predation.

Gobiomorphus species are a major prey item of introduced salmonids, particularly brown trout (Franklin et al., 2014; Ling et al., 2014; West et al., 2014). The spread of introduced invasive fish species changes fish assemblages which will likely have an impact on mussels, as not only are the number of suitable hosts reduced, for example through predation and competition, but also glochidia may attach to but not transform on non-native hosts resulting in wasted reproductive effort (Moore et al., 2019). Moore et al. (2019) found that New Zealand freshwater mussels are most likely to be affected by interactions with non-native species in lowland lakes and waterways as this is where connectivity for diadromous fish hosts and high bioinvasion potential intersect. Risks of future fish species invasion in New Zealand is high, particularly in lakes along the east coast, via natural dispersal along waterways or through human-assisted movement (Leathwick et al., 2016). Species that have the most potential for future spread include the brown bullhead catfish (*Ameiurus nebulosus*), perch (*Perca fluviatilis*), and rudd (*Scardinius erythrophthalmus*) (Collier et al., 2016a; Leathwick et al., 2016). In future, biosurveillance and if necessary invasive fish control may be required in high value sites to ensure host fish populations are not swamped by unsuitable species.

There is no protection of native freshwater fish in New Zealand currently, however, the Conservation (Indigenous Freshwater Fish) Amendment Bill seeks to update the Conservation Act 1987 to manage the conservation of indigenous freshwater fish and threats to those fish (New Zealand Parliament, 2018). This will go some way to ensuring future viability of host fish populations, and is particularly important for migratory hosts which require connectivity across marine and freshwater environments to pass through mussel beds and potentially disperse juveniles to favourable habitats upstream (Neves & Widlak, 1988; Barnhart et al., 2008). Although this research indicated that smaller, naïve migrating hosts (e.g., juvenile redfin bullies) are not ecologically favoured over larger fish (e.g., adult bullies), freshwater mussel survival is dependent on the continuing recruitment of diadromous fish, so fish passage will be an integral part of mussel conservation (see Franklin & Gee, 2019).

Conservation efforts for mussels often include propagation and stocking programs which can help jump-start species recovery. This can include artificially infesting and releasing fish hosts into target sites, or through in-vitro (no host) laboratory cultivation of juveniles (Patterson et al., 2018). Redfin and common/Crans bullies were identified as the most important hosts for *E. menziesii* in the field, and common bully transformed glochidia into juvenile mussels in the laboratory, suggesting it could be a target fish species used in propagation, although transformation rates were low. However, aiming for a high glochidia load on fish hosts released into the wild may not be the best option in terms of host fitness and successful glochidia attachment (Thomas et al., 2014). In fact, aiming for natural infestation rates of a few dozen glochidia or less (Neves & Widlak, 1988; Barnhart et al., 2008; Chapter 4) may be more beneficial, as the chances of transformation success and juvenile fitness are higher, even though the numbers produced are lower.

Finally, restoration of mussel habitat and improvements to water quality are also key elements to any multifaceted conservation effort. For example, water temperature is likely a key factor influencing the transformation success of juveniles and potentially their fitness, and should be considered when selecting sites for introduction of infested fish. In streams, alteration of the natural water temperature regime may be a result of mixing effluent with stream water, flow modifications and changes in the surrounding riparian zones (Taeubert et al., 2014). An increase in water temperatures prematurely in a season will likely induce an early mussel release, and Taeubert et al. (2014) suggests increasing shading by bank vegetation to decrease or stabilise water temperatures. Riparian planting may therefore be an important component of longer term mussel conservation to enhance recruitment, especially in the face of a future warming climate.

5.4 Future work

This thesis has increased the understanding of *E. menziesii* fish-host relationships in both natural and laboratory settings. My research is the first comprehensive study in New Zealand looking at the temporal patterns of glochidia release and attachment to native fish hosts. Although the focus of this research was on streams, mussels

also inhabit lakes and therefore future research should encapsulate lentic habitats to assess whether similar host relationships and glochidia release and attachment patterns occur. Furthermore, this study was temporally limited as sampling occurred for five months over the main glochidial release season. Future studies should extend this period of sampling and cover consecutive seasons to increase the understanding of how fish host dynamics may change over time. In addition, future field studies could expand the current range of non-native fish species tested by Moore and Clearwater (in press) to determine the range of invasive species that may threaten mussel recruitment by serving as glochidia sinks. Unfortunately, once mussel populations have declined, it can take decades for freshwater mussels to recolonize restored habitats, as they are a long-lived, sessile and have a complex life history. These factors highlight the need for active conservation now, to ensure these filter-feeding ecosystem engineers remain in future freshwater ecosystems.

References

Aldridge, D. C. (1999). Development of European bitterling in the gills of freshwater mussels. *Journal of Fish Biology, 54*(1), 138-151.

Aldridge, D. C., Fayle, T. M., & Jackson, N. (2007). Freshwater mussel abundance predicts biodiversity in UK lowland rivers. *Aquatic Conservation: Marine and Freshwater Ecosystems, 17*(6), 554-564.

Allen, D. C., Vaughn, C. C., Kelly, J. F., Cooper, J. T., & Engel, M. H. (2012). Bottom-up biodiversity effects increase resource subsidy flux between ecosystems. *Ecology, 93*(10), 2165-2174.

American Society for Testing and Materials. (2006). *Standard guide for conducting laboratory toxicity tests with freshwater mussels*. E2455-06. ASTM Committee E47 on Biological Effects and Environ Fate, West Conshohocken, PA.

Atkins, L. (1979). Observations on the glochidial stage of the freshwater mussel *Hyridella* (*Hyridella*) *drapeta* (Iredale) (Molluscs: Pelecypoda). *Australian Journal of Marine and Freshwater Research, 30*(3), 411-416.

Barnhart, M. C., Haag, W. R., & Roston, W. N. (2008). Adaptations to host infection and larval parasitism in Unionoida. *Journal of the North American Benthological Society, 27*(2), 370-394.

Bauer, G., & Vogel, C. (1987). The parasitic stage of the freshwater pearl mussel (*Margaritifera margaritifera* L.) I. Host response to glochidiosis. *Archiv für Hydrobiologie Supplement, 76*, 393-402.

Bauer, G., & Wächtler, K. (2001). *Ecology and evolution of the freshwater mussels Unionoida*. Berlin Heidelberg, Germany: Springer.

Beckett, D. C., Green, B. W., Thomas, S. A., & Miller, A. C. (1996). Epizoic invertebrate communities on upper Mississippi River unionid bivalves. *American Midland Naturalist, 135*(1), 102-114.

Benson, J., Close, P., Stewart, B., & Lymbery, A. (2017). Upstream recolonization by freshwater mussels (Unionoida: Hyriidae) following installation of a fishway. *Aquatic Conservation: Marine and Freshwater Ecosystems, 28*, 512-517.

Beveridge, A., & Daniel, M. J. (1965). Observations on a high population of brown rats (*Rattus norvegicus* Berkenhout 1767) on Mokoia Island, Lake Rotorua. *New Zealand Journal of Science, 8*, 174-189.

Blakeslee, C. J., Silldorff, E. L., & Galbraith, H. S. (2018). Changes in freshwater mussel communities linked to legacy pollution in the lower Delaware River. *Northeastern Naturalist, 25*(1), 101-117.

Bogan, A. E. (1993). Freshwater bivalve extinctions (Mollusca: Unionoida): A search for causes. *American Zoologist, 33*(6), 599-609.

