Dietary overlap between the non-native mosquitofish (*Gambusia affinis*) and native common bully (*Gobiomorphus cotidianus*):

Evidence for interspecific competition?

A thesis

submitted in partial fulfilment

of the requirements for the degree

of

MSc (Research) in Ecology and Biodiversity

at

The University of Waikato

by

Flavian Ember

THE UNIVERSITY OF
WAIKATO
Te Whare Wānanga o Waikato

2023

Abstract

Since their establishment in New Zealand in the 1930s, the globally invasive western mosquitofish (*Gambusia affinis*) has become widespread throughout lowland freshwaters, primarily in the North Island. Despite co-occurring in many ecosystems, interactions between native common bullies (*Gobiomorphus cotidianus*) and *G. affinis* are presently unknown. Like *G. affinis*, *G. cotidianus* are abundant in lowland waterways, tolerant of a wide range of environmental conditions and can consume similar prey. My research investigated the potential dietary overlap between these two species using a controlled mesocosm experiment and a stable isotope study of co-occurring *G. affinis* and *G. cotidianus* in natural lake habitats.

Zooplankton were selected as the prey source for the mesocosm experiment. Twenty-one 100 L mesocosms were assigned either four *G. cotidianus*, four *G. affinis*, or were left as a control group (i.e., with no fish) and monitored for 14 days. Zooplankton community composition in the fish treatments was significantly different from the control tanks by Day 14 of the experiment yet did not differ significantly from one another. Both *G. cotidianus* and *G. affinis* reduced the abundances of larger crustacean zooplankton relative to the controls. This is likely to have reduced the effects of predation, competition, and physical interference by the crustaceans on rotifers, allowing rotifer species to reach numerical dominance in the treatment mesocosms by the end of the experiment. In contrast, zooplankton assemblages in the control mesocosms were dominated by cladocerans by Day 14, indicating that these had a competitive advantage in the absence of zooplanktivorous fish.

Four Waikato lakes were selected as sites for the stable isotope study. *Gambusia affinis*, *G. cotidianus*, and basal food web taxa were collected from each lake during the austral winter of 2022. Samples were analysed for the stable carbon and nitrogen isotopes ^{13}C and ^{15}N, and results were compared to data published by Collier et al. (2018) from the same taxa and lakes during an austral summer. Mean δ^{13}C values of *G. affinis* were significantly enriched compared to *G. cotidianus* at three lakes in both summer and winter, while the mean δ^{15}N of *G. affinis* was significantly enriched at three lakes in summer and all lakes in winter. These differences were greatest in winter, even when the summer dataset was censored to match the winter fish length distribution. My results indicate that *G. affinis* and *G. cotidianus* are likely consuming different prey sources and that their diet is most similar during summer. Additionally, my results showed seasonal variation between lakes in the isotopic enrichment of both fish and basal food web taxa, suggesting food webs and trophic interactions may differ between sites.

Overall, my thesis demonstrates the potential for interspecific competition between native *G. cotidianus* and non-native *G. affinis* in New Zealand through an example of zooplankton dietary overlap in a controlled experimental context. The stable isotope experiment provided mixed evidence for dietary overlap across both summer and winter in sympatric lake populations. Both experiments offer numerous future research opportunities to investigate the mechanisms behind the findings.

Acknowledgements

I would first like to acknowledge my supervisor, Ian Duggan. I appreciate the extensive amount of work you have put into helping me complete this thesis, ranging from planning and conducting experiments, identifying zooplankton, analysing data, and of course your detailed and constructive feedback when it came to writing it all up. I have learned a lot over the last two years, and I am grateful for your experience, patience, and quick replies whenever I asked for help. My thanks also go to my supervisor Nick Ling, particularly for your help with planning the stable isotope study, lab work, and interpreting the results. Kat and Warrick, thank you for your help in both the lab and field, especially with some of the less pleasant tasks such as digging through lake detritus for invertebrates in the middle of winter. My thanks go to the landowners who gave me access through their property to my study sites.

Thanks to the University of Waikato for the scholarships that supported my learning over the last five years, the Research Masters Scholarship in particular helped me focus my time on this thesis. I also appreciate staff at the University of Waikato who helped me fit my enrolment around my job at the Waikato Regional Council, and my WRC colleagues for being flexible with work while I progressed with my studies.

I would like to thank my fellow students in R-block and from my postgraduate papers, it was great being able to share experiences, struggles, and advice as we progressed through our studies. Special thanks to Francis, for always being happy to read my work, and to Melissa, for your help with formatting. Helena, Josh, Hannah, Matthew, Matt, Shay, Lev, and all my other friends, thank you for listening to my frequent thesis status updates and being ready with sympathy, advice or much needed distractions. Köszönöm and danke to my mum and dad, and thank you to Nicola. Your ongoing support and encouragement over the last two years helped me focus on the bigger picture whenever it seemed there was no end in sight.

A big thank you again, you all have helped make this thesis possible.

Table of Contents

List of Figures

List of Tables

Appendix Tables

Chapter 1.

Introduction and literature review

1.1 *Gambusia*: a global context to invasive freshwater fish

1.1.1 Invasive fish at a global scale

The establishment of non-indigenous fish species is a significant threat to freshwater ecosystems worldwide, which along with other anthropogenic pressures, contribute to a global trend of biodiversity decline (Collins et al., 2002; Reid et al., 2019). While some non-native species have positive or neutral social, economic or ecological effects (Copp et al., 2005), many classed as invasive are able to rapidly spread and proliferate, contributing to overwhelmingly adverse consequences for native biota (Valéry et al., 2007). Impacts of invasions vary in scope and severity due to the complex ecological relationships produced by the diverse range of habitats, species, and biological, physical, and chemical processes in freshwaters. The mechanism by which non-native fish species are introduced and the stage of the invasion process also play an essential role in determining whether their presence poses an immediate ecological threat or whether consequences become apparent in the long term (Kolar & Lodge, 2001; García-Berthou et al., 2005). For example, high propagule pressure combined with rapid reproductive rates can result in the numerical dominance of an introduced fish within a very short timeframe. This can occur due to deliberate introductions where large quantities of fish are bred, transported and acclimated, such as brown trout (*Salmo trutta*) introduced from Britain to New Zealand (McDowall, 2010) or grass carp (*Ctenopharyngodon idella*) from Asia to the United States (Mitchell & Kelly, 2006). In other cases, fish escape (or are released from) aquaria and aquaculture facilities, gradually building up feral populations as is the case of the near-globally distributed goldfish (*Carassius auratus*) (García-Berthou et al., 2005; Chan et al., 2019). The traits that support the survival and proliferation of these species in such a diverse range of freshwater ecosystems frequently also contribute towards negative impacts on native species. Impacts include predation, competition, trophic shifts and cascades, habitat alteration, and hybridisation (Gallardo et al., 2016). Introduced piscivores such as South American peacock bass (*Cichla* spp.) have been linked to several of these impacts, such as a 45-year decline of sympatric prey fish populations and diversity in Central America (Sharpe et al., 2017) as well as the extirpation of native fish in South America (Pelicice & Agostinho, 2009). Smaller-bodied invasive predators such as European round gobies (*Neogobius melanostomus*) have also been linked to significant changes to their prey populations, reducing macroinvertebrates such as snails and amphipods at much higher rates than native fish in North America (Pagnucco et al., 2016; Pennuto et al., 2018). This was demonstrated to have significant cumulative effects, as the rapid loss of these grazing and shredding species triggered increases in phytoplankton and periphyton in both mesocosm and stream studies (Pagnucco et al., 2016; Pennuto et al., 2018). Furthermore, *N. melanostomus* predation produced trophic cascades that affected crucial ecosystem processes, such as the decomposition rate of detritus. Common carp *(Cyprinus carpio)* originating from Eurasia also had habitat-altering effects in Australia by increasing nutrients, algae and turbidity while reducing macrophytes through feeding and spawning behaviour (Marshall et al., 2019).

A lack of widespread evidence for interspecific competition-induced extinctions of native species suggests that intertrophic interactions from invasive species may be a greater threat to global biodiversity overall (Davis, 2003). However, interference and exploitative competition are well-documented consequences of freshwater fish invasions, as the niche filled by the invader is likely already occupied by native species. For example, the common carp is a globally invasive species with a niche overlap with European native crucian carp (*Carassius carassius*), and hybridisation is prevalent (Sayer et al., 2011). This produces a threat from the superior competitive ability of common carp, which occupies a larger isotopic niche, can consume higher volumes of food and grow faster (Almela et al., 2021), as well as from interbreeding, which can produce unviable or sterile offspring (Hulata, 1995).

1.1.2 Ecology and biology of *Gambusia*

The name ‘mosquitofish’ is commonly used to refer to two morphologically and ecologically similar species within the genus *Gambusia*: *Gambusia holbrooki*/eastern mosquitofish and *Gambusia affinis*/western mosquitofish (Pyke, 2005). Both species have been classified as invasive throughout their introduced range, extending across all continents except Antarctica, as far south as New Zealand, and north as Canada and central Europe (Jourdan et al., 2021). Henceforth referred to as *Gambusia*, the life history traits and biology of these fish facilitate their invasion of a range of freshwater ecosystems and increase their competitive ability against native species. *Gambusia* are small fish from the family Poeciliidae reaching up to ~60 mm in length, with males generally not growing much past sexual maturity at ~25 mm (Vondracek et al., 1988). Their native distribution covers shallow temperate and tropical waters in central and eastern parts of the USA and Mexico, from approximately 40°0′N to 22°0′N (Rosen & Bailey, 1963). While they are generally restricted to lower elevations outside their native range (Pyke, 2008), *Gambusia* have successfully established in over 60 countries, often due to intentional introductions for mosquito control (Walton, 2007). This reflects the highly tolerant and adaptable nature of these fish, both at an individual physiological and at a population scale. *Gambusia* are highly adaptable to a range of environmental conditions, including fluctuating temperature, salinity, dissolved oxygen (Pyke, 2005; Callaghan et al., 2021) and the presence of pollutants (Coffin et al., 2022). Preferred conditions include calm water with a dark substrate and lateral cover, such as submerged macrophytes (Casterlin & Reynolds, 1977). Preferred water temperatures range between 25°C and 35°C (Swanson et al., 1996; Pyke, 2005) and ideal water depths lie between 8 cm and 15 cm (Pyke, 2005). *Gambusia* achieve maximum growth at 5 ppt salinity (Meffe & Snelson, 1989). Their tolerance of the extremes of these variables is well-researched. Swanson et al. (1996) identified a thermal minimum (LC50) of 0.5°C and a maximum of 42°C, though Meffe et al. (1995) found variation in critical thermal maxima (CTM) among different populations. This was attributed to genetic differences arising after each population was subjected to different temperature ranges for 60-90 generations. Salinity tolerance reached up to 19.5 ppt following an immediate immersion from freshwater, though acclimating fish gradually led to 65% surviving 39 ppt conditions for a 7-day experiment duration (Chervinski, 1983). *Gambusia* can survive in low dissolved oxygen conditions, exhibiting 100% survival rates when confined underwater at dissolved oxygen conditions of 1.3 mg/L and surviving at 0.28 mg/L when given access to the surface (Odum & Caldwell, 1955). *Gambusia* diet and feeding behaviour can also be very flexible; therefore, they occupy different trophic positions in different environments. For example, an Australian study of a river ecosystem containing smaller-bodied native fish species found that *Gambusia holbrooki* acted as an opportunistic carnivore, feeding on both benthic and water surface macroinvertebrates (Pen et al., 1993). Both small and large individuals had a wide dietary breadth in spring, although large individuals decreased dietary breadth in summer and winter in response to food availability and niche overlap (Pen et al., 1993). A study of *G. holbrooki* stomach contents found that they primarily fed on cladoceran and copepod zooplankton, with a higher dietary breadth in summer to include surface insects (Gkenas et al., 2012). This was described as a specialist feeding strategy, with consistent dietary preferences in most seasons, though there were major differences between the food sources of smaller and larger individuals (Gkenas et al., 2012). *Gambusia* are effective predators in standing water environments, finding a high proportion of prey among submerged vegetation (Linden & Cech, 1990) and targeting the most profitable prey when satiated, though feed indiscriminately when starved (Bence & Murdoch, 1986).

The morphology of *Gambusia* makes them best adapted to feeding at the surface of still water environments, with an upward-facing mouth and flattened head and relatively poor swimming performance compared to similar-sized fish species (Srean et al., 2017). A factor often considered in the diet of *Gambusia* is cannibalism, with evidence of this reducing population densities via an impeding of recruitment in habitats with low proportions of shelter (Benoît et al., 2000). However, this may be largely an artefact of higher-density laboratory studies, with gut content analyses revealing cannibalism rates in nature of only 1% (Specziár, 2004) and 0.48% (Nesbit & Meffe, 1993). *Gambusia* are capable of reproduction at under 20 days of age, though this is heavily dependent on available resources and environmental conditions, particularly temperature (Meffe, 1992). Fertilisation and gestation are both internal, with females giving birth to as many as 300 young in under 35 days (Walton, 2007). Female *Gambusia* can store sperm over winter (Haynes, 1993) allowing fertilisation without males and an intrinsic rate of population increase much greater than many other species (Tonkin et al., 2014). Reproduction in temperate regions is seasonal, with males ceasing sexual activity at about 10°C water temperature (Haynes, 1993), while reproduction can continue year-round in warmer climates (Martin, 1975). In temperate climates, females can stop reproducing in late summer to instead build up fat reserves, which can serve as an advantage for overwintering. This can help *Gambusia* breed earlier in the spring, with offspring capable of reproducing in the same year (Reznick & Braun, 1987). However, there is significant variation in brood and offspring sizes, the minimum size at first reproduction, and reproductive frequency among different populations, all of which are largely associated with climatic conditions (Haynes & Cashner, 1995). While the reproductive rate is greatest at temperatures as high as 30°C, young grow the fastest when there is diel temperature variation, with a mean of 25°C (Vondracek et al., 1988). Immature *Gambusia* achieve growth rates of 1-2 mm per week for both sexes, though females gain mass at a greater rate (Vargas & de Sostoa, 1996). There is a measurable difference in the characteristics of fish born early and late in the breeding season, with males born later taking longer to mature and achieving larger body sizes (Hughes, 1985). There is also evidence for social inhibition of male maturation, with males reared individually maturing sooner and at a smaller size and weight than those in groups (Campton & Gall, 1988). The complete development of the male gonopodium and the gravid spot on females signals that the fish have reached sexual maturity (Norazmi-Lokman et al., 2016). *Gambusia* display a range of behavioural traits, often varying with their environment. Aggression towards conspecifics and other fish is well documented (Ling & Willis, 2005; Pyke, 2005). This includes the establishment of dominance hierarchies in both sexes via chasing and exclusion (McPeek, 1992; Chen et al., 2011), which influence the access of males to females, and which females are preferred by males (Bisazza & Marin, 1991; Chen et al., 2011). Aggressive behaviour is especially prominent in small spaces such as aquaria, where males will seek out confrontation, whereas males tend to avoid one another in natural environments (Itzkowitz, 1971). Similarly, Martin (1975) found female territoriality, female same-sex aggression, and inter-sex aggression to be generally limited to laboratory conditions and explained by stocking density. *Gambusia* are usually social fish, frequently joining same-sex and mixed-sex groups to reduce sexual aggression (Pilastro et al., 2003) and for predator avoidance (Ward & Mehner, 2010). Individuals can vary in sociability, with active fish schooling more often, while less social individuals dispersed further (Cote et al., 2010). While *Gambusia* may move between habitat areas daily (Winkler, 1979), they generally do not migrate (Pyke, 2005) aside from special circumstances such as from seasonally dry wetlands (Hoch et al., 2020).

1.1.3 *Gambusia* as invasive species

The biology and traits of *Gambusia*, combined with deliberate introductions starting from the beginning of the 20th century (Krumholz, 1944), have led to numerous ecological impacts outside of their native range. This includes competitive effects on fish that occupy similar ecological niches, predation on fish larvae and invertebrates (Pyke, 2008), as well as larger-scale ecosystem impacts ranging across trophic levels (Hurlbert et al., 1972; Drenner & Smith, 1991; Blanco et al., 2004). For example, rapid reproductive capability and high adaptability to a range of conditions have supported the spread of *G. holbrooki* across the Iberian Peninsula, where negative consequences for native species are well documented (Vargas & de Sostoa, 1996; Vila-Gispert et al., 2005; Ruiz-Navarro et al., 2011). Female *G. holbrooki* were able to produce, on average, one brood per month in the Ebro Delta of the Iberian Peninsula for up to six months of the year, each with more offspring than typically possible in their native range (Vargas & de Sostoa, 1996). Furthermore, *Gambusia* have effectively adapted to local conditions, including high salinity and variable salinity streams (Ruiz-Navarro et al., 2011). The maintenance of normal life history traits and behaviours in these physiologically stressful conditions poses a major risk to native species previously thought to be protected from *Gambusia* by elevated salinity (Alcaraz et al., 2008). Native Iberian Peninsula freshwater fish species such as Valencian and Iberian toothcarps (*Valencia hispanica*, *Aphanius iberus*) were outcompeted for food by *Gambusia* in mesocosm experiments and aquaria (Caiola & de Sostoa, 2005). Caiola and de Sostoa (2005) found that even when the toothcarps were numerically dominant, they were not able to outcompete *Gambusia* for food. The exploitative competition effect exerted by *Gambusia* became more significant as their relative density increased, evidenced by more toothcarps identified with empty guts in the experiment (Caiola & de Sostoa, 2005). This aligns with findings of population increases in native Iberian Peninsula fish, which correlated with the gradual removal of sympatric *Gambusia* through trapping and netting (Ruiz-Navarro et al., 2013a). Interference and exploitative competition were found to be more prevalent at higher temperatures, with *Gambusia* capturing more food and performing more aggressive actions towards Iberian toothcarp at temperatures above 24°C (Rincón et al., 2002; Carmona-Catot et al., 2013). Aggressive feeding behaviour also has implications for native toothcarp population fitness and recruitment, as *G. holbrooki* have been found to prey on juvenile *A. iberus* and inhibit the growth of survivors in mesocosms (Rincón et al., 2002). Similar impacts were found in least chub (*Iotichthys phlegethontis*) populations coexisting with invasive *G. affinis* in Utah, USA (Mills et al., 2004; Ayala et al., 2007). Least chub juveniles had survival rates negatively correlated with increasing *Gambusia* densities in mesocosm experiments, which was attributed to predation (Mills et al., 2004). Larger least chub were also impacted by aggression from *Gambusia*, frequently being chased away from food and open water habitat in laboratory experiments, which culminated in a six-fold decreased growth rate (Mills et al., 2004). In the wild, this caused a behavioural shift in the least chub to avoid the shallow and warm habitats preferred by *Gambusia*, particularly during the summer when both species are most active (Ayala et al., 2007). Furthermore, least chub altered their diel movements by spending more time in cooler and deeper water to avoid *Gambusia* during the day (Ayala et al., 2007), which demonstrates an attempt at avoiding spatiotemporal niche overlap with a superior competitor. The impacts of *Gambusia* on a single fish species can radiate through entire food webs, influencing the presence and abundance of other organisms. For example, the presence of *G. holbrooki* in Australian wetlands was correlated with the presence, abundance, and condition of native species, such as a decrease in the fin condition of Australian smelt (*Retropinna semoni*) (Macdonald et al., 2012). Many of the wetlands in this study exerted environmental stressors on species due to summer drying, which combined with the biotic pressures from *Gambusia* to restrict the diversity of native fish to more generalist, resilient species (Macdonald et al., 2012; Tonkin et al., 2014). Thus, while having a major niche overlap with many species, it is those with traits such as specialist life histories and with reproduction coinciding with high *Gambusia* numbers that are at elevated risk (Macdonald & Tonkin, 2008). *Gambusia* also have the potential to trigger trophic cascades, particularly via top-down effects on prey such as larger cladoceran and copepod zooplankton (Margaritora et al., 2001). Predation in a mesocosm study reduced cladoceran and copepod taxa to the degree that allowed phytoplankton to proliferate, increasing primary production more significantly than nutrient addition (Rettig & Smith, 2021). While this effect of *Gambusia* on chlorophyll-*a* level was not observed in some studies (Geyer et al., 2016), in others, evidence points towards similar trophic cascades through predation on grazing invertebrates and amphibian larvae (Ho et al., 2011; Preston et al., 2018). The top-down impacts of *Gambusia* vary in different ecosystems, as limiting factors, food sources, and food web linkages differ also. For example, although *Gambusia* significantly reduced zooplankton numbers in a coastal wetland, this did not influence lower trophic levels as primary production was dominated by submerged macrophytes rather than phytoplankton (Cardona, 2006). Additionally, the density and population structure of *Gambusia* can affect the outcome of invasions, as female-biased populations altered their environment to a greater degree (Fryxell et al., 2015).

The range of studies documenting the negative effects of *Gambusia affinis* and *Gambusia holbrooki* contrasts with the very little research showing invasiveness in the other 43 species of the genus *Gambusia* (Rauchenberger, 1988). These species share a similar appearance and many life history traits with the two invasive species, such as viviparity (Thibault & Schultz, 1978), dentition and jaw structure, carnivory, surface/subsurface feeding, and a general preference for slow-flowing waters (Rivas, 1963). However, non-invasive *Gambusia*, such as *Gambusia geiseri*, are less effective competitors in most cases, reaching only half the population densities of *G. affinis*, growing more slowly, and producing fewer offspring (Rehage et al., 2020). *Gambusia geiseri* are well adapted for specific environments such as springheads but lack the flexibility of invasive *Gambusia* that allows for survival and rapid reproduction in novel habitats (Rehage et al., 2020). This highlights that subtle differences among the genus can produce very different interactions with other species in an ecosystem and limit the spread and invasion potential of some *Gambusia*. *Gambusia geiseri* were less tolerant of temperature variability and preferred a diet of benthic invertebrates, and so were not found to create trophic cascades through zooplankton predation, unlike *G. affinis* (Rehage et al., 2020). Differences in predation capability and voracity also favoured *G. affinis* and *G. holbrooki* over non-invasive congeners, though other components of behaviour, such as responses to novel predators and competitors, were similar among all *Gambusia* assessed in a study by Rehage et al. (2005). Traits such as the capability and potential for dispersal have been suggested as key factors differentiating invasive and non-invasive *Gambusia* (Rehage & Sih, 2004). *Gambusia affinis* and *G. holbrooki* were significantly more likely to disperse to new areas in an artificial stream experiment than *G. hispaniolae* and *G. geiser*i, while comparing the invasiveness of *G. affinis* with *G. holbrooki* remained inconclusive (Rehage & Sih, 2004).

1.2 A New Zealand context to native and introduced fish species

1.2.1 Invasive fish species in New Zealand

Island ecosystems, particularly those isolated from immigration, experience unique evolutionary trajectories that can lead to ecological niches occupied by species very different from more homogeneous continental systems (Case & Cody, 1987; Mittelbach & Schemske, 2015). New Zealand's biogeographical history is reflected in the unique assemblages of species present and is characterised by high endemicity across most taxa such as reptiles (Daugherty et al., 1994), birds (Wilson, 1997), plants (Cooper & Millener, 1993), and fish (Allibone et al., 2010). This has elevated the threat of anthropogenic pressures and non-indigenous species on native ecosystems, with many species lacking competitive adaptations against invasive organisms. Native freshwater fish have been significantly affected by habitat loss and alteration, as well as predation and competition from invasive species since the introduction of brown trout (*Salmo trutta*) in 1867 (MacCrimmon & Marshall, 1968). Notably, 28% of native fish are classified as 'threatened' and 40% as 'at risk' (Allibone et al., 2010). This is of particular concern as New Zealand already has a low species diversity in fish, with 51 native species compared to 211 in Japan (Yuma et al., 1998), a similar-sized island. In a survey of 38 medium-large rivers, an average of only five native fish species were caught per site, with species distributions largely affected by elevation and catchment land use (Jowett & Richardson, 1996). The major role of elevation relates to the high incidence of diadromy among New Zealand fish, which reflects the historical dispersal of fish to the area (McDowall, 2010). Many of the country's freshwater habitats are relatively young, as frequent disturbances from volcanism and plate tectonics have created, modified, and destroyed lakes and rivers. Thus, the diadromous nature of native fish has been an important factor in the recolonisation of fresh waters (McDowall, 2010). This has created unique species assemblages, often low in diversity and lacking key trophic groups found internationally, such as larger-bodied pelagic piscivores

(Collier et al., 2017). The introduction of non-native fish, primarily throughout the 19th and 20th centuries, quickly led to the establishment of high trophic-level predatory fish, and native fish experienced rapid and ongoing declines as a result. There are at least 20 non-native fish species established in New Zealand (Allibone et al., 2010), with the most widespread including *G. affinis*, brown bullhead catfish (*Ameiurus nebulosus*), goldfish, perch (*Perca fluviatilis*), rudd (*Scardinius erythrophthalmus*), koi carp (*Cyprinus carpio*) and tench (*Tinca tinca*) (Rowe & Wilding, 2012). The degree and mechanism by which they impact ecosystems vary, and classifications such as the Fish Risk Assessment Model are used to categorise threats of predation, interference and exploitative competition, bioturbation and herbivory (Rowe & Wilding, 2012). This metric combines the risk of establishment and risk of the impact of each species, with the highest scores produced for perch, koi carp, *G. affinis* and brown bullhead catfish (Rowe & Wilding, 2012). Catfish and perch also scored highest in lake-specific invasion risk and impact assessments (Collier et al., 2017), as they effectively deplete native fish and invertebrate populations (Ludgate & Closs, 2003; Collier et al., 2018) with few native competitors or predators. Another species scoring highly for similar reasons is the brown trout (*Salmo trutta*), which despite suppressing populations of galaxiids (McDowall, 1968; Townsend, 1996) and altering their behaviour (McIntosh et al., 2010), has been spread and protected by law for their economic and cultural benefits (Jones & Closs, 2017). Carp, goldfish, rudd, and tench are largely planktivorous, larvivorous, and herbivorous, with varying degrees of risk determined by traits such as the highly destructive feeding behaviour and rapid growth exhibited by carp (Tempero et al., 2006; Hicks & Ling, 2015). However, the true present and future impact of invasive fish is often difficult to predict, as complex interactions of both native and introduced species with each other as well as with processes such as climate change (Macinnis-Ng et al., 2021) and changing land use (Ling, 2010; Joy et al., 2019) can contribute to the ongoing biodiversity decline in New Zealand.

