Partitioning along reproductive niche dimensions in sympatric New Zealand freshwater bivalve species

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Abstract

Sympatric species of freshwater mussels in the order Unionida may need to partition resources to enable coexistence due to their relatively sedentary life-style and complex symbiotic life-phase dependent on host-fish. Although new data on their reproductive biology is being increasingly documented, particularly in the Northern Hemisphere, major gaps remain in New Zealand where two threatened Hyriidae, *Echyridella aucklandica* and *E. menziesii*, can co-occur. This thesis combines multiple approaches to elucidate the reproductive ecology of the previously unstudied *E. aucklandica* compared to the more widespread *E. menziesii*, and to examine mechanisms enabling their successful coexistence. I use field and laboratory investigations to compare reproductive niche parameters along three major resource use dimensions: host-fish species, reproductive phenology (time), and habitat use (space), and show that these two sympatric congeneric species have evolved sharply contrasting reproductive strategies.

Complex adaptations were identified among the two *Echyridella* species through contrasting use of larval (glochidia) host-fish infestation strategies and contrasting glochidia morphometry. Female *E. aucklandica* were found to produce conglutinates, mucus packages containing miniature glochidia thought to lure specific fish to them by resembling fish prey. This is thought to be one of the first Unionida species outside of North America reported to be using functional conglutinates to mimic host diet as an infestation strategy. Miniature glochidia produced by *E. aucklandica* were around three times smaller than those of *E. menziesii*, which along with other features such as shape and buoyancy, were consistent with morphological features found in host-specific unionid species elsewhere.

For the first time, the brooding phenology of *E. aucklandica* is reported, filling an important data gap on the basic biology of this poorly known and threatened species. Though *E. aucklandica* began brooding earlier and remained gravid for longer than *E. menziesii*, the brooding onset for both species generally occurred in winter (*E. aucklandica* in May–July, *E. menziesii* in August), reaching peak brooding (and thus glochidia release) in late austral spring to austral summer (November and December). High temporal overlap in glochidia development of these two species was observed, particularly during peak brooding when mature glochidia are expected to be released.

Intra- and interspecific differences in reproductive timing identified key thermal cues (particularly accumulated degree days) associated with brooding onset and glochidia maturation in both species. The importance of water temperature suggests that changes in climatic conditions have the potential to cause negative effects on both species by causing mismatches between mussel reproduction and host phenology.

Field and laboratory studies of glochidia attachment and development on fish confirmed host specificity for *E. aucklandica* and host species partitioning between the two *Echyridella* species. *Echyridella menziesii*, was found to infest a wide range of fish species, being particularly prevalent on benthic *Gobiomorphus* species and *Anguilla dieffenbachii*, in contrast to *E. aucklandica* which produced viable juveniles only on the pelagic *Retropinna retropinna*. However, this Retropinnidae species was found only in low numbers across both study sites investigated and found to be infested with consistently low numbers of *E. aucklandica* glochidia, suggesting it may be a secondary rather than the primary host. Laboratory investigations confirmed that *E. aucklandica*'s miniature glochidia encysted exclusively on the gills of *R. retropinna*, in comparison to *E. menziesii* which attached not only on the gills but also externally on the fins and skin of its host. Miniaturised glochidia of *E. aucklandica* grew nearly five times their original size on *R. retropinna* before maturing as juveniles, compared to the larger *E. menziesii* glochidia which stayed the same size throughout metamorphosis. As a result, metamorphosis duration for *E. aucklandica* glochidia was significantly longer than for the larger *E. menziesii*, but only by two to three weeks.

Passive integrator transponder (PIT) tagging was used in a coastal Waikato stream in combination with electrofishing to track movements of both mussel species and determine host fish species locations within mapped habitats, to better understand spatial and temporal movement patterns of both sexes in relation to species-specific timing of fertilisation, glochidia release and host fish infestation. During the glochidia release season, results showed evidence of relatively high net horizontal movement rates and active bank-ward cluster formation in tagged individuals of both species. Spatial overlap between mussel species and their respective host fish was partially observed for *E. menziesii*, but could not be confirmed for the host-specific *E. aucklandica* due to only one *R. retropinna* being captured. Furthermore, vertical positions of mussels varied throughout the onset brooding period for both species, but generally, proportions of female mussels increased at the sediment surface during respective reproductive onsets. A moderate, bed-moving flood

event that occurred during this experiment was associated with downstream displacement of both mussel species, with potential flood-flow refugia associated with riparian vegetation and debris in bank habitats apparently providing resistance to dislodgement.

This research provides critical information on the reproductive biology and partitioning of reproductive niche dimensions associated with the coexistence of sympatric *E. aucklandica* and *E. menziesii*. The findings will assist in the development of conservation strategies and stream management interventions to enhance freshwater mussel recruitment and survival in northern New Zealand streams.

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Echyridella aucklandica pointillism illustration by Art of Poots

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Chapter One

General Introduction

1.1 Biotic interactions and the ecological niche

Ecological communities comprise not only diverse species but also a multitude of interspecific interactions that connect them. Biotic interactions can occur either directly or indirectly, be beneficial or detrimental, and cover a wide array of associations, with the most prevalent including predation, resource competition, and symbiosis (Holomuzki et al., 2010). Together with abiotic environmental conditions, biotic interactions have implications for the survival and reproduction of individuals, and ultimately shape the diversity, structure and function that underlie communities (Post & Palkovacs, 2009). Investigation of species interactions, and the ways in which species coexist at local scales, is essential to improve understanding of the ecological principles underlying ecosystem structure and function (Thompson, 2005).

The overall concept of the niche has been debated for decades, with the definition shifting over time and interpreted differently among fields. Grinnell (1917) first described it as a behavioural response of a species to a given set of abiotic and biotic variables within its habitat. Elton et al. (1927) further defined the functional concept of a niche as the trophic position of a species in a community and its place in the biotic environment, particularly its relation to species interactions. More recently, the ecological niche of a species has been described as the volume that is occupied in n-dimensional space within an ecosystem (Hutchinson, 1959; Whittaker et al., 1972; Devictor et al., 2010). Hutchinson's (1959) work inspired ecologists to develop models of coexisting species within a community, leading to the concepts of niche partitioning (resource differentiation by coexisting species; Schöner, 1974; 1989), and niche overlap (overlap of resources used by different species; MacArthur & Levins, 1967; Pianka, 1974; Pocheville, 2015).

1.2 Resource partitioning

The concepts described above have fuelled interest in mechanisms by which sympatric species with apparently similar ecological needs partition resources, thereby enabling them to coexist. Freshwater mussels of the order Unionida are long-lived, filter-feeding benthic organisms with limited mobility that can occur in multi-species aggregations in

lakes, rivers and streams, attributes that make it a suitable model group to examine mechanisms enabling resource partitioning in natural settings (Strayer, 2008). For example, in parts of the USA mussels can form locally dense populations, sometimes comprising as many as 40 species inhabiting a single riffle (Strayer, 2008; Haag, 2012; Ford et al., 2014). However, despite the potentially important role of resource use in determining coexistence in freshwater mussel species, relatively few studies have investigated this concept, notably the partitioning of reproductive resources (see Sections 1.2.4 and 1.3).

When resources are limited, theory holds that closely related species may coexist by minimising niche overlap via exploitative competition (MacArthur, 1958; Hutchinson, 1959; Schöner, 1965; Pianka, 1974; Chesson, 2000; Chase & Leibold, 2003). Reductions in overlap can occur by species utilising one or multiple niche partitioning mechanisms, or by having low overlap in one niche dimension and high overlap in another (i.e., 'niche-complementarity hypothesis') (Jimenéz et al., 1996; Platell et al., 1998; Vieira & Port, 2007; Fossette et al., 2017). In the absence of any resource partitioning, one or all competing species may suffer detrimental effects (e.g., 'competitive exclusion'; Gause, 1934).

Traditionally, resource partitioning falls into three main dimensions that involve: (1) habitat partitioning, (2) diet partitioning and/or (3) temporal partitioning (Schöner, 1974). For instance, competing species can coexist in one region by using different habitats (*spatial partitioning*; Silverman et al., 1997; Chesson, 2000), or species may also avoid competition by exploiting different food resources (*diet partitioning*; Schöner, 1974; Takahashi et al., 2020). Finally, should species rely on the same resource, be it space, food or both, coexistence can be facilitated by exploiting this resource at different times (*temporal partitioning*, Chesson, 2000; Valeix et al., 2007; Mori et al., 2019). As an extension to these three primary niche dimensions, the concept of the reproductive niche has recently been proposed by Wessel (2012) and Bykova et al. (2012) to describe the relationship between the distribution and abundance of plants and their reproductive success.

1.2.1 Spatial partitioning

Spatial partitioning, which can result from structural partitioning of the habitat (i.e., habitat heterogeneity) and can occur at different scales (e.g., microhabitat partitioning), is viewed as one of the major mechanisms of species coexistence (Schöner, 1974; Jones et al., 2001). Habitat is often divided into horizontal and vertical components. Horizontal spatial segregation can occur by means of mutually exclusive selection for different

microhabitat types by species whose habitats otherwise overlap (Edington & Edington, 1972). Vertical stratification may occur when different species live within the same horizontal habitat type, but inhabit different layers for resource utilisation (e.g., vertical segregation of the substrate profile in sympatric freshwater mussels; Allen & Vaughn, 2009). In freshwater mussel communities, few studies, other than Allen & Vaughn, (2009) and Quinn et al. (2014), have found evidence to support competition for space between adult mussel species within stream reaches, mussel beds, or patches of substrate (Strayer, 1981; Holland-Bartels, 1990; Strayer & Ralley, 1993; Vaughn & Pyron, 1995; Spooner & Vaughn, 2009). Although different ecosystems may support a range of different mussel communities, habitat and environmental variables may poorly explain presence and coexistence of multiple mussel species at a particular location (e.g., Holland-Bartels, 1990; Strayer & Ralley, 1993; Strayer et al., 1994).

1.2.2 Diet partitioning

Partitioning of trophic resources can occur at several levels, including through diet selection (e.g., generalised or specialised feeding), and differences in temporal foraging and feeding behaviours, with segregation in preferred time of day of feeding and foraging activity patterns among species (Cody & Walter, 1976; see also Section 1.2.3). Diet partitioning resulting from behavioural differences in foraging strategies or feeding mechanisms may allow co-existing species to acquire nutrition from different habitats or at different trophic levels (Weir et al., 2009; Voight, 2013; Albo-Puigserver et al., 2015).

The study of freshwater mussel competition for food resources (mainly algae and bacteria) has been the subject of some debate (Vaughn & Hakenkamp, 2008). While some studies have suggested that food-resource competition probably does not occur or is negligible in importance due to high diet overlap between species (Brönmark & Malmqvist, 1982; Kat, 1982; Bauer et al., 1991; Collier & Melchior, 2020), other studies have indicated that some freshwater mussel species partition food resources by feeding selectively on particles of a certain size and quality, at least in laboratory experiments under static (Silverman et al., 1997; Baker & Levinton, 2003; Dionisio Pires et al., 2004; Atkinson et al., 2011) and natural (Tran & Ackerman, 2019) water conditions.

1.2.3 Temporal partitioning

Time may be seen as a stand-alone factor that when partitioned promotes coexistence between closely related species. For example, differing activity patterns of sympatric species have been viewed as ways to reduce interspecific resource competition by

enabling exploitation of specific resources at different times (Johnston & Zucker, 1983). Temporal partitioning can also occur when there is a seasonal change in certain habitats or food supplies (Schöner, 1974; Gutman & Dayan, 2005; Fossette et al., 2017).

A key time-related concept that plays a significant role in influencing the structure of and dynamics within communities is 'phenology', the predictable timing of biological activities in plants and animals, such as migration and hibernation, in response to seasonal cues (Schwartz, 2003; Visser et al., 2010). Any modification in timing cues, or reduction in the availability of suitable conditions resulting from the occurrence of abiotic constraints, that alter the onset or duration of a pheno-phase may have implications for population success and ultimately biodiversity (Wolkovich & Cleland, 2011). Accordingly, the seasonal patterns of species' life-history events can be arrayed along a temporal axis (Wolkovich & Cleland, 2011; Post, 2019). In particular, the timing, duration, and synchrony of reproduction and development in relation to environmental conditions (i.e., reproductive phenology) is widely considered to have significant fitness consequences in species (Forrest & Miller-Rushing, 2010; Visser et al., 2010; Helm et al., 2013; Post, 2019). Variations in reproductive phenology of coexisting species are common, and have, at times, been reported in congeneric sympatric species (e.g., Watters & O'Dee, 2001; Lau et al., 2016; Pakanen et al., 2016; Scriven et al., 2016).

1.2.4 Reproductive partitioning

Recently, Wessel (2012) proposed that the reproductive niche should be included as part of the traditional niche dimensions, to describe the intrinsic mechanisms used for fertilisation, and the development of gametes within the gonads since each of these features impinge on the overall ecological, developmental and cellular spaces used to reproduce. This term was based on Bykova et al.'s (2012) description of reproductive niche developed from observations of overlap in production of offspring and germination in plants in relation to temperature. Much of the research to date in this realm has focussed on the reproductive segregation of plants (e.g., Fernández-Pascual et al., 2017; Pironon et al., 2018), often including their symbiotic relationship with pollinators (Lau et al., 2016; Scriven et al., 2016; Iwasaki et al., 2018), but few studies have focussed on the reproductive niches of aquatic species.

Unionid mussels have a complex reproductive cycle involving larvae (glochidia) with characteristics of symbionts that depend on the distribution and abundance of hosts as a highly important resource base (Price, 1990; Rashleigh & DeAngelis, 2007; Barnhart et al., 2008).

The niche concept as applied to freshwater mussels needs to include the reproductive niche dimension and encompass the potential for partitioning of the host fish resource. Determining reproductive partitioning resulting from the segregation in host-glochidia interactions, along with understanding basic reproductive ecology, mechanisms of glochidia attachment strategies and implications of glochidia morphometry, may be key for understanding species' ecology and explaining species coexistence (Haag & Warren, 1998; Rashleigh & DeAngelis, 2007).

1.3 Reproductive ecology of unionid mussels

1.3.1 Unionid mussel life-cycle

To complete their complex life-cycle, the larvae of unionid mussels undergo a critical obligate symbiotic period of development on host fish, primarily on the epithelial cells of gills and fins (Kat, 1984). During reproduction, sperm aggregations (spermatozeugmata) are broadcast by male mussels into the water column and are carried by currents to nearby females, allowing for the uptake of sperm through their inhalant apertures (Fergusson et al., 2013). Fertilisation occurs internally, and fertilized embryos develop into mature glochidia within specialised brood chambers inside the gill demibranchs of the female (for several weeks to months, see section 1.3.3; Bauer & Wächtler, 2001).

Released glochidia typically can survive only a few days and therefore must encounter and infest a suitable host to continue development (Zimmerman & Neves, 2002; Hastie & Young, 2003; Melchior, 2017). Upon attachment to a suitable host, glochidia encyst and eventually metamorphose into juveniles. This symbiotic period of development may last from weeks to months, depending on mussel species and water temperature (Schneider et al., 2018). Once this metamorphosis is complete, the mussels drop from the fish and eventually settle as free-living juveniles which bury into a suitable substratum until they emerge at the surface several years later, developing into sexually mature adults (see section 1.3.4; Bauer, 1988; Geist & Auerswald, 2007).

Fish-mussel interactions are predominately thought to be phoretic, a type of commensalism where the phoront (in this case a glochidium) uses a host (fish species) for dispersal rather than nutrition (Houck & O' Connor, 1991; Watters, 2001; St. John White et al., 2017). The reason for this strategy is that most glochidia do not grow on their host and require relatively short-lived attachment durations that cause little impact on host behaviour or fitness (Lefevre & Curtis, 1912; Watters, 2001). For some unionid species,

however, phoresis may have evolved into parasitism, notably where particularly small glochidia (i.e., <150 μm, termed 'miniature glochidia';see Barnhart et al., 2008) derive nutrients and grow considerably for extended periodson fish to reach sizes suitable for metamorphosis (Fritts et al., 2013; Denic et al., 2015; Chowdhury et al., 2017).

1.3.2 Mussel glochidia-host relationships

The ability of glochidia to attach and encyst on fish is critical, as more than 99.99% of glochidia fail to reach a suitable host (Young & Williams, 1984; Ćmiel et al., 2018). Successful attachment requires gravid females to produce and release a large quantity of potential recruits as a survival strategy to compensate for failed attachment (Walker et al., 2001). Glochidia of different mussel species range in their host specificity, from metamorphose only on one or a few closely related fish species (see Haag, 2012). However, globally, many mussel species have glochidia that can survive only on a narrow range of host fish species, so that their reproduction can be limited by the availability of suitable hosts (Zale & Neves, 1982; Barnhart et al., 2008; Haag, 2012).

To increase the likelihood of obtaining important host resources and offset mortality, unionids have evolved a wide range morphological features and behaviours that are thought to attract and facilitate attachment to compatible fish species (Kat, 1984; Barnhart et al., 2008; Haag, 2012). For example, many specialists perform mimicry by using modified mantle margins as lures that resemble host prey, such as larval and adult fish (Barnhart et al., 2008). Displays such as these have been observed to elicit fish attacks or feeding strikes, upon which the mussel expels its glochidia onto or near the fish (Barnhart et al., 2008).

Alternatively, glochidia release strategies may involve the production and release of conglutinates (mucus aggregates containing glochidia) that mimic host prey items such as fish eggs or aquatic macroinvertebrates (Hartfield & Hartfield, 1996; Watters, 2002). Unionids that are host specific also tend to (but not always, see Haag, 2012) produce smaller glochidia that usually lack hooks and are often found to encyst only on fish gills where they grow substantially, therefore requiring longer encystment, as seen in *Margaritifera margaritifera* (Young & Williams, 1984; Bauer, 1994; Nezlin et al., 1994; Ziuganov et al., 1994; Taeubert et al., 2012; Stoeckl et al., 2015). Unionid species with host-specific glochidia infestation strategies display a narrow immunological compatibility, which means they can only metamorphose on a limited range of host species (Haag, 2012).

Commonly, host generalists are found to simply broadcast their offspring into the water column, usually attached to mucus strands or webs that may serve to indiscriminately attach to or entangle potential host fish (Haag & Warren, 1998). Generalist glochidia have broad host compatibility enabling them to transform on a wide taxonomic range of fish species or feeding-guilds. Typically, host generalists produce larger glochidia with hooks that assist with attachment on the fins and body, as well as gills, for short encystment periods (e.g., *Unio crassus*, 250 µm diameter, encysts for a few weeks on at least 12 host fish species; see Taeubert et al., 2012) (Bauer, 1994).

1.3.3 Reproductive phenology

Unionids were traditionally classified into short-term brooders (tachytictic) or long-term brooders (bradytictic) (Graf & Foighil, 2000; Watters, 2006). Tachytictic breeding mussels usually spawn in spring and release batches of glochidia in mid-to-late summer. In contrast, bradytictic groups spawn in summer or autumn, develop glochidia and brood these in their marsupia over the winter until the next spring or summer when single batches of larvae are released (Zale & Neves, 1982; Graf & Foighil, 2000). A third reproductive pattern is termed 'host-overwintering', by which mussels release glochidia in autumn or winter, and these remain dormant on the host until a threshold temperature is reached the following spring, initiating increases in the dispersal of glochidia by fish hosts (Watters, 2006).

Partitioning in reproductive phenology may be a mechanism whereby sympatric species can reduce the risk of interspecific competition to avoid competitive exclusion, thus promoting the likelihood of coexistence (Gause, 1934; Hardin, 1960; Schöner, 1965, 1974; Post, 2019). As thermoconformers (i.e., species whose body temperatures fluctuate according to external temperature [Sanborn, 2008]), unionids will directly experience any changes in the environmental thermal regime to which they have adapted (Spooner & Vaughn, 2008; Pandolfo et al., 2012).

Patterns in the timing of brooding and larval release can vary among unionid species, due in part to differences in prevailing water temperature regimes (Young & Williams, 1984; Watters & O'Dee, 2001; Walker et al., 2001; Hastie & Young, 2003; Österling, 2015; Melchior, 2017), as well as seasonal changes in photoperiod, water flow and food availability (Hastie & Young, 2003; Barnhart et al., 2008; Galbraith & Vaughn, 2009). In particular, measures of accrued degree days (Davenport & Warmuth, 1965; Galbraith & Vaughn, 2008; Schneider et al., 2018; Melchior, 2017; Dudding et al., 2019) and thermal

thresholds (Watters & O'Dee, 2001; Schneider et al., 2018) are reported to have species-specific effects on the timing of unionid recruitment in laboratory and field studies.

1.3.4 Habitat use and movement

Unionids often occur as species-rich aggregations in lakes and streams (Strayer et al., 2004, Vaughn & Spooner, 2006) and are found in a wide range of habitats, from soft sediment bottoms in lakes to sandy, gravel and cobble substrates in fast-flowing, highly oxygenated streams and rivers (Grabarkiewicz & Davis, 2008). As adults, unionid mussels live at the sediment-water interface, usually partly buried within substrates or entirely buried in the top layers of sediment. At the surface, they filter feed on suspended phytoplankton, bacteria, detritus, and other organic matter (Strayer et al., 2004), or use a muscular foot to pedal feed within the interstitial water of the substrate (Vaughn & Hakenkamp, 2008). Juvenile mussels are thought to live primarily below the surface within the sediment, where they pedal and siphon filter feed (Yeager et al., 1994; Lavictoire et al., 2018) until they reach sexual maturity and emerge at the substrate surface (Bauer, 1988; Geist & Auerswald, 2007). Adult unionids are generally considered sedentary, moving relatively short vertical and horizontal distances in response to seasonal or environmental cues (Balfour & Smock, 1995; Schwalb & Pusch, 2011) and to reproduce (Amyot & Downing, 1998). The muscular foot of adult mussels enables them to move on the substrate surface, with movement rates of up to 0.54 m wk^{-1} reported in the literature (Burla, 1971; Burla et al., 1974; Balfour & Smock, 1995; Amyot & Downing, 1997; Schwalb & Pusch, 2007).

Due to their (mostly) sedentary lifestyle, adult freshwater mussels rely on passive encounters of gametes from within their surrounding environment for successful fertilisation. Movement and aggregation exhibited by freshwater mussels has been suggested to be connected to the timing of reproductive activity, particularly during spawning events (Piechocki, 1969; Burla et al., 1974; Amyot & Downing, 1998; Schwalb & Pusch, 2007). Although studies have reported contrasting results on the effects of density on fertilisation success in freshwater mussels (see Bauer, 1987; Downing et al., 1993; Fergusson et al., 2013; Mosley et al., 2014), examples exist of species aggregating, thereby increasing density and proximity of conspecific mussels during this critical time of reproduction (e.g., Piechocki, 1969; Burla et al., 1974; Downing et al., 1993; Amyot & Downing, 1998; Schwalb & Pusch, 2007).

Most studies of aggregation behaviour in unionid mussels have focussed on movement related to the timing of spawning, but few studies have addressed questions

regarding movement or aggregation related to glochidia release and host fish spatial overlap. The spatial coincidence of mussels with their hosts is important because reduced host encounters during the glochidia release period can have negative fitness consequences for mussel populations (Paull & Johnson, 2014; Modesto et al., 2018). Movement by freshwater mussels into habitats that overlap with host fish may be particularly important for unionids that broadcast glochidia and do not use attraction strategies such as lures (e.g., Barnhart et al., 2008; Haag, 2012), requiring brood-releasing females to be positioned in habitats optimal for host encounter by released glochidia. One example is the observation whereby female *U. crassus* migrated horizontally to river margins, spurting jets of glochidia into the mid-channel, apparently to attract host fish (Vicentini, 2005; Aldridge et al., 2018).

1.4 Threats to freshwater mussel reproduction

As long-lived filter feeders, mussels play key ecological roles and fulfil crucial ecosystem services including water purification, food provision for other biota through bio-deposition of faeces and pseudo-faeces, and nutrient cycling (Vaughn & Hakenkamp, 2008; Vaughn & Spooner, 2006; Vaughn, 2018). Large aggregations of mussels can therefore improve water quality leading to an ultimate increase in the health of aquatic ecosystems (Strayer et al., 1994; Olgivie & Mitchell, 1995; Walker et al., 2001; Chowdhury et al., 2016;). Despite their importance to the structure, functioning and health of freshwater ecosystems, in recent decades many freshwater mussel species have suffered severe declines and range reductions across freshwater ecosystems around the globe. Indeed, they are now considered one of the most threatened animal groups, with 45% of assessed species being listed as near-threatened, threatened or extinct in the IUCN Red List (IUCN, 2019). General causes for the steep declines in diversity and abundance of freshwater mussels have been reviewed elsewhere (Dudgeon et al. 2006; Lopes-Lima et al. 2018), and include direct habitat loss and degradation due to flow modification and pollution, as well as indirect pressures on the catchment, such as increased sedimentation following deforestation.

More specifically, unionid mussels are vulnerable to human-induced change in part because of their complex reproductive strategy, in particular their unusual reliance on specific hosts (Lefevre & Curtis, 2012; Barnhart et al., 2008; Haag, 2012; Modesto et al., 2018). Accordingly, some of the declines mentioned above have been attributed to the disruption of recruitment in key fish host species which they need to complete their

reproductive cycle (Modesto et al., 2018). Host specialisation in particular, may come with a cost, as it ties the fate of the unionid species with that of its specific host, which may be vulnerable to a wider range of anthropogenic pressures such as passage disruption, leading to recruitment failure for mussel populations (McNichols et al., 2011; Douda et al., 2012). The same fate is less probable for generalist glochidia that metamorphose on a range of fish and can continue to use remaining host fishes, even after one of the species within their host range declines (Watters & O' Dee, 1998; Douda et al., 2012; Haag, 2012).

Other aspects of mussel reproduction can be directly threatened by local or regional-scale changes in water temperature and flow regimes that can affect the timing of brooding onset and glochidia release, and the survival of juvenile or adult mussels. For example, shifts in the timing of flood events and occurrences of critical water temperature thresholds linked to climate change may delay or bring forward important phenological events, such as the migration of amphidromous fish species, or glochidia release events dependent on temperature thresholds and accumulated degree days in unionids (see Chapter 3; Hastie & Young, 2003; Parmesan, 2006; Cosgrove et al., 2012; Paull & Johnson, 2014).

1.5 New Zealand freshwater mussels

Three extant unionid mussel species belonging to the family Hyriidae, *Echyridella menziesii*, *E. aucklandica* and *E. onekaka*, are found in waterbodies throughout New Zealand. *Echyridella aucklandica* can occur sympatrically with the more common *E. menziesii* in lotic environments in the northern regions of the North Island. Small, outlyingpopulations of *E. aucklandica* are distributed in some lakes of the southern North and South Island (Lake Hauroko and Lake Manapouri; see Walker et al., 2001; Marshall et al., 2014). *Echyridella menziesii* is more widely distributed across a range of aquatic environments, occurring in small fast-flowing streams, to rivers and lakes throughout New Zealand. Lastly, the less common *E. onekaka* is only found in northern areas of the South Island (Fenwick & Marshall, 2006).

Echyridella menziesii has been reported to live up to up to 55 years (Grimmond, 1968; Roper & Hickey, 1994), but the longevity and general biology of the other extant species remain unknown. Several workers have investigated feeding and reproduction of *E. menziesii* which involves an intermediate glochidia stage reported on several host fish species (Percival, 1931; Hine, 1978; Clearwater et al., 2014; Brown et al., 2017; Hanrahan, 2019; Pearson & Duggan, 2019; Collier & Melchior, 2020). Some populations of

E. menziesii and northern populations of *E. aucklandica* have been reported to comprise adult-skewed size structures, with few juveniles found (e.g., Chapter 3; James, 1985; Roper & Hickey, 1994; Rainforth, 2008; McEwan, 2012; Catlin et al., 2018). This apparent geriatric population structure is consistent with the global pattern of decline in most unionid populations (Lopes-Lima et al., 2018).

The three species of New Zealand freshwater mussels have been ranked for conservation purposes as, At risk-Declining (*E. menziesii*), Threatened-Nationally vulnerable (*E. aucklandica*) and 'Data deficient' (*E. onekaka*) (Grainger et al., 2018). Disappearances of freshwater mussel species from some lakes and waterways which they once dominated have been reported (James, 1985; Rainforth, 2008). Known threats include ecosystem changes, water pollution and invasive species (Roper & Hickey, 1995; Clearwater et al., 2014; Hare et al., 2019). In addition, fragmentation of mussel populations or reduced connectivity with their hosts, in part due to clashes with economic and development priorities that adversely affect habitats and fish passage, are also thought to be contributing to freshwater mussel decline in New Zealand (Hare et al., 2019).

1.6 Thesis aims and objectives

Given the continuing threats and the lack of knowledge on the comparative ecology and reproductive biology of native freshwater mussels, research is required ensure the long-term survival of sympatric species. In particular, unravelling the host fish associations, phenological timing of reproductive events and habitat use by spawning *E. aucklandica* and *E. menziesii* may help to explain partitioning of resources enabling their coexistence and, more broadly, provide insights into the role of interspecific interactions in structuring aquatic communities (Vaughn et al., 2018). Reducing reproductive overlap, through contrasting reproductive events such as brooding, larval release and timing of infestation, may mitigate competition for diadromous fish hosts that enter streams at different times as they move from the sea to freshwater (McDowall, 1990). Enhanced knowledge of the reproductive niche dimensions will assist in the development of conservation strategies and stream management interventions that aid in arresting the decline of these species by enhancing freshwater mussel recruitment and survival.

The only previous work on reproduction in New Zealand freshwater mussels prior to this thesis was for the more common mussel *E. menziesii*, with unpublished studies having researched its life cycle in Lake Taupo in 1994 (Clearwater, 2011) and mussel-host fish associations in the laboratory (Brown et al., 2017). More recently,

Hanrahan (2019) examined host-fish associations with *E. menziesii* in the field, and Moore (2020) investigated glochidia infestation and maturation on three non-native fish species, finding them to be unsuitable hosts. Prior to the present study being undertaken, there was no information on the biology of *E. aucklandica*. Accordingly, the overall aims of this thesis were to (1) determine the reproductive ecology of *E. aucklandica*, and (2) understand the mechanisms enabling successful coexistence of *E. aucklandica* with its sympatric congener *E. menziesii* within small Waikato streams. Using a range of laboratory and field studies, this thesis addresses four specific objectives related to resource partitioning along reproductive niche dimensions:

1) Identify differences in glochidia morphometry and release strategies in *E. aucklandica* compared to *E. menziesii.*
2) Characterise the reproductive phenology of the two *Echyridella* species in relation to water temperature regimes to determine whether temporal differences in glochidia brooding onset and peak brooding may enable the two mussel species to partition host fish resources.
3) Determine host-specificity, and glochidia attachment locations, encystment durations and growth on fish in both *Echyridella* species
4) Measure horizontal and vertical net movements of the two *Echyridella* species over the breeding season in relation to reproductive phenological phase and spatial and temporal patterns of host fish species occurrence.

1.7 Thesis outline

This thesis comprises six chapters of which research chapters 2-5 were developed as a series of four stand-alone studies, one of which has been published (Melchior et al., 2021) and the others in preparation for submission to peer-reviewed scientific journals (see Figure 1.1).

- **Chapter 2** describes contrasting use of glochidia release strategies and morphometry between sympatric *E. aucklandica* and *E. menziesii*, providing important background to further explore reproductive resource partitioning along three different niche dimensions in Chapter 3-5. Chapter 2 was published by Hydrobiologia (2021) in the special issue on Freshwater Mollusks under the title "First record of complex release strategies and morphometry of glochidia in sympatric *Echyridella* species (Bivalvia: Unionida: Hyriidae)" by M Melchior, K. J. Collier & S. J. Clearwater

- **Chapter 3** investigates partitioning of the reproductive phenological (temporal) niche dimension by comparing intra- and interspecific variation in the timing of reproduction and identifying key thermal cues (particularly accumulated degree days) associated with brooding and glochidia maturation among *E. aucklandica* and *E. menziesii* in four Waikato streams.
- **Chapter 4** explores partitioning of host fish species between the two mussel species and identifies previously unknown host fish associations for *E. aucklandica.* Field surveys were used to quantify glochidia infestation on fish present throughout the peak reproductive periods in of both mussel species two Waikato streams. Additionally, laboratory host fish trials were conducted to validate field results, determine location of glochidia attachment, and compare encystment duration and juvenile growth rates in both species.
- **Chapter 5** tracks spatial and temporal movement patterns of both sexes in *E. menziesii* and *E. aucklandica*, and relates these to species-specific timing of fertilisation, glochidia release and host fish species occurrence at fine spatial scales within mapped habitats of one coastal Waikato stream. Additionally, this chapter examines effects of a serendipitous flood event during the reproductive period on both mussel species.

As the chapters are written as stand-alone studies, there may be some repetition in the methodological details and context provided in the introductions. I assumed responsibility for the fieldwork, laboratory analyses, data analysis and writing of this thesis, and confirm that the material within was produced from my own ideas except where referenced. Additionally, I contributed to a publication that accompanies but is not included in this thesis, published by New Zealand Journal of Marine and Freshwater Research (2020) under the title "Congruence in stable isotope values among two sympatric freshwater mussel species in northern New Zealand streams" by K. J. Collier & M Melchior (see Appendix A1).

Figure 1.1. Conceptual diagram illustrating the structure of this thesis. The findings of contrasting glochidia release strategies and morphometry in sympatric *Echyridella aucklandica* and *E. menziesii* (Chapter 2) provide the basis for investigations within the following chapters (3, 4, 5) to explore mechanisms of coexistence through partitioning of independent reproductive niche axes that involve phenology (Chapter 3), host fish species (Chapter 4) and space (habitat; Chapter 5). Temporal partitioning cuts across the host fish and space axes (in red), followed by a synthesis of the findings and future research and management recommendations (Chapter 6). An investigation on diet partitioning between both species is included in the Appendix.

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Chapter Two

First record of complex release strategies and morphometry of glochidia in sympatric *Echyridella* species (Bivalvia: Unionida: Hyriidae)

Adapted from video footage with permission from Bob Brown, 2020

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2.1 Abstract

Larvae (glochidia) of the freshwater mussel order Unionida undergo a brief parasitic phase by attaching to and metamorphosing on suitable host fish. Here, novel observations of complex glochidia release strategies and glochidia morphometry are reported and compared in two sympatric New Zealand hyriid species, *Echyridella menziesii* and *Echyridella aucklandica*. *Echyridella menziesii* produced glochidia averaging 277 ± 0.7 (SE) µm in diameter which were broadcast individually and bound to mucus threads into the water column. In contrast, the sympatric *E. aucklandica* produced miniature glochidia (99 ± 0.3 µm SE) embedded in functional conglutinates, thought to facilitate host fish attraction. To our knowledge, this is the first Unionida species, outside of North America, reported to be using functional conglutinates to mimic host diet as an infestation strategy. The production of miniature glochidia that were morphologically distinguishable from those of *E. menziesii*, coupled with contrasting release strategies, highlights the potentialfor partitioning of host resources through contrasting attachment strategies and infestation times. Additionally, these findings provide the basis for distinguishing glochidia of the two *Echyridella* species in field studies of host fish infestation and highlight the need to develop novel methods for captive propagation of *E. aucklandica* to support restorationof declining populations.

2.2 Introduction

Specialised larvae (glochidia) of the freshwater mussel order Unionida undergo a critical period during which they must parasitise suitable host fish to complete their life-cycle (Kat, 1984). During reproduction, sperm aggregations (spermatozeugmata) are broadcast by unionid males into the water column and are carried by currents to nearby females, allowing for the uptake of sperm through their inhalant apertures (Ferguson et al., 2013). Fertilisation occurs internally, and fertilised embryos develop into mature glochidia within specialised brood chambers inside the gill demibranchs of the female (Bauer & Wächtler, 2001). Once mature, parasitic glochidia are eventually released into the water column and may survive only a few days before they must encounter and infest a host (Zimmerman & Neves, 2002; Hastie & Young, 2003; Melchior, 2017). Upon attachment to a suitable host, glochidia encyst and metamorphose into juveniles. These then drop from the fish and eventually settle, buried within a suitable substratum until they emerge at the surface several years later, developing into sexually mature adults (Bauer, 1988; Geist & Auerswald, 2007).

Strategies for transmitting parasitic glochidia to host fish are diverse in unionids (Haag, 2012). The type of release strategy employed by gravid female mussels is generally consistent with certain patterns of host fish use (i.e., specific host use with specialised lures), and unionids are often classified as either host fish infestation generalists or specialists (Barnhart et al., 2008; Haag, 2012). Generalist glochidia may transform on a wide taxonomic range of fish species or feeding-guilds. Commonly, host generalists are found to simply broadcast their offspring into the water column, usually attached to mucus strands or webs that may serve to indiscriminately attach to or entangle potential host fish (Haag & Warren, 1998). Unionid species with host-specific glochidia infestation strategies have a narrower immunological compatibility with host fish, being able to only metamorphose on a limited range of host species (Haag, 2012).

Specialist unionids have therefore evolved complex adaptations to increase the chance of attracting and attaching to an immunologically-compatible host (Haag, 2012). For example, many specialists may use aggressive mimicry, a form of deception often used by parasites, to attract their target host species by exhibiting adaptations that imitate the host's prey (Pasteur, 1982). In North America, in particular, examples of aggressive mimicry are commonly observed in gravid female unionids releasing parasitic glochidia. Specialist unionids may perform mimicry lure displays, using modified mantle margins

that resemble host prey, such as larval and adult fish (Barnhart et al., 2008). Displays such as these have been observed to elicit attacks or feeding strikes, upon which the mussel expels its glochidia onto, or near the fish (Haag et al., 1995; Haag & Warren, 1998). Alternatively, glochidia release strategies may involve the production and release of conglutinates (mucus-aggregates containing glochidia) that mimic host prey items such as fish eggs and aquatic macroinvertebrates (Hartfield & Hartfield, 1996; Watters, 2002; Watters, 2008).

Studies also suggest that host species range may increase with glochidia size. Larger glochidia are often found on a wider range of hosts (e.g., *Westralunio carteri*, L = >300 μm) and generally have shorter encystment times than smaller glochidia (e.g., *Margartifera margaritifera* L = <100 μm), where the length of encystment may range from weeks to months during which time glochidia also grow substantially (Bauer, 1994; Nezlin et al., 1994; Ziuganov et al., 1994; Klunzinger, 2013). Knowledge of the mechanisms of glochidia release and implications of glochidia morphometry are therefore critical for understanding species ecology and host-mussel interactions, and for developing conservation measures targeting threatened species of freshwater mussels.

Although widely documented around the globe, particularly in the Northern Hemisphere (Barnhart et al, 2008; Haag, 2012), unionid release mechanisms and morphometry of glochidia are poorly understood in New Zealand's freshwater mussels (Bivalvia: Unionida: Hyriidae) (traditionally known as kākahi or kāeo). Two of the three species native to New Zealand, *Echyridella menziesii* and *Echyridella aucklandica*, co-occur in waterways in the northern half of the North Island (Marshall et al., 2014) where they have been ranked for conservation management as, At risk-Declining (*E. menziesii*) and Threatened-Nationally vulnerable (*E. aucklandica*) (Grainger et al., 2018). Geriatric population-size structures are often recorded in New Zealand waters (e.g., Roper & Hickey, 1994), reflecting global trends of decline in mussel populations (Lopes-Lima et al., 2017) and inferring the existence of recruitment bottlenecks. Often, the conservation of declining species such as these is challenged by scant knowledge of their reproductive strategies and basic biology. In this paper, we report for the first time, glochidia morphometry and release strategies in *E. aucklandica* compared to *E. menziesii*. These data contribute to the basic understanding of the contrasting reproductive biology of two sympatric New Zealand freshwater mussel species, assisting future conservation interventions that facilitate population recovery, such as captive propagation and waterway restoration.

2.3 Methods

2.3.1 Mussel collection and maintenance

Gravid *E. aucklandica* and *E. menziesii* were hand-collected from populations in (1) the Ohautira Stream (-37.762392, 174.98124), a short coastal stream in western Waikato, in November 2017 and January 2019, and (2) the Mangapiko Stream (-37.982022, 175.473541), a tributary of the Waikato River, in February 2018 and January 2019. Brooding female mussels were identified by *in vivo* examination and classification of gill demibranch pigmentation and volume (Plate 2.1; Table 2.1). The procedure involves a non-destructive visual inspection using a nasal speculum to pry the mussel valves apart (ca. 0.5 – 1 cm). Gravid and fully charged (assumed to contain the entire brood) *E. menziesii* (Mangapiko n = 6, Mean (SE) length (L) = 60 ± 5.6 mm); Ohautira Stream n = 6, L = 57 ± 2.9 mm) and *E. aucklandica* (Mangapiko Stream n = 6, L = 77 ± 5.0 mm; Ohautira Stream n = 6, L = 84 ± 4.4 mm) were separated into buckets containing aerated 18°C water and sediment from the source location, and were then transported to The University of Waikato laboratory.

Mussels were held within five L aerated aquaria containing dechlorinated tap water and three cm depth of silica sand (one mussel per aquarium). Ten per cent of the water in each aquarium was changed every other day to minimise build-up of ammonia and other waste products. Mussels were fed a mixture of Reed Mariculture Nanno 3600 and Mariculture Shellfish diet diluted with 1 L dechlorinated tap water to provide ca. 4700 cells/mL/mussel/day (Ganser et al., 2015). Controlled temperatures (18°C) and a light:dark cycle (16:8 h) matched ambient conditions, allowing adult females to release their broods naturally.

Table 2.1. Brood pouch characteristics used to determine gravidity stage by anatomical examination (gill colour and volume) of sampled *Echyridella menziesii* (modified from Melchior,2017) and *E. aucklandica*.

			Echyridella menziesii	*Echyridella aucklandica*
Sex	***Gravidity Stage***	***Gill volume***	***Brood pouch colour***	
Female	Ripe (Stage 3)	Inflated	Yellow - Orange	Orange: Purple
	Ripe (Stage 4)	Inflated	Dark Orange	100% Purple
	Spent (Stage 5)	Deflated	Pale yellow	Orange
Male	-	Flat	Dark Orange	Dark Orange

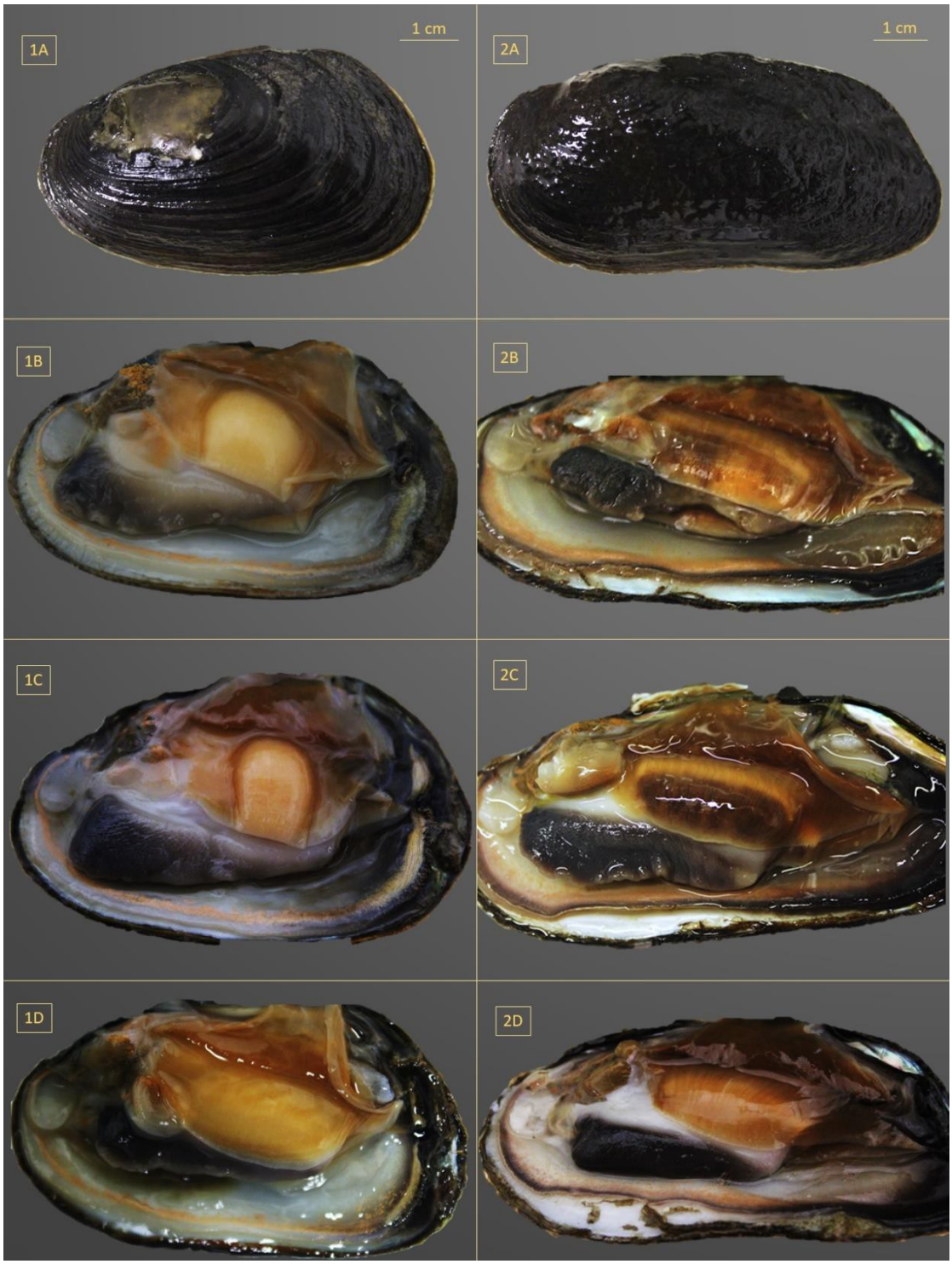

Plate 2.1. Marsupia of female *Echyridella menziesii* (1A: outer valve, 1B: gravidity stage 3, brooding unviable glochidia 1C: Gravidity stage 4, brooding viable glochidia, 1D: Gravidity stage 5, spent) and *Echyridella aucklandica* (2A: outer valve, 2B: Gravidity stage 3, brooding unviable glochidia 2C: Gravidity stage 4, brooding viable glochidia; 2D: Gravidity stage 5, spent) at various stages of gravidity, complementing Table 2.1.

2.3.2 Glochidia release

Adult *E. menziesii* and *E. aucklandica* were observed daily for release of glochidia. If glochidia were found to be released, these were retrieved using a 5 mL pipette and placed on a petri dish or watch glass under the Olympus SZ-6045 dissecting microscope to determine whether they were released as individual glochidia, embedded in mucus strands, or as conglutinates. Samples of glochidia were analysed for maturity, characterised by 1) the presence of hooks on opposing valves, 2) translucent valves, free of their vitelline membrane, and 3) rapid opening and closing of the glochidia valves. All glochidia collected from each mussel were preserved in 70% ethanol for later analysis. If the sample contained conglutinates, distinctions were made between puerile or non-functional (containing only premature glochidia) and functional (structures containing mature glochidia) conglutinates (Barnhart et al., 2008). Maturity of glochidia was assessed as above. All conglutinates released by each mussel were preserved in 10% formalin.

Fecundity was defined as the total number of glochidia brooded by a female during a single brooding event, with the assumption that mussels produced one clutch per year (this is unknown for the studied species). Estimations of fecundity were made using the collected glochidia or conglutinate content from 3 females per species per stream population. If at the completion of the trial, mussels had not released their entire gill contents, these were then flushed using dechlorinated tap water to remove remaining glochidia material. *Echyridella menziesii* glochidia were diluted to a 100 mL homogenised dechlorinated tap water solution. The number of glochidia were then counted in 5 aliquots of 1 mL under the Olympus SZ-6045 dissecting microscope. Fecundity was estimated by multiplying the mean number of glochidia in the 5 sub-samples by dilution volume. *Echyridella aucklandica* fecundity estimations were made by counting glochidia attached to a sub-sample of 5 conglutinates and multiplying the mean number of glochidia by total released conglutinates (excluding conglutinate fragments).

2.3.3 Glochidia valve morphometry

A sub-sample of preserved glochidia were photographed and measured using the Leica DM RD equipped with the Olympus DP70 Digital Camera system and Olympus image analysis software. Length (widest part of the shell between anterior and posterior edges, parallel to the hinge), height (widest part of the shell perpendicular to the hinge) and hinge length were measured to the nearest 1 µm (see Plate 2.2) to compare morphometric differences among individuals, populations and species. For *E. menziesii*, 10 glochidia from 3 adult mussels per stream population were measured for a total sample of 60 glochidia. For *E. aucklandica*, 3 glochidia from 3 conglutinates of 3 mussels were measured for a total of 27 glochidia per stream population (total n = 54). Height, length, size (average of height and length; Barnhart et al., 2008) and height/length ratio (shape) were compared among populations and species using *t*-tests for independent groups (STATISTICA version 13; Stat Soft Inc. Oklahoma, U.S.A.).

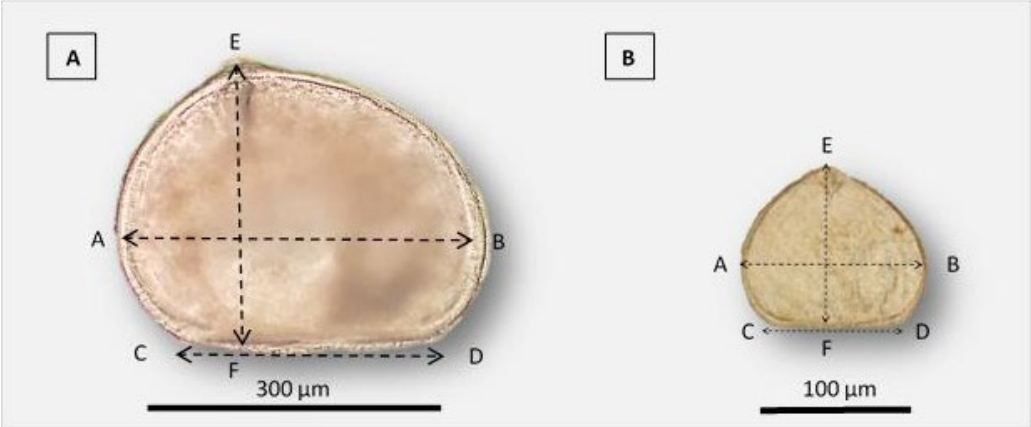

Plate 2.2. Morphometric measurements of mature (A) *Echyridella menziesii* and (B) *E. aucklandica* glochidium valves: A – B, length; C - D, hinge length;E – F, height (modified from Klunzinger et al., 2013). Anatomical orientation descriptions are labelled according to Hoggarth (1987).

2.4 Results

Echyridella menziesii marsupia were found to be positioned in the inner one-third of the inner demibranchs. The marsupia increased in volume with developmental stage and changed in pigmentation from transparent yellow with immature glochidia (Stage 3) to dark orange, containing mature glochidia before glochidial parturition (Stage 4, Plate 2.1; Table 2.1). *Echyridella aucklandica* had marsupia located at the posterior end of the inner demibranchs. These occupied a greater surface area (two-thirds) of the gills than those of *E. menziesii*, and the pigmentation of the demibranchs in *E. aucklandica* ranged from orange and purple (Stage 3) to fully purple (Stage 4) for gravid females (Plate 2.1; Table 2.1).

2.4.1 Glochidia release strategies

Within laboratory aquaria, all female *E. menziesii* from Mangapiko and Ohautira Stream populations exhibited dual host infestation methods by releasing translucent glochidia both individually and bound to mucus threads (up to 4-5 cm in length) attached temporarily tothe exhalant aperture of the adult (Plate 2.3). Rhythmic contractions of the adult female'sexhalant aperture were observed to cause the mucus threads to drift and suspend glochidia in the water column, as shown in the video (Supplementary material 1: https://doi.org/10.1007/s10750-019-03995-3). Glochidia strands often became entwined with one another, leading to mucus masses containing glochidia that were observed lying on the sediment next to adult female *E. menziesii*.