Bogan, A. E. (2008). Global diversity of freshwater mussels (Mollusca: Bivalvia) in freshwater. In E. V. Balian, C. Lévêque, H. Segers & K. Martens (Eds.), *Freshwater animal diversity assessment* (pp. 139-147). Dordrecht Netherlands: Springer.

Brown, E. J. (2010). Flow regime and water use. In K. J. Collier, D. P. Hamilton, W. N. Vant & C. Howard-Williams (Eds.), *The waters of the Waikato: Ecology of New Zealand's longest river*. Hamilton, New Zealand: Environment Waikato and the Centre for Biodiversity and Ecology Research, The University of Waikato.

Brown, M. E., Kowalewski, M., Neves, R. J., Cherry, D. S., & Schreiber, M. E. (2005). Freshwater mussel shells as environmental chronicles: Geochemical and taphonomic signatures of mercury-related extirpations in the North Fork Holston River, Virginia. *Environmental Science & Technology, 39*(6), 1455-1462.

Brown, R. L., Clearwater, S. J., Thompson, K. J., Martin, M. L., & Jellyman, P. G. (2017). *Comparison of host fish suitability for larvae (glochidia) of the native freshwater mussel, Echyridella menziesii*. Poster presentation to the Integrating Multiple Aquatic Values, 5th Biennial Symposium of the International Society for River Science in association with the IPENZ/Water NZ Rivers Group and the New Zealand Freshwater Sciences Society, Hamilton, New Zealand, 19-24 November 2017.

Catlin, A., Collier, K., Pingram, M., & Hamer, M. (2018). *Regional guidelines for ecological assessments of freshwater environments - standardised protocol for adult freshwater mussel monitoring in wadeable streams*. Waikato Regional Council: Hamilton, New Zealand.

Chappell, P. R. (2013). *The climate and weather of Waikato*. NIWA Science and Technology Series, 61: New Zealand.

Chowdhury, G. W., Zieritz, A., & Aldridge, D. C. (2016). Ecosystem engineering by mussels supports biodiversity and water clarity in a heavily polluted lake in Dhaka, Bangladesh. *Freshwater Science, 35*(1), 188-199.

Christian, A. D., Monroe, E. M., Asher, A. M., Loutsch, J. M., & Berg, D. J. (2007). Methods of DNA extraction and PCR amplification for individual freshwater mussel (Bivalvia: Unionidae) glochidia, with the first report of multiple paternity in these organisms. *Molecular Ecology Notes, 7*(4), 570-573.

Clarke, K. R., Gorley, R. N., Somerfield, P. J., & Warwick, R. M. (2014). *Change in marine communities: An approach to statistical analysis and interpretation* (3rd ed.). Plymouth, England: Plymouth Marine Laboratory.

Clearwater, S. J., Roper, D. S., Hickey, C. W., Martin, M. L., & Williams, E. K. (2011). *The reproductive cycle of the freshwater mussel Echyridella menziesii (Unionacea: Hyriidae) (Gray, 1842) in Lake Taupō, New Zealand*. Unpublished manuscript, National Institute of Water and Atmospheric Research: Hamilton, New Zealand.

Clearwater, S. J., Thompson, K. J., & Hickey, C. W. (2014). Acute toxicity of copper, zinc, and ammonia to larvae (glochidia) of a native freshwater mussel *Echyridella menziesii* in New Zealand. *Archives of Environmental Contamination and Toxicology, 66*(2), 213-226.

Clements, E., Thomas, R., & Adams, C. (2017). An investigation of salmonid host utilization by the endangered freshwater pearl mussel (*Margaritifera margaritifera*) in north-west Scotland. *Aquatic Conservation: Marine and Freshwater Ecosystems, 28*(3), 1-5.

Ćmiel, A., Zając, K., Lipińska, A., & Zając, T. (2018). Glochidial infestation of fish by the endangered thick-shelled river mussel *Unio crassus*. *Aquatic Conservation: Marine and Freshwater Ecosystems, 28*(3), 535-544.

Coffey, B. (1997). *A contribution to a biological description of instream community structure along nine permanent transects across Lake Whakamaru, middle Waikato River*. Brian T Coffey and Associates: Hamilton, New Zealand.

Coffey, B., Coffey, O., & Welch, M. (1998). *A contribution to a biological description of instream community structure in Lake Arapuni, middle Waikato River. Part A: Permanent transects 1 to 5, summer 1997-98*. Brian T Coffey and Associates: Hamilton, New Zealand.

Collier, K. J., & Grainger, N. P. (2015). *New Zealand invasive fish management handbook*. Hamilton, New Zealand: Lake Ecosystem Restoration New Zealand (LERNZ: The University of Waikato) and Department of Conservation.

Collier, K. J., Hamer, M. P., & Moore, S. C. (2014). Littoral and benthic macroinvertebrate community responses to contrasting stressors in a large New Zealand river. *New Zealand Journal of Marine and Freshwater Research, 48*(4), 560-576.

Collier, K. J., Hamilton, D. P., Vant, B., & Howard-Williams, C. (2010). *The waters of the Waikato: Ecology of New Zealand's longest river*. Hamilton, New Zealand: Environment Waikato and the Centre for Biodiversity and Ecology Research, The University of Waikato.

Collier, K. J., & Hogg, I. D. (2010). Macroinvertebrates. In K. J. Collier, D. P. Hamilton, W. N. Vant & C. Howard-Williams (Eds.), *The waters of the Waikato: Ecology of New Zealand's longest river* (pp. 173-192). Hamilton, New Zealand: Environment Waikato and the Centre for Biodiversity and Ecology Research, The University of Waikato.

Collier, K. J., Leathwick, J. R., & Rowe, D. K. (2016a). Assessing vulnerability of New Zealand lakes to loss of conservation value from invasive fish impacts. *Aquatic Conservation: Marine and Freshwater Ecosystems, 27*(2), 534-546.

Collier, K. J., Probert, P. K., & Jeffries, M. (2016b). Conservation of aquatic invertebrates: Concerns, challenges and conundrums. *Aquatic Conservation: Marine and Freshwater Ecosystems, 26*(5), 817-837.

Cope, G. W., Bringolf, R. B., Buchwalter, D. B., Newton, T. J., Ingersoll, C. G., Wang, N., Augspurger, T., Dwyer, J. F., Barnhart, C. M., Neves, R. J., &

Hammer, E. (2008). Differential exposure, duration, and sensitivity of unionoidean bivalve life stages to environmental contaminants. *Journal of the North American Benthological Society, 27*(2), 451-462.

Culp, J. J., Haag, W. R., Arrington, D. A., & Kennedy, T. B. (2011). Seasonal and species-specific patterns in abundance of freshwater mussel glochidia in stream drift. *Journal of the North American Benthological Society, 30*(2), 436-445.

Cyr, H., Collier, K. J., Clearwater, S. J., Hicks, B. J., & Stewart, S. D. (2017). Feeding and nutrient excretion of the New Zealand freshwater mussel *Echyridella menziesii* (Hyriidae: Unionida): Implications for nearshore nutrient budgets in lakes and reservoirs. *Aquatic Sciences, 79*(3), 557-571.

David, B. O., Bourke, C., Hamer, M. P., Scothern, S., Pingram, M., & Lake, M. D. (2016). *Incorporating fish monitoring into the Waikato Regional Councils' Regional Ecological Monitoring of Streams (REMS): Preliminary results for wadeable streams 2009-2015*. Waikato Regional Council: Hamilton, New Zealand.