1.2.2 *Gambusia affinis* in New Zealand

The widespread global impacts of *Gambusia* have also been observed in New Zealand, where *G. affinis* was introduced in 1930 (McDowall, 2000). While it is the only member of its genus in New Zealand, *G. affinis* has become widespread in the upper and central North Island, with populations established throughout the rest of the North Island and northern South Island (Chadderton et al., 2001; Ling, 2004). *Gambusia affinis* are typically found in lowland streams, rivers, and lakes, and are restricted by elevation, water velocity, and temperature. While they prefer the warmer temperatures of the coastal North Island, *G. affinis* have the potential to colonise waterways across most of the South Island (Rowe et al., 2007), as *G. affinis* have successfully established breeding populations in colder climates such as Cape Cod, Massachusetts, USA (Wood et al., 2020). Furthermore, their tolerance of brackish water (Chervinski, 1983) may enable dispersal along coastal waters to new catchments. *Gambusia affinis* tend to be more prevalent in catchments dominated by agricultural land use, as these create environmental conditions favourable for their life history traits (Lee et al., 2017). Streams flowing through pasture often have higher suspended solids, nutrients, and temperatures, while having decreased clarity (Quinn & Stroud, 2002). This supports the establishment of macrophytes and algae (Parkyn et al., 2003), which provide lower velocity shelter and sustain food sources preferred by *Gambusia*. Therefore, the spread and intensification of agriculture to cover over 40 % of New Zealand's land area (Daigneault et al., 2018) has coincided with, and likely facilitated, the proliferation of *G. affinis*.

Impacts of *G. affinis* in New Zealand have mirrored findings internationally, particularly in impacts on zooplankton, and competition and aggression towards other small fish. A study of *G. affinis* diet in North Island lakes showed a broad generalist feeding strategy, with differences between size classes and seasonal variation in primary food sources (Mansfield & McArdle, 1998). Diet of all sizes of *G. affinis* was dominated by cladocerans, copepods, and chironomids in lakes (Mansfield & McArdle, 1998), whereas in streams, large *Gambusia* preferred Culicidae, amphipods, and terrestrial invertebrates (Lee et al., 2018). This dietary flexibility shown by *Gambusia* supports their survival in different New Zealand ecosystems, particularly those exhibiting degradation from catchment land use (Lee et al., 2018). The physiological and behavioural adaptability of *G. affinis* makes them effective competitors against many native fish, including the black mudfish (*Neochanna diversus*). The presence of *G. affinis* in wetland sites coincided with reduced mudfish recruitment, with mesocosm and laboratory experiments pointing towards the occurrence of both predation and competition (Ling & Willis, 2005). Mudfish most at risk are fry (Barrier & Hicks, 1994) and juveniles in near-permanent water habitats, whereas larger mudfish and fish living in ephemeral areas don't appear greatly threatened by *G. affinis* (Ling & Willis, 2005). The overall threat of *G. affinis* is further lowered by mudfish behavioural and life history traits such as occupying a different temporal niche (Ling, 2001) and reproducing during periods of lowered *G. affinis* abundance (Barrier & Hicks, 1994). Other native New Zealand species have potentially greater niche overlap with *Gambusia*, such as inanga (*Galaxias maculatus*) and bullies (*Gobiomorphus* spp.) (McDowall, 2000). Inanga are a widespread native fish growing up to three times longer than the average *G. affinis* and exhibit adaptable behaviour and life history traits (Chapman et al., 2006). However, despite having a size and swimming speed advantage, inanga mortality increased significantly when placed in aquaria with *G. affinis*, particularly at temperatures >15°C where *Gambusia* are more active (Baker et al., 2004; Rowe et al., 2007). This was largely attributed to agonistic behaviour such as chasing and fin-nipping (Rowe et al., 2007), which has been documented in several *Gambusia* laboratory studies in New Zealand and internationally (McPeek, 1992; Mills et al., 2004; Macdonald et al., 2012). These findings are yet to be supported by field evidence of large-scale inanga population decline following *Gambusia* invasion. The two species are capable of coexisting (Ling, 2004), particularly in habitats where variability in water depth and flow provides conditions favourable to inanga (Rowe et al., 2007).

1.2.3 *Gobiomorphus cotidianus*: biology, ecology, and threats

The common bully (*Gobiomorphus cotidianus*) is a small benthic fish endemic to New Zealand (McDowall, 2000). It is one of nine native bully species in New Zealand, with two congeners in Australia (Thacker et al., 2021a). *Gobiomorphus* is a genus of the family Eleotridae, which includes coastal and inland species across most continents, several with diadromous life histories (Stevens & Hicks, 2009). This is reflected in the biogeographical history and present distribution of *G. cotidianus*, as it is suggested that the common ancestor of all New Zealand bullies dispersed across the ocean from Australia in the early Miocene (Stevens & Hicks, 2009). This was followed by adaptive radiation, where the reproductive isolation of populations contributed to speciation into strictly diadromous, facultatively diadromous, and non-diadromous species (Stevens & Hicks, 2009). *Gobiomorphus cotidianus* exhibits facultative diadromy, where fish undertake amphidromous migration to the ocean but may also form nonmigratory populations when landlocked or when favourable stream and river habitats are present (Closs et al., 2003; Hicks et al., 2017). *Gobiomorphus cotidianus* are the most widely distributed native bully, prevalent throughout New Zealand’s lowland streams, rivers, and lakes (Thacker et al., 2021b).

Gobiomorphus cotidianus are adaptable and resilient to a range of conditions, including depth, temperature, turbidity, and dissolved oxygen gradients. Acclimated *G. cotidianus* survived to upper lethal temperatures of 34°C, higher than many other native freshwater fish such as torrentfish (*Cheimarrichthys fosteri*), common smelt (*Retropinna retropinna*), kōaro (*Galaxias brevipinnis*), and kōkopu (*Galaxias fasciatus*, *Galaxias postvectis*, *Galaxias argenteus*) (Richardson et al., 1994). Similarly, *G. cotidianus* were more tolerant to acute hypoxia than common smelt and rainbow trout (*Oncorhynchus mykiss*) (Landman et al., 2005). *Gobiomorphus cotidianus* exhibit phenotypic plasticity along depth gradients, such as decreased eye size and body width, and an increased body length in deep lakes (Ingram et al., 2020). Their foraging is also not limited by darkness or high turbidity; therefore, they can be found in high-productivity waterways (Rowe, 1999). Primary food sources vary with bully size, habitat, and seasonality. *Gobiomorphus cotidianus* diet in eutrophic Lake Okaro was dominated by chironomid larvae (*Polypedilum pavidus* and *Chironomus zealandicus*) for most of spring, summer, and autumn, while cladocerans such as *Ceriodaphnia dubia* were the primary prey in winter (Forsyth & James, 1988). Similar seasonal diet differences have been found at Lake Taupō, where bully dietary niche narrowed when zooplankton abundance increased, then widened to include other prey items as zooplankton experienced a seasonal decline (Stewart et al., 2017). Likewise, Stephens (1978) demonstrated a seasonal influence on the diet of *G. cotidianus* at Lake Waahi, where they consumed almost exclusively chironomid larvae during spring and summer, whereas aquatic snails, oligochaetes and zooplankton became important prey items during autumn and winter. Additionally, a shallow coastal lake's juvenile bully population predominantly fed on an amphipod, *Paracalliope fluviatilis* (Wilhelm et al., 2007). Changes in *G. cotidianus* habitat, such as the invasion of macrophytes (e.g., *Lagarosiphon major* at Lake Dunstan), can shift their diet to the most abundant prey sources; in this case, invertebrates inhabiting the vegetation (Bickel & Closs, 2008). *Gobiomorphus cotidianus* are largely diurnal, which allows temporal dietary niche differentiation with native fish feeding on similar food sources (Sagar & Glova, 1994), though juvenile fish, in particular, can exhibit active nocturnal feeding behaviour (Rowe & Chisnall, 1996).

The gonads of *G. cotidianus* mature from April to July (austral autumn to winter), during which time both sexes undergo physiological and behavioural changes (Bleackley et al., 2009). The pigmentation and territoriality of males increases as they approach maturity, with female fecundity peaking from August (austral winter) (Stephens, 1982). Throughout spring and summer, males clear an area of the benthos to produce a nest, into which one or more females attach eggs. Males then fertilise, guard, and fan the eggs until larvae hatch and disperse, either to inhabit the pelagic zone in landlocked populations or to be washed to sea (McDowall & Whitaker, 1975). During this life stage, freshwater bully larvae feed on zooplankton, with the potential to exert top-down control on cladocerans during the summer months (Jeppesen et al., 1997). The diet of juveniles shifts as they grow to include prey species inhabiting macrophytes and the benthos. *Gobiomorphus cotidianus* begin inhabiting the benthos in shallow water from about 18 mm in length, transitioning to deeper water as the summer progresses and they reach an adult size of 30-120 mm (Rowe, 1999; Wilhelm et al., 2007). *Gobiomorphus cotidianus* that hatch earlier in the season achieve a greater growth rate, and growth is faster in warmer and more eutrophic lakes (Taylor et al., 2000). *Gobiomorphus cotidianus* reach a sexually mature adult size of 30-40 mm within a year, continuing to grow for another one to two years before experiencing declining condition and increasing mortality (Stephens, 1982), though they have been found to live up to five years (McDowall, 2000).

As *G. cotidianus* are a widespread fish species in New Zealand lowland freshwaters, they co-occur with many native and introduced species. This includes predators, competitors, and morphologically similar species, such as other bully species. For example, bluegill bullies (*Gobiomorphus hubbsi*), upland bullies (*Gobiomorphus breviceps*) and *G. cotidianus* are all present in the Rakaia River, though there appears to be niche separation between *G. cotidianus* and the other two species. Sagar and Eldon (1983) found *G. cotidianus* to primarily feed on chironomid larvae, whilst bluegill bullies and *G. breviceps* incorporated larger quantities of *Deleatidium* (mayfly) larvae into their diets. *Gobiomorphus cotidianus* targeted chironomid larvae disproportionately to their abundance in the substrate and had a wider food niche breadth, thus avoiding competition for food. Sagar and Eldon (1983) also did not find evidence for competition for habitat, as *G. cotidianus* were restricted to a slower velocity site closer to the coast, which seems to be a reflection of their poorer swimming and climbing capabilities (McDowall, 2000). Likewise, there appears to be some structuring of *G. cotidianus* and *G. breviceps* populations along stream and river reaches, whereby the latter occupies higher elevations. This was supported in an artificial channel experiment, where Glova (1999) did not find evidence for negative interspecific competition effects of the two species coexisting. Introduced competitors may include *G. affinis* and other small-bodied zooplanktivorous or benthic fish, though *G. cotidianus* demonstrated high dietary flexibility and shifted away from zooplankton prey when sympatric with non-native grass carp (*Ctenopharyngodon idella*) (Mitchell, 1986). *Gobiomorphus cotidianus* are preyed on by trout, perch, and eels, with an increased vulnerability to predators such as brown trout when males are guarding nests during the breeding season (McCarter, 1986). Perch can suppress *G. cotidianus* populations, with predation being the most significant interaction (Closs et al., 2001). Eels, including the New

Zealand longfin (*Anguilla dieffenbachii*) and the short-finned eel (*A. australis*), are native predators of bullies and were found to control *G. cotidianus* populations to a greater degree than introduced rainbow trout (Rowe, 1999). Predator detection includes visual identification of threats and use of vantage points (Jellyman et al., 2012), recognising injured conspecifics via alarm substances released by broken skin, and the capability to learn the odour of predators such as perch (Kristensen & Closs, 2004). This is followed by avoidance behaviour, including rapid dashing to new cover and hiding among the substrate (Kristensen & Closs, 2004).

1.3 Methods for assessing the impacts of an invasive fish species

1.3.1 Assessing impacts of invasive species

The complexity of ecological relationships and ecosystem scale research makes it difficult to determine the true impact of an introduced species, particularly when key aspects of the biology, physiology and behaviour of species are unknown. Thomsen et al. (2011) categorise invasion research into studies that look at the ecological impacts of a novel species on the environment and studies researching factors facilitating invasion in a particular environment. Field studies quantifying the former category are possible in later stages of an invasion, where a species has overcome ecological filters preventing dispersal and survival of individuals, the establishment of a self-recruiting population, and growth of this population to the degree that impacts on native species can occur (Catford et al., 2009). Conversely, researching variables that make establishment possible may target earlier stages of the invasion process, where numerous hypotheses exist around key mechanisms involved. This includes relative contributions of factors affecting the likelihood of successful dispersal and establishment, such as propagule pressure, species richness in the receiving region, temporarily favourable environmental and ecological conditions, and the ability of a species to survive in the absence of other species from its native range. Hypotheses around the possibility of population growth must also be investigated. These may be favourable life history traits and adaptability of the invading species, the lack of an important population control variable such as a predator or pathogen, facilitation or inhibition of prey, predators, and competitors, low niche saturation, as well as external factors such as human activity (Catford et al., 2009). Any quantified impact may also change significantly over time due to the depletion of a temporary resource, natural selection on both invaders and natives or fluctuating environmental conditions (Thomsen et al., 2011). This is relevant to almost any species invasion context, with attributes of the invading species, native species, resources and environmental conditions being factors that can be investigated as unique to a specific invasion or as universally influencing the impacts of nearly any invading species (Thomsen et al., 2011). Therefore, freshwater fish invasion research must look at these drivers of invasion and the immediate and long-term outcomes whilst accounting for scientific uncertainty.

As research resources are limited, the challenge is to identify the invasions with the highest probability of ecological impacts and identify the primary mechanisms responsible in order for management to be possible (Leprieur et al., 2009). However, there is a considerable absence of clear data describing the consequences of many introduced species, due in part to the potential of differing impacts in different ecosystems, combined with funding and focus bias towards certain species and geographical areas. Introduced fish that cause major declines in native populations make up only 8-14% of invasions at a global scale, yet some ecosystems are more vulnerable than others, and some invaders can be close to universally harmful (Ricciardi & Kipp, 2008). The importance of both field and laboratory-based experiments is highlighted in their strengths and drawbacks in providing answers to the wide range of possible research questions that can be formed to investigate the aforementioned factors and relationships (Leprieur et al., 2009). Laboratory and field experiments are frequently combined to assess interspecific relationships such as competition, predation, aggression, and herbivory. Laboratory studies are particularly useful for assessing behaviour, as many variables can be controlled, and observation is easy. Almela et al. (2021) studied competition between introduced common carp and native crucian carp in England using aquaria to measure comparative functional responses to food. This was then supported by a controlled natural pond study and a wild pond survey with no experimental manipulation. The use of these three degrees of experimental manipulation provides insight into behaviour that is difficult to assess in the wild while avoiding results biased by laboratory conditions. The use of at least two of these methods is a common experimental design in invasion ecology, such as a mesocosm study combined with a Before-After-Control-Impact (BACI) field study to assess the degree to which invasive *Gambusia* affect native amphibians in California, USA (Preston et al., 2017). In that study, the mesocosm experiment provided insight into larger-scale ecosystem processes found to be occurring in the field study and revealed potential invasive fish management strategies, though the authors noted drawbacks, including a possibly insufficient experiment length (Preston et al., 2017). Numerous studies use only laboratory or field experiments and still provide strong results, as certain research questions are more appropriate for aquarium studies with a shorter period or with multiple treatments and replicates (e.g., Britton et al., 2011; Beggel et al., 2016). Likewise, research into community structure and trophic linkages in the presence of invasive fish is realistically investigated with exclusively field-based sampling (Lowe et al., 2008; Jackson et al., 2016). Mesocosms can be a good middle ground where key parts of an ecosystem can be recreated to test certain hypotheses that require more controlled variables than possible in nature, such as density-dependent impacts of an invasive fish (Jackson et al., 2015). Within each of these study methods are further techniques that can be used to research invasive fish, such as indirect sampling to conduct stable isotope analysis or create dietary overlap indices and direct observation of behaviour and interactions. These have their own limitations and advantages, with best practices being determined by the environment being studied, research resources available, and the type of interaction being investigated (Almeida & Grossman, 2012). Finally, meta-analyses and predictive modelling can be used to integrate environmental and ecological data to assess the susceptibility of ecosystems to invasion, traits of successful invaders, and general patterns in competitive and trophic interactions (Connell, 1983; Kolar, 2004; Gherardi, 2007).

1.3.2 Zooplankton community composition to assess interspecific competition

Interspecific competition is a common consequence of the establishment of invasive fish, as it is likely that a native species is already occupying some of its niche. Non-indigenous fish species can directly threaten native species for food and habitat through interference and exploitative competition, as well as indirectly through apparent competition. For studies investigating competition for food resources, the changes to the population dynamics, community composition or behaviour of the shared prey species can provide insight into both the top-down control exerted by the predators, and the degree of dietary niche overlap. This can take place via research incorporating zooplankton, which comprise a major food source for many smaller fish species, as well as for the juveniles of larger predators. Studies can use direct and indirect methods to quantify whether fish compete for zooplankton. This includes measuring the relative growth rates of the fish in sympatric treatments, but also gut content, stable isotope analysis, and changes in zooplankton community composition. For example, Angeler et al. (2002) investigated the impacts of three introduced fish on wetland environments and used the changing abundances of key zooplankton groups to explain not just zooplanktivory, but the response of the wetland ecosystem to changing water quality from these fish. The methods used are well-developed and fairly consistent across studies. Mesocosms are useful for observing changes to zooplankton community composition across treatments while including replicates, with sizes ranging from 0.08 m^3 tanks using fish larvae (Breitburg et al., 1997; Lacerot et al., 2013) to larger 3 m^3 tanks with three replicates (Matveev et al., 2002), and to 14.6 m^3 mesocosms set into ponds (Schrank et al., 2003). Larger mesocosms are possible, though field studies are also viable for assessing dietary overlap and impacts of zooplanktivorous fish (e.g. Sampson et al., 2009; Sass et al., 2014). Field studies can achieve results using techniques such as fish foregut analysis to identify their main prey, combined with zooplankton sampling pre- and post- invasive fish establishment to measure their top-down impact. This was possible in the Illinois River, USA, where the bighead carp (*Hypophthalmichthys nobilis*) and silver carp (*Hypophthalmichthys molitrix*) gradually invaded and established breeding populations over the 1990s and 2000s (Irons et al., 2007). Researchers had existing data on zooplankton community composition and thus could measure trends in the abundance and proportion of rotifers, copepods, and cladocerans as the fish invaded (Sass et al., 2014). However, this is not viable in most situations due to the difficulty of predicting when and where invasive fish will establish and ethical issues surrounding the deliberate experimental introduction of non-native species. Therefore, mesocosms become a valuable method for controlling these variables. For example, USA native paddlefish (*Polyodon spathula*) and introduced bighead carp were found to overlap in preferred zooplankton prey sources, and a 34-day mesocosm study showed negative impacts of bighead carp presence on paddlefish growth (Schrank et al., 2003). Likewise, a mesocosm study of two competing zooplanktivorous fish larvae, gizzard shad (*Dorosoma cepedianum*) and bluegill (*Lepomis macrochirus*), found a difference in the importance of zooplankton abundances to the growth and survival of the fish. Gizzard shad were found to deplete zooplankton to a significant degree but were also more developmentally limited by the reduced food sources than the bluegills (Welker et al., 1994). These studies demonstrate the utility of zooplankton counts to competition research as an effective, low-cost method provided that biases in sample collection, equipment, and identification are appropriately minimised (Mack et al., 2012).

1.3.3 Stable Isotope Analysis to measure interspecific competition

Stable isotope analysis (SIA) is an important tool for many fields of science, including archaeology, geochemistry, forensic science, geology and ecology (Chesson et al., 2014). The technique was developed in the 1940s and 1950s, following the description of carbon, hydrogen, sulphur, and nitrogen isotope ratios and the invention of effective isotope ratio mass spectrometry (Ehleringer & Rundel, 1988). ‘Stable isotope’ refers to non-radioactive forms of an atom and are typically isotopes where the neutron number is the same or slightly higher than the number of protons (Fry, 2006). The abundance and proportion of isotopes is determined by physical processes such as radioactive decay and are also influenced by unique conditions on Earth, such as life processes. In ecology, isotope pairs of interest are $^{2}H/^{1}H$, $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$, and $^{34}S/^{32}S$, due to their presence in organic matter, as well as being nonradiogenic (Newton, 2016). These isotopes are also useful because the elements are relatively light; thus, the additional neutron(s) alter the relative mass of the isotope much more than a heavier element. This is important for tracking the mixing and fractionation of isotopes through natural processes and measuring changes to the ratio of each against international reference standards. These standards are substances with an established isotopic ratio that can be used to calibrate equipment globally, such as standard mean oxygen water (SMOW), PeeDee belemnite (PDB) and atmospheric air for hydrogen and oxygen, carbon and oxygen, and nitrogen, respectively (Ehleringer & Rundel, 1988). The carbon isotope ratio is useful for hydrocarbon geochemistry (Fuex, 1977), climate research (Bauska et al., 2016) as well as biology, due to the significance of the element for organic matter. The uptake and fractionation of carbon can be tracked through the various plant photosynthetic pathways (Smith & Epstein, 1971) and through differences in carbon ratios between marine and terrestrial systems (Raven et al., 2002). $\delta^{13}C$ increases from plant to herbivore to predator at a measurable, fairly constant rate due to trophic discrimination during assimilation and excretion of tissue, which makes carbon isotopes useful for identifying and tracking trophic relationships (Ben-David & Flaherty, 2012). The stable isotope pair of $^{15}N/^{14}N$ is similar to carbon in that it generally enters the biosphere from the atmosphere, and the heavier isotope becomes enriched with increasing trophic level (Newton, 2016). However, there is little to no discrimination during nitrogen fixation, and the enrichment rate from prey to predator is approximately three times that of carbon (Deniro & Epstein, 1981). Therefore, carbon and nitrogen are frequently used together for the dietary analysis of an ecological community. Conversely, hydrogen and oxygen isotope levels in organisms are indicators of the water they consume (Ehleringer & Rundel, 1988), with uses including hydrogeology and climate research (Clark & Fritz, 1997) and migration studies (Vander Zanden et al., 2016). Lastly, sulphur isotopes ^{32}S, ^{33}S, and ^{34}S are often used in marine and estuarine studies, as there is a major difference in enrichment between bacterial reduction of sulphates and sulphur compounds taken up by plants from rainwater or the water column (Connolly et al., 2004).

Applications of stable isotopes to fish competition and invasion studies are numerous. It is a time- and cost-saving technique when compared to stomach content based dietary analysis, as SIA incorporates food sources from up to seven months in liver samples and over one year for white muscle (Skinner et al., 2017). Therefore, a single sampling event for species of interest, incorporating samples from key size classes for longer-lived species, is often sufficient for an understanding of general diet and trophic position. In freshwater fish invasion studies, this can be very useful for determining gradual changes to the food web as the invader becomes established. For example, Vander Zanden et al. (1999) demonstrated a clear drop in the trophic position of native lake trout (*Salvelinus namaycush*) following the invasion of smallmouth bass (*Micropterus dolomieu*) and rock bass (*Ambloplites rupestris*). The enrichment of ^{13}C and ^{15}N in trout tissue was lower in invaded lakes, which indicates a change in trout diet to prey at lower trophic levels in response to competition, such as consuming more pelagic zooplankton than littoral fish (Vander Zanden et al., 1999). The gathering of baseline data for such a study is important, as species from different freshwater ecosystems can vary in stable isotope enrichment. SIA can provide insight into detail otherwise not possible when used together with traditional field or laboratory studies such as the measurement of growth rates of sympatric and allopatric populations. In France, Blanchet et al. (2007) found little to no negative impact of introduced brook trout (*Salvelinus fontinalis*) on native brown trout growth rate and survival using field recapture and laboratory observational studies. Despite this, SIA of the same species in a similar stream ecosystem revealed that brown trout shifted their diet to be more similar to that of brook trout when sympatric (Cucherousset et al., 2007), which poses further research questions such as potential changes in trout impact on lower trophic levels. In New Zealand, SIA has been undertaken on many native and introduced fish species, providing insight into dietary change in single species (Ingram et al., 2020), food web data and patterns (McHugh et al., 2010), and differences in energy sources between freshwater environments (Hicks, 1997). Collier et al. (2018) demonstrated the trophic position and primary diet of native shortfin eels and invasive brown bullhead catfish using SIA of muscle tissue, which showed a broader trophic niche in the eels, therefore mitigating the impacts of competition. However, SIA does present challenges and limitations that must be addressed in a study. Firstly, site-specific baseline data is required to establish isotopic enrichment, particularly at lower trophic levels that reflect energy input sources, as this can vary significantly with environmental factors (Gladyshev, 2009). Additionally, the methodology involved in obtaining and processing samples must be consistent between organisms in an ecosystem, as well as between studies, to ensure accurate comparisons can be made (Gladyshev, 2009). Quantitative comparisons between food webs using stable isotope values alone are therefore challenging, as is discerning complex trophic relationships such as omnivory and cases of high prey diversity (Phillips et al., 2014; Glibert et al., 2019).