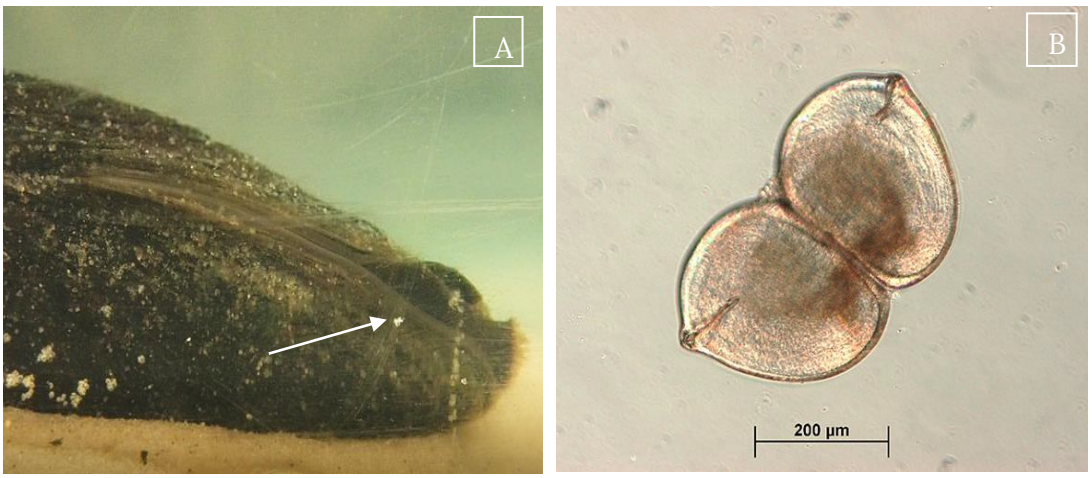

Plate 2.3. Adult *Echyridella menziesii* releasing glochidia bound to mucus strands attached to the exhalant siphon (white arrow) (A), and photo-microscopy of mature and viable hooked *E. menziesii* glochidia (B).

All *E. aucklandica* from both the Mangapiko Stream and Ohautira Stream populations released glochidia attached to conglutinates (Plate 2.4; see video, Supplementary material 2: https://doi.org/10.1007/s10750-019-03995-3). Individual conglutinates were expelled through the exhalant aperture, and mussels were sometimes observed to discharge conglutinates that were attached temporarily to an adhesive mucus strand protruding from between the extended tissues of the mantle margin (as shown in the video: Supplementary material 3: https://doi.org/10.1007/s10750-019-03995-3). Detached conglutinates (intact and fragmented) were then observed floating with wavelike motions in water currents created by aeration or tumbling on the bottom of the tank.

Echyridella aucklandica produced two types of conglutinates: functional conglutinates, which contained mature glochidia attached externally to the conglutinate material (Plate 2.4b, c); and puerile (or non-functional) conglutinates (Plate 2.4d). Very few puerile conglutinates were released by adults from both populations (n = <5-10 per mussel), commonly at the end of the release period. Puerile conglutinates differed from functional conglutinates in shape, size and colour. These structures were entirely brown material containing immature glochidia (encased in vitelline membranes) embedded within the conglutinate material. Lengths for puerile conglutinates for mussels from both Mangapiko and Ohautira Stream populations ranged from 5 to 10 mm.

Functional conglutinates varied in morphology and size (L: 4-9 mm; W: 2-3 mm) from both Mangapiko and Ohautira Stream populations, however, all were dorso- ventrally flattened with a vermiform appearance (Plate 2.4a, b). All functional conglutinates were composed of solid, spongy mucus and shared similarities in colour, with a white membrane and the dorsal surface partly covered with tan-coloured material to which translucent mature glochidia were attached by their hinge or outer valves (Plate 2.4b, c). Functional conglutinates were spontaneously discharged by individuals one-at- a-time at a rate of 12-21 per day over a period of 1-2 weeks. At times, releases were also observed as single conglutinates, occurring in response to stimulation by touch of the pipette (reflexive release; Barnhart, 2008). The number of functional conglutinates released ranged from 49 to 239 per mussel ($\bar{x}$ = 136.5 ± 59.4 SE) for both populations (n = 12) with 141 ± 50.6 for Mangapiko adults (n = 6) and 131 ± 36.8 for Ohautira adults (n = 6). Fragments of conglutinates were not quantified.

The number of glochidia per conglutinate (n = 15) ranged from 61 to 280 ($\bar{x}$ = 173.7 ± 19). Estimated fecundity was found to be greater in *E. menziesii* (n = 6; L: 59 ± 3.6), with some females producing broods that contained nearly twice the number of glochidia ($\bar{x}$ = 44,016; range: 28,840-72,000) than *E. aucklandica* (n = 6; L: 85 ± 1.8), which only released an estimated mean of 17,840 glochidia (range: 1737-34570), although this difference was not statistically significant ($t_{(11)}$ = 2.5, p = 0.3). No significant differences in fecundity were found between Ohautira and Mangapiko stream populations in *E. menziesii* ($t_{(5)}$ = 0.4, p = 0.7) or *E. aucklandica* ($t_{(5)}$ = 0.5, p = 0.6).

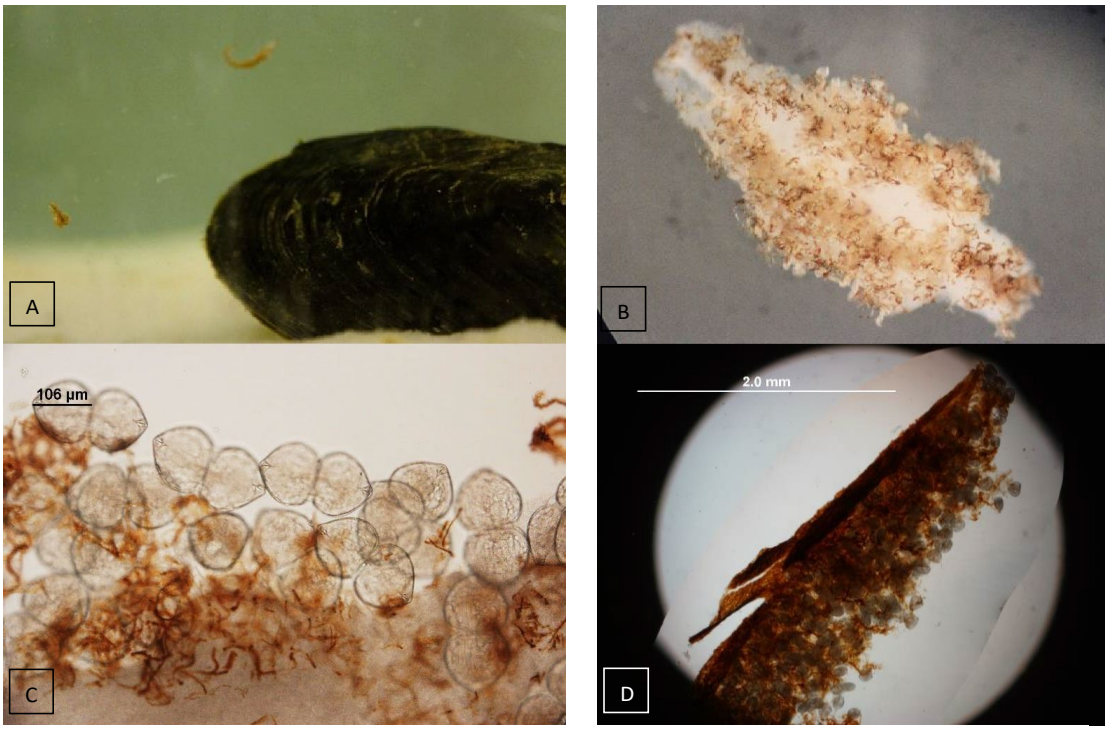

Plate 2.4.. Two conglutinates released by adult *Echyridella aucklandica* (A). Photomicrograph of *E. aucklandica* conglutinate (L: 7mm) containing mature and viable glochidia (B). Compound photomicrograph of *E. aucklandica* glochidia attached to the outer layer of the conglutinate (C). Puerile conglutinate containing unviable (closed) glochidia (D).

2.4.2. Glochidia description

Released glochidia from both *E. menziesii* and *E. aucklandica* were sub-triangular in shape with a straight hinge and hooks proximal to the apex of each valve. Valves of *E. menziesii* glochidia compared to *E. aucklandica* were distinctly more inequilateral (i.e., the ventral hooked edge of each valve was off-centre and displaced posteriorly with the anterior edge being longer and having a more prominent curve) (Plate 2.2). Glochidia shape (shell height: length ratio) differed significantly between the species ($t_{(112)}$ = 14.2, p<0.01), with *E. menziesii* glochidia having a lower height to length ratio (0.83 ± 0.0), resulting in more elongated valve shapes compared to those of *E. aucklandica* (0.92 ± 0.01) which had an almost equal height to length ratio. Further significant differences between species were found in glochidia shell size (mean shell height and length; Barnhart et al., 2008), with *E. menziesii* (277 ± 0.7 µm) nearly 3 times the size of *E. aucklandica* (99 ± 0.3 µm SE) ($t_{(112)}$ = 220.9, p <0.01). When comparing glochidia valve sizes within species between Mangapiko and Ohautira stream populations, no significant differences were found (*E. menziesii*: $t_{(59)}$ = 1.3, p = 0.19; *E. aucklandica*: $t_{(53)}$ = 0.44, p = 0.66). However, *E. aucklandica* glochidia were found to have significant differences in shape between stream populations, with Mangapiko Stream populations having a greater length to height ratio than glochidia from Ohautira Stream populations ($t_{(53)}$ = 3.51, p = <0.01). *Echyridella menziesii* valve shapes were found not to be significantly different ($t_{(59)}$ = 1.3, p =0.20) between stream populations (Table 2.2). No larval threads were observed in any of the studied *E. menziesii* or *E. aucklandica* glochidia samples.

Table 2.2. Mean (± standard error; ranges in parentheses) length, height and hinge length of glochidia and size measured in µm. Size is the mean of length (L) and height (H) and shape is the H:L ratio.

			Glochidia				
Species	**Locality**	***N***	**Length (µm)**	**Height (µm)**	**Hinge length (µm)**	**Size (µm)**	**Shape (H:L ratio)**
E. menziesii	Mangapiko	30	303 ± 1.3 (293 – 320)	252 ± 1.1 (240 – 266)	205 ± 1.1 (196 – 222)	278 ± 1.1	0.84 ± 0.0
E. menziesii	Ohautira	30	301 ± 1.1 (294 – 314)	251 ± 0.9 (243 – 261)	209 ± 1.6 (198 – 233)	276 ± 0.9	0.83 ± 0.0
Both *E. menziesii* populations		60	302 ± 0.7 (293 – 320)	252 ± 0.7 (240 – 266)	207 ± 1.0 (196 – 233)	277 ± 0.7	0.83 ± 0.0
E. aucklandica	Mangapiko	27	103 ± 0.4 (94 - 106)	94 ± 0.5 (90 - 99)	68 ± 0.5 (63 - 73)	98 ± 0.3	0.91 ± 0.01
E. aucklandica	Ohautira	27	102 ± 0.7 (95 - 105)	96 ± 0.7 (85 - 98)	65 ± 0.6 (59 - 69)	99 ± 2.9	0.94 ± 0.05
Both *E. aucklandica* populations		54	103 ± 0.4 (95 - 109)	95 ± 0.5 (85 - 99)	66 ± 0.5 (58 - 73)	99 ± 0.3	0.92 ± 0.01

2.5 Discussion

2.5.1 Host infestation strategies

Host infestation strategies described in this study for both *Echyridella aucklandica* and *Echyridella menziesii* represent a contrasting set of behavioural and morphometrical adaptations for the two species to facilitate transmission of glochidia to hosts. To our knowledge, the Australasian hyriid, *E. aucklandica* is the first non-North American mussel species reported to use a host attraction strategy through the release of functional conglutinates that resemble vermiform macroinvertebrate prey items of fish, such as some Diptera larvae, Hirudinea or Turbellaria. In North America, host-attracting, functional conglutinates are a common feature in unionids (Haag, 2012), whereas in Europe, the conglutinate production reported to date appears to be non-functional (consisting of unviable glochidia or eggs and not serving as a host attractant) and induced by stress (Aldridge & McIvor, 2003; Lopes-Lima et al., 2017). In contrast the functional conglutinates released by *E. aucklandica* were embedded with mature and viable glochidia, suggesting that the structures are adapted to attract and increase infestation rates on fish (Haag, 2012).

Although structurally variable, all *E. aucklandica* conglutinates were dorso-ventrally flattened and composed of solid, spongy mucus bodies to which glochidia were attached. The North American Creeper mussel *Strophitus undulatus*, produces a similar type of conglutinate, described as a translucent, milky, rod-shaped (3-7 mm) conglutinate that contains 1-15 glochidia, probably mimicking maggots and other insect larvae (Watters, 2002; Watters, 2008; Haag, 2012). Watters (2008) categorised these structures by morphology and composition into the meso-conglutinates (mature larvae attached to solid mucus conglutinates). The description of the meso-conglutinate produced by *S. undulatus* parallels with the description of *E. aucklandica* conglutinates within our study. However, in contrast to *E. aucklandica* which was found to produce miniature glochidia, *S. undulatus* is known to release the largest glochidia known (>200–500 μm) and unlike other conglutinate producers, *S. undulatus* are host generalists, using at least 15 unrelated hosts for the transformation of their glochidia (Watters, 2002). *Echyridella aucklandica* conglutinates are also similar in shape to the leech like (2-5 mm) conglutinates of the Dromedary pearly mussel (*Dromus dromas*) (Jones et al., 2004). However, unlike the *E. aucklandica* conglutinates, which did not display colour variability and only contained viable glochidia, *D. dromas* conglutinates range in colour from red to white and contain immature eggs that hold together the centre of the

conglutinate with mature glochidia on the outer margin (Jones et al., 2004), and are thus grouped into composite conglutinates (Watters, 2008). Glochidia released by *D. dromas* have been successfully transformed on 10 benthic-feeding fish species in the laboratory, including 9 species of darter (Percidae) and one sculpin (Cottidae).

Haag (2012) divides North American unionid conglutinate releasers into several sub-categories: 1) pelagic; 2) mucoid; and 3) demersal. Pelagic conglutinates are forcefully expelled into the water column and immediately enter the drift, targeting drift-feeding fish such as minnows (*Fucsonia* sp.; Patterson et al., 2018). Most pelagic conglutinates seem to be generalised mimics that do not resemble specific organisms but rely on their motion in stream drift to attract fishes (W. Haag, Center for Mollusk Conservation, Kentucky Department of Fish and Wildlife Resources, pers. comm.). Mucoid conglutinates are associated with benthic habitat, and do not resemble specific prey groups, lacking a clear structure. These conglutinates are often found in species that target generalist feeders as a host (e.g., *Cyclonaias pustulosa* targeting catfish; Patterson et al., 2018). Demersal conglutinates, on the other hand, are structured packages of larvae that can be very elaborate. These packages resemble a variety of food items, including leeches, worms and aquatic insects such as *Ptychobranchus* blackflies. Conglutinates either quickly settle to the bottom, stay adhered to the parent mussel, or in the case of *Cyprogenia* sp. stay attached to the mussel for a period of time before settling to the substrate. The demersal conglutinate release strategy is strongly oriented towards parasitism of benthic host fish, targeting small invertivores (Patterson et al., 2018).

Our laboratory observations of *E. aucklandica* conglutinate release suggest the potential for both pelagic and demersal release strategies. When first released, conglutinates were seen floating with a rippling motion in the aquaria, which may be a useful strategy to attract pelagic or drift-feeding fish. After a short time, however, conglutinates settled to the bottom of the tank in the absence of flow, which would place conglutinates in prime locations to be preyed upon by benthic feeders in the wild (Patterson et al., 2018). The use of conglutinates as a host attraction strategy for *E. aucklandica*, in comparison to a passive attachment strategy for *E. menziesii*, suggests that *E. aucklandica* glochidia may require different host fish species to *E. menziesii* and that *E. aucklandica*'s glochidia may parasitise internal structures due to possible consumption by attracted host. Furthermore, *E. aucklandica* may be a potential host fish specialist as other studies of species using similar release strategies suggest that these evolved to suitthe feeding habits of a host species or a suite of fish feeding-guilds adapted

to attract andfacilitate glochidia transfer to a specific feeding guild (Barnhart et al., 2008; Haag, 2012; Patterson, 2018). Field research is currently underway to identify host fish infestation by *E. aucklandica* and *E. menziesii*, with initial findings identifying pelagic species of Retropiniidae as host fish for *E. aucklandica* but not benthic Anguillidae or Eleotridae (see Chapter 4; Melchior et al., 2021).

It is important to note that the observations of glochidia release behaviour in this study have been of mussels held in a laboratory setting, and release behaviours may vary in the wild. Previous studies found that some unionids (e.g., *Quadrulini* sp.) produce puerile conglutinate-like structures, often composed of eggs or developing embryos (Barnhart et al., 2008) that may be released as a stress-response under hypoxia (e.g., *Unio pictorum* and *Unio tumidus*; Aldridge & McIvor, 2003). In the present study, aerators were used in all aquaria to reduce stress to adult mussels and only a small number (<5 per mussel) of puerile conglutinates were released. Ideally, future studies would include field observations of conglutinate release to fully elucidate host attraction and infestation strategies for this New Zealand unionid species.

Unlike *E. aucklandica*, which uses mimicry in the form of conglutinates as a strategy presumed to attract host fish, *E. menziesii* uses a passive form of infestation by broadcasting both individual glochidia, and glochida-laden threads of mucus that probably serve to suspend larvae into the water column and entangle passing hosts. Passive entanglement is most often found in generalist mussel species (including Australasian hyriids *Westralunio carteri* (Haag, 2012; Klunzinger et al., 2013) and *Hyridella drapeta* (Jupiter & Byrne, 1997), as a variety of fish may be infested indiscriminately through contact with the released mucus webs (Haag & Warren, 2003). *Echyridella menziesii*, is now well-known to parasitise a range of native New Zealand fish, and can thus be categorised as a host-generalist based on existing information. In the wild, glochidia have been found on fish species such as kōaro (*Galaxias brevipinnis*) and small benthic-feeding fish such as the giant bully (*Gobiomorphus gobioides*) (Percival, 1931; Hine, 1978). More recently, successful laboratory glochidia attachment and transformation was demonstrated on rainbow trout (*Oncorhynchus mykiss*) (Clearwater et al., 2014), common bully (*G. cotidianus*), native banded kōkopu (*Galaxias fasciatus*), longfin eel (*Anguilla australis*), shortfin eel (*A. dieffenbachii*), and Canterbury galaxias (*G. vulgaris*), with the highest transformation rates on common bully (91%) followed by banded kōkopu (69%) (Brown et al., 2017). However, host infestation and transformation success is currently unknown for *E. aucklandica*.

In this study, estimated fecundity varied within and among species, which is a consistent finding in unionids generally (Haag & Staton, 2003). In North American mussels, the number of glochidia produced annually ranges from <2000 up to 10 million (Haag, 2013). Body size has been suggested to be a strong predictor in fecundity of freshwater mussels within and among species, with large individuals generally found to have a higher reproductive output (Haag, 2013). This is inconsistent with the data collected within this study, although sample size was small. Based on the material collected, estimated clutch size per adult was considerably lower in the larger-sized *E. aucklandica* in comparison to *E. menziesii*. Furthermore, both species produced a considerably lower number of offspring than some North American mussels (Haag, 2013) and the hyriid *H. drapeta* (Byrne, 1998). The low fecundity estimated in both species may reflect complex relationships with host fish due to the efficiency of host infestation through the production of conglutinates in *E. aucklandica* and the wide range of hosts available for infestation for the host generalist, *E. menziesii*. Haag (2013) indicates that the release of conglutinates and mucus webs are generally associated with low length-standardised fecundity. The production of conglutinates and release of puerile conglutinates (containing immature glochidia) may significantly increase energetic costs and therefore lower fecundity, whereas the production of mucus strings associated with host generalists increases the chance of glochidia infestation, allowing for low fecundity.

2.5.2 Glochidia morphometry

Glochidia of both *Echyridella* species were morphometrically distinct from each other in size and shape. *Echyridella menziesii* glochidia were larger than *E. aucklandica* and distinctly larger than the size reported for most Australasian Hyriidae, with the exception of *Westralunio carteri* (309 µm) (Klunzinger et al., 2013) and *Hyridella drapeta* (320 µm) (Atkins, 1979). The ability to identify species in the field using morphometrics is important, particularly in riverine systems where species co-occur, as is the case for *E. menziesii* and *E. aucklandica*. Kennedy & Haag (2005) used morphometrics to identify 72–79% of total glochidia from the Sipsey River system (in west central Alabama) that is inhabited by 21 unionid species. As with our research, the study found low within-population variability, with the exception of one species, supporting the general use of morphometry to differentiate between unionid species. Though within-population variability for glochidia morphometry was low for our study, variability between populations seems to occur throughout New Zealand with glochidia dimensions of

E. menziesii in this study being distinctly smaller than in previous studies by Percival (1931) and McMichael & Hiscock (1958) who measured glochidia lengths of 360 µm (Lake Sarah, West Canterbury, South Island) and 310 µm (Waikato River, Hamilton, North Island), respectively.

Small glochidia, such as those produced by *E. aucklandica*, have been reported for other unionid species (e.g., the pearl mussel *Margartifera margaritifera* - size <100 µm). These were observed to attach to the gills of host fish, whereas larger glochidia are found primarily on the fins of their hosts (Bauer, 1994). Attachment to skin and fins is commonly found in triangular-hooked glochidia of Unionidae and Hyriidae (Bauer & Wächtler, 2001). The combination of larger size and a low height:length ratio, as found in *E. menziesii*, are traits that improve leverage and gripping force (Hoggarth & Gaunt, 1988), allowing the glochidia to easily attach externally to the fish (Bauer, 1994). Furthermore, larger glochidia have been reported to have shorter host retention times than smaller glochidia that may be retained for a longer period, enabling them to not only develop internal organs but also grow in size while on their host (Bauer, 1987; Nezlin et al., 1994).

Host specificity is associated with selective encounters of host fish taxa or due to the dominance of a particular host species in certain habitats. The relationship between unionids and host fish is easily disrupted, particularly in New Zealand, where a significant proportion of the potential host fish pool is diadromous, adding to the risk of recruitment disruption for *E. aucklandica*, in particular. Contrasting glochidia release and attachment strategies by both species, suggest that sympatric populations of *E. menziesii* and *E. aucklandica* may co-exist through partitioning of host resources. Furthermore, differences in release strategies and morphology in both glochidia and adults also suggest that *E. menziesii* and *E. aucklandica* may not be as closely related as previously reported and should be further investigated using integrative approaches that combine molecular, morphological and ecological data. As well as providing the basis for recognising and distinguishing glochidia of these two *Echyridella* species in field studies of host fish infestation, our novel finding of probable host fish attraction through mimicry of conglutinates in *E. aucklandica* highlights the importance of understanding the fundamental biology of host-mussel relationships and the need to develop species-specific methods for captive propagation to support restoration of declining populations.

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Chapter Three

When the time is ripe: phenological overlap among two freshwater bivalve species in northern New Zealand streams

3.1 Abstract

The timing and synchrony of reproduction and development in relation to environmental conditions (reproductive phenology) is widely considered to have significant fitnessconsequences for many species. For freshwater mussels (Unionida), which undergo a symbiotic larval phase on fish, reducing phenological overlap, through contrasting reproductive events including brooding, larval release and timing of fish infestation, may mitigate competition for diadromous fish hosts that enter streams at different times. This chapter compared intra- and interspecific variation in reproductive timing, and identified key thermal cues (particularly accumulated degree days) associated with brooding and glochidia maturation among sympatric populations of the New Zealand Hyriidae *Echyridella aucklandica* and *E. menziesii* in four Waikato streams for the annual reproductive seasons. For the first time, the brooding phenology of *E. aucklandica* is reported, filling an important data gap on the basic biology of this poorly known and duration of their threatened species. Though *E. aucklandica* began brooding earlier and remained gravid for longer (9-11 months) than *E. menziesii* (6-7 months) in this study, the brooding onset for both species generally occurred in winter (*E. aucklandica* in May – July, *E. menziesii* in August), reaching peak brooding (and thus glochidia release) in late austral spring to summer (November and December). Brooding phenology of *E. menziesii* and *E. aucklandica* demonstrated high temporal overlap, particularly during the critical peak brooding period when mature glochidia are expected to be released to infest host fish. Onset of brooding required on average 440 accumulated degree days for *E. aucklandica* compared to 502 accumulated degree days (ADD) for *E. menziesii*, while brooding peak required on average 763 and 478 ADD, respectively. Although weak relationships were found between degree days and timing of peak brooding, relatively narrow ranges across sites in degree days required to reach onset brooding were found particularly in *E. aucklandica*. This, coupled with a strong relationship between ADD and timing of onset of brooding in both species, suggest that ADD is key in regulating the timing of brooding onset in the two coexisting species.

3.2 Introduction

Time represents a fundamental dimension upon which ecological patterns are shaped (Post, 2019; Wolkovich et al., 2014), and has long been recognised as a major niche axis along which species may partition resource use to coexist (Hutchinson 1959; Schöner, 1974; Pianka, 1981; Kronfeld-Schor & Dayan, 2003). A time-related concept that plays a keyrole in influencing the structure and dynamics within communities is phenology — that is, the predictable timing of biological activities in plants and animals, such as migration, hibernation, flowering and reproduction in response to seasonal cues (Schwartz, 2003; Visser et al., 2010). In particular, the timing, duration, and synchrony of reproduction and development in relation to environmental conditions (i.e., reproductive phenology) is widely considered to have significant fitness consequences in species (Forrest & Miller- Rushing, 2010; Visser et al., 2010; Helm et al., 2013; Post, 2019). Variations in reproductive phenology of coexisting species are common, and have, at times, been reported in congeneric sympatric species (e.g., Watters & O'Dee, 2001; Pakanen et al., 2016). However,these patterns are often not fully understood, likely due to being driven by a complex interplay between abiotic and biotic variables (Thackeray et al., 2010; Pekkonen et al., 2013).

Abiotic factors such as water temperature are important in regulating phenology in temperate aquatic environments where they may serve as predictable cues for timing of life-cycle stages (such the onset of reproduction) to match with resource availability (Stachowics et al., 2002; Goldman et al., 2004; Westerman et al., 2009), and to optimise offspring growth and survival (McNamara et al., 2011). Biotic variables, on the other hand,reflect the evolutionary processes behind the timing of events and can influence reproductive phenological variations in species through interactions with otherorganisms. For example, biotic interactions, such as interspecific competition and parasitism, are often tightly linked to resource use and partitioning (Post, 2019). Temporal partitioning of limited resources may be an important mechanism whereby sympatric species can reduce the risk of interspecific competition to avoid competitive exclusion, thus promoting the likelihood of coexistence (Gause, 1934; Hardin, 1960; Schöner, 1965, 1974; Tokeshi, 1986; Post, 2019).

Freshwater mussels of the order Unionida are characterised by a life-cycle that includes a symbiotic larval (glochidia) stage dependent on suitable host fish (Strayer et al., 2004; Haag, 2012, a potentially limited resource that coexisting species may need to

compete for to successfully recruit juveniles (Price, 1990; Haag & Warren, 1998; Rashleigh & DeAngelis, 2007; Haag, 2012). As thermoconformers (i.e., species whose body temperatures fluctuate according to external temperature [Sanborn, 2008]), unionids will directly experience any changes in the environmental thermal regime to which they haveadapted (Spooner & Vaughn, 2008; Pandolfo et al., 2012). Patterns in the timing of brooding and larval release can vary among unionid species, due in part to differences inprevailing water temperature regimes (Young & Williams, 1984; Watters & O'Dee, 2000; Walker et al., 2001; Hastie & Young, 2003; Österling, 2015; Melchior, 2017), as well as photoperiod, water flow and food availability (Hastie & Young, 2003; Barnhart et al., 2008; Galbraith & Vaughn, 2009). In particular, measures of accrued degree days (Davenport & Warmuth, 1965; Galbraith & Vaughn, 2008; Schneider et al., 2018; Melchior, 2017; Dudding et al., 2019) and thermal thresholds (Watters & O'Dee, 2000; Schneider et al., 2018) have been reported to have species-specific effects on the timing of unionid recruitment in laboratory and field studies.

Partitioning in reproductive phenology may be important for the coexistence of sympatric unionid species, such as the New Zealand Hyriidae *Echyridella menziesii* and the congeneric *E. aucklandica* which can co-occur in waterways throughout the northern regions of the North Island (Marshall et al., 2014). *Echyridella menziesii* is a known host fish generalist, capable of infesting a range of native diadromous and non-diadromous species, as well as some non-native species (Percival, 1931; Hine, 1978; Clearwater et al., 2014; Brown et al., 2017; Hanrahan, 2019). By comparison, host fish relationships for *E. aucklandica* have up to now been unknown (but see Chapter Four). However, recent evidence of contrasting larval release strategies and infestation behaviours of the two species in a laboratory setting (Melchior et al., 2021) highlights the potential for host- resource partitioning. Reducing phenological overlap, through contrasting reproductive events such as brooding, larval release and timing of infestation, may mitigate competition for diadromous fish hosts that enter streams at different times as they move from the seato freshwater (McDowall, 1990).

In this study, I compared intra- and interspecific variation in reproductive timing among sympatric populations of *E. aucklandica* and *E. menziesii* in four Waikato streams to determine whether phenological partitioning occurs between the two species. I characterised reproductive phenology in both species in relation to water temperature regimes for the duration of their annual reproductive season to test the hypotheses that,

(i) based on the ecological theory of resource partitioning (Schöner, 1974), brooding onset and peak brooding for *E. aucklandica* do not overlap temporally with *Echyridella menziesii*, and (ii) water temperature is a key driver regulating the timing and duration of brooding in both species (Young & Williams, 1984; Watters & O'Dee, 2000; Walker et al., 2001; Hastie & Young, 2003; Österling, 2015). Temporal partitioning of brooding onset and peak brooding through contrasting thermal thresholds and/or degree days may enable the two mussel species to optimise use of host fish resources over their respective reproductive periods (see Chapter 4 for host fish relationships).

3.3 Methods

3.3.1 Field sampling sites

Sampling was conducted from March 2018 to March 2019 in four Waikato, North Island, streams (Strahler order 3-5) known to support relatively large populations of the two study species, *E. aucklandica* and *E. menziesii* (Table 3.1). Three of the four study sites aresituated on short coastal waterways draining into the Tasman Sea (Ohautira Stream [37°45'43.0"S, 174°58'49.0"E], Kahururu Stream [37°41'11.2"S, 174°58'45.0"E], Pakoka River [37°55'42.0"S, 174°52'13.0"E]), while the fourth site, the Mangapiko Stream, (37°58'.54.2"S, 175°28'26.3E) is an inland Waipa River tributary draining a predominantly agricultural landscape (Figure 3.1; Table 3.1).

Ancient volcano activity has shaped the region's landscapes, including the dormant volcanoes Karioi and Pirongia dominating the west, whilst Maungatautari in closer proximity to the inland Mangapiko Stream site. Moreover, the three coastal stream sites are surrounded by a mixture of moderately to well-drained allophanic, brown and ultic soils posing moderate to high erosion risk, while the inland Mangapiko site is poorly drained and characterised by gley soils (Lowe, 2010; Waikato Regional Council, 2020). The climate in the region is typically warm, with humid summers and mild winters; western areas are exposed to extreme weather conditions brought in by prevailing westerly winds (Chappell, 2013).

A

B

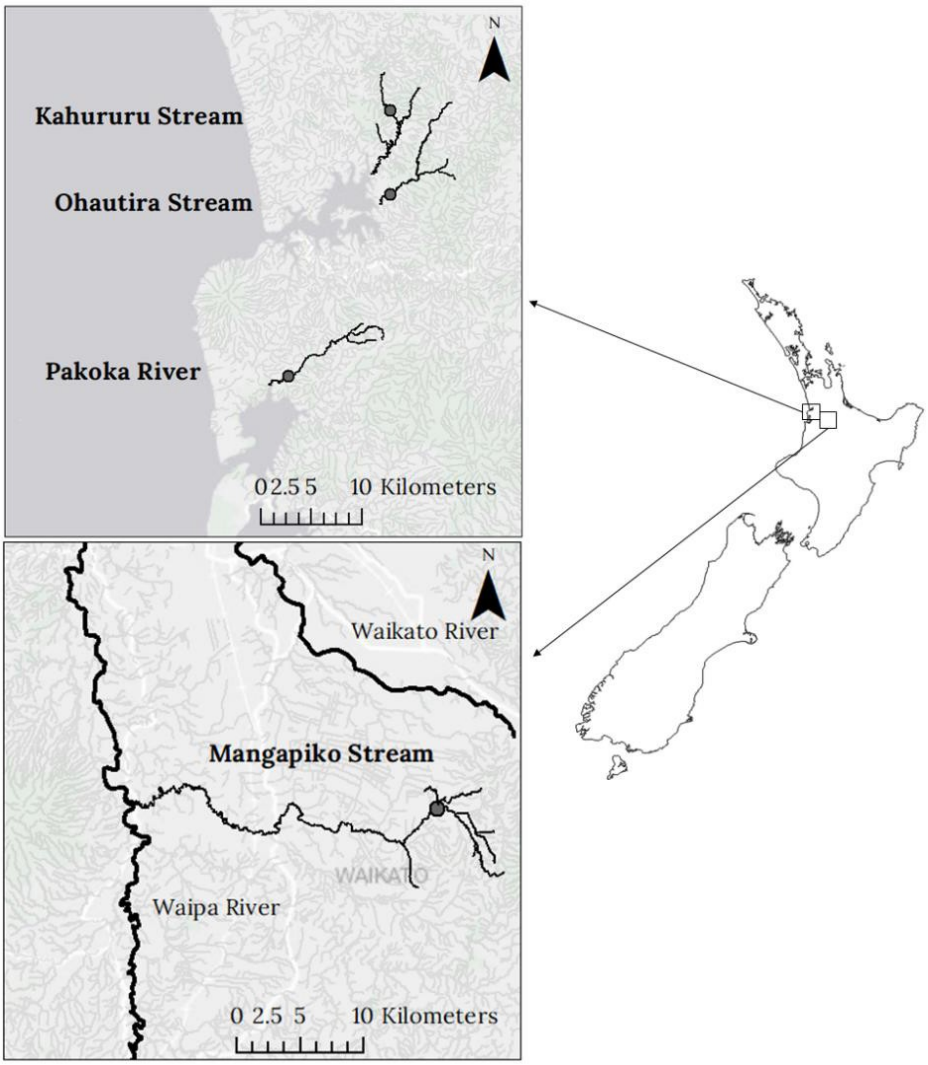


Figure 3.1. Locations of sampling sites (*filled circles*) showing coastal sites Kahururu Stream, Ohautira Stream and Pakoka River (A), and the inland site Mangapiko Stream (B).

Ohautira has extensive indigenous forest upstream (61% of catchment area) while nearby Kahururu has around 9% of its upper catchment area in native forest, in addition to a significant area of mature pine forest which occurred alongside the site over the time of sampling. Pakoka's upstream catchment area is comprised of 20% indigenous forest with most of the remaining land use developed for sheep and beef farming. Inland Mangapiko runs through a low-lying rural catchment with dairy farming the most common land use, including alongside the sampling reach, and native forest comprising 12% of upstream catchment area (FENZ database, Leathwick et al., 2010; Table 3.1).

The streambeds for three of the study reaches are characterised by unconsolidated bottom substrates dominated by silt at Kahururu, small gravel at Mangapiko and large gravel at Ohautira. The channel bottom at Pakoka is dominated by cobbles with pockets of fine sediments which mussels inhabit (Waikato Regional Council, unpubl. data). During sampling, the study reaches' mean wetted channel widths ranged from 3.9-5.6 m, and overhead shade was between 7% at Pakoka and 69% at Ohautira (Table 3.). Mean monthly temperatures ranged from 13.5-14.7°C, dissolved oxygen was between 10.4-11.3 mg/L and specific conductivity 110.7-143.7 µS/cm (Table 3.1).

Table 3.1. Upstream catchment and sample reach characteristics of the four streams in the study. Physicochemical data are means of point measurements (± standard deviation) taken during the year-long sampling period. Total number of unionid individuals per species collected at the start of the sampling period is listed in parentheses.

	Ohautira	Kahururu	Pakoka	Mangapiko
E. aucklandica density (no./m^2)	0.81 (51)	0.34 (32)	0.43 (20)	0.15 (23)
E. menziesii density (no./m^2)	0.49 (31)	0.43 (40)	1.01 (50)	0.32 (50)
Catchment area (km^2)[1]	38.8	42.6	24.7	40.1
Upstream native forested catchment area (%)[1]	61.2	9.1	20.1	12.0
Sample reach length (m)	15	20	15	40
Distance to sea (km)[2]	2.7	13.6	2.2	195.0
Strahler order[2]	4	4	3	5
Reach scale canopy cover (%)[3]	69	44	7	14
Wetted width (m)[4]	4.2±0.19	4.7±1.10	5.6±0.20	3.9±0.20
Water temperature (°C)[5]	13.5±3.6	14.5±3.9	14.3±4.1	14.7±3.3
Dissolved oxygen (mg/L)[5]	11.1±1.0	10.5±2.5	11.3±0.9	10.4±0.8
Dissolved oxygen (%)[5]	105±6.4	88.7±23.3	108.3±6.0	102.3±7.9
Conductivity (uS/cm)[5]	137.9±17.7	141.6±40.5	143.7±24.7	110.7±9.7

[1], data obtained from FENZ (Freshwater Ecosystems of New Zealand, Leathwick et al. 2010);
[2], data obtained from NZ Freshwater Fish Database (accessed May 2020);
[3], densiometer measurement at the thalweg midway within each sample reach;
[4], ± SD of transects evenly-spaced every 5 m along sampling reach;
[5], ± SD measured fortnightly - monthly at thalweg (YSI 2030 Pro meter; Yellow Springs Instruments, Ohio,USA) (see Appendix Table A.3.1 for details).

3.3.2 Field assessment of brooding status

At all sites, *E. menziesii* and *E. aucklandica* sampling was undertaken monthly, except during late spring-summer when female larval brooding activity is known to increase and mussels were sampled fortnightly (excluding December 2019 due to high flow events). Tactile and visual (via bathyscope) searches were conducted along the same reaches (15-40 m) on each occasion until 20-40 individuals of each species were collected. All individuals were measured to the nearest mm (length) using calipers, and sexed based on marsupium absence (male) or presence (female) by observation of the gill morphology using a speculum for opening of valves. In sexually mature female hyriids, the marsupium is clearly visible throughout the year on the inner demibranchs as opaque thickened columns perpendicular to the hinge of the shell (Jones et al., 1986; Jupiter & Byrne, 1997; Walker et al., 2001; see Plate 3.1). Female brooding status was assessed using the procedure outlined by Melchior et al. (2021) based on classification of gill volume and pigmentation. During this process, individuals were held at ambient water temperatures in 2.5 L buckets and/or in mesh nets within the stream. Individual mussels were returned to their original location at the end of each sampling event.

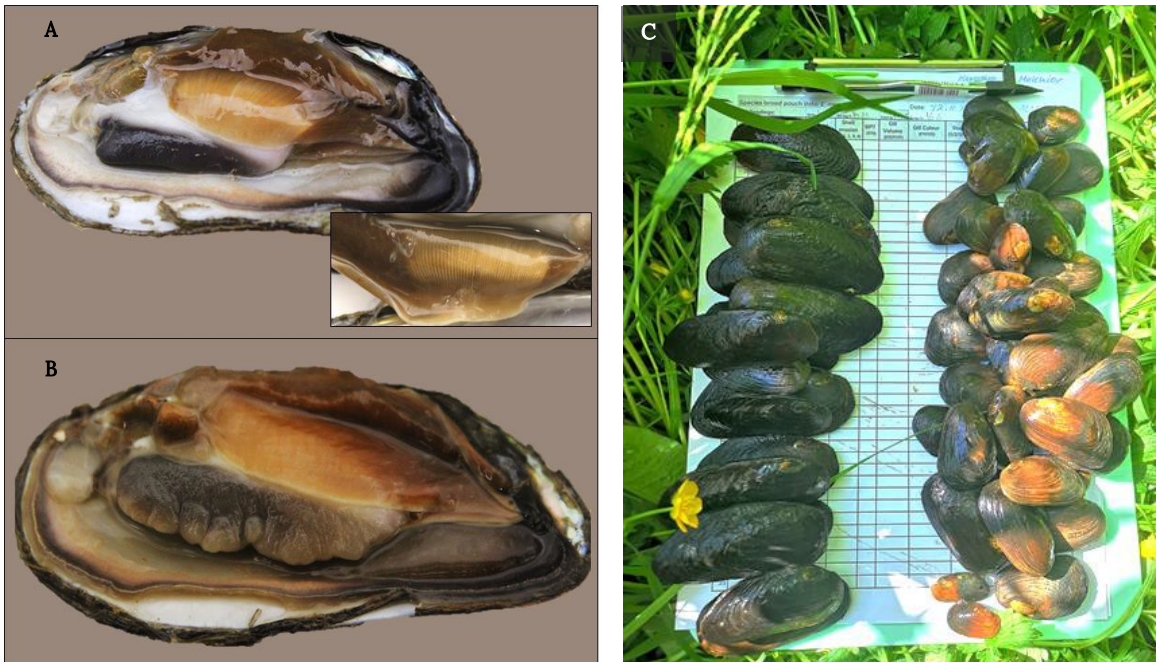
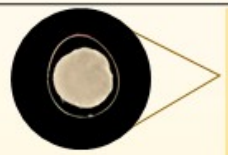
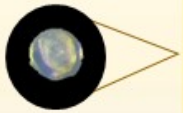


Plate 3.1. Non-brooding *Echyridella aucklandica* female (A) with empty marsupium clearly visible on ventral inner demibranch (insert), and male *E. aucklandica* (B) with no marsupium present. Mussels shown (C) are from Kahururu Stream, with *E. aucklandica* (left) and *E. menziesii* (right) showing differences in size.

Six additional individuals per species collected upstream of each sampling reach during each sampling event (except March 2018) were used for validation of *in vivo* brooding stage via extraction of eggs or glochidia from gill marsupia (Table 3.2). The extracted gill contents were examined in the laboratory on a petri dish under the Olympus SZ-6045 dissecting microscope (20-40 x) and characterised into one of the five developmental stages partially recognised by Melchior et al. (2021) and refined here (see Table 3.2). Microphotographs of the gill content samples were taken using the Leica DM RD microscope equipped with the Olympus DP70 Digital Camera system and Olympus image analysis software.

Table 3.2. Larval stage microphotography, gill pigmentation gradient and description for staging validation in *Echyridella aucklandica* and *E. menziesii* (adapted from Melchior, 2017 and Melchior et al., 2021).

Larval stage	Microphotograph and Gill Pigmentation *E. aucklandica*	 *E. menziesii*	Description
1: Egg			Opaque spherical mass within vitelline membrane, may be split into multiple spherical masses during early cell division with no clear glochidia shape.
2: Immature glochidia			Bivalve shaped mass formation contained within the vitelline egg membrane.
3: Developing glochidia			Valves closed with fuzzy non-translucent appearance, hooks not fully developed, vitelline membrane present at times (Melchior et al. 2019).
4: Mature and viable glochidia			Presence of hooks on opposing valves,translucent valves, free of their vitelline membrane (Melchior et al. 2019).
5: Spent			Empty brood pouch (Melchior et al. 2019).

3.3.3 Environmental parameters

Water temperature (°C) was recorded daily at 15-minute intervals at all sites for the duration of the study using temperature loggers (Hobo ® Tidbit) submerged close to the streambed. Accumulated degree-days (ADD hereafter) were calculated with the University of California's State-wide Integrated Pest Management Program online degree-day calculator (Baskerville & Emin, 1969; UC IPM, 2020) which uses maximum and minimum daily temperatures to calculate the number of ADD from set dates based on the single sine method (Higley et al., 1986). The method produces a sine curve over a 24-hour period, and then estimates ADD for that day by calculating the area above a threshold and below the curve. In this study, two start dates were chosen to for calculation of ADD. First, start dates were set from the end of March 2018, the end of the previous brooding period for *E. aucklandica* and *E. menziesii*, to determine ADD required to reach the next season's brooding onset. Second, to determine the ADD required to reach peak brooding proportion as well as the duration of the brooding period, start dates were set to the sample date at which brooding mussels were first observed for each species within each stream (see Results).

The minimum thermal limit used for calculating ADD for each species was defined as 10°C, based on laboratory trials (Appendix Text A.3.1) which tested for low thermal tolerance in brooding *E. aucklandica* and *E. menziesii* females. Here, the endpoint was defined as the temperature at which reproduction was likely halted and larvae prematurely aborted. The minimum threshold temperature used in this study corresponds to that found in similar international studies of unionids of the genera *Quadrula*, *Cyclonaias* and *Fucsonia* for ADD calculations based on metabolic rate data (Spooner & Vaughn, 2008; Galbraith & Vaughn, 2009; Dudding et al., 2019).

3.3.4 Statistical analyses

All analyses were performed using software R (version 4.0.0; R Project for Statistical Computing, Vienna, Austria). Data were assessed for normality (Shapiro-Wilk's test and Q-Q plots) and homogeneity of variances (Levene's test). Where parametric assumptions could not be confirmed, even after transformations, non-parametric tests were used. Statistical significance for all tests was defined at $p = 0.05$.

Both species' median valve lengths were compared between sites using Kruskal- Wallis tests, followed by Dunn's post-hoc multiple comparisons tests (with Bonferroni correction to reduce risk of Type I error), while Mann-Whitney U tests

were performed to test for differences between male and female lengths for each species within a site. Differences in sex ratios among sites were assessed for each species with binomial exact tests to determine the deviation from a 1:1 sex ratio. Following on from this, two-way Kolmogorov–Smirnov two-sample goodness of fittests were used to detect differences in cumulative distribution of brooding proportions over time between each species within each site. Kruskall-Wallis tests (followed by Dunn's post-hoc multiple comparisons tests with Bonferroni correction) were performed totest for differences between sites in ADD required to reach onset brooding, peak brooding, and duration of brooding for each species.

To determine the strength of relationships between water temperature, specifically thermal summation (i.e., ADD), and brooding onset and brooding peak in *E. menziesii* and *E. aucklandica*, Beta regressions with logit link function were implemented using the *betareg* package (Cribrari-Neto & Zeileis, 2010), with ADD as the single predictor variable and the following response variables analysed separately for *E. menziesii* and *E. aucklandica* based on pooled data from multiple sites: (i) proportion of mussels with glochidia development at Stage 1 (i.e., onset of brooding), and (ii) proportion of mussels with glochidia development at Stage 4 (peak brooding). The coefficient of determination (r^2) calculated using beta regression with logit function are given for each model. I used Beta regression to analyse these data, instead of a Gaussian generalised linear model, as the response variables (brooding onset and brooding peak) were expressed as proportions with range from 0 to1 and had variances that are usually not constant across the range of the predictor (Douma & Weedon, 2019). Beta regressions can account for the heteroskedasticity and skewness that are common with proportional data (Cribrari-Neto & Zeileis, 2010). To help interpret the magnitude of the effects of the Beta regressions, average marginal effects (which measure the change in a response given a change in a covariate) were calculated for each model using the *margins* package (Williams, 2012; Leeper, 2016). Model assumptions, including normality and heteroskedasticity of residuals, were verified and assessed visually by diagnostic plots.

3.4 Results

3.4.1 Stream temperature regimes

Water temperatures varied slightly between sites, with Ohautira experiencing the lowest average temperatures every month throughout the entire sampling period (Appendix Table A.3.1). Lowest recorded temperatures ranged from 6.4°C (Ohautira) to 7.1°C (Mangapiko), occurring from May to June at each of the sites, while the warmest temperatures were experienced in February with maxima at Kahururu (24.2°C) followed closely by Pakoka (24.1°C), and then Mangapiko (22.9°C) and Ohautira (22.1°C) (Figure 3.3; Appendix Table A.3.1). Mean temperatures in February varied between 18.9°C (Ohautira) and 20.2°C (Kahururu), while lowest average temperatures ranged from 9.8°C (Ohautira)to 10.9°C (Mangapiko) (Appendix Table A.3.1).

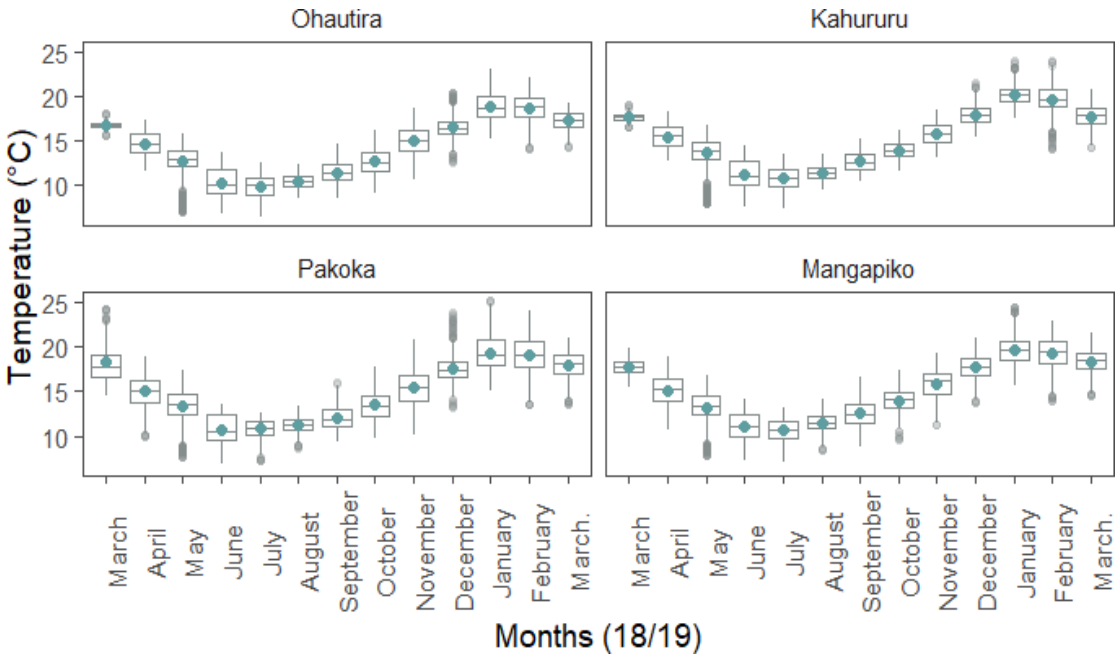


Figure 3.2. Water temperatures (°C) within each sample site throughout March 2018 to March 2019. Box boundaries indicate interquartile ranges; within each box are the median (line) and mean (point); whiskers are 10th and 90th percentiles, while outer circles denote outliers.

3.4.2 Mussel population characteristics

Valve length varied significantly between sites within both species (*E. aucklandica*: K-W: n = 528, H = 424.2, p <0.001; *E. menziesii*: n = 242, H = 96.42, p <0.001). For *E. aucklandica*, significant pairwise size differences occurred between all sites except for Pakoka and Ohautira where *E. aucklandica* were largest on average, and for Mangapiko and Kahururu

where they were smallest. For *E. menziesii*, significant size differences were found between all sites, except for Mangapiko and Pakoka (Figure 3.3). At all sites, valve lengths were skewed towards larger mussels, particularly for *E. aucklandica* with few mussels below 40 mm collected (Figure 3.3).

Field observations showed no obvious signs of sexual size dimorphism in *E. aucklandica* and *E. menziesii*, which was confirmed statistically with both species having similar-sized males and females within each site (Appendix Table A.3.2; Appendix Table A.3.3). Additionally, sex ratios in each species were consistent among sites with no significant deviations from 1:1 (Appendix Table A.3.2). Gravid females were identified across a range of size classes within each species. The smallest brooding female found in this study was 23 mm long for *E. menziesii* (Pakoka) and 40 mm for *E. aucklandica* (Mangapiko), while the largest brooding *E. aucklandica* collected was 110 mm (Ohautira) compared to 91 mm for *E. menziesii* (Pakoka) (Appendix Table A.3.2).