David, B. O., Hamer, M. P., Collier, K. J., Lake, M. D., Surrey, G. M., McArthur, K., Nicholson, C., Perrie, A., & Dale, M. (2010). A standardised sampling protocol for robust assessment of reach-scale fish community diversity in wadeable New Zealand streams. *New Zealand Journal of Marine and Freshwater Research, 44*(3), 177-187.

Dodd, B. J., Barnhart, M. C., Rogers-Lowery, C. L., Fobian, T. B., & Dimock, R. V. (2005). Cross-resistance of largemouth bass to glochidia of unionid mussels. *Journal of Parasitology, 91*(5), 1064-1072.

Dodd, B. J., Barnhart, M. C., Rogers-Lowery, C. L., Fobian, T. B., & Dimock, R. V. (2006). Persistence of host response against glochidia larvae in *Micropterus salmoides*. *Fish and Shellfish Immunology, 21*(5), 473-484.

Douda, K., Sell, J., Kubikova-Pelakova, L., Horky, P., Kaczmarczyk, A., & Mioduchowska, M. (2014). Host compatibility as a critical factor in management unit recognition: Population-level differences in mussel-fish relationships. *Journal of Applied Ecology, 51*(4), 1085–1095.

Downing, J. A., Rochon, Y., Pérusse, M., & Harvey, H. (1993). Spatial aggregation, body size, and reproductive success in the freshwater mussel *Elliptio complanata*. *Journal of the North American Benthological Society, 12*(2), 148-156.

Downing, J. A., Van Meter, P., & Woolnough, D. A. (2010). Suspects and evidence: A review of the causes of extirpation and decline in freshwater mussels. *Animal Biodiversity and Conservation, 33*(2), 151-185.

Dudgeon, D., Arthington, A., Gessner, M., Kawabata, Z., Knowler, D., Leveque, C., Naiman, R., Prieur-Richard, A., Soto, D., Stiassny, M., & Sullivan, C. (2006). Freshwater biodiversity: Importance, threats, status and conservation challenges. *Biological Reviews, 81*(2), 163–182.

Dunn, N. R., Allibone, R., Closs, G. P., Crow, S. K., David, B. O., Goodman, J. M., Griffiths, M., Jack, D. C., Ling, N., Waters, J. M., & Rolfe, J. R. (2018). *Conservation status of New Zealand freshwater fishes, 2017*. Department of Conservation: Wellington, New Zealand.

Fenwick, M. C., & Marshall, B. A. (2006). A new species of *Echyridella* from New Zealand, and recognition of *Echyridella lucasi* (Suter, 1905) (Mollusca: Bivalvia: Hyriidae). *Molluscan Research, 26*(2), 69-76.

Ferguson, C. D., Blum, M. J., Raymer, M. L., Eackles, M. S., & Krane, D. E. (2013). Population structure, multiple paternity, and long-distance transport of spermatozoa in the freshwater mussel *Lampsilis cardium* (Bivalvia: Unionidae). *Freshwater Science, 32*(1), 267-282.

Ferreira-Rodríguez, N., Akiyama, Y. B., Aksenova, O. V., Araujo, R., Barnhart, M. C., Bespalaya, Y. V., Bogan, A. E., Bolotov, I. N., Budha, P. B., Clavijo, C., Clearwater, S. J., et al. (2019). Research priorities for freshwater mussel conservation assessment. *Biological Conservation, 231*, 77-87.

Filipsson, K., Petersson, T., Höjesjö, J., Piccolo, J. J., Näslund, J., Wengström, N., & Österling, M. E. (2018). Heavy loads of parasitic freshwater pearl mussel (*Margaritifera margaritifera* L.) larvae impair foraging, activity and dominance performance in juvenile brown trout (*Salmo trutta* L.). *Ecology of Freshwater Fish, 27*(1), 70-77.

Fisher, G. R., & Dimock, R. V. (2002). Ultrastructure of the mushroom body: Digestion during metamorphosis of *Utterbackia imbecillis* (Bivalvia: Unionidae). *Invertebrate Biology, 121*(2), 126-135.

Franklin, P., & Gee, E. (2019). Living in an amphidromous world: Perspectives on the management of fish passage from an island nation. *Aquatic Conservation: Marine and Freshwater Ecosystems*, special issue.

Franklin, P., Ling, N., David, B., Allibone, R., Crow, S., West, D., & Closs, G. (2014). *Gobiomorphus basalis*. The IUCN Red List of Threatened Species 2014: e.T197317A2481973. Downloaded on 17 June 2019.

Fritts, A., Barnhart, C. M., Bradley, M., Liu, N., Cope, W. G., Hammer, E., & Bringolf, R. (2014). Assessment of toxicity test endpoints for freshwater mussel larvae (glochidia): Glochidia toxicity test endpoints. *Environmental Toxicology and Chemistry, 33*(1), 199–207.

Fritts, A. K., Fritts, M. W., Peterson, D. L., Fox, D. A., & Bringolf, R. B. (2012). Critical linkage of imperiled species: Gulf sturgeon as host for purple bankclimber mussels. *Freshwater Science, 31*(4), 1223-1232.

Gascho Landis, A. M., Mosley, T. L., Haag, W. R., & Stoeckel, J. A. (2012). Effects of temperature and photoperiod on lure display and glochidial release in a freshwater mussel. *Freshwater Science, 31*(3), 775-786.

Geist, J. (2010). Strategies for the conservation of endangered freshwater pearl mussels (*Margaritifera margaritifera*): A synthesis of conservation genetics and ecology. *Hydrobiologia, 644*(1), 69-88.

Geist, J., & Auerswald, K. (2007). Physicochemical stream bed characteristics and recruitment of the freshwater pearl mussel (*Margaritifera margaritifera*). *Freshwater Biology, 52*(12), 2299-2316.

Geist, J., Porkka, M., & Kuehn, R. (2006). The status of host fish populations and fish species richness in European freshwater pearl mussel (*Margaritifera margaritifera*) streams. *Aquatic Conservation: Marine and Freshwater Ecosystems, 16*(3), 251-266.

Graf, D. L., & Cummings, K. S. (2007). Review of the systematics and global diversity of freshwater mussel species (Bivalvia: Unionoida). *Journal of Molluscan Studies, 73*, 291-314.

Graf, D. L., & Cummings, K. S. (2011). Freshwater mussel (Mollusca: Bivalvia: Unionoida) richness and endemism in the ecoregions of Africa and Madagascar based on comprehensive museum sampling. *Hydrobiologia, 678*(1), 17-36.

Graham, E., Franklin, P., Croker, G., Reeve, K., & Smith, J. (2016). *Waihou and Piako ecological monitoring 2015*. National Institute of Water and Atmospheric Research: Hamilton, New Zealand.

Grainger, N., Harding, J. S., Drinan, T., Collier, K. J., Smith, B. J., Death, R., Makan, T., & Rolfe, J. R. (2018). *Conservation status of New Zealand freshwater invertebrates, 2018*. Department of Conservation: Wellington, New Zealand.

Graynoth, E., Bonnett, M., & Jellyman, D. (2012). Estimation of native fish density in lowland streams by repeated electric fishing during the day and following night. *New Zealand Journal of Marine and Freshwater Research, 46*(2), 243-261.

Grimmond, N. M. (1968). *Observations on growth and age of Hyridella menziesii (Mollusca: Bivalvia) in a freshwater tidal lake.* Masters thesis, University of Otago: Dunedin, New Zealand.

Haag, W. R. (2013). The role of fecundity and reproductive effort in defining life-history strategies of North American freshwater mussels. *Biological Reviews, 88*(3), 745-766.