1.4 Thesis purpose and objectives

1.4.1 Thesis purpose

Research on the ecology and impacts of invasive fish such as *G. holbrooki* and *G. affinis* is extensive (Vargas & de Sostoa, 1996; Mills et al., 2004; Vila-Gispert et al., 2005; Ayala et al., 2007; Ruiz-Navarro et al., 2011). These include interference and exploitative competitive interactions with similar-sized pelagic and benthic native fish as well as aggression in confined spaces. The literature is primarily focused on several major geographical regions, such as the Iberian Peninsula (Caiola & de Sostoa, 2005; García-Berthou et al., 2005; García-Berthou, 2007; Alcaraz et al., 2008), Australia (Pen et al., 1993; Bool et al., 2011; Macdonald et al., 2012) and the USA (Lydeard & Belk, 1993; Mills et al., 2004; Rehage et al., 2020). However, in New Zealand, agonistic and competitive interactions between *G. affinis* and native fish have primarily been investigated in only black mudfish (Ling & Willis, 2005; McDonald, 2007), dune lakes galaxias (*Galaxias* sp.) (Pingram, 2005) and inanga (Rowe et al., 2007). New Zealand case studies have produced mixed results, with mesocosm and laboratory studies generally finding aggressive and competitive behaviour from *G. affinis*, leading to decreased condition and survival rates in native fish (McDonald, 2007; Nygard, 2007; Rowe et al., 2007). Conversely, field studies have generally cast doubt on the interspecific competition and aggression related impacts of *G. affinis*, as they are likely exploiting a unique niche and have relatively poor swimming performance, allowing native fish to avoid direct competition and escape aggression (Ling, 2004). *Gambusia* prefer slow-flowing streams, as well as pond and lake habitats (Rivas, 1963), which in New Zealand are often occupied by various bully species, particularly redfinned (*Gobiomorphus huttoni*), upland (*Gobiomorphus breviceps*) and common (*Gobiomorphus cotidianus*) bullies (Jowett & Richardson, 1996; McDowall, 2010). No studies at present have investigated competition, predation, or aggression between any of these species and *G. affinis*, despite the significant overlap in their distributions (Thacker et al., 2021b). *Gobiomorphus cotidianus* are among the most widespread and disturbance-tolerant native freshwater fish (McDowall, 2000) and thus pose an interesting comparison to the globally distributed, resilient, and physiologically and behaviourally adaptable *G. affinis*. While the conservation status of *G. cotidianus* is ‘Not Threatened’ (Dunn et al., 2018), the presence of competition between the two species could produce trophic shifts, changes in the abundance and distribution of *G. cotidianus*, and top-down pressure on their prey. Likewise, the presence of competition could serve as a limiting factor to the abundance and spread of *G. affinis*, protecting more vulnerable species.

1.4.2 Objectives and hypotheses

The objectives of this study were to identify any dietary overlap occurring between invasive *G. affinis* and native *G. cotidianus*, and to provide insight into potential interspecific competition. A mesocosm study was used to investigate the degree of overlap in zooplankton predation by these two species. This was supplemented by a field study of four lakes investigating stable isotope ratios in the tissues of sympatric *G. cotidianus* and *G. affinis*, in addition to stable isotope analysis of their prey. The field study provides insight into the dietary overlap of these fish in a natural context, whilst the mesocosm study shows the degree to which each species alters zooplankton communities through preferential predation of key taxa. Based on existing research on the preferred habitats, diet, and behaviour of both fish, I hypothesised that there would be overlap in zooplankton predation in allopatric mesocosms, as well as overlap in primary prey sources in the sympatric lake populations. Within the mesocosm experiment, I also hypothesised that both fish would exhibit a preference for larger zooplankton taxa, depleting these in relation to the control. In the stable isotope study, additional predictions were that isotopic enrichment of the fish would be most similar in the winter, reflecting greater dietary overlap.

1.4.3 Thesis outline

Chapter 1 provided a broad-scale literature review on the impacts of introduced freshwater fish and introduced the species and geographical context to the thesis. Additionally, the methodology used in later chapters has been introduced. Chapter 2 uses a mesocosm experiment to assess the dietary overlap of *G. affinis* and *G. cotidianus*, by investigating changes to zooplankton community composition. Chapter 3 is a stable carbon and nitrogen isotope study of *G. affinis* and *G. cotidianus* during the austral winter in four lakes in the Waikato Region. The focus is on dietary overlap between the two fish species, with isotopic enrichment of each fish species and basal food web taxa compared with data collected from the same species in the austral summer by Collier et al. (2018). This supports an analysis of the influence of season on the similarity of the diet of each fish species. Chapter 4 incorporates each chapter's key findings and recommendations in a summary and conclusion. Chapters 2 and 3 are written as two standalone papers prepared for publication; therefore, there is minor repetition between the chapters of this thesis, particularly for introductory and background information.

Chapter 2. Zooplankton prey selection by the native common bully (*Gobiomorphus cotidianus*) and non-native mosquitofish (*Gambusia affinis*): evidence for dietary overlap in a mesocosm context

2.1 Introduction

Aquatic ecosystems are highly vulnerable to biological and environmental change, and non-native species have contributed to biodiversity loss in most biogeographical regions of the world (Ricciardi & Rasmussen, 1999; Gurevitch & Padilla, 2004; Gallardo et al., 2016). Numerous interacting factors mean that outcomes of a particular invasion can prove highly challenging to predict and quantify (Büyüktahtakın & Haight, 2018). Once a non-indigenous species becomes established, management or eradication attempts are costly, if not practically impossible (Simberloff et al., 2005). Freshwater fish are frequently introduced to areas outside of their native range for their social and economic benefits (Gozlan, 2008). A small subset of non-native freshwater fish species are considered invasive at a global scale, and these often share characteristics such as high physiological tolerances and fecundity, omnivory, a small adult body size, and value to human interests (Ruesink, 2005; García-Berthou, 2007). These are among several predictor traits for fish species that have successfully overcome the ecological filters of each invasion stage in multiple environments (García-Berthou, 2007).

Gambusia affinis, the western mosquitofish, is a small freshwater fish of the family Poeciliidae that has become one of the ten most widely introduced aquatic species globally (García-Berthou et al., 2005). The impacts of *G. affinis* and its congener *G. holbrooki* have primarily been observed as competitive and agonistic behaviours towards small-bodied native fish species that exhibit some degree of niche overlap, or on the prey of *Gambusia* (Rupp, 1996; Pyke, 2008). *Gambusia* are effective competitors in many ecosystems for their preferred food sources and habitats. Consequences of interspecific competition have been established in both laboratory and field studies, with evidence for aggressive chasing and fin-nipping behaviour towards similar-sized fish when investigated sympatrically in confined spaces (Rincón et al., 2002; Mills et al., 2004; Carmona-Catot et al., 2013). In field studies, native fish species responded to the presence of *G. affinis* by exhibiting spatial and temporal avoidance strategies (Galat & Robertson, 1992; Ayala et al., 2007), or communities becoming restricted to generalist, resilient species (Macdonald et al., 2012; Tonkin et al., 2014). These impacts have the potential to occur in a large proportion of the world's freshwater lakes, ponds, and slow-moving lotic waterways, as *Gambusia* are highly tolerant of temperature, salinity, depth, and dissolved oxygen fluctuations (Odum & Caldwell, 1955; Chervinski, 1983; Swanson et al., 1996). Indeed, *Gambusia* are present in over 60 countries (Walton, 2007) and exhibit population-level physiological adaptations to novel habitats. Prey of *G. affinis* include the eggs and hatchlings of amphibians (Komak & Crossland, 2000; Smith & Smith, 2015), larvae of native fish (Rincón et al., 2002) and terrestrial invertebrates (Mansfield & McArdle, 1998). *Gambusia affinis* are also prolific consumers of zooplankton, significantly reducing or eliminating cladocerans such as *Daphnia pulex* and *Chydorus sphaericus* in experimental pond studies (Hurlbert et al., 1972; Hurlbert & Mulla, 1981). This can produce cascading effects of increased rotifer abundance as crustacean predators and competitors are consumed by *Gambusia* (Hurlbert & Mulla, 1981). However, *G. affinis* have also been observed to deplete rotifer populations once larger zooplankton were removed, leading to phytoplankton proliferation (Hurlbert et al., 1972).

Understanding the threats posed by *G. affinis* in New Zealand is important, in particular due to the high level of endemicity in native fish species (Allibone et al., 2010). Likewise, the widespread distribution of *G. affinis* throughout much of the North Island and their establishment in restricted areas of the South Island (Chadderton et al., 2001; Ling, 2004) confers a degree of urgency, as the species commonly becomes highly prolific where established. Despite this, research on interspecific interactions with *G. affinis* is mainly limited to three native New Zealand fish species; inanga (*Galaxias maculatus*) (Baker et al., 2004; Rowe et al., 2007), dune lake galaxias (*Galaxias gracilis*) (Pingram, 2005) and black mudfish (*Neochanna diversus*) (Barrier & Hicks, 1994; Ling & Willis, 2005). Very little research exists on interactions between *G. affinis* and native bullies (Eleotridae: *Gobiomorphus* spp.), despite both inhabiting the littoral zones of lakes. Species such as the upland bully (*Gobiomorphus breviceps*), common bully (*Gobiomorphus cotidianus*), and Cran's bully (*Gobiomorphus basalis*) are widespread throughout the North and South Island and can reach high abundances in freshwater streams, rivers and lakes (McDowall, 2000; GBIF.org, 2022). The abundance of each of these species primarily varies with elevation and distance inland, though they have been observed to co-occur (Jowett & Richardson, 2003). Several bully species are likely to have dietary and habitat overlap with *G. affinis*. Larval *G. cotidianus* are often pelagic (McDowall & Whitaker, 1975) and reach a similar size to adult *G. affinis* within their first year

(Stephens, 1982). They can be important zooplanktivores in native ecosystems, and small bullies have been documented to induce significant population shifts in *Daphnia* and calanoid copepods in a shallow, brackish water lagoon (Jeppesen et al., 1997). Although adults are primarily benthic and feed on chironomid larvae (Rowe, 1999), they also consume zooplankton, insect larvae, and terrestrial invertebrates (McDowall, 2010). *Gobiomorphus cotidianus* are resilient, surviving thermal stress better than many other native fish species (Richardson et al., 1994), and can forage effectively even in turbid, eutrophic conditions (Rowe, 1999). This is aided by phenotypic plasticity in several traits, such as eye size, body width and length (Ingram et al., 2020). Therefore, for at least part of their life histories, landlocked populations of *G. cotidianus* may compete with *G. affinis* for food and other resources.

Interspecific competition for food sources such as zooplankton can be assessed through mesocosm studies. This allows for a controlled environment where preferred diet can be examined by studying changes in prey community composition. I aimed to determine whether there is dietary overlap for zooplankton in *G. cotidianus* and *G. affinis* in a mesocosm context. To achieve this, my study investigated changes in zooplankton community composition in the presence of *G. affinis* and *G. cotidianus*. I predicted that the zooplankton community composition of fish mesocosms would differ significantly from the controls by the end of the experiment due to top-down control of preferentially consumed prey. I also predicted that both species would target larger zooplankton, as demonstrated by other studies on *Gambusia* (Margaritora et al., 2001; McDonald, 2007; Špoljar et al., 2019) and *G. cotidianus* (Stephens, 1978; Forsyth & James, 1988).

2.2 Methods

2.2.1 Fish collection

A total of 60 adult *Gambusia affinis* and 60 adult *Gobiomorphus cotidianus* were collected on 17 September 2021 from Oranga Lake (37°47'12.4"S, 175°18'57.6"E) and Chapel Lake (37°47'17.73"S, 175°18' 54.2"E), both located on the University of Waikato campus in Hamilton, New Zealand. Fish were captured using a combination of minnow traps and hand nets. *Gambusia affinis* and *G. cotidianus* were placed in separate 450 L capacity outdoor plastic tubs (105 cm (L) x 72 cm (W) x 60 cm (D)), each filled with approximately 300 L of dechlorinated water and aerated with an air stone (Figure 2.1). The *G. cotidianus* tub was treated with 2.5 ppt sodium chloride to reduce transport-associated stress (Harmon, 2009). The fish were then left for 10 days to acclimate to these conditions, with no feeding taking place. A total of 28 *G. affinis* and 28 *G. cotidianus* were randomly selected for the experiment from this group.

Figure 2.1. Aerated acclimation tubs containing *G. cotidianus* (left) and *G. affinis* (right).

2.2.2 Zooplankton and phytoplankton collection

Zooplankton and phytoplankton were collected from seven urban lake and pond sites in Hamilton on 20 September 2021 (austral spring) using a 40 µm mesh net; Knighton Lake (37°47'8.4"S, 175°18'52.9"E), Oranga Lake (37°47'12.4"S, 175°18'57.6"E), Chapel Lake

(37°47'17.7"S, 175°18' 54.2"E), the Faculty of Māori and Indigenous Studies decorative pond (37°47’20.6”S, 175°18’56.8”E), a small private goldfish pond (37°47'59.6"S, 175°19' 41.8"E), Turtle Lake (37°48'17.5"S, 175°18'13.8"E), Lake Rotokaeo (37°46'24.0"S,175°15'4.4"E) and four agricultural troughs in Morrinsville (37°40'24.5"S, 175°31'58.3"E; 37°40'23.4"S, 175°31'58.7"E; 37°40'22.5"S, 175°31'57.0"E; 37°40'22.1"S, 175°31'56.9"E). Collection sites included artificial and heavily modified habitats, stagnant water, and other small waterbodies, to obtain a diverse range of zooplankton species with respect to ecology, size, and taxonomic group, that were likely pre-adapted to experimental mesocosm conditions. A further 600 mL of water containing non-indigenous North American *Daphnia pulex* was sourced from Global Goldfish Fish Farm, Te Aroha (37°31'42.8"S, 175°42'42.7"E).

2.2.3 Experimental setup

The experimental setup used 21 cylindrical plastic drums with 125 L capacity (484 mm (D) x 875.5 mm (H)) as mesocosms (Figure 2.2). The exterior of the mesocosms was painted white to reduce solar heating, and they were arranged in a 3 x 7 pattern approximately 800 mm apart in an open field. On 19 September 2021, each mesocosm was filled with 100 L of tap water and left for 48 hours to dechlorinate. On the same day, three temperature loggers were attached to two mesocosms on opposite ends of the experimental setup, which collected continuous data at 10-minute intervals from 19 September 2021 to 11 October 2021. These consisted of one logger on both mesocosms capturing temperature data every 10 minutes at 5 cm below the water surface and one logger on one mesocosm capturing temperature data every 10 minutes from the air adjacent to the water surface. On 21 September 2021, each mesocosm was dosed with a nutrient mix (0.54 g NH_3Cl, 0.60 g $K_2HPO_4.3H_2O$, 4.8 g $NaHCO_3$, 3.8 g $CaSO_4$ and 3.0 g $MgSO_4$), which was adapted from a ‘synthetic pond water’ formulation by Hebert and Crease (1980). These served the purpose of providing the phytoplankton with nutrient levels similar to the eutrophic water bodies from where they were sourced. The collected zooplankton concentrate and *Daphnia pulex* were distributed evenly among the 21 mesocosms on the same day. Mesocosms were randomly assigned as *G. affinis* treatments, *G. cotidianus* treatments, or left as fishless controls. On 27 September 2021, four *G. affinis* were added into each of seven mesocosms, four *G. cotidianus* were added into each of seven mesocosms, and the remaining seven mesocosms were left without fish. This marked the commencement of the monitoring period, referred to as Day 0.

Figure 2.2. Mesocosms used in the experiment.

Zooplankton were sampled immediately prior to fish addition using a 70 mm diameter PVC integrated plankton cylinder with a total height of 1.12 m, which was vertically submerged into the water covering the entire water column. This was then capped and lifted from the drum, sampling 2.4 L of water. This water was filtered through a 40 μm sieve over each tank, with filtered water returned to the mesocosm. Zooplankton retained on the filter were washed into a sample container and preserved in ethanol (>50% final concentration). This sampling process was repeated on 30 September 2021, 4 October 2021, and 11 October 2021, referred to as Days 3, 7, and 14. On each sampling day, two samples were collected from each mesocosm for chlorophyll-*a* analysis, using a syringe to filter 50 mL of water at low vacuum through a 0.2 μm Whatman GF/C glass microfiber filter. Filters were stored frozen until analysed. All equipment was washed thoroughly with tap water between mesocosms to avoid cross-contamination during sampling. Temperature and pH were measured using a Thermo Scientific pHTestr 30 meter from each mesocosm on each sampling day. Dissolved oxygen and specific conductance were measured in each mesocosm on each sampling day using a YSI ProSolo 2030 Dissolved Oxygen and Conductivity Meter. All water samples were taken at approximately 15 cm below the water surface. Measurements were taken at 10:00 am on Day 0 and 2:00 pm on Days 3, 7, and 14. On 12 October 2021, the fish in each mesocosm were measured, weighed, and then euthanised.

2.2.4 Laboratory analysis

Chlorophyll-*a* was extracted from the filter papers using a DLAB D-160 electric tissue grinder to produce a slurry in 20 mL of buffered acetone (10% saturated $MgCO_3$). Samples were steeped at 4°C for 24 hours, then centrifuged at 700 *g* for 10 minutes. Following this, a Turner Designs 10-AU fluorometer (Turner Designs, Sunnyvale, CA, USA) was used to measure chlorophyll-*a* concentration in each mesocosm on each sampling day. Preserved zooplankton were counted in 5 mL aliquots in a gridded Perspex sorting tray under a dissecting microscope (Olympus SZ60) at approximately 30x magnification until a minimum of 300 individuals per sample were counted or until the entire sample was completed. Taxa were identified to species level where possible using an Olympus BH2 compound microscope at up to 400x magnification, using standard taxonomic keys (Shiel, 1995; Chapman et al., 2011). Copepod nauplii could not be identified to species level but were retained in counts for statistical analysis.

2.2.5 Statistical analyses

Zooplankton counts were expressed to numbers per litre for all analyses. ANOVA tests were undertaken to investigate differences between environmental variables among the two treatments and control on each sampling date. ANOVA tests were also undertaken to investigate differences in total copepod, cladoceran, and rotifer counts per L between each treatment and control. Results were considered significant where $p < 0.0125$ in accordance with a Bonferroni correction due to multiple comparisons. Where ANOVA indicated significant results, these were followed by Bonferroni *post-hoc* tests. All ANOVA and associated analyses were conducted using STATISTICA (v.14.0.0.15 TIBCO Software Inc.; Palo Alto, CA, USA). Non-metric multidimensional scaling (nMDS) and analysis of similarities (ANOSIM) were undertaken using PRIMER v.7.0.17 to assess changes in zooplankton community composition among treatments and controls on each sampling day. One analysis was run for each sampling day, using zooplankton count data for all species found in three or more samples to reduce the influence of low-abundance species sampled by chance. Species excluded were *Cephalodella catellina* and *Euchlanis meneta* from Day 3, *Asplanchna priodonta* from Day 7, and *Keratella tecta* and *Polyarthra dolichoptera* from the Day 14 analyses. Taxon data were log (x+1) transformed to reduce undue influence of highly abundant species. A similarity matrix based on the Bray-Curtis similarity coefficient was constructed on the transformed abundance data.

Non-metric multidimensional scaling plots were constructed based on the similarity matrices to produce two-dimensional plots of the data, where the distance between samples reflects similarity in community composition between mesocosm samples. ANOSIM was conducted on the Bray-Curtis similarity matrix (999 permutations executed) to investigate whether differences in community composition among treatments and controls were significant on each sampling day. Where significant ANOSIM results were found, these were followed by similarity percentage (SIMPER) *post-hoc* analyses to determine the importance of taxa to the dissimilarity between *G. cotidianus* and control, and *G. affinis* and control mesocosms. P-values of <0.0125 were considered statistically significant in accordance with a Bonferroni correction to account for the four comparisons.

2.3 Results

2.3.1 Environmental data

Air temperatures fluctuated between a night-time minimum of 3.0°C and a daytime maximum of 27.9°C for the duration of the experiment (Figure 2.3). Diel variation affected water temperature less, with mesocosm water temperature generally remaining between 10°C and 25°C at the surface. The mean difference between the two mesocosms with permanent temperature loggers was 0.06°C, and the average temperature throughout the experiment was 15°C (Figure 2.3).

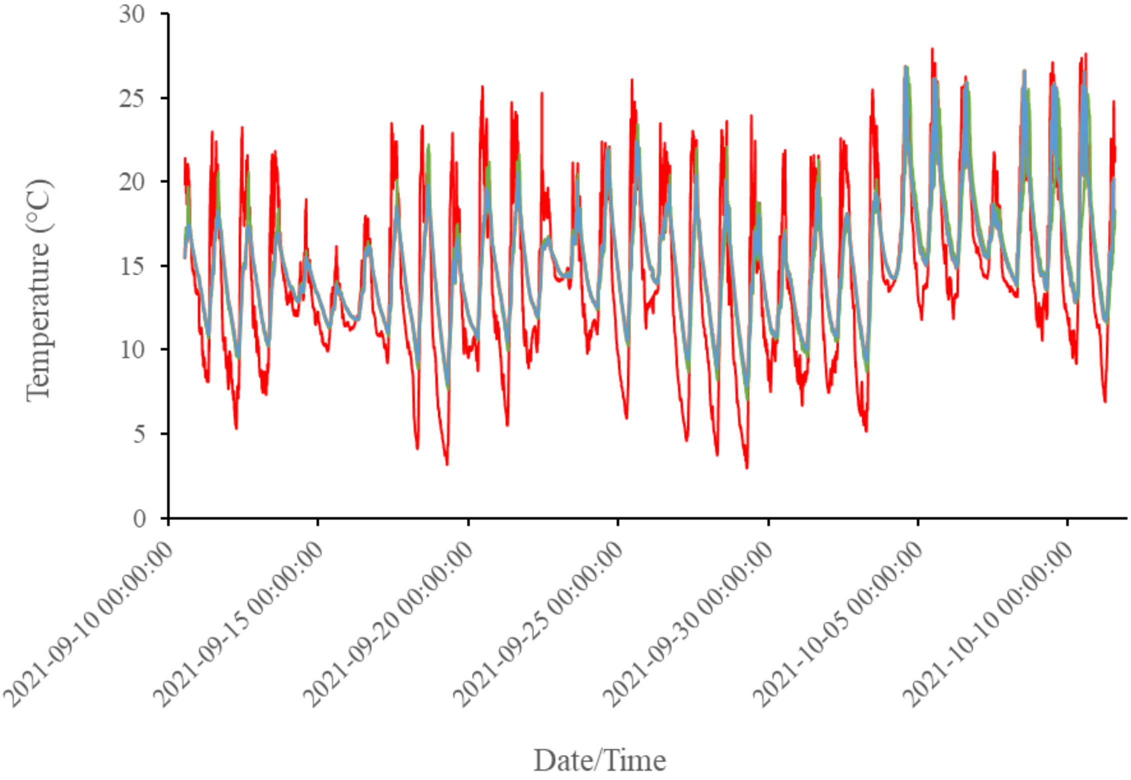

Figure 2.3. Temperature logger measurements taken every 10 minutes at 5 cm water depth (green line and blue line) and the air near the surface (red line) of two mesocosms for the duration of the experiment.

Environmental variables were most similar between control and treatment tanks on Day 0 of the study and differed most greatly on Day 7 and Day 14. No significant differences were found in mean temperatures of treatment and control mesocosms on Day 0 (F=3.57; p=0.050), Day 3 (F=4.29; p=0.030), Day 7 (F=2.48; p=0.112) or Day 14 (F=2.44; p=0.115). On Day 0, no significant difference was found between the means for chlorophyll-*a* (Figure 2.4; F=1.27; p=0.302). Mean chlorophyll-*a* concentrations rose in all treatments on Day 3, further increased by Day 7, and then dropped in all treatments on Day 14. The greatest increases from Day 0 to

Day 3 were in the fish treatment tanks, with mean chlorophyll-*a* increasing to 41.8 µg/L in the *G. cotidianus* mesocosms and increasing to 45.5 µg/L in the *G. affinis* mesocosms. No significant difference was found among the treatments and control on Day 3 (F=2.19; p=0.142). Chlorophyll-*a* concentrations were greatest on Day 7 in both fish mesocosms and the controls, and while variation between individual mesocosms was the greatest on Day 7 (SE=73.93), the differences in concentrations among the control and treatments did not differ significantly (F=0.32; p=0.731). By Day 14, chlorophyll-*a* concentrations dropped across both treatments and the control mesocosms, with the greatest average decrease in the control. No significant difference was found between treatments on Day 14 (F=0.52; p=0.614). The standard deviations within each treatment and control were lowest on Day 0 (SD=4.51) and highest on Day 7 (SD=195.60). Specific conductance did not differ significantly between treatments on Day 0 (F=0.50; p=0.613), Day 3 (F=0.19; p=0.831), Day 7 (F=0.45; p=0.643) and Day 14 (F=1.02; p=0.381) (Figure 2.4). On Day 14, there was a significant difference in pH among mesocosms (Global F=6.42; p=0.008). A Bonferroni *post-hoc* test indicated a significant difference in pH between the control and *G. cotidianus* treatment (p=0.007), though the means only differed by 0.25. Average dissolved oxygen increased with each sampling day in both treatments and control (Figure 2.4). Throughout the experiment, average dissolved oxygen concentration increased to 16.4 mg/L in the control mesocosms, 16.6 mg/L in *G. cotidianus* mesocosms, and 17.3 mg/L in *G. affinis* mesocosms. On Day 0, treatments and control varied on average by 0.05 mg/L (F=0.10; p=0.903) or 0.76% (F=0.21; p=0.813), with variance among the treatments and control increasing with each sampling day. However, no significant difference was found among the means on any day.