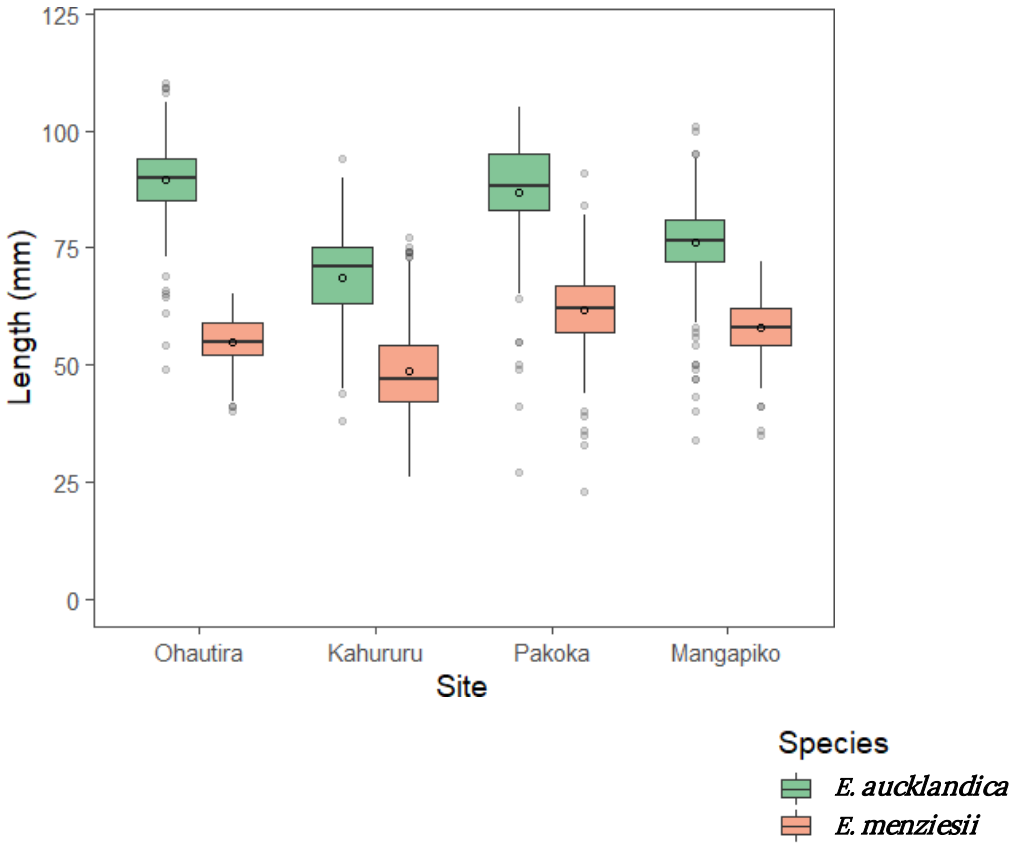


Figure 3.3. Valve length (mm) boxplots of sampled *Echyridella aucklandica* and *E. menziesii* at four Waikato stream sites. Box boundaries indicate interquartile ranges; within each box are the median (line) and mean (point); whiskers are 10th and 90th percentile, while outer circles denote outliers.

3.4.3 Spatio–temporal brooding patterns

A total of 1813 assessments of female brooding status were made over all sites, species and dates combined. Brooding season varied among species with brooding across the four sites starting 1–3 months earlier in *E. aucklandica* (austral autumn) than *E. menziesii* which initiated brooding in late winter–early spring. In *E. aucklandica*, the timing of brooding onset varied between sites, ranging from late May to mid-July, unlike *E. menziesii* which consistently began brooding larvae at all sites in August when water temperatures startedto steadily increase (Figure 3.4).

Brooding proportions at all sites except Mangapiko increased gradually over time following the onset, with females of *E. aucklandica* and *E. menziesii* reaching ~90% gravidity at peak brooding within each site (Figure 3.4). Both *E. menziesii* and *E. aucklandica* were observed have unimodal brooding peaks, with only one peak reproductive event at each site. Peak brooding occurred slightly earlier in October–November (spring) and remained high for longer in *E. aucklandica* than in *E. menziesii* which reached maximum brooding proportions in November and December (late spring to early summer) (Figure 3.4). Gradual patterns of decline in brooding proportions following the peak at each site suggest the start of glochidia release in both species, with declines to minimum brooding proportion in February to March (mid-summer to autumn) for *E. aucklandica* and January to February (summer) for *E. menziesii*, coinciding with maximum stream temperatures (Figure 3.4).

Within all sites, the overall brooding season for female *E. menziesii* was shorter (6–7 months) in comparison to female *E. aucklandica* which remained gravid for a 9-11 monthperiod (Figure 3.4). Though brooding periods for *E. aucklandica* occurred earlier and continued for longer in comparison to those for *E. menziesii*, no statistically significant variations were detected in the overall brooding distributions between species at each site (Ohautira: $D = 0.3$, $p = 0.7$; Kahururu: $D = 0.2$; $p = 0.99$; Pakoka: $D = 0.3$ $p = 0.6$, Mangapiko: $D = 0.3$, $p = 0.6$), in part due to temporal overlaps among sites in peak and non-brooding periods.

Brooding stages determined *in vivo* (and verified by *in vitro* gill examinations) found that glochidia development within both species across all sites appeared synchronous throughout the brooding season, with early embryos making up the highest proportions of brooding stages for several months from brooding onset, proceeding gradually onto later developmental stages throughout the brooding season (Figure 3.5). Over time, development became more asynchronous with individuals of

various developmental stages appearing throughout each sample month. Mature glochidia (Stage4) within *E. aucklandica* marsupia first occurred in September or October at all sites, while mature glochidia in *E. menziesii* marsupia were observed one month later in October and November (Figure 3.5).

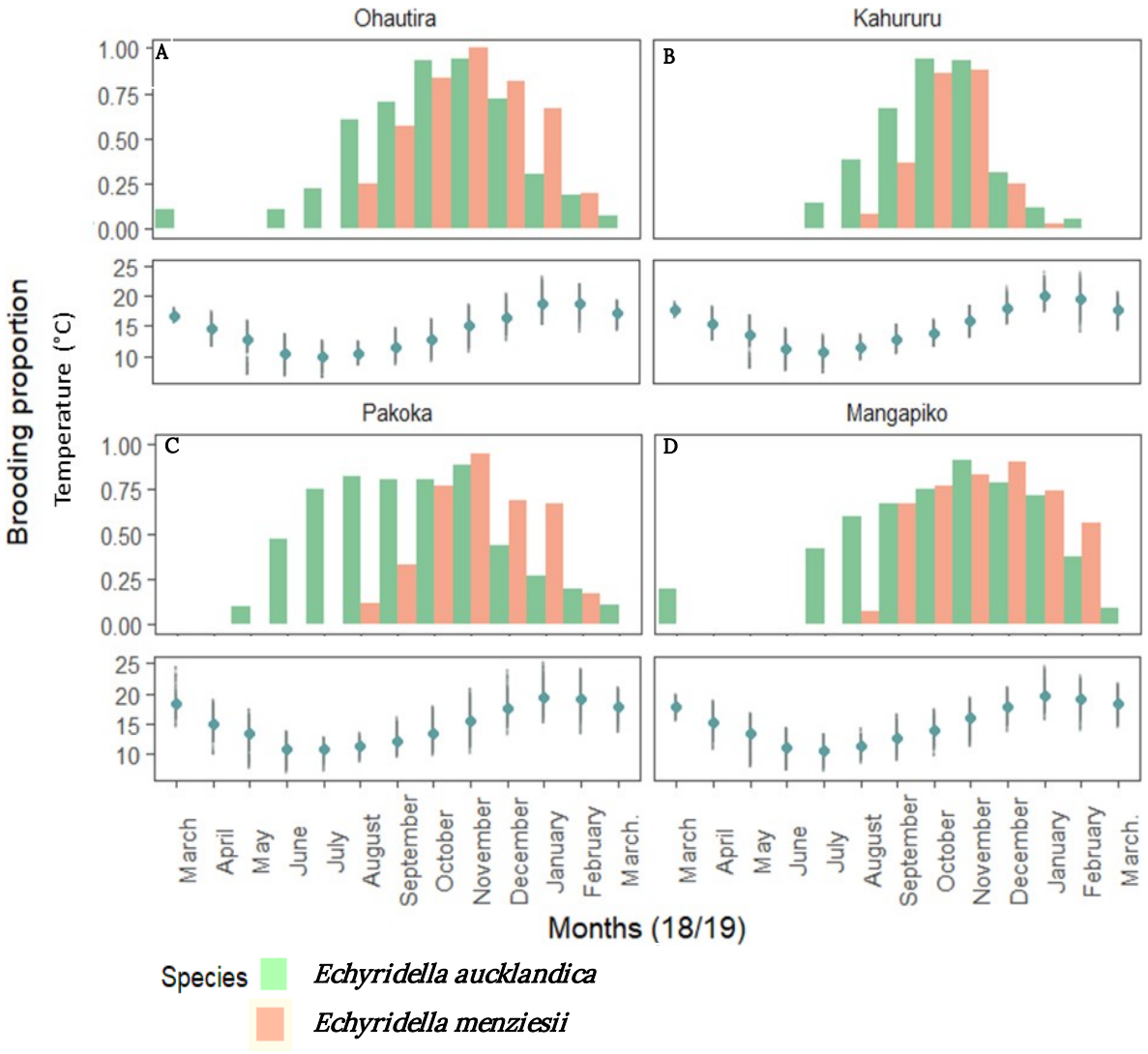


Figure 3.4. Brooding proportions (A-D; upper panels) in female *Echyridella aucklandica* and *E. menziesii* over 1 year (March 2018 – March 2019) at the four sampling sites (see Figure 3.6 for more detail). Monthly temperature plots (A-D; lower panels) are shown for comparison; points indicate means and vertical lines indicate 10th and 90th percentiles (see Figure 3.3 for more detail).

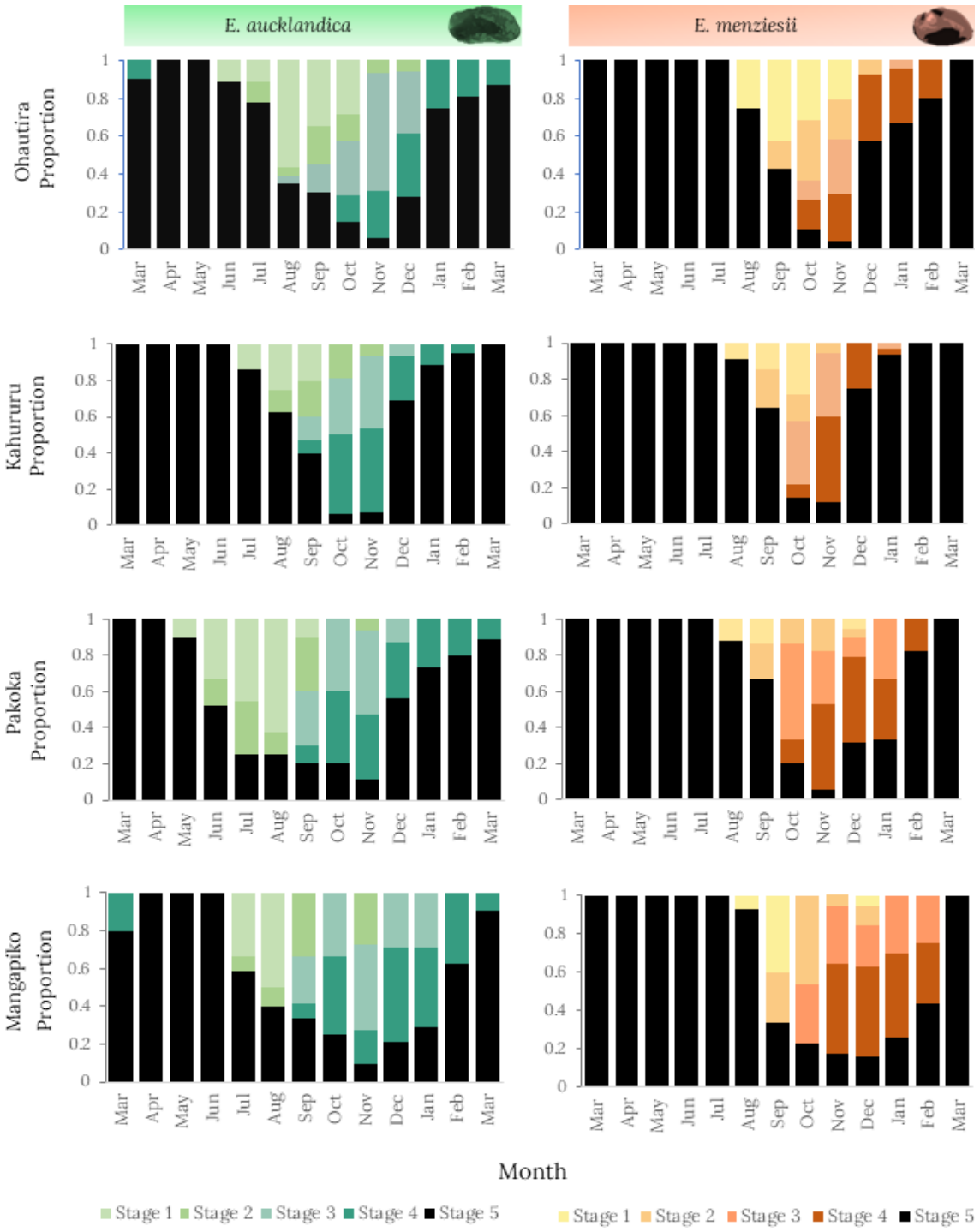


Figure 3.5. Glochidia brooding stages (1–5; see Table 3.2) over time (March 2018 to March 2019) in female *Echyridella aucklandica* (left) and *E. menziesii* (right) at four sites.

3.4.4 Effects of temperature on brooding phenology

Examination of cumulative stream temperatures as ADD relative to phenologically "sensitive" periods (i.e., brooding onset and peak) showed variable thermal regimes experienced by *E. aucklandica* and *E. menziesii* over time among sites. The general upward trend in ADD steadied throughout the cooler months (May to July), and then increased from August and September onward when water temperatures began to increase (Figure 3.). Despite being widely separated geographically, Kahururu (coastal) and Mangapiko (inland) had ADD regimes that were synchronous throughout almost the entire sample year, with both streams experiencing similar increasing rates in ADD from October to November onwards. Pakoka, too, followed similar patterns to Kahururu and Mangapiko, but experienced a slowing in ADD from November, while Ohautira's forested headwaters and shaded reach meant that the ADD curve was lower throughout the entire study periodin comparison to the other sites.

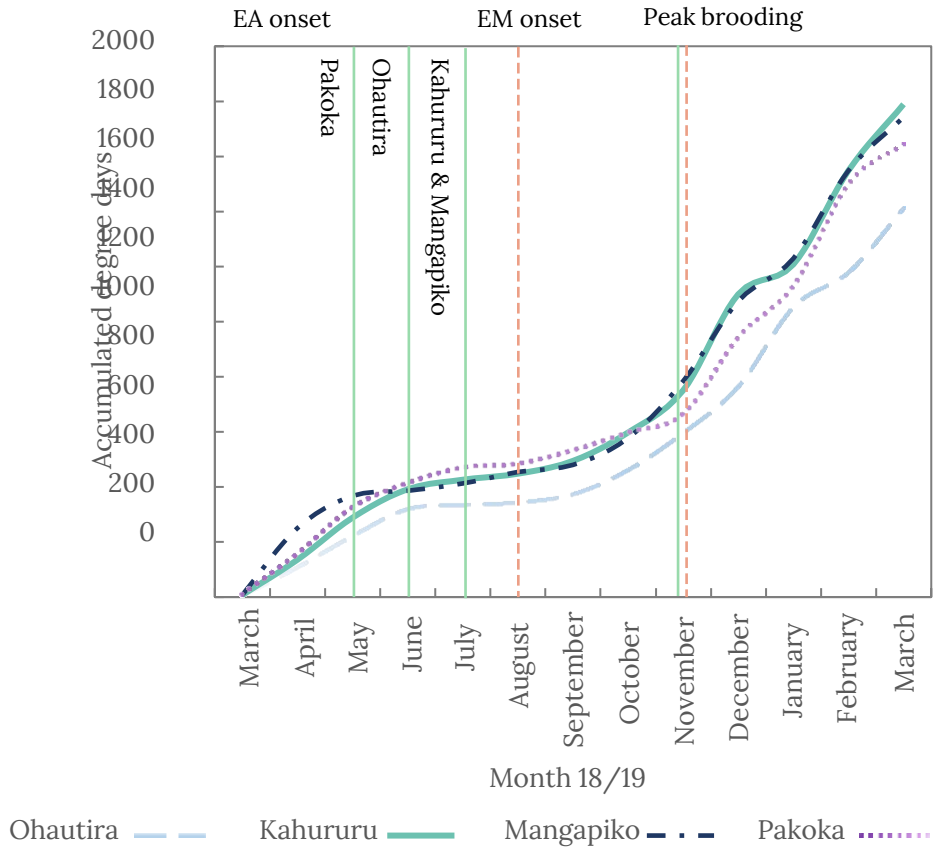


Figure 3.6. Cumulative stream temperature curves as degree days for each stream showing penologically sensitive time periods: EA onset = first observation of brooding in *Echyridella aucklandica* (solid vertical line) at each site; EM onset = first observation of brooding in *E. menziesii* (dotted vertical line); Peak brooding = month at which brooding proportions were highest for each species.

Significant differences between *E. aucklandica* and *E. menziesii* in median ADD required to reach onset brooding were found across all sites (Ohautira: p = 0.018, Kahururu: p = 0.01, Pakoka: p<0.001, Mangapiko: p<0.001; Appendix Table A.3.4). First observations of brooding in *E. aucklandica* varied substantially in terms of ADD between sites (321, 326, 412 and 426 ADD at Ohautira, Pakoka, Mangapiko and Kahururu, respectively) (Figure 3.6). Median ADD required to reach brooding (Stage 1) for each *E. aucklandica* varied significantly between sites (K-W: n = 81, H = 424.2, p = 0.04), with differences occurring between Ohautira ($\tilde{x}$ = 431 ADD) and Pakoka ($\tilde{x}$ = 471 ADD adj p =0.03), and between Ohautira and Kahururu ($\tilde{x}$= 494 ADD, adj p = 0.05), but not between the other sites. *Echyridella menziesii* showed a similar wide range among sites in terms of ADD for onset of brooding (375, 448, 454, and 484 ADD at Ohautira, Kahururu, Pakoka and Mangapiko, respectively) (Figure 3.6). Median ADD required to reach brooding (Stage 1) for each *E. menziesii* varied significantly among sites (K-W: n = 70, H = 30.9, p <0.001), with Ohautira requiring lower ADD to reach onset brooding than all of the other sites (Kahururu: $\tilde{x}$ = 494 ADD, adj p <0.001; Pakoka: $\tilde{x}$ = 533 ADD = adj p <0.001; Mangapiko: $\tilde{x}$ = 480 ADD, adj p = 0.04) (Table 3.3).

Significant differences between *E. aucklandica* and *E. menziesii* in median ADD to reach peak brooding (Stage 4) were found across all sites (Ohautira: p = 0.018, Kahururu: p = 0.01, Pakoka: p<0.001, Mangapiko: p = 0.002). In *E. aucklandica*, median ADD for peak brooding varied significantly between sites (K-W: n = 162, H = 26.60, p<0.001), with significant differences between Ohautira (lower ADD at peak brooding) and each of the other sites (Kahururu: p = 0.002, Pakoka: p <0.001, Mangapiko: p<0.001) (Table 3.4). MedianADD for peak brooding in *E. menziesii* also varied significantly among sites (K-W: n = 184, H = 31.95, p <0.001), with significant differences only occurring between Mangapiko (higher ADD at peak brooding) and each of the other sites (Ohautira: adj p <0.001; Kahururu: adj p <0.001; Pakoka: adj p = 0.01; Table 3.3). The ADD required to complete the brooding period ranged from 1411 (Ohautira) to 1500 (Pakoka) for *E. aucklandica*, and from 851 (Kahururu) to 1107 (Mangapiko) for *E. menziesii* (Table 3.3; Appendix Table A.3.4).

Table 3.3. Summary of accumulated degree-days (ADD) required to reach brooding onset and peak brooding, and required for the entire brooding period in *Echyridella aucklandica* and *E. menziesii*. Minimum and maximum ADD across the four sample sites are in parentheses.

	Ohautira	Kahururu	Pakoka	Mangapiko	Mean (±SD)
E. aucklandica					
Onset	431 (321-484)	494 (426-494)	471 (326-471)	454 (412-480)	440±44
Peak	481 (431-1411)	715 (494-1208)	665 (533-1500)	783 (489-1548)	763±301
Brooding duration*	1411	1208	1500	1548	1424±159
E. menziesii					
Onset	431 (345-484)	494 (448-597)	533 (484-597)	480 (454-577)	502±68
Peak	242 (118-931)	288 (96-851)	523 (168-1066)	549 (190-1107)	478±289
Brooding duration*	931	851	1066	1107	988±118

* first brood observation to last brood observation during the brooding season 18/19

Beta regressions used to determine the strength of relationships between water temperature expressed as ADD and brooding onset and brooding peak in *E. menziesii* and *E. aucklandica* revealed variable results. Significant relationships for onset of brooding (Stage 1) were stronger in *E. aucklandica* (β = 0.010, SE = 0.004, p = 0.001) than *E. menziesii* (β = 0.005, SE = 0.002, p = 0.047), with average marginal effect sizes averaging 0.2 percentage points in *E. aucklandica* and 0.1 percentage point in *E. menziesii* for every increase in ADD (Appendix Table A.3.5). Peak maturity (Stage 4) was negatively related to ADD in *E. aucklandica* (β = -0.001, SE = 0.001, p = 0.003), but there was no significant relationship for *E. menziesii* (β = - 0.001, SE= 0.001, p = 0.2; Figure 3.7). Here, average marginal effect sizes were -0.03 percentage points for every increase in ADD for both species (Appendix Table A.3.5). Percent variance explained by ADD for significant relationships ranged from 28% to 47% (Figure 3.7).

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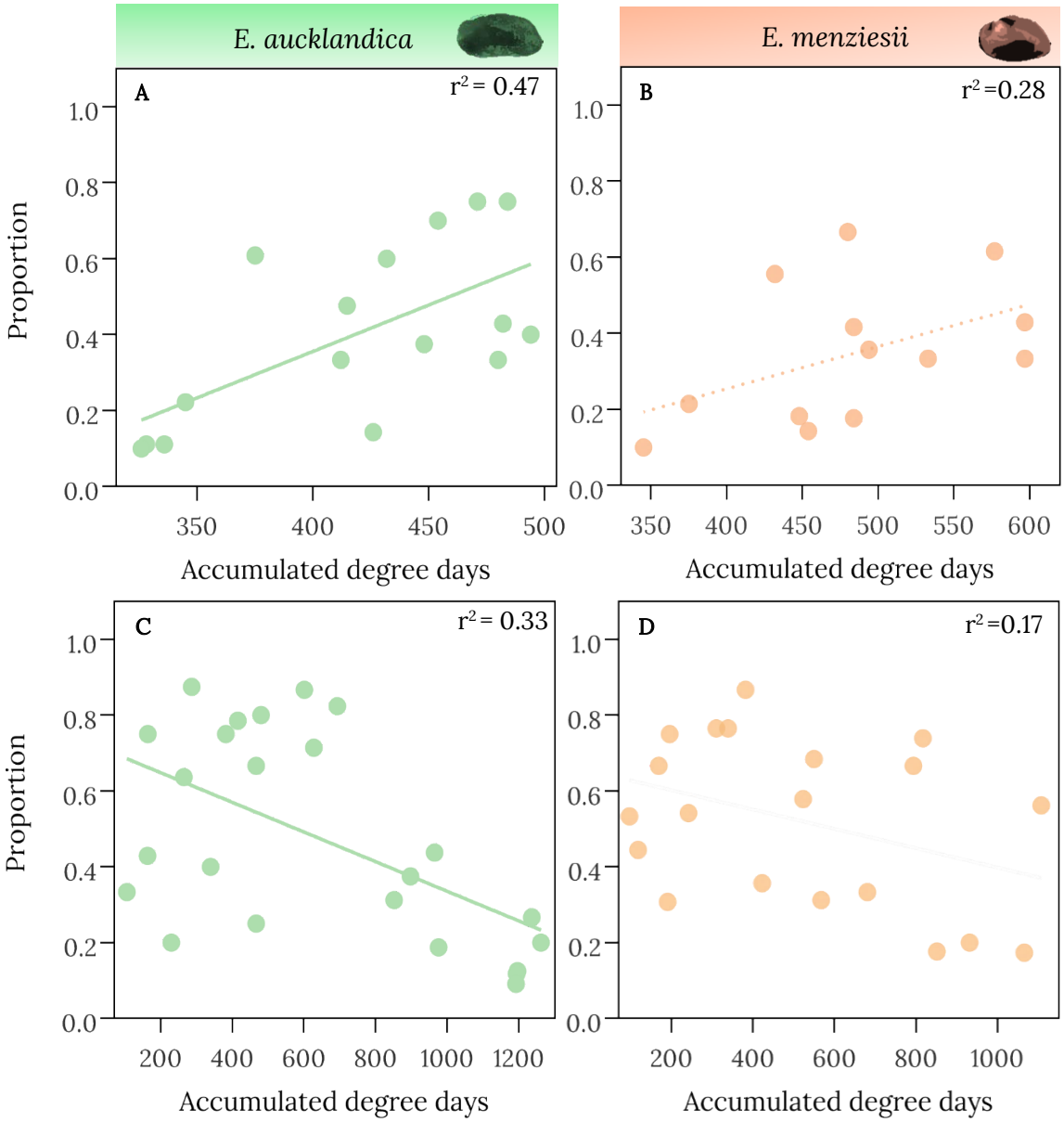

Figure 3.7. Relationship between 'onset brooding' proportion (glochidia Stage 1) (A-B) and between 'peak brooding' proportion (glochidia Stage 4) (C-D) with number of accumulated degree-days in *Echyridella aucklandica* and *E. menziesii*. Solid lines show beta regression relationships that are statistically significant at $p<0.01$, while the dashed line for $p<0.05$. Pseudo r^2 values calculated using beta regression are given for each model.

3.5 Discussion

This chapter resolves the reproductive phenology of two sympatric New Zealand freshwater mussel species, *E. aucklandica* and *E. menziesii*, in four Waikato streams, and identifies key thermal cues associated with brooding and glochidia maturation. Notably, for the first time, the brooding phenology of *E. aucklandica* is reported, filling an important data gap on the basic biology of this poorly known and 'threatened' species of conservation concern. Overall, the brooding phenology of *E. menziesii* and *E. aucklandica* demonstrated high temporal overlap, particularly during the critical peak brooding period when mature glochidia are expected to be released from females to infest host fish (see Chapter 4). This high degree of synchrony during the important peak brooding phase does not support the hypothesis of temporal partitioning in peak brooding between both mussel species as a means of avoiding competition for fish hosts. Nonetheless, water temperature regime was identified as an important factor influencing the timing of phenological phases between sites, particularly brooding onset in both species, in part supporting the second hypothesis. ADD appeared less critical for timing of the important peak brooding phase which may be influenced by endogenous and/or exogenous factors other than thermal regime within the streams studied.

3.5.1 Unionid population characteristics

The balanced sex ratios observed in this study for both *E. menziesii* and *E. aucklandica* are typical for other stream-dwelling populations of Australasian hyriids, including *Cucumerunio novaehollandiae*, *Hyridella australis*, *H. depressa*, *Alathyria jacksoni* and *Velesunio ambiguous* (Jones et al., 1986; Walker et al., 2001). I found no evidence of skewedsex ratios within my study, although these have been reported in other unionid populations where mussel densities are low (Heard, 1975; Byrne, 1998; Walker et al., 2001),with some species changing sex or developing hermaphroditic gonads to enable self- fertilisation (Heard, 1975; Byrne, 1998; Watters, 2007). Streams in the present study were selected because they had relatively dense populations of both mussel species, so balanced sex ratios were not unexpected.

Although valve lengths of both species varied between sites, it was apparent that all sites were dominated by older individuals with no small mussels found below 40 mm for *E. aucklandica* or below 23 mm for *E. menziesii*. The presence of geriatric, senescing populations dominated by large individuals, such as in the study streams, has

been observed within waterways throughout New Zealand (James, 1985' Roper & Hickey, 1994; Catlin et al., 2018) and abroad (Vaughn & Spooner, 2004; Geist et al., 2006; Österling, 2006; Hastie, 2011; Stöckl et al., 2014), and provides evidence of recruitment constraints that could lead to 'extinction debt' (Tilman et al., 1994; Vaughn, 2012). Accordingly, the longevity of mussels (e.g., >50 years for *E. menziesii*, see Grimmond, 1968; unknown for *E. aucklandica*) coupled with the scarcity of smaller mussels in these Waikato streams, could indicate that both species may not be recruiting sufficient numbers of juveniles to sustain viable populations over the long term. However, the mechanisms that affect unionid recruitment are complex and further studies are required to understand recruitment dynamics in both species, including host fish specificity (see Chapter 4).

In this study, the smallest female mussels collected in both species were found to be gravid. Thus, the minimum sizes for brooding maturity (23 mm in *E. menziesii* and 40 mm in *E. aucklandica*) inferred here are considered conservative estimates as the lack of juveniles found in the field meant that smaller females were not assessed. Nevertheless, the minimum sizes reported in this study fall within the normal range for age at maturity of unionids elsewhere in New Zealand and globally. For example, histological analyses of *E. menziesii* in Lake Taupo found the smallest sexually mature female to be 37 mm long, estimated to be approximately four years old (Clearwater et al., unpublished). Small size at sexual maturity has also been reported in females of an Australian Hyriidae species *Velesunio angasi* which attained maturity at 1.5 years of age (40 mm; cf in 1 year or 30 mm length in males), while *Hyridella* species have been reported to mature in 2-3 years (40 mm) and *C. novaehollandiae* at 3-4 years (60 mm) (Jones et al., 1986; Byrne, 1998).

3.5.2 Interspecific variations in brooding phenology

Echyridella aucklandica began brooding earlier in early to mid-austral winter (compared to late winter in *E. menziesii*) and remained gravid for longer (9-11 months) than *E. menziesii* (6-7 months) in this study, reaching peak brooding (and thus glochidia release) in late austral spring to summer. Despite differing brooding onsets between species, both *E. aucklandica* and *E. menziesii* displayed synchrony in the peak brooding period across all sites, such that mature glochidia of both species were ready for release throughout the warmer part of the year in November and December. Prior studies on *E. menziesii* suggestsimilarities in the timing of peak brooding in spring and summer, as

indicated by the presence of glochidia on fish in October and November (Percival, 1931; Hine, 1978). A further study by Clearwater et al. (unpublished) found Lake Taupo *E. menziesii* populations with peak brooding in January. This general brooding seasonality by both species is consistent with other hyriids found in temperate coastal Australia (New South Wales), including *A. profuga*, *H. australis*, *H. depressa* and *H. drapeta* which coexist in the Macleay River, and in the sympatric *V. ambiguous* and *A. jacksoni* found in the Murray River (Jones et al., 1986; Walker, 2017). As with *E. menziesii* and *E. aucklandica*, brooding inthe above-mentioned hyriids was initiated in winter and glochidia were released in springto summer (extending into autumn in the case of *H. australis*) (Jones et al., 1986).

The extended brooding durations of both mussel species in the present study (although shorter for *E. menziesii* than *E. aucklandica*) are generally consistent with 'bradyticty' or a long-term brooding strategy, rather than 'tachyticty' or a short-term brooding strategy as recognised and described in many North American freshwater mussels (Sterki, 1895, 1898; Ortmann, 1911; Graf & Foighil, 2000; Price & Eads, 2011). In tachytictic mussels, fertilisation occurs in spring, with larvae brooded until they are fully developed and released within weeks to a few months over summer (Graf & Foighil, 2000). Contrastingly, in braditycticmussels, fertilisation usually starts in autumn, with broodingexpanding over the winter and the release of mature glochidia in spring (Graf & Foighil, 2000). The important distinction between these patterns is that bradytictic mussels complete fertilisation and ontogenesis earlier and continue to brood long after glochidia are mature and infectious (Kat, 1984). Bradyticty, known only from temperate lineages of freshwater mussels, is hypothesised to be advantageous because earlier ontogeny permits earlier infestation and metamorphosis on hosts in spring, allowing more time for growth injuveniles before temperature reductions in winter (Graf, 1997; Graf & Foighil, 2000).

Although the bradyticty and tachyticty model is used widely in the literature to broadly classify unionid brooding strategies, some researchers have pointed out that thetachytictic/bradytictic classification is too simplistic and needs to be re-evaluated as brooding periods among unionid species can be highly variable (Kondo, 1987; Watters & O'Dee, 2000; Haag, 2012). Indeed, the brooding durations exhibited in both *E. aucklandica* and *E. menziesii* showed large interspecific variations across all sites, a common feature found among coexisting species within the Hyriidae in Australia and South America (Joneset al., 1986; Callil et al., 2012; Walker, 2017). Earlier brooding onsets

and longer brooding durations in *E. aucklandica* compared to *E. menziesii* were found to lead to earlier onset of glochidia maturation and longer duration of mature glochidia present in the marsupia of *E. aucklandica*. Though brooding peaks in both species overlapped, early brooding onset may have facilitated the release of mature glochidia over a longer period, potentially increasing the chance of successful host fish attachment (Haag & Warren, 2000).

Varying developmental stages were present within the marsupia of both *E. menziesii* and *E. aucklandica* throughout their respective brooding seasons. Mature glochidia were present for up to five months in *E. menziesii* and seven months in *E. aucklandica* at varying proportions, and the number of empty dembibranchs was observed to decrease toward the end of the reproductive season, indicating glochidia release was not a one-off synchronous event and that release occurred over a longer period. This finding is consistent with other Hyriidae, including the Australian *A. jacksoni* and *V. ambiguous* which release their larvae throughout spring and summer (Jones, 1986; Walker, 2017). However, it contrasts with findings for the northern hemisphere *M. margaritifera* by Hastie and Young (2003) and *M. auriculata* by Soler et al. (2019), who reported releases to occur as sudden synchronised events triggered by environmental cues such as water temperature and/ or river levels.

Longer glochidia release durations in unionids, as observed in *E. aucklandica* in Waikato streams, may occur for a number of reasons. First, it may reduce the risk of temporal mismatches with host abundances (Cushing, 1990; Hochwald, 1997; see Chapter 4 for further details). Second, asynchronous, brooding (and thus glochidia release), found in both *E. aucklandica* and *E. menziesii*, may make both species less vulnerable to environmental disruptions such as seasonal pulsed flow events (floods; see Chapter 5). Last, early-stage embryos present within marsupia late in the brooding season, as observed in some *E. menziesii* within some sites, may suggest the production of multiple broods per season, a trait that has been witnessed in a study on an *E. menziesii* population from in Lake Taupo (Clearwater et al., unpublished) and other unionid species (Byrne, 1998; Price & Eads, 2011). The homogeneity of late developmental glochidia stages at the peak and end of the brooding period within individuals of the two species in this study indicates that females brooded only a single clutch at any one time within the studied streams.

3.5.3 Intraspecific variations in brooding phenology

Intraspecific variations in life history traits documented in freshwater mussel species are attributed to phenotypic plasticity in response to local environmental conditions (Bauer, 1992; Johnson & Brown, 1998; Hochwald, 2001). Variation of brooding patterns within species has commonly been recorded in other unionid species globally (Jones, 1986; Haggerty & Garner, 2000; Hastie & Young, 2003; Price & Eads, 2011; Walker, 2017). For example, Haggerty and Garner (2000) attributed within species brooding variations to geographic location, with longer brooding periods occurring at higher latitudes due to colder temperatures. Similarly, McIvor and Aldridge (2007) suggested that *Pseudanodonta complanata* brooding periods were shorter and glochidia release occurred earlier in regions with warmer temperatures (Pekkarinen, 1993; Zhadin, 1952), and Hastie and Young (2003) documented between catchment variations across Scotland in onset brooding in *M. margaritifera*, also ascribing these to variations to water temperature.

Variations in brooding phenology within species between sites in the present study, particularly onset brooding and brooding durations, may in part be related to specific local adaptations to thermal regimes, as suggested for *M. margaritifera* by Hastie and Young (2003) (see Section 3.5.4). In addition, the observed between-site intraspecific variations in onset of brooding as well as brooding durations may also partly reflect 1) low sample sizes collected at some sites during the winter months when water levels were high, and 2) the low frequency of sampling (monthly, except for fortnightly in the summer period). Future studies on brooding patterns in *E. menziesii* and *E. aucklandica* should include wider geographic ranges and thermal regimes. Additionally, increasing frequency of sampling, particularly during the onset, peak and end of the brooding season, may be useful to establish more precise and accurate phenological patterns.

Although not examined in the present study, interannual variations in brooding phenology may also occur. As spring weather patterns and stream conditions can vary from year to year, the reproductive phenology driven by these variables may be altered as a result. For example, the timing of brooding and glochidia release has been shown to vary up to several weeks between years in *M. margaritifera* (Young & Williams, 1984; Ross, 1992; Hastie & Young, 2003) and *Anodonta grandis* (Lewis, 1985) populations. Further studies are needed to elucidate long-term phenological trends in brooding in both *E. menziesii* and *E. aucklandica* at sites with varying thermal regimes.

3.5.4 Thermal cues associated with brooding

Both field and laboratory investigations indicate thermal cues as important in regulating the timing of brooding onset, brooding duration, and peak brooding in both *E. aucklandica* and *E. menziesii*. Water temperature may influence the timing of phenological events by effects of 1) thermal summation (i.e., ADD) (Davenport & Warmuth, 1965; Young & Williams, 1984; Hastie & Young, 2003; Galbraith & Vaughn, 2009; Dudding et al., 2019) a minimum threshold temperature (Trübsbach, 1998; Watters & O'Dee, 2000; Blažek & Gelnar, 2006; Schneider et al., 2018), or 3) a thermal shock (Jungbluth & Lehmann, 1976; Hastie & Young, 2003). However, it is possible that more than one of these processes is involved at different reproductive phenological phases (Jungbluth & Lehmann, 1976;Hastie & Young, 2003).

Generally, the calculated ADD required to reach each of the phenological phases varied between species, with *E. menziesii* requiring broadly more ADD to reach onset of brooding but less ADD for peak brooding and brooding duration in comparison to *E. aucklandica*. Relatively narrow ranges across sites in ADD required to reach onset of brooding, particularly in *E. aucklandica*, coupled with a strong relationship between ADD and onset of brooding in both species (although the strength of the relationship was weaker in *E. menziesii*), suggest that ADD is key in regulating the timing of brooding onset in both species. *Echyridella aucklandica* brooding began in early to mid-winter when water temperatures were lowest among sites, while *E. menziesii* brooding onset occurredsimultaneously across all sites at the end of winter when stream temperatures were increasing, potentially indicating temperature thresholds as a secondary process involved in *E. menziesii* brooding initiation. The negative relationship between peak brooding and ADD in *E. aucklandica*, showing a gradual decline in the proportion of Stage 4 brooding unionids (carrying mature glochidia) with increasing ADD, probably indicated the onset of gradual larval release.

The proportion of brooding females in both species among all rivers increased gradually, reaching peak brooding in synchrony in November and December, before gradually declining until February and March. This pattern suggests that thermal summation (ADD) played a key role in determining brooding duration more so than a minimum threshold or sudden temperature shock which would have triggered sudden and simultaneous brooding or release events. The wide ADD ranges within species and between sites, particularly for peak brooding and brooding duration, may be attributed to phenotypic plasticity in response to local environmental conditions (Bauer, 1992;

Johnson & Brown, 1998; Hochwald, 2001). However, the wide ranges in ADD along with large amounts of unexplained variation seen in the relationships of peak brooding and onset brooding with ADD could also suggest that processes other than thermal summation playa role the unionid phenological phases. For example, fish presence has been suggested toinfluence the timing of glochidia release, particularly in bradytictic brooding mussels, with some studies suggesting that host recognition mechanisms mediated via physical and chemical interactions may be important for the stimulation of glochidia release (Haag& Warren, 2003; Barnhart et al., 2008; Schneider et al., 2018 and references therein).

High overlap in brooding phenology in both *E. menziesii* and *E. aucklandica*, particularly during the important peak brooding season, raises questions on whether partitioning for resources occurs along niche dimensions other than phenology. In other words, sympatric species with high overlap along one dimension often overlap relatively little along another dimension, reducing overall effective niche overlap (i.e., niche- complementarity hypothesis) and promoting co-existence (Pianka, 1976). Rather than partitioning temporally, it seems likely that the two unionid species examined in the present study may instead partition fish species required for metamorphosis, given the contrasting use of reproductive strategies between both species (i.e., conglutinate use versus broadcast release, see Melchior et al., 2021). Evidence presented in Chapter 4 shows that *E. aucklandica* uses only *Retropinna retropinna* as a host fish while *E. menziesii* appears to be a generalist. Timing glochidia release to coincide with host presence may be particularly important in New Zealand unionid species where a large proportion of theknown host fish species pool is diadromous.

As freshwater mussels are predominantly sedentary, with limited dispersal abilities as adults, they are especially vulnerable to the effects of climate change (Archambault et al., 2018). Because some aspects of the reproductive phenology of freshwater mussels appear sensitive to environmental cues such as water temperature, as indicated in this chapter, accelerated changes in climate, specifically thermal fluctuations, may potentially disrupt or shift reproductive timing in these species (Forrest& Miller-Rushing, 2010; Schneider et al., 2018). Additionally, sudden fluctuations in temperature from a thermal regime to which species have adapted may impair mussel recruitment through abortions of immature and unviable larvae in early brooding females(Van Vrede et al., 1999; Melchior, 2017; Schneider et al., 2018; Appendix Text. A.3.1). It is therefore imperative to understand the impacts of climate change on

community-level patterns in the reproductive phenology of unionid mussels and their reproductive synchrony with host fish phenology.

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Chapter Four

Contrasting host-symbiont interactions among fish and larvae in sympatric freshwater mussel species (Hyriidae)

Retropinna retropinna and *Echyridella aucklandica*

4.1 Abstract

To complete their life-cycle, larvae (glochidia) of Unionida mussels must form obligate phoretic relationships with host fish, a critical resource that closely-related species may compete for. Partitioning host fish at the glochidia stage may be a mechanism by which unionid species avoid competition and facilitate coexistence. To investigate host resource partitioning in the two threatened sympatric New Zealand *Echyridella aucklandica* and *E. menziesii*, and identify previously unknown host fish associations for *E. aucklandica*, field surveys were used to quantify glochidia infestation on fish present of both mussel species throughout their peak reproductive periods in two Waikato streams. Additionally, laboratory host fish trials were conducted to validate field results, determine location of glochidia attachment, and compare encystment duration in both species. Combined field and laboratory results showed evidence of hostpartitioning by both unionid species, with *E. menziesii* glochidia most prevalent on benthic *Gobiomorphus* species and *E. aucklandica* for the first time confirmed to attach and encyst on only the gills of *Retropinna retropinna*. Although few *R. retropinna* were captured in these streams, glochidia infestations levels were similar at the two sites (6.3 and 6.5 glochidia fish^{-1}) and higher than infestation levelsfor laboratory trials. *Echyridella aucklandica* glochidia had peak metamorphosis at 34 dayspost-infestation, and grew on *R. retropinna* gills from $\bar{x}$ = 99.5 ± 4.7 μm SD to $\bar{x}$ = 449.2 ± 28.2 μm SD. In contrast, *E. menziesii* peak metamorphosis ranged from 16 to 18 days during which they did not grow on host fish. These results improve the understanding of host– glochidia interactions in sympatric unionid species, and advance knowledge for integrated conservation management strategies that protect both unionid communities and host fish populations.

4.2 Introduction

Biotic interactions are central to shaping community dynamics and are often tightly linked to resource use and partitioning (Gause, 1934; Hardin, 1960; Schöner, 1974). As adults, freshwater mussels in the order Unionida can be found in diverse communities where species coexist in dense patches over long periods of time (Strayer et al., 2004; Vaughn et al., 2008). During their larval (glochidia) stage, unionids form complex relationships with fish, on which they are obligate symbionts, to complete their life cycle (Kat, 1984; Geist & Auerswald, 2007). Host relationships are believed to have evolved primarily as a means of dispersal for otherwise relatively immobile mussel larvae (Kat, 1984; Wächtler et al., 2001).

Fish-glochidia interactions are predominately thought to be phoretic, a form of commensalism where an organism (phoront) with limited mobility uses a host for dispersal rather than nutrition or development (Houck & O' Connor, 1991; Watters, 2001; St. John White et al., 2017). The reason for this strategy is that most glochidia do not grow on their host and require relatively short-lived attachment durations that cause little impact on host behaviour or fitness (Lefevre & Curtis, 1912; Watters, 2001). For some unionid species, however, phoresis may have evolved into parasitism, notably where particularly small glochidia (i.e., <150 µm, termed 'miniature glochidia'; see Barnhart et al., 2008) derive nutrients and grow considerably for extended periods on fish to reach sizes suitable for metamorphosis (Fritts et al., 2013; Denic et al., 2015; Chowdhury et al., 2017). An example of this is provided in *Margaritifera margaritifera* glochidia which grow from 70 µm to 500 µm diameter over up to 12 months on *Salmo trutta* (see Bauer & Vogel, 1987).

Prior to host attachment, glochidia are generally released into the water from spring to summer, following development within the gills of a parent mussel (Haag, 2012; see Chapter 3). Drifting glochidia that find themselves attached to compatible hosts successfully encyst on the epithelial cells of the fins, body and/or gills where they undergo metamorphosis into viable juveniles that drop off to assume a sedentary life within benthic sediments (Bauer, 1988; Nezlin et al., 1994). However, incapable of active host selection, glochidia survival rates are low, with up to 99.9% of released glochidia estimated not to survive to metamorphosis (Young & Williams, 1984; Strayer, 2008; Haag, 2012). Factors controlling successful metamorphosis are complex and include attachment to and rejection by unsuitable hosts, the timing of seasonal host migrations, host abundance, as well as potential competition for host resources as a

result of temporal overlap in glochidia release among species (Kat, 1984; Zimmermann & Neves, 2001; Hastie& Young, 2003; Rogers-Lowery & Dimock, 2006; Chapter 3).

Consequently, unionids have evolved complex adaptations to offset high mortality and to increase the likelihood of obtaining important host resources. While glochidia of some species have evolved traits enabling them to attach to a wide range of fish taxa (host fish generalists), others are dependent on only a limited number of species or lineages (host fish specialists) (Trdan & Hoeh, 1982; Yeager & Saylor, 1995; Barnhart et al., 2008; Haag, 2012). Host fish generalists typically produce larger glochidia with hooks that assist with attachment on the fins and body, as well as gills, for a short encystment period (e.g., *Unio crassus*, 250 µm diameter, which encysts for a few weeks on at least 12 host fish species Taeubert et al., 2012). Unionids that are host specific tend to (but not always, see Haag, 2012) produce smaller glochidia that usually lack hooks and are often found to encyst on fish gills where they grow substantially, therefore requiring longer encystment times as noted earlier for *M. margaritifera* (Young & Williams, 1984; Bauer, 1994; Nezlin et al., 1994; Ziuganov et al., 1994; Taeubert et al., 2012; Stoeckl et al., 2015). Further, to improve the chance of fish encounters, some mussels lure specific hosts to them via conglutinates comprising mucus packages of glochidia that mimic fish prey (e.g., macroinvertebrates). These may include, for example, pelagic conglutinates that are more likely to encounter drift-feeding fish or demersal conglutinates that settle to the benthos targeting benthic- feeding fish (Hartfield & Hartfield, 1996; Watters, 2002, 2008; Haag, 2012; Melchior et al., 2021). Host generalist glochidia, on the other hand, are commonly broadcast, sometimes in mucus webs, into the water to passively attach to any nearby fish (Barnhart et al., 2008; Haag, 2012).

New data on overlap in glochidia brooding phenology (spring to summer, see Chapter 3) in two sympatric New Zealand *Echyridella* species (*E. menziesii* and *E. aucklandica*) suggests there is potential for interspecific competition for host resources. Until now, host fish relationships in *E. aucklandica* were unknown, but recent research on contrasting larval release and morphometry between *E. aucklandica* and *E. menziesii* has highlighted the potential for partitioning in host resources due to contrasting larval development modes (i.e., glochidia encystment sites, encystment duration and glochidia growth) (Melchior et al., 2021).

In that study, *E. aucklandica* was found to produce pelagic conglutinates in combination with miniature glochidia around three times smaller than *E. menziesii* which broadcast release glochidia, often on mucus strings. Conglutinate features (shape, buoyancy; see Melchior et al., 2019) in *E. aucklandica* suggest that this unionid species potentially targets pelagic hosts, in comparison to *E. menziesii* which is a known host fish generalist with the ability to infest a range of native diadromous and non-diadromous species, as well as some non-native fish (Percival, 1931; Hine, 1978; Clearwater et al., 2014; Brown et al., 2017; Hanrahan, 2019; Moore & Clearwater, 2019). Furthermore, the production of miniature glochidia suggests specialised attachment sites for *E. aucklandica* with the potential for glochidia growth and longer encystment times, in contrast to *E. menziesii* which is known to attach to multiple sites on individual fish (gills, body and fins) and requires a short encystment duration of up to three weeks, depending on water temperature (Clearwater et al., 2014, Hanrahan, 2019; Moore & Clearwater, 2019).

Unionids are among the most threatened faunal groups globally and species present in New Zealand are no exception (Collier et al., 2016; Lopes-Lima et al., 2017; Lopes-Lima et al., 2018). Partitioning of host fish species and developmental modes may be a key survival strategy facilitating coexistence in *E. menziesii* and the congeneric *E. aucklandica* (Rashleigh & DeAngelis, 2007; Marshall et al., 2014), which have been classified as 'At Risk, Declining' and 'Threatened, Nationally Vulnerable', respectively (Grainger et al., 2018). Because unionids are entirely dependent on hosts to complete their life cycle, a lack of suitable hosts (particularly in specialists with a small host range) may lead to a lack of juvenile recruitment, reducing population densities, and increasing vulnerability to co- declines should the host-glochidia relationship be disrupted (Young & Williams, 1984; Haag & Warren, 1998; Arvidsson et al., 2012; Modesta et al., 2018). Unionid conservation in New Zealand cannot disregard the role of fish species, particularly with around three- quarters of native New Zealand freshwater fish species recognised as threatened with risk of extinction (39 of 53, see Dunn et al., 2018; Ministry for the Environment & Statistics NZ, 2019; Joy et al., 2019). Therefore, improved understanding of host–glochidia interactions is critical, particularly in advancing integrated conservation management strategies that protect both unionid and host fish populations.

The focus of Chapter 4 is to determine whether partitioning of host fish resources occurs between *E. aucklandica* and *E. menziesii* using field and laboratory investigations, and to document the previously unknown host-glochidia associations of *E. aucklandica*. Specific field objectives were to: 1) quantify host attachment by glochidia of both unionidspecies on fish present in two of the four Waikato streams, and 2) assess relationships between temporal changes in fish species abundance and unionid reproduction periods. Laboratory objectives were to: 1) validate field results by conducting trials of host fish attachment by glochidia and analysis of encystment duration, 2) determine the location of glochidia attachment and evidence of glochidia growth on host fish, 3) identify host fish infestation methods in *E. aucklandica* using individual glochidia exposure versus conglutinate feeding trials (mimicking natural interactions between *E. aucklandica* and potential host fish), and 4) measure post-parasitic stage juvenile growth rates. *Echyridella menziesii*, a known host fish generalist, was used as a contrasting sympatric species to compare with *E. aucklandica* for relevant laboratory and field objectives.

Based on findings from Melchior et al. (2021) and Chapter 3, that *E. aucklandica* glochidia are markedly smaller than those of *E. menziesii* and are released as conglutinates, coupled with knowledge of reproductive strategies by similar northern hemisphere unionids, it was hypothesised that: 1) *E. aucklandica* are host specialists relying on pelagic, drift-feeding fish species for glochidia attachment, 2) to increase the chance of host encounters, glochidia release periods would coincide with seasonal peaks in host fish abundance, 3) *E. aucklandica* glochidia will preferentially attach to and grow on host fish gills for an extended period to reach maturity, as observed in several northern hemisphere unionids with miniature larvae, and 4) given the contrasting glochidia release and development strategies, there will be species partitioning in use of host fish resources between the two sympatric mussel species.

4.3 Methods

4.3.1 Field sampling

4.3.1.1 Initial host fish survey

Host-symbiont interactions in both *E. aucklandica* and *E. menziesii* were initially investigated at four sites (Ohautira, Pakoka, Kahururu and Mangapiko; see Chapter 3 for site descriptions) in mid-summer (January 2019) on one sampling occasion coinciding with the glochidia release periods of both species determined in Chapter 3. The central focus was on detecting *E. aucklandica* glochidia, for which host fish had not been determined prior to this study, and thus four sites were selected as they were known to have large populations of coexisting and reproducing *E. menziesii* and *E. aucklandica*, andsimilar physical and chemical characteristics (see Table 3.1).