Haag, W. R., & Staton, L. J. (2003). Variation in fecundity and other reproductive traits in freshwater mussels. *Freshwater Biology, 48*(12), 2118-2130.

Haag, W. R., & Stoeckel, J. A. (2015). The role of host abundance in regulating populations of freshwater mussels with parasitic larvae. *Oecologia, 178*(4), 1159-1168.

Haag, W. R., & Warren, M. L. (2000). Effects of light and presence of fish on lure display and larval release behaviours in two species of freshwater mussels. *Animal Behaviour, 60*(6), 879-886.

Harding, J., Clapcott, J., Quinn, J., Hayes, J., Joy, M., Storey, R., Greig, H., Hay, J., James, T., Beech, M., Ozane, R., et al. (2009). *Stream habitat assessment*

protocols for wadeable rivers and streams of New Zealand. Christchurch, New Zealand: School of Biological Sciences, University of Canterbury.

Hare, K. M., Borrelle, S. B., Buckley, H. L., Collier, K. J., Constantine, R., Perrott, J. K., Watts, C. H., & Towns, D. R. (2019). Intractable: Species in New Zealand that continue to decline despite conservation efforts. *Journal of the Royal Society of New Zealand*, 1-19.

Hastie, L., & Boon, P. (2001). Does electrofishing harm freshwater pearl mussels? *Aquatic Conservation: Marine and Freshwater Ecosystems, 11*(2), 149-152.

Hastie, L., & Young, M. (2003). Timing of spawning and glochidial release in Scottish freshwater pearl mussel (*Margaritifera margaritifera*) populations. *Freshwater Biology, 48*(12), 2107–2117.

Hine, P. M. (1978). Distribution of some parasites of freshwater eels in New Zealand. *New Zealand Journal of Marine and Freshwater Research, 12*(2), 179-187.

Hiroa, T. R. (1921). Māori food supplies of Lake Rotorua, with methods of obtaining them, and usages and customs appertaining thereto. *Transactions of the Royal Society of New Zealand, 52*, 433-451.

Houghton, W. (1862). On the parasitic nature of the fry of *Anodonta cygnea*. *Journal of Cell Science, 2*(7), 162-168.

James, M. R. (1985). Distribution, biomass and production of the freshwater mussel, *Hyridella menziesii*, in Lake Taupō, New Zealand. *Freshwater Biology, 15*(3), 307-314.

Jansen, W., Bauer, G., & Zahner-Meike, E. (2001). Glochidial mortality in freshwater mussels. In G. Bauer & K. Wächtler (Eds.), *Ecology and evolution of the freshwater mussels Unionoida* (pp. 185-211). Berlin Heidelberg, Germany: Springer.

Jansen, W., & Hanson, M. J. (1991). Estimates of the number of glochidia produced by clams (*Anodonta grandis simpsoniana* Lea), attaching to yellow perch (*Perca flavescens*), and surviving to various ages in Narrow Lake, Alberta. *Canadian Journal of Zoology, 69*, 973-977.

Jing, W., Lang, L., Lin, Z., Liu, N., & Wang, L. (2019). Cadmium bioaccumulation and elimination in tissues of the freshwater mussel *Anodonta woodiana*. *Chemosphere, 219*, 321-327.

Joy, M. K., Foote, K. J., McNie, P., & Piria, M. (2019). Decline in New Zealand's freshwater fish fauna: Effect of land use. *Marine and Freshwater Research, 70*(1), 114-124.

Kelly, C. B., & Watters, G. T. (2010). Distribution and prevalence of glochidia-infested wild-caught fishes at a Muskingum River site in southeastern Ohio. *Journal of Freshwater Ecology, 25*(1), 119-126.

Kettle, M. (2013). *Waikato spatial plan*. Waikato Regional Council: Hamilton, New Zealand.

Klunzinger, M. W., Beatty, S. J., Morgan, D. L., Thomson, G. J., & Lymbery, A. J. (2012). Glochidia ecology in wild fish populations and laboratory determination of competent host fishes for an endemic freshwater mussel of south-western Australia. *Australian Journal of Zoology, 60*(1), 26-36.

Klunzinger, M. W., Morgan, D. L., Lymbery, A. J., Ebner, B. C., Beatty, S. J., & Thomson, G. L. (2010). Discovery of a host fish for glochidia of *Velesunio angasi* (Sowerby, 1867) (Bivalvia: Unionoida: Hyriidae) from the Fortescue River, Pilbara, Western Australia. *Australian Journal of Zoology, 58*(4), 263-266.

Kuussaari, M., Bommarco, R., Heikkinen, R. K., Helm, A., Krauss, J., Lindborg, R., Öckinger, E., Pärtel, M., Pino, J., & Roda, F. (2009). Extinction debt: A challenge for biodiversity conservation. *Trends in Ecology and Evolution, 24*(10), 564-571.

Land and Water Aotearoa. (2017). Mangapapa Stream at Henry Watson Road. Retrieved from https://www.lawa.org.nz/explore-data/waikato-region/river-quality/piako-river/mangapapa-stm-matamata-at-henry-watson-rd-mci/

Larsson, M. (2015). *Interaction between fish and freshwater mussels: Determination of functional hosts for the thick-shelled river mussel, Unio Crassus.* Masters thesis, University of Gothenburg: Sweden.

Leathwick, J., Elith, J., Chadderton, W., Rowe, D., & Hastie, T. (2008). Dispersal, disturbance and the contrasting biogeographies of New Zealand's diadromous and non-diadromous fish species. *Journal of Biogeography, 35*(8), 1481-1497.

Leathwick, J. R., Collier, K. J., Hicks, B. J., Ling, N., Stichbury, G., & de Winton, M. (2016). Predictions of establishment risk highlight biosurveillance priorities for invasive fish in New Zealand lakes. *Freshwater Biology, 61*(9), 1522-1535.

Lellis, W. A., White, B. S., Cole, J. C., Johnson, C. S., Devers, J. L., Gray, E. V., & Galbraith, H. S. (2013). Newly documented host fishes for the eastern elliptio mussel *Elliptio complanata*. *Journal of Fish and Wildlife Management, 4*(1), 75-85.

Lieschke, G. J., & Trede, N. S. (2009). Fish immunology. *Current Biology, 19*(16), 678-682.

Lima, P., Lima, M. L., Kovitvadhi, U., Kovitvadhi, S., Owen, C., & Machado, J. (2012). A review on the "in vitro" culture of freshwater mussels (Unionoida). *Hydrobiologia, 691*(1), 21-33.

Ling, N., Allibone, R., Franklin, P., David, B., Crow, S., & Closs, G. (2014). *Gobiomorphus huttoni*. The IUCN Red List of Threatened Species 2014: e.T197322A2482207. Downloaded on 17 June 2019.

Lopes-Lima, M., Teixeira, A., Froufe, E., Lopes, A., Varandas, S., & Sousa, R. (2014). Biology and conservation of freshwater bivalves: Past, present and future perspectives. *Hydrobiologia, 735*(1), 1-13.

Lowe, D. J. (2010). *Introduction to the landscapes and soils of the Hamilton Basin*. In D.J. Lowe, V.E. Neall, M. Hedley, B. Clothier & A. Mackay (Eds.), Guidebook for pre-conference North Island New Zealand "Volcanoes to ocean" 26th-30th July, 2010, 19th World Congress of Soil Science: soil solutions for a changing world: Brisbane Australia 1-6 August 2010. (pp. 1.14-1.61). Palmerston North, New Zealand: New Zealand Society of Soil Science.