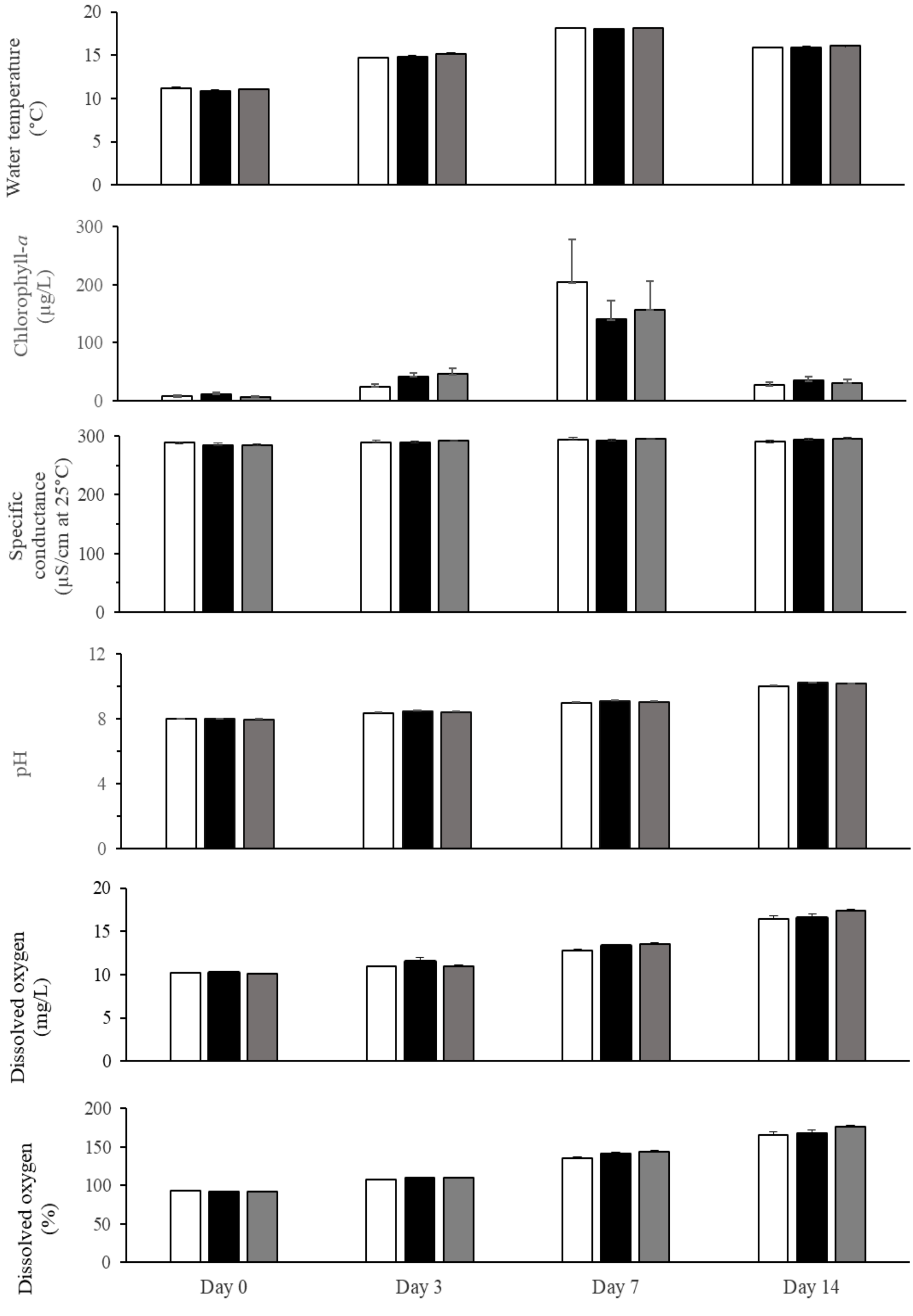

Figure 2.4. Mean environmental variables + SEM of control, *G. cotidianus*, and *G. affinis* mesocosms on sampling days 0, 3, 7, and 14. The white bars are control, black bars are *G. cotidianus*, and grey bars are *G. affinis*.

2.3.2 Zooplankton assemblages

The nMDS plot for Day 0 showed no separation in zooplankton composition between the *G. cotidianus* or *G. affinis* mesocosms from the control, or from each other (Figure 2.5). ANOSIM for Day 0 indicated no significant difference among groups (Global r=0.009; p=0.409). The nMDS plots for Days 3 and 7 also showed no clear separation of either treatment from the control, or from each other, and ANOSIM indicated no significant differences in zooplankton community composition on either Day 3 (Global r=0.12; p=0.063) or Day 7 (Global r=0.05; p=0.216). On Day 14, the nMDS plot showed overlap in *G. cotidianus* and *G. affinis* mesocosms on the right side of the ordination and a clear separation in zooplankton composition in each of the fish treatments from the control mesocosms (with control samples distributed on the left side of the ordination). ANOSIM indicated a significant difference among groups (Global r=0.44; p=0.001), and pairwise tests showed statistically significant differences in zooplankton composition between the control and *G. cotidianus* mesocosms (r=0.52; p=0.002) and control and *G. affinis* mesocosms (r=0.76; p=0.002). Pairwise tests indicated no significant difference in zooplankton community composition between the two fish treatments (r=-0.05; p=0.642).

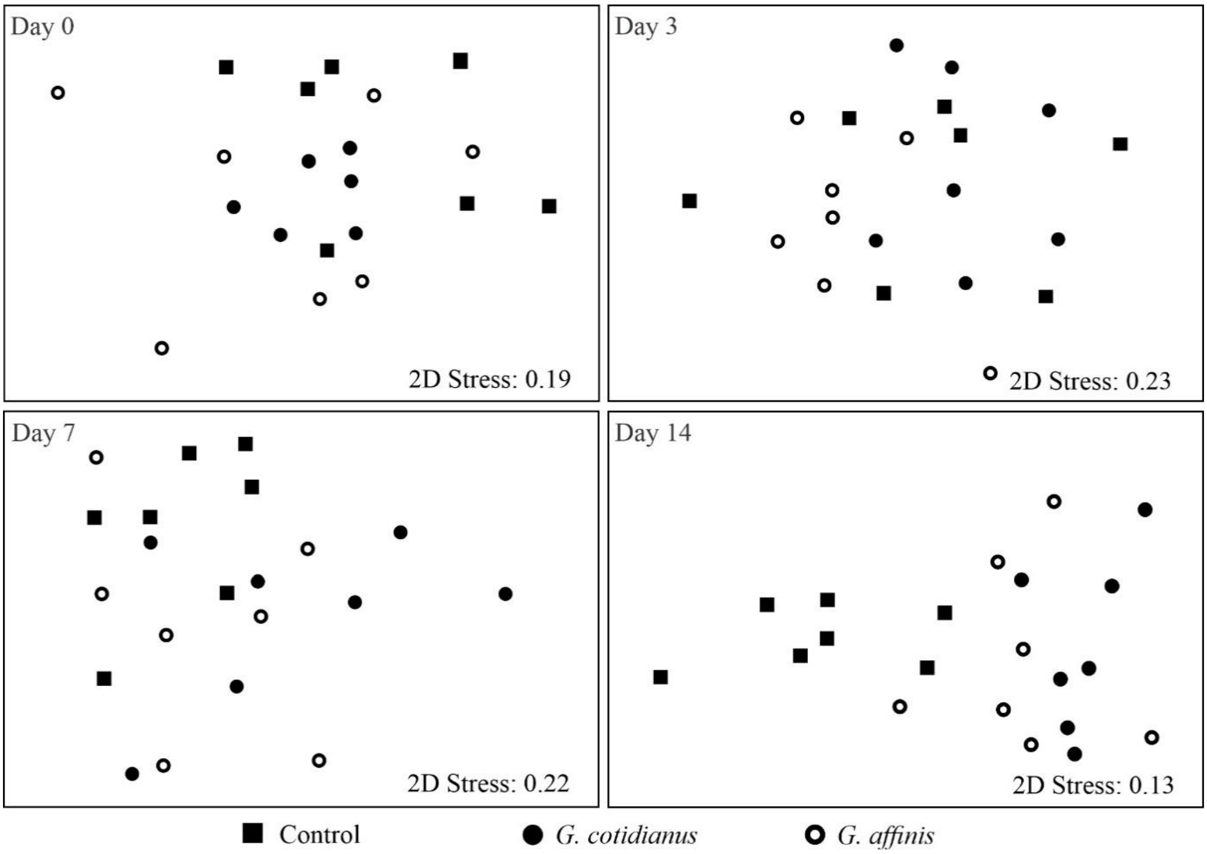

Figure 2.5. Multidimensional scaling (MDS) plots depicting similarities between zooplankton assemblages in *G. cotidianus*, *G. affinis*, and control treatments on sampling Days 0, 3, 7, and 14.

SIMPER analysis between the control and the *G. cotidianus* mesocosms on Day 14 indicated that the rotifers *Cephalodella catellina*, *Euchlanis dilatata*, *Euchlanis meneta*; *Proales* sp. and *Lecane closterocerca* occurred in greater abundances in the *G. cotidianus* treatment tanks than in the control tanks (Table 2.1). Taxa present in greater abundances in control mesocosms that contributed most to dissimilarity were cladocerans such as *Bosmina meridionalis*, *Daphnia pulex*, and the copepod *Mesocylops australiensis* (Table 2.1). Assessment of dissimilarity between the control and *G. affinis* mesocosms revealed similar patterns. The rotifers *Cephalodella catellina*, *Euchlanis dilatata*, and *Euchlanis meneta* were once again found in greater abundances in the *G. affinis* treatment than in controls. In contrast, species with greater abundances in the control mesocosms were cladocerans and copepods (Table 2.1). Taxa with greater abundances in the controls than *G. affinis* mesocosms were the cladocerans *Bosmina meridionalis*, *Daphnia pulex* and *Chydorus* sp., the copepods *Mesocyclops australiensis* and *Calamoecia lucasi*, and copepod nauplii.

Table 2.1 SIMPER analysis results of the main (>5% contribution to the total) zooplankton taxa on Day 14 between *G. cotidianus* and control, and *G. affinis* and control treatments. Taxa are ordered by contribution to dissimilarity (%) and are identified as rotifers (rot), cladocerans (cla) and copepods (cop).

Taxon	**Average abundance (control)**	**Average abundance (fish treatment)**	**Average dissimilarity**	**Contrib. %**	**Cum. %**
***G. cotidianus* and control**					
Cephalodella catellina (rot)	2.16	5.52	6.55	14.14	14.14
Bosmina meridionalis (cla)	4.93	2.50	4.51	9.74	23.87
Daphnia pulex (cla)	2.83	0.40	4.38	9.44	33.32
Mesocyclops australiensis (cop)	2.28	0.26	3.70	7.99	41.31
Euchlanis dilatata (rot)	1.66	3.40	3.67	7.92	57.19
Euchlanis meneta (rot)	0.33	2.26	3.69	7.99	49.27
Proales sp. (rot)	1.97	3.19	2.49	5.38	62.57
Chydorus sp. (cla)	1.82	1.82	2.44	5.27	67.84
Lecane closterocerca (rot)	2.05	2.43	2.32	5.02	72.86
***G. affinis* and control**					
Cephalodella catellina (rot)	2.16	6.00	7.57	17.72	17.72
Bosmina meridionalis (cla)	4.93	2.87	4.52	10.58	28.31
Euchlanis dilatata (rot)	1.66	3.11	3.58	8.39	36.70
Mesocyclops australiensis (cop)	2.28	0.46	3.47	8.13	44.83
Daphnia pulex (cla)	2.83	1.73	3.35	7.86	52.68
Chydorus sp. (cla)	1.82	0.93	2.64	6.19	58.88
Euchlanis meneta (rot)	0.33	1.36	2.40	5.61	64.49
Copepod nauplii (cop)	2.34	1.80	2.35	5.50	69.99
Calamoecia lucasi (cop)	1.60	0.95	2.31	5.41	75.40

At a coarser scale, the numerical composition of rotifers, copepods and cladocerans in each treatment and the control showed considerable variation by Day 14 (Figure 2.6). The first three sampling days showed zooplankton counts below 150 individuals per L, with similar proportions of rotifers, cladocerans and copepods in each treatment. On Day 14, cladoceran densities across the *G. affinis* and *G. cotidianus* treatments were their lowest for the whole experiment, whereas rotifers were the highest. On the same day, the opposite trend was apparent in the control, where cladocerans were at their greatest abundance, and rotifers were reduced (Figure 2.6). ANOVA tests showed significant differences between control and *G. affinis* mesocosms for rotifer (p=0.005) and cladoceran (p=0.038) numbers. Likewise, ANOVA tests between control and *G. cotidianus* mesocosms showed statistically significant differences between the means for cladoceran numbers (p=0.004) and rotifer numbers (p=0.006). No significant difference was found in copepod counts between the fish treatments and the control.

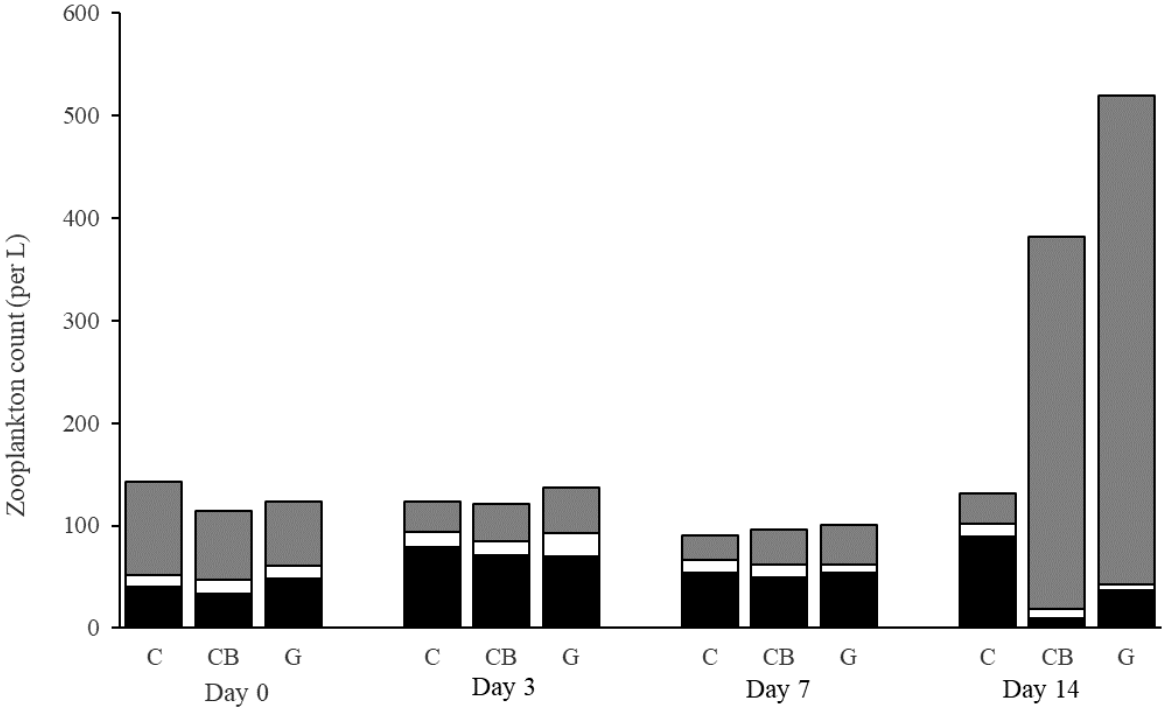

Figure 2.6. Broad-scale zooplankton composition of control (C), *G. cotidianus* (CB) and *G. affinis* (G) mesocosms throughout the experiment. Black bars represent the count data for cladocerans, white bars copepods, and grey bars rotifers.

2.4 Discussion

Similar changes in zooplankton community composition were observed in the *G. cotidianus* and *G. affinis* mesocosms through time relative to the controls, indicating that the two species had similar dietary preferences. The impact of fish predation became evident on Day 14 for both fish species. No statistically significant differences were found between the fish treatments and control mesocosms on any previous sampling day. The Day 14 results indicated an overall numerical dominance of rotifers in the *G. cotidianus* and *G. affinis* mesocosms, and a reduction in crustaceans relative to the control mesocosms. This suggests that both fish were primarily targeting cladocerans and copepods, with the smaller rotifers released from competition or predation by the reduction of larger zooplankton taxa in the fish treatments. SIMPER analysis indicated that both *G. cotidianus* and *G. affinis* prefer larger crustacean taxa such as *Bosmina meridionalis*, *Daphnia pulex*, *Chydorus* sp., and *Mesocyclops australiensis*. No significant differences were observed between the mesocosms for any environmental variable on Day 0, and temperatures remained consistent among mesocosms on all sampling days. This suggests that there was no bias from the placement of mesocosms, allocation of treatments, or other external inputs. The results align with both of my hypotheses and with the findings of other studies investigating zooplanktivory in each of the studied species.

2.4.1 Diet of *G. cotidianus*

My findings align with general trends in the dietary preference of *G. cotidianus* and other *Gobiomorphus* species and are in accordance with optimal foraging theory for planktivorous fish (Townsend & Winfield, 1985). Fish tend to select larger zooplankton prey when prey density is high, as these are usually the most visible and energetically profitable (Werner & Hall, 1974). For example, upland bullies (*Gobiomorphus breviceps*) preferentially selected larger over smaller oligochaete worms in an aquarium study both in the presence and absence of conspecific competitors (James & Poulin, 1998). Likewise, Staples (1975) used stomach content analysis of 1039 lacustrine *G. breviceps* to demonstrate a preference for oligochaetes, cladocerans and copepods rather than rotifers, particularly in their first year of life. Both *G. breviceps* and *G. cotidianus* display ontogenetic dietary shifts in their natural habitats as they transition from pelagic to primarily benthic habitat use (Staples, 1975; Wilhelm et al., 2007). Nevertheless, the prey targeted at each life stage can be highly dependent on the specific lake or river environment, and *G. cotidianus* show a high dietary breadth (Wilhelm et al., 2007).

Juvenile *G. cotidianus* typically select the most abundant and easy-to-capture pelagic prey, which can be amphipods such as *Paracalliope fluviatilis* (Wilhelm et al., 2007), cladocerans such as *Bosmina meridionalis* (Rowe & Chisnall, 1996), or copepods (Rowe et al., 2001). Adult *G. cotidianus* tend to prefer benthic food sources, including chironomid larvae, such that *Polypedilum pavidus* comprised up to 97.5% of bully gut weight in a eutrophic lake (Forsyth & James, 1988). However, my study did not provide benthic prey sources, thus emulating conditions where zooplanktivory occurs even in adult fish. This has a strong seasonal component, as zooplankton abundance can increase significantly in response to food availability (Chapman et al., 1985; Chapman & Green, 1999) and climatic conditions (Burns, 1992), altering the availability of key prey species of fish (Forsyth & James, 1991). For example, zooplankton in oligotrophic Lake Taupō were estimated to contribute to the diet of *G. cotidianus* between a minimum of 8 ± 8% and a maximum of 52 ± 19% over an 11-month stable isotope study (Stewart et al., 2017). Similar variability was found in eutrophic Lake Ōkaro, where benthic *G. cotidianus* fed on a pelagic cladoceran, *Ceriodaphnia dubia*, between June and December (austral winter, spring, and early summer) (Forsyth & James, 1988). This seasonal shift to pelagic prey was attributed to decreased abundances of benthic chironomid larvae over the winter months and demonstrates opportunistic feeding behaviour (Forsyth & James, 1988). As such, *G. cotidianus* selected the largest prey in the mesocosms, Crustacea, and avoided less profitable rotifers. This finding aligns with the results of a lake enclosure study by Jeppesen et al. (1997), where the addition of small common bullies significantly reduced the abundances of *Daphnia* and the copepod *Boeckella hamata*, but not rotifer abundance.

2.4.2 Diet of *G. affinis*

The results showing a preference for larger zooplankton by *G. affinis* follows many prey selection studies of *G. affinis* and *G. holbrooki* globally. These fish actively seek out certain types and sizes of prey, often aligning with the most profitable items as suggested by optimal diet theory (Bence & Murdoch, 1986). Prey selection appears to be influenced by numerous factors, including *Gambusia* size (Wurtsbaugh et al., 1980), satiation level (Bence & Murdoch, 1986), sex, and habitat type (Blanco et al., 2004). However, zooplankton appear consistently among the primary prey of *Gambusia*, and within this category, research points towards elevated predation on medium to large species of cladocerans and copepods. For example, Špoljar et al. (2019) found that cladocerans and copepods were heavily depleted in small

Mediterranean ponds containing *G. holbrooki*, whereas these species dominated zooplankton biomass in ponds without *G. holbrooki*. Furthermore, rotifer abundance was reduced in ponds without *Gambusia* (Špoljar et al., 2019), which was also observed in the current mesocosm study. Similar patterns were found in a pond study in Italy, where zooplankton surveys in the presence and absence of *G. holbrooki* showed a significant difference in community composition (Margaritora et al., 2001). Large-bodied daphniids became more abundant and taxonomically diverse in the absence of *Gambusia*, whereas zooplankton were restricted to taxa smaller than 1 millimetre when exposed to fish predation. Likewise, the increase of large cladocerans and copepods coincided with a decrease in the proportion of rotifers present, from 47% of the sampled zooplankters to only 20% in that study (Margaritora et al., 2001). Similarly, rotifers comprised 22% of the numerical zooplankton abundance in control mesocosms and 92% of the community in the *G. affinis* treatments at the end of my study, showing a strong impact of the fish within 14 days.

The primary reasons for these findings are likely explained through size-selective predation by *Gambusia*, combined with the superior competitive ability of large over small zooplankton in the absence of predation. Brooks and Dodson (1965) introduced the size-efficiency hypothesis for zooplankton feeding, which predicts that small herbivorous species, including many rotifers, cannot effectively compete for food with larger zooplankton due to reduced food filtration efficiency. Instead, rotifers can be reliant on top-down control of competitors such as larger-bodied cladocerans and copepods, often from planktivorous fish such as *Gambusia* (Brooks & Dodson, 1965). Further research by Dodson (1974) suggested that this trend was more likely due to predation by the larger zooplankton instead of exploitative competition. Thus, top-down control of cladocerans and copepods by fish could be releasing rotifers from predation pressure. Interference competition is another mechanism by which larger taxa may have influenced rotifer abundance. Gilbert and Slemberger (1985) demonstrated that *Daphnia* species can sweep rotifers such as *Keratella cochlearis* through their branchial chambers, causing mechanical damage to the rotifer. In my mesocosm study, the primary species consumed by *Gambusia* included both omnivores (e.g., the copepods *Mesocyclops australiensis* and *Calamoecia lucasi*) and filter feeders (e.g., the cladocerans *Daphnia pulex* and *Bosmina meridionalis*), so the relative significance of competition and predation remains unclear. However, these were among the largest zooplankton in the mesocosms, which aligns with existing research on prey size selection by *Gambusia*. Wurtsbaugh et al. (1980) found *G. affinis* to attack the largest mosquito larvae available, even when these were too large for small

G. affinis to capture efficiently. Increasing fish size was positively correlated with increased preferred prey size, though the movement of the mosquito larvae was also considered an important factor for triggering an attack response (Wurtsbaugh et al., 1980). The fourth instar of *Culex tarsalis* larvae in the Wurtsbaugh et al. (1980) study are typically larger than the zooplankton present in this mesocosm study, and were found to be preferred over *D. pulex* by Linden and Cech (1990). Therefore, no zooplankton in my study could be considered too large for *G. affinis* to target. However, there are exceptions to these findings. Large cladocerans such as *Daphnia lumholtzi* may increase in the presence of *Gambusia* due to the development of morphological defensive features (i.e., long head and tail spines) that are detrimental to their competitive ability in *Gambusia*-free waterways (Hinchliffe et al., 2017), leading to a depletion of medium-sized zooplankton species when fish are present. Haiahem et al. (2017) also found an increase in smaller cladocerans and copepods in ponds with *G. holbrooki*, while large ostracods, daphniids, and branchiopods were rapidly consumed. Other studies have found *Gambusia* to consume large quantities of algae and detritus, some chironomids (Blanco et al., 2004), significant quantities of *Chaoborus* (not present in New Zealand) and some Corixidae (Hinchliffe et al., 2017), which were not present in the mesocosms.

2.4.3 Potential for competition between *G. affinis* and *G. cotidianus*

No significant difference was found between zooplankton assemblages in the *G. affinis* and *G. cotidianus* treatments on Day 14, despite both having diverged from the controls. The taxa identified by SIMPER analysis as contributing to the greatest amount of variation between the *G. cotidianus* and control mesocosms, and the *G. affinis* and control mesocosms, showed considerable similarity. For example, by the end of the experiment, both *G. cotidianus* and *G. affinis* treatments had elevated average proportions and counts (per L) of rotifers and significantly fewer cladocerans. Overall, copepod counts did not differ significantly between the control in both fish treatments. However, SIMPER analysis detected reductions in two copepod taxa and nauplii contributing to dissimilarity between the control and the *G. affinis* mesocosms, and one copepod taxon contributing to the dissimilarity between the control and the *G. cotidianus* treatment. Although no other studies have directly compared the diet of these two fish, comparisons can be made with research into the dietary overlap of each species with other morphologically or physiologically similar fish to assess the significance of this finding. Interspecific competition studies involving the diet of *G. cotidianus* are much fewer than research on *G. affinis*, though there is evidence for the coexistence of different bully species.