Single-pass electrofishing was performed using the EFM300 (NIWA Instrument Systems, Christchurch, New Zealand) along a 50-meter reach at each site to determine relative abundance of fish (standardised to number per 100 m^2). Three fine-mesh (2 mm) minnow traps (unbaited) were also deployed at each site upstream or downstream of the reach over the electrofishing period to augment electrofishing specimens for assessmentof glochidia infestation. Upon capture, fish were held in 20 L buckets filled with source water and with battery powered aerators until identified to species level and measured for total length (TL) to the nearest millimeter.

Each fish was visually assessed for external glochidia attachment (*E. menziesii* glochidia length: ~300 µm, *E. aucklandica* length: ~100 µm; Melchior et al., 2021) using a 40x hand-held magnifying glass (Magnifiers New Zealand Ltd., 12 mm lens). During this process, fish <100 mm were viewed in a 150 mm acrylic plastic fish viewer (Dynamic Aqua Supply, Canada Ltd.) while larger fish were assessed on a 50 cm viewing tray containing source water. The number of glochidia attached (not enclosed by fish epidermal tissue) or encysted (enclosed by fish epidermal tissue) on external surfaces of each fish was recorded. Approximately five fish of each native species and all non-native fish captured were euthanised (AQUI-S, 0.8 mg/L) for further internal assessment. The remaining fish were returned to streams after external glochidia analysis. In the laboratory, euthanised fish were re-examined for attachment and encystment on external surfaces to confirm field observations before mouths and gills were examined internally for presence or absence of glochidia encystment. Gills were removed via micro-dissecting scissors for closer examination under the Olympus SZ-6045 dissecting microscope.

4.3.1.2 Temporal survey

Once glochidia associations had been identified in the *initial* field examination, two sites that had the high glochidia prevalence and supported fish infested by both *E. aucklandica* and *E. menziesii* were selected for a temporal study (i.e., Ohautira and Pakoka; see Results). Lower densities of *E. menziesii* (0.29 m^{-2}) than *E. aucklandica* (0.77 m^{-2}) occur at Ohautira, while at Pakoka densities of *E. menziesii* (1.58 m^{-2}) are much greater than for *E. aucklandica* (0.08 m^{-2}) (Waikato Regional Council, 2017). Monthly sampling was conducted at these sites in the months when the highest glochidia infestation prevalence was expected (October 2019 – February 2020; see Chapter 3). Single pass electro-fishing was conducted along sampling reaches to determine relative abundance of fish over time, supplemented with spot electrofishing and three additional unbaited fine mesh minnow traps (2 mm) to target key species for glochidia assessment. Glochidia were counted as described in the previous section.

Concurrently with fish sampling, brooding activity for *E. aucklandica* and *E. menziesii* was determined to assess the relationships between the glochidia release period and infestation rates of different fish species. Up to 40 mussels of each species were evaluated for reproductive status on each occasion, as outlined in Chapter 3. During brood pouch evaluations, mussels were held at 18 °C in 1 L water buckets and/or in mesh nets in the stream, until they could be returned to their original location.

4.3.2 Laboratory study

4.3.2.1 Collection and acclimation

Gravid *E. menziesii* and *E. aucklandica* females were collected from Ohautira Stream (water temperature 18°C) in November 2019. Gravidity stage in females was recorded in the field by observing inflation of inner gills and changes in gill colour (see Melchior, 2017; Melchior et al., 2021). Individuals of *E. aucklandica* (n = 10) and *E. menziesii* (n = 9) that were brooding mature glochidia were placed separately into 5 L buckets containing aerated stream water and source sediment to allow for burrowing and reduce thelikelihood of stress induced premature glochidia release.

In the laboratory, buckets with gravid mussels were placed in a controlled temperature room set to a light:dark photoperiod of 16:8 h and an initial air temperature of 18°C which was gradually decreased to 15°C to assist in extending the brooding period by approximately 14 days until the beginning of the experiment. Water was changed every other day, incrementally replacing the stream water to 100%

dechlorinated tap water. Dissolved oxygen and water temperature were measured every other day (YSI Pro 2030 meter) and mussels were fed daily with an algal diet consisting of Nanno 3600™ (*Nannochloropsis*) instant algae and Shellfish diet 1800™ at a 1:2 ratio (Ganser et al., 2013). *Retropinna retropinna*, *Galaxias maculatus*, and *Gobiomorphus cotidianus* were selected for analysis of host compatibility based on observations of glochidia infestation in field surveys (see Field Results 4.4.1). *Gobiomorphus huttoni* was originally chosen for laboratory analyses as it was among the most abundant species caught during the field survey with evidence of glochidia attachment. However, I was unable to source/capture sufficient numbers of naiive fish from waterways without unionids to avoid potential issues with host resistance to glochidia infestation, so G. *cotidianus* was used for the main experiment instead as it has been previously confirmed as a common *E. menziesii* host (e.g., Hanrahan 2019). In late November – early December 2019, all trialfish species were collected in sections of the lower Waikato River where mussels are not known to occur, to avoid testing fish with acquired immunity to glochidia (Dodd et al., 2006; Zale & Neves, 1982). *Retropinna retropinna* (n = 40) and G. *cotidianus* (*n* = 36) were collected from the same location (- 37.806775, 175.305300) using a seine net. G. *maculatus* (*n* = 37) were captured using 6 fine mesh (2 mm) Gee minnow traps and a fine-meshed (4 mm) fyke net deployed overnight along the littoral zone at a different location within the river (-37.281487, 175.0459901). A smaller number of *G. huttoni* (n = 10) were also collected from the upper Ohautira Stream (-37.758258, 174.987181) where densities of both mussel species are low, for observation of host use by *E. aucklandica*. Individuals of all fish species collected were checked to confirm no glochidia were attached externally prior to transportation to the laboratory.

After each collection, fish (all >30 mm TL) were returned to the laboratory in a 50 L chilli bin lined with plastic and filled with source river water (18–19°C) and 10 mL of API stress coat for stress reduction. During a 1-2 week-long laboratory acclimation period, fish species were housed separately, with the exception of G. *cotidianus* and G. *huttoni*, within shade cloth-covered 100 L tanks in a constant temperature room (16:8h [dimmed] light:dark photoperiod, air temperature 18.5°C). Fish were gradually transitioned from stream water to dechlorinated tap water with daily water changes adjusted to a 3 ppt saline solution of filtered natural seawater added to reduce risk of disease. Each tank contained two aerators, a biofiltration system (Fluval 206, Performance Canister Filter), and two rectangular PVC pipes (11 cm x 7 cm x 5.5 cm) as

shelter. Tank water was changedevery other day, and water temperature (°C), dissolved oxygen (DO; % and mg/L) (YSI Pro 2030 meter) and ammonia (mg/L) were measured daily (see Table 4.1 for data). Fish were considered acclimated once they had been housed for at least one week, were consumingrations of at least 5% of their body weight per day of frozen chironomid larvae (Advanced Hatchery Technology, Inc), and showed no evidence of external disease (Piper, 1982; OECD, 2019).

Table 4.1 Water quality variables (mean ± SD except for NH_3) measured daily within three tanks throughout the respective acclimation periods for four fish species. Temp = water temperature; DO = dissolved oxygen.

Fish species (acclimation period)	Temp. (°C)	DO (%)	DO (mg/L)	NH_3 (mg/L)	Salinity (ppt)
Retropinna retropinna (13 days)	18.7±0.6	97.5±1.4	8.9±0.2	0.25	3.2±0.5
Galaxias maculatus (8 days)	18.6±0.4	98.4±0.6	9.0±0.2	0	2.9±0.5
Gobiomorphus cotidianus/huttoni (8 days)	18.5±0.4	97.8±0.4	9.1±0.2	0	2.8±0.6

4.3.2.2 Glochidia viability test

Glochidia viability tests were performed prior to each infestation experiment. Throughoutlaboratory acclimation, no female mussels of either species showed signs of abortion, and thus glochidia viability for all collected females from each species was able to be quantified. Glochidia attached to conglutinates (*E. aucklandica*) and individual glochidia (*E. menziesii*) were obtained via natural release stimulated by placing females in 500 mL beakers containing dechlorinated tap water and increasing temperature gradually from 15 to 22°C. Prior to viability tests, *E. aucklandica* glochidia were detached from conglutinates by spraying conglutinates with dechlorinated water using a pressure sprayer through 85 µm mesh filter screens (Dodd et al., 2006).

Subsamples of released glochidia (n = 100) for both mussel species were first checked visually in a petri dish for maturity (i.e., indicated by hooks on opposing valves, translucent colour, visible adductor muscle, emergence from vitelline membrane; following Melchior et al., 2021 and applying prior knowledge of *E. menziesii* viability in culture), and were then exposed to 1 mL of 98 ppt concentrated sodium chloride (NaCl) solution. The number of open and closed glochidia before and after 1-minute exposure to NaCl was quantified, with % viability determined from the number of mature glochidia that closed their valves after exposure. Batches of glochidia with >90%

viability (mean ± SD viability for *E. menziesii* = 92 ± 3.4% and *E. aucklandica* = 95 ± 2.9%) were pooled (n = 3 per species) and diluted to produce a solution containing ~2000 glochidia L^{-1} for each fish infestation trial involving three tanks for each fish species/mussel species combination (total tanks = 24). Mean (± SD; n = 3) lengths of mussels used to harvest glochidia were 52.6 ± 3.6 mm for *E. menziesii* and 74.6 ± 4.1 mm for *E. aucklandica*.

4.3.2.3 Fish infestation procedure

Three infestation trials were performed in December 2019 in an 18.5°C controlled temperature room to confirm and assess glochidia–host relationships, as follows:

- Exposure 1: Confirmation of metamorphosis success and time to metamorphosis on *R. retropinna*, *G. maculatus* and *G. cotidianus* for *E. aucklandica* in comparison to *E. menziesii*.
- Exposure 2: *E. aucklandica* only glochidia 'broadcast exposures' to monitor glochidia development on *R. retropinna*, *G. maculatus*, *G. cotidianus* and a small number of *G. huttoni*.
- Exposure 3: *E. aucklandica* only conglutinate 'feeding exposures' to attempt to replicate natural interactions between conglutinate-producing species and their potential host fishes (*R. retropinna*, *G. maculatus*, *G. cotidianus* and *G. huttoni*).

4.3.2.4 Exposure 1

Fifteen-minute exposures were conducted using six infestation baths, each containing an aerator submerged in 5 L dechlorinated tap water with ~2000 glochidia L^{-1} (i.e., 10,000 glochidia). Each bath contained 10 individuals of *R. retropinna*, *G. maculatus* or *G. cotidianus* that were exposed to either *E. menziesii* or *E. aucklandica* glochidia. Throughout each infestation, water was carefully mixed to ensure a homogenous suspension of glochidia. Following each glochidia exposure, two fish from each bath were anesthetised and examined for attachment success using a Leica stereo microscope at 25x magnification.

The remaining fish were transferred into randomly assigned 3 L (*G. maculatus*, *G. cotidianus*) or 10 L (*R. retropinna*, a larger shoaling species) self-cleaning tanks (Pentair Aquatic Eco-Systems) (= 2 n fish/tank and 4 tanks per fish/mussel species combination). Filter cups (85 µm mesh: *E. aucklandica*; 100 µm mesh: *E. menziesii*) received the outflow of each tank to collect unattached glochidia and excysted juveniles.

Each tank was supplied with 18°C dechlorinated tap water (adjusted to 3 ppt salinity; Long et al., 1977) internally recirculated using a pump (Aqua One, 4000 L/hr). A single rectangular PVC pipe (dimensions as above) was provided for each fish as cover. Individual tanks were flushed every second day for 1 hour to wash detached glochidia or juveniles into outflow filter cups over 36 days.

Detached juveniles or glochidia retained in filter cups were counted using a Bogorov tray under the Leica digital stereo microscope at 25x magnification. Observed glochidia were considered viable based on whether valve movement occurred, whereas juveniles were considered viable based on pedal movement. Inactive juveniles were held for several days and rechecked to confirm viability. Juvenile excystment was considered complete when no juvenile mussels were collected in their respective filter cup for ≥4 days, upon which fish were euthanised (>0.8 mg/L AQUI-S) and checked externally and internally for any remaining encysted glochidia.

4.3.2.5 Exposures 2 and 3

Two infestation trials were conducted using *E. aucklandica* glochidia only. For the 'broadcast exposures' (Exposure 2), *E. aucklandica* glochidia were detached from conglutinates, as described earlier, and exposed to four fish species (*R. retropinna* n = 15, *G. maculatus n* = 12, *G. cotidianus n* = 15, *G. huttoni n* = 5) following the Exposure 1 infestation bath protocol. In contrast, 'feeding exposures' (Exposure 3) with intact conglutinates (6-7 mm long) collected from three releasing *E. aucklandica* females were conducted by injecting one conglutinate per fish using a 3 mL pipette into infestation baths with vigorous aeration (*R. retropinna n* = 9, *G. maculatus n* = 6, *G. cotidianus* n = 5, and *G. huttoni n* = 5). Fish behaviour was recorded using a Canon 550D camera for 15 minutes.

Following Exposure 2 and Exposure 3, fish were transferred into 100 L 'broadcast' or 'conglutinate feeding' tanks sectioned off into three sub-tanks (with mesh walls) separating individuals by species (*n* = 15 fish per sub-tank, *n* = 3 tanks per 'broadcast' or 'conglutinate feeding' tank). Each tank contained a biofiltration system (Fluval 206, Performance Canister Filter), and each quadrat had aerators and two rectangular PVC pipes (11 cm x 7 cm x 5.5 cm) as shelter. On day 2 post-infestation and weekly thereafter, fish (*n* = 4 per species except *G. huttoni* where *n* = 1) were euthanised (>0.8 mg/L AQUI- S) for examination of attachment and transformation success, and glochidia development on internal and external body structures.

4.3.2.6 Post-excystment juvenile growth rates

Newly metamorphosed juvenile *E. aucklandica* and *E. menziesii* individuals from Exposure 1 were placed into separate glass jars (containing autoclaved and air dried 250 μm sand) within an automated 'grow out' recirculating water and feeding system set at 19°C. Natural lake algae at a density of approximately 40,000 algal cells/mL (dominant species included *Cryptomonas* sp; *Monoraphidium* sp; *Nephrocytium* sp; *Gonium pectoral* and *Nitzschia* sp.) was fed at 4-hour intervals (Patterson et al., 2018). Juvenile length was measured weekly for three weeks under a Leica digital stereo microscope at 25x magnification to compare post-parasitic growth rates between species.

4.3.3 Statistical Analyses

All data were assessed for normality (Shapiro Wilk test and Q-Q plots) and homoscedasticity (Levene's test). Where parametric assumptions could not be confirmed, even after transformations, non-parametric tests were used. Significance for all tests was defined at $p = 0.05$. Analyses were conducted in R (R Core Development Team, 2020) unless otherwise stated.

4.3.3.1 Field study

To determine host-glochidia interactions in the initial field examinations, three response variables were calculated for each site using Quantitative Parasitology software (Reiczigel et al. 2019): 1) prevalence of infestation (proportion of fish infested) (mean ± 95% Clopper – Pearson confidence limits), 2) abundance of glochidia (mean number of glochidia per total fish sample ± 95% confidence limits from 2000 bootstrap replications), and 3) intensity of infestation (number glochidia per infested fish) (mean ± 95% confidence limits from 2000 bootstrap replications).

Following on from this, host-glochidia associations were further investigated at two sites using monthly data as replicates. Each monthly sample was assumed to be independent due to average glochidia infestation lasting approximately two weeks, at least for *E. menziesii* (Hanrahan, 2019). The same three response variables were used as in the initial field examination. Attached glochidia density (mean ± 95% confidence limits) was also calculated by multiplying glochidia abundance by the species-specific fish density per m^2 of stream fished, following Schneider et al. (2019). This response variable represents the number of glochidia potentially transforming to juvenile mussels per unit area of river.

Independent two sample *t*-tests were used to determine differences for

E. menziesii glochidia abundance, infestation intensity and glochidia density between frequently infested fish species (only two species had sufficient data for comparisons) and within species between the two sites. For these pairwise comparisons, data were square root-transformed when necessary. As *E. aucklandica* glochidia were rarely encountered in field sampling (see Results), glochidia abundance, intensity and density were compared between sites using independent *t*–tests on data pooled by date.

4.3.3.2 Laboratory study

In the trials to determine potential host fish for *E. aucklandica* and *E. menziesii* (Exposure 1), the mean number of rejected glochidia (i.e., those that either did not attach or initially attached and then dropped off) were calculated and compared between fish species using one-way ANOVA. Peak rejection time (days post-exposure at which the highest number of glochidia were rejected) and rejection period duration (i.e., day range) were also determined. For fish species that produced viable juveniles (only two species), the non-parametric Mann-Whitney *U* test was used to compare median excystment time, peak excystment time (days post-exposure at which the greatest number of juveniles excysted) and excystment period duration. Metamorphosis success (%), determined for each fish as number of metamorphosed juveniles divided by rejected glochidia plus metamorphosed juveniles x 100, was compared between fish species using unconditional exact test, replacing Fisher's exact test, with the advantage that the former test is more sensitive at detecting differences of binomial proportions data of two small samples ($n < 30$) (Reizgiel et al., 2009). Independent *t*-tests were used to compare three-week post-excystment juvenile lengths between *E. aucklandica* and *E. menziesii*.

4.4 Field Results

4.4.1 Initial fish-host survey

Members of the genus *Anguilla* (A. *dieffenbachii*, A. *australis*) and *Gobiomorphus* (*G. huttoni*, *G. cotidianus*, *G. basalis*) were the most abundant fish species caught by electrofishing across the four sites, representing 74% of the 159 individuals collected (excluding G-minnow traps). The least abundant species caught were *R. retropinna* with one specimen captured at each of Pakoka and Ohautira, and one adult *S. trutta* captured at Mangapiko. Species present in all three of the coastal sites at the time of sampling were *A. dieffenbachii*, *G. huttoni*, and the galaxiid *G. maculatus*. The only site with non-indigenous species was Mangapiko with *S. trutta* and *Gambusia affinis* (Appendix 4.1).

The only fish species on which *E. aucklandica* glochidia occurred were the two *R. retropinna* specimens caught. On these specimens, glochidia were only found encysted on the gills (Plate 4.1). Infestation abundance and intensity were equally low in infested specimens caught with one glochidia found on each fish (Table 4.2). Both glochidia showed signs of growth with one having grown three times its original size of approximately 100 μm (indicated by the glochidia scar) to 393 μm in length (Plate 4.1) while the other had grown to 291 μm.

In contrast, *E. menziesii* glochidia were found attached to and/or encysted on the gills or epidermis of 7 of the 10 fish species examined, including on the non-indigenous *G. affinis* (attached, not encysted) and *S. trutta* (attached, not encysted). *Echyridella menziesii* glochidia were not found attached to *R. retropinna*, *A. australis*, or *Geotria australis* (only larval ammoecetes were caught). Mean prevalence for *E. menziesii* glochidia ranged from 0 to 67% with the greatest prevalence found on *Gobiomorphus* species. (>43% of fish examined at all sites; Table 4.2). Although *E. menziesii* glochidia were found on *A. dieffenbachii* and *G. maculatus*, mean prevalences for these species was low (Table 4.2). Across sites, mean *E. menziesii* glochidia abundance and infestation intensity were greatest in *Gobiomorphus* species, with highest infestations in *G. basalis* (captured at Mangapiko) followed by *G. huttoni* (captured at all sites except Mangapiko) (Table 4.2). Out of the ten species captured, *A. australis* (n = 14) and *G. australis* (n = 12) were the only ones without infestations by either unionid species.

Table 4.2. Results of the initial investigation of two mussel species at four sites (Ohautira, Pakoka, Kahururu and Mangapiko), showing the number of sites with infested fish, the number of fish examined and infested, and mean infestation prevalence, glochidia abundance and infestation intensity for attached and/or encysted glochidia on each fish species. 95% confidence intervals are shown in parentheses where appropriate. Other species (not infested) that were caught are shown in Table A.4.1. See Section 4.3.3.1 for definition of each parameter.

Species	*N* sites	*N* fish examined	*N* fish infested	$\bar{x}$ prevalence (% fish infested)	$\bar{x}$ abundance (total no. glochidia infested for total infested fish^{-1})	$\bar{x}$ intensity (no. glochidia infested per fish^{-1})
E. menziesii						
Anguilla dieffenbachii	3	36	6	16.7 (0.6-33)	0.3 (0,0.6)	1.5 (1,2)
Gambusia affinis[1]	1	15	5	33.3 (12-62)	0.3 (0,0.5)	1
Gobiomorphus basalis	1	6	4	66.7 (22-96)	2.2 (0.3,4.2)	3.3 (1,4.8)
	2	16	10	62.5 (23-66)	1.2 (0.7,1.8)	1.9 (1.3,2.5)
G. cotidianus						
G. huttoni	3	38	24	63.2 (47-78)	1.5 (0.9,2.3)	2.5 (1.8,3.6)
Galaxias maculatus	3	26	2	7.7 (0.9-25)	0.1 (0,0.4)	1.5 (1,1.5)
Salmo trutta[1]	1	1	1	100	9	9
E. aucklandica						
Retropinna retropinna	2	2	2	10 0(16-100)	2	2

[1] Non-indigenous fish species

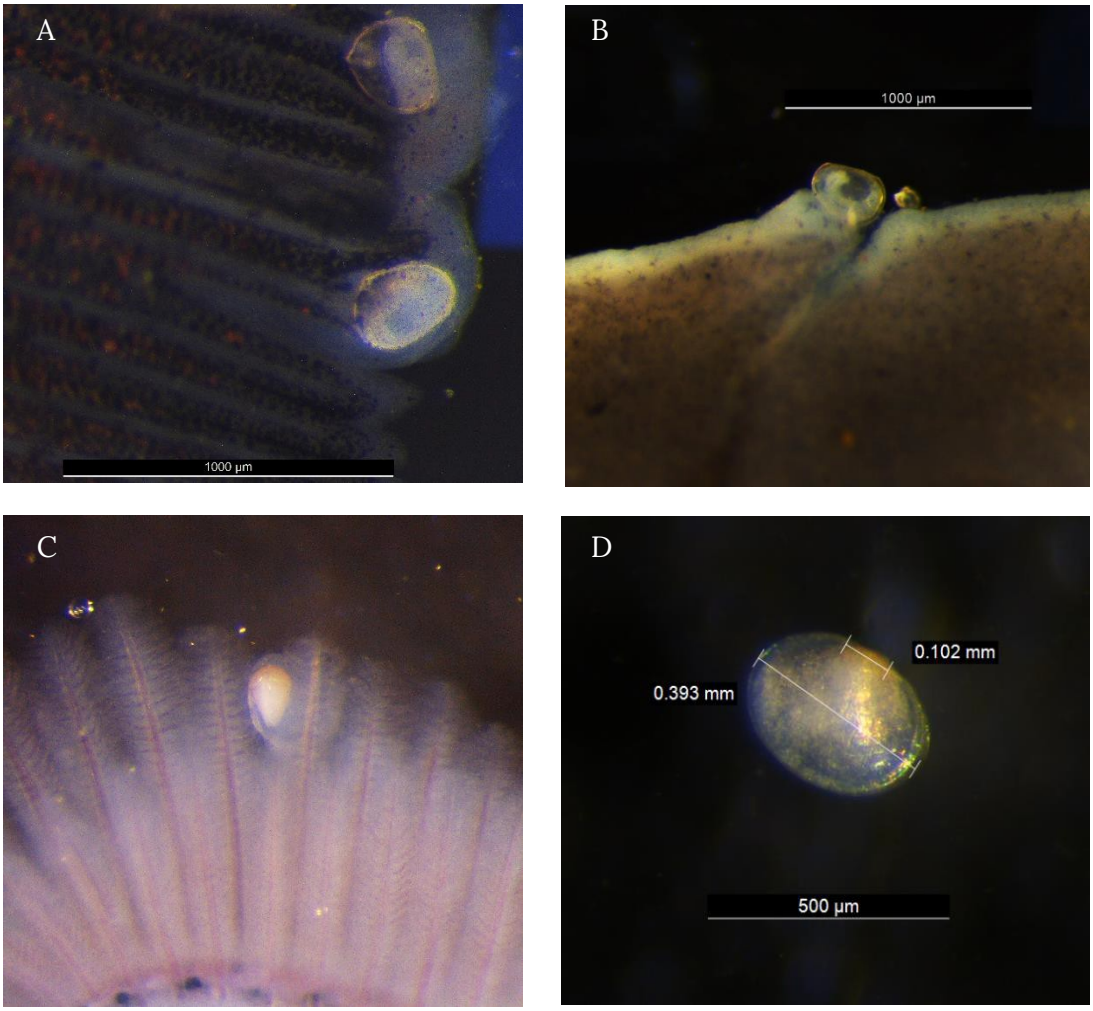


Plate 4.1. Encysted *Echyridella menziesii* glochidia on the caudal fin of *G. huttoni* (A); attached *E. menziesii* glochidia on the dorsal fin of *A. dieffenbachii* (B); encysted *E. aucklandica* glochidia on the gill of *R. retropinna* (C); and a glochidia removed from *R. retropinna* gill (D; dimensions shown are total length and glochidia scar length).

4.4.2 Temporal survey

4.4.2.1 Unionid brooding and fish density

Unionid brooding activity was evident throughout October to February for both mussel species. On average, 59% of *E. aucklandica* and 56% of *E. menziesii* females inspected (*n* = 101 and 111, respectively) were found to be brooding larvae (all sites and months combined). Maximum percentages of female mussels carrying broods occurred in November across both sites for both species, with 83% of *E. aucklandica* and 82% of *E. menziesii* brooding at Ohautira, and 100% of *E. aucklandica* and 94% of *E. menziesii* at Pakoka (Appendix Figure A.4.1). Some temporal synchrony was evident at Ohautira in November between peak brooding in both unionid species and peak abundance of *G. maculatus*. At Pakoka, peak brooding activity in both mussel species also occurred in November, with peak fish abundances occurring one month later in December except for *R. retropinna* which had consistently low relative abundances throughout the sample period (Appendix Figure A.4.1).

When averaged across October to February, the most abundant fish species caught by electrofishing at Ohautira were *G. maculatus* (9.1 per 100 m^2) followed by *A. dieffenbachii* and *G. huttoni* (5.7 and 5.2 per 100 m^2, respectively), while the species with the greatest abundances at Pakoka were *A. dieffenbachii* (6.9 per 100 m^2) followed by *G. huttoni* and *G. maculatus* (4.1 and 1.9 per 100 m^2, respectively; Appendix Table A.4.1). These species represented 83% of the 491 fish caught at both sites. *Anguilla australis*, *G. australis*, *R. retropinna* and *Cheimarrichthys fosteri* accounted for the remaining 17% of fish caught. *Retropinna retropinna* was one of the least abundant species (0.4 per 100 m^2 at both Ohautira and Pakoka) and was captured only in November through to February at Pakoka compared to December to February at Ohautira (Appendix Table A.4.3; Appendix Figure A.4.1). Densities of *A. dieffenbachii*, *G. huttoni* and *G. maculatus* at Pakoka showed similar temporal patterns over time, increasing to maximum abundance in December followed by declines throughout January and February. At Ohautira, *G. maculatus* density peaked in November, *A. dieffenbachii* increased in December, while *Gobiomorphus huttoni* remained at low relative abundance throughout the sampling period (Appendix Figure A.4.1).

4.4.2.2 Infestation prevalence

Overall, 17% of all 491 fish examined were found to have glochidia of either *E. menziesii* or *E. aucklandica* attached or encysted. *Retropinna retropinna* was the only fish species infested with encysted *E. aucklandica* larvae during the temporal survey at both Pakoka (n = 4 of 6 fish fish which were infested) and Ohautira (n = 3 of 4 fish which were infested),with glochidia first detected on fish in November and December, respectively (Appendix Figure A.4.1). Notwithstanding the low abundances of *R. retropinna*, prevalence for *E. aucklandica* glochidia on this species was high with 70% of fish caught infested (Appendix Figure A.4.1). Unlike the initial study, infestations of *E. aucklandica* were additionally detected on one *G. huttoni* and one *G. maculatus* on one sampling occasion in January, however, glochidia were only found attached but not encysted on these species.

Echyridella menziesii glochidia were encysted and/or attached on three fish species across the two sites during the temporal survey, namely *G. huttoni* (47% prevalence), *A. dieffenbachii* (17%), and *G. maculatus* (2%, one individual infested at each site) (Appendix Table A.4.3). At Pakoka, *E. menziesii* prevalence on *G. huttoni* remained high for 4 of the 5 months sampled with a sharp decrease at the end of the brooding season (Appendix Figure A.4.1). A similar pattern was shown in the prevalence of *E. aucklandica* on *R. retropinna* at both Pakoka and Ohautira, with high prevalence from November or December followed by a sharp decrease in February. *Anguilla dieffenbachii* and *G. maculatus* infestation prevalence remained uniformly low throughout the season at both Pakoka and Ohautira. No *E. menziesii* glochidia were found on *A. australis*, *G. australis*, *R. retropinna* and *Cheimarrichthys fosteri* during the temporal survey.

4.4.2.3 Glochidia abundance and infestation intensity

Echyridella aucklandica glochidia abundance (glochidia fish^{-1}) was highly variable on the few *R. retropinna* on which glochidia were found; from 1 to 21 glochidia were found encysted on the gills of these fish. Average infestation rates did not differ between sites ($t_{(8)}$ = 0.03, p = 0.9), with Pakoka and Ohautira showing similar levels of infestation (Pakoka 6.3 and Ohautira 6.5 glochidia fish^{-1}) (Figure 4.1A). Infestation for *E. aucklandica* on *R. retropinna* occurred only internally on the gills rather than externally on the fins and body, which was more commonly observed for *E. menziesii* infestation on other species.

Due to low prevalence of *G. maculatus* with *E. menziesii* glochidia, the following results only focus on *A. dieffenbachii* and *G. huttoni*. Examination of subsamples in the laboratory found *E. menziesii* glochidia attached both internally and externally on *G. huttoni* but only externally on *A. dieffenbachii*. Overall, for *E. menziesii*, average glochidia abundance was significantly greater on *G. huttoni* (2.7 glochidia fish^{-1}) than *A. dieffenbachii* (0.3 glochidia fish^{-1}; $t_{(18)}$ = 2.5, p = 0.02; Figure 4.1). Abundance on *G. huttoni* was significantly greater at Pakoka (0.5 glochidia fish^{-1}) than Ohautira (0.06 glochidia fish^{-1}; $t_{(8)}$ = 11.6, p <0.001) with no significant differences in *A. dieffenbachii* between sites ($t_{(8)}$ = 6.1, p =0.05).

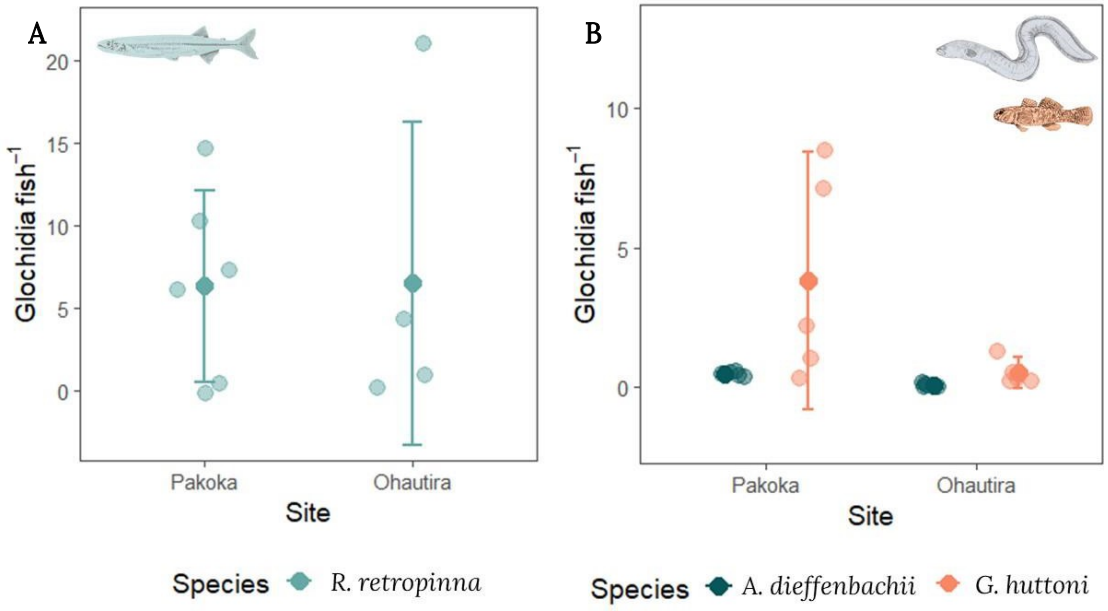


Figure 4.1 Glochidia abundance (number fish^{-1}) of *Echyridella aucklandica* on *Retropinna retropinna* (A) and of *E. menziesii* on *Anguilla dieffenbachii* and *Gobiomorphus huttoni* (B). Dark points are means while light points are individual data points and error bars are the 95% confidence intervals. Note different y-axis scales.

Mean infestation intensity of *E. aucklandica* on *R. retropinna* across both sites was 9.1 glochidia per infested fish (Figure 4.2A; Appendix Table A.4.3) but it was highly variable between fish, with no significant difference in intensity between sites ($t_{(3)}$ = 7.1, p = 0.10). Glochidia infestation intensity on *R. retropinna* at Pakoka was 9.5 and was highest in November and December. At Ohautira, mean intensity was 8.7 glochidia per infested fish and was greatest in December, although variability between dates was high (note: no *R. retropinna* were captured in October or November; Figure 4.2 A; Appendix Figure A.4.1).

E. menziesii infestation intensity did not differ significantly between species ($t_{(16)}$ = 1.6, p = 0.13) with no significant differences found between sites in either *G. huttoni* ($t_{(6)}$ = 1.5, p = 0.19) or *A. dieffenbachii* ($t_{(8)}$ = 1.9, p = 0.08; FigureB). Although, not statistically different, mean *G. huttoni* infestation intensity was considerably higher at 6.2 infested fish^{-1} than *A. dieffenbachii* (1.8 infested fish^{-1}) at Pakoka and greater than both *G. huttoni* (2.2 infested fish^{-1}) and *A. dieffenbachii* (1.3 infested fish^{-1}) intensities at Ohautira (Figure 4.2B). For *G. huttoni*, infestation intensity was higher early in the unionid brooding season (November, December), while for *A. dieffenbachii* intensity was uniformly low through time (Appendix Figure A.4.1).

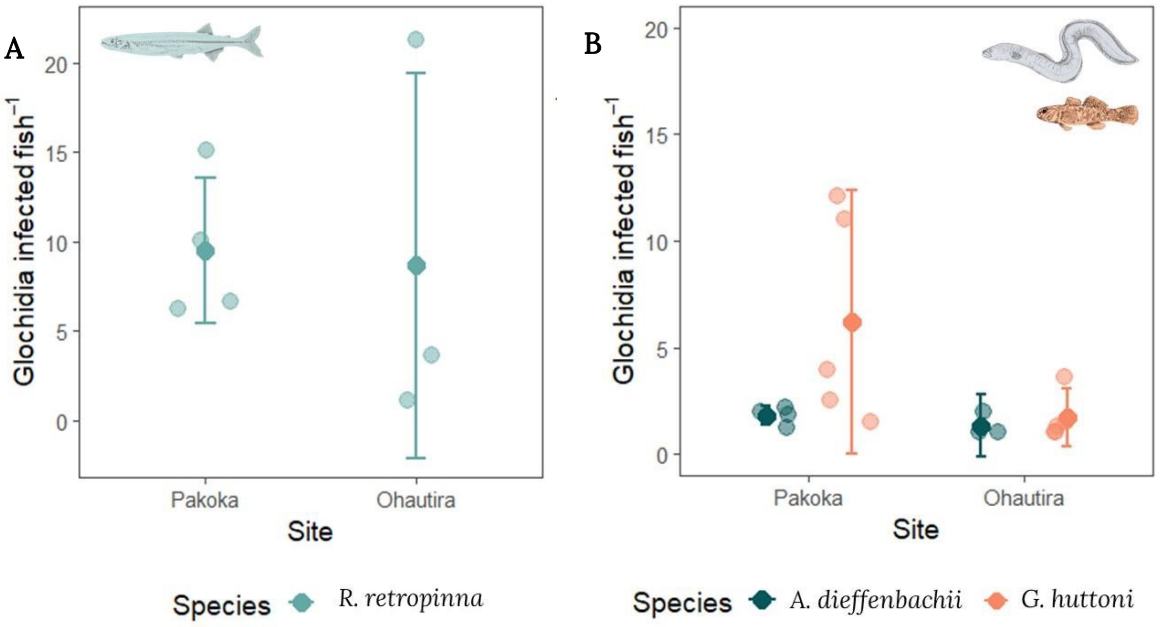


Figure 4.2. Glochidia infestation intensity (number infested fish^{-1}) of *Echyridella aucklandica* on *Retropinna retropinna* (A), and of *E. menziesii* on *Anguilla dieffenbachii* and *Gobiomorphus huttoni* (B). Dark points are the means while light points are individual data points and error bars are the 95% confidence intervals.

4.4.2.4 Glochidia density

Overall, glochidia densities, calculated as glochidia abundance multiplied by fish density per m^2 of streambed, were low for both *E. menziesii* and *E. aucklandica* in the two streams studied over time. Particularly low abundances of *E. aucklandica* glochidia on fish, in combination with low densities of *R. retropinna* captured, yielded low glochidia densities at both Ohautira (0.025 glochidia m^{-2}) and Pakoka (0.024 glochidia m^{-2}), with no significant differences between sites ($t_{(7)}$ = 0.19, p = 0.9) (Table 4.3A). Overall *E. menziesii* glochidia densities differed significantly between fish species, being significantly greater on *G. huttoni* (0.07 glochidia m^{-2}) than *A. dieffenbachii* (0.02 glochidia m^{-2}; $t_{(18)}$ = 2.1, p = 0.047). Further significant differences occurred between study sites in both *A. dieffenbachii* ($t_{(8)}$ = 4.1, p = 0.003) and *G. huttoni* ($t_{(7)}$ = 2.3, p = 0.049), with significantly greater densities on both species at Pakoka than Ohautira (Figure 4.3B).

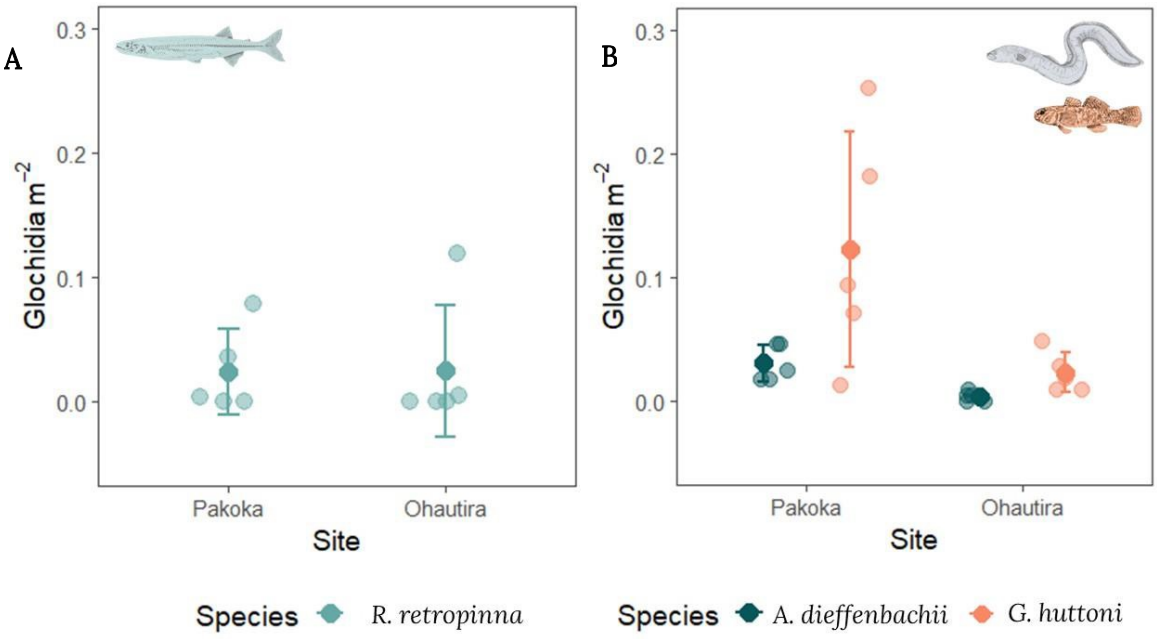


Figure 4.3. Glochidia density(m^{-2}) of *Echyridella aucklandica* on *Retropinna retropinna* (A) and *E. menziesii* on *Anguilla dieffenbachii* and *Gobiomorphus huttoni* (B). Dark points are the means while light points are individual data points and error bars are the 95% confidence intervals.

4.4.3 Metamorphosis success and timing (Exposure 1)

The number of glochidia (detached from conglutinates) rejected after infestation trials using the 'broadcast' method was generally low in *E. aucklandica* due to the low number of glochidia initially attached to each of the three fish species (relative to *E. menziesii* infestation trials), with no significant differences between fish species (one-way ANOVA: $F_{(2,9)} = 1.2$, $p = 0.3$). In contrast, *E. menziesii* glochidia rejection differed significantly among fish species infested (one-way ANOVA: $F_{(2,9)} = 26.0$, $p = 0.03$), with a greater mean number of glochidia rejected from *G. cotidianus* than from either *R. retropinna* (adj $p < 0.001$) or *G. maculatus* (adj $p < 0.001$). There were no differences between *R. retropinna* and *G. maculatus* (adj $p = 0.7$), which both lost relatively few glochidia (possibly because of the low number of glochidia that initially attached on the fish specimens in the trial) (Table 4.3). The periods of peak rejection for both *E. menziesii* and *E. aucklandica* glochidia were similar in all fish species, with highest rejection occurring 2–4 days (equivalent to 37–74 accumulated degree days) post-infestation. The species with the longest glochidia rejection period range was *G. cotidianus* infested with *E. menziesii*, sloughing off glochidia for 18 days (333 accumulated degree days) post-infestation (Table 4.3).

Of all combinations tested, successful fish infestations indicated by excystment of juvenile mussels occurred only for *E. menziesii* on *G. cotidianus*, and *E. aucklandica* on *R. retropinna*. For successful fish infestations, median excystment time was significantly longer for glochidia of *E. aucklandica* (34 days, 629 accumulated degree days) than for *E. menziesii* (20 days, 370 accumulated degree days; Mann-Whitney $U = 0$ $n_1 = 14$, $n_2 = 26$, $p < 0.001$; Table 4.3). The first *E. menziesii* juvenile excysted from *G. cotidianus* on day 14 (252 accumulated degree days) post-infestation, with a peak in excystment on days 16–18 and continuing for 12 more days. In contrast, *E. aucklandica* metamorphosis lasted twice as long, with the first observation of excystment 28 days post-infestation (504 accumulated degree days) and continuing for 8 more days (Table 4.3). Metamorphosis success did not differ significantly between unionid species, with success rates of 45% in *E. aucklandica* on *R. retropinna* and 41% in *E. menziesii* on *G. cotidianus* ($p = 0.8$), despite the large difference in absolute numbers attaching to each species (Table 4.3). Mean number of juveniles excysted were significantly lower in *E. aucklandica* on *R. retropinna* compared to *E. menziesii* on *G. cotidianus* ($t_{(4)} = 5.7$ $p = 0.009$; Table 4.3).

Table 4.3. Summary of measured response variables for each fish-mussel combination. Time periods and ranges are shown as degree days (days), with accumulated degree days (add) provided in parentheses. - = no data. $\bar{x}$ is the mean and CI is the confidence interval. Peak excystment is the time at which the highest number of juveniles excysted. Metamorphosis success is the percentage juveniles that excysted from all attached glochidia.

		R. retropinna	*G. maculatus*	*C. cotidianus*
E. aucklandica				
No. glochidia rejected	$\bar{x}$ ± 95 % CI	4±2.6	12.6±4.6	9.8±5.8
Peak rejection period	Days	2	2	4
	(add)	(37)	(37)	(74)
Rejection period range	Days	2-6	2-8	2-4
	(add)	(37–111)	(37-148)	(37-148)
Peak excystment period	Days	34	-	-
	(add)	(629)		
Excystment period range	Days	28-36	-	-
	(add)	(518-666)		
Median excystment time	Days	34	-	-
	(add)	(629)		
Metamorphosis success %	$\bar{x}$ ± 95 % CI	45±2.94	0	0
No. juveniles excysted	$\bar{x}$ ± 95 % CI	3.5±2.55	0	0
E. menziesii				
No. glochidia rejected	$\bar{x}$ ± 95 % CI	29.7±10.2	15.7±6.2	80.1±30.2
Peak rejection period	Days	2	2	2
	(dd)	(37)	(37)	(37)
Rejection period range	Days	2-4	2-12	2-18
	(dd)	(37–148)	(37-222)	(37–333)
Peak excystment period	Days			16–18
	(dd)	-	-	(296–333)
Excystment period range	Days			14–26
	(dd)	-	-	(259–481)
Median excystment time	Days			20
	(dd)	-	-	(370)
Metamorphosis success %	$\bar{x}$ ± 95 % CI	0	0	41±0.9
No. juveniles excysted	$\bar{x}$ ± 95 % CI	52.6±14.5	-	-

4.4.4 *Echyridella aucklandica* broadcast infestation of fish (Exposure 2)

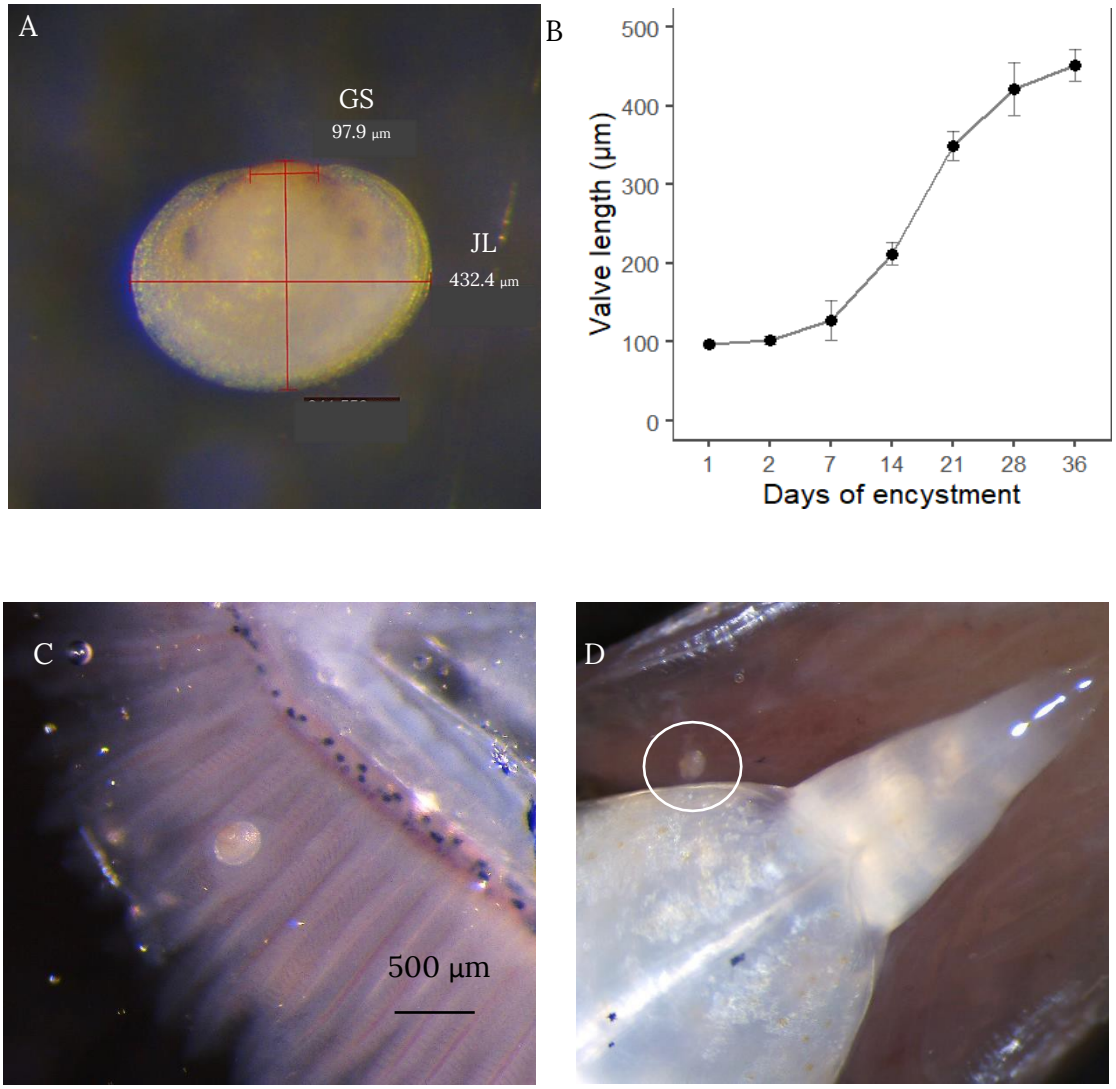
The results of Exposure 2 support the results of the previous trial, in that *E. aucklandica* glochidia were unable to encyst and metamorphose into viable juveniles on either *G. maculatus* or *G. cotidianus*, as well as indicating lack of metamorphosis on *G. huttoni*. *E. aucklandica* successfully metamorphosed on gills of *R. retropinna* and were observed to grow steadily on the gills over the entire encapsulation period of up to 36 days (666 accumulated degree days at a constant room temperature of 18.5°C; Plate 4.2 A, B). *Echyridella aucklandica* glochidia length increased from $\bar{x}$ = 99.5 ± 4.7 μm SD when first released from parent mussels to a length of $\bar{x}$ = 449.2 ± 28.2 μm SD at excystment, equivalent to a growth rate of 9.7 μm per day. Analogous to the field study findings, glochidia encystment occurred only internally on the gills (Plate 4.2 C, D), with infestations present on all gill arch segments (dorsal, medial, and ventral).

Prevalence of *E. aucklandica* on *R. retropinna* in the bulk experiment was 80% (95%CI [52, 96] n = 15). Mean abundances of glochidia on the total fish sample was 1.3 (95% CI [0.8, 1.6]), with a low but relatively uniform infestation intensity on all infested individuals ($\bar{x}$ = 1.6 [95% CI: 1.2, 1.9] glochidia per infested fish). Median lengths of infested fish ($\bar{x}$ = 64 mm) and un-infested fish ($\bar{x}$ = 63 mm) did not significantly differ (Mann-Whitney U = 15.5 n_1 = 12, n_2 = 3, p = 0.75).

4.4.5 *Echyridella aucklandica* conglutinate infestation of fish (Exposure 3)

Video observations showed that the only fish species to feed on and/or inhale conglutinates during the 'feeding trials' were individuals of *R. retropinna*. These findings were confirmed through gill dissections of the exposed fish, with encysted and metamorphosing *E. aucklandica* glochidia prevalent in 44% (95% CI [0.1, 0.8]) of the nine *R. retropinna* specimens exposed to conglutinates. No glochidia were present (attached or encysted) on any internal or external surfaces on the three other fish species (*G. maculatus*, *G. cotidianus* and *G. huttoni*) exposed to conglutinates. Average glochidia infestation intensity and abundance was, again, relatively low on *R. retropinna* (given that 50-100 glochidia are typically enclosed within a single conglutinate [Melchior et al. 2021]) at 0.9 (95% CI: 0.2,2.1) glochidia per total fish and 2 (95% CI [1,3.3]) glochidia per infested fish. No significant differences were found between lengths of infested ($\bar{x}$ = 73 ± 4.9 μm SD) and uninfested ($\bar{x}$ = 72.6 ± 10.5 μm SD) *R. retropinna* ($t_{(7)}$ 0.07, p = 0.95).