Lydeard, C., Cowie, R. H., Ponder, W. F., Bogan, A. E., Bouchet, P., Clark, S. A., Cummings, K. S., Frest, T. J., Gargominy, O., Herbert, D. G., Hershler, R., et al. (2004). The global decline of nonmarine mollusks. *Bioscience, 54*(4), 321-330.

Maceda-Veiga, A., Green, A. J., Poulin, R., & Lagrue, C. (2016). Body condition peaks at intermediate parasite loads in the common bully *Gobiomorphus cotidianus*. *PloS one, 11*(12), 18.

Mahon, R. (1980). Accuracy of catch-effort methods for estimating fish density and biomass in streams. *Environmental Biology of Fishes, 5*(4), 343-363.

Markich, S. J. (2017). Sensitivity of the glochidia (larvae) of freshwater mussels (Bivalvia: Unionida: Hyriidae) to cadmium, cobalt, copper, lead, nickel and zinc: Differences between metals, species and exposure time. *Science of the Total Environment, 601*, 1427-1436.

Marshall, B. A., Fenwick, M. C., & Ritchie, P. A. (2014). New Zealand recent Hyriidae (Mollusca: Bivalvia: Unionida). *Molluscan Research, 34*(3), 181-200.

Marwaha, J., Aase, H., Geist, J., Stoeckle, B. C., Kuehn, R., & Jakobsen, P. J. (2019). Host (*Salmo trutta*) age influences resistance to infestation by freshwater pearl mussel (*Margaritifera margaritifera*) glochidia. *Parasitology Research, 118*(5), 1519-1532.

Marwaha, J., Jensen, K. H., Jakobsen, P. J., & Geist, J. (2017). Duration of the parasitic phase determines subsequent performance in juvenile freshwater pearl mussels (*Margaritifera margaritifera*). *Ecology and Evolution, 7*(5), 1375-1383.

Matheson, F., Clayton, J., & Smith, J. (2010). *Lake Karāpiro weed control from April to June 2010*. National Institute of Water and Atmospheric Research: Hamilton, New Zealand.

McDowall, R. M. (1990). *New Zealand freshwater fishes: A natural history and guide.* Auckland, New Zealand: Heinemann Reed.

McDowall, R. M. (2002). Decline of the kākahi: Identifying cause and effect. *Water and Atmosphere, 10*(4), 8-9.

McEwan, A. (2015). *Kākahi monitoring guide*. Greater Wellington Regional Council: Wellington, New Zealand.

Melchior, M. (2017). *Effects of water temperature on the release and viability of glochidia of the freshwater mussel, Echyridella menziesii.* Honours dissertation, The University of Waikato: Hamilton, New Zealand.

Melchior, M., Collier, K., & Clearwater, S. (in press). First record of complex release strategies and morphometry of glochidia in sympatric *Echyridella* species (Bivalvia: Unionida: Hyriidae). *Hydrobiologia*.

Ministry for the Environment and Stats NZ. (2019). *New Zealand's environmental reporting series: Environment Aotearoa 2019 summary*. Ministry for the Environment and Stats NZ: Wellington, New Zealand.

Moore, T., Collier, K., & Duggan, I. (2019). Interactions between Unionida and non-native species: A global meta-analysis. *Aquatic Conservation: Marine and Freshwater Ecosystems*, special issue.

Moore, T. P., & Clearwater, S. J. (in press). Non-native fish as glochidial sinks: Elucidating disruption pathways for *Echyridella menziesii* recruitment. *Hydrobiologia*.

Neves, R. J., & Widlak, J. C. (1988). Occurence of glochidia in stream drift and on fishes of the upper North Fork Holston River, Virginia. *American Midland Naturalist, 119*(1), 111-120.

New Zealand Parliament. (2018). Protecting New Zealand's indigenous freshwater fish. Retrieved from https://www.parliament.nz/en/get-involved/topics/all-current-topics/protecting-new-zealand-s-indigenous-freshwater-fish/

Nezlin, L. P., Cunjak, R. A., Zotin, A. A., & Ziuganov, V. V. (1994). Glochidium morphology of the freshwater pearl mussel (*Margaritifera margaritifera*) and glochidiosis of Atlantic salmon (*Salmo salar*): A study by scanning electron microscopy. *Canadian Journal of Zoology, 72*(1), 15-21.

Nobles, T., & Zhang, Y. (2011). Biodiversity loss in freshwater mussels: Importance, threats, and solutions. In O. Grillo (Ed.), *Biodiversity loss in a changing planet* (pp. 138-162). San Marcos, TX: InTech.

Nowak, J., & Kozlowski, M. (2013). *Mussels: Ecology, life habits and control*. New York, NY: Nova Science Publishers.

O'Connell, M. T., & Neves, R. J. (1999). Evidence of immunological responses by a host fish (*Ambloplites rupestris*) and two non-host fishes (*Cyprinus carpio* and *Carassius auratus*) to glochidia of a freshwater mussel (*Villosa iris*). *Journal of Freshwater Ecology, 14*(1), 71-78.

O'Shea, B., Mordue-Luntz, A. J., Fryer, R. J., Pert, C. C., & Bricknell, I. R. (2006). Determination of the surface area of a fish. *Journal of Fish Diseases, 29*(7), 437-440.

O'Donnell, C. F., Weston, K. A., & Monks, J. M. (2017). Impacts of introduced mammalian predators on New Zealand's alpine fauna. *New Zealand Journal of Ecology, 41*(1), 1-22.

Ogilvie, S. C., & Mitchell, S. F. (1995). A model of mussel filtration in a shallow New Zealand lake, with reference to eutrophication control. *Archiv für Hydrobiologie, 133*(4), 471-482.

Ooue, K., Terui, A., Urabe, H., & Nakamura, F. (2017). A delayed effect of the aquatic parasite *Margaritifera laevis* on the growth of the salmonid host fish *Oncorhynchus masou masou*. *Limnology, 18*(3), 345-351.

Österling, M. E. (2015). Timing, growth and proportion of spawners of the threatened unionoid mussel *Margaritifera margaritifera*: Influence of water temperature, turbidity and mussel density. *Aquatic Sciences, 77*(1), 1-8.

Österling, M. E., Arvidsson, B. L., & Greenberg, L. A. (2010). Habitat degradation and the decline of the threatened mussel *Margaritifera margaritifera*: Influence of turbidity and sedimentation on the mussel and its host. *Journal of Applied Ecology, 47*(4), 759-768.

Österling, M. E., Ferm, J., & Piccolo, J. J. (2014). Parasitic freshwater pearl mussel larvae (*Margaritifera margaritifera* L.) reduce the drift-feeding rate of juvenile brown trout (*Salmo trutta* L.). *Environmental Biology of Fishes, 97*(5), 543-549.

Österling, M. E., Greenberg, L. A., & Arvidsson, B. L. (2008). Relationship of biotic and abiotic factors to recruitment patterns in *Margaritifera margaritifera*. *Biological Conservation, 141*(5), 1365-1370.

Österling, M. E., & Larsen, B. M. (2013). Impact of origin and condition of host fish (*Salmo trutta*) on parasitic larvae of *Margaritifera margaritifera*. *Aquatic Conservation: Marine and Freshwater Ecosystems, 23*(4), 564–570.

Patterson, M. A., Mair, R. A., Eckert, N. L., Gatenby, C. M., Brady, T., Jones, J. W., Simmons, B. R., & Devers, J. L. (2018). *Freshwater mussel propagation for restoration*. Cambridge, England: Cambridge University Press.

Percival, E. (1931). A note on the life history of *Diplodon lutulentus* Gould. *Transactions of the Royal Society of New Zealand, 62*, 86-91.