For example, Sagar and Glova (1994) studied a sympatric population of *G. cotidianus* and upland bullies (*Gobiomorphus breviceps*), revealing considerable similarities in their diet, despite temporal niche overlap. Both species were most active during the day and primarily targeted chironomid larvae during this period, resulting in a significantly correlated diet (Sagar & Glova, 1994). The diet of *G. cotidianus* and *G. breviceps* is more similar than that of *G. cotidianus* and *G. affinis* in most situations, as both *Gobiomorphus* species were found to primarily feed on the benthos, with *G. cotidianus* also targeting fish eggs and fish remains where available (Sagar & Eldon, 1983). Glova (1999) found that *G. cotidianus* and *G. breviceps* co-occurring in artificial channels avoided interspecific competition by occupying different longitudinal sections of the waterway and that intraspecific competition in the control groups had a more negative impact on *G. breviceps* body weight than the presence of *G. cotidianus*. These studies demonstrate that bully coexistence is widespread and that *G. cotidianus* is unlikely to have a strong negative effect on physiologically similar sympatric species. Conversely, the ability of *Gambusia* to competitively exclude other small fish is well documented (Caiola & de Sostoa, 2005; Ling & Willis, 2005; Ruiz-Navarro et al., 2013a), in addition to evidence for aggressive behaviour when sufficient space and refugia are not available (Rincón et al., 2002; Mills et al., 2004; Macdonald et al., 2012; Carmona-Catot et al., 2013). The impacts of *Gambusia* on other species are correlated with factors such as available cover, habitat, and diet overlap. *Gambusia holbrooki* did not appear to outcompete a similar-sized zooplanktivore (*Heterandria formosa*) that occurs within its native range but did alter its population size and sex structure through predation on juveniles (Belk & Lydeard, 1994). However, studies on *Gambusia* outside its native range have consistently showed traits such as increased prey capture rates and voracity (Caiola & de Sostoa, 2005), habitat dominance (Ayala et al., 2007), higher dietary flexibility (Kalogianni et al., 2014) and aggression (Laha & Mattingly, 2007). Some of these traits appear to be reflected in the changes to zooplankton communities in this study, from which possible interactions when *G. affinis* co-occur with bullies can be hypothesised. Both *G. affinis* and *G. cotidianus* produced similar zooplankton assemblages through predation after 14 days, yet *G. affinis* achieved this with much lower fish biomass, average length, and average weight (Appendix 2.1). *Gobiomorphus cotidianus* were 3.7 times heavier and 1.6 times longer on average (Appendix 2.1), thus could be expected to consume more prey to meet their metabolic needs. Though this was not directly investigated in this research, the similar degree of depletion of cladocerans and copepods suggests that *G. affinis* can induce changes to zooplankton community structure at lower population levels and body sizes than *G. cotidianus*. This, in conjunction with their life history traits such as ovoviviparity, high fecundity, lower interbrood interval, and faster growth rates (Stephens, 1982; Vondracek et al., 1988), suggests that in sympatric populations, *G. affinis* could easily exceed the abundance and biomass of *G. cotidianus* in the absence of targeted predation. *Gambusia* populations can rapidly reach very high densities on a seasonal basis (Hughes, 1985) and were found to suppress microcrustaceans at densities above 0.8 g m^2, and both microcrustaceans and rotifers when at densities greater than 2.5 g m^2 (Ning et al., 2009). My study used *G. affinis* densities of approximately 3.5 g m^2 but also had artificially elevated initial zooplankton concentrations, while Ning et al. (2009) used naturally occurring zooplankton concentrations and juvenile *G. holbrooki*.

2.4.4 Extrapolating to freshwater systems

Zooplankton predation patterns identified in this experiment could be used to make predictions about the differential impacts of each fish species on zooplankton communities in natural habitats. Zooplankton can be affected in various ways, as predation may disproportionately target larger, easier to capture individuals, such as ovigerous females (Conde-Porcuna & Declerck, 1998; Ning et al., 2009). Planktivorous fish species such as *G. affinis* and larval *G. cotidianus* have the potential to structure zooplankton communities, which can often lead to cascading effects in other parts of the food web. For example, a pond study in the USA showed that *G. affinis* depleted zooplankton to the degree that major phytoplankton blooms developed, leading to decreased water clarity, altered dissolved phosphorus dynamics, and contributed to the loss of an annelid species (Hurlbert et al., 1972). This *Gambusia*-mediated release of grazing pressure on phytoplankton was also observed by Ning et al. (2009) and was reflected by elevated chlorophyll-*a* levels. My mesocosm study did not find a significant difference among the fish treatments and controls for chlorophyll-*a*, which peaked on Day 7, dropping near Day 0 levels on Day 14. Although the significant depletion of cladocerans and copepods in the fish mesocosms was only found on Day 14, it is possible that the subsequent proliferation of rotifers effectively offset the loss of grazing crustaceans. Total zooplankton counts were highest on Day 14, where the 46% decrease of cladocerans and 36% decrease of copepods in the *G. affinis* tanks was balanced by a 1150% increase in rotifer numbers. The differential response of rotifers to *G. affinis* may be due to the study environment. Špoljar et al. (2019) found macrophyte cover to positively affect zooplankton density and biomass in small ponds, and macrophytes are also a preferred habitat of *Gambusia* (Casterlin & Reynolds, 1977). The use of macrophytes may have altered the results in that experiment through the provision of shelter and shading. However, the absence of a significant increase in phytoplankton suggests that any alteration to primary production or nutrient dynamics may only be relevant over longer periods. Margaritora et al. (2001) found a similar result in an enclosure-based mesocosm study of *G. holbrooki*, as despite significant decreases in cladoceran numbers due to predation, there was no cascading effect on phytoplankton. This was attributed to the dominance of submerged macrophytes in the ecosystem, thus providing resilience to the removal of filter feeding zooplankton by *Gambusia* (Margaritora et al., 2001). Comparatively, a three-month study in New Zealand stocking mesocosms with *G. affinis* and black mudfish (*N. diversus*) along with peat and emergent macrophytes found an increase in phytoplankton, followed by a significant increase in rotifers due to the elimination of larger zooplankton by fish predation (McDonald, 2007). *Gobiomorphus cotidianus* zooplanktivory identified in this study is unlikely to directly translate to similar depletion of larger copepods and cladocerans in most New Zealand waterways. The bullies in this study were adult fish, which prefer benthic prey such as chironomid larvae for most of the year (Forsyth & James, 1988; Stewart et al., 2017). However, they are flexible in feeding on food sources both on the benthos and in the water column, depending on the availability and abundance of each prey item (Wilhelm et al., 2007; Bickel & Closs, 2008). Top-down control of zooplankton by bullies would primarily be attributed to juvenile fish, which are pelagic and prefer amphipods or cladocerans such as *Bosmina meridionalis* (Rowe & Chisnall, 1996; Wilhelm et al., 2007). The degree to which fish deplete zooplankton in New Zealand freshwaters has been debated, with older studies, in the absence of non-native fishes, generally suggesting zooplankton are food limited (Jolly & Brown, 1975; Chapman et al., 1985; Burns, 1992). These trends and the prevalence of species such as the copepod *Calamoecia lucasi* were explained by the lack of adult zooplanktivorous fish and large invertebrates in many New Zealand lakes (Chapman et al., 1985). This is due to the propensity for diadromy in the majority of native freshwater fish species, which means the zooplanktivorous life stages of many species consume marine food sources (McDowall, 2007). This is contrasted by evidence of top-down control of zooplankton by fish internationally, and many lakes in New Zealand now possess similar characteristics in fish density and zooplankton: phytoplankton ratios (Jeppesen et al., 1997; Jeppesen et al., 2000). Although such zooplanktivorous fish densities may only be reached seasonally through bully spawning, this mesocosm study demonstrates that adult *G. cotidianus* can shift zooplankton community composition in a comparable way to *G. affinis*, at least in ponds and shallow lake margins. Environmental changes and the spread of *G. affinis* to more New Zealand freshwaters could lead to changes in the feeding behaviour of both *Gobiomorphus cotidianus* and *Gambusia* *affinis*. *Gambusia* are documented to affect both pelagic and benthic prey species depending on the habitat (Blanco et al., 2004; Hinchliffe et al., 2017), which could indicate competition with both adult and juvenile *G. cotidianus* on a seasonal basis.

2.4.5 Future research and conclusions

While this study provides interesting findings on the degree to which *G. cotidianus* and *G. affinis* prey selection overlap in a mesocosm setting, it also opens future avenues of research. These include expanding on behavioural and dietary research of both fish in a laboratory or mesocosm context to reveal the factors contributing to interspecific competition, and field studies to understand better how these relationships translate to ecosystem-level outcomes. Firstly, the experimental design could be easily altered to investigate several variables that may influence dietary overlap between the two fish species. For example, switching or adding to the response variable to include different prey sources such as a benthic-focused invertebrate assemblage, would provide better insight into the impacts of *G. affinis* on adult *G. cotidianus*. Adult *G. cotidianus* primarily feed on the benthos (Forsyth & James, 1988; Sagar & Glova, 1994), while *Gambusia* do so occasionally (Hurlbert et al., 1972; Schaefer et al., 1994). Therefore, it may prove interesting to study whether *G. affinis* can produce as great a shift in benthic invertebrates as they have been demonstrated to do in pelagic zooplankton. Additionally, the sizes and abundances of fish can be varied. Juvenile *G. cotidianus* are primarily pelagic, are generally closer in size to small *Gambusia*, and have been demonstrated to exert top-down control on lake zooplankton (Rowe & Chisnall, 1996; Wilhelm et al., 2007). Thus, a mesocosm study could investigate whether juvenile or larval *G. cotidianus* are likely to exhibit significant dietary niche overlap with *G. affinis* and the outcomes of any interaction between the two species. Likewise, more research could be undertaken with bullies and *G. affinis* in sympatric enclosures, investigating whether there is evidence for avoidance behaviour, spatial or temporal segregation, dietary niche shifts, or population level changes over time. Both fish are highly adaptable, with *G. cotidianus* demonstrating phenotypic plasticity (Ingram et al., 2020) and tolerance to habitat and food source change (Mitchell, 1986). Likewise, *G. affinis* are considered one of the most globally invasive freshwater fish for their individual and population level adaptability to novel environmental conditions, rapid reproduction, opportunist diet, and aggressive behaviour (Pen et al., 1993; Vargas & de Sostoa, 1996; Pyke, 2005). Therefore, it may be useful to assess the diet and interaction of the two species in a range of habitat types, as their behaviour and their prey can differ considerably.

Zooplankton survival and abundance can positively correlate with macrophyte cover in the absence of predatory macroinvertebrates and zooplanktivorous fish (Eivers et al., 2018; Špoljar et al., 2019), but macrophytes are often avoided by larger zooplankton species (Castro et al., 2007; Sagrario et al., 2009; Sagrario et al., 2010). *Gambusia* are effective predators in macrophyte beds and have been found to consume more zooplankton, particularly rotifers, when these are present (Blanco et al., 2004). Therefore, the provision of shelter and habitats through macrophytes could be an interesting research avenue, along with testing more extensive, more natural mesocosms.

In summary, this mesocosm experiment has indicated that *G. cotidianus* and *G. affinis* have a significant degree of dietary overlap in zooplankton. Both *G. affinis* and *G. cotidianus* displayed a preference for larger cladocerans and copepods, with shared prey items including *Bosmina meridionalis, Daphnia pulex, Chydorus* sp. and *Mesocylops australiensis*. At the fish densities tested, both *G. affinis* and *G. cotidianus* have the ability to significantly alter zooplankton assemblages through predation. There remain uncertainties about whether the same findings would occur if the fish were provided one or multiple different prey sources, with the addition of experimental habitat structure, or in sympatry.

2.5 Appendix

Appendix 2.1 Lengths (mm) and weights (g) of *G. affinis* and *G. cotidianus* used in the mesocosm experiment, and number of fish retrieved at the end of the experiment.

G. affinis				***G. cotidianus***			
Mesocosm number	N (fish retrieved)	Length (mm)	Weight (g)	Mesocosm number	N (fish retrieved	Length (mm)	Weight (g)
3	4	24	0.12	2	4	40	0.55
		22	0.10			42	0.72
		20	0.11			29	0.22
		26	0.16			35	0.41
5	4	22	0.08	9	4	40	0.56
		27	0.17			44	0.67
		33	0.32			36	0.38
		20	0.09			38	0.47
12	4	30	0.3	10	4	39	0.61
		26	0.1			40	0.78
		19	0.08			43	0.74
		30	0.24			39	0.56
13	4	18	0.07	11	4	36	0.49
		25	0.17			40	0.52
		20	0.16			37	0.5
		22	0.15			35	0.38
17	4	20	0.1	14	4	38	0.66
		22	0.11			39	0.6
		21	0.11			26	0.23
		19	0.09			35	0.41
18	4	20	0.08	15	4	36	0.59
		29	0.21			45	0.77
		21	0.1			34	0.49
		19	0.07			36	0.56
21	3	22	0.16	19	4	37	0.59
		26	0.17			33	0.39
		30	0.22			25	0.24
						39	0.56
Mean		**23.51**	**0.14**	**Mean**		**37.00**	**0.52**

Chapter 3. Seasonal variation in dietary overlap between the mosquitofish (*Gambusia affinis*) and common bully (*Gobiomorphus cotidianus*) using stable carbon and nitrogen isotopes in four Waikato, New Zealand lakes.

3.1 Introduction

The threats posed by non-native freshwater fish to ecosystems is a growing ecological and economic issue worldwide, heightened by land use intensification and the influence of climate change (Gozlan et al., 2010; Cucherousset & Olden, 2011). Threats from non-native fish species include direct interactions with native fish, such as predation or competition and also ecosystem-wide impacts that reach across trophic levels and alter food webs, biochemical cycles, and energy fluxes of freshwaters (Cucherousset & Olden, 2011). The western mosquitofish (*Gambusia affinis*) and eastern mosquitofish (*Gambusia holbrooki*) are two morphologically and ecologically similar small freshwater fish species belonging to the family Poeciliidae, which have wide-ranging negative impacts outside their native ranges (Pyke, 2008). Invasive *Gambusia* are highly adaptable to novel environments due in part to their physiological resilience to fluctuations and extremes in temperature (Swanson et al., 1996), dissolved oxygen (Odum & Caldwell, 1955) and salinity (Chervinski, 1983). Additionally, they are effective opportunistic omnivores, exploiting diverse food sources, including algae (Capps et al., 2009), detritus, zooplankton (Blanco et al., 2004), macroinvertebrates (Harmon & Smith, 2020), and the larvae of fish and amphibians (Mills et al., 2004; Zeiber et al., 2008). These traits and a high reproductive rate and typically aggressive temperament (Meffe, 1992; Warburton & Madden, 2003) make *Gambusia* strong competitors for food and space against similar-sized native fish. Deleterious competitive effects of *Gambusia* on small-bodied fish have been documented in studies from the USA (Galat & Robertson, 1992), the Iberian Peninsula in Europe (Ruiz-Navarro et al., 2013b), Australia (Howe et al., 1997), and New Zealand (Rowe et al., 2007).

Stable Isotope Analysis (SIA) is a time- and cost-effective method for investigating the long-term diet of fish and comparing the dietary overlap of co-occurring species. SIA is a timeintegrative alternative to gut content analysis that uses the ratio of stable isotope pairs associated with key life processes to conceptualise food webs and determine trophic levels (Ehleringer & Rundel, 1988). The isotope pairs most pertinent to freshwater trophic ecology are carbon ($^{12}C/^{13}C$) and nitrogen ($^{14}N/^{15}N$) (Grey, 2006). The ratio of the heavier isotope to the lighter isotope is expressed in relation to a constant and is referred to as $\delta^{13}C$ and $\delta^{15}N$, which varies among organisms due to isotopic discrimination occurring in enzymatic and physical processes (Marshall et al., 2007). For example, the concentration of $\delta^{13}C$ in the tissues of primary producers varies due to differences in photosynthetic pathways (Fry & Sherr, 1989) and the ^{13}C:^{12}C ratio gradually increases up the food chain due to the conservation of ^{13}C from prey to predator tissue. The trophic increase can be highly site- and ecosystem- specific, with average $\Delta\delta^{13}C$ increase per trophic level in freshwaters variously identified as +0.2‰ (France & Peters, 1997), +0.4‰ (Post, 2002) and +0.47±1.23‰ (Nawrocki et al., 2020). Therefore, one of the primary uses of $\delta^{13}C$ for freshwater ecology is to determine the energy sources of an organism or ecosystem rather than trophic position (Peterson & Fry, 1987). Benthic and planktonic primary production in lakes differs in $\delta^{13}C$ (France, 1995), so SIA can reveal the contribution of each to the diet of consumers. The enrichment of ^{15}N in the tissues of consumers tends to increase with each trophic level by 3.4‰ in freshwater ecosystems, which makes it a better indicator of trophic position (Nawrocki et al., 2020). When combined, $\delta^{13}C$ and $\delta^{15}N$ can be used to predict the diet of fish provided appropriate conditions are met, such as the collection of sufficient baseline data for the study location (Kjeldgaard et al., 2021). Baseline data is important due to the isotopic variation that can be present between the primary producers among freshwater sites, which is influenced by abiotic conditions as well as biological differences between species (Vander Zanden & Rasmussen, 1999). Likewise, sufficient sample size must be obtained to minimise individual variation, with five to ten replicates recommended per trophic group (Kjeldgaard et al., 2021). Numerous formulae and models are used for estimating trophic position, so studies must consider the most appropriate methodology for the research aim, and pair SIA with other techniques where possible (Kjeldgaard et al., 2021).

SIA research on the diet of *Gambusia* is extensive and includes invasion ecology case studies on a global scale, which provide insight into the dietary flexibility of these fish. *Gambusia* have been documented to incorporate highly variable proportions of primary producers in their diet, ranging from <10% of gut contents in New Zealand and Australia (Mansfield & McArdle, 1998; Remon et al., 2016) to 25%-37% in Europe (Blanco et al., 2004; Specziár, 2004), to 80%

in Hawaii (Capps et al., 2009). Additionally, terrestrial food sources can make up significant proportions of *Gambusia* diet, which can be isotopically distinct from dietary items derived from aquatic sources (Tsang & Dudgeon, 2021). This makes δ^{13}C analysis useful for differentiating between species that may occupy similar trophic positions but do not overlap in diet. For example, a mesocosm study in the USA by Rehage et al. (2020) found no significant difference in $\Delta\delta^{15}$N between invasive *G. affinis* and lower invasive potential conspecific *Gambusia geiseri*, yet variance in δ^{13}C indicated that their diet was sourced from different primary producers. SIA has been used to investigate the dietary overlap of *Gambusia* with native fish in the USA (Rehage et al., 2020), Japan (Sakano & Iguchi, 2009), Hong Kong (Tsang & Dudgeon, 2021) and Italy (Andolina et al., 2022). Still, there is a notable absence of similar studies centred around the native fish of New Zealand. New Zealand's freshwater ichthyofauna is characterised by high levels of endemicity and diadromy, which have contributed to an increased risk from introduced species and habitat loss (Allibone et al., 2010; McDowall, 2010). Therefore, the spread and proliferation of invaders such as *G. affinis* may pose a threat to a range of native fish, with evidence of deleterious effects of this species on black mudfish (*Neochanna diversus*) (Ling & Willis, 2005; McDonald, 2007) and inanga (*Galaxias maculatus*) (Rowe et al., 2007). Contrarily, Ling (2004) questioned the threat *G. affinis* poses to New Zealand's native fish due to the absence of a similar small, top-feeding carnivorous species that would overlap in niche. This is supported by evidence for the coexistence of *Gambusia* with small native Australian and New Zealand fish (Ling, 2004; Macdonald et al., 2012). The common bully (*Gobiomorphus cotidianus*) is the most widespread of the nine bully species in New Zealand and is an abundant facultatively diadromous fish inhabiting the benthos of lowland waterways (Stevens & Hicks, 2009; Thacker et al., 2021a). They are also resilient fish with a flexible diet and behaviour, frequently co-occurring with similar-sized native fish (Sagar & Glova, 1994) as well as with *G. affinis* (Hayes & Rutledge, 1991; Swales & West, 1991; Wu et al., 2013b; Collier et al., 2019). Dietary overlap, and therefore potential interspecific competition between co-occurring *G. affinis* and *G. cotidianus* is likely, as both species share seasonally significant prey items. Forsyth and James (1988) demonstrated that pelagic zooplankton provide an important winter food source to some *G. cotidianus* populations, and Stewart et al. (2017) found dietary contributions of zooplankton and benthic macroinvertebrates to vary seasonally with zooplankton abundance. Studies of *Gambusia* have also revealed seasonal variability in the importance of food sources such as conspecifics and amphibians (Remon et al., 2016) and phytoplankton, terrestrial insects, and zooplankton (Singh & Gupta, 2010). Baseline isotopic signatures of lakes fluctuate seasonally, with the greatest temporal changes seen in the δ13C and δ15N of particulate organic matter occurring in eutrophic lakes at higher latitudes (Gu, 2009; Gu et al., 2011). Baseline isotopic fluctuations influence the isotopic enrichment of higher trophic levels, such as fish. Therefore, understanding seasonal isotopic changes in *G. affinis*, *G. cotidianus*, and basal food web taxa from their habitats is important for investigating the presence of trophic overlap and interspecific competition, as the preferred food sources of each fish may align more closely during certain periods of the year.

The primary aim of my study was to investigate whether there was dietary overlap between *G. affinis* and *G. cotidianus* by comparing the similarity in isotopic enrichment of fish muscle tissue in summer and winter. Secondly, I aimed to assess the effects of season on the carbon and nitrogen isotopic enrichment of sympatric *G. affinis* and *G. cotidianus*, in addition to identifying any seasonal isotopic variation in basal food web taxa that are likely to be components of their diet. I selected two riverine lakes and two peat lakes in the Waikato Region of New Zealand as study sites, at which Collier et al. (2018) conducted research into dietary overlap between invasive brown bullhead catfish (*Ameiurus nebulosus*) and native shortfin eels (*Anguilla australis*) using SIA of white muscle and fin tissue samples. The Collier et al. (2018) study also collected stable isotope data on species at lower trophic levels, including both *G. affinis* and *G. cotidianus* and basal food web taxa, with samples collected during the austral spring and summer. My study aimed to collect stable isotope data of *G. affinis*, *G. cotidianus*, and basal food web taxa during the austral winter and compare the two datasets for seasonal differences. Winter sampling was selected due to the reduced rates of isotopic turnover in fish muscle tissue at this time (Busst & Britton, 2018), which, combined with estimated lag times in isotopic turnover (Colborne et al., 2017), means that samples are likely to integrate fish diet from the austral autumn to winter seasons. I hypothesised that there would be some isotopic overlap between *G. affinis* and *G. cotidianus* and that mean stable isotope values of both fish would be most similar in the winter due to an increased contribution of pelagic zooplankton to the diet of *G. cotidianus* as benthic food sources become less abundant. I also predicted that summer data would vary significantly in isotopic enrichment from the winter data due to seasonal differences in primary production and the food sources of the fish.

3.2 Methods

3.2.1 Study sites

The four lakes sampled by both Collier et al. (2018) and my study were Lake Rotomanuka, Lake Rotopiko (a.k.a. Serpentine North), Lake Okowhao and Lake Ohinewai, located in the Waikato Region of New Zealand’s North Island (Figure 3.1). Of these, the former two are peat lakes, with an age of approximately 20,000 years, while the latter two were formed by riverine processes and are about 2000 years old (Dean-Speirs et al., 2014; Lowe, 2014). While the lakes are all under 0.2 km^2 in surface area and situated no more than 30 km south and north of Hamilton, Waikato, they differ in several physical parameters, water quality, and the fish species present.

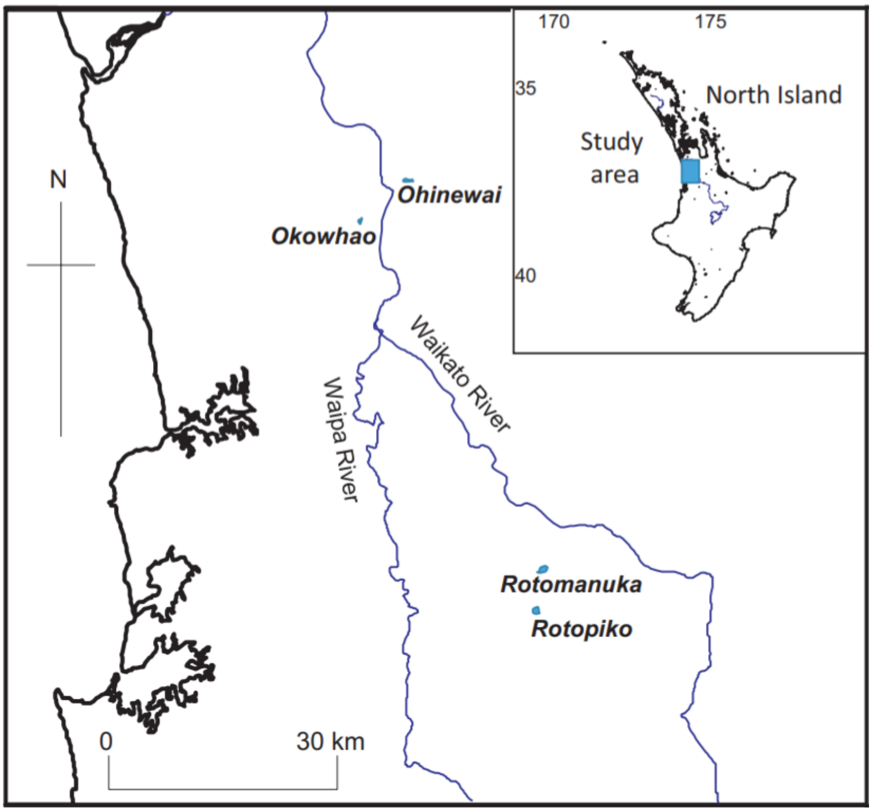

Figure 3.1. Map showing the locations of Lakes Rotomanuka, Rotopiko, Ohinewai and Okowhao, which were sites used in the collection of stable isotope samples by my study and by Collier et al. (2018). Figure adapted from Collier et al. (2018).

3.2.2 Lake Rotomanuka

Lake Rotomanuka is the largest and deepest site (Table 3.1) and has ‘Poor’ water quality (Collier et al., 2018), largely due to catchment land use consisting of 90% dairy and dry stock, and 10% urban (Lehmann et al., 2017). The high external nutrient load is compounded by connectivity to a southern lake remnant, which is hypertrophic and hydrologically connected to the Lake Rotopiko complex (Lehmann et al., 2017). Introduced fish species found at Lake Rotomanuka include brown bullhead catfish (*Ameiurus nebulosus*), goldfish (*Carassius auratus*), rudd (*Scardinius erythrophthalmus*) and *G. affinis,* whereas native fish species are dominated by shortfin and longfin eels (*Anguilla australis*, *A. dieffenbachi*), *G. cotidianus,* and common smelt (*Retropinna retropinna*) (Lehmann et al., 2017; Collier et al., 2018). Chironomid larvae are abundant throughout most of the year, though oligochaetes were found at lower densities than Lake Rotopiko (Collier et al., 2019). The most abundant zooplankton include calanoid copepods such as *Calamoecia lucasi* and *Boeckella delicata*, with cladocerans and cyclopoid copepods also present (Edgar & Green, 1994).