Plate 4.2. Summary of *Echyridella aucklandica* metamorphosis and growth. A, fully developed and metamorphosed juvenile *E. aucklandica* (GS = glochidia scar length, i.e., glochidia length at time of infestation; JL = juvenile valve length at excystment. B, plot of mean ± SD glochidia valve length over encystment time at 18.5°C. C, developing *E. aucklandica* encapsulated on a gill filament of *R.retropinna*. D, ventral view of head region in *R. retropinna* with encapsulated *E. aucklandica* (white circle) on the left side (under operculum) on ventral gill segment (glochidia length is 432 µm*).*



4.4.6 Post-parasitic juvenile growth rates

Three weeks after successful transformation trials, juvenile post-excystment growth lengths significantly differed between mussel species after 3-weeks in a culture system providing them with natural lake water and algae, with algae present within the guts of most juveniles examined indicating that juveniles were feeding. *Echyridella aucklandica* valve growth was significantly greater than *E. menziesii* ($t_{(18)} = 7.1$, $p < 0.001$, *E. aucklandica*: $\bar{x} = 105.3 \pm 10.7$ µm per week, *E. menziesii*: $\bar{x} = 71.1$ per week ± 10.9 µm). Indeed, over three weeks post-excystment, *E. aucklandica* grew on average 316.1 ± 32.7 µm or 1.8 times their initial valve length at excystment (length at excystment: 442.5 ± 12.5 µm, to 3 weeks post-excystment: 777.9 ± 40.8 µm), in comparison to *E. menziesii* which grew 213.3 ± 31.9 µm or 1.6 times their initial valve length (length at excystment: 298.4 ± 3.54 µm , to 3 weeks post-excystment: 518.9 ± 21.9 µm Figure 4.4).

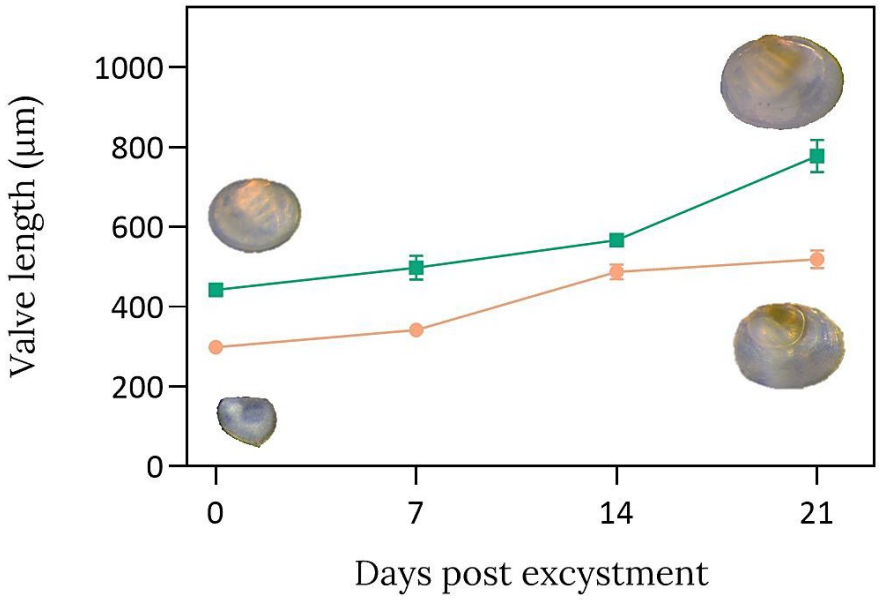


Figure 4.4. Mean (± standard deviation, n = 27-32) valve lengths over time in *Echyridella menziesii* (○) and *E. aucklandica* (■) juveniles after excystment from fish host.

4.5 Discussion

4.5.1 Fish host – glochidia interactions

Collectively, field and laboratory findings in the present study support the hypothesis of partitioning of host fish species between two sympatric unionid species studied in Waikato streams. This study also provides new information on glochidia-host interactions for the 'Threatened - Nationally Vulnerable *E. aucklandica*, for which host-relationships were previously unknown, indicating that this species is a host specialist on *R. retropinna*. For *E. menziesii*, however, glochidia infested several fish species present within the study streams at the same time at various abundances and intensities, confirming the status of this species as a host generalist. Although initial field investigations on wild fish also found *E. aucklandica* glochidia attached to single specimens of *G. huttoni* and *G. maculatus*, laboratory trials later indicated that neither of these species nor *G. cotidianus* were suitable hosts for *E. aucklandica*. Accordingly, the analysis of the ten fish species caught within the stream study sites confirms for the first time that the pelagic drift-feeding fish *R. retropinna* is the only known competent host for *E. aucklandica*.

Low abundances of *R. retropinna* captured at the stream sites throughout the study may partly reflect capture methodology limitations as well as inter and intra- annual differences in fish recruitment and movement patterns. While electrofishing can be highly efficient for catching larger benthic fish that are more vulnerable to the electric current, it can be less effective for capturing shoaling species within the water column (Graynoth et al., 2012; pers. comm., Bruno David, Waikato Regional Council, 2020). Although fyke nets and Gee minnow traps were employed to address this issue and were successful in capturing large numbers of shoaling *G. maculatus*, only low numbers of *R. retropinna* were captured. Nevertheless, electrofishing data records in the New Zealand Freshwater Fish Database (NZFFD) from several sample years report *R. retropinna* as the most dominant catch during one-off sampling events at Ohautira (November 1989: $n = 56$, declining to $n = 10$ in January 2002), highlighting potential efficacy of electric fishing in small streams in years when *R. retropinna* occurs in high numbers. Given the longevity of unionids, recruitment for host-specific species such as *E. aucklandica* may be patchy over time and highly dependent on interannual recruitment patterns of the host species.

In contrast to *E. aucklandica*, *E. menziesii* glochidia prevalence was highest on three benthic species within the *Gobiomorphus* genus; *G. huttoni*, *G. cotidianus* and *G. basalis*, followed by *A. dieffenbachii* and the pelagic *G. maculatus*. The non-native

species *S. trutta* and *G. affinis*, too, were infested with *E. menziesii* glochidia in the field, although transformation into juveniles has not been confirmed on these fish (but see below). In addition to some of the species confirmed within the present study, other research has reported *E. menziesii* glochidia in the wild attached to the galaxiid *G. brevipinnis*, the anguillid *A. australis*, and the benthic-feeding bully *G. gobioides* (Percival, 1931; Hine, 1978; Hanrahan, 2019). Although Jolly (1967) identified *Hyridella* glochidia (*E. menziesii*) attachedto some *R. retropinna* individuals in Lake Rotorua, where this fish species was introducedby humans (Burstall, 1980; McDowall, 1990), there was no evidence of *E. menziesii* encystment or transformation on *R. retropinna* there.

Artificial transformation in other laboratory studies include confirmation of *E. menziesii* glochidia transformation on the introduced salmonid *Oncorhynchus mykiss* (Clearwater et al., 2014), and metamorphosis on native *G. cotidianus*, *G. fasciatus*, *G. vulgaris*, *A. australis*, *A. dieffenbachii* (Brown et al., 2017; pers. comm. Bob Brown, Landcare Research, 2020). Highest transformations were reported on *G. cotidianus* (91% of attached glochidia), followed by *G. fasciatus* (69%), but low transformation on *G. maculatus* with most glochidia rejected from this species within thefirst three days of the trial. In the present study, the laboratory trials of infestation by *E. menziesii* on *G. cotidianus*, *G. maculatus* and *R. retropinna* found successful metamorphosis only on *G. cotidianus*. Differences between this study and the other findings reported above for *E. menziesii* hosts, both infested in the wild and in the laboratory, could be due to potential variations in host species suitability from different geographic locations, as has been demonstrated for a range of unionids globally (Salonenet al., 2017; St. John White et al., 2017). For example, for the unionid *Alasmidonta heterodon* host use differed among Atlantic coastal river basins in the USA, whereby individuals that co-occurred with unionids were able to transform more juvenile unionids than allopatric species (St John White et al., 2017).

The method of broadcasting glochidia individually and via mucus entanglement in *E. menziesii* is consistent with findings elsewhere for host fish generalists which are believed to use this non-selective strategy to facilitate contact with a range of benthic and mid-water inhabiting fish species (Barnhart et al., 2008; Haag, 2012; Melchior et al., 2021). While generalists are rare in the highly biodiverse North American unionid fauna, they are common among the southern Hyriidae (Bonetto & Ezcurra, 1963; Humphrey, 1984; Widarto, 1996; Walker et al., 2001; Haag, 2012; Klunzinger et al., 2012). In contrast, specialist release strategies such as conglutinates are rare in the Hyriidae, with

E. aucklandica being a notable exception among reported studies (op. cit.; see also Melchior et al., 2021). Conglutinates appear to have evolved to target the feeding habits of closely related host species or a suite of fish feeding-guilds (Barnhart et al., 2008; Haag, 2012; Patterson et al., 2018). *Echyridella aucklandica* conglutinates that resemble the shape of macroinvertebrates, such as Hirudinea or Diptera, are thought to have adapted to attract pelagic fish species because the conglutinates drift in the water-column with a rippling motion when first released, although sometimes they can also be found attached to the substrate or the mother unionid shell (see Melchior et al., 2021). In the current laboratory experiment, conglutinates floated in the vigorously aerated laboratory aquaria for some time (hours) before eventually settling to the bottom, so would have been available to bothpelagic and benthic test species.

The use of conglutinates by *E. aucklandica* is consistent with host specificity for *R.retropinna*, a shoaling, drift-feeding species that is well adapted to feed on a range of benthic, pelagic and surface prey in a variety of water quality conditions (Boubee & Ward, 1997; Rowe et al., 2002). Reported studies indicate that juvenile fish feed predominantly on copepods and cladocerans, while the adults feed on macroinvertebrates including chironomids and mysid shrimps (Boubee & Ward, 1997; Ward et al., 2005). During the 'conglutinate feeding trials' (Exposure 2), some individuals of *R. retropinna* were observed repeatedly attacking, regurgitating, and consuming whole conglutinates, whereas *G. maculatus* and *G. cotidianus* did not. Similar fish-feeding behaviours of repeated attacks have been reported in 'conglutinate feeding' experiments on *Ptychobranchus jonesi* where attacks were found to be important for initiating eruption of glochidia from within the conglutinate, allowing attachment on the gills of the host (McLeod et al., 2017). Although *R. retropinna* were observed to feed on conglutinates, 'feeding trials' revealed low glochidia prevalence on fish, suggesting that in the present study, not all fish contacted conglutinates or glochidia, or, that some conglutinates were consumed by fish before glochidia erupted or otherwise detached.

Both *E. aucklandica* and *E. menziesii* glochidia were observed in the wild attached to fish which were later confirmed to be unsuitable hosts for juvenile transformation, at least under laboratory conditions (e.g., *G. maculatus*). This finding is not uncommon in unionids, as once released from the parent, many glochidia will attach to any fish that they encounter (Kat, 1984; Dodd et al., 2005; Moore & Clearwater et al., 2019). Unsuitable fish species may consequently act as glochidia sinks, whereby glochidia attach to the fish but do not metamorphose into juveniles, reducing the number of glochidia available to

infest compatible hosts while at the same time reducing overall recruitment (Bauer & Vogel, 1987; Tremblay et al., 2016; Moore & Clearwater et al., 2019). Although not observed to have fed on conglutinates in the fish 'feeding trial', high abundances of shoaling and pelagic-feeding *G. maculatus*, which are ecologically similar to *R. retropinna*, have the potential to be a recruitment sink for this mussel species if they actively feed on *E. aucklandica* glochidia in natural settings.

4.5.2 Temporal and spatial patterns in fish–unionid relationships

Synchronising critical life history events (reproduction) with resource availability is an important evolutionary trait (Visser et al., 1998; Bradshaw et al., 2004). However, within the present study, little evidence was found to support temporal synchrony between peak unionid brooding and peaks in host fish abundances, at least for the two coastal streams sampled over time. At Pakoka, a temporal disconnect was evident between peak brooding (which overlapped with timing of peak glochidia release, as indicated by observations of increased infestation prevalence, abundance and intensity on fish), and host abundance which peaked one month later, coinciding with the migration period of *G. huttoni* (McDowall, 1990), one of the main hosts for *E. menziesii* within both study streams. Furthermore, decreasing glochidia abundance and intensity on fish during peak fish abundances may also indicate a potential mismatch between the critical period of glochidia release and suitable host fish availability (Hastie & Young, 2003; Brooks & Hoberg, 2007; Cosgrove et al., 2012).

At Ohautira, there were no obvious patterns of synchrony between peak brooding and peak fish abundance in any potential host species through time except for *G. maculatus*. Peak abundance of *G. maculatus* coincided with peak brooding in both unionid species, further supporting the earlier suggestion that *G. maculatus* could act as a recruitment sink for *E. aucklandica* (for which it is an incompatible host), particularly if their densities are considerably greater than compatible host species at the time of peak glochidia release within streams. Although results are reported for only for one brooding season (2018/19), continued sampling for another year (2019/20; data not shown) at Ohautira confirmed lack of coincident temporal peaks in preferred host fish abundance and glochidia release. Notwithstanding this, more intensive sampling of a larger number of streams over multiple years would be required to assess temporal relationships more fully between recruitment of migratory host fish and mussel glochidia release.

Influences controlling temporal synchrony between brooding, glochidia infestation and host fish abundance likely involve a combination of environmental factors, such as water temperature, coupled with host factors such as seasonal migration cues and overall abundance of host fish (Schneider, 2017). For example, mussel species may use increasing water temperatures in spring to time reproduction with peaks of resource availability, while diadromous fish may time upstream migration with spring flood eventsas well as resource availability (McDowall, 1995; Visser et al., 1998; Bradshaw et al., 2004; Schneider, 2017). These natural processes may be easily disrupted in host-unionid relationships, where temporal mismatches between mussel reproduction and host abundance at critical times may be caused through phenological shifts (Cushing et al., 1990; Poulin, 2007; Schneider et al., 2016). For example, shifts in the timing of flood events and occurrences of critical water temperature thresholds linked to climate change may delay or bring forward important phenological events, such as the migration of amphidromous fish species, or glochidia release events dependent on temperature thresholds and accumulated degree days in unionids (See Chapter 3; Hastie & Young, 2003; Parmesan, 2006; Cosgrove et al., 2012; Paull & Johnson, 2014).

Site-specific factors can influence glochidia prevalence, abundance and intensity on fish, such as unionid adult density combined with host behaviour and availability (Strayer, 2008; Österling et al., 2008; Arvidsson et al., 2012; Haag & Stoeckel, 2015). For example, Österling et al. (2008) found a positive relationship between *M. margaritifera* glochidia abundance on *S. trutta* and adult mussel density. Furthermore, Downing et al. (1993) found that reproductive success decreased with declining unionid density, with very little fertilisation occurring at densities <10 individuals m^{-1} in a 6–7 m segment of Lac de l'Achigan, Canada. Despite this, Schneider et al. (2019) and Scheder et al. (2014) suggested that relatively low unionid densities can have high glochidia infestation and reproduction potential if host abundance remains high, emphasising that mussel density alone does not affect glochidia infestation and recruitment success, and that other factors such as host suitability in time and space can also be important (Jansen et al., 2001; Levine et al., 2012; Schneider, 2017). This was evident within the present study where higher *E. menziesii* adult mussel densities at Pakoka, in combination with relatively higher abundances of compatible hosts, may have led to higher *E. menziesii* glochidia prevalence, abundance, and intensity on *G. huttoni* than found at Ohautira. However, no relationship like this was observed for *E. aucklandica* at either site, probably due to consistently low abundances of the host *R. retropinna* despite relatively high adult *E. aucklandica* densities.

Although densities of *G. huttoni* and *A. dieffenbachii* were similar among sample dates and between sites, overall prevalence and degree of infestation were greater on *G. huttoni* at both sites. Differences between these species may be due to habitat utilisation (as benthic fish are more closely associated with mussel beds), including behavioural and life-history traits of hosts, such as swimming, spawning and feeding behaviours which may be critical for successful glochidia infestation (Humphrey, 1984; Widarto, 1996; Jansen et al., 2001; Strayer, 2008). Although *G. huttoni* and *A. dieffenbachii* are both associated with the benthos (McDowall, 1990), the behaviour of *G. huttoni*, notably males guarding nests during the austral spring reproductive period (McDowall, 1990), may increase the chance of glochidia encounter for this species. Moreover, infestation differences could be because of clear differences in species physical features such as host shape, size and glochidia accessibility to *G. huttoni* fins in comparison to *A. dieffenbachii*. Further research is needed to elucidate factors underpinning differences in metamorphosis success of *E. menziesii* on *A. dieffenbachii* and *G. huttoni*.

4.5.3 Contrasting glochidia development on compatible hosts

Glochidia of *E. menziesii* and *E. aucklandica* appear to not only partition host use through contrasting attraction and infestation strategies, but also differing life-history traits, including modes of development (growth and longer metamorphosis duration in *E. aucklandica*) and attachment location. The current study found *E. aucklandica* to encyst exclusively on gills of their host, in comparison to *E. menziesii* which attached not only on the gills but also externally on the fins and skin. Attachment on the gills appears to be a common feature in smaller glochidia with marginal appendages (hooks) weakly-developed or absent, while larger glochidia with well-developed hooks are adapted to attach to harder tissues (e.g., margin of the gill operculum) and fins (Bauer, 1994; Wächtler et al., 2001). Unlike other unionid species with small glochidia, *E. aucklandica* does contain appendages, however, how functional these are in terms of attachment to host fish is unknown. Furthermore, Wootten (1974) suggested that glochidia are not selective for attachment site but clamp on to the first fish tissue encountered, while Paling (1968) demonstrated attachment on the gills is significantly influenced by the route of the respiratory current, suggesting that gill-parasitising glochidia need to be inhaled by the host for contact with the gills to occur (Blazek et al., 2006). This mode of infestation is facilitated by the use of conglutinates that resemble fish prey, like those produced by *E. aucklandica*.

Miniaturised glochidia of *E. aucklandica* were observed to grow nearly five times their original size compared to the larger *E. menziesii* which stayed the same size throughout metamorphosis. Growth of glochidia on gills during encystment is a rare feature in unionid species (Lefevre & Curtis, 1912). However, it appears that glochidia growth may be a trait of miniaturised glochidia (<100 μm) which have evolved multiple times in Unionida (i.e., Margaritiferidae, *Quadrula quadrula* species group, *Leptodea*, and *Truncilla*, reviewed in Barnhart et al., 2008). Miniaturised glochidia that grow on their host often share the trait of an extended encystment period, which sometimes includes overwintering on hosts (Young & Williams, 1984; Haag, 2012), although this was not observed in *E. aucklandica*.

Metamorphosis duration was significantly longer in miniature *E. aucklandica* glochidia than for the larger *E. menziesii*, but only by two to three weeks. Timing of transformation from glochidia to the juvenile stage appears to be largely water temperature-dependent, with metamorphosis occurring more rapidly at warmer temperatures related to degree days (Walker, 1981; Dudgeon & Morton, 1984; Humphrey, 1984; Hruska, 1992; Hastie & Young, 2003; Moore & Clearwater, 2019). The relationship between metamorphosis and temperature is supported by the laboratory studies carried out using *G. cotidianus* on which juvenile excystment peaked at 16–18 days at a constant temperature of 18.5°C. Studies of the same species undertaken at slightly higher temperatures (20°C) resulted in excystment times of 14–16 days (Moore & Clearwater, 2019), while a constant 22°C resulted in even shorter excystment times of 6–14 days (Hanrahan, 2019). Accounting for differences in temperature using degree days demonstrates 280–388 degree days is required to reach peak excystment. Fluctuating temperature regimes in the wild are likely to cause encystment durations that differ from these laboratory studies which used constant temperature regimes. For example, Steingraeber et al. (2007) reported that at constant laboratory temperatures excystment peak ranged from 28 to 37 days at a constant thermal regime of 19°C, but this extended to 70 days in a varied thermal regime of 12–19°C. In contrast, broadscale fluctuations in temperature, such as increases associated with global climate change, have been observed to significantly shorten excystment periods (by up to 5 months in the case of *M. margaritifera*; pers. comm. Louise Lavictoire, Freshwater Biological Association, 2021), whereby juveniles excyst in conditions that may impede growth and survival, especially ifjuveniles excyst prior to cold winter temperatures (Marhawa et al., 2017).

Other than water temperature, factors that may determine glochidia growth

while encysted are poorly known. Selective pressures may exist for juvenile mussels to reach a minimum size before they are released from their host, increasing survival rates in unpredictable and fluctuating environments whereby larger sized juveniles settle more readily in flowing water after release from the host (Barnhart et al., 2008). Rapid settlement post-excystment could decrease predation risk or increase the likelihood of reaching suitable habitat, as perhaps smaller juveniles would settle in habitats with lower velocities where dissolved oxygen is likely to be lower. Further, prolonged excystment periods could be advantageous as larger juveniles may have greater nutritional reserves and resilience than smaller individuals (Douda, 2015), allowing for the dispersal of juvenile mussels over a larger area through host migration (Watters & O'Dee, 1999; Taeubert et al., 2013).

Following successful excystment in laboratory trials, *E. aucklandica* juveniles exhibited significantly greater average post-excystment growth (over three weeks) than *E. menziesii*. Intraspecific growth disparities within excysted juveniles from within the same cohort are apparently not uncommon (Marhawa et al., 2017). In the present study, variations in juvenile post-excystment development between the two mussel species may be related a number of factors, including pre- and post-excystment conditions (Douda, 2015; Jones et al., 2005). For instance, nutrition reserves obtained during metamorphosis on host fish are thought to have key consequences on the vitality and development of juveniles in the early post-excystment stage (Douda, 2015). Moreover, Marhawa et al. (2017) found that juvenile mussels with long encystment phases had increased post-excystment growth rates compared to those with shorter phases. Additionally, species-specific preferences in rearing conditions required by each species may differ (including water temperature, substrate types, feeding regime and water quality; see Jones et al., 2005), and need to be further investigated.

4.5.4 The downside of fish host specialisation

Host specificity comes with risks as it ties the fate of the unionid species with that of its host, such that a decline in the specific host may result in recruitment failure for the mussel (McNichols et al., 2011; Douda et al., 2012). The same is less probable for glochidia that can metamorphose on a range of fish species, enabling them to spread the risk among host species should one or more of those species within their host range decline or undergo interannual population fluctuations across multiple fish species (Douda et al., 2012; Haag, 2012; Watters & O'Dee, 1999). Currently, the only identified host for *E. aucklandica*, *R. retropinna*, is found to inhabit a range of habitats throughout Waikato

waterways (Speirs et al., 2001), and it is one of the few native fish species in New Zealand recognised as "Not threatened" (Dunn et al., 2018; Joy et al., 2019), with an IUCN Red List status of "Least concern" (Franklin et al., 2014). Although widespread, *R. retropinna* may be vulnerable to local population declines, particularly due to a range of increasing environmental changes induced by anthropogenic activities. In particular, *R. retropinna* can be highly sensitive to pollutants such as ammonia, water turbidity, and stressors such as high-water temperature and low dissolved oxygen (Simons, 1986; Dean & Richardson, 1999; Richardson et al., 2001; Rowe et al., 2004; Ward et al., 2005). As coastal stream populations of *R. retropinna* tend to be diadromous (McDowall, 1990) with poor climbing ability, migration both upstream and downstream may be disrupted or limited by river mouth closures and other obstructions to fish passage (Ward et al., 1987; McDowall, 1995; Franklin & Gee, 2019). The longer development time of *E. aucklandica* glochidia may make it even more vulnerable to the effects of anthropogenic pressures on this host species because of the more extended time for exposure to pollutants etc (but see Gillis et al., 2008).

Although *R. retropinna* was the only fish species on which *E. aucklandica* glochidia were able to successfully metamorphose in the present study, glochidia abundances and infestation intensities in laboratory and field conditions were equally low. One interpretation of generally low attachment and metamorphosis success of *E. aucklandica* may be that *R. retropinna* is not the primary host but rather is serving as a secondary host. Primary hosts have consistently high levels of infestation (intensity) and prevalence whereas secondary hosts, although allowing metamorphosis, yield lower numbers of juveniles (Haag & Warren, 1998; O'Brien & Williams, 2002; McNichols et al., 2011). Other suitable hosts may therefore be required to produce sufficient juveniles to sustain populations.

One potential candidate is another Retropinnidae species *Stokellia anisodon*, but this is restricted to the east coast South Island of New Zealand and its distribution does not overlap with distributions with *E. aucklandica*. A more likely candidate for compatibility with *E. aucklandica* based on phylogeny, distribution, habitat and feeding ecology is the closely-related but extinct grayling, *Prototroctes oxyrhynchus* (Retropinnidae), an amphidromous, shoaling species endemic to New Zealand (Allen, 1949; McDowall, 1976). Prior to rapid declines, *P. oxyrhynchus* was widespread and abundant throughout New Zealand, inhabiting lowland rivers and coastal streams (McDowall, 1978; Lee & Perry, 2019) with a distribution that likely overlapped with that of *E. aucklandica*.

P. oxyrhynchus went extinct, probably in the early 1900s (Dunn et al., 2018; Lee & Perry, 2019), at a time at which New Zealand waterways were undergoing rapid environmental change due to deforestation, industrial development and non-native species introductions, coupled with mass harvest of freshwater fish (Ewers et al., 2006; Townsend & Simon, 2006). Feeding habits of *P. oxyrhynchus* were likely omnivorous or herbivorous, with reports of fishes grazing on periphyton, as well as fish gut content examinations containing caddisfly larvae (Graham, 1956; McDowall, 1978). Similarly, the extant and closely-related *P. maraena* in Australia is omnivorous, feeding on aquatic insect larvae and small plant material, including macrophytes and filamentous algae (Jackson, 1976; Cadwallader & Backhouse, 1983; Berra et al., 1987). Studies have yet to explore interactions between native unionid species and *P. maraena* in Australia, but the extinction of potential hosts like *P. oxyrhynchus* for *E. aucklandica* might help explain the concurrently observed aging *E. aucklandica* populations. If *R. retropinna* is serving as a secondary, suboptimal host unable to produce enough juveniles to sustain *E. aucklandica* populations over the long term, then urgent action (e.g., captive breeding) may be required to ensure the survival ofthis threatened unionid species.

4.6 References

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Chapter Five

Slowly but surely: Seasonal movement patterns in two sympatric freshwater mussel species

5.1 Abstract

Freshwater mussel species (Unionida) can exhibit active horizontal and vertical locomotion patterns that may be connected to the timing of reproductive activity. This chapter quantified movement and positioning of two sympatrically-occurring species, *Echyridella aucklandica* and *E. menziesii*, with the aim of relating spatial and temporal movement patterns of both sexes to species-specific timing of fertilisation, glochidia release and host fish species occurrence in a coastal Waikato stream. Additionally, I examined the effects of flooding on mussel positioning and movement following a large bed-moving flood event that occurred during the critical glochidia release period. Passive integrator transponder tagging was used to track movements of both mussel species, and electrofishing was conducted to determine host fish species locations within mapped habitats. Throughout the glochidia release period (October – December), results showed evidence of relatively high net horizontal movement rates (3.9 ± 1.7 SD m for *E. aucklandica* [n = 56, both sexes] and 4.7 ± 2.0 m for *E. menziesii* [n = 43, both sexes]), and active bank-ward cluster formation in tagged individuals of both species. Spatial overlap between mussel species and their respective host fish was partially observed for *E. menziesii*, but could not be confirmed for the host-specific *E. aucklandica* due to only one *Retropinna retropinna* being captured. Vertical positions varied throughout the onset brooding period for both species, but generally proportions of female mussels increased at the sediment surface during respective reproductive onsets. Downstream displacements of up to 349 m for *E. menziesii* and 46 m for *E. aucklandica* were observed following a bed-moving flood event, but there was mixed evidence that larger and more firmly - anchored *E. aucklandica* were, on average, more resistant to a flood of this magnitude. Rather, flood flow refugia associated with riparian vegetation and debris in bank habitats appeared to provide resistance for both species to the effects of the moderate flood that occurred in this study.

5.2 Introduction

Reproduction is a crucial and potentially limiting life-history event in organisms that release gametes into their surrounding environment, particularly for freshwater mussels of the family Unionida that use spermcasting for fertilisation (McMahon & Bogan, 2001; Bishop & Pemberton, 2006). Unlike broadcast spawning, whereby both sexes synchronously release gametes into their surroundings, spermcasting only requires males to broadcast their sperm into the water column, while females retain their ova internally. Thus, rather than external fertilisation, sperm carried by currents must be captured by inhalant apertures of female mussels, after which fertilisation and subsequent larval brooding occurs within the female gill demibranchs (Ishibashi et al., 2000; McMahon & Bogan, 2001; Fergusson et al., 2013).

Factors affecting the success of this type of reproductive strategy are not well understood but may include the distance between fertilising mussels, population density and water flow regimes (Bauer, 1987; Downing et al., 1993; Schwalb & Push, 2007). All these factors are widely considered to directly affect sperm availability (Yund, 2000; Gascoigne et al., 2009), potentially limiting fertilisation rates and subsequent larval production (but see Fergusson et al., 2013 and Mosley et al., 2014). To counteract limiting factors and improve fertilisation success, some spermcasting benthic organisms are thought to have adopted various behavioural strategies, including aggregation of conspecific males and females during the reproduction period (Levitan & Peterson, 1995; Yund, 2000; Downing et al., 1993).

Unionid mussels often aggregate spatially in patches in terms of distribution and density, and sometimes comprise multiple species (Strayer et al., 2008; Sansom et al., 2018). Though often considered sedentary, adult freshwater mussels have been reported to engage in active locomotion by cyclical extension and retraction of their muscular foot (Trueman, 1983), moving both vertically within and horizontally along the beds of lakes and rivers (Balfour & Smock, 1995; Amyot & Downing, 1997, 1998; Watters et al., 2001; Perles et al., 2003; Allen & Vaughn, 2009). Locomotion in unionids has been related to changes in water levels, water temperatures and flow conditions (Balfour & Smock, 1995; Di Maio & Corkum, 1995; Amyot & Downing, 1997; Schwalb & Pusch, 2007; Lymbery et al., 2020). Movement has also been observed in response to displacement by disturbances such as floods, with subsequent aggregations developing in more suitable habitats with reduced shear stress and higher dissolved oxygen concentrations (Trueman, 1983; Strayer, 1999; Allen & Vaughn, 2009).

Additionally, movement and aggregation exhibited by freshwater mussels has been suggested to be connected to the timing of reproductive activity, particularly during spawning events (Piechocki, 1969; Burla et al., 1974; Amyot & Downing, 1998; McLain & Ross, 2004; Schwalb & Pusch, 2007). Although studies have reported contrasting results on the effects of density on fertilisation success in freshwater mussels (see Bauer, 1987; Downing et al., 1993; Fergusson et al., 2013; Mosley et al., 2014), many examples exist of species aggregating, thereby increasing density and proximity of conspecific mussels during this critical time of reproduction. For instance, *Anodonta* and *Unio* species have been observed to move horizontally to form aggregations during the spawning period, presumably to enhance fertilisation success by increasing the density of conspecific males and females (Stansbery, 1966; Burla et al., 1974; Engel, 1990; Amyot & Downing, 1998; Vicentini, 2005). In contrast, species within the genera *Quadrulini*, *Ptybranchus*, *Elliptio* and *Amblema* have been observed to migrate vertically within the bed, emerging at the sediment surface at different times during each species' respective reproductive periods, apparently triggered by rising water temperatures (Balfour & Smock, 1995; Watters et al., 2001). Schwalb and Pusch (2007) also found that *Unio tumidus* populations moved vertically, increasing population densities at the sediment surface during their reproductive period.

In unionid mussels, reproductive success is further complicated by a symbiotic larval (glochidia) phase which many species must complete on fish to progress into adulthood (Kat, 1984; Bauer, 2001; Barnhart et al., 2008; Haag, 2012). Reduced host encounters during the glochidia release period may have negative fitness consequences for freshwater mussel populations (Paull & Johnson, 2014; Altman et al., 2016; Modesto et al., 2018). Most studies of aggregation behaviour in unionid mussels have focussed on movement related to the timing of spawning, but few studies have addressed questions regarding movement or aggregation related to glochidia release and host fish spatial overlap. One example is the observation whereby female *U. crassus* migrated horizontally to river margins, spurting jets of glochidia into the mid-channel, a behaviour thought to attract host fish (Vicentini, 2005; Aldridge et al., 2018). Movement by freshwater mussels into habitats that overlap with host fish may be particularly important for unionids that broadcast glochidia and do not use attraction strategies such as lures (e.g., Barnhart et al., 2008; Haag, 2012), requiring brood-releasing females to be positioned in habitats optimalfor host encounter by released glochidia.

Understanding movement and aggregation patterns is fundamental to effectively manage and conserve unionid populations, particularly during critical reproductive events. This chapter focusses on quantifying movement and aggregation patterns in space and time related to two critical life-cycle events of the threatened freshwater mussel species *Echyridella aucklandica* and *E. menziesii* in a small coastal Waikato stream. Recent research on these two species has found that their brooding seasons overlap in Waikato streams, particularly during peak brooding in November and December (austral summer) (Chapter 3), although *E. aucklandica* initiated brooding a few months earlier than *E. menziesii* (austral winter) (Chapter 3). The two species use different reproductive release strategies (Melchior et al., 2021; Chapter 2) and use different host fish species for glochidia attachment (Chapter 4). *E. menziesii* is classified as a host fish generalist utilising several benthic-dwelling fish species for glochidia metamorphosis, while *E. aucklandica* is currently classified a host fish specialist known only to use pelagic *Retropinna retropinna* for metamorphosis.

The specific aims of this study were to (1) quantify horizontal and vertical movements of two sympatrically-occurring unionid species over the breeding season in a Waikato stream, and (2) relate spatial and temporal movement patterns for both sexes to species-specific timing of fertilisation, glochidia release and host fish species occurrence. During the course of this study, a large bed-moving flood event occurred, allowing me to examine effects of flooding during the reproductive period on both musselspecies. I hypothesised that:

- H1: Based on knowledge of glochidia release strategies and host fish species, gravidfemale *E. menziesii* and *E. aucklandica* densities will be greater in habitats that overlap with their host fish. Accordingly, host-generalist *E. menziesii* females thatbroadcast glochidia will disperse across a broader range of instream habitats, while host-specific *E. aucklandica* that produce conglutinates will remain near where itsonly known host is most likely to occur.
- H2: The proportion of female *E. aucklandica* and *E. menziesii* will be greater downstream of conspecific male aggregations during their respective brooding periods, and most females will occur at the sediment surface to increase the chance of fertilisation.
- H3: Larger and more firmly-anchored *E. aucklandica* will be more resistant to the flood event than the smaller *E. menziesii* which will persist in refugia habitats post-flood (Levine et al., 2014).

5.3 Methods

5.3.1 Study site sampling

This study was carried out in Ohautira Stream (-37.762392, 174.98124), a short 4th order coastal stream in western Waikato, chosen for the presence of relatively high densities ofboth mussel species (*E. menziesii*: 0.29 m^{-2}, *E. aucklandica*: 0.77 m^{-2}). General details of thestudy stream can be found in Chapter 3. The two 60-meter stream reaches chosen for the analysis of movement and aggregation patterns were similar physically with relatively homogenous substrate size composition, being generally dominated by gravels embedded in sand/silt sediments, and silt and clay along edges of undercut banks where mussels were commonly found (see Table 5.1). Wetted and bankfull widths at sampling locations ranged from 4.0-5.8 m and 5.0-6.8 m, respectively (Table 5.2).

Tagged mussels (see Section 5.3.2) in the first experimental reach ('summer' reach) were sampled monthly during the peak brooding season of both species from October 2018 to January 2019 (austral spring to summer; see Chapter 3) for assessment of changesin spatial aggregation related to glochidia release and fish habitat use. Mean water temperatures (measured using YSI 2030 Pro meter; Yellow Springs Instruments, Ohio, USA) over this period were 17.08 ± 1.91 (SD) °C. The study was disrupted in December by a moderate flood event with a putative return period of 1 year based on Waikato Regional Council discharge data available from a neighbouring site (Waingaro River; peak discharge: 27.4 m^{3-s}, which caused bedload movement and scouring along some sections of the study reach and lead to displacement of some tagged specimens (see Results). Therefore, a second reach ('winter' reach) approximately 100 m upstream from the summer reach was chosen for a subsequent analysis of movement patterns related to timing of onset brooding. Thisexperiment was conducted initially from May to October 2019 (austral winter to spring; see Chapter 3), with additional sampling in February 2020 to enable comparisons with February 2019 post-flood data. Mean temperatures for the winter experiment (excluding February) were 10.35 ± 1.80°C.

5.3.2 Experimental design

Within both 60-m 'summer' and 'winter' reaches, three 10-m by approximately 5-m (channel length by wetted width; see Table 5.1) sub-reaches were delineated, each separated by 10 m of channel. Within each sub-reach, 1 m^2 grid cells were staked out using alphanumeric tagged pegs pushed into the substrate. Movement of mussels within each reach and between reaches was unrestricted.

Visual estimates within each grid cell were made of substrate composition (%) using the following particle size scale based on b-axis dimensions: silt (<0.06 mm), sand (0.06-2 mm), fine gravel (2-10 mm), large gravel (10-64 mm), cobble (64-256 mm), boulder(>256 mm), and bedrock (solid rock surfaces). A substrate size index was then calculated based on the sums of the weighted substrate percentages (Jowett et al., 1991). Weighting values were slightly modified from the original substrate codes (Bovee, 1982) to allow for two gravel categories, large and fine, as follows: Substrate size index = 0.08*bedrock% + 0.07*boulder% + 0.06*cobble% + 0.05*large gravel% + 0.04*fine gravel% + 0.03*sand% + 0.02*silt%. Mean substrate size index values within sub-reaches ranged from 3.19 ± 0.63 (SD) to 4.73 ± 0.46, indicating dominance by fine-large gravels (Table 5.1).

Presence of potential flood-flow refugia (undercut bank, trailing riparian vegetation, woodymaterial, debris) mapped within grid cells prior to each experiment comprised mostly wood. Water depth and velocity (at 0.6 of the depth using Rickly USGS 4' Wading Rod and Marsh McBirney Flo-Mate 2000, set to 0.01 m/s accuracy) measured in the centre of eachgrid cell on each sampling occasion averaged 0.26 ± 0.03 m and 0.27 ± 0.05 m/s,respectively, across the three summer sub-reaches, compared to mean water depth of 0.20 ± 0.11 m and velocity of 0.23 ± 0.08 m/s for the winter sub-reaches (see Table 5.2 forsub-reach means).

Table 5.1. Summary of physical stream characteristics within each sub-reach (see Section 5.3.2 for explanation) for 'summer' and 'winter' study reaches.

	Sub-reach	± SD substrat esize index	Dominant substrate type(s)	Dominant floodflow refugia type(s)
Summer	1	4.73±0.46	Large gravel	Wood
	2	4.39±0.83	Fine/Large gravel	Wood
	3	4.15±0.42	Fine /Large gravel	Wood/Vegetation
Winter	1	3.51±0.25	Fine gravel	Wood
	2	3.26±0.33	Fine gravel	Wood
	3	3.19±0.63	Fine gravel/Sand	Wood

Table 5.2. Mean (SD) widths (n = 10), and water depths and velocities (n = 140-147) within each sub-reach (see Section 5.3.2 for explanation) for 'summer' and 'winter' study reaches. Ranges are shown in parentheses for depths and velocities (negative values indicate backflow).

	Sub-reach	wetted width (m)	bankful width (m)	water depth (m)	velocity (m/s)
Summer	1	4.01±0.69	5.05±0.23	0.27±0.14 (0.02-0.65)	0.31±0.29 (-0.08-1.0)
	2	4.78±0.54	5.03±0.0	0.22±0.11 (0.01-51)	0.29±0.24 (-0.06-0.87)
	3	5.55±0.58	5.66±0.61	0.28±0.14 (0.01-0.64)	0.21±0.16 (-0.04-0.57)
Winter	1	5.80±0.42	6.42±0.22	0.12±0.06 (0.01-0.32)	0.27±0.17 (0-0.70)
	2	5.77±0.29	6.77±0.33	0.16±0.06 (0.006=0.28)	0.25±0.18 (-0.01-0.89)
	3	5.18±0.09	5.82±1.24	0.33±0.12	0.14±0.09

5.3.3 Mussel collection and tagging

In September 2018, mussels of both species were collected from the summer reach using aquascope and tactile searches. Collected individuals were separated by sex and the sexual maturity status of females was determined by brood pouch examination using reproductive development indices described in Melchior et al. (2021; Chapter 3). The same number of male and female mussels in each species were selected (i.e., 1:1 male to female ratio), and their lengths measured using callipers. Mussels of similar lengths were chosen for the study: *E. menziesii* (SD) length 52.9 ± 3.9 (n = 66), and *E. aucklandica* (SD) length 77.0 ± 16.9 (n = 66).

External surfaces of valves were cleaned and dried prior to tagging using two approaches. Passive integrator transponder (PIT) tags (FDX B, 12x2.15 mm; Oregon RFID, Portland, USA) were affixed externally on the left valve of each individual using "Loctite super glue" and embedded in dental cement (Fuji Glass Ionomer Luting Cement, Japan) (Plate 5.1.A,B). PIT tags produce high radio-frequency signals with a unique digital code that was detected by a Data Tracer reader (Oregon RFID, Portland, USA; read range 16 cm) attached with a submersible antenna (0.75 m). In addition, numbered 8x4 mm FPN glue-on tags (Hallprint, Hindmarsh, Australia; *E. aucklandica*: green, *E. menziesii*: yellow) were attached with super glue to the anterior end on the right valve of each mussel. Glue-on tags enabled individual

identification, for example in the event that PIT tags detached or where individuals were clumped at the surface. To reduce stress, all tagging on individual mussels was completed within approximately 2 minutes.

Once tagging was complete, individuals were placed in containers filled with stream water or held in mesh bags within the stream until their reintroduction (see below). Prior to placement of tagged mussels, sub-reaches were cleared of visible untagged mussels to standardise conditions and reduce potential attraction to hosts by other mussels present. Monthly brood surveys coinciding with mussel movement monitoring were carried out in an adjacent reach to determine the stage of glochidia development for each species. Summer brooding surveys revealed peak brooding proportions in November, declining in December and January, indicating the initiation ofglochidia release for females of both species, while brooding onsets were observed in June for *E. aucklandica* and August in *E. menziesii* (see also Chapter 3).

5.3.4 Movement survey

Movement patterns by both species were investigated by tracking pit tagged individuals over time (based on related studies including Zajac & Zajac, 2011). For the summer survey, mussels were released in October 2018 by hand-planting 22 individuals per species (1:1 male to female ratio) on the substrate surface (at an approximate 50% burrowing depth for each individual mussel) within a central grid cell of each of the three sub-reaches. The grid cells occupied by individual mussels within sub-reaches were mapped monthly until February by a single researcher systematically moving upstream in a zig-zag fashion with the PIT tag reader scanning the entire bed. A copy of the stream map was carried on each sampling occasion and the location of each recorded tag entered in the corresponding grid cell on the map, allowing for mussel locations to be matched up with the previously recorded locations. Grid locations (x-y alphanumeric position) within each of the three sub-reaches were mapped using both thePIT tag reader and aquascope to verify tag identity, along with the exact position within the grid using a measuring tape to record the distance from the true left and downstreamborders of each sub-reach.

After each sub-reach had been surveyed, the 10 m above the most upstream sub-reach, the 10 m below the most downstream sub-reach, and the 10 m between each sub- reach were further scanned using the PIT tag reader and aquascope to recover any individuals that had moved outside of their original assigned sub-reach. The locations of these individuals were recorded (distance downstream of sub-reach

and from true-left), along with their vertical position and habitat type. These individuals were left *in-situ* but considered to have left the study area for the purposes of data analysis.

At the end of the summer survey in February, all detectable individuals were collected from the sampling reach, and an approximate 400 m downstream PIT tag survey was conducted to recapture detectable mussels that had been displaced after the flooding event in late December. Downstream areas included pools which were too deep to survey. Mussel location, water flow and depth, and substrate size composition were all measured as described above for individuals that remained in the study reach or that had been dislodged downstream.

Collected individuals were held within a deep pool until the subsequent winter survey began. As not all individuals from the summer reach were recaptured, a lower number of individuals (14 per species at 1:1 male to female ratio) was used in the winter survey. These were hand-planted into the substrate within the central grid cell of each of the three sub-reaches and monitored as described above. Sampling for the winter survey occurred fortnightly (from May to August 2019 to capture brooding onset) and then monthly (September to October), with a final sampling event and collection in February 2020. At each sample event, the vertical position of individuals was also recorded, measured as shell height above surface (on a scale of 0%, 25%, 50%, 75%, 90%, and 100% [detected by PIT tag reader but not visible on surface]). Detected mussels were never removed and care was taken to cause as little physical disturbance to each surveyed reach as possible.

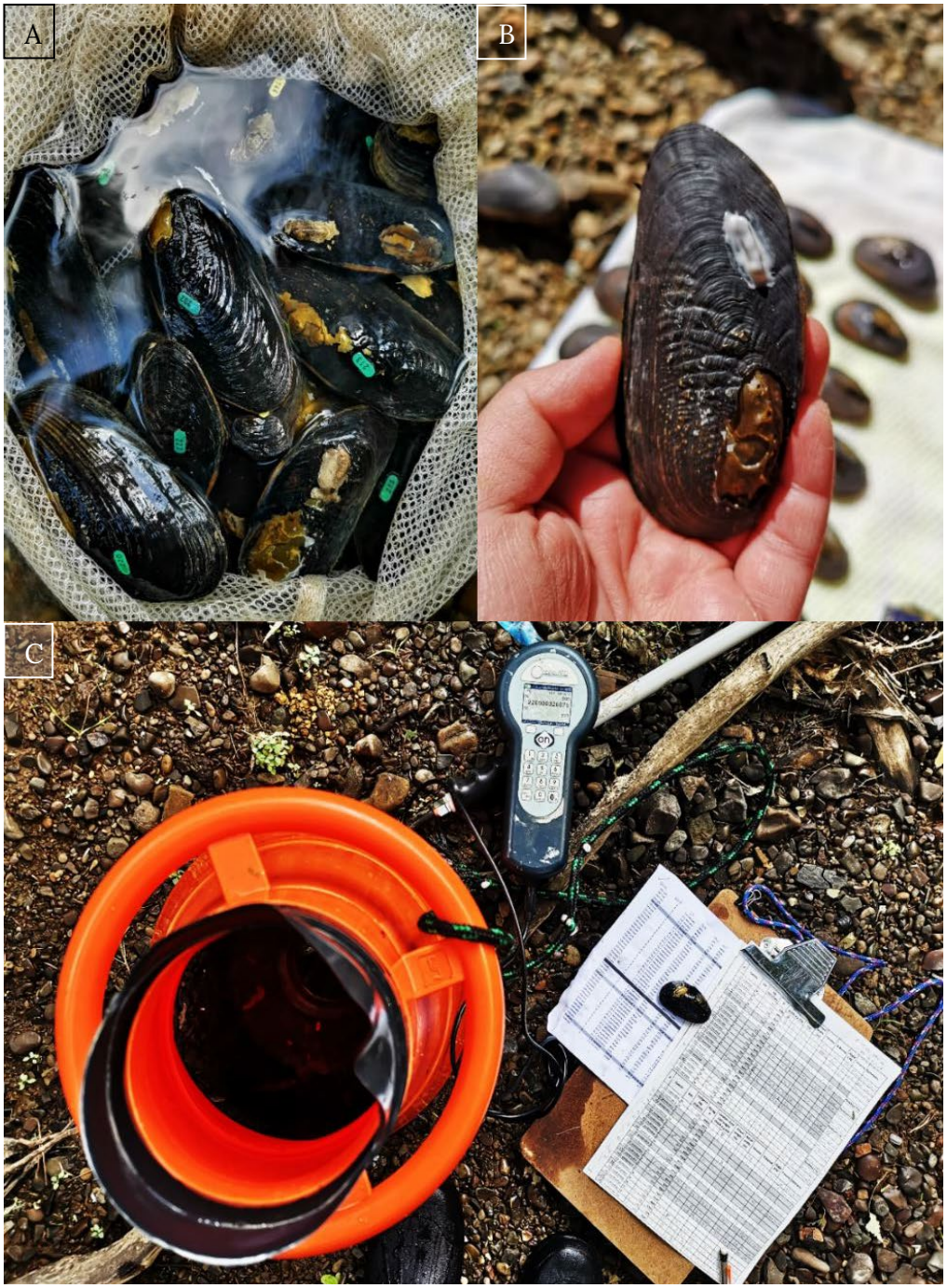

Plate 5.1. Tagged mussels showing (A) glue-on tags within mesh bag (green and yellow tags for *Echyridella aucklandica* and *E. menziesii*, respectively), (B) PIT tag enclosed in dental cement on *E. aucklandica*, C, aquascope, tag reader, wand and survey form used for PIT tagged mussel detection.

5.3.5 Fish surveys

Fish sampling was undertaken on three occasions overlapping with the summer mussel monitoring period (November and December 2018, January 2019), aligning with the known glochidia release periods of both *E. menziesii* and *E. aucklandica* (see Chapter 3 and Chapter 4). Single-pass electrofishing was performed along the entire 60 m mapped summer reach using the EFM300 machine set to 200 volts (NIWA Instrument Systems, Christchurch, New Zealand). Upon capture, fishes were held in 20 L buckets (kept separately for each grid-cell and sub-reach) filled with source water and with battery powered aerators until identified to species level and measured for total length to the nearest millimeter.

5.3.6 Statistical analyses

Analyses were carried out using the software R (version 4.0.0; R Project for Statistical Computing, Vienna, Austria). Data were assessed for normality (Shapiro-Wilk's test and Q-Q plots) and homogeneity of variances (Levene's test). Where parametric assumptions could not be confirmed, even after transformations, non-parametric tests were used.

5.3.6.1 'Summer' survey

I used the *spatstat* (Baddeley & Turner, 2020) and *maptools* (Bivand et al., 2014) packages to determine coincidence between the locations of each mussel species and their respective host fish for the summer sample period. First, I created point pattern data sets from x-y locations of each mussel species and each fish species as a data exploration method to visualise spatial patterns of mussel species and depict any areas of high fish densities per m^2 for grid cells within each 10x5 m sub-reach. Maps created for November and December contain data points for males and females of *E. menziesii* and *E. aucklandica*, as well as displaying density map (with 1 m bandwidths) overlays showing all fish species pooled together. A second set of maps was created for visualisation of spatial distribution patterns using individual fish locations.

Point process models were fit to test the effect of host fish covariates on the spatial density per m^2 of mussels for each species, i.e., the hypothesis that mussel species point patterns are related to host fish density patterns. Models were fit for each mussel species and their respective hosts among the three sub-reaches. The fish data set was separated into host fish species for *E. menziesii* (combined spatial location densities per m^2 of *A. dieffenbachii* and *G. huttoni*) and a surrogate host species for *E. aucklandica* as only one *R. retropinna* was caught. Accordingly, adult *G. maculatus* was used for the purposes of

this analysis because it has shoaling post-larvae that co-occur with *R. retropinna* and has similar adult feeding habits. After creating null models (using the observed density of each unionid species), likelihood ratio tests were used to compare the alternate models to the null models. Goodness-of-fit for all models was evaluated using diagnostic plots for fitted point process models and Berman's tests (Berman, 1986) which compares the observed distribution of the values of a spatial covariate at the data points, and the predicted distribution of the same covariate under the model. December reproductive sampling of brooding mussels showed declines in peak brooding proportions in *E. menziesii* and *E. aucklandica* females, indicating the initiation of glochidia release in both species (see Chapter 3). Because of this, and the flood event in late December leading to reduced sample sizes in January, I chose to focus on the spatial positions of female mussels for the December sample period only.

To test if *E. menziesii* travelled further distances and occurred across a broader range of instream habitat types where they were likely to encounter fish, I first summed the net Manhattan horizontal distances travelled (sum of the absolute differences between two or more point coordinates) by recaptured individuals (which included movements upstream, downstream and bank-ward [perpendicular to the flow]) over the November and December sample events combined. These distances were then compared using a nested ANOVA to determine any differences in the distances travelled between *E. menziesii* and *E. aucklandica* (non-nested factors) using the three summer sub-reaches as replicates (nested factors). I used the same test to determine differences in weekly movement rates between sexes for each species. Next, one-tailed two proportion z-tests for directional movements in the December sample period were used to compare between species, expressed as 1) the proportion of individuals that were found downstream (versus upstream) of the release point, and 2) the proportion of individuals that were found bank-ward (within true-left or true-right bank cells) compared to the mid-channel (defined as the three perpendicular central grid cells). I expected that a greater proportion of *E. menziesii* individuals compared with *E. aucklandica* individuals would move downstream and bank-ward, in linewith hypothesis 1.

Two-sample Kolmogorov-Smirnov (K-S) tests were performed to compare univariate distributions of habitat use and availability in female *E. menziesii* and *E. aucklandica* for velocity, water depth and the substrate size index. A chi-squared test was used on categorical potential refugia type data (categories: 'none', 'undercut bank', 'vegetation', 'debris', 'woody material') to test for non-random use of refugia. Values

from all available grids were pooled from each 10-m sub-reach. As the data collected were arranged in contiguous grid cells across the 60-m summer grid, with the potential for spatial autocorrelation producing more significant results than are justified (Fortin & Dale, 2005), significance thresholds for comparisons between used grid cells and available gridcells were set at $p<0.01$ while all other comparisons were set to thresholds of $p<0.05$.