Peterson, J. T., Thurow, R. F., & Guzevich, J. W. (2004). An evaluation of multipass electrofishing for estimating the abundance of stream-dwelling salmonids. *Transactions of the American Fisheries Society, 133*(2), 462-475.

Phillips, N., Parkyn, S., Kusabs, I., & Roper, D. (2007). *Taonga and mahinga kai species of the Te Arawa lakes: A review of current knowledge - kākahi.* National Institute of Water and Atmospheric Research: Hamilton, New Zealand.

Rainforth, H. J. (2008). *Tiakina kia ora: Protecting our freshwater mussels.* Masters thesis, Victoria University: Wellington, New Zealand.

Roberts, A. D., & Barnhart, M. (1999). Effects of temperature, pH, and CO_2 on transformation of the glochidia of *Anodonta suborbiculata* on fish hosts and in vitro. *Journal of the North American Benthological Society, 18*(4), 477-487.

Rogers-Lowery, C. L., & Dimock, R. V. (2006). Encapsulation of attached ectoparasitic glochidia larvae of freshwater mussels by epithelial tissue on fins of naïve and resistant host fish. *Biological Bulletin, 210*(1), 51-63.

Rogers, C. L., & Dimock, R. V. (2003). Acquired resistance of bluegill sunfish *Lepomis macrochirus* to glochidia larvae of the freshwater mussel *Utterbackia imbecillis* (Bivalvia: Unionidae) after multiple infections. *Journal of Parasitology, 89*(1), 51-56.

Roper, D. S., & Hickey, C. W. (1994). Population structure, shell morphology, age and condition of the freshwater mussel *Hyridella menziesii* (Unionacea: Hyriidae) from seven lake and river sites in the Waikato River system. *Hydrobiologia, 284*(3), 205-217.

Roper, D. S., & Hickey, C. W. (1995). Effects of food and silt on filtration, respiration and condition of the freshwater mussel *Hyridella menziesii* (Unionacea: Hyriidae): Implications for bioaccumulation. *Hydrobiologia, 312*(1), 17-25.

Rowe, D. K. (1999). Factors influencing the abundance of the common bully, *Gobiomorphus cotidianus* McDowall, in small North Island, New Zealand lakes. *Fisheries Management and Ecology, 6*(5), 377-386.

Salonen, J. K., & Taskinen, J. (2016). Electrofishing as a new method to search for unknown populations of the endangered freshwater pearl mussel *Margaritifera margaritifera*. *Aquatic Conservation Marine and Freshwater Ecosystems, 27*, 115-127.

Schneider, L. D., Nilsson, P. A., Höjesjö, J., & Österling, M. E. (2017). Local adaptation studies and conservation: Parasite–host interactions between the endangered freshwater mussel *Unio crassus* and its host fish. *Aquatic Conservation: Marine and Freshwater Ecosystems, 27*(6), 1261-1269.

Schneider, L. D., Nilsson, P. A., Höjesjö, J., & Österling, M. E. (2019). Effects of mussel and host fish density on reproduction potential of a threatened unionoid mussel: Prioritization of conservation locations in management trade-offs. *Biodiversity and Conservation, 28*(2), 259-273.

Schneider, L. D., Nilsson, P. A., & Österling, M. E. (2018). Evaluating temperature- and host-dependent reproduction in the parasitic freshwater mussel *Unio crassus*. *Hydrobiologia, 810*(1), 283-293.

Schofield, J. (1967). Geology of the Hamilton region. *Earth Science Journal, 1*(1), 82-88.

Schwalb, A. N., Cottenie, K., Poos, M. S., & Ackerman, J. D. (2011). Dispersal limitation of unionid mussels and implications for their conservation. *Freshwater Biology, 56*(8), 1509-1518.

Slapansky, L., Jurajda, P., & Janac, M. (2016). Early life stages of exotic gobiids as new hosts for unionid glochidia. *Freshwater Biology, 61*(6), 979-990.

Smith, J. (2014). *Freshwater fish spawning and migration periods*. National Institute of Water and Atmospheric Research: Hamilton, New Zealand.

Speirs, D. (2001). *The diversity and distribution of freshwater fish and their habitat in the major rivers of the Waikato Region*. Waikato Regional Council: Hamilton, New Zealand.

Spooner, D. E., & Vaughn, C. C. (2008). A trait-based approach to species' roles in stream ecosystems: Climate change, community structure, and material cycling. *Oecologia, 158*(2), 307-317.

Stenseth, N. C., & Mysterud, A. (2002). Climate, changing phenology, and other life history traits: Nonlinearity and match–mismatch to the environment. *Proceedings of the National Academy of Sciences, 99*(21), 13379-13381.

Stöckl, K., Taeubert, J. E., & Geist, J. (2014). Fish species composition and host fish density in streams of the thick-shelled river mussel (*Unio crassus*): Implications for conservation. *Aquatic Conservation: Marine and Freshwater Ecosystems, 25*(2), 276-287.

Strayer, D. L. (2008). *Freshwater mussel ecology: A multifactor approach to distribution and abundance*. London, England: University of California Press.

Strayer, D. L. (2014). Understanding how nutrient cycles and freshwater mussels (Unionoida) affect one another. *Hydrobiologia, 735*(1), 277-292.

Strayer, D. L., Downing, J. A., Haag, W. R., King, T. L., Layzer, J. B., Newton, T. J., & Nichols, J. S. (2004). Changing perspectives on pearly mussels, North America's most imperiled animals. *BioScience, 54*(5), 429-439.

Strayer, D. L., & Dudgeon, D. (2010). Freshwater biodiversity conservation: Recent progress and future challenges. *Journal of the North American Benthological Society, 29*(1), 344-358.

Taeubert, J. E., El-Nobi, G., & Geist, J. (2014). Effects of water temperature on the larval parasitic stage of the thick-shelled river mussel (*Unio crassus*). *Aquatic Conservation: Marine and Freshwater Ecosystems, 24*(2), 231-237.

Taeubert, J. E., Gum, B., & Geist, J. (2012a). Host-specificity of the endangered thick-shelled river mussel (*Unio crassus*, Philipsson 1788) and implications for conservation. *Aquatic Conservation: Marine and Freshwater Ecosystems, 22*(1), 36-46.

Taeubert, J. E., Gum, B., & Geist, J. (2013). Towards standardization of studies into host relationships of freshwater mussels. *Biological Conservation, 159*, 550-551.

Taeubert, J. E., Martinez, A. M. P., Gum, B., & Geist, J. (2012b). The relationship between endangered thick-shelled river mussel (*Unio crassus*) and its host fishes. *Biological Conservation, 155*, 94-103.

Terui, A., Miyazaki, Y., Yoshioka, A., Kadoya, T., Jopp, F., & Washitani, I. (2014). Dispersal of larvae of *Margaritifera laevis* by its host fish. *Freshwater Science, 33*(1), 112-123.

Theobald, S., & Coad, N. (2002). *Den control of stoats (Mustela erminea) in Trounson Kauri Park, Northland*. Department of Conservation: Wellington, New Zealand.

Thomas, G. R., Taylor, J., & de Leaniz, C. G. (2014). Does the parasitic freshwater pearl mussel *Margaritifera margaritifera* harm its host? *Hydrobiologia, 735*(1), 191-201.

Tremblay, M. E. M., Morris, T. J., & Ackerman, J. D. (2016). Loss of reproductive output caused by an invasive species. *Royal Society Open Science, 3*(4), Article no. 150481.