3.2.3 Lake Rotopiko

Lake Rotopiko is the smallest study site (Table 3.1) and has a largely pastoral catchment. Key nutrient inputs include groundwater and surface runoff, while an outflow runs into Lake Serpentine South (Wu et al., 2013a). Native fish biomass per hectare is 23 times greater than non-native fish in the lake, with native biomass dominated by shortfin eels and introduced fish biomass primarily consisting of brown bullhead catfish (Wu et al., 2013a). Other fish species found at Lake Rotopiko over the last 20 years include *G. cotidianus*, *G. affinis,* common smelt, goldfish and rudd (GBIF.org, 2022). The lake has abundant invertebrates, including chironomid larvae and oligochaetes, with Collier et al. (2019) finding the highest total invertebrate density out of the four lakes investigated in this study. Surrounding vegetation includes wetlands composed of sedges and rushes, as well as woody vegetation (Thompson & Champion, 1993), while natural macrophyte beds in the lake are mostly intact (Edwards et al., 2010), contributing to one of the lowest levels of Total Suspended Solids (TSS) of the four sites (Table 3.1).

3.2.4 Lake Okowhao

Lake Okowhao is a small riverine lake in a catchment dominated by agricultural land use. The lake is fully fenced and surrounded by a band of native and non-native riparian vegetation, though inputs include direct flow from farm drains in the south in addition to surface runoff (Dean-Speirs et al., 2014). Lake outflows include culverts running towards the Waikato River; however, flap gates are present to prevent water from returning to the lake. This also prevents native fish species other than eels and *G. cotidianus* from entering from the river, leading to a high proportion of non-migratory non-native fish such as koi carp, goldfish, catfish and *G. affinis* (Dean-Speirs et al., 2014). Non-native species, along with excess nutrient inputs and the loss of submerged macrophyte beds (Edwards et al., 2005), have contributed to a degradation in water quality since measurements commenced in 1981 (Dean-Speirs et al., 2014).

3.2.5 Lake Ohinewai

Lake Ohinewai is a hypertrophic lake currently dominated by algae, which followed a near-complete loss of aquatic macrophytes throughout the 1980s (Edwards et al., 2005). Pest fish such as koi carp were found to disturb the substrate, partially contributing to the high nutrient loads present in the lake (Allan, 2016). The high Trophic Level Index (TLI) is often reflected in summer cyanobacteria blooms (Daniel & Morgan, 2011), and TSS are high compared to the peat lakes (Table 3.1). Fish biomass and abundance are dominated by non-native species such as *G. affinis*, goldfish, koi carp, catfish, and goldfish-koi hybrids, while native fish are dominated by shortfin eels and *G. cotidianus* (Hicks et al., 2015). Other species include snails, chironomid larvae and oligochaetes, though the latter two are less abundant than at Lake Rotomanuka and Lake Rotopiko (Collier et al., 2019).

Table 3.1 Physical, hydrological and water quality characteristics at the four study lakes, using data adapted from Edgar and Green (1994), Dean-Speirs et al. (2014), Collier et al. (2019) and Allan (2017).

Lake	**Lake type**	**Surface area (km^2)**	**Maximum depth (m)**	**Mixing regime**	**Trophic state (TLI)**	**Total suspended solids (mg L^{-1})**
Rotomanuka	Peat	0.17	8.7	Monomictic	Eutrophic (4.7)	8.75
Rotopiko	Peat	0.05	4.0	Polymictic	Eutrophic (4.9)	9.47
Okowhao	Riverine	0.12	2.2	Polymictic	Supertrophic (6.2)	24.10
Ohinewai	Riverine	0.16	4.5	Polymictic	Hypertrophic (6.3)	23.65

3.2.6 Sample collection

Lakes were sampled over the austral winter of 2022 for fish, chironomid larvae, aquatic snails, and zooplankton. Lake Rotomanuka was visited on 4 July 2022 and 5 July 2022, and ten *G. cotidianus* and ten *G. affinis* were caught using a combination of sweeping shallow water with a hand net, and Gee-minnow and collapsible minnow traps. Fifty aquatic snails (*Potamopyrgus antipodarum*) were caught by searching the underside of submerged vegetation, wood, and debris. Zooplankton were collected using a 100 µm mesh plankton net by performing 15 hauls of approximately 5 m from a dinghy, replicated three times. A Ponar grab sampler was used to sample sediment from several points on the lake for chironomid larvae, but only five small larvae were obtained from twelve hauls, so these were discarded. Lake Rotopiko was visited on 4 July 2022, 5 July 2022, 18 July 2022, and 19 July 2022. Using the same equipment and methods mentioned above, 10 *G. cotidianus* and 10 *G. affinis* were collected, in addition to 15 snails, 30 chironomid larvae, and 3 samples of zooplankton collected through 15 hauls each. Lake Okowhao was visited on 21 July 2022 and 22 July 2022, and 10 *G. cotidianus*, 10 *G. affinis*, 15 snails, and 3 samples of zooplankton via 15 hauls each were collected, while only one chironomid larva was obtained from 6 Ponar samples, so this was discarded. Lake Ohinewai was visited on 29 August 2022 and 30 August 2022, and 10 *G. cotidianus*, 10 *G. affinis*, 10 chironomid larvae, 3 samples of zooplankton via 15 hauls each, and 12 snails were collected.

3.2.7 Sample processing

Fish, snail, zooplankton, and chironomid larvae samples were chilled in sample containers over ice immediately upon capture and were kept chilled until they were processed on the same day. *Gobiomorphus cotidianus* were measured, then filleted and skinned to obtain a white muscle tissue sample, which was placed in individual sample tubes. *Gambusia affinis* were also measured, but were too small to fillet, so were prepared by removing the head, internal organs, and fins, with the remainder then placed in individual sample tubes. Snails were extracted from their shells and pooled in groups of three per sample tube. Zooplankton samples were briefly observed under a microscope, then filtered through a 250 µm mesh to collect a concentrated sample of crustacean zooplankton, which were distributed into 3-5 sample tubes. Chironomid larvae were rinsed using Milli-Q water and then placed in groups of five per sample tube. All sample tubes were placed into an oven set to 60°C for 24 hours to dry. Once dry, samples were ground using a mortar and pestle, and then 1.500 mg (± 0.200 mg) of powder was measured into one tin capsule per sample. The tin capsules were then sealed by folding and sent to the University of California, Berkeley Centre for Stable Isotope Biogeochemistry for analysis. Samples were combusted in a IsoPrime100 mass spectrometer (Isoprime Ltd) calibrated against the standard reference materials of the Pee Dee Belemnite for C, and atmospheric N_2 for N. Stable isotope ratios were expressed as delta (δ) of parts per thousand (‰).

3.2.8 Statistical analysis

Independent sample t-tests with equal/unequal variance estimates were conducted to determine whether there were significant seasonal differences in $\delta^{13}C$ or $\delta^{15}N$ between each sample taxon at each lake. These were repeated to compare $\delta^{13}C$ and $\delta^{15}N$ between *G. affinis* and *G. cotidianus* in the same season. Two-way factorial ANOVA were performed to investigate the impact of season and lake on $\delta^{13}C$ and $\delta^{15}N$ values of *G. affinis* and *G. cotidianus*. Dependent variables were $\delta^{13}C$ or $\delta^{15}N$, and categorical factors were lake and season. Results were considered significant where $p<0.05$. Where ANOVA indicated significant results, these were followed by Tukey's *post-hoc* tests. Following initial analysis, the summer dataset was censored to assess the influence of the wider fish size distribution of the summer data on the statistical analyses and inter-season comparisons. All *G. affinis* and *G. cotidianus* $\delta^{13}C$ and $\delta^{15}N$ data sourced from summer samples that fell outside of the length range of the same species at the same lake in the winter data was removed, and all statistical analyses were then repeated with this modified dataset. Statistical analyses were conducted in STATISTICA (v.14.0.0.15 TIBCO Software Inc.; Palo Alto, CA, USA).

3.3 Results

Fish collected during the winter had significantly greater mean lengths than fish collected by Collier et al. (2018) during spring and summer at Lakes Rotopiko, Okowhao, and Ohinewai (Table 3.2). Censoring of the summer dataset to remove data that fell outside the winter fish length distributions increased the mean lengths and decreased standard deviations of both species at all lakes. Within the censored summer data, the mean lengths of *G. cotidianus* at Lake Rotopiko remained significantly shorter compared to the winter data. The greatest changes to the censored summer data were at Lake Ohinewai, where the stable isotope values of 43 *G. cotidianus* and 23 *G. affinis* were removed and at Lake Okowhao where 36 *G. cotidianus* and 24 *G. affinis* were removed. The majority of the fish removed from the summer dataset were smaller than the minimum lengths recorded in the winter data.

Table 3.2 Minimum, maximum and mean fish lengths in mm (SD) at each lake, in summer (a) and winter (b). ‘N (a) censored’ is the number of fish in the summer data that fall within the length range of the winter data, and ‘Mean length (a) censored’ is the average length and SD of these fish. Significant differences (p< 0.05) between ‘(a)’ and ‘(b)’, and ‘(a) censored’ and ‘(b)’ are shown with *.

Lake	**Sample taxa**	**N (a)**	**N (a) censored**	**N (b)**	**Min length (a)**	**Min length (b)**	**Max length (a)**	**Max length (b)**	**Mean length (a)**	**Mean length (a) censored**	**Mean length (b)**
Rotomanuka	*G. cotidianus*	64	42	10	14	30	73	60	35.9 (13.32)	41.4 (7.52)	42.9 (10.60)
	G. affinis	42	40	10	20	20	38	34	20.5 (4.53)	27.6 (2.83)	28.5 (4.20)
Rotopiko	*G. cotidianus*	44	38	10	22	35	71	71	41.5 (8.96) *	43.3 (8.17) *	52.5 (10.88)
	G. affinis	44	12	10	9	28	39	35	25.2 (5.71) *	30.4 (2.07)	31.9 (2.47)
Okowhao	*G. cotidianus*	46	10	10	16	45	58	56	38.1 (9.89) *	48.1 (3.24)	50.3 (3.56)
	G. affinis	36	12	10	15	24	36	31	23.6 (5.28) *	27.0 (2.63)	26.9 (2.88)
Ohinewai	*G. cotidianus*	65	22	10	14	40	50	46	32.4 (10.54) *	42.6 (1.82)	43.3 (2.21)
	G. affinis	47	24	10	6	25	38	36	24.3 (7.97) *	29.6 (3.24)	30.3 (3.27)

The censored mean $\delta^{13}C$ and $\delta^{15}N$ values for *G. cotidianus* and *G. affinis* showed little within-season overlap, and *G. affinis* was more enriched in both isotopes at every site except $\delta^{13}C$ at Lake Okowhao in the winter (Figure 3.2). The $\delta^{13}C$ values were more similar between fish species in the winter, and only differed significantly at Lake Ohinewai ($p=0.02$) and Lake Okowhao ($p<0.01$). In the summer $\delta^{13}C$ data, significant differences were found between the means of both species at Lakes Rotomanuka, Rotopiko and Ohinewai ($p<0.01$) but not Lake Okowhao ($p=0.28$). The within-lake and within-season $\delta^{13}C$ variation between *G. cotidianus* and *G. affinis* was greatest at Lake Rotopiko in the winter (2.77‰) and lowest at Lake Okowhao in the summer (0.41‰). The $\delta^{15}N$ values were significantly different ($p<0.01$) at every lake in the winter and at Lakes Rotomanuka, Rotopiko, and Okowhao in the summer. No significant difference was found between fish in the Lake Ohinewai summer data ($p=0.54$). The within-lake and within-season $\delta^{15}N$ variation between *G. cotidianus* and *G. affinis* was greatest at Lake Rotomanuka in the winter (3.41‰) and lowest at Lake Ohinewai in the summer (0.13‰).

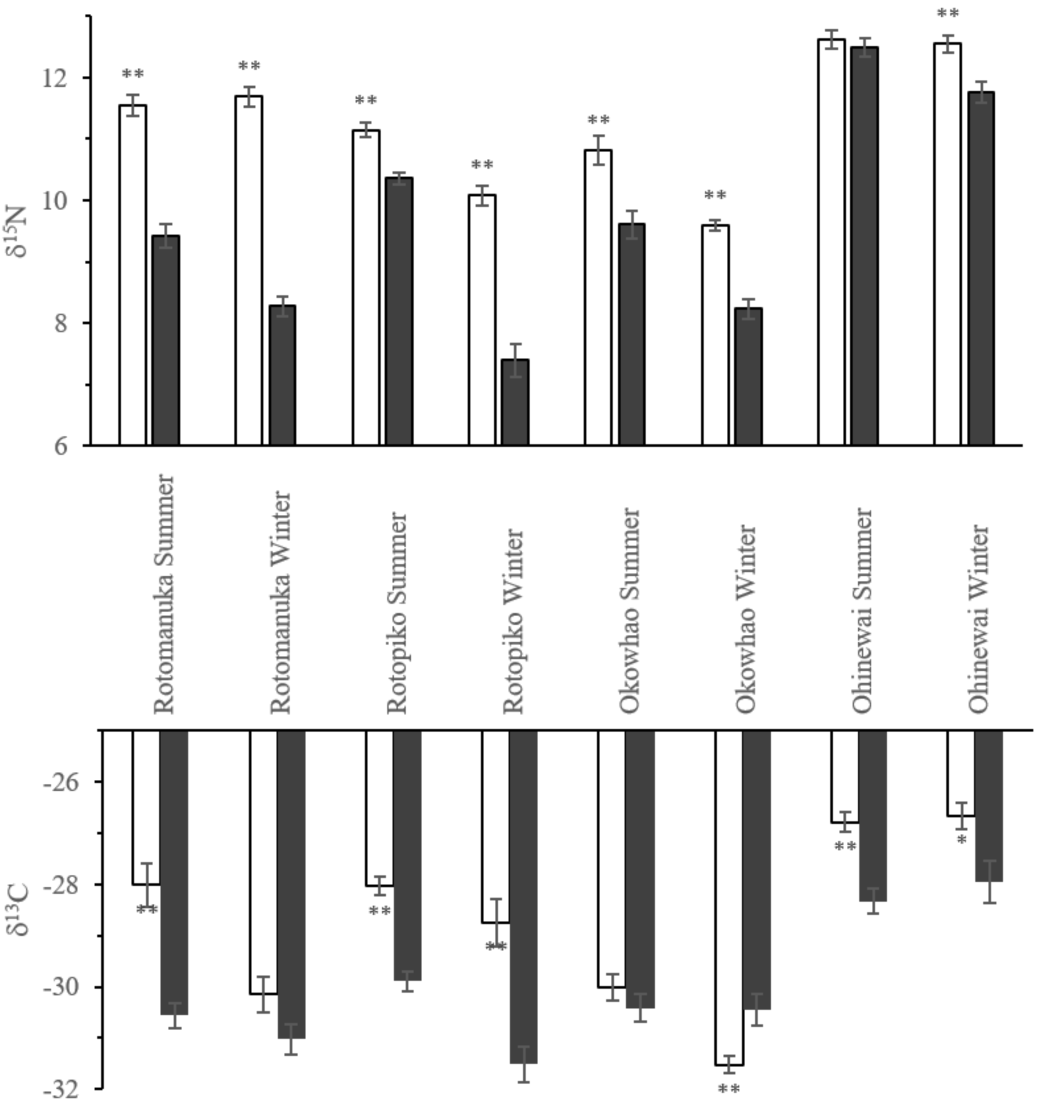

Figure 3.2 Mean (±SEM) $\delta^{15}N$ and $\delta^{13}C$ values of *G. affinis* (white bars) and *G. cotidianus* (grey bars) at each lake in each season. The summer data is censored to only include fish that fall within the length distribution of the winter fish data. Significant differences between the mean stable isotope values of *G. affinis* and *G. cotidianus* are indicated with (*) for p<0.05 and (**) for p<0.01.

The mean isotopic enrichment of each fish species at each lake did not differ significantly between the original and the censored data (Figure 3.3). The greatest changes to $\delta^{13}C$ in *G. affinis* were at Lake Ohinewai (+0.34‰), and greatest changes to $\delta^{15}N$ were at Lake Okowhao (+0.30‰). For *G. cotidianus*, the greatest shift in $\delta^{13}C$ in the censored data was at Lake Rotomanuka (-0.79‰) and the greatest change in $\delta^{15}N$ was at Lake Ohinewai (-0.04‰).

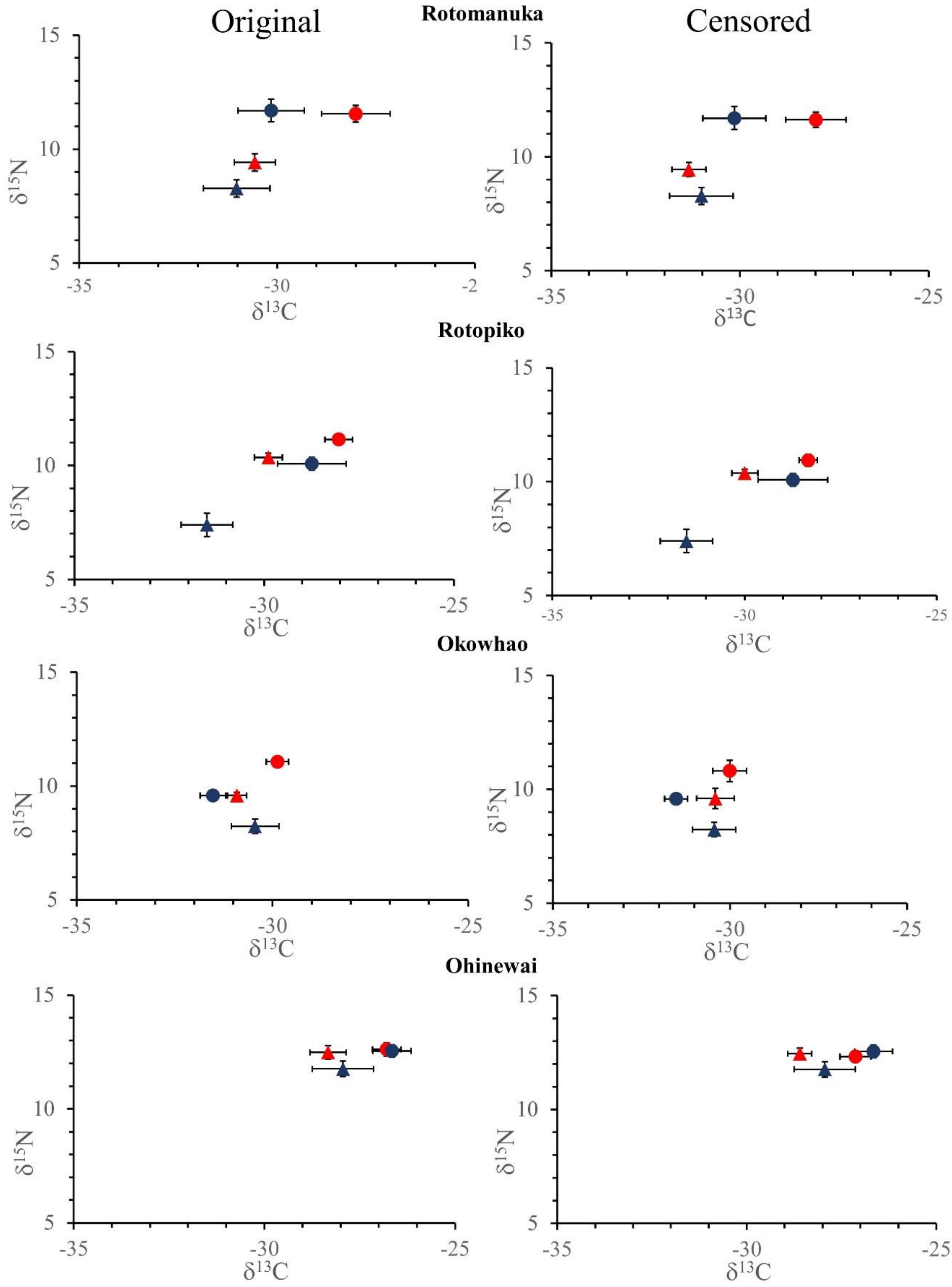

Figure 3.3 Dual isotope plots showing mean values for *G. cotidianus* (triangles) and *G. affinis* (circles) in the summer data (red) and winter data (blue). The error bars represent 95% confidence intervals. The summer data in the censored column is adjusted to only include fish that fall within the length distribution of the winter fish data.

Inter-lake comparison of mean isotopic enrichment of *G. affinis* and *G. cotidianus* indicated that fish from Lake Ohinewai had the greatest enrichment in δ^{13}C and δ^{15}N in both seasons, while the mean stable isotope values for fish from other lakes showed more variability (Figure 3.4). The greatest within-species seasonal difference in δ^{13}C was in Lake Rotopiko *G. cotidianus* (2.8‰), and the greatest difference in δ^{15}N was found in Lake Rotomanuka *G. cotidianus* (3.4‰). Stable isotope values of *G. cotidianus* and *G. affinis* varied most greatly at Lake Rotopiko in winter and were most similar at Lake Ohinewai in summer.

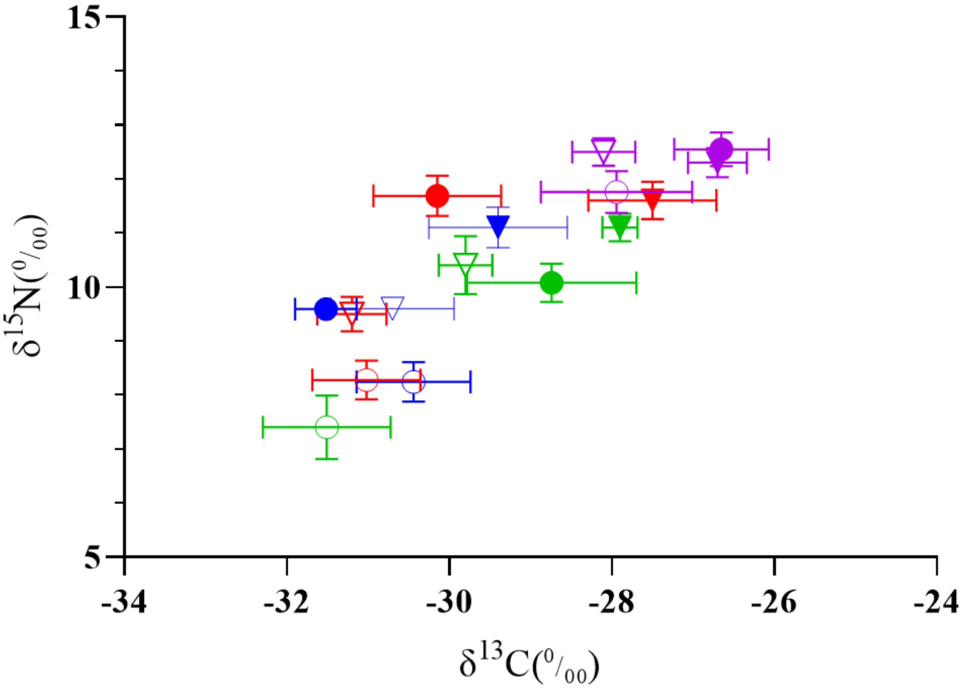

Figure 3.4 Dual isotope plot of mean *G. cotidianus* (open symbols) and *G. affinis* (closed symbols) δ^{13}C and δ^{15}N values (‰) at Lakes Rotomanuka (red), Rotopiko (green), Okowhao (blue) and Ohinewai (purple) during summer (triangles) and winter (circles). The summer fish data is not censored to match the length distributions of the winter fish data.

Across both fish species and stable isotopes, seasonal variation was lowest at Lake Ohinewai (Figure 3.5). The two-way factorial ANOVA indicated a statistically significant interaction between the effect of lake and season on δ^{13}C ($p=0.03$) and δ^{15}N ($p<0.01$) values of *G. cotidianus* but no significant difference between seasons for δ^{13}C ($p=0.96$; $F=0.00$). The δ^{13}C values of *G. cotidianus* were not significantly different between seasons at Lake Ohinewai ($p=0.83$), Lake Okowhao ($p=0.97$) and Lake Rotomanuka ($p=0.99$) but were significantly different at Lake Rotopiko (p=0.03) (Figure 3.5A). The seasonal $\delta^{15}N$ values of *G. cotidianus* were most similar at Lake Ohinewai (p=0.31) and differed significantly at all other lakes (Figure 3.5B). Both lake (p<0.01; F=111.50) and season (p<0.01; F=101.3) contributed to significant differences between $\delta^{15}N$ data points. The ANOVA of *G. affinis* results indicated a significant interaction existed between the effect of lake and season on the $\delta^{13}C$ (p<0.01; F=6.91) and $\delta^{15}N$ (p=0.02; F=5.03) values. There were significant contributions of both lake (p< 0.01; F=42.97) and season (p=0.01; F=11.42) to the variance of $\delta^{13}C$ values of *G. affinis* (Figure 3.5C). A significant seasonal difference in $\delta^{13}C$ was found at Lake Okowhao (p=0.05) and Lake Rotomanuka (p<0.01) but not at Lake Ohinewai (p=0.98) and Lake Rotopiko (p=0.99). The effect of lake (p<0.01) and season (p=0.01) was significant in the variance of $\delta^{15}N$ in *G. affinis* samples (Figure 3.5D). Seasonal differences in $\delta^{15}N$ were significant at Lake Okowhao (p<0.01) and Lake Rotopiko (p=0.04) but were not present between other lakes.