5.3.6.2 'Winter' survey

I used *spatstat* (Baddeley & Turner, 2020) to 1) determine interpoint spatial patterns of males and females during each species' respective onset brooding time (*E. menziesii*: August; *E. aucklandica*: June), and 2) determine whether male and female relative distances changed over time throughout the sample duration based on Ripley's K cross function (Ripley 1979, 1981; Baddeley & Turner, 2020). Ripley's K-function is a hypothesis test based on the distance of points from each other, and counts the expected number of points of a mark (K) within a given distance (r). The point patterns are then compared to the true value of K (Lambda K(r)) for a completely random (Poisson) point process. Deviations between estimated K(r) and theoretical Lambda K(r) indicate significant spatial clustering or dispersal, comparing the observed point patterns to simulated distributions of points based on an assumption of complete spatial randomness. The resulting plots indicate the spatial pattern (random, clustered, over-dispersed) of marked groups (males vs females) relative to each other at various scales within each sub-reach. From the above point patterns, average nearest-neighbour distances between males and females of each species were calculated for each sample month. Spatial maps contain pooled data points due to low sample sizes within each sub-reach.

As above, monthly horizontal net distance rates were compared between species using a nested ANOVA to determine any differences in the distances travelled between *E. menziesii* and *E. aucklandica* with the three winter sub-reaches as replicates. Because of the smaller sample sizes, Fisher's exact tests (one-tailed) were then used to determine differences in proportions of males and females that had moved downstream or bank- ward during their respective brooding onsets, testing for the hypothesis (H2) that a greater proportion of females than males in both species would occur downstream (rather than upstream) and bank-ward (rather than mid-channel). I conducted Mann-Whitney U tests to investigate whether females of each species travelled further distances downstream than males. Mean percentage shell height protruding above the sediment surface was analysed over time (excluding day 0 [April]) for males and females

of each species using repeated measures ANOVA (including six sampling occasions excluding April) with Geisser-Greenhouse corrections as assumptions of sphericity were not met.

5.3.6.3 Flood effect analysis

To test for differential responses between mussel species to the effects of the flood event, I conducted Mann-Whitney U tests to compare post-flood distances travelled between the two species using distance data from dates October 2018 – February 2019 (post-flood) and from October 2019 - February 2020 (no preceding flood). As above,two-sample Kolmogorov-Smirnov (K-S) tests were performed to compare univariate distributions of habitat use and availability in *E. menziesii* and *E. aucklandica* that were found within each sub-reach after the flood event. A chi-squared test was used on categorical refugia type data to test for non-random use of refugia based on values from all available grid cells pooled from each 10-m sub-reach.

5.4 Results

5.4.1 'Summer' survey

5.4.1.1 Mussel recaptures

Of the 132 marked individuals released within the 60-m summer reach, 109 (76% of *E. menziesii*: *n* = 50, and 89% of *E. aucklandica*: *n* = 59) were recaptured on the first sample event in November (Table 5.3). Recaptures declined for *E. menziesii* to 65% (*n* = 43) in December but remained steady for *E. aucklandica* with 88% (*n* = 58; including two outside of sub-reaches). Following the flood event in late December, recaptures within each sub- reach decreased further for *E. menziesii* to 47% in January. Declines in recaptures also occurred for *E. aucklandica* after the flood event to 66% in January Table 5.3). During the final collection of mussels in February, 44% of *E. menziesii* and 62% *E. aucklandica* individuals were collected within the summer reach, with an additional 14 *E. menziesii* and seven *E. aucklandica* recovered during the 400-m downstream survey (outside sub - reaches and downstream of the boundary of the 60-m summer reach), representing 21% *E. menziesii* and 11% *E. aucklandica* of the originally marked individuals recovered during the downstream survey. Shells of two deadPIT tagged *E. aucklandica* were collected at different locations on dry gravel banks downstream in February. Effects of the summer flood on mussel movement and habitat are analysed further in Section 5.4.3, including comparisons of February data 2 months (2019) and 14 months (2020) after the flood.

Table 5.3. Total recapture percentages and numbers (*n*) of PIT tagged *Echyridella menziesii* and *E. aucklandica* within all three 'summer' sub-reaches, and outside (up or downstream) sub-reach boundaries in November to February 2018/19.

Within sub-reaches		November	December	January	February
E. menziesii	%	76%	65%	47%	44%
	n	50	43	28	25
E. aucklandica	%	89%	84%	62%	61%
	n	59	56	41	40
Outside reach*					
E. menziesii	*n*	0	0	4	14
E. aucklandica	*n*	0	2	3	7
Total*					
E. menziesii	%	76%	65%	47%	59%
	n	50	43	31	39
E. aucklandica	%	89%	88%	66%	71%
	n	59	58	44	47
Dead	*n*	-	-	-	2

Includes final downstream (outside of grid boundaries) surveys for collection of mussels in February.

5.4.1.2 Fish surveys

Through November to January, the most abundant fish species caught by electrofishing within each of the surveyed sub-reaches were *G. maculatus* (mean 6.1 ± 4.7 (SD) per sub-reach), followed by *A. dieffenbachii* and *G. huttoni* (3 ± 1.0 and 2.7 ± 1.1 per sub-reach, respectively). Single *Retropinna retropinna* and *Geotria australis* were captured in November and December, respectively. The number of total fish captured showed little variability across sub-reaches within dates, except for higher densities in sub-reaches 2 and 3 in November due to large shoals of *G. maculatus*. Densities for the most abundant species caught varied over time, with *A. dieffenbachii* and *G. maculatus* showing declines over time within sub-reaches, but *G. huttoni* abundances remaining steady with similar temporal patterns (Appendix A.5.1).

5.4.1.3 Horizontal summer movements

Throughout the summer sampling events, mussels of both species were frequently observed to be moving along the substrate surface (Plate 5.2). Net horizontal distances travelled within all sub-reaches over the peak brooding period (from October to December, for individuals that were recaptured alive at least once prior to the flooding event in late December) averaged 3.9 ± 1.7 (SD) m for *E. aucklandica* (1.5 ± 1.1 m month^{-1}, n = 56, both sexes) and 4.7 ± 2.0 m for *E. menziesii* (1.6 ± 1.2 m month^{-1}, n= 43, both sexes). The two *E. aucklandica* individuals that were recaptured outside of the sub-reach boundaries in December (Table 5.3) travelled 4m and 6 m downstream of sub-reaches 2 and 3, respectively. No statistically significant differences were found in net distances travelled between *E. menziesii* and *E. aucklandica*(nested ANOVA: $F_{(1,4)} = 3.6$, $p = 0.13$), or among sub-reaches for marked individuals of bothspecies ($\chi^2_{(1)} = 0.05$, $p = 0.50$).

Weekly movement rates from the same period for *E. aucklandica* varied between 0.04 and 1.0 m wk^{-1}, averaging 0.50 ± 0.21 m wk^{-1} with no statistically significant differences among sexes ($F_{(1,4)} = 0.45$, $p = 0.54$) or among sub-reaches ($\chi^2_{(1)} = 0.8$, $p = 0.37$). Similarly, *E. menziesii* movement rates varied between 0 and 1.23 m wk^{-1}, averaging 0.59 ± 0.26 m wk^{1}, again, with no evidence of differences between sexes ($F_{(1,4)} = 0.35$, $p = 0.56$) or among sub-reaches ($\chi^2_{(1)} = 0.001$, $p = 0.97$). Across all sub-reaches, the proportion of individuals that moved downstream (versus upstream) in December, prior to the flood, was not found to be significantlygreater in *E. menziesii* compared to *E. aucklandica*, with 93% of *E. menziesii* and 89% of *E. aucklandica* individuals retrieved downstream from their original positions (z^1_1 =0.08. $p = 0.38$, $n_1 = 43$, $n_2 = 56$). Furthermore, across all sites, bank-

ward movements (i.e., towards the true right or true left of sub-reaches) were exhibited in both species, with mean bank-ward displacements of 1.63 ± 1.09 m for marked *E. aucklandica* and 2.17 ± 1.28 m for *E. menziesii* individuals across all sites. As above, proportions that moved bank-ward were not found to be significantly greater in *E. menziesii* (73%) than *E. aucklandica* (63%; $z^1_1 = 1.08$, $p = 0.15$ $n_1 = 43$, $n_2 = 56$).

5.4.1.4 Summer spatial patterns of mussels and fish

Spatial sampling of unionids in November showed dispersed distributions from their original release positions (red X within each sub-reach map, Figure 5.1) in males and females of both species, with particularly high dispersion in sub-reaches 2 and 3 (Figure 5.1, G2a, G3a). Sub-reach 1 showed highest densities of individuals remaining clustered around the original point of release, with some dispersion towards the true-right bank, particularly by some female *E. menziesii* (Figure 5.1 G1a). During December sampling, individuals in sub-reaches 1 and 3 exhibited bank-ward aggregations while in sub-reach 2 individuals remained more scattered with minimal clusters of female *E. menziesii* foundto be positioned near bank habitats (Figure 5.1 G1b, G2b, G3b).

Plate 5.2. *Echyridella menziesii* moving along the substrate surface within one of the three sub-reaches during the summer survey.

Fish captures varied spatially within each sub-reach, occurring in various densities both in mid-channel and bank grid cells, with some spatial overlap observed between densities of fish and both mussel species (Figure 5.1. G1c, G2c, G3c). The two known host fish species analysed for *E. menziesii*, *G. huttoni* and *A. dieffenbachii*, were captured in higher average densities along bank grid cells than mid-channel cells within all sub-reaches (*G. huttoni*: $t_{(4)} = 7.5$, $p = 0.002$; *A. dieffenbachii*: $t_{(4)} = 3.5$, $p = 0.03$). *G. maculatus* was found in both mid-channel and bank habitats within all grids, with no differences in average fish densities between locations ($t_{(4)} = 0.22$, $p = 0.83$; Appendix A.5.2). The one specimen of *R. retropinna* caught was found within a mid-channel cell in sub-reach 2 (Figure 5.1, G2c).

Likelihood ratio tests from univariate models containing host fish densities as covariates within all three sub-reaches showed significant positive associations and better fit over the null model for *E. menziesii* within all sub-reaches in December (Table 5.4) prior to the flood, supporting the hypothesis that female *E. menziesii* densities would be greater in habitats that overlap with the density of their host fish, in this case *G. huttoni* and *A. dieffenbachii*. Significant associations between *G. maculatus* and *E. aucklandica* point patterns were found within sub-reach 1 but not sub-reaches 2 and 3 (Table 5.4).

Table 5.4. Inhomogeneous point Poisson process model intercepts, coefficients, and likelihood ratio test results (deviance and p-value) for each mussel species' spatial association with the density of host fish (*Echyridella menziesii* – *A. dieffenbachii* and *G. huttoni*; E. *aucklandica* – *G. maculatus* [surrogate]). Significant relationships are shown in bold.

Sub-reach	Species	Intercept	Coefficient	Deviance	*p*-value
1	*E. menziesii*	-0.22	0.71	8.82	**0.002***
	E. aucklandica	-2.22	1.86	8.44	**0.03***
2	*E. menziesii*	-0.61	1.24	5.81	**0.02***
	E. aucklandica	-1.37	1.65	2.40	0.49
3	*E. menziesii*	-0.79	1.29	5.97	**0.01***
	E. aucklandica	-0.42	1.42	8.95	0.34

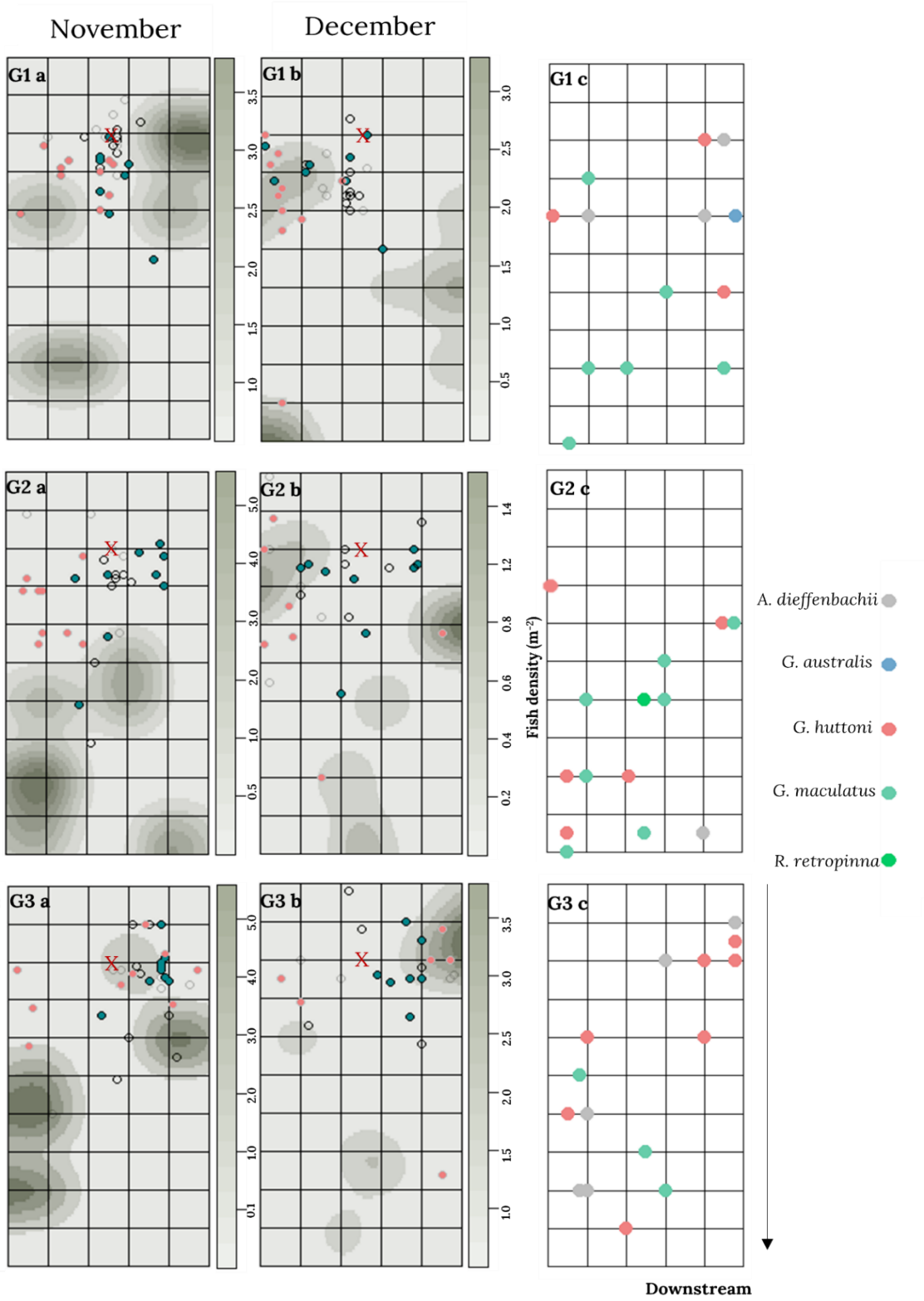

Figure 5.1. Location maps of *Echyridella aucklandica* males (black open circle) and females (turquoise points), and *E. menziesii* males (grey open circles) and females (pink points) in November (a) and December (b) within three 10x5 m sub-reaches (G1-G3), with each square representing a m^2 grid cell. X marks the cell location at which individual mussels were released in October. Each map contains a layer of calculated isotropic kernel (1 m bandwidth) density estimates of all fish (m^{-2} in grey with darker shades indicating higher density). Mapped point patterns of fish species present within each of the three sub-grids pooled across November and December are shown in c (some overlapping points not shown due to being found in same location).

Univariate frequency distributions of habitats used by females of both mussel species were significantly different to corresponding distributions of available habitat for depth (*E. aucklandica* $D = 0.44$ $n_1 = 21$, $n_2 = 147$, $p<0.001$; *E. menziesii* $D = 0.52$ $n_1 = 25$, $n_2 = 147$, $p = 0.001$) and refugia type (*E. aucklandica* $\chi^2 = 31.2_{(4)}$ $n_1 = 21$, $n_2 = 147$, $p<0.001$; *E. menziesii* $\chi^2 = 20.5_{(4)}$ $n_1 = 25$, $n_2 = 147$, $p<0.001$). In contrast, neither velocity nor substrate size significantly affected spatial occurrence of females for either species compared to the habitat available (all $p>0.07$; Figure 5.2; Appendix Table A.5.3). Comparing between mussel species, similar habitat distributions were observed for both *E. menziesii* and *E. aucklandica* females for all variables assessed. Accordingly, females were mainly found

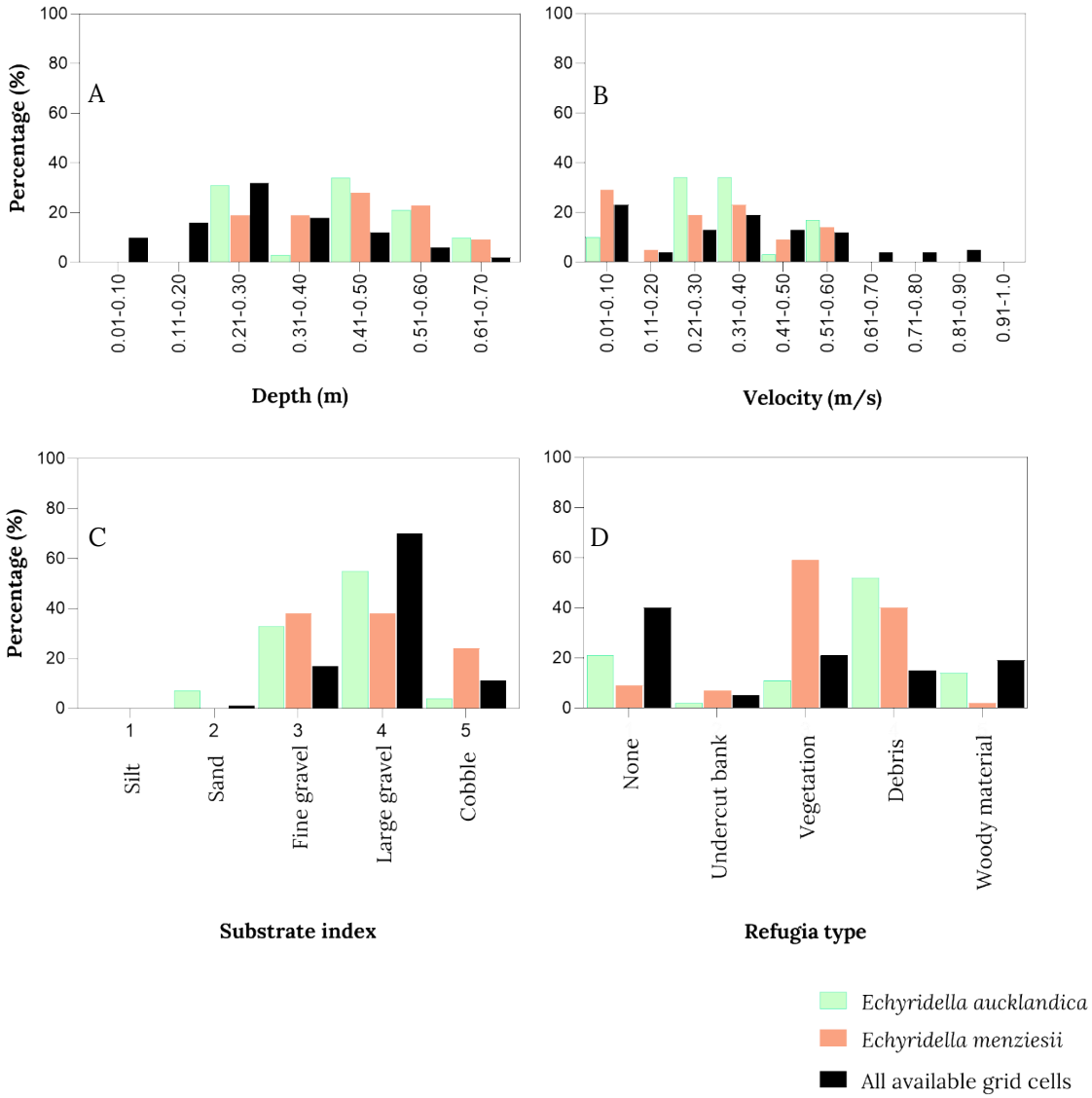

Figure 5.2. Habitat data distributions for *Echyridella aucklandica* and *E. menziesii* comparing (A) depth (m), (B) velocity (m/s), (C) substrate type, and (D) refugia type in which both species were found with all grid cells available.

within fine and large gravel substrates, more frequently within refugia types associated with overhanging vegetation and debris, only at depths above 0.2 m and in velocities ranging between 0.01 m/s and 0.60 m/s.

Individuals of G. *huttoni* and A. *dieffenbachii* were caught in similar substrate types to E. *menziesii*, dominated by fine to large gravel, and within velocities <0.60 m/s, but both fish species were found spread among all refugia types. Contrasts in depth distributions between G. *huttoni* and E. *menziesii* were apparent, with G. *huttoni* distributed in much shallower habitats in comparison to E. *menziesii* (Figure 5.3). Habitat use by G. *huttoni* did not significantly differ for velocity and substrate size index (both variables $p>0.05$; Appendix Table A.5.4), but differences in depth ($D = 0.72$ $n_1 = 21$, $n_2 = 147$, $p = 0.01$) and refugia type ($\chi^2 = 56.5_{(4)}$ $n_1 = 18$, $n_2 = 147$, $p<0.0001$) were significant when compared to use of those habitats in E. *menziesii* (Figure 5.3). Except for refugia type ($\chi^2 = 34.4_{(4)}$ $n_1 = 18$, $n_2 = 147$, $p<0.0001$; Figure 5.3), habitat use in A. *dieffenbachii* did not differ when compared to E. *menziesii* (all $p>0.05$; Appendix Table A.5.4).

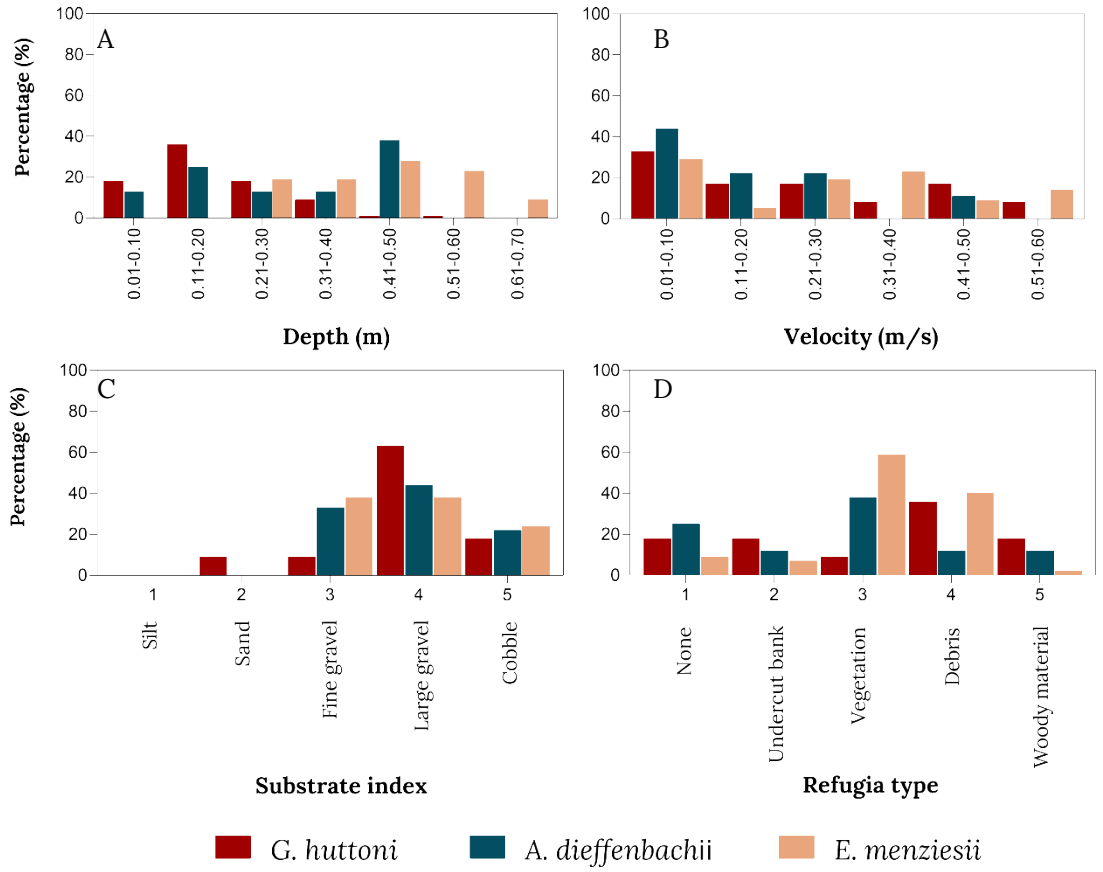

Figure 5.3. Habitat data distributions for host fish species of *Echyridella menziesii*, comparing (A) depth (m), (B) velocity (m/s), (B) substrate index, and (D) refugia type in which species were found with all grid cells available.

Habitat distributions of the surrogate host *G. maculatus* were significantly different when compared to habitat use in *E. aucklandica* for all variables assessed (depth, velocity and refugia type $p<0.0001$; substrate type $p = 0.01$; Figure 5.4;). *Galaxias maculatus* were found in shallower depths, associated with a narrower substrate range, and spread across a wider range of velocities. This fish also occurred within grid cells that contained either no apparent refugia type or in grid cells with overhanging vegetation, while *E. aucklandica* was most associated with debris (Figure 5.4).

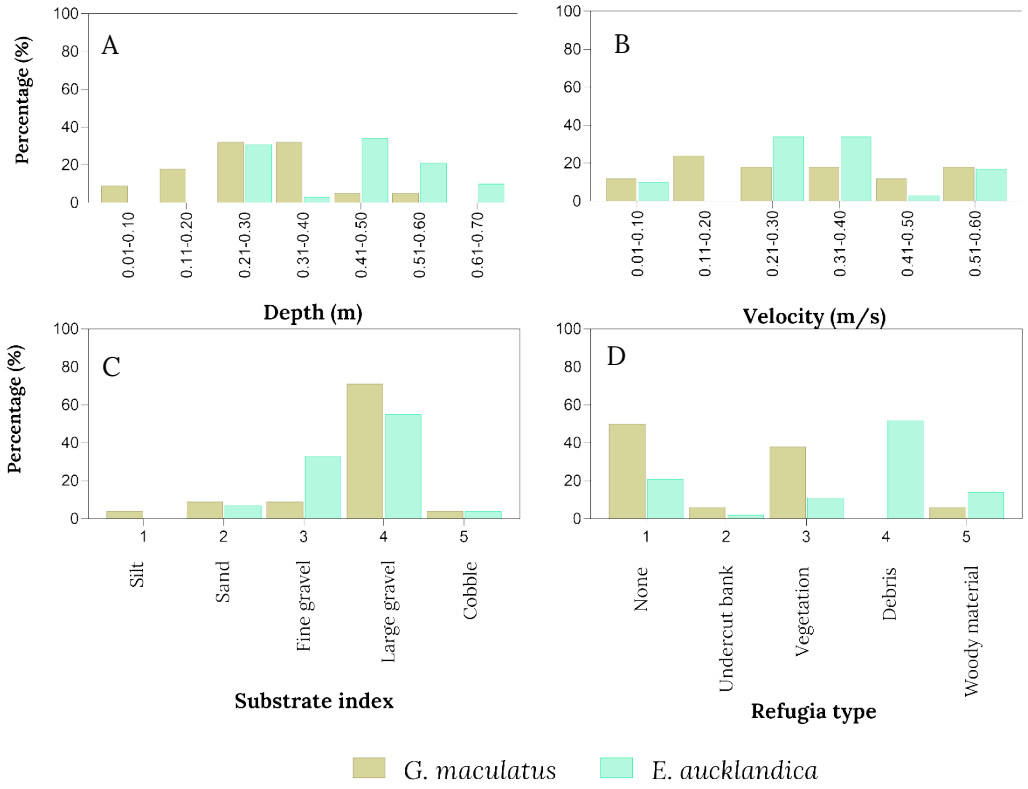

Figure 5.4. Habitat data distributions for surrogate host fish *Galaxias maculatus* and *Echyridella aucklandica* comparing (A) depth (m), (B) velocity (m/s), (B) substrate index, and (D) refugia type in which species were found with all grid cells available.

5.4.2 'Winter' survey

5.4.2.1 Mussel recaptures

Of the 84 marked individuals released within the three winter sub-reaches, a total of 67 (79% of *E. menziesii*: $n = 33$, and 81% of *E. aucklandica*: $n = 34$) were recaptured (including outside of the three sub-reaches) on the first sample event in May, with recaptures steadily declining in October to 69% for both species (Table 5.5). For the February 2020 collection, higher percentages of both species were recaptured up to 83 m downstream

of the 60-m reach than within each of the sub-reaches (Table 5.5), despite there being no bed-moving flood event prior to sampling (see Section 5.4.3).

Table 5.5. Total recapture percentages and numbers (*n*) of PIT tagged *Echyridella menziesii* and *E. aucklandica* within all three 'winter' sub-reaches, and outside (up or downstream) sub-reach boundaries from May to October 2019, and a final collection inFebruary 2020.

Within sub-reaches		May	June	July	August	September	October	February
E. menziesii	%	76%	57%	55%	52%	55%	50%	19%
	n	32	24	23	22	23	21	8
E. aucklandica	%	81%	74%	79%	76%	69%	62%	29%
	n	34	31	33	32	29	26	12
Outside sub-reach*								
E. menziesii	*n*	1	8	9	10	8	8	18
E. aucklandica	*n*	0	0	0	1	1	3	21
Total*								
E. menziesii	%	79%	78%	76%	76%	74%	69%	62%
	n	33	32	32	32	31	29	26
E. aucklandica	%	81%	74%	79%	79%	71%	69%	78%
	n	34	31	33	33	30	29	33

*Includes final ~ 400 m downstream collection of tagged mussels

5.4.2.2 Winter spatial patterns of mussels

Recaptured individuals of both sexes in *E. aucklandica* showed minimal dispersion throughout the brooding season, with few individuals recovered far from their original release site, except for three females and one male that had travelled up to 4 m downstream over the May-October sample period (i.e., not in February when some had moved longer distances downstream) (Figure 5.5). Ripley's K cross function for interpoint interaction patterns detected clusters at all distances between male and female *E. aucklandica* that were significantly different from complete spatial randomness (Figure 5.6). *Echyridella aucklandica* remained aggregated throughout the entire brooding onset period (June-October), with low male-female pair mean nearest neighbour distances (0.08 ± 0.01 (SD) m, range 0.01 m to 1.6 m), and only slight increases in distances from April, when mussels were first released, to June (Figure 5.5; Figure 5.7).

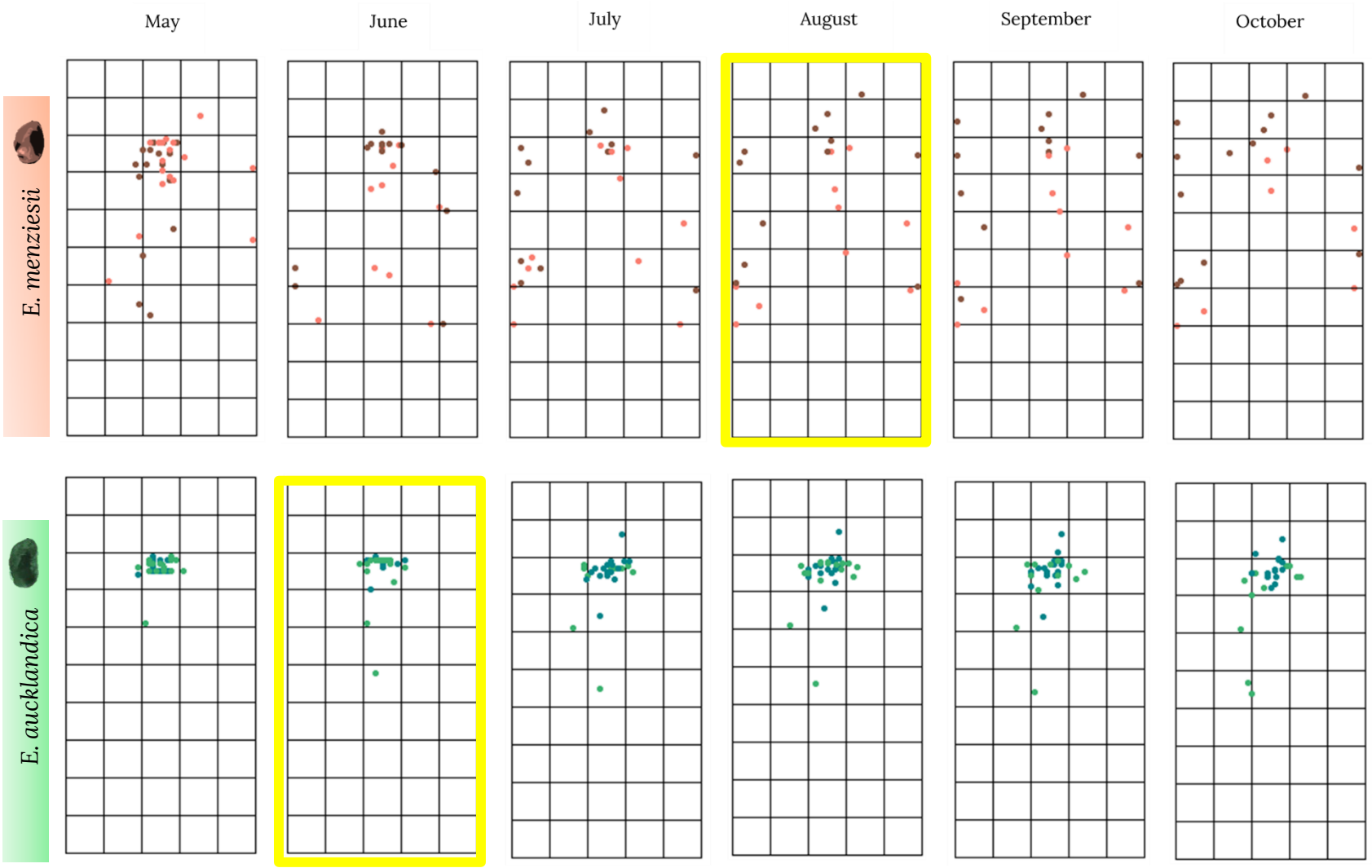

Figure 5.5. Location maps of *Echyridella aucklandica* males (dark turquoise points) and females (light turquoise points), and *E. menziesii* males (dark red points) and females (light red points) from May to October pooled across three 10x5 m sub-reaches, with each square representing a m^2 grid cell. Timing of brooding onset highlighted in yellow for each species. Some points in some plots are not visible due to overlap with other points.

Both sexes in *E. menziesii* showed increasing spatial dispersion over time from their original release location, with individuals recovered upstream, downstream and bank-ward from May through to October (Figure 5.5). Nevertheless, Ripley's K cross function detected clusters that were significantly different from complete spatial randomness for male and female *E. menziesii* points during brooding onset in August (Figure 5.6). Average nearest neighbour interpoint distances between male and female *E.menziesii* increased through time from April to October, with the exception of a decline in June when overall mean distances between *E. menziesii* males and female remained relatively low at 0.13 ± 0.03 m (range 0.01-0.75 m; Figure 5.7).

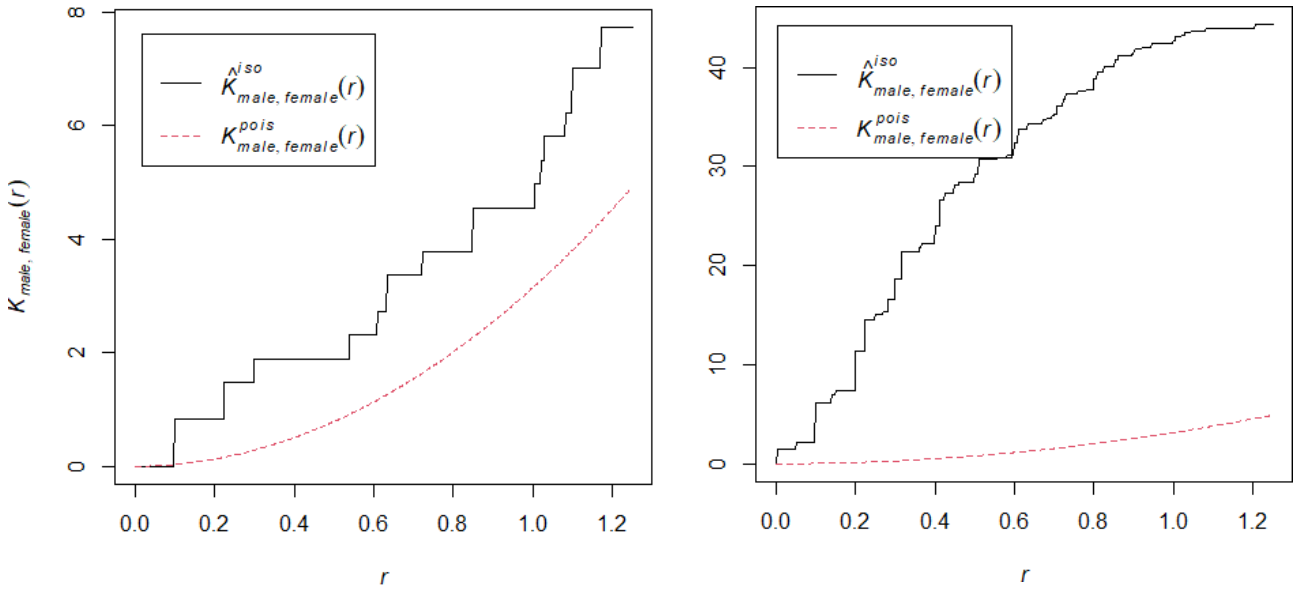

Figure 5.6. Estimated Ripley's K cross function for interpoint patterns of female and male *Echyridella menziesii* in August (left) and *E. aucklandica* in June (right), with isotropic edge correction implemented for rectangular sub-grids. Lambda K(r) equals the expected number of additional random points within a distance r for a typical random point of X. Deviations between estimated K (black line) and theoretical K (red dotted line) suggest significant spatial clustering or dispersal. Lines above the red dotted line suggest clusters between sexes while lines below equal dispersion between sexes.

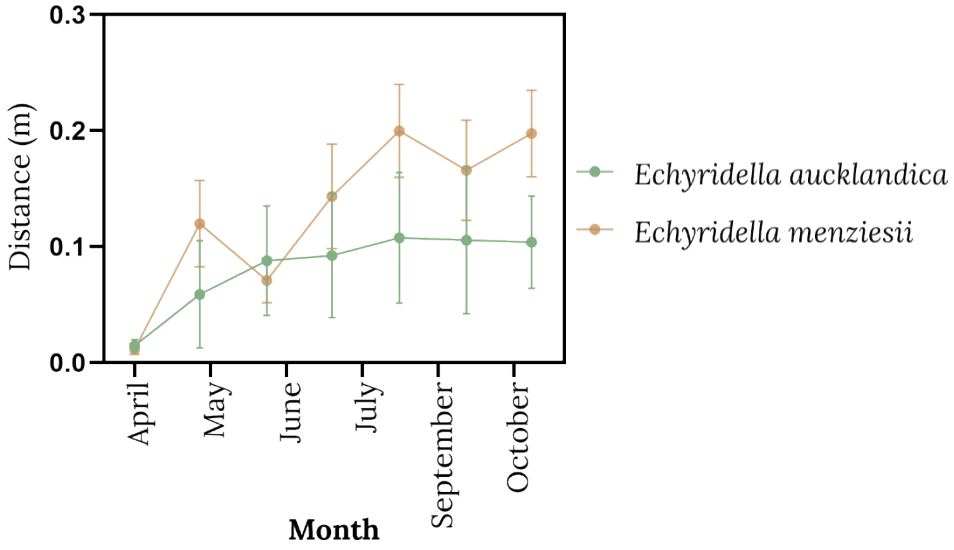

Figure 5.7. Mean (± SD) nearest neighbour distances between point pairs of *Echyridella aucklandica* and *E. menziesii* (males and females combined) from April to October 2019.

5.4.2.3 Vertical and horizontal winter movements

Mean percentage shell height above the sediment surface for recaptured individuals varied over time in both species and sexes following initial decreases in the first sample month following the April introduction of tagged individuals at 50% above the surface. Percentage shell heights remained <50% for *E. aucklandica*, with the highest shell height percentages at the surface found in October for both males (44 ± 8.3 SD %) and females (45 ± 3.8%) (Figure 5.8). Shell heights in *E. menziesii* showed greater variability over time with more males typically protruding from the surface (Figure 5.8). In contrast, *E. menziesii* shell height percentages in females protruded the most in October (50 ± 10.5%). Although both species and sexes (except male *E. menziesii*) showed general increases in mean shell height over September and October, repeated measures ANOVA did not detect statistically significant differences between months (from May to October) in male or female *E. aucklandica* (males: $F_{(1.3,2.6)} = 2.97$, $p = 0.20$; female: $F_{(1.3,2.6)} = 2.82$, $p = 0.21$) or *E. menziesii* (male: $F_{(1.2,2.4)} = 1.4$, $p = 0.35$; female: $F_{(1.2,2.4)} = 0.97$, $p = 0.44$)

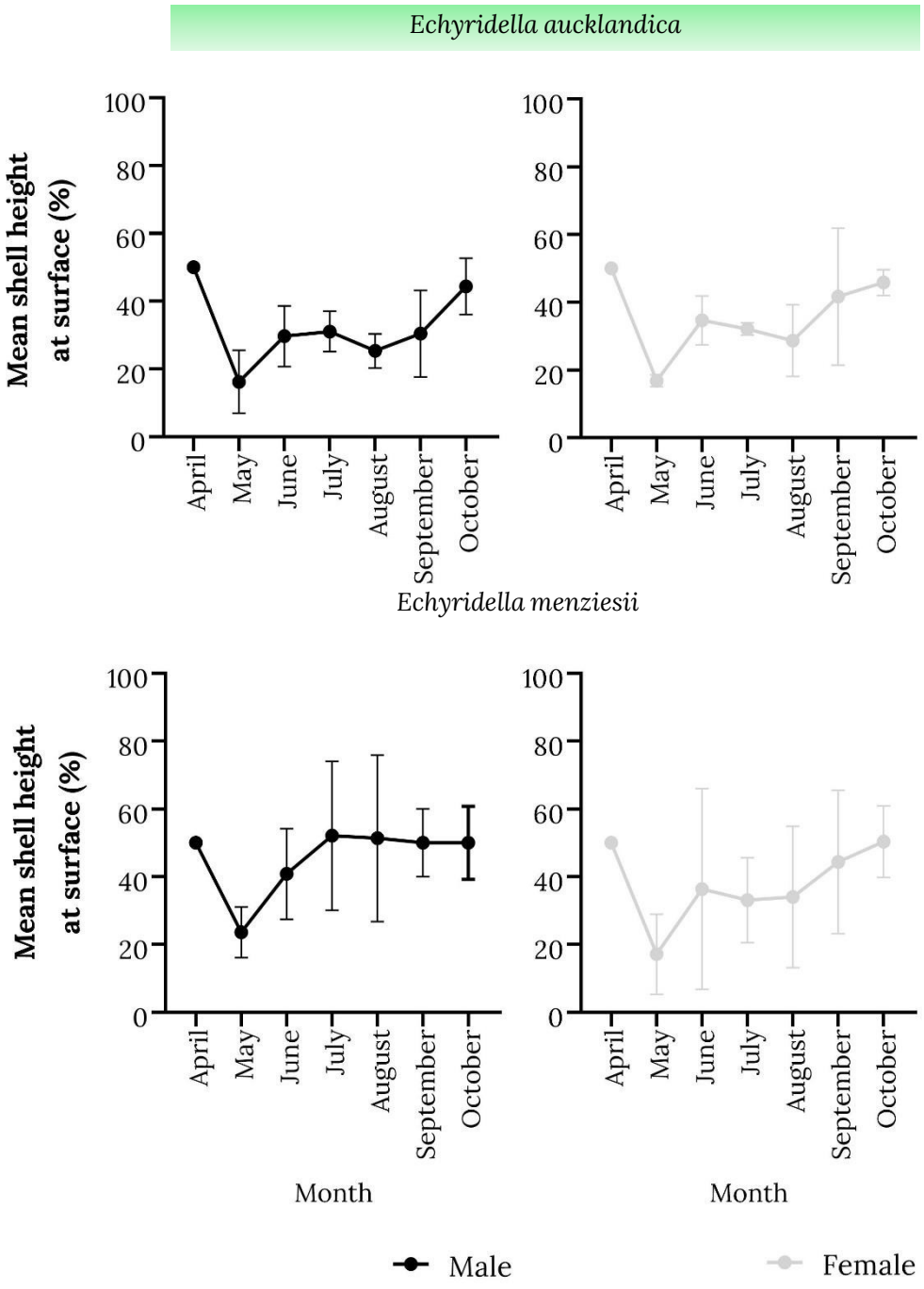

Figure 5.8. Mean (± SD) percentage of shell height above the sediment surface within each sub-reach for *Echyridella aucklandica* and E. menziesii males and females from April (when they were placed at 50% depth) to October.

In terms of horizontal movement, both mussel species, particularly *E. menziesii*, were observed to be relatively mobile (although much less than in summer) during the cooler winter sample months, at times showing long sediment tracks with erratic horizontal movement patterns along the sediment surface (Plate 5.3). Average net horizontal monthly travel rates differed significantly between species (nested ANOVA: $F_{(1,56)}$ = 15.9, p = 0.002) but not among sub- reaches (p>0.9) for marked individuals. *E. menziesii* travelled further per week (0.29 ± 0.18m) than *E. aucklandica* (0.12 ± 0.17 m per week) which remained closer and more aggregated around their original points of release throughout the entire winter sample period (Figure 5.5).

During their respective brooding seasons, starting in June for *E. aucklandica* and August in *E. menziesii*, no differences were detected between the proportion of males or females of either species found upstream or downstream from their original point of release (Fisher's exact test: *E. aucklandica*, p>0.9; *E. menziesii*, p = 0.29). Similarly, no significant differences were found between the proportions of males and females of either species found in bank versus mid-channel locations (Fisher's exact test: *E. aucklandica*, p = 0.53; *E. menziesii*, p = 0.59). Thus, similar proportions of *E. menziesii* males (41.2%) and females (36.8%) moved bank-ward (rather than remaining in mid-channel), while the opposite was observed for *E. aucklandica* which had low percentages of both males (6.7%) and females (10.5%) recorded in bank-ward grid cells during the winter onset brooding period.

Plate 5.3. Tracking pattern by an individual female *Echyridella menziesii* observed during the winter survey.

5.4.3 Flood event effects

The flood event in December 2018 was associated with reduced January recaptures of mussels from 50% prior to the flood, down to 19% in *E. menziesii*, and 62% to 29% in *E. aucklandica*. No significant differences were detected between species in the distance rates travelled by recovered mussels post- flood (January and February) compared to pre-flood (December) (Mann-Whitney U, 1070, $p<0.18$, n = 68), with recovered *E. menziesii* found at median distances of 3.2 m downstream compared to *E. aucklandica* median distances of 2.45 m after the flood (compared to median movement patterns of 1.6 m month^{-1} in *E. menziesii* and 1.5 m month^{-1} in *E. aucklandica*. One individual *E. menziesii* was displaced 349 m downstream, with the furthest *E. aucklandica* individual found 46 m downstream in February 2019.

Recaptures from October 2019 to February 2020 when there was no preceding flood, declined from 69% to 62% for *E. menziesii* and increased from 69% to 78% for *E. aucklandica*. Notwithstanding the recovery of an individual mussel 83 m downstream of its sub-reach in February 2020, median distances travelled by both species were significantly lower in during the time period October 2019 to February 2020, when there was no preceding flood event, compared to the period October 2018 to February 2019 when a flood event occurred (*E. aucklandica*: Mann Whitney U = 317.5, $p<0.001$, $n_1 = 33$, $n_2 = 47$; *E. menziesii* = U: 950.5, $p = 0.02$, $n_1 = 26$, $n_2 = 39$).

Of the mussels that did not get displaced downstream and were recaptured within the summer reach during January and February 2019, significantly more individuals of both species were found in bank habitats than in mid-channel (98% for *E. aucklandica*; z^1_1 =35.2. $p<0.001$, n = 41) and 97% for *E. menziesii* ($z^1_1 = 23.3$, $p<0.001$, n = 28). Furthermore, habitat frequency distributions for individuals that withstood the flood showed that both species occupied significantly different habitats relative to those that were available for depth, velocity, substrate composition, and refugia type (all $p<0.03$; Appendix Table A.5.3). Accordingly, most post-flood *E. menziesii* were found at 0.5-0.6 m depth, velocity of 0.01- 0.1 m/s, and in fine gravel substrates associated with debris, while most *E. aucklandica* was found post-flood at depths of 0.3-0.4 m, velocity of 0.01-0.1 m/s, and in finer gravel substrates associated with a range of refugia including undercut banks, overhanging vegetation and debris (Figure 5.9).

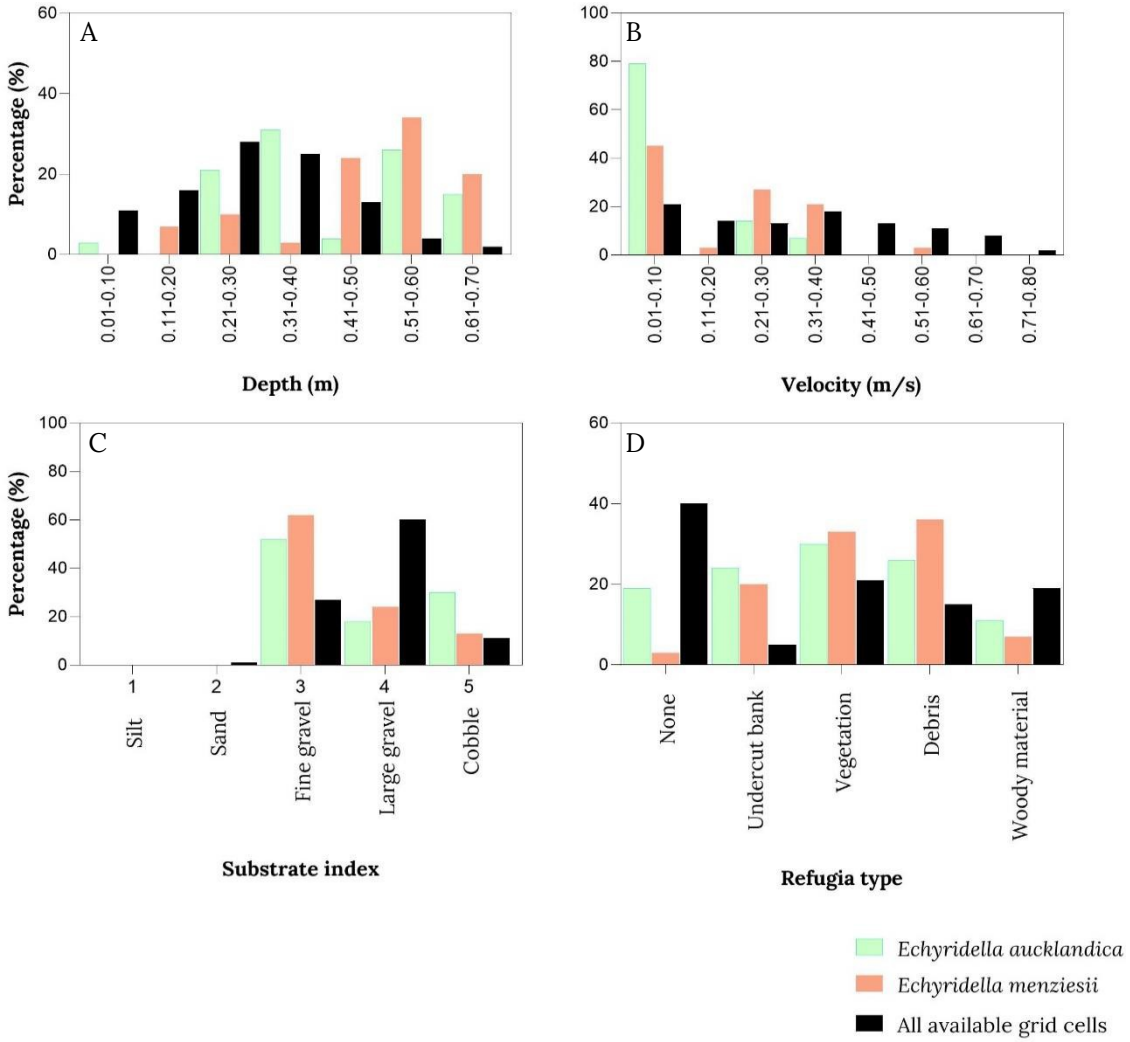

Figure 5.9. Post-flood habitat frequency distributions for *Echyridella aucklandica* and *E. menziesii* comparing (A) depth (m), (B) velocity (m/s), (C) substrate type, and (D) refugia type in which both species were found with all grid cells available.