Tulagi, A. (2017). *Waikato River water quality monitoring programme: Data report 2016*. Waikato Regional Council: Hamilton, New Zealand.

Van Vreede, K. B., MacIntosh, D. L., & Black, M. C. (1999). Estimating time-to-gravid for a freshwater mussel, *Utterbackia imbecillis* (Unionidae), after temperature conditioning in the laboratory. *Environmental Toxicology and Chemistry, 18*(7), 1469-1473.

Vant, B. (2018). *Trends in river water quality in the Waikato region, 1993-2017*. Waikato Regional Council: Hamilton, New Zealand.

Vaughn, C. (2017). Ecosystem services provided by freshwater mussels. *Hydrobiologia, 810*(1), 15-27.

Vaughn, C. C., Nichols, S. J., & Spooner, D. E. (2008). Community and foodweb ecology of freshwater mussels. *27*(2), 409-423.

Vaughn, C. C., & Spooner, D. E. (2006). Unionid mussels influence macroinvertebrate assemblage structure in streams. *Journal of the North American Benthological Society, 25*(3), 691-700.

Vaughn, C. C., & Taylor, C. M. (2000). Macroecology of a host-parasite relationship. *Ecography, 23*(1), 11-20.

Wächtler, K., Dreher-Mansur, M. C., & Richter, T. (2001). Larval types and early postlarval biology in naiads (Unionoida). In G. Bauer & K. Wächtler (Eds.), *Ecology and evolution of the freshwater mussels Unionoida* (pp. 93-125). Berlin Heidelberg, Germany: Springer.

Wacker, S., Larsen, B. M., Jakobsen, P., & Karlsson, S. (2018). High levels of multiple paternity in a spermcast mating freshwater mussel. *Ecology and Evolution, 8*(16), 8126-8134.

Waikato Regional Council. (2018). Soil risk for farm dairy effluent. Retrieved from https://waikatomaps.waikatoregion.govt.nz/Viewer/?map=1aa9c952a38949a68cbe3ca7aed48270

Waikato Regional Council. (2019). Waikato flows map. Retrieved from https://www.waikatoregion.govt.nz/services/regional-services/river-levels-and-rainfall/floods-and-flow/waikato-flows-map/

Walker, K., Byrne, M., Hickey, C. W., & Roper, D. S. (2001). Freshwater mussels (Hyriidae) of Australasia. In G. Bauer & K. Wächtler (Eds.), *Ecology and evolution of the freshwater mussels* (pp. 5-31). Berlin Heidelberg, Germany: Springer.

Watters, G. T. (2001). The evolution of the Unionacea in North America, and its implications for the worldwide fauna. In G. Bauer & K. Wächtler (Eds.), *Ecology and evolution of the freshwater mussels Unionoida* (pp. 281-307). Berlin Heidelberg, Germany: Springer.

Watters, G. T., & O'Dee, S. H. (1996). Shedding of untransformed glochidia by fishes parasitized by *Lampsilis fasciola* Rafinesque, 1820 (Mollusca: Bivalvia: Unionidae): Evidence of acquired immunity in the field? *Journal of Freshwater Ecology, 11*(4), 383-389.

Watters, G. T., & O'Dee, S. H. (2000). *Glochidial release as a function of water temperature: Beyond bradyticty and tachyticty.* Paper presented at the In *Freshwater Mollusk Symposia Proceedings, Special Publication of the Ohio Biological Survey* (pp. 135-140).

West, D., David, B., Closs, G., Allibone, R., Crow, S., Franklin, P., & Ling, N. (2014). *Gobiomorphus cotidianus*. The IUCN Red List of Threatened Species 2014: e.T197319A2482075. Downloaded on 11 June 2019.

Wood, P. J., & Armitage, P. D. (1997). Biological effects of fine sediment in the lotic environment. *Environmental management, 21*(2), 203-217.

Wu, R. W., Chen, T. H., Zanatta, D. T., Ouyang, S., & Wu, X. P. (2018). Reproductive traits of nine freshwater mussel species (Mollusca: Unionidae) from Poyang Lake, China. *Journal of Molluscan Studies, 84*, 324-332.

Yeager, M. M., Cherry, D. S., & Neves, R. J. (1994). Feeding and burrowing behaviors of juvenile rainbow mussels, *Villosa iris* (Bivalvia: Unionidae). *Journal of the North American Benthological Society, 13*(2), 217-222.

Young, M., & Williams, J. (1984). The reproductive biology of the freshwater pearl mussel *Margaritifera margaritifera* (Linn) in Scotland: Field studies. *Archiv für Hydrobiologie, 99*(4), 405-422.

Yusseppone, M. S., Rocchetta, I., Sabatini, S. E., Luquet, C. M., de Molina, M. D. R., Held, C., & Abele, D. (2018). Inducing the alternative oxidase forms part of the molecular strategy of anoxic survival in freshwater bivalves. *Frontiers in Physiology, 9*, 1-12.

Appendices

Appendix 1: Field sheet developed for stream-side glochidia infestation assessments.

FIELD GLOCHIDIA ASESSMENT — **Koura:**

Date: _____ **Samplers:** _____ **Stream:**_____ **Start time:**_____ **End time:** _____

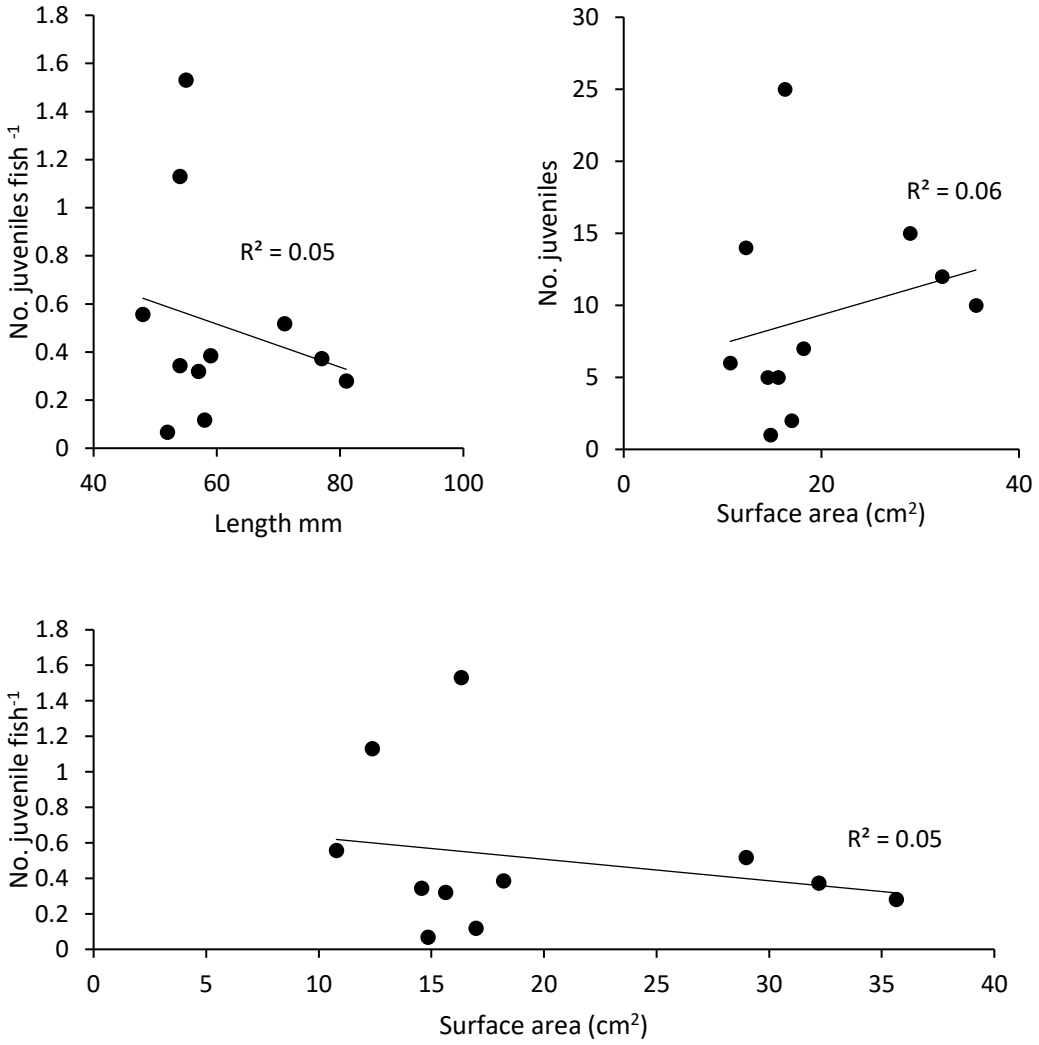
Total number samples brought back to lab: _____