The censored summer data did not result in major changes to the ANOVA results for *G. cotidianus* but influenced the ANOVA results for *G. affinis*. The interaction between lake and season, and the effect of lake individually remained significant in variation of the *G. cotidianus* $\delta^{13}C$ and $\delta^{15}N$ data (p<0.01). Significant seasonal variation in $\delta^{13}C$ remained restricted to Lake Rotopiko (p<0.01), and seasonal variation in $\delta^{15}N$ remained significant at all lakes but Lake Ohinewai (p=0.32). The censoring of data for *G. affinis* reduced the significance of the interaction of lake and season on $\delta^{13}C$ (p=0.09), though interaction effects remained significant for lake and season for $\delta^{13}C$, and all tests for $\delta^{13}C$. Significant seasonal variation in $\delta^{13}C$ values of *G. affinis* was now found at Lake Okowhao (p=0.05) as well as Lake Rotomanuka (p<0.01). Changes to $\delta^{15}N$ results included an absence of significant seasonal variation at Lake Rotopiko (p=0.08), while Lake Okowhao remained significantly different between summer and winter (p<0.01).

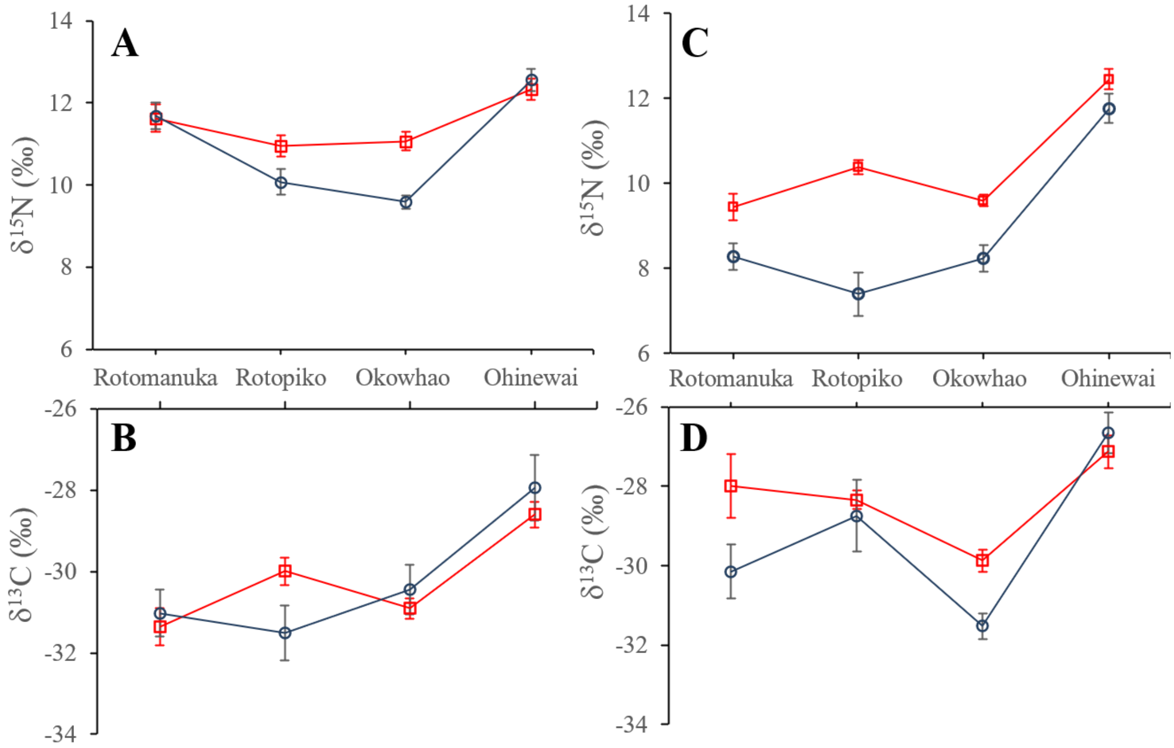

Figure 3.5 Isotope plots comparing seasonal $\delta^{13}C$ and $\delta^{15}N$ data of both fish at each lake. 'A' and 'B' are *G. affinis*, and 'C' and 'D' are *G. cotidianus*. Square markers with a red line indicate summer, and the round markers with a blue line indicate winter. Error bars represent 95% confidence intervals.

Significant differences were found between the seasonal mean $\delta^{13}C$ or $\delta^{15}N$ values of fish as well as the basal food web taxa at each study lake (Table 3.3). These differences followed a general trend of isotopic enrichment in $\delta^{13}C$ and $\delta^{15}N$ within the summer samples. Seasonal samples of *G. cotidianus* were significantly different at only Lake Rotopiko, where the summer data was enriched in $\delta^{13}C$ ($p<0.001$; $F=1.10$) and $\delta^{15}N$ ($p<0.001$; $F=0.43$) (Table 3.3). For *G. affinis*, Lake Okowhao was the only site where significant enrichment was found in seasonal means of $\delta^{13}C$ ($p<0.001$; $F=2.71$) and $\delta^{15}N$ ($p<0.001$; $F=0.15$), both of which occurred in the summer. Out of the eight lake-grouped fish samples, both standard deviation and variance was higher in the summer $\delta^{13}C$ data for six samples. For $\delta^{15}N$, the summer data also had higher standard deviations for six samples, and greater variance for six samples (Table 3.3). The censored data followed a similar trend: six of the eight summer lake-grouped fish samples had higher standard deviations, and six had greater variance.

Summer stable isotope values of Mollusca were significantly higher in $\delta^{13}C$ at Lake Rotomanuka ($p=0.05$; $F=64.33$), and in both $\delta^{13}C$ ($p<0.01$; $F=0.46$) and $\delta^{15}N$ ($p<0.01$; $F=0.48$) at Lake Ohinewai (Table 3.3). Zooplankton summer samples were significantly enriched in $\delta^{13}C$ at Lake Okowhao ($p<0.01$; $F=1.14$) and Lake Ohinewai ($p<0.01$; $F=0.75$) and in $\delta^{15}N$ at Lake Okowhao ($p<0.01$; $F=0.66$), and Lake Ohinewai ($p=0.01$; $F=4.66$). In the winter data, zooplankton samples at Lake Rotopiko were significantly enriched in $\delta^{15}N$ ($p<0.01$; $F=8.00$). Mean stable isotope values for Chironomidae were only significantly different at Lake Rotopiko for $\delta^{15}N$ ($p=0.008$; $F=0.08$), where the summer data was more enriched.

Table 3.3 Mean (SD) δ^{15}N and δ^{13}C values of the summer data collected by Collier et al. (2018) (a) compared to the mean (SD) δ^{15}N and δ^{13}C values of the winter data (b). Columns marked '(a) censored' are summer data that has been adjusted to match the fish length distributions of the winter dataset. Data is not corrected for tissue type or lipids. Significant differences between '(a)' and '(b)', and '(a) censored' and '(b)' ($p < 0.05$) are shown with *****.

Lake	**Sample taxa**	**N (a)**	**N (a) censored**	**N (b)**	**Mean δ^{13}C (‰) (a)**	**Mean δ^{13}C (‰) (a) censored**	**Mean δ^{13}C (‰) (b)**	**Mean δ^{15}N (‰) (a)**	**Mean δ^{15}N (‰) (a) censored**	**Mean δ^{15}N (‰) (b)**
Rotomanuka	*G. cotidianus*	64	42	10	-31.4 (1.80)	-30.6 (1.63)	-31.0 (0.93)	9.5 (1.27) *****	9.4 (1.20) *****	8.3 (0.51)
	G. affinis	42	40	10	-28.0 (2.64) *****	-28.0 (2.65) *****	-30.1 (1.10)	11.6 (1.11)	11.6 (1.12)	11.7 (0.53)
	Mollusca	9		5	-29.0 (2.85) *****		-31.8 (0.36)	7.1 (1.17)		7.9 (2.14)
	Zooplankton	7		3	-29.6 (0.94)		-33.0 (0.36)	5.9 (1.53)		5.62 (0.10)
Rotopiko	*G. cotidianus*	44	38	10	-30.0 (1.15) *****	-29.9 (1.16) *****	-31.5 (1.10)	10.4 (0.54) *****	10.4 (0.58) *****	7.4 (0.83)
	G. affinis	44	12	10	-28.3 (0.85)	-28.0 (0.64)	-28.7 (1.46)	10.9 (0.93) *****	11.1 (0.39) *****	10.1 (0.50)
	Chironomidae	9		2	-31.2 (2.62)		-33.6 (0.08)	6.6 (1.32) *****		5.3 (0.02)
	Mollusca	6		5	-29.5 (3.12)		-31.5 (0.68)	6.2 (1.57)		6.1 (0.63)
	Zooplankton	3		3	-30.8 (3.50)		-37.7 (0.30)	4.9 (1.23) *****		10.2 (0.43)
Okowhao	*G. cotidianus*	46	10	10	-30.91 (0.85)	-30.4 (0.86)	-30.4 (0.98)	9.59 (0.47) *****	9.6 (0.72) *****	8.2 (0.51)
	G. affinis	36	12	10	-29.9 (0.87) *****	-30.0 (0.84) *****	-31.5 (0.53)	11.1 (0.70) *****	10.8 (0.82) *****	9.6 (0.27)
	Mollusca	6		3	-30.2 (2.37)		-31.8 (1.24)	5.8 (0.59)		4.9 (0.72)
	Zooplankton	7		4	-32.3 (0.98) *****		-34.9 (0.92)	5.6 (0.48) *****		3.89 (0.59)
Ohinewai	*G. cotidianus*	65	22	10	-28.6 (1.31)	-28.3 (1.14)	-27.9 (1.30)	12.5 (1.04) *****	12.5 (0.69) *****	11.8 (0.54)
	G. affinis	47	24	10	-27.1 (1.44)	-26.8 (0.93)	-26.7 (0.81)	12.3 (0.92)	12.6 (0.74)	12.3 (0.91)
	Chironomidae	3		2	-30.2 (1.29)		-30.3 (2.50)	7.60 (0.07)		9.0 (0.24)
	Mollusca	4		3	-28.0 (0.47) *****		-30.5 (0.61)	10.2 (0.36) *****		8.8 (0.52)
	Zooplankton	6		3	-29.9 (1.36) *****		-35.9 (1.57)	8.4 (2.02) *****		5.1 (0.94)

Dual isotope plots of mean values for fish and basal food web taxa showed seasonal variation in both $\delta^{13}C$ and $\delta^{15}N$ (Figure 3.6). Chironomidae, Mollusca, and zooplankton were more enriched in $\delta^{13}C$ in the summer samples, and data points are more closely clustered than in winter. *Gambusia affinis* were more enriched in both stable isotopes than *G. cotidianus* in all lakes in both seasons, aside from the Lake Ohinewai summer data, where *G. cotidianus* was slightly more enriched in $\delta^{15}N$ (Figure 3.6). The error bars for the fish data are consistently low across lakes and seasons, while data for Chironomidae, Mollusca, and zooplankton shows greater variability. While these basal food web taxa were generally clustered together in most plots, zooplankton data for Lake Rotopiko in the winter shows a distinctly high $\delta^{15}N$ value of 10.1‰, representing the highest mean $\delta^{15}N$ out of all taxa in the lake (Figure 3.6).

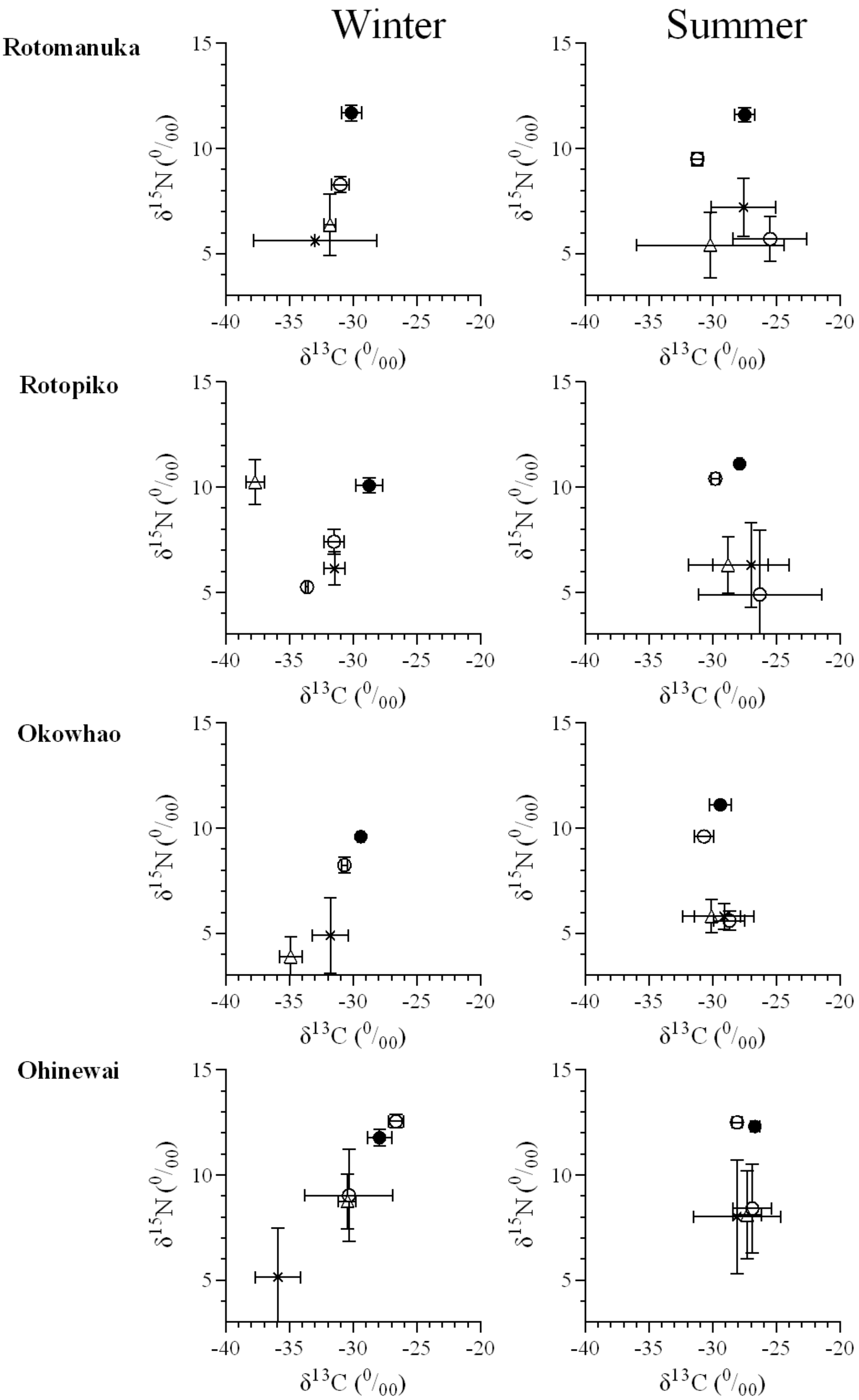

Figure 3.6 Dual isotope plots for mean $\delta^{13}C$ and $\delta^{15}N$ values (‰) of *G. affinis* (filled circles), *G. cotidianus* (open circles), Mollusca (open triangles), zooplankton (asterisks) and Chironomidae (open diamonds) in summer and winter at Lakes Rotomanuka, Rotopiko, Ohinewai and Okowhao. The summer fish data is not censored to match length distributions of the winter fish data.

3.4 Discussion

The results showed consistently greater isotopic enrichment in *G. affinis* compared to *G. cotidianus* across most lakes and both seasons, and these differences were significant at three lakes in the summer and all four lakes in the winter. This indicates that dietary overlap between the two fish species is likely minimal, particularly during winter. Additionally, elevated mean $\delta^{13}C$ and $\delta^{15}N$ values were found during summer throughout the sampled invertebrate taxa. Comparison of mean stable isotope values also revealed significant differences in $\delta^{13}C$ and $\delta^{15}N$ between the four lakes for both fish and invertebrates (Table 3.3, Figure 3.6). Seasonal variation was greater at some sites and within some taxa, suggesting that there are differences present among lakes in isotopic fractionation, trophic interactions, and nutrient and energy sources. Variation in $\delta^{15}N$ is indicative of trophic position due to an increase of approximately +3.4‰ occurring from prey to consumer in aquatic food webs (Vander Zanden & Rasmussen, 2001). However, seasonal change in $\delta^{15}N$ can have several causal factors, such as starvation (Bowes et al., 2014), a shift in diet, and changes to the $\delta^{15}N$ of existing food sources due to environmental factors (Lau et al., 2009) or external inputs (Wen et al., 2010). Likewise, there were differences in seasonal mean $\delta^{13}C$ among sites and species, which can also be a product of several interacting factors and processes. Although $\delta^{13}C$ trophic discrimination is relatively minor (Peterson & Fry, 1987), $\Delta\delta^{13}C$ can vary with trophic group, isotopic routing (Vander Zanden & Rasmussen, 2001), temporal nutrient dynamics, and changes to primary production (Taipale et al., 2008).

3.4.1 Seasonal dietary variation of *G. affinis* and *G. cotidianus*

3.4.1.1 Isotopic overlap between *G. cotidianus* and *G. affinis*

Comparison of stable isotope values within the same ecosystem can provide insight into the similarity of the diet and trophic position of two or more species. However, accurate comparisons require a large quantity of baseline data to discern the relative contribution of $\delta^{13}C$ and $\delta^{15}N$ sources (Post, 2002). Assessment of summer and winter $\delta^{13}C$ and $\delta^{15}N$ values of *G. cotidianus* and *G. affinis* suggests that dietary overlap at most of the four lakes is minor or absent. The significant differences in the $\delta^{15}N$ values of *G. cotidianus* and *G. affinis* indicate that adult *G. affinis* feed on more $\delta^{15}N$ enriched prey sources at all lakes in both seasons, except for Lake Ohinewai. The $\Delta\delta^{15}N$ between *G. cotidianus* and

G. affinis was >3‰ at Lake Rotomanuka in the winter, which suggests that the fish may be feeding at adjacent trophic levels at this site, though it is highly unlikely that predation is occurring. However, $\Delta\delta^{15}N$ was lower at other lakes, which means that it is likely there are some shared prey sources. Contrary to expectations of increased zooplanktivory in both species in the winter, the summer $\delta^{15}N$ data showed less interspecific variation and thus greater potential dietary overlap. Crustacean zooplankton are one of the most likely prey items consumed by both *G. affinis* and *G. cotidianus* but tend to only become significant food sources of *G. cotidianus* when zooplankton increase in abundance relative to the preferentially targeted chironomid larvae (Sagar & Eldon, 1983; Forsyth & James, 1988). Likewise, *G. cotidianus* display dietary flexibility when the availability of zooplankton is reduced (Mitchell, 1986), which also makes it unlikely that there is strong competition for this food resource. Stephens (1978) found that the winter diet of *G. cotidianus* in eutrophic Lake Waahi did not contain significant contributions of zooplankton and rather consisted of terrestrial earthworms, gastropods, and mysid shrimp (*Tenagomysis chiltoni*). This range of potential winter dietary sources unique to *G. cotidianus* may have contributed to the absence of winter isotopic overlap between fish species at the study sites. The potential summer dietary overlap between fish at Lakes Rotopiko, Okowhao and Ohinewai may be in chironomid larvae. Although not a benthic fish, *Gambusia* have been found to consume the eggs, larvae, and adults of Chironomidae (Cabral et al., 1998). Chironomid larvae have been found to comprise up to 21% of the spring diet of small-intermediate sized *G. affinis* in some New Zealand lakes, though this value can be much lower at other lakes (Mansfield & McArdle, 1998). Predation on Chironomidae generally peaks during oviposition and emergence of adults (Bay & Anderson, 1966; Merkley et al., 2015). Therefore, even if the two fish species are not directly competing for chironomid larval prey, the similarity in the summer $\delta^{15}N$ values could be explained by the predation of *G. affinis* on other life stages of Chironomidae. Of the Mollusca samples, snails such as *P. antipodarum* are an important prey source of many *G. cotidianus* populations (Rowe et al., 2001; Holomuzki & Biggs, 2006), particularly during winter (Stephens, 1978). Snail predation by *G. affinis* is documented (Sulieman et al., 2016) though less common, and primarily hatchling snails and eggs are consumed (Acra et al., 1986; McCarthy & Fisher, 2000). Mollusca were, on average, between 0.4‰ to 4.2‰ depleted in $\delta^{15}N$ compared to *G. cotidianus* and 2.1‰ to 5.3‰ depleted in $\delta^{15}N$ compared to *G. affinis*. Therefore, it remains a possible that aquatic snails are also a food resource competed for in some or all study lakes.

3.4.1.2 *Gambusia affinis*

The mean summer δ^{13}C of *G. affinis* was significantly higher at Lakes Rotomanuka and Okowhao than in the winter, while no significant difference was present elsewhere. This suggests that the basal food sources differ seasonally at these sites, or the δ^{13}C of the same basal food sources vary seasonally. *Gambusia* are globally widespread and follow a generalist opportunistic feeding strategy (Pen & Potter, 1991; Pyke, 2005), thus their preferred prey and isotopic signature varies greatly by location. For example, *Gambusia* diet has been found to range from largely herbivorous (Specziár, 2004; Capps et al., 2009), to completely carnivorous, feeding on invertebrates, conspecifics, and amphibians (Remon et al. (2016). There is evidence for δ^{13}C depletion in *Gambusia* during autumn and winter (Fadda et al., 2016; Remon et al., 2016) and seasonal changes to the contribution of different basal organic carbon sources to their biomass (Hladyz et al., 2012). However, the isotopic turnover rate in fish muscle can be variable, with increased turnover associated with periods of growth (Vander Zanden et al., 1998) and tissue replacement (Weidel et al., 2011). The decreases in δ^{13}C seen in some of the winter data reflect past shifts in basal food resources due to the lag in turnover, which may be enhanced by reduced growth rates and changes to population size and sex structure that occur between seasons (Sloterdijk et al., 2015). Seasonal changes to δ^{15}N can be an indicator of changes to *Gambusia* diet and trophic position, which has been documented to vary with location (Pyke, 2008), fish size (Crivelli & Boy, 1987; Lee et al., 2018), and season (Gkenas et al., 2012; Remon et al., 2016). Mean δ^{15}N was significantly lower in winter at Lakes Rotopiko and Okowhao, which is contrary to predicted seasonal patterns. *Gambusia* population size and sex ratios vary seasonally, which produces patterns of larger mean body sizes and predominantly female fish over winter (Gkenas et al., 2012; Sloterdijk et al., 2015). This is due to reproduction being limited to warmer spring and summer conditions, and smaller-sized males having lower overwinter survival rates than larger males and females (Zulian et al., 1995; Vargas & de Sostoa, 1996). In my study, the mean lengths of *Gambusia* were significantly greater in winter at both Lake Rotopiko and Lake Okowhao (Table 3.2). Larger *Gambusia* are more likely to consume larger prey due to juveniles being gape limited while adult fish exhibit a preference for larger food items (Metzke & Pederson, 2006; Gkenas et al., 2012). These larger food sources are likely to be more enriched in δ^{15}N due to sitting higher in the food web, such as surface insects, large cladocerans, or conspecifics. In a gut content study of eight New Zealand lakes, Mansfield and McArdle (1998) found that *G. affinis* >25mm primarily consumed cladocerans, terrestrial fauna and copepods, while smaller fish consumed greater proportions of rotifers and chironomid larvae. The size-adjusted seasonal comparisons supported the trend of δ^{15}N enrichment of larger *G. affinis*, as the Lake Rotopiko summer δ^{15}N increased further upon the censoring of values outside the winter distribution. This is due to the exclusion of smaller-sized *Gambusia*, which are most abundant in the summer, and generally feed at lower trophic levels (Lee et al., 2018). Small *G. affinis* may have contributed to the diet of the larger fish in summer, which could explain the elevated δ^{15}N of the data. Cannibalism has been documented to increase in autumn (Remon et al., 2016) and with the depletion of other food sources (Dionne, 1985), though has been rare or absent in several studies (Blanco et al., 2004; Specziár, 2004; Singh & Gupta, 2010). In New Zealand, Mansfield and McArdle (1998) found that cannibalism contributed to less than 3% of the diet of large *G. affinis* and peaked in summer. It is possible that summer cannibalism was occurring at Lake Okowhao and Lake Rotopiko, but the winter depletion of δ^{15}N could also be attributed to seasonal changes to the δ^{15}N of preferred prey sources, such as zooplankton and chironomid larvae. Mean δ^{15}N was significantly lower for winter zooplankton samples at Lake Okowhao and for Chironomidae at Lake Rotopiko. However, the significantly δ^{15}N enriched zooplankton at Lake Rotopiko in the winter are not reflected in the *G. affinis* data.

3.4.1.3 *Gobiomorphus cotidianus*

The seasonal patterns for *G. cotidianus* were significant δ^{13}C enrichment in the summer at Lake Rotopiko, and significant summer δ^{15}N enrichment at every lake. The δ^{13}C data does not show a clear seasonal trend, which suggests that the carbon sources contributing to *G. cotidianus* diet do not fluctuate significantly. Adult *G. cotidianus* primarily feed on the benthos of the littoral zone, with the most significant prey sources typically being chironomid larvae (Forsyth & James, 1988; James et al., 2000) and snails (Ingram et al., 2020). These reflect δ^{13}C sourced from sedimented detritus and particulate organic matter (Jones & Grey, 2004) but also from biogenic methane under low oxygen conditions (Jones et al., 2008). Mean $\Delta\delta^{13}$C between chironomid larvae and *G. cotidianus* at Lake Rotopiko was +2.1‰ in winter and +1.2‰ in summer, and $\Delta\delta^{15}$N was +2.1‰ in winter and +3.8‰ in summer. The summer data is more closely aligned with expected trophic enrichment values if chironomid larvae were a primary prey source, while fish may be feeding on other sources, such as zooplankton, in the winter. A seasonally significant contribution of zooplankton to *G. cotidianus* diet may be reflected by the δ^{15}N data. In each lake, *G. cotidianus* was most depleted in δ^{15}N during the winter, which aligns with the lowest availability of chironomid larvae (Graham & Burns, 1983). Furthermore, zooplankton have been found to become more significant prey sources in eutrophic lakes (Rowe, 1999) and in winter (Forsyth & James, 1988; Stewart et al., 2017). These results may be influenced by *G. cotidianus* size differences between seasons, as the mean winter lengths were significantly greater than summer at all sites except Lake Rotomanuka. The censoring of data to reflect the same *G. cotidianus* length distributions in both seasons did not alter the trend of isotopic enrichment in the summer. The exception to this was Lake Ohinewai, which had a high degree of seasonal isotopic overlap in both fish species. While cannibalism has been observed in captivity (Kerr, 2017), this has only been found in very large (>80 mm) individuals in the natural habitats of *G. cotidianus* (Stephens, 1978) and therefore is unlikely to have influenced stable isotope results.