5.5 Discussion

This chapter analyses fine spatial scale (1 m^2 grid cells) movement patterns of tagged *E. aucklandica* and *E. menziesii* during two critical phenological phases of brooding onset in winter and glochidia release in summer within a Waikato stream. Overall, I foundevidence in both species of 1) relatively high net horizontal movement rates (compared to other mussel species, see below) and active bank-ward cluster formation during the glochidia release season, and 2) increasing percentage shell height above the sediment surface over time during the winter brooding period, although shell height changes were not statistically significant. There was, however, no evidence of downstream aggregations of females relative to males during each species' respective brooding periods. Nonetheless, throughout the duration of the winter survey, *E. aucklandica* and *E. menziesii* males and females remained densely aggregated, with lowest male-female distances detected during brooding onset. Generally, these findings are consistent with movement patterns that increase chance of fertilisation during brooding onset and host fish encounter during glochidia release, at least for *E. menziesii*. I was unable to effectively test host spatial association for *E. aucklandica* because catches of its only known host were low.

Changes in mussel distribution observed following a moderate flooding event provided mixed evidence for interspecific differences in resistance to bed-moving floods, in particular the hypothesis that larger and more firmly-anchored *E. aucklandica* would be more resilient to a flood of this magnitude. Greater resistance in *E. aucklandica* comparedto *E. menziesii* was supported in terms of the maximum distances individuals were recovered post-flood, but not in terms of distances travelled pre- and post-flood or in years with and without a preceding flood. Post-flood habitat use mapping for both mussel species supported the role of flood flow refugia associated with riparian vegetation and debris in bank habitats in providing resistance to the effects of the bed- moving flood that occurred in this study.

These findings should be interpreted with a number of caveats. Firstly, observations in the present study are based on fine-scale movements within relatively homogenous sub-reaches for tagged individuals of *E. menziesii* and *E. aucklandica* that were part of a larger mussel population in an open system. The sub-reaches selected were broadly representative of wadeable sections of the stream generally but did not include deeper pools or areas with high wood accumulations. Secondly, this intensive study was conducted during the day over single seasons for two critical phenological periods in one tream, so the spatial and temporal applicability of these results is limited. Finally, an

important aspect of such studies is understanding the detectability and recapture of tagged individuals. Although recapture rates remained relatively high throughout both summer (prior to the flood) and winter surveys, steady declines were observed for both species over time, yielding recapture rates of 76-89% initially, to 56-78% at the end of the study. These recapture results were lower in comparison to other studies that have used PIT tagging as a method for mark and recapture, for example recaptures of 72-80% for individuals of *Lampsilis radiata radiata* between 10 and 23 months after release (Kurth et al., 2007), near 100% detection of individuals during seven sample events over a 2-year period (Hua et al., 2015), and 83% recovery during 17 sample events over 3 years following a short distance relocation experiment (Tiemann et al., 2016). Other than downstream displacement due to high flow events, mussels that remained undetected in the present study may have either buried deeper in the substrate or moved into crevices where they were not detectable by the PIT tag reader, as suggested in the study by Zajac and Zajac (2011).

5.5.1 Male-female vertical and horizontal aggregation during brooding

To ensure fertilisation, feeding, growth and glochidia release, mussels that have burrowed into sediments need to emerge to the surface (Negus, 1966; Watters et al., 2001; Saarinen & Taskinen, 2003; Schwalb & Pusch, 2007). In terms of vertical movement, the pattern observed in the present study of *E. menziesii* and *E. aucklandica* protruding at greater heights from the sediment surface during their respective brooding onsets and later glochidia development stages is consistent with a behaviour aimed at increasing reproductive success, as reported elsewhere (Burla et al., 1974; Amyot & Downing, 1997; Rogers et al., 2001; Watters et al., 2001; Perles et al., 2003). A comparable seasonal trend in vertical movement, particularly for females from winter (brooding onset) to spring (onset of glochidia release) has as reported in other species (Saarinen & Taskinen, 2003; Schwalb & Pusch, 2007; Balfour & Smock, 1995; Amyot & Downing, 1997; Negishi et al., 2011; Zieritz et al., 2014).

Burrowing ability may be affected by abiotic factors such as substrate type and temperature. For example, a number of laboratory and field studies have found that burrowing abilities were greater in smaller substrate types such as sand in comparison to larger gravel in several unionid species (Lewis & Riebel, 1984; Lara & Parada, 2009; Hernandez, 2016). The study reaches within the current study had little sand substrate available, but both mussels were found to move to or stay within smaller

substrate types than were available. Several studies have also suggested that temperature affects burrowing ability. Block et al. (2013) found that burrowing activity was halted at colder temperatures in *Potamilus alatus* in Lake Kentucky, while Hernandez (2016) found that burrowing stopped for A. *plicata* and Q. *aurea* between 12 and 15°C, suggesting a significant cost of burrowing at low temperatures which would be expected during the winter brooding sampling in the present study (see Chapter 3).

Although temporal changes in vertical movement were observed in the present study they were not statistically significant, in part due to female *E. aucklandica* and *E. menziesii* burrowing and re-emerging several times from June to October, a behaviour that has also been observed in other species. For example, this behaviour was reported for *Unio timidus*, *U. pictorum* and *Anadona anatina* in the River Spree, where it was attributed to timing of egg fertilisation and glochidia release (Schwalb & Pusch, 2007). *E. menziesii* males displayed consistently higher shell height percentages above the surface throughout the brooding season, potentially due to a lack of distinct seasonality in gametogenesis, as seen in Lake Taupo where continuous spawning and sperm storage throughout the year was observed in male *E. menziesii* (Clearwater et al., unpublished), and reported elsewhere for other unionids (Byrne, 1998; Wacker et al., 2018). Unlike other species with distinct seasonal spawning patterns, *E. menziesii* males may have developeda more opportunistic strategy that involves mussels protruding above the substrate surface for longer rather than emerging and re-emerging vertically at certain times of the year. Gametogenesis in *E. aucklandica* is yet to be investigated and may shed a light on these contrasting behaviours.

Throughout the duration of the winter survey, horizontal aggregations remained stable for both sexes of recaptured *E. aucklandica*, while the distribution of *E. menziesii* males and females became more random over time. However, both *E. menziesii* and *E. aucklandica* males and females appeared to exhibit spatial clustering, with the lowest male-female nearest neighbour distances detected during each species' respective brooding onsets, supporting the proposition that aggregation behaviour may be an adaptation that facilitates fertilisation success during the spawning period (Amyot & Downing, 1998; Rogers et al., 2001; Watters et al., 2001; Perles et al., 2003). While *E. aucklandica* remained highly aggregated during the initial sample periods and stayed clustered through time, *E. menziesii* increasingly dispersed over time and throughout the early stages of brooding in females. Increasingly random and erratic spatial dispersion throughout the winter survey may have been an artefact of the habitat heterogeneity andavailability of sub-optimal habitat within the study reaches, potentially

leading to movement in search of suitable habitat conditions.

Such erratic movement patterns have been attributed to habitat acquisition elsewhere, with mussel species actively seeking flow refugia (Strayer, 1999; Stegmann, 2020) and deeper water (Newton et al., 2015; Sullivan & Woolnough, 2021), as was evident for *E. menziesii* which was found in deeper waters than *E. aucklandica* (see Figures 5.2 and 5.9). Other studies have related erratic movement patterns to food resource acquisition (Bovbjerg, 1957) as part of the feeding mechanism in some species (McMahon, 1991; Schwalb & Pusch, 2007). Whether related to habitat or food acquisition, seasonal patterns in horizontal movement behaviour for both species were largely concordant with previous studies (Amyot & Downing, 1998; Zieritz et al., 2014) in that horizontal movement remained high during high temperatures in summer during glochidia release and decreased throughout brooding onset in winter, even with *E. menziesii*'s high activity in the winter period.

5.5.2 Horizontal movement patterns in relation to glochidia release

Horizontal movements were analysed in relation to net distance moved and direction travelled between sampling events. During the summer glochidia release period, females of both *Echyridella* species travelled comparable net distances, mostly towards stream banks from their mid-channel introduction sites. Net movement rates averaging 0.5-0.6 m wk^{-1} over the summer for *E. aucklandica* and *E. menziesii* are at the higher end in comparison to other studies which have reported movements rates from 0.15–0.54 m wk^{1} (Burla, 1971; Burla et al., 1974; Balfour & Smock, 1995; Amyot & Downing, 1997; see Schwalb& Pusch, 2007 for summary of distances travelled by six other species). As with vertical movement patterns, active horizontal movements in both *E. menziesii* and *E. aucklandica* during glochidia release are plausibly associated with reproduction (Amyot & Downing, 1998; Asher & Christian, 2012). Accordingly, results of the present study support part of the original hypothesis (H1) that, due to its broadcast glochidia release strategy, host–generalist *E. menziesii* would move into habitats that spatially overlap with host fish to maximise probability of glochidia infestation. As noted above (Section 5.5.1), horizontal movement may also be partly attributable to short-term habitat searching behaviour (Dunn et al., 2000; Bolden & Brown, 2002; Morales et al., 2004; Stegmann, 2020).

Movement of *E. menziesii* generally occurred towards bank habitats that overlapped with high host fish densities (*G. huttoni* and *A. dieffenbachii*). However, specific depth, velocity, substrate and refugia habitats selected by *E. menziesii* overlapped more with *A. dieffenbachii* habitat than with *G. huttoni* which was only found to overlap

with refugia type and water depth used by *E. menziesii*. Although *A. dieffenbachii* is a recorded host for *E. menziesii*, *G. huttoni* has in previous studies been found to have higher infestation prevalences and intensities in comparison to *A. dieffenbachii* (see Chapter 4 of this thesis and Hanrahan, 2019). However, it must be remembered that sampling was conducted during the day when many native fish are less active. Notably, *G. huttoni* is known to undergo a diel shift in habitat use, selecting a wider range of habitats when studied both at night and during the day (McEwan & Joy, 2015), highlighting that results of fish positions acquired for the present study only partly reflects actual diel habitat use. As noted above, mussel-host spatial coincidence could not be tested for *E. aucklandica* due to low catches of its host *R. retropinna*. The use of *G. maculatus* as a surrogate host fish species, because of its similar feeding and swimming behaviours, showed no significant spatial coincidence with gravid *E. aucklandica*, suggesting that this species was not a suitable surrogate or other factors are likely involved in the interaction of this mussel species with *R. retropinna*.

Alignment of spatial patterns in habitat use between host fish and mussel species have been reported elsewhere, including in *Unio crassus* (Vicentini, 2005; Taeubert et al., 2012) and *Theliderma cylindrica* (Fobian, 2007) where females were observed to move towards the banks to eject larvae during their reproductive periods, apparently to maximise the probability of infesting host fish. Similarly, host and habitat specialist *Simpsonaias ambigua* has been observed to actively move into specialised shaded habitats under rocks where its salamander host *Necturus maculosus* is most frequently found (Stegmann, 2020). Host-recognition mechanisms that have been described for some unionid species (Jokela & Palokangas, 1993; Teutsch, 1997; Trübsbach, 1998; Welte, 1999) involve chemical and mechanical cues from nearby suitable host fish, reported to alter unionid behaviour and trigger glochidia release (Meyers & Millemann, 1977; Jokela & Palokangas, 1993; Welte, 1999; Haag & Warren, 2000). However, very few studies have focussed on chemotaxis in freshwater mussels toward a host, with only one known study showing insignificant findings (Stegmann, 2020).

5.5.3 Resistance to floods

Effects of the flood event that serendipitously occurred during summer were interpreted based on changes in the % of the two mussel species recovered post-flood, net distances travelled by recovered mussels, and differences between years with and without a large preceding flood event. Overall, recaptured mussel abundances declined from 84% to 65% post-flood in *E. aucklandica*, and from 64% pre-flood to 47 % post-flood in *E. menziesii*, associated with downstream movements averaging 2.5-3.2 m, (with one recaptured *E. menziesii* found 349 m downstream). As high flow events have theability mobilise areas of unconsolidated substrate with low stability (Hastie et al., 2001), downstream displacement of both mussel species following the flood event was likely attributable to bed scouring observed in the mid-channel where mussels were originally released.

In order to persist through high flow events, mussels often position themselves in areas of flow refugia that remain stable during floods (Strayer, 1999, Howard & Cuffey, 2003). Indeed, observations in the present study of mussel movement toward bank habitats where undercutting presumably reduced flood-flow stress, along with the significant association of mussels that withstood the flood with refugia, including instream debris, overhanging vegetation, and low velocity and deeper water habitats, ultimately support the role of flood flow refugia in providing resistance to the effects of this bed-moving flood for both species. Due to being artificially positioned into areas that were relatively exposed to hydraulic effects (mid-channel) with minimal refugia types available (e.g., no accumulations of wood), those individuals that did not move bank-ward or to nearby flow refugia prior to the flood were probably more likely to get displaced, even if they moved vertically deep enough into the substrate to escape high velocities.

Several other factors have been suggested to explain the differential effects of peak flow events on mussel populations, including stream geomorphology, substrate type and stability, and species-specific attributes such as shell morphology and burrowing behaviour (Allen & Vaughn, 2009; Gangloff & Feminella, 2007; Meador et al., 2011; Morales et al., 2006; Randklev et al., 2019; Strayer, 1999; Zigler et al., 2008). Mussels with sculptured shells are thought to have enhanced anchoring abilities in comparison to species with smoother valves (Watters, 1994; Allen & Vaughn, 2009; Hornbach et al., 2010; Goodding et

al., 2019; Sotola et al., 2020). Furthermore, mussel size has also been suggested to increaseanchoring ability, as larger mussels have greater muscle mass that might help to resist dislodgement. However, larger mussels were found to need less force for dislodgment from the substrate as a result of the decrease in cross-sectional area (Levine et al., 2014),possibly explaining the lack of difference in flood resistance between unionid

species in the present study.

Despite *E. aucklandica* having shells that are moderately sculptured (ridges on outer valves) and being large and heavy in comparison to unsculptured, small and lightweight *E. menziesii*, the hypothesis that larger and therefore more firmly-anchored *E. aucklandica* would be more resistant to a flood of such a magnitude was only weakly supported. Recaptured mussels of both species were found to travel similar distances post-flood, indicating comparable displacement in both species, although the fate of unrecovered mussels is not known and differences among species in response to smaller floods cannot be discounted. Indeed, sampling in February 2020, when there was no preceding flood event, found higher percentages of both species up to 83 m downstream than within the sub-reaches, possibly due to movement induced by smaller flow events, although mean distance travelled was less than for February 2019.

Periodic flood events that are a part of the natural flow regime and not exacerbated by anthropogenic alterations are thought to provide beneficial ecosystem services and functions (Konrad & Booth, 2005). These may include stimulation of life-cycle cues including glochidia release, removal of accumulated sediments from interstitial spaces potentially increasing juvenile survival, and initiation of host fish migrations as found in some southern Galaxiidae (McDowall, 1995; Poff et al., 1997; Hastie & Young, 2003; Inoue et al., 2014). However, flood events, depending on the size and frequency, may also have detrimental effects on local mussel populations (Hastie et al., 2001). An extreme example includes the mortality of >50 000 mussels (5-10% of the total population) during a 100-year flood event in the River Kerry in Scotland (Hastie et al., 2001). The displacement of mussels as observed in the present study in late December, at the height of reproductive timing (i.e., glochidia release) has the potential to disrupt reproduction either by displacement of adult aggregations into unsuitable areas (stranding or smothering), or by causing released glochidia to be washed downstream potentially limiting attachment onto fish. Knowledge of the ability of freshwater mussels to resist or recover from flood events is important to their conservation and this study adds to the growing body of knowledge on the responses of aquatic organisms to floods, particularly with increasing extreme weather events predicted to occur with climate change (Hastie et al., 2001).

5.6 References

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Chapter Six

Thesis synthesis

6.1 Contextual overview

Interactions among species are fundamental to shaping community dynamics and are closely linked to the use and partitioning of available resources (Gause, 1934; Hardin, 1960; Schöner, 1974). Such interactions are key to biodiversity, since most biotic communities and the organisms within are involved in multiple interaction types with other species for resources to ensure reproduction and survival (Thompson, 2005). Comparative studies of multi-species assemblages, and the ways in which species coexist and partition potentially limiting resources, are therefore essential to further our insight into the ecology of individual species (Telfer et al., 2008). Sympatrically-occurring species within the freshwater mussel order Unionida engage in multiple interaction types brought about by their relatively sedentary life-style, as well as a complex symbiotic life-phase dependent on host-fish as a resource required for reproduction. A thorough understanding of life-history and biological interactions is key to conserving self-sustaining populations of threatened species, in particular, for unionid mussels which can deliver important ecosystem services and have been experiencing unprecedented declines around the globe (Geist, 2010; Modesto et al., 2018; Lopes-Lima et al., 2018; Vaughn, 2018).

New data on their complex reproductive biology is being increasingly documented, particularly in the Northern Hemisphere (Barnhart et al, 2008; Haag, 2012), but major gaps still remain in our knowledge and understanding of these threatened and valuable species, particularly in New Zealand (Hare et al., 2019). The two sympatrically-occurring New Zealand unionids, *Echyridella aucklandica* and *E. menziesii* (Hyriidae), represent examples of one such major knowledge gap. Prior to this PhD thesis research, nothing was known about the biology of *E. aucklandica*, and information on the reproductive ecology of *E. menziesii* was limited and inconsistent. Accordingly, knowledge about how the use of resources and interactions with host fish enables the two *Echyridella* species to coexist in sympatrically-occurring populations was limited, information that is important for effective conservation and management. As with many unionids globally, the species present in New Zealand are considered at threat of extinction (Collier et al., 2016; Lopes-Lima et al., 2017; Grainger et al., 2018; Lopes-Lima et al., 2018).

Partitioning of host fish species themselves, or reproductive resources in time and space, may be a key survival strategy facilitating coexistence in *E. menziesii* and the congeneric *E. aucklandica*, as reported in other unionid species (Haag & Warren, 1998; Rashleigh & DeAngelis, 2007; Marshall et al., 2014). In this thesis I combine multiple approaches within four research chapters to fill knowledge gaps on the reproductive ecology of *E. aucklandica* (in particular) and *E. menziesii* to understand mechanisms enabling their successful coexistence within small Waikato streams. I show that these two sympatric congeneric *Echyridella* species have evolved sharply contrasting reproductive strategies within species-poor mussel communities. I compare reproductive niche parameters along three major resource use dimensions: host-fish as a dimension itself, reproductive phenology (time), and habitat use (space), thereby providing insight into resource overlap and partitioning.

In this concluding chapter, I provide a synthesis of the key findings of each research chapter, focussing on reproductive ecology and reproductive resource use to identify overlap and partitioning among these *Echyridella* species in Waikato streams. I also provide conservation management and future research recommendations in the hope that these insights will be useful to aid future conservation and restoration efforts of New Zealand freshwater mussel communities.

6.2 Research chapter highlights

Chapter 2 identified complex adaptations among the two *Echyridella* species through contrasting use of larval (glochidia) host-fish infestation strategies and contrasting glochidia morphometry. These findings filled crucial knowledge gaps in the life-history strategies of these species, and highlighted the potential for host-fish specialisation in *E. aucklandica* leading to partitioning among the two sympatric *Echyridella* species. Female *E. aucklandica* were found to produce conglutinates, mucus packages containing miniature glochidia thought to lure specific fish to them by resembling fish prey. To my knowledge, this is one of the first Unionida species outside of North America reported to be using functional conglutinates to mimic host diet as an infestation strategy. Conglutinate features (shape, buoyancy), as found Chapter 2 for *E. aucklandica*, suggest that this unionid species potentially targets pelagic fish (Haag, 2012). These findings further indicate that *E. aucklandica* may be a host specialist as other studies of similar species suggest that these features have evolved to attract and facilitate glochidia transfer to specific fish feeding guilds (Barnhart et al., 2008; Haag 2012; Patterson et al. 2018). In comparison *E. menziesii*, a known host fish generalist with the ability to infest a

range of native diadromous and non-diadromous species as well as some non-native fish (Percival, 1931; Hine, 1978; Clearwater et al., 2014; Brown et al., 2017; Hanrahan, 2019; Moore & Clearwater, 2019), released their glochidia through a broadcast release strategy.

Additionally, Chapter 2 demonstrated that *E. aucklandica* produced miniature glochidia around three times smaller than those of *E. menziesii*, again, highlighting a morphological feature often found in host-specific unionid species (but not always, see Haag, 2012). Small glochidia typically encyst on fish gills where they grow substantially, therefore requiring longer encystment times, as reported for *M. margaritifera* (Bauer & Vogel, 1987). In contrast, host-generalist mussels typically produce larger glochidia with hooks that assist with attachment on the fins and body, as well as gills, for a short encystment period (e.g., *Unio crassus*, 250 µm diameter, which encysts for a few weeks on at least 12 host fish species) (Young & Williams, 1984; Bauer, 1994; Nezlin et al., 1994; Ziuganov et al., 1994; Taeubert et al., 2012; Stoeckl et al., 2015). Findings of contrasting infestation strategies and glochidia morphometry among both species reinforced the potential for host resource partitioning. This provided the context for Chapters 3 and 4 to further explore the partitioning and use of reproductive niche dimensions as mechanisms of coexistence in the two sympatric *Echyridella* species.

Chapter 3 investigated inter- and intraspecific reproductive phenological niche dimensions among the two *Echyridella* species (Figure 6.1). Results indicated extended brooding durations in both mussel species, although there were shorter for *E. menziesii* than *E. aucklandica*. This finding was generally consistent with 'bradyticty' or a long-term brooding strategy, rather than 'tachyticty' or a short-term brooding strategy as recognised and described in many North American freshwater mussels (Sterki, 1895, 1898; Ortmann, 1911; Graf & Foighil, 2000; Price & Eads, 2011). Furthermore, Chapter 3 found overlaps in the timing of larval maturity and peak brooding between both species, and uncovered thermal (accumulated degree days and temperature thresholds) relationships with the onset of brooding. The latter finding suggests that changes in climatic conditions have the potential cause negative effects on both species via mismatches between mussel reproduction and host phenology.

During the sampling conducted in Chapter 3, it was apparent that all sites were dominated by older individuals with no small mussels found below 40 mm for *E. aucklandica* or below 23 mm for *E. menziesii*. Although this may partly reflect the challenges in collecting small mussels, the presence of geriatric, senescing populations dominated by large individuals, such as in the study streams, has been observed within waterways throughout New Zealand (James, 1985; Roper & Hickey, 1994; Catlin et al., 2018)

and abroad (Vaughn & Spooner, 2004; Geist et al., 2006; Hastie, 2011; Stöckl et al., 2014), providing evidence of recruitment constraints that could lead to 'extinction debt' (Tilman et al., 1994; Vaughn, 2012; see below for further discussion).

Findings in Chapter 3 of overlap in peak brooding phenology combined with contrasting use of reproductive strategies in Chapter 2 further indicated for potential competition and partitioning of host fish species themselves among *E. menziesii* and *E. aucklandica*. Accordingly, Chapter 4 examined host fish attachment and development by glochidia through field and laboratory studies, and confirmed host fish species partitioning between the two *Echyridella* species. *Echyridella menziesii*, was found to infest a wide range of fish species and be most prevalent on benthic *Gobiomorphus* species and *Anguilla dieffenbachii*, in contrast to *E. aucklandica* which produced viable juveniles only on the pelagic *Retropinna retropinna*, confirming the status of *E. aucklandica* as a host specialist (Figure 6.1; Figure 6.2). However, this Retropinnidae species was found to be infested with consistently low numbers of E. aucklandica glochidia.

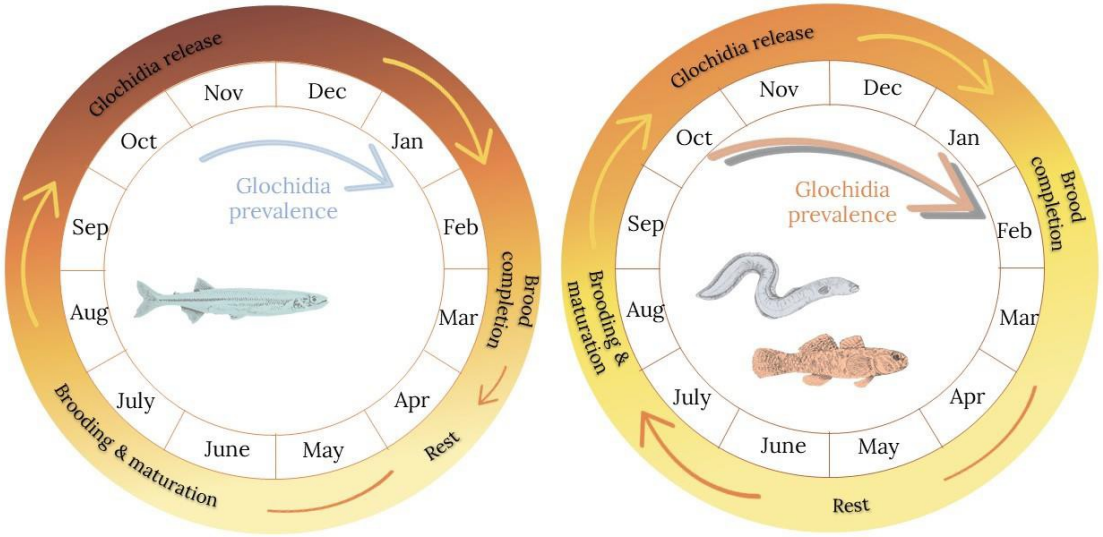

Figure 6.1. Conceptual diagram of the brooding phenology and timing of glochidia prevalence on the respective host fish of *E. aucklandica* (left) and *E. menziesii* (right).

Host infestation on the above-mentioned fish species was confirmed in the laboratory, where contrasting modes of development and attachment location between the two *Echyridella* species on their respective hosts were also determined, in line the expectations based on glochidia morphometry (Chapter 2). The current study found *E. aucklandica* to encyst exclusively on gills of their host, in comparison to *E. menziesii* which attached not only on the gills but also externally on the fins and skin. Attachment on the gills appears to be a common feature in smaller glochidia with marginal appendages (hooks) that are weakly-developed or absent, while larger glochidia with well-developed hooks are adapted to attach to harder tissues (e.g., margin of the gill operculum, fins; Bauer, 1994; Walker et al., 2001). Miniaturised glochidia of *E. aucklandica* were observed to grow nearly five times their original size on *R. retropinna* before maturing as juveniles, compared to the larger *E. menziesii* glochidia which stayed the same size throughout metamorphosis. Indeed, it appears that glochidia growth may be a trait of miniaturised glochidia (<100 µm) which have evolved multiple times in Unionida (i.e., Margaritiferidae, *Quadrula quadrula* species group, *Leptodea*, *Truncilla*; reviewed in Barnhart et al., 2008). Miniaturised glochidia that grow on their host often share the trait of an extended encystment period (Young & Williams, 1984; Haag, 2012), consistent with metamorphosis duration for *E. aucklandica* glochidia which was significantly longer than for the larger *E. menziesii*, but only by two to three weeks. Timing of transformation from glochidia to the juvenile stage appears to be largely water temperature-dependent, with metamorphosis occurring more rapidly at warmer temperatures related to degree days (Walker, 1981; Dudgeon & Morton, 1984; Humphrey, 1984; Hruska, 1992; Hastie & Young, 2003; Moore & Clearwater, 2019).

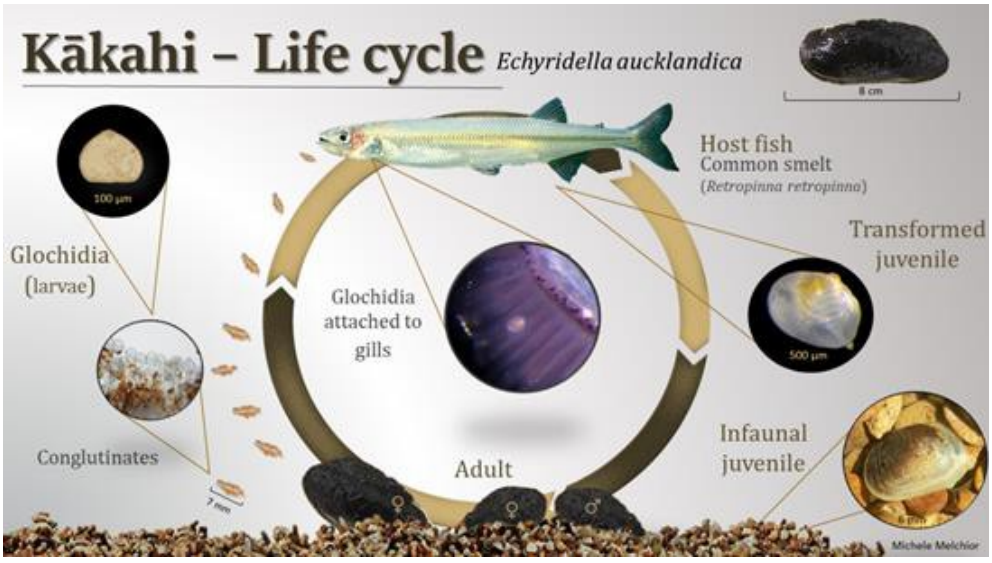

Figure 6.2. *Echyridella aucklandica* life-cycle diagram with filled in life-historygaps based on this current thesis.

Research showing that peak brooding seasons of the two species overlapped in November and December (austral summer) (Chapter 3), combined with the two species' use of different reproductive release strategies (Chapter 2) and different host fish species for glochidia development (Chapter 4), provided the context for Chapter 5 on spatial and temporal movement patterns among sexes of both species to investigate spatial partitioning in relation to fertilisation and glochidia infestation on host fish. Chapter 5 found evidence of relatively high net horizontal movement rates compared to other unionid species overseas, and active bank-ward cluster formation in passive integrator responder tagged individuals of both species. Cluster formation and vertical movement varied throughout the onset brooding period in winter for both species, although there was no apparent partitioning of temporal horizontal movement patterns in male-female aggregations as hypothesised to occur based on different brooding onset times (Chapter 3). Generally, proportions of female mussels increased at the sediment surface during their respective brooding onsets and later glochidia development stages, although statistically significant temporal patterns were not detected. This vertical movement pattern, potentially associated with fertilisation and glochidia release, is consistent with a behaviour aimed at increasing reproductive success as reported elsewhere (Burla et al., 1974; Amyot & Downing, 1997; Rogers et al., 2001; Watters et al., 2001; Perles et al., 2003).

Although, some spatial partitioning during the glochidia release period occurred, I wasn't able to link this to host fish habitat use, due in part to the low numbers of fish available for analysis and the potential for changes in habitat use during nocturnal activity periods. Nevertheless, spatial overlap between mussel species and their respective host fish was partially observed during the day-time for *E. menziesii*, but could not be determined for the host-specific *E. aucklandica* due to only one *R. retropinna* being captured. Analysis of the pelagic and more abundant *G. maculatus* as a surrogate species for *R. retropinna* in the study stream did not help resolve the potential for habitat overlap with *E. aucklandica*.

The Chapter 5 study was disrupted by a moderate flood event. Downstream displacements of up to 349 m for *E. menziesii* and 46 m for *E. aucklandica* were detected following this bed-moving flood, but there was limited evidence to suggest that larger and more firmly-anchored *E. aucklandica* were, on average, more resilient to a flood of this magnitude during their reproductive period. Rather, flood flow refugia associated with riparian vegetation and debris in bank habitats appeared to provide resistance for both species to the effects of the bed-moving flood. This study has added to the growing body of knowledge on the responses of aquatic organisms to moderate floods and

highlighted the role of flood-flow refugia in preventing dislodgement.

6.3 Evidence for reproductive niche partitioning

Collectively, the research chapters of this thesis, underpinned by concepts of niche theory and resource partitioning, highlight important mechanisms whereby sympatric species can reduce the risk of interspecific competition to avoid competitive exclusion, thus promoting the likelihood of coexistence (Gause, 1934; Hardin, 1960; Schöner, 1965, 1974; Tokeshi, 1986; Post, 2019). Freshwater mussels of the order Unionida are long-lived, filter-feeding benthic organisms with limited mobility that can occur in multi-species aggregations in lakes, rivers and streams, attributes that make it a suitable model group to examine mechanisms enabling resource partitioning in natural settings (Strayer, 2008). However, despite the potentially important role of resource use in determining coexistence in freshwater mussel species, relatively few studies have investigated this concept, notably the partitioning of reproductive resources. Because of their complex reproductive cycle involving larvae with characteristics of symbionts that depend on the distribution and abundance of hosts as a highly important resource base (Price, 1990; Watters, 2001; Rashleigh & Dimock, 2007; Barnhart et al., 2008), the niche concept as applied to freshwater mussels needs to include the reproductive niche dimension (Bykova et al., 2012; Wessel, 2013) and the potential for partitioning of the host fish resource.

For inter-specific resource competition to occur, there must be niche overlap between species in their need for limited resources (Hutchinson, 1959). Greater ecological overlap generally implies a greater tendency to compete for resources (Schöner, 1974). In Chapter 3, high overlap was found in the peak brooding phenology of both species suggesting the potential for competition for limited host resources, particularly diadromous fish hosts that enter streams at different times as they move from the sea to freshwater (McDowall, 1990). High overlap in brooding phenology in both *E. menziesii* and *E. aucklandica*, particularly during the important peak brooding season, raised questions on whether partitioning for resources occurs along niche dimensions other than phenology, as sympatric species with high overlap along one dimension often overlap relatively little along another dimension (i.e., niche-complementarity hypothesis), reducing overall effective niche overlap and promoting co-existence (Pianka, 1974). Rather than partitioning temporally (Chapter 3) and spatially in relation to host fish (Chapter 5), the two unionid species examined in the present study partitioned fish species required for metamorphosis (Chapter 4) through contrasting glochidia release strategies (conglutinate versus broadcast release; Chapter 2).

6.3.1 Host fish specialisation

Ecologically similar animals may facilitate coexistence by acting as generalists or specialists when resource availability is limited (MacNally, 1995). Ecological specialisation is the process of adaptation to a narrow range of available environmental conditions (Poisot et al., 2011), and can have consequences for the abundance and distribution of species (Brown, 1984) and their persistence (Julliard et al., 2004; Devictor et al., 2008). Host specificity can affect how a mussel population responds to a reduction in abundance of specific fish-host species, and may come with a cost as it ties the fate of the unionid species with that of its host, such that a decline in the specific host may result in recruitment failure for the mussel (McNichols et al. 2011.; Douda et al., 2012). The same is less probable for glochidia that metamorphose on a range of fish which can continue to use remaining host fishes, even after one of the species within their host range declines (Douda et al., 2012; Haag, 2012). For example, declines in the specialist unionid *Fucsonia ebena* in the upper Mississippi River have been attributed to the local extinction of its specialist host, *Alosa chrysochloris*, caused by impoundment in the Upper Mississippi River (Kelner & Sietman, 2000; Hart et al., 2018), while host generalists *Elliptio complanata* and *E. slotidianus* that co-exist with *F. ebena* were less affected (Kneeland & Rhymer, 2008; Lellis et al., 2013).

One interpretation of generally low attachment and metamorphosis success of *E. aucklandica* may be that *R. retropinna* is not the primary host but rather is serving as a secondary host. Primary hosts have consistently high levels of infestation (intensity) and prevalence whereas secondary hosts, although allowing metamorphosis, yield lower numbers of juveniles (Haag & Warren, 1998; O'Brien & Williams, 2002; McNichols et al., 2011), as observed for *E. aucklandica* glochidia on *R. retropinna*, at least at the sites sampled in my study. Other suitable hosts may therefore be required to produce sufficient juveniles to sustain populations. One potential candidate, based on phylogeny, distribution, habitat and feeding ecology is the closely-related but extinct grayling, *Prototroctes oxyrhynchus*, which belonged to the same family (Retropinnidae) as *R. retropinna* and was an amphidromous, shoaling species endemic to New Zealand (Allen, 1949; McDowall, 1976). The extinction of *P. oxyrhynchus* and the necessity to use a sub-optimal host might explain the observed aging population structure of *E. aucklandica*. It is clearly not possible to conduct host trials on an extinct species, so I conducted non-destructive analysis of *P. oxyrhynchus* museum specimens, some of which had been collected close to the location of the coastal streams that I sampled. Gills of preserved

P. oxyrhynchus (n = 7) were examined for presence of glochidia or glochidia scars left behind post-encystment. While none was evident based on preliminary visual examination, further analyses are being conducted.

6.4 Conservation management

Often, the conservation of declining species is challenged by scant knowledge of their reproductive strategies and basic biology. Furthermore, due to the longevity and slow-growth of freshwater mussels, time lags occur between an action (whether it be positive or negative) and a detectable biological response (e.g., population collapse or discovery of recruitment), potentially masking the current health status of populations. The longevity of freshwater mussels may also buffer populations from a disturbance if perturbations are short-term, enabling reproduction after recovery, but if perturbations are continuous the risk of population declines increases (Raimondo & Donaldson, 2003; Morris et al., 2008). Thus, conservation management actions with rapid positive effects on unionid population, and the interactions with other species and the complete ecosystem should be given priority.

The new information in this thesis on the reproductive phenology of sympatric *E. menziesii* and *E. aucklandica* provides crucial life-history data that can be incorporated into conservation management strategies for freshwater mussels in New Zealand. Critical time periods such as peak brooding and glochidia release in both mussel species, as indicated in Chapter 3, coupled with knowledge of species environmental tolerances (e.g., Clearwater et al., 2014; Melchior, 2017), need to be taken into consideration in future developments that could adversely affect key populations and recruitment. Additionally, maintaining and restoring riparian buffer zones could be used to create lower water temperatures in streams (Feld et al., 2018), particularly those with low proportions of upstream forested catchments. Because some aspects of the reproductive phenology of *E. menziesii* and *E. aucklandica* are sensitive to environmental cues such as accumulated degree days and thermal thresholds (Chapter 3), any accelerated changes in climate, specifically thermal fluctuations, may potentially disrupt or shift reproductive timing in these species (Forrest & Miller-Rushing, 2010; Schneider et al., 2018).

Management approaches for freshwater mussels with symbiotic life stages require identification of bottlenecks related to conservation targets, including knowledge about the biotic interactions and life-histories of both freshwater mussels and their host fish (Geist, 2010, Modesto et al., 2018). Indeed, given their reliance on host fish, declines in unionid populations have been attributed to reductions of host fish species (Douda et al.,

2012; Modesto et al., 2018; Dias et al., 2020). Accordingly, as noted earlier, host specificity can affect how a mussel population responds to a reduction in abundance of specific fish-host species. Currently, the only identified host for *E. aucklandica*, *R. retropinna* (Chapter 4), is found to inhabit a range of habitats throughout Waikato waterways and New Zealand in general (Speirs et al., 2001; McDowall, 1990). It is one of the few native fish species in New Zealand currently recognised as "Not Threatened" (Dunn et al., 2018; Joy et al., 2019), with an IUCN Red List status of "Least concern" (Franklin et al., 2014). Although, widespread throughout New Zealand waterways, very few specimens were caught in the present study (Chapter 4) and *R. retropinna* may be vulnerable to local population declines due to a range of increasing environmental changes induced by anthropogenic activities. Unionid conservation in New Zealand cannot disregard the role of fish species, particularly with around three-quarters of native New Zealand freshwater fish species recognised as threatened with risk of extinction (39 of 53, see Dunn et al., 2018; Ministry for the Environment & Statistic NZ, 2019; Joy et al., 2019). Therefore, improved understanding of host–glochidia interactions is critical, particularly in advancing integrated conservation management strategies that protect both unionid and host fish populations.

Freshwater mussel propagation, augmentation, reintroduction and introduction has a great potential for conservation but is recommended as a last-minuterescue tool in many recovery plans for species, globally (Gum et al., 2011; Srayer et al., 2019).Pursuing restoration management such as this for threatened species like *E. menziesii*, and particularly for *E. aucklandica* which is currently known to only successfully encyst (in low numbers) on *R. retropinna*, should be considered to conserve declining species. However, the risks, for example in the context of genetic and ecological traits, should be carefully evaluated prior to using propagation as conservation tool (Geist,2010; Gum et al., 2011). Development of *in vitro* protocols (which preclude the need for host fish) for *E. menziesii* is currently underway in New Zealand, with varying levels of success in the survival of juveniles beyond a certain age (Thompson et al., in prep), but has not yet been considered for *E. aucklandica* (but see research priorities below).

The leading intrinsic (species condition) and extrinsic (state of the environment) research priorities as proposed by Ferreira-Rodríguez et al. (2019) for assessing unionid conservation status globally, align with the work carried out in this thesis. Intrinsic research priorities supported within the current thesis include studies of demography (recruitment, population size structure) and life-history traits (reproductive strategy, timing of reproductive cycle, fecundity, age at sexual maturity), while the top extrinsic

priorities include identifying primary mussel-host relationships including metamorphosis success and host availability. The results of this thesis has filled critical knowledge gaps and research priorities regarding the life-history, demography and host partitioning mechanisms between *E. aucklandica* and *E. menziesii* in both natural and laboratory settings. However, many questions still remain to be answered regarding biotic interactions and life-history of freshwater mussels. The results presented within this thesis highlight seven priority avenues for future research that will aid with freshwater mussel conservation management:

1. Future field and laboratory glochidia infestation studies urgently need to expand the current known range of native fish species that are found living in sympatry with *E. aucklandica* to better understand the degree of host specificity in *E. aucklandica* and confirm host fish specialisation on *R. retropinna*.
2. If *R. retropinna* is serving as a secondary, suboptimal host unable to produce enough juveniles to sustain *E. aucklandica*, then urgent actions including captive breeding programmes and *in* vitro mussel propagation protocols are urgently required. Protocols are still lacking globally for mussel species like *E. aucklandica* that grow substantially during their phoretic stage on fish, and fail to complete metamorphosis under *in vitro* conditions (Taskinen et al., 2011; Calderon et al., 2019).
3. Further investigations into adult and juvenile habitat requirements within lotic habitats among a range of geographic locations are required, particularly to aid in habitat restoration management of both species.
4. As this study was temporally limited to only two brooding cycles in a limited number of streams, future studies on *E. aucklandica* brooding and glochidia attachment should extend over multiple years and over a larger range of sites to understand how the brooding phenology and fish host dynamics may change over time and geographically.
5. Determining environmental tolerances of *E. aucklandica* in comparison to *E. menziesii* would inform identification of sites suitable for reintroduction.
6. Determining the longevity and accurate information on age and growth of *E. aucklandica* is important for the effective management and conservation of the species.

7. If further studies show sub-optimal host use in *R. retropinna* with *E. aucklandica* glochidia, testing host compatibility of the closely related New Zealand *Stokellia anisodon* and Australian *P. maraena*, the extant and close relative of New Zealand extinct *P. oxyrhynchus*, may help to shed light on their potential role as surrogate hosts in capture rearing programmes.

6.5 Implications of climate change

Global climate change is a looming threat for freshwater ecosystems leading to warming waters, higher amplitude of thermal fluctuations and increased frequency of extreme weather events such as droughts, floods and heat waves (Woodward et al., 2010). The consensus scenarios of the Intergovernmental Panel for Climate Change (IPCC) forecast a 1.5 °C increase in mean temperature worldwide, with freshwater bodies warming significantly faster than most terrestrial and marine habitats (Hoegh-Guldberg et al., 2018). As freshwater mussels are predominantly sedentary with limited dispersal abilities as adults, they are especially vulnerable to the effects of climate change (Archambault et al., 2018).

Because some aspects of the reproductive phenology of freshwater mussels are sensitive to environmental cues such as water temperature, as indicated in Chapter 3, accelerated changes in climate, specifically thermal fluctuations, may potentially disrupt or shift reproductive timing in these species (Forrest & Miller-Rushing, 2010; Schneider et al., 2018). Additionally, sudden fluctuations in temperature from a thermal regime to which species have adapted may impair mussel recruitment through abortions of immature and unviable larvae in early brooding females (Melchior, 2017; Schneider et al., 2018).

Differential effects of temperature change may cause mismatches between freshwater mussel reproduction and host availability (Hastie & Young, 2003; Cosgrove et al., 2012; Pandolfo et al., 2012). For example, both earlier release of glochidia in response to warming temperatures and delays in glochidia release at lower temperatures can have consequences for later life cycle stages, and could result in a temporal mismatch with host fish phenology, particularly migratory fish which move through different areas of waterways in response to seasonal cues (Klemetsen et al., 2013). Mismatches in mussel-host interactions (as suggested in Chapter 4) may also occur as host populations decline as a result of climate change (Pandolfo., 2012), however, this depends on species-specific thermal tolerances of host fish species (Reyjol et al., 2009).

Additionally, extreme weather events causing floods and droughts that lead to large fluctuations in flow and temperature have been reported to affect reproduction as gravid females are particularly sensitive to anything that interferes with respiration processes (Hastie & Young, 2003; Gascho Landis et al., 2013). It is, therefore, imperative to understand the impact of climate change and the likelihood of altering community- level patterns in reproductive phenology and reproductive synchrony with host fish phenology. Flood events, depending on the size and frequency, may also have detrimental effects on local mussel populations through physical displacement (Hastie et al., 2001). An extreme example includes the mortality of >50 000 mussels (5-10% of the total population) during a 100-year flood event in the River Kerry in Scotland (Hastie et al., 2001). The displacement of mussels as observed in the present study in late December, at the height of glochidia release, had the potential to disrupt reproduction by disrupting adult spawning aggregations, displacing them into unsuitable areas, or by causing released glochidia to be washed downstream potentially limiting attachment onto fish. Knowledge of the ability of freshwater mussels to resist or recover from flood events and the role of refugia in providing flood-flow resistance, as informed by Chapter 5, adds to the growing body of knowledge on the responses of aquatic organisms to large floods, particularly with increasing extreme weather events predicted to occur with climate change (Hastie et al., 2001), particularly with increasing extreme weather events predicted to occur with climate change (Hastie et al., 2001).

6.6 Concluding remarks

Extinction risk assessments have largely ignored biotic interactions and have mostly been based on analyses of species habitat relationships and population sizes (Vaughn, 2012). However, species interactions have important implications for extinction processes, and symbiotic interaction types between species may be especially important (Dunn et al., 2009; Kiers et al., 2010; Spooner et al., 2011). The complete dependence upon their host to complete their life-cycle, puts freshwater mussels at a higher risk of coextinctions (Modesto et al., 2017; Dias et al., 2019). Furthermore, since freshwater mussels are long-lived and slow-growing, they are prone to recruitment bottlenecks with the potential for extinction debt (future extinction of species owing to past events; Tilman et al., 1994; Strayer, 2008; Haag, 2009; Vaughn, 2012). The results in this thesis have provided evidence for potential recruitment bottlenecks, particularly for *E. aucklandica* and highlighted important pathways for future management to ensure their continued survival. As a host-specialist, *E. aucklandica*'s risk of coextinction is high due to their dependence on a small

host range. The extinction of potential hosts like *P. oxyrhynchus* for *E. aucklandica* might help explain the concurrently observed aging *E. aucklandica* populations, highlighting the need for urgent action (host-trials, and captive breeding programmes) to ensure their survival.

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Appendices

A1: Published co-authored manuscript

Kevin J. Collier & Michele Melchior (2020): Congruence in stable isotope values among two sympatric freshwater mussel species in northern New Zealand streams, New Zealand Journal of Marine and Freshwater Research, DOI: 10.1080/00288330.2020.1833947

SHORT COMMUNICATION

Congruence in stable isotope values among two sympatric freshwater mussel species in northern New Zealand streams

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ABSTRACT

We measured $\delta^{15}N$, $\delta^{13}C$, and percent carbon (C) and nitrogen (N) on foot and remaining soft tissue of the two native freshwater mussel species *Echyridella aucklandica* and *E. menziesii* (Hyriidae: Unionida) from three Waikato, northern New Zealand, streams to investigate differences among sites, sexes and species. Mean differences in $\delta^{15}N$ and $\delta^{13}C$ between foot and remaining soft tissue (mainly guts, gills and gonads) were <0.5‰, whereas %C and %N were higher in foot tissue and C:N values were lower. No differences were detected between mussel species, but $\delta^{15}N$ and $\delta^{13}C$ displayed marked differences among sites for both species, with $\delta^{15}N$ variations consistent with the level of catchment land-use intensification. Variation in $\delta^{13}C$ and $\delta^{15}N$ among replicate mussels was low indicating few individuals are required to precisely characterise isotope signatures of local populations. The lack of evidence of trophic resource partitioning among species, and consistent differences in isotope ratios between sites, suggests that the more widespread and abundant *E. menziesii* may provide an effective baseline integrator of catchment activities that alter carbon supplies and nitrogen sources.

KEYWORDS: Unionida, *Echyridella aucklandica*, *Echyridella menziesii*, $\delta^{15}N$, $\delta^{13}C$, carbon:nitrogen ratio

Introduction

Freshwater mussels perform important functions in aquatic ecosystems where they occur in high densities, providing ecosystem services such as filtering of suspended particulate matter, bioturbation of sediments and provision of habitat for other biota (Vaughn and Spooner 2004; Vaughn 2010). In many parts of the world, mussels belonging to the order Unionida occur in multispecies assemblages that are widely considered under threat due to multiple anthropogenic pressures (Collier et al. 2016; Lopes-Lima et al. 2018). In New Zealand, three extant species of freshwater mussel (Hyriidae: Unionida) are recognised with threat statuses ranging from Data Deficient to Nationally Vulnerable (Grainger et al. 2018). Two of these species, *Echyridella menziesii* and *E. aucklandica*, occur sympatrically in some northern Waikato region streams, where studies are underway to resolve how they partition resources (e.g., Melchior et al. 2021).

One resource sympatric species of mussels potentially share is suspended particulate matter filtered from the overlying water column, often referred to as seston. However, it is not clear which components of seston are assimilated into tissue and whether this differs among species and sexes. These questions can be addressed through stable isotope analysis which uses changes in natural abundances of carbon and nitrogen isotopes ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) to provide insights into (i) sources of nutrition and their propagation through to consumers (Pingram et al. 2012), and (ii) anthropogenic nitrogen enrichment of aquatic food webs (e.g., Trochine et al. 2017). In addition, associated analyses of tissue nitrogen (N) and carbon (C) composition yield information on nutritional quality expressed as carbon:nitrogen (C:N) ratios (Marcarelli et al. 2011; Sullivan et al. 2014).

Studies that have applied stable isotope analyses to mussels have also used them to infer historical changes in food-web pathways by comparing contemporary samples with museum-preserved specimens (Delong and Thorp 2009; Fritts et al. 2017), to confirm parasitic associations of mussel glochidia with host fish (Fritts et al. 2013), and to characterise historical changes in water oxygen isotope ratios based on analysis of shells (Pfister et al. 2018). Here we present initial results of $\delta^{13}C$, $\delta^{15}N$, %C, %N and C:N analyses for sympatric populations of *E. menziesii* and *E. aucklandica* in three Waikato streams to help understand the effects of soft tissue type (foot versus remaining tissue), species and sex on these parameters. We also explore differences between sites that contrast in level of catchment land-use intensity, and report on within site variability to inform sampling for future studies characterising stable isotope signatures of mussel populations.