Count (L/R= left/right fins) | A= attached, E= encysted

Sub reach #	Fish #	Fish species (code)	Length (mm)	Lips/ mouth/snout	Gill cover	1st Dorsal fin	Adipose fin (2nd dorsal)	Pectorals	Pelvic fins	Anal fin	Tail (Caudal fin)	Overall fish (external)	Notes (*= brought to lab for ID)
	1				L- R-			L- R-	L- R-				
	2				L- R-			L- R-	L- R-				
	3				L- R-			L- R-	L- R-				
	4				L- R-			L- R-	L- R-				
	5				L- R-			L- R-	L- R-				
	6				L- R-			L- R-	L- R-				
	7				L- R-			L- R-	L- R-				
	8				L- R-			L- R-	L- R-				
	9				L- R-			L- R-	L- R-				
	10				L- R-			L- R-	L- R-				
	11				L- R-			L- R-	L- R-				
	12				L- R-			L- R-	L- R-				
	13				L- R-			L- R-	L- R-				
	14				L- R-			L- R-	L- R-				
	15				L- R-			L- R-	L- R-				
	16				L- R-			L- R-	L- R-				
	17				L- R-			L- R-	L- R-				
	18				L- R-			L- R-	L- R-				
	19				L- R-			L- R-	L- R-				
	20				L- R-			L- R-	L- R-				
	21				L- R-			L- R-	L- R-				
	22				L- R-			L- R-	L- R-				
	23				L- R-			L- R-	L- R-				
	24				L- R-			L- R-	L- R-				
	25				L- R-			L- R-	L- R-				

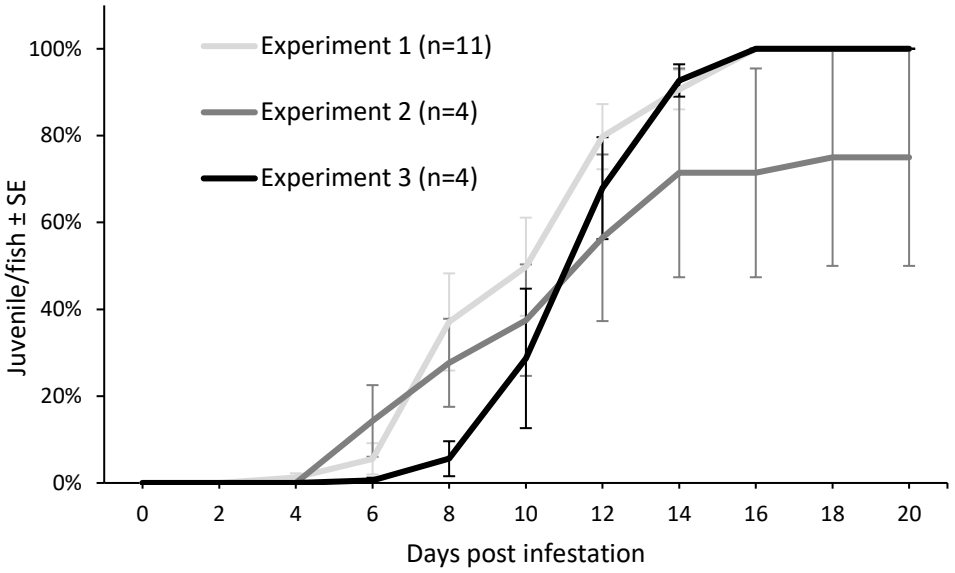
Appendix 1; Figure 1: Field sheet for the stream-side approach developed in this study to quantify external glochidia attachment on a range of native fish.

Appendix 2: Preliminary data analyses exploring relationships between juvenile mussels produced vs length and surface area.

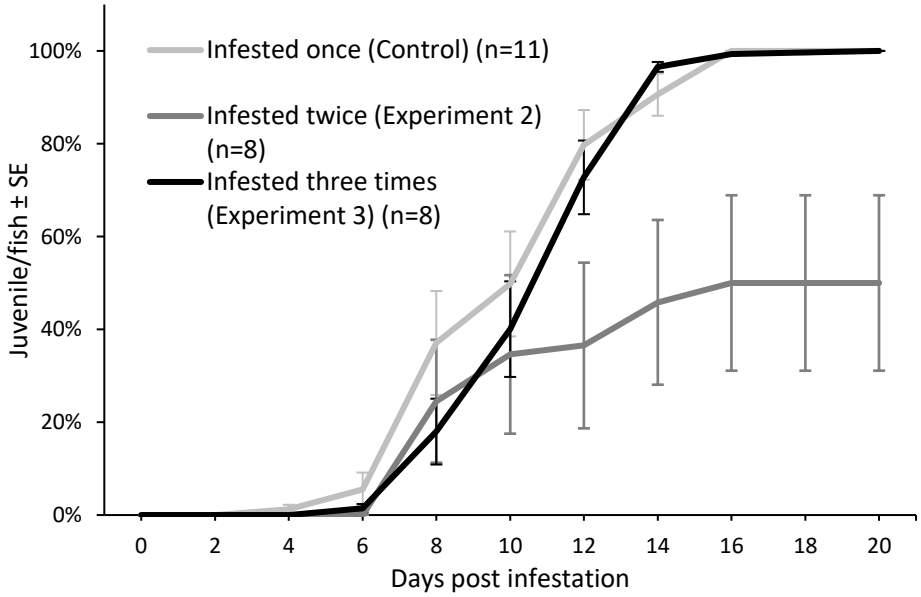


Appendix 2; Figure 1: Examples of exploring relationships between: the number of juveniles fish cm^{-2} vs length (top left); total number of juvenile mussels vs surface area (top right); and the number of juveniles fish cm^{-2} vs surface area; for experiment one control fish. R^2 values <0.1 were considered not significant and were therefore not presented in the main text.

Appendix 3: Preliminary cumulative analyses including fish that were not regarded as 'juvenile-producing fish'.



Appendix 3; Figure 1: The cumulative mean (± SE) % of juveniles produced over 20 days for control fish in each experiment (i.e., fish infested once). Note: One fish in experiment two did not produce any juveniles hence the curve plateaus at 75%.



Appendix 3; Figure 2: The cumulative mean (± SE) % of juveniles produced over 20 days for treatment fish infested once, twice and three times. Note: Four fish in experiment two did not produce any juveniles hence the curve plateaus at 50%.