3.4.2 Seasonal isotopic variation in basal food web taxa

The isotopic signatures of basal food web taxa of lakes integrate terrestrial and atmospheric inputs as well as biological and hydrological processes within lakes. Pingram et al. (2020) investigated the effects of multiple stressors on various trophic and community metrics at six lakes, which included my study sites, and found a strong contribution of littoral and benthic aquatic resources to carbon in fish. Therefore, understanding the isotopic enrichment and trends of freshwater basal food web taxa is important for assessing trophic relationships of *G. affinis* and *G. cotidianus*. Despite geographic, hydrological, and ecological similarities between the lakes, there were distinct inter-lake and inter-season differences in the Chironomidae, Mollusca, and zooplankton mean δ^{13}C and δ^{15}N values (Table 3.3, Figure 3.6). Zooplankton were generally the least enriched in δ^{15}N throughout most lakes in both seasons, indicating they are near the bottom of each food web. Similar zooplankton taxa are present at each lake, with a combination of smaller-bodied filter feeders and larger cladocerans and copepods (Duggan, 2008). At Lake Rotomanuka, no significant difference was found between summer and winter zooplankton data for either mean δ^{13}C or δ^{15}N, indicating that this lake does not have major seasonal variation in food sources of zooplankton. However, significant seasonal differences in δ^{15}N at all other lakes and significant differences in δ^{13}C at two other lakes suggests that the food sources and trophic position of zooplankton may vary between summer and winter at these sites. Lacustrine zooplankton can exhibit seasonal isotopic signature shifts due to increases in aquatic primary production in summer months (Grey et al., 2001; Taipale et al., 2008; Rautio et al., 2011). Terrestrial inputs can form an important component of zooplankton diet year-round, ranging from 20-37% for cladocerans in two small mesotrophic/oligotrophic lakes in the USA (Cole et al., 2011), with similar values found for cladocerans and copepods in 18 oligotrophic-mesotrophic lakes in Canada (Berggren et al., 2014). Allochthonous inputs tend to contribute more significantly to organisms in low nutrient lakes (Grey et al., 2000), which could reduce seasonal variation in zooplankton carbon sources compared to eutrophic lakes. Although the four study lakes were all eutrophic, Lake Rotomanuka had the lowest TLI, and the absence of a significant seasonal difference may be due to a strong terrestrial contribution to zooplankton food sources. Likewise, Lake Rotopiko had the second lowest TLI, and did not vary significantly in seasonal zooplankton δ^{13}C. Temporal change in zooplankton δ^{15}N has been attributed to seasonal variation in nitrogen uptake, fixation, and sources used by primary producers (Lehmann et al., 2004), as well as selective feeding and changes to the relative abundance of food sources of zooplankton (Matthews & Mazumder, 2007; Lee et al., 2013). The findings of zooplankton δ^{15}N enrichment during summer by Matthews and Mazumder (2007) and Syväranta et al. (2008) were supported by the results of my study, as three of the four lakes had higher mean summer δ^{15}N values. This generally reflects phytoplankton within their diet, which is typically more enriched in δ^{15}N than terrestrial vegetation (Cole et al., 2011). The exception to this is Lake Rotopiko, where winter mean δ^{15}N value of zooplankton was 5.31‰ higher than the summer mean. As the Lake Rotopiko winter zooplankton data consisted of three samples consisting of 15 hauls each, and variance between each sample was low, it is unlikely that this elevated δ^{15}N is due to sampling error. This change is also unlikely to be a result of a major shift in δ^{15}N within all of the basal resources of the lake, as the δ^{15}N of *G. cotidianus*, *G. affinis*, Mollusca and Chironomidae do not indicate any unusual increase compared to the summer data. Chironomidae were only collected at two lakes during winter sampling due to difficulty in capture. Collier et al. (2019) found 22 times greater chironomid biomass at Lakes Rotomanuka and Rotopiko than at the more nutrient enriched riverine lakes over summer. This was attributed to factors such as increased turbidity and decreased oxygen potentially limiting algal food sources of the riverine lake populations, or increased impact of benthic feeding fish. However, sufficient Chironomidae could only be captured in Lake Rotopiko and Lake Ohinewai during winter, which limits the seasonal trend analysis, but is also an indication of reduced abundance and activity of chironomid larvae in winter months. New Zealand chironomid larvae are associated with summer conditions, particularly with increased temperature

(Woodward & Shulmeister, 2006; Rees et al., 2021). A stable isotope study of eutrophic lakes documented a strong seasonal shift in δ^{13}C of chironomid larvae, which was attributed to the influence of biogenic methane on their diet during summer thermal stratification (Grey et al., 2004). Of the four lakes in my study, Lake Rotomanuka stratifies once in summer (Dean-Speirs et al., 2014), Lake Ohinewai does so occasionally (Tempero & Hicks, 2017), while Lakes Okowhao and Rotopiko are likely to be polymictic. As the δ^{13}C values of chironomid larvae do not vary significantly between summer and winter samples at Lakes Rotopiko and Ohinewai, it is likely that the summer samples were not taken at a time of thermal stratification. Mollusc samples varied significantly between seasons for δ^{13}C at only Lakes Rotomanuka and Ohinewai, which suggests that winter food sources differ at these sites. The species collected in the winter samples were restricted to *Potamopyrgus antipodarum* mud snails, which graze on algae, diatoms, detritus and decaying plant material (James et al., 2000). The population density of these snails peaks during summer (Geist et al., 2022), which was reflected in the difficulty of capturing sufficient individuals during the winter particularly at Lakes Rotopiko, Okowhao and Ohinewai. Changes to water parameters and reduced primary production and algal food resources may have contributed to the pattern of lower δ^{13}C in snail samples at each lake in the winter. The δ^{13}C of seston and periphyton was lowest during winter in nutrient enriched mesocosms, with this pattern associated with fluctuations in pH, temperature and nutrient availability (Trochine et al., 2017).

3.4.3 Evaluation of study and future research

This study demonstrates seasonal differences between stable isotopes of basal food web taxa and two fish across four Waikato lakes. However, it does not identify the causal mechanisms behind these differences, thus presenting a diverse range of potential future research. Additionally, evaluation of the methodology used in this study reveals several considerations for interpreting the findings. Firstly, the sample sizes between the summer and winter datasets were highly variable, and isotopic analysis methods may affect the accuracy of some seasonal comparisons. The summer samples were assessed at facilities of the University of Waikato, and winter samples were assessed at the University of California Berkeley, due to the shutdown of the University of Waikato Stable Isotope Unit in 2021. The two facilities used different mass spectrometers; however, samples tested at both locations have revealed minimal variation in δ^{13}C and δ^{15}N (personal communication, B. J. Hicks, University of Waikato Stable Isotope Unit, September 15, 2022). Ten *G. cotidianus* and *G. affinis* were collected at each lake for the winter samples, which is likely sufficient for population mean estimates of $\delta^{13}C$ and $\delta^{15}N$ in freshwater organisms (Gustafson et al., 2007; Mazumder et al., 2008). However, the summer data used sample sizes of up to 64 individuals, which was also associated with greater standard deviations on average. Differences in the datasets may influence comparisons because the winter data had less variation in both fish lengths and isotope values, which can be an important factor in the diet and trophic position of *G. affinis* in particular (Mansfield & McArdle, 1998). *Gambusia affinis* can vary in isotopic signature with size and sex (Crivelli & Boy, 1987), and had significantly greater mean lengths at three of the four winter lake samples. Size-correction of the summer dataset corrected this issue and altered the results, while also disproportionately decreasing the summer sample sizes at some lakes. Seasonal differences between the sizes of fish are also aligned with expected seasonal population dynamics, where smaller fish and males become less abundant (Crivelli & Boy, 1987; Zulian et al., 1995; Pyke, 2008). Fish capture was not size selective as it was challenging to trap sufficient individuals at some lakes, but also because random sampling can provide a more accurate population-level estimate on the trophic relationships of *G. cotidianus* and *G. affinis*. Chironomid larvae were also difficult to capture in sufficient numbers, meaning that there was no winter data at two lakes. The lowered densities of larval Chironomidae are typical of winter conditions in New Zealand freshwaters (Forsyth & James, 1988; Winterbourn, 1990; Burns, 1992), and it is unclear whether they remained a significant prey of *G. cotidianus*. There was minor variation in sample gathering and processing methods of the summer and winter studies. The summer fish samples were frozen following euthanasia, after which white muscle tissue was analysed (Collier et al., 2018), while in the winter samples the fish were processed on the day of capture, and bones were left in *G. affinis* but not in *G. cotidianus*. This is a potential source of variance in the isotope data, as Feuchtmayr and Grey (2003) demonstrated $\delta^{13}C$ decreases of 0.9‰ and $\delta^{15}N$ enrichment following freezing, which was attributed to mechanical breakdown of cells. Additionally, Feuchtmayr and Grey (2003) found that including the gut content of zooplankton in stable isotope analysis could produce variances of up to 0.7‰ in *Daphnia* and 3.6‰ in copepods. Although this was avoided in the fish samples, the accuracy of Mollusca samples could also be enhanced by incorporating gut evacuation, which can be achieved by keeping the zooplankton and aquatic snails in empty freshwater enclosures for 24 hours prior to processing (Lopez van Oosterom et al., 2016; Wang et al., 2021). The zooplankton data can be further improved by more thorough separation of zooplankton from algae and detritus in the sample. This could include manual separation into smaller subgroups, such as cladocerans and copepods (Matthews & Mazumder, 2003) to reveal seasonal trends and fish dietary preferences, though such work is very time consuming. Lipid correction is another technique that was absent from the methods but can prove useful in reducing uncertainty in stable isotope results. Lipids within samples can influence $\delta^{13}C$ results depending on whether the lipid is accumulated from the diet or is synthesized within the organism (Grey, 2006), thus this is accounted for via extraction or mathematical corrections (Logan et al., 2008). This was done in the summer data but not winter, so seasonal comparisons in this study used the original, non-corrected data for consistency.

In addition to modifying the methods as outlined above, future experiments could broaden the scope of the study. Firstly, the expansion of trophic groups collected would allow for the creation of mixing models of the winter food sources of both fish at each lake. This would enable statistical categorisation of the estimated proportions of each diet source of *G. cotidianus* and *G. affinis*, and of the uncertainties and variability associated with the model (Phillips et al., 2014). Next, the potential trophic overlap between *G. cotidianus* and *G. affinis* could be investigated with greater precision, such as collecting different size and sex classes in each season. Larval *G. cotidianus* can exert top-down control on zooplankton (Jeppesen et al., 1997), and do not transition to benthivory until above ~18mm in length (Rowe & Chisnall, 1996). Similar sized juvenile *G. affinis* could then compared to assess the presence of any competition. Likewise, this study suggests that both fish may have been consuming Chironomidae over the summer, but it is unclear whether both fish were targeting larvae, or whether *G. affinis* were consuming other life stages. A combination of stable isotope mixing models and other techniques such as gut content analysis (Davis et al., 2012) and fatty acid marker analysis (Napolitano, 1999) can more accurately determine the food sources of all species of interest. The interpretation of these results is assisted by strong baseline data on the physicochemical and biological processes occurring at each lake. Collier et al. (2019) assessed pelagic and benthic trophic resources at each lake, such as various size classes of seston, benthic particulate organic matter, and invertebrates at the four study sites but did not collect samples in the winter, which provides an opportunity to add to seasonal comparisons presented here. This could also include tracing terrestrial inputs into the lakes, which may change seasonally or over larger time scales. Lastly, the study sites are all shallow eutrophic peat and riverine lakes, and therefore are not indicative of the full range of ecosystems in which *G. affinis* and *G. cotidianus* co-occur. A more complete understanding of trophic interactions between these two fish species and the ecological impact of *G. affinis* in New Zealand can be achieved through further research in different locations and under different conditions.

Chapter 4. Research summary and conclusions

The overarching goal of this thesis was to investigate the dietary overlap between non-native *G. affinis* and native *G. cotidianus*, using the following research aims:

1. To determine whether *G. cotidianus* and *G. affinis* consume similar zooplankton taxa using a mesocosm study.
2. To assess the impact of season on the dietary similarity of co-occurring *G. cotidianus* and *G. affinis*, using stable carbon and nitrogen isotope analysis of fish and invertebrate taxa at four Waikato lakes

In Chapter 2, I addressed the first aim by analysing changes to zooplankton community composition among 100 L mesocosms containing either *G. affinis*, *G. cotidianus*, or no fish. The most significant finding of this experiment was that zooplankton community composition did not differ significantly between the *G. affinis* and *G. cotidianus* treatments but differed significantly between the fish treatments and fishless controls. The results suggest that the two fish species were feeding on similar taxa. This was strongly supported by the SIMPER analysis, which showed that the five zooplankton species contributing most to dissimilarity between the fish treatments and controls were the same in both the *G. cotidianus* and *G. affinis* treatments. Additionally, broad-scale zooplankton composition followed a similar trend in the fish treatments, where a significant reduction in the abundance of cladocerans and copepods was found by Day 14 of the experiment. This aligns with my hypothesis that zooplankton composition would overlap between the fish mesocosms, and the findings are concurrent with expectations when comparing my experiment to existing research on zooplanktivorous fish. *Gobiomorphus cotidianus* have been documented to feed on zooplankton when other food sources become scarce (Forsyth & James, 1988; Stewart et al., 2017), while zooplankton are commonly consumed by *Gambusia* year-round (Margaritora et al., 2001; Špoljar et al., 2019). The experiment also highlighted the preference shown by both fish for the larger cladocerans and copepod prey sources over smaller rotifers, which is a documented phenomenon within adult fish of the genera *Gambusia* (Wurtsbaugh et al., 1980; Cabral et al., 1998; Špoljar et al., 2019) and *Gobiomorphus* (Staples, 1975; Jeppesen et al., 1997). The findings of this chapter suggest a strong likelihood of dietary overlap between the fish when they co-occur in freshwater habitats, though the presence of interspecific competition cannot be determined from the results. The primary limitations of this experiment are that the conditions under which fish dietary preferences were assessed were highly specific, and both fish can have a wide dietary breadth. Zooplankton prey has been documented to only make up a small proportion of *G. cotidianus* diet for much of the year (Forsyth & James, 1988; Wilhelm et al., 2007), while *Gambusia* diet can also include large proportions of primary producers (Capps et al., 2009), amphibians (Preston et al., 2018) and surface macroinvertebrates (Pen et al., 1993).

In Chapter 3, the second research aim was addressed through the collection of winter stable isotope data on *G. affinis*, *G. cotidianus,* aquatic snails, chironomid larvae, and zooplankton in Lakes Rotomanuka, Rotopiko, Okowhao, and Ohinewai. The comparison of these stable isotope values to summer data collected by Collier et al. (2018) revealed that *G. affinis* was significantly more enriched in δ^{13}C and δ^{15}N compared to *G. cotidianus* across most lakes in both seasons. The isotopic differences were more pronounced in the winter data, which suggests that the diet of the two fish was most similar during summer. The differences in δ15N were not great enough to place *G. affinis* and *G. cotidianus* on adjacent trophic levels, therefore it is likely they shared some food sources at the four lakes. The findings also highlighted distinct inter-lake differences, such as a high similarity in isotopic enrichment between the fish at Lake Ohinewai. This suggests that dietary overlap, and therefore interspecific competition, are more significant in some locations. My hypothesis that lake taxa would vary seasonally in δ^{13}C and δ^{15}N was supported by the results. However, the significant differences in isotopic enrichment in the two fish species during winter are contrary to my expectations that the diet of *G. affinis* and *G. cotidianus* would be most similar in this season. The lack of a clear ecological explanation for this finding highlights some of the limitations of this study. Relative contributions of each food source to fish tissue could not be determined. As such, it is not clear which dietary sources of *G. affinis* and *G. cotidianus* fluctuated in significance seasonally or the degree of dietary overlap that was present in the summer and winter data.

The results of the two experiments as well as their limitations reveal a wide range of questions for future research to expand on these findings. The mesocosm study methodology can be replicated to assess the dietary preferences of both fish using other food sources, and the scope of the experiment can widen to include multiple prey sources or more natural mesocosms. The stable isotope study serves as a broad-scale overview of seasonal isotopic variation in the two fish and basal food web taxa at peat and riverine lakes, and future research can enhance sample processing and integrate gut content analysis and mixing models to provide more clear explanations for the trends observed.

References

Acra, A., Milki, R., Raffoul, Z., Karahagopian, Y., & Fletcher, M. (1986). Laboratory evaluation of *Gambusia affinis* fish as predators of the schistosome-bearing snails *Bulinus truncatus*. *The American Journal of Tropical Medicine and Hygiene*, *89*(1), 7-12. http://europepmc.org/abstract/MED/3755766

Alcaraz, C., Bisazza, A., & García-Berthou, E. (2008). Salinity mediates the competitive interactions between invasive mosquitofish and an endangered fish. *Oecologia*, *155*, 205-213. https://doi.org/10.1007/s00442-007-0899-4

Allan, M. (2016). *A coupled hydrodynamic-ecological model of management options for restoration of Lake Ohinewai* (Environmental Research Institute Report 68). University of Waikato, Hamilton, New Zealand

Allibone, R., David, B., Hitchmough, R., Jellyman, D., Ling, N., Ravenscroft, P., & Waters, J. (2010). Conservation status of New Zealand freshwater fish, 2009. *New Zealand Journal of Marine and Freshwater Research*, *44*(4), 271-287. https://doi.org/10.1080/00288330.2010.514346

Almeida, D., & Grossman, G. D. (2012). Utility of direct observational methods for assessing competitive interactions between non-native and native freshwater fishes. *Fisheries Management and Ecology*, *19*(2), 157-166. https://doi.org/https://doi.org/10.1111/j.1365-2400.2012.00847.x

Almela, V. D., South, J., & Britton, J. R. (2021). Predicting the competitive interactions and trophic niche consequences of a globally invasive fish with threatened native species. *Journal of Animal Ecology*, *90*(11), 2651-2662. https://doi.org/https://doi.org/10.1111/1365-2656.13571

Andolina, C., Signa, G., Cilluffo, G., Iannucci, S., Mazzola, A., & Vizzini, S. (2022). Coexisting with the alien: Evidence for environmental control on trophic interactions between a native (*Atherina boyeri*) and a non-indigenous fish species (*Gambusia holbrooki*) in a Mediterranean coastal ecosystem. *Frontiers in Ecology and Evolution*, *10*. https://doi.org/10.3389/fevo.2022.958467

Angeler, D. G., Álvarez-Cobelas, M., Sánchez-Carrillo, S., & Rodrigo, M. A. (2002). Assessment of exotic fish impacts on water quality and zooplankton in a degraded semi-arid floodplain wetland. *Aquatic Sciences*, *64*(1), 76-86. https://doi.org/10.1007/s00027-002-8056-y

Ayala, J., Rader, R., Belk, M., & Schaalje, G. (2007). Ground-truthing the impact of invasive species: spatio-temporal overlap between native least chub and introduced western mosquitofish. *Biological Invasions*, *9*, 857-869. https://doi.org/10.1007/s10530-006-9087-4

Baker, C., Rowe, D., & Smith, J. (2004). *Gambusia* - a biodiversity threat? *Water & Atmosphere*, *12*, 24-25.

Barrier, R. F. G., & Hicks, B. J. (1994). Behavioral interactions between black mudfish (*Neochanna diversus* Stokell, 1949: Galaxiidae) and mosquitofish (*Gambusia affinis* Baird & Girard, 1854). *Ecology of Freshwater Fish*, *3*(3), 93-99. https://doi.org/https://doi.org/10.1111/j.1600-0633.1994.tb00110.x

Bauska, T. K., Baggenstos, D., Brook, E. J., Mix, A. C., Marcott, S. A., Petrenko, V. V., Schaefer, H., Severinghaus, J. P., & Lee, J. E. (2016). Carbon isotopes characterize rapid changes in atmospheric carbon dioxide during the last deglaciation. *Proceedings of the National Academy of Sciences*, *113*(13), 3465-3470. https://doi.org/10.1073/pnas.1513868113

Bay, E. C., & Anderson, L. D. (1966). Studies with the mosquitofish, *Gambusia affinis*, as a chironomid control. *Annals of the Entomological Society of America*, *59*(1), 150-153. https://doi.org/10.1093/aesa/59.1.150

Beggel, S., Brandner, J., Cerwenka, A. F., & Geist, J. (2016). Synergistic impacts by an invasive amphipod and an invasive fish explain native gammarid extinction. *BMC Ecology*, *16*(1), 32. https://doi.org/10.1186/s12898-016-0088-6

Belk, M. C., & Lydeard, C. (1994). Effect of *Gambusia holbrooki* on a similar-sized, syntopic Poeciliid, *Heterandria formosa*: competitor or predator? *Copeia*, *1994*(2), 296-302. https://doi.org/10.2307/1446979

Ben-David, M., & Flaherty, E. A. (2012). Stable isotopes in mammalian research: a beginner's guide. *Journal of Mammalogy*, *93*(2), 312-328. https://doi.org/10.1644/11-MAMM-S-166.1

Bence, J. R., & Murdoch, W. W. (1986). Prey size selection by the mosquitofish: relation to Optimal Diet Theory. *Ecology*, *67*(2), 324-336. https://doi.org/https://doi.org/10.2307/1938576

Benoît, H. P., Post, J. R., & Barbet, A. D. (2000). Recruitment dynamics and size structure in experimental populations of the mosquitofish, *Gambusia affinis*. *Copeia*, *2000*(1), 216-221. http://www.jstor.org/stable/1448252

Berggren, M., Ziegler, S. E., St-Gelais, N. F., Beisner, B. E., & Del Giorgio, P. A. (2014). Contrasting patterns of allochthony among three major groups of crustacean zooplankton in boreal and temperate lakes. *Ecology*, *95*(7), 1947-1959. https://doi.org/10.1890/13-0615.1

Bickel, T. O., & Closs, G. P. (2008). Fish distribution and diet in relation to the invasive macrophyte *Lagarosiphon major* in the littoral zone of Lake Dunstan, New Zealand. *Ecology of Freshwater Fish*, *17*(1), 10-19. https://doi.org/https://doi.org/10.1111/j.1600-0633.2007.00249.x

Bisazza, A., & Marin, G. (1991). Male size and female mate choice in the eastern mosquitofish (*Gambusia holbrooki*: Poeciliidae). *Copeia*, *1991*(3), 730-735. https://doi.org/10.2307/1446400

Blanchet, S., Loot, G., Grenouillet, G., & Brosse, S. (2007). Competitive interactions between native and exotic salmonids: a combined field and laboratory demonstration. *Ecology of Freshwater Fish*, *16*(2), 133-143. https://doi.org/https://doi.org/10.1111/j.1600-0633.2006.00205.x

Blanco, S., Romo, S., & Villena, M.-J. (2004). Experimental study on the diet of mosquitofish (*Gambusia holbrooki*) under different ecological conditions in a shallow lake. *International Review of Hydrobiology*, *89*(3), 250-262. https://doi.org/https://doi.org/10.1002/iroh.200310684

Bleackley, N. A., Landman, M. J., & Ling, N. (2009). Ecology of common bully (*Gobiomorphus cotidianus*) in the Tarawera and Rangitaiki rivers: isolation by inland distance or anthropogenic discharge? *New Zealand Journal of Marine and Freshwater Research*, *43*(4), 889-899. https://doi.org/DOI:10.1080/00288330909510047

Bool, J. D., Witcomb, K., Kydd, E., & Brown, C. (2011). Learned recognition and avoidance of invasive mosquitofish by the shrimp, *Paratya australiensis*. *Marine and Freshwater Research*, *62*(10), 1230-1236. https://doi.org/10.1071/MF11140

Bowes, R. E., Lafferty, M. H., & Thorp, J. H. (2014). Less means more: nutrient stress leads to higher δ^{15}N ratios in fish *Freshwater Biology*, *59*(9), 1926-1931. https://doi.org/https://doi.org/10.1111/fwb.12396

Breitburg, D. L., Loher, T., Pacey, C. A., & Gerstein, A. (1997). Varying effects of low dissolved oxygen on trophic interactions in an estuarine food web. *Ecological Monographs*, *67*(4), 489-507. https://doi.org/https://doi.org/10.1890/0012-9615(1997)067[0489:VEOLDO]2.0.CO;2

Britton, J. R., Cucherousset, J., Grey, J., & Gozlan, R. E. (2011). Determining the strength of exploitative competition from an introduced fish: roles of density, biomass and body size. *Ecology of Freshwater Fish*, *20*(1), 74-79. https://doi.org/https://doi.org/10.1111/j.1600-0633.2010.00460.x

Brooks, J. L., & Dodson, S. I. (1965). Predation, body size, and composition of plankton. *Science*, *150*(3692), 28-35. https://doi.org/10.1126/science.150.3692.28

Burns, C. W. (1992). Population dynamics of crustacean zooplankton in a mesotrophic lake, with emphasis on *Boeckella hamata* Brehm (Copepoda: Calanoida). *Internationale Revue der gesamten Hydrobiologie und Hydrographie*, *77*(4), 553-577. https://doi.org/https://doi.org/10.1002/iroh.19920770403

Busst, G. M. A., & Britton, J. R. (2018). Tissue-specific turnover rates of the nitrogen stable isotope as functions of time and growth in a cyprinid fish. *Hydrobiologia*, *805*(1), 49-60. https://doi.org/10.1007/s10750-017-3276-2

Büyüktahtakın, İ. E., & Haight, R. G. (2018). A review of operations research models in invasive species management: state of the art, challenges, and future directions. *