Methods

Individual mussels were collected in February 2019 from three Waikato, North Island, streams supporting populations of *E. aucklandica* and *E. menziesii*. Two of the sites are on coastal waterways draining into the Tasman Sea (Ohautira Stream [37°45'43.0"S, 174°58'49.0"E], Kahururu Stream [37°41'11.2"S, 174°58'45.0"E]), and the third site, Mangapiko Stream (37°58'.54.2"S, 175°28'26.3E), is an inland tributary of the Waipa River. Ohautira has extensive indigenous forest upstream (58% of catchment area) and extensive riparian shading along the sampling reach. Around 9% of the Kahururu catchment area is native forest in addition to a significant area of mature pine forest which occurred alongside the sampling reach. For the Mangapiko site, 12% of upstream catchment area was in native forest with most of the catchment upstream developed for dairy farming. Accordingly, predicted nitrogen concentrations in the Freshwater Environments of New Zealand (FENZ) database (Leathwick et al. 2010) were highest in Mangapiko and lowest in Ohautira. Mean wetted channel widths at sampling locations ranged from 3.9–4.7 m. For the three months prior to and during sampling (January–March 2019), monthly average summer temperatures were between 18.1 and 19.2°C (Hobo® Tidbit loggers, Onset, Massachusetts, USA), dissolved oxygen concentrations were between 9.8 and 11.1 mg/L, and specific conductivity ranged from 104.9 to 136.4 µS/cm (both YSI 2030 Pro meter, Yellow Springs Instruments, Ohio, USA) (M. Melchior unpubl. data).

Three mussels of each species from each site were frozen after collection. Length and sexwere determined prior to shucking; as mussels were collected at the end of the brooding season glochidia were unlikely to be present in female mussels. The foot was separated from remaining soft tissue using a scalpel. The excised foot and remaining tissue were dried separately at 60°C and later ground in a mortar and pestle. Up to 40 mg of each tissue type (±0.01 mg) were placed in separate aluminium cups for analysis of $\delta^{13}C$, $\delta^{15}N$,
%C and %N relative to the leucine standard ($\delta^{15}N$ value 1.00‰, $\delta^{13}C$ -13.32‰) on a fully automated Europa Scientific 20/20 isotope analyser at The University of Waikato stable isotope facility. The instrument precision (standard deviation) was ±0.3‰ and ±0.5‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively.

Stable isotope ratios presented using the δ notation represent per mille (‰) deviations from atmospheric nitrogen for $\delta^{15}N$ and from Vienna Pee Dee Belemnite for $\delta^{13}C$, calculated as:

$\delta X = [R_{sample}/R_{standard}) - 1]x\ 10^3$

where: X = $\delta^{15}N$ or $\delta^{13}C$; and R is the respective $^{15}N/^{14}N$ or $^{13}C/^{12}C$ ratio.

Parametric data assumptions were not consistently met so non-parametric tests were used for all statistical analyses. Wilcoxon matched pairs test was used for pairwise comparisons of individual foot and remaining soft tissues. Z-test was used to investigate differences between species within and across sites, and between sexes within species. Effects of site were analysed using Kruskall-Wallis test separately for each species. Relationships between isotope values and mussel length were explored using Spearman correlation analysis.

Results

Measured $\delta^{13}C$ and $\delta^{15}N$ values ranged from -28.5 to -25.0‰ and 5.1 to 9.5‰, respectively. Although values were consistently higher in foot than remaining soft tissue for both isotopes (Z = 3.72 and 3.55, respectively, P < 0.001), overall differences were small (mean difference = 0.47‰ for $\delta^{13}C$ and 0.32‰ for $\delta^{15}N$see Table S1 for raw data) and the slopes of relationships were close to 1 (see Figure S1). Significantly larger pairwise values were detected in foot than remaining soft tissue for %C (mean 43.9 and 34.8%, respectively; Z = 3.72, P < 0.001) and %N (10.9 and 7.4%; Z = 3.72, P < 0.001), and smaller values for C:N (4.09 and 4.76; Z = 3.64, P < 0.001). Species did not significantly influence any of the variables measured, but sex did for *E. menziesii* with %N in foot tissue significantly higher in males than females (male:female ratio 1.25 cf 2.0 for *E. aucklandica*), although the difference was small (mean 11.1 versus 10.4%, respectively; Z = 2.21, P < 0.05). No sex-related differences were evident when analysing remaining soft tissue.

%C and C:N of foot tissue were not affected by site for either species, while %N differed between sites for *E. aucklandica* at P = 0.05 (H = 5.96) with lowest % values at Mangapiko (see Table 1 for site means). $\delta^{13}C$ and $\delta^{15}N$ were significantly and markedly affected by site (Figure 1). Pairwise comparisons of *E. menziesii* were significant for Ohautira versus Kahururu (H = 7.20, P < 0.05 for both isotopes), while for *E. aucklandica* Ohautira versus Mangapiko differences were significant ($\delta^{13}C$ H = 7.20, P < 0.05; $\delta^{15}N$ H = 6.49, P < 0.05) (see Figure 1). Collection of three mussels per site yielded coefficients of variation within sites

of <1% for $\delta^{13}C$ and <5% for $\delta^{15}N$ (overall means of 0.4% and 3.4%, respectively), with both species showing similar levels of within-site variation. Mussel length for *E. menziesii* (range 38-63 mm; see Table S1) was not significantly related to any of the measured response variables, while a significant positive rank correlation was detected for *E. aucklandica* length (61-95 mm; see Table S1) with $\delta^{13}C$ ($r_s = 0.790$, $P < 0.01$, $n = 9$).

Discussion

Results of the present study indicate predictable small variations in isotope signatures among foot and remaining soft tissues, and no differences between species or sexes, with consistent patterns for both species across sites. This finding supports comparisons among studies that have analysed different tissue types, sex ratios and mussel species. LaFrancois et al. (2018) concluded that foot and mantle tissues from two North American mussel species provided interchangeable $\delta^{13}C$ and $\delta^{15}N$ values, while Gustafen et al. (2007) found that haemolymph provided a suitable substitute for foot tissue in $\delta^{15}N$ analyses of another North American unionid species. As we collected samples on only one date and after the peak brooding period (M. Melchior, unpubl. data), it is possible that sex-related differences may occur at other times due to the physiological demands of brooding (e.g., Fritts et al. 2013). Low metabolic rates of freshwater mussels may affect temporal variation due to long tissue turnover times ranging from 113 days for $\delta^{15}N$ haemolymph to over 300 days for $\delta^{15}N$ foot tissue, and a number of years for $\delta^{13}C$ (Raikow and Hamilton 2001; Gustafen et al. 2007; Weber et al. 2017).

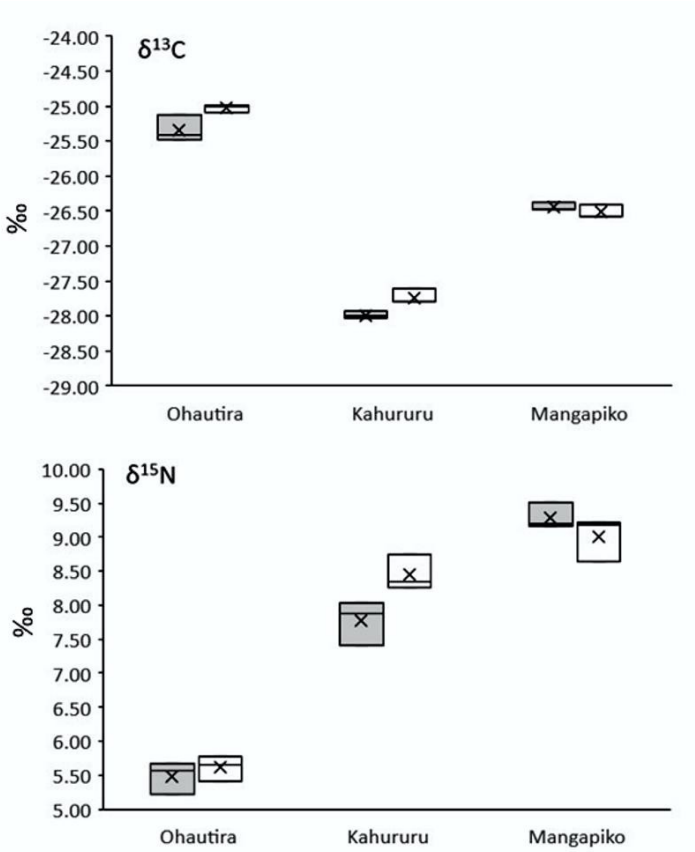

Figure 1. Box plots of $\delta^{13}C$ and $\delta^{15}N$ values of foot tissue for *Echyridella menziesii* (grey) and *E. aucklandica* (white) collected from three Waikato streams in order of increasing catchment development. The middle line of the box represents the median, X represents the mean, and the box delineates the 1st and 3rd quartiles.

Isotope values reported for *E. aucklandica* and *E. menziesii* were generally within the range of those summarised for a range of North American mussel species by Weber et al. (2017), although mean values for both $\delta^{13}C$ and $\delta^{15}N$ from Ohautira were lower, potentially reflecting southern temperate conditions in this minimally-disturbed stream. $\delta^{15}N$ for both species increased in relation to upstream land-use intensity, supporting the value of mussels as baseline integrators of nitrogen enrichment in streams, similar to findings of Clapcott et al. (2010) who reported a strong relationship between $\delta^{15}N$ of primary consumers and land-use gradients for New Zealand streams. Overseas studies have also highlighted that mussels can provide long-term and spatially-integrated indicators of episodic nitrogen inputs to waterways (Cabana and Rasmussen 1996; Gustafen et al. 2007; Atkinson et al. 2014; Fritts et al. 2017). In contrast, the site-related differences we detected in $\delta^{13}C$ may reflect a range of factors, potentially including differences in underlying geology affecting dissolved inorganic carbon signatures, and/or the relative importance

of autochthonous versus allochthonous contributions to the seston which mussels primarily feed on (Post 2002).

Not only do mussels filter, process and sequester multiple sources of particulate material from the water column, and potentially also from surrounding sediments (Weber et al. 2017), they can also provide a potentially important nutritional resource for opportunistic predators, including native and non-native species (Moore et al. 2019; B. Farnworth, The University of Waikato, unpubl. data). Mean C:N ratios across all sites in the present study were similar to the five North American species (mean = 4.1) analysed by Weber et al. (2017). Isotope analyses of mussels and potential predators may provide novel insights into aquatic-terrestrial food-web linkages, and highlight threats posed by non-native species to native aquatic biodiversity values and ecosystem services.

Despite an apparent relationship between length of one mussel species and $\delta^{13}C$ in the present study, more data are needed from a broader size range to confirm the generality of this finding for both species. Elsewhere, $\delta^{15}N$ has been reported to increase by 2‰ as *Margaritifera falcata* aged over 1 to 30 years, potentially reflecting access to different food resources (Howard et al. 2005). Notwithstanding this, collection of similar-sized mussels across different sites should help limit any effects of age as a factor affecting comparisons. For the size range sampled in the present study, within-site variation in $\delta^{13}C$ and $\delta^{15}N$ among replicate mussels was low indicating few individuals are needed to precisely characterise isotope signatures of local populations.

Congruence in isotope signatures among species means that the more common and widespread *E. menziesii* can be used to represent mussels generally in ecological studies aimed at tracking trophic pathways or monitoring long-term catchment-scale impacts on filtering biota in New Zealand streams. Indeed, based on Gustafen et al. (2007), it may even be possible to use minimally invasive and non-destructive haemolymph sampling for future stable isotope studies of freshwater mussels in New Zealand, although the nature of any temporal and physiological variations in isotopic signatures among various tissue types need to be investigated for these species. In this regard, foot muscle may provide some advantages as a long-term integrator of carbon flow and catchment activities as it likely is less influenced by organism physiological changes and effects of inorganic carbon and lipid content which can affect interpretation of trophic pathways (e.g., Post 2002).

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Disclosure statement

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Supplementary material

Table S1. Raw data used in analyses of two mussel species from three Waikato stream sites. M = male;F = female.

Site	Sex	Length (mm)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Carbon (%)	Nitrogen (%)	Carbon: Nitrogen
Foot tissue							
E. aucklandica							
Ohautira	M	95	-24.98	5.77	43.68	11.67	3.74
Ohautira	M	77	-25.00	5.65	43.62	11.72	3.72
Ohautira	M	81	-25.10	5.40	45.55	11.13	4.09
Kahururu	M	79	-27.62	8.74	43.81	11.54	3.80
Kahururu	F	61	-27.80	8.35	43.71	10.82	4.04
Kahururu	F	75	-27.79	8.26	46.72	11.52	4.06
Mangapiko	M	76	-26.58	8.64	42.57	10.51	4.05
Mangapiko	M	81	-26.41	9.19	42.42	9.96	4.26
Mangapiko	F	77	-26.57	9.21	44.85	10.08	4.45
E.menziesii							
Ohautira	M	61	-25.42	5.66	44.27	10.66	4.15
Ohautira	M	55	-25.13	5.57	44.48	11.22	3.96
Ohautira	F	51	-25.47	5.21	45.44	10.28	4.42
Kahururu	F	38	-28.04	7.40	43.14	9.42	4.58
Kahururu	F	41	-27.93	8.04	42.18	10.52	4.01
Kahururu	M	46	-28.00	7.88	43.28	11.54	3.75
Mangapiko	M	59	-26.47	9.16	42.24	10.92	3.87
Mangapiko	M	63	-26.49	9.20	44.71	11.48	3.89
Mangapiko	F	58	-26.37	9.50	43.58	10.78	4.04
Other soft tissue							
E. aucklandica							
Ohautira	M	95	-25.35	5.30	24.49	4.85	5.05
Ohautira	M	77	-25.67	5.38	35.43	7.51	4.72
Ohautira	M	81	-25.64	5.15	34.12	7.05	4.84
Kahururu	M	79	-28.10	8.12	29.86	6.45	4.63
Kahururu	F	61	-28.29	7.84	36.03	6.75	5.34
Kahururu	F	75	-28.27	7.86	32.57	7.25	4.49
Mangapiko	M	76	-27.13	8.38	36.34	7.50	4.85
Mangapiko	M	81	-26.75	8.55	31.84	6.53	4.88
Mangapiko	F	77	-27.08	8.62	33.22	6.28	5.29
E.menziesii							
Ohautira	M	61	-25.46	5.37	33.78	7.48	4.52
Ohautira	M	55	-25.28	5.41	36.71	8.94	4.11
Ohautira	F	51	-25.94	5.10	39.14	8.65	4.53
Kahururu	F	38	-28.42	7.50	36.10	8.12	4.44
Kahururu	F	41	-28.53	7.70	36.39	7.39	4.92
Kahururu	M	46	-28.39	7.94	39.48	9.58	4.12
Mangapiko	M	59	-27.09	8.80	35.82	7.23	4.95
Mangapiko	M	63	-26.95	9.11	35.67	7.35	4.86
Mangapiko	F	58	-27.26	9.06	39.25	7.52	5.22

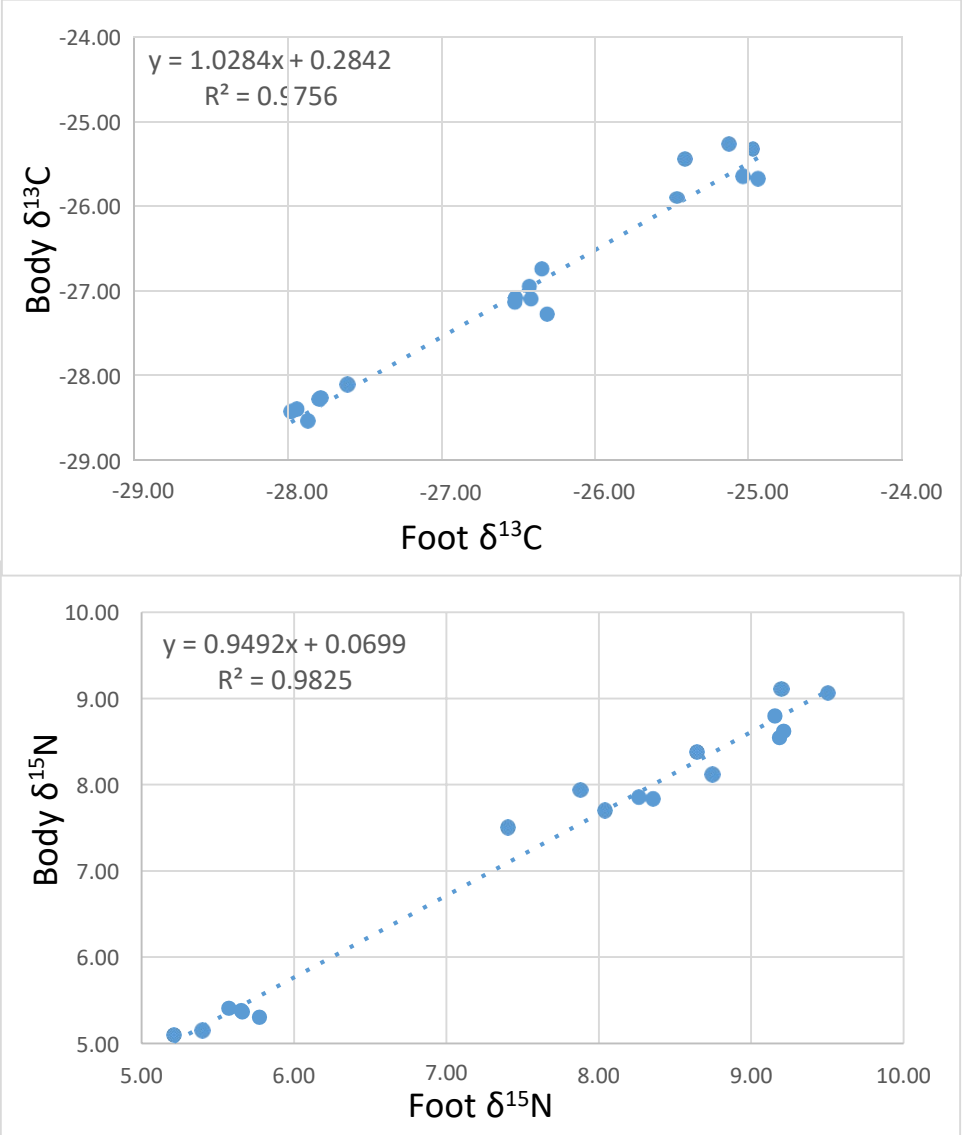

Figure S1 Relationships between $\delta^{13}C$ and $\delta^{15}N$ (‰) in foot tissue and remaining soft tissue for *Echyridella menziesii* and *E. aucklandica* from three Waikato stream sites

A2: Co-authorship form for Chapter 2

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First record of complex release strategies and morphometry of glochidia in sympatric Echyridella species (Bivalvia: Unionida: Hyriidae)

Nature of contribution by PhD candidate	Designed study with input from supervisors; conducted field sampling and developed appropriate methods; analysed data and wrote draft manuscripts for input from co-authors
Extent of contribution by PhD candidate (%)	90

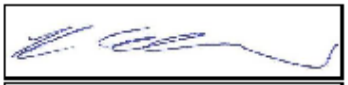
CO-AUTHORS

Name	Nature of Contribution
Kevin J. Collier	Provided science advice; field assistance; comments on draft manuscript.
Susan J. Clearwater	Advised on study design and methods; comments on draft manuscript

Certification by Co-Authors

The undersigned hereby certify that:

- the above statement correctly reflects the nature and extent of the PhD candidate's contribution to this work, and the nature of the contribution of each of the co-authors; and

Name	Signature	Date
Kevin Collier		26/3/21
Susan Clearwater		26/3/21

July 2015

A3: Supplementary material from Chapter 3

Text A.3.1.: Laboratory temperature study

Context

Two separate laboratory experiments were conducted to support field observations of temperature regimes and for ADD calculations. For the first experiment, minimum thermal tolerance was tested in gravid *E. menziesii* and *E. aucklandica* to determine at what temperatures reproduction was likely disrupted and abortions of unviable larvae occurred. Procedures followed Melchior (2017) that examined differences in larval release timing in *E. menziesii* at constant laboratory temperatures of 8°C, 12°C and 18°C. Based on those findings, that early larvae were aborted at 8°C for *E. menziesii* while at 12°C and 18°C mussels released mature glochidia, a narrower gradient of constant water temperatures of 9°C, 10°C and 11°C was used to refine minimum thermal thresholds for *E. menziesii* compared with *E. aucklandica*.

6.7.1 Methods

During the austral mid-summer (2019) at Ohautira Stream, 15 gravid (Stage 3-4) females of each species were collected for the first experiment and kept separately in two aerated buckets containing stream water (17°C) and substrate for transport to the laboratory to examine species-specific minimum thermal thresholds. Individuals were randomly divided into three static temperature treatments (n = 5 of each species at 9°C, 10°C and 11°C; light:dark cycle of 16:8 hours), and held separately in 2.5 L glass aquaria containing 3 cm silica sand and oxygenated with aerators (see Plate below). Individuals were fed every other day with a 2:1 algae mixture using Reed Marine shellfish diet and *Nannochloropsis* (Reed Mariculture, Campbell California, USA). All mussels were acclimated to the same thermal regime of 12 °C for 7 days before decreasing temperatures to their assigned treatment by moving the aquaria to separate controlled temperature rooms set at the assigned temperature. Observations of glochidia release were recorded daily. If glochidia were released, they were removed, counted, and analysed for viability, characterised by (i) the presence of hooks on opposing valves, (ii) translucent valves, free of their vitelline membrane, and (iii) rapid opening and closing of the glochidia valves viewed under a binocular microscope (40 x magnification).

The second laboratory trial was undertaken to test for maximum thermal thresholds for the first release of glochidia and peak release, and to detect any differences

in the influence of temperature on the timing of larval release between each species. Here, thermal limits of initiation in larval release were estimated using a dynamic thermal threshold method (Lutterschmidt & Hutchison, 1997) in which individuals of both species were exposed to a constant increase in temperature. For this experiment, 6 gravid females (Stage 4; see Melchior et al., 2021) of each species were collected in summer following the procedures described above. Individuals were acclimated to laboratory conditions for 7 days at 12 °C with a 16:8 hour light dark cycle. Twelve degrees Celsius was chosen as this was a temperature at which unionids are known to delay brood timing (Melchior, 2017). Individuals were held separately, monitored daily for potential glochidia abortions, and fed as in Experiment 1. At the onset of the trial, temperatures were increased at a rate of 1° C per day until glochidia release by each mussel peaked. The threshold for larval release was then determined as the mean thermal point at which individuals had released at least (i) 100 viable glochidia for *E. menziesii*, or (ii) one conglutinate containing viable glochidia in *E. aucklandica*. ADD required for glochidia release were calculated for each species.

Laboratory trial set-up for testing thermal tolerance in gravid *Echyridella menziesii* and *E. aucklandica*.

6.7.2 Results - Minimum Temperature Trial

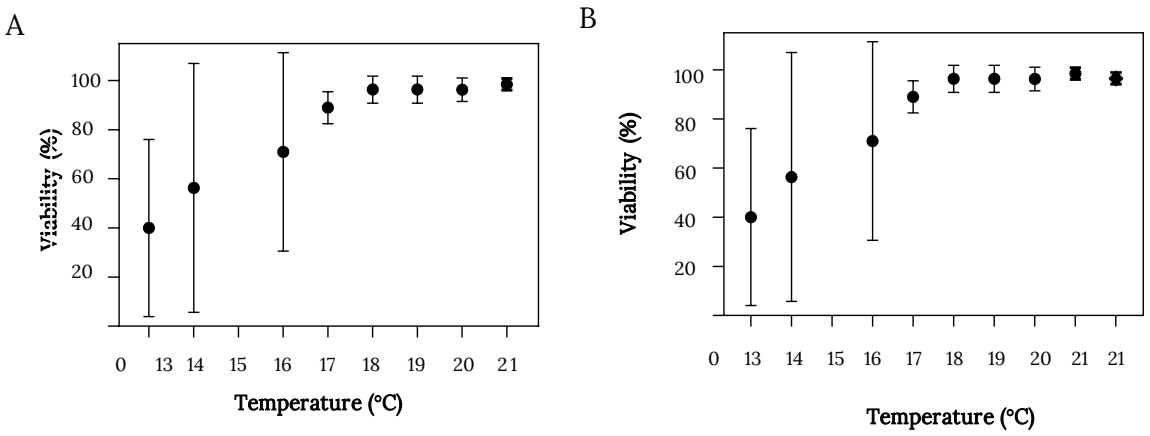
The 9 °C treatment resulted in premature glochidia release in both species, with the expulsion of individual unviable larvae in *E. menziesii* (released larvae per individual, mean ± SD = 1063 ± 973.6, n = 5, range: 175–2785) and conglutinates containing closed (unviable) larvae in *E. aucklandica* (mean number of released conglutinates per individual = 89 ± 93.6, n = 5, range: 3–240). Larvae of both species were aborted within the first 5 days of the 9 °C treatment, with no significant differences in the timing of premature release between species ($t_{(4)}$ = 0.172, p = 0.87). At the end of the experiment, no major brood releases were observed by individuals of both species exposed to 10 and 11 °C treatments, although there was minor release by one *E. menziesii* held at 10 °C which occurred 18 days into the experiment. Brood inspections after 20 days exposure to low temperatures confirmed no or minimal larval release at these treatments, while within the 9 °C treatment only empty or half empty broods were found. Given that 10 °C was the threshold temperature below which abortions occurred in this study (except for one occurrence), and considering the glochidia abortions reported at 8 °C by Melchior (2017), 10 °C was chosen as the minimum temperature that was used for the calculation of ADD for use in exploring developmental thresholds for both *E. menziesii* and *E. aucklandica* among sites.

6.7.3 Results - Maximum Temperature Trial

During the acclimation period at a constant 12 °C, one individual *E. menziesii* was observed to abort part of its brood and was therefore excluded from the remainder of the trial. In *E. aucklandica*, glochidia release occurred at the first instance of temperature increase by 1 °C at 13 °C (equivalent to 97 ADD including the 7-day acclimation period), with 3 out of 6 individuals observed to have released conglutinates. Peak release (6 out of 6 individuals releasing viable conglutinates) occurred between 17 and 18 °C (159-177 ADD), however, conglutinates were still gradually being released at 21 °C (237), 8 days after initial release. Mean number of conglutinates released per individual was 164.8 ± 65.3 (range: 91 – 241). Viability of released glochidia remained high (>85%) with each temperature increase.

In *E. menziesii*, peak release occurred at 18°C (177 ADD) (5 out of 5 individuals) with viable glochidia continuing to be released for 9 days post initial release at 22°C (259 ADD). Mean number of glochidia released at the peak was 2178 ± 2256.3 (range: 1000 – 6200). Mean viability in released glochidia at the first increases in temperature was low, but

gradually increased at 17 °C (See Figure below), indicating possible stress release within the first days rather than the reaching a natural maximum threshold for larval release.



Mean viability for Echyridella aucklandica (A) and *E. menziesii* (B) at each temperature increase throughout the laboratory trial.

Table A.3.1. Physicochemical data showing monthly temperature (°C), dissolved oxygen (% and mg/L), specific conductivity (µs/cm @25°C-) among sites with temperature ranges in parantheses (refer to Table 3.1 for annual summaries).

		Month (2018-19)												
Site	Parameter	March	April	May	June	July	August	September	October	November	December	January	February	March
Ohautira	Water temperature (°C)	16.8±0.3 (15.6 -18.1)	14.5±2.1 (11.6-17.4)	12.5±1.7 (6.9-15.9)	10.2±1.7 (6.8-13.7)	9.8±1.3 (6.4-13.7)	10.4±0.8 (8.5-12.5)	11.5±1.5 (8.6-16.1)	12.4±1.5 (9.2-14.7)	14.7±1.5 (10.6-18.2)	16.4±1.2 (12.6-20.5)	18.3±1.5 (15.2-23.2)	18.9±1.5 (13.9-22.1)	17.1±1.1 (14.2-19.2)
	Dissolved oxygen (%)	111.8	117.4	99.0	110.4	105.1	95.5	102.9	103.2	97.0	110.7	104.8	101.8	108.6
	Dissolved oxygen (mg/L)	10.8	12.4	11.1	13.0	12.2	9.7	11.4	11.2	10.3	10.9	10.1	9.4	11.0
	Specific conductivity (µs/cm @t 25°C)	157.0	154.0	137.0	151.4	106.1	130.0	121.0	146.0	152.1	129.0	138.6	110.4	160.1
Kahururu	Water temperature (°C)	17.6±0.3 (17.2-18.2)	15.5±1.4 (12.7-18.3)	13.8±1.7 (7.9-16.7)	11.1±1.7 (7.6-14.6)	10.7±1.3 (7.3-13.5)	11.2±0.8 (9.4-13.5)	12.7±1.2 (10.5-15.2)	13.5±0.9 (15.4-21.8)	15.5±1.1 (13.1-18.4)	17.8±1.1 (15.4-21.8)	19.6±1.3 (16.1-22.7)	20.2±1.3 (17.3-24.2)	17.4±1.2 (14.0-20.7)
	Dissolved oxygen (%)	108.0	80.5	94.3	99.7	100.6	93.5	96.5	91.7	106.7	104.1	101.7	88.2	69.5
	Dissolved oxygen (mg/L)	10.3	8.4	10.2	11.6	11.6	10.5	10.6	9.2	10.3	9.9	10.7	8.7	6.9
	Specific conductivity (µs /cm @ 25°C)	165.0	166.0	124.2	161.9	130.0	150.0	147.0	154.0	154.0	140.2	153.4	160.8	181.6
Pakoka	Water temperature (°C)	18.4±1.9 (14.5-24.4)	14.9±1.8 (9.9-18.9)	13.4±1.5 (8.1-17.4)	10.7±1.6 (6.9-13.6)	10.7±1.2 (7.2-12.7)	11.1±0.8 (8.7-13.4)	12.1±1.7 (9.4-15.9)	13.1±1.3 (9.7-17.8)	15.0±1.8 (10.1-20.7)	17.6±1.7 (13.2-23.9)	18.9±1.8 (15.2-25.3)	19.7±2.0 (13.4-24.1)	17.8±1.6 (13.6-20.9)
	Dissolved oxygen (%)	115.8	108.0	98.0	118.7	105.1	103.5	-	104.8	107.2	113.2	114.9	99.0	111.2
	Dissolved oxygen (mg/L)	11.5	11.2	10.5	12.9	12.2	11.7	-	11.5	11.3	10.8	10.8	9.3	11.5
	Specific conductivity (µs /cm @ 25°C)	138.0	193.0	166.5	128.7	106.1	134.0	-	143.8	117.0	130.7	133.7	166.3	166.5
Mangapiko	Water temperature (°C)	17.8±0.6 (15.5-19.9)	15.1±1.6 (10.7-18.8)	13.4±1.7 (7.2-14.2)	11.1±1.6 (7.7-16.2)	10.9±1.6 (7.1-13.3)	11.2±1.0 (8.3-14.1)	12.6±1.5 (8.8-16.6)	13.7±1.4 (9.6-17.3)	15.5±1.6 (11.2-19.2)	17.7±1.3 (13.7-20.9)	19.2±1.9 (15.7-24.9)	19.9±1.6 (13.8-22.9)	18.1±1.6 (14.4-21.6)
	Dissolved oxygen (%)	95.6	95.2	95.3	99.0	101.3	117.4	100.0	93.4	102.6	112.6	101.8	115.8	99.6
	Dissolved oxygen (mg/L)	9.5	10.1	10.2	10.8	11.3	12.4	10.5	10.0	9.9	10.8	9.4	10.1	9.9
	Specific conductivity (µs /cm @ 25°C)	102.0	122.0	124.2	122.4	103.0	124.0	106.0	107.0	107.3	107.1	110.4	93.4	110.8

Table A.3.2. Minimum and median valve lengths of all female and male mussels collected (interquartile ranges in parentheses), sex ratio and total number of mussels sampled at four sites.

	Min. brooding length (mm) ♀	Sex ratio ♂:♀[1]	length (mm) ♀	length (mm) ♂	*N*
E. menziesii					
Ohautira	41	1.02:0.98 *p*=0.999	53 (49-58)	54 (50-56)	124
Kahururu	26	1.12:0.88 *p*=0.690	47 (36-47)	41 (43-50)	43
Pakoka	23	1.04:0.98 *p*=0.855	66 (59-73)	64 (59-72)	43
Mangapiko	36	0.80:1.20 *p*=0.203	59 (53-62)	60 (56-62)	50
E. aucklandica					
Ohautira	54	1.0:0.95 *p*=0.262	88 (82-90)	86 (82-93)	150
Kahururu	44	1.40:0.60 *p*=0.115	71 (62-75)	72 (66-76)	45
Pakoka	49	1.18:0.82 *p*=0.392	87 (82-93)	93 (87-97)	51
Mangapiko	40	1.12:0.88 *p*=0.405	77 (69-80)	76 (71-79)	47

[1] Binomial exact tests assessed deviation from a 1:1 sex ratio.

Table A.3.3. Mann-Whitney U test statistics comparing valve lengths between males and females for each species across each site.

Species and sites	Mann-Whitney *U*	*p*
E. menziesii		
Ohautira	1900	0.96
Kahururu	94	0.09
Pakoka	126.5	0.58
Mangapiko	275.5	0.63
E. aucklandica		
Ohautira	2436	0.20
Kahururu	200	0.34
Pakoka	222	0.07
Mangapiko	185	0.69

Table A.3.4. Mann-Whitney U test statistics analysing differences in accumulated degree days required to reach brooding onset (Stage 1) and brooding peak (Stage 4) between *Echyridella menziesii* and *E. aucklandica*.

Site	Mann-Whitney *U*	*p*
Brooding onset		
Ohautira	221	0.018
Kahururu	30	0.01
Pakoka	0	<0.001
Mangapiko	35	<0.001
Brooding peak		
Ohautira	400	<0.001
Kahururu	99	<0.001
Pakoka	448	<0.001
Mangapiko	651	0.002

Table A.3.5. Summary of generalized linear models (beta regression) explaining relationship between accumulated degree days on patterns of brooding proportions of E. *aucklandica* and E. *menziesii* is the beta coefficient (which is the degree of change in the outcome variable for every 1-unit of change in the predictor variable), χ^2 is a partial Wald Chi-Squared test to assess that the coefficient is significant. AME are the average marginal effects (calculation of marginal effects at every observed value of X and averaged across the resulting effect estimates) for each model.

Species and Model	β	SE	X^2	*p*	*AME*	SE
E. aucklandica						
Onset brooding	0.010	0.004	3.202	**0.006**	0.002	0.0006
Peak brooding	-0.001	0.001	-3.110	**0.002**	-0.0003	0.0001
E. menziesii						
Onset brooding	0.005	0.002	1.979	**0.048**	0.001	0.0005
Peak brooding	-0.001	0.001	-1.875	0.061	-0.0003	0.0001

A4: Supplementary material from Chapter 4

Table A.4.1. Number of fish caught (N), catch per unit effort (CPUE: fish per 100 m^2) and mean length for each species electro-fished at each site for the initial investigation.

Site	Species	*N*	CPUE (fish/100m^2)	$\bar{x}$ length ± SD (mm)
Ohautira	Longfin eel (*Anguilla dieffenbachii*)	15	7.14	200±166
	Shortfin eel (*Anguilla australis*)	1	0.48	63±6
	Redfin bully (*Gobiomorphus huttoni*)	11	5.24	62±14
	Inanga (*Galaxias maculatus*)	5	2.38	57±8
	Lamprey ammocoete (*Geotria australis*)	5	2.38	95±5
	Smelt (*Retropinna retropinna*)	1	0.48	57
	Total	**38**	**18.09**	
Kahururu	Longfin eel (*Anguilla dieffenbachii*)	2	0.85	200±59
	Redfin bully (*Gobiomorphus huttoni*)	15	6.38	31±8
	Common bully (*Gobiomorphus cotidianus*)	7	2.98	35±6
	Inanga (*Galaxias maculatus*)	3	1.28	63±9
	Total	**27**	**11.49**	
Pakoka	Longfin eel (*Anguilla dieffenbachii*)	19	6.79	387±153
	Shortfin eel (*Anguilla australis*)	6	2.14	125±76
	Redfin bully (*Gobiomorphus huttoni*)	12	4.29	77±10
	Inanga (*Galaxias maculatus*)	4	1.43	52±3
	Lamprey ammocoete (*Geotria australis*)	7	2.50	79±8
	Smelt (*Retropinna retropinna*)	1	0.36	67
	Total	**49**	**17.50**	
Mangapiko	Shortfin eel (*Anguilla australis*)	7	3.59	115±44
	Common bully (*Gobiomorphus cotidianus*)	16	8.21	40±7
	Cran's bully (*Gobiomorphus basalis*)	6	3.08	56±5
	Brown trout* (*Salmo trutta*)	1	0.51	95
	Gambusia* (*Gambusia affinis*)	15	7.69	27±3
	Total	**45**	**23.08**	

*Non-indigenous fish species

Table A.4.2. Glochidia infestation intensity (total glochidia per fish) and prevalence (percentage of fish infested by *Echyridella menziesii* or *E. aucklandica*) within and across sites in the temporal survey. Infestation type is indicated as attached (A) or encysted (E), and as internal (In) and/or external (Ex). Fish species are divided into benthic and pelagic species. CI = 95% confidence intervals.

Species	Site	No. fish	*E. menziesii* x̄ (CI) prevalence (%)	*E. aucklandica* x̄ (CI) prevalence (%)	x̄ (CI) intensity (no./fish)	Infestation type
Benthic						
Anguilla dieffenbachii	Ohautira	15	6.7 (0.2,32)	0	1	A(Ex)
	Pakoka	19	21.1 (6,46)	0	1.75 (1.0, 2.5)	A(Ex)
	Kahururu	2	50 (0.1,99)	0	1	A(Ex)
	Total	**36**	**16.7 (0.6,33)**	**0**	1.5 (1, 2)	
Gobiomorphus basalis	Mangapiko	6	67.7 (22,96)	0	2 (1, 3.7)	E(In/Ex)
	Total	**6**	**67.7 (22,96)**	0	3 (1, 3.7)	
Gobiomorphus cotidianus	Kahururu	7	0	0	-	
	Mangapiko	16	62.5 (35, 85)	0	1.9 (1.3, 2.6)	E(In/Ex)
	Total	**23**	**43.5(23, 66).**	0	1.9 (1.3, 2.6)	
Gobiomorphus huttoni	Ohautira	11	90.9 (59, 100)	0	2 (1.2, 3.5)	E(In/Ex)
	Pakoka	12	83.3 (52, 98)	0	3.4 (2, 4.3)	E(In/Ex)
	Kahururu	15	26.7 (0.8, 55)	0	3 (1, 5)	E(In/Ex)
	Total	**38**	**63.2(48, 78)**	**0**	2.7 (2, 3.9)	
Pelagic						
Gambusia affinis	Mangapiko	15	33.3 (12, 62)	0	1	A(Ex)
	Total	**15**	**33.3 (12, 62)**	**0**	1	
Galaxias maculatus	Ohautira	13	7.7 (0, 36)	0	2	A(In)
	Pakoka	10	10 (0, 45)	0	1	A(In)
	Kahururu	3	0	0	-	
	Total	**26**	**7.7 (0.9, 25)**	**0**	1.5 (1, 1.5)	
Retropinna retropinna	Ohautira	1	0	100	1	E(In)*
	Pakoka	1	0	100	1	E(In)*
	Total	**2**	**0**	**100**	1	
Salmon trutta	Mangapiko	1	100	0	9	A(In)
	Total	**1**	**100**	**0**	9	

**E. aucklandica*

Table A.4.3 Summary of infestation parameters from captured fish infested with either *Echyridella menziesii* or *E. aucklandica* glochidia at sites Ohautira and Pakoka in the temporal study. Values are means of sampling dates with 95% confidence limits in parentheses. Abundance and intensity confidence intervals were calculated using the BCa (bias-corrected and accelerated) method with 2000 bootstrap replication.

	Site	CPUE (fish 100 m^{-2})	Infestation prevalence (%)	Abundance (# glochidia total $fish^{-1}$)	Intensity (# glochidia infested $fish^{-1}$)	Density (# glochidia m^{-2})	Number of fish infested	Number of fish examined
E. menziesii								
G. huttoni	Ohautira	5.2 (4.1,6.3)	27.4 (17,40)	0.6 (0.3,1.2)	2.2 (1.4,3.8)	0.022 (0,0.04)	17	62
	Pakoka	4.1 (1.8,6.4)	68.4 (55,8)	4.9 (3.3,7.9)	6.2 (3.3,7.9)	0.123 (0,0.24)	39	57
	Total	4.7 (3.6,5.7)	47.1 (40, 59)	2.7 (1.8,4.1)	5.7 (4.1,8.4)	0.073 (0,0.13)	56	119
A. dieffenbachii	Ohautira	5.7 (1.5,9.8)	5 (1,13)	0.06 (0,0.2)	1.3 (1.0,1.7)	0.003 (0,0.004)	3	64
	Pakoka	6.9 (3.2,10.6)	24.7 (17,35)	0.5 (0.3,0.7)	1.8 (1.4,2.4)	0.03 (0,0.008)	24	97
	Total	6.3 (4.1,8.5)	16.8 (11, 24)	0.3 (0.2,0.4)	1.8 (1.4,2.3)	0.016 (0,0.03)	27	161
G. maculatus	Ohautira	9.1 (0.8,19)	0.9 (0,5)	0.02 (0,0.05)	2*	0.007*	1	113
	Pakoka	1.7 (0,4.0)	8.3 (1,27)	0.08 (0,0.2)	1*	0.003*	1	24
	Total	5.4 (0.6,10.3)	2.2 (0,6.3)	0.03 (0,0.08)	1.3 (1,1.7)	0.002 (0,0.15)	2	137
E. aucklandica								
R. retropinna	Ohautira	0.38 (0,0.88)	75 (19,99)	6.5 (0.8,16.8)	8.7*	0.025 (0,0.2)	3	4
	Pakoka	0.4 (0,0.8)	67 (22,96)	6.3 (2.2,10.8)	9.5 (6.5,13.8)	0.024 (0,0.09)	4	6
	Total	0.4 (0.2,0.6)	70 (35,93)	6.4 (3.1,11.2)	9.1 (4.9,14.4)	0.024 (0,0.08)	7	10
G. maculatus	Ohautira	9.1 (0.8,19)	0.9 (0,5)	0.008 (0,0.03)	1*	0.0008*	1	113
	Pakoka	1.7 (0,4.0)	0	0	0	0	0	24
	Total	5.4 (0.6,10.3)	0.7 (0,4)	0.007 (0,0.02)	1*	0.0004*	1	137
G. huttoni	Ohautira	5.2 (4.1,6.3)	1.8 (0,9.7)	0.1 (0,0.3)	6*	0.005*	1	55
	Pakoka	4.1 (1.8,6.4)	0	0	0	0	0	58
	Total	4.7 (3.6,5.7)	0.09 (0,4.8)	0.05 (0,0.2)	6*	0.002*	1	113

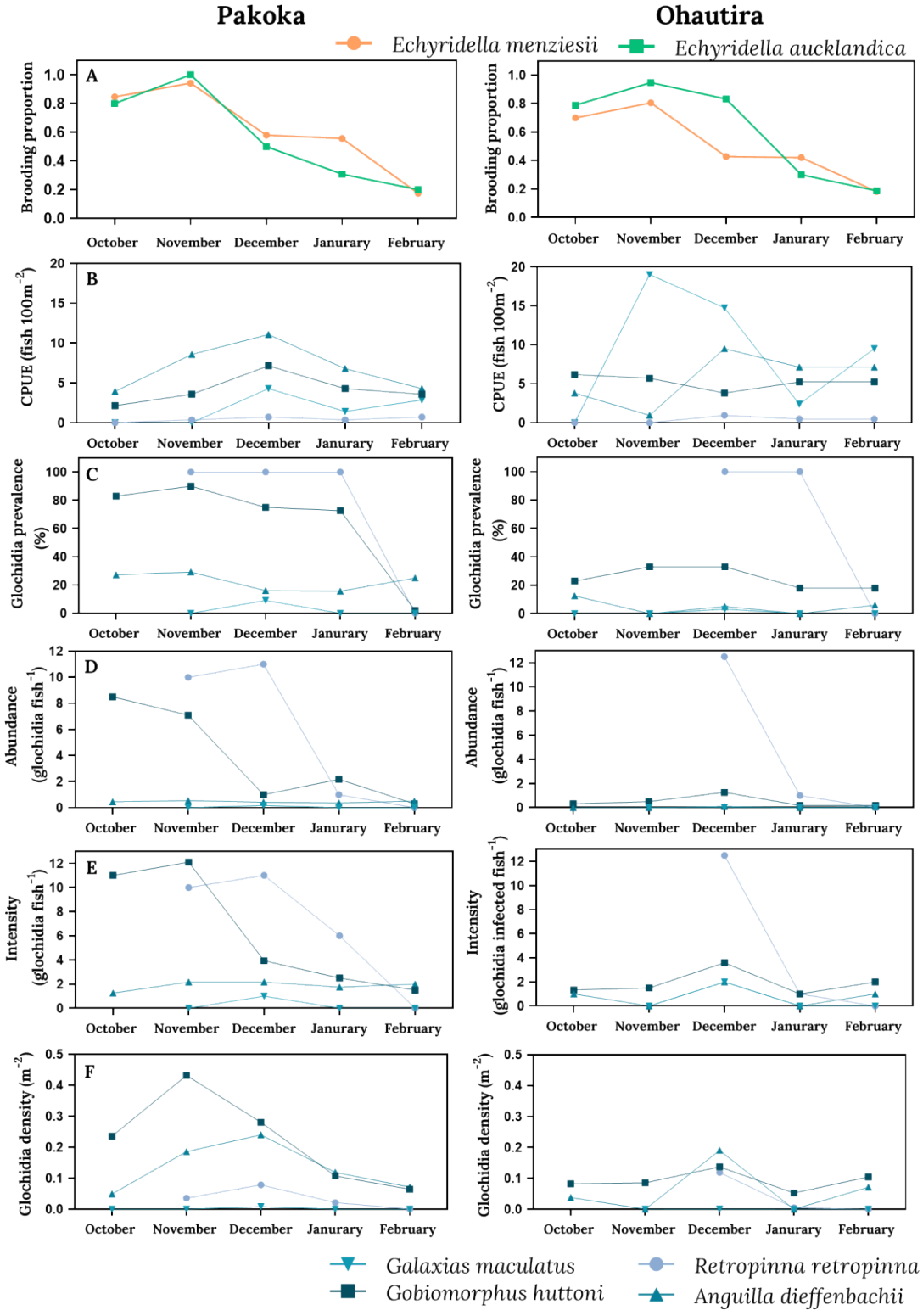


Figure A.4.1. A) Mussel brooding proportions in *Echyridella menziesii* and *E. aucklandica*, B) CPUE (fish 100 m^{-2}), C), glochidia prevalence (% infested), D) glochidia abundance (mean number of glochidia on all fish), E) glochidia intensity (mean number of glochidia per infested fish), and F) average glochidia density (glochidia m^2 of fish surface area) at sites Pakoka and Ohautira, from October to February 2018-2019 on four native fish species.

Table A.4.4. Fish wet weight, total length and total number used in each laboratory fish infestation trial (see Section 4.3.2.3).

Fish - mussel species	Wet weight $\bar{x}$ + SD (g)	Length $\bar{x}$ + SD (mm)	Number of fish
Per Individual tanks			
R. retropinna - *E. menziesii*	2.2 ± 0.9	66.5 ± 7.9	8
R. retropinna - *E. aucklandica*	1.9 ± 0.3	62.2 ± 4.4	8
G. cotidianus - *E. menziesii*	1.4 ± 0.7	46.8 ± 6.5	8
G. cotidianus - *E. aucklandica*	2.5 ± 1.7	54.8 ± 11.1	8
G. maculatus - *E. menziesii*	1.2 ± 0.5	51.0 ± 3.8	8
G. maculatus - *E. aucklandica*	1.7 ± 0.3	58.0 ± 4.3	8
'Broadcast' – *E. aucklandica* tank			
R. retropinna	2.7 ± 1.4	67.2 ± 9.3	15
G. cotidianus	1.9 ± 1.3	58.4 ± 8.4	15
G. maculatus	1.4 ± 0.8	56.7 ± 5.8	15
G. huttoni	2.4 ± 0.3	53.3 ± 3.9	5
'Feeding' – *E. aucklandica* tank			
R. retropinna	3.7 ± 0.9	72.2 ± 7.9	9
G. cotidianus	2.0 ± 1.1	51 ± 7.9	5
G. maculatus	1.5 ± 0.7	53.8 ± 7.6	6
G. huttoni	1.8 ± 0.9	54.2 ± 2.9	5

A5: Supplementary material from Chapter 5

Table A.5.1. Mean (±SD) lengths and number of fish (N) captured within each 10-m sub-reach from November 2018 to January 2019.

Sub-reach	Species	November		December		January	
		N	Length	N	Length	N	Length
1	Longfin eel (*Anguilla dieffenbachii*)	4	147.5±53.6	0	-	2	175.5±94.0
	Redfin bully (*Gobiomorphus huttoni*)	2	58.5±19.1	3	41±8.8	3	34±8.8
	Inanga (*Galaxias maculatus*)	4	59.5±13.7	6	53.5±5.5	3	59.3±10.1
	Lamprey ammocoete (*Geotria australis*)	0	-	1	91	0	-
	Sub-reach Total	**10**		**10**		**8**	
2	Longfin eel (*Anguilla dieffenbachii*)	3	255±185.7	1	207	0	-
	Redfin bully (*Gobiomorphus huttoni*)	3	62.7±8.1	3	42±7.5	3	37.3±3.5
	Inanga (*Galaxias maculatus*)	12	53.2±8.9	7	58.7±14.1	4	50.2±2.5
	Smelt (*Retropinna retropinna*)	1	85	0	-	0	-
	Sub-reach Total	**19**		**11**		**7**	
3	Longfin eel (*Anguilla dieffenbachii*)	4	233.7±167.7	3	150.3±87.2	2	101.5±4.9
	Redfin bully (*Gobiomorphus huttoni*)	2	52±9.9	5	53.7±13.0	3	45±18.0
	Inanga (*Galaxias maculatus*)	9	52.8±7.8	2	63±2.8	4	57.5±14.5
	Sub-reach Total	**17**		**10**		**9**	
	Total	**46**		**31**		**24**	

Table A.5.2. Mean (±SD) mussel and fish densities within bank and mid-channel grid cells. Significant values shown in bold.

Species	Bank cell density (no./m²)	Mid-channel cell density (no./m²)	*p*-value
E. aucklandica	0.23 ± 0.03	0.10 ± 0.01	**0.002**
E. menziesii	0.33 ± 0.09	0.02 ± 0.02	**0.006**
A. dieffenbachii	0.24±0.10	0.02±0.03	**0.03**
G. huttoni	0.25±0.05	0.02±0.01	**0.002**
G. maculatus	0.35±0.32	0.31±0.07	0.83

Table A.5.3. Statistical comparisons of female *Echyridella aucklandica* and female *E. menziesii* microhabitat use and availability for continuous (Kolmogorov–Smirnov two-sample (D) test; all variables except refugia) and categorical (chi-squared test; refugia) variables pooled across all sub-reaches for the summer survey (peak brooding) and winter survey (onset brooding). Subscripts represent degrees of freedom for chi-squared tests. Significant values shown in bold.

	Echyridella aucklandica		*Echyridella menziesii*	
Variable	Statistic	*p*	Statistic	*p*
Summer				
Velocity (m/s)	0.23	0.15	0.17	0.65
Depth (m)	0.44	**0.0001**	0.52	**0.0001**
Substrate index	0.26	0.075	0.21	0.38
Refugia type	$31.24_{(4)}$	**<0.0001**	$20.51_{(4)}$	**0.0004**
Post-flood				
Velocity (m/s)	0.66	**<0.0001**	0.35	**0.003**
Depth (m)	0.39	**0.0007**	0.61	**<0.0001**
Substrate index	0.42	**0.0002**	0.46	**<0.0001**
Refugia type	$23.71_{(4)}$	**<0.0001**	$39.48_{(4)}$	**<0.0001**

Table A.5.4. Statistical comparisons of host fish (*Gobiomorphus huttoni, Anguilla dieffenbachii* and *Galaxias maculatus*) microhabitat use compared to their respective mussel species for continuous (Kolmogorov–Smirnov two-sample (D) test; all variables except refugia type) and categorical (chi-squared test; cover) variables pooled across all sub-reaches for the summer survey (peak brooding). Subscripts represent degrees of freedom for chi-squared tests. Significant values shown in bold.

	Echyridella menziesii				*Echyridella aucklandica*	
	Gobiomorphus huttoni		*Anguilla dieffenbachii*		*Galaxias maculatus*	
Variable	Statistic	*p*	Statistic	*p*	Statistic	*p*
Summer						
Velocity (m/s)	0.17	0.98	0.23	0.93	0.86	**<0.0001**
Depth (m)	0.72	**0.001**	0.40	0.43	0.91	**<0.0001**
Substrate index	0.45	0.06	0.23	0.78	0.45	**0.01**
Refugia type	$56.5_{(4)}$	**<0.0001**	$34.4_{(4)}$	**<0.0001**	$83.9_{(4)}$	**<0.0001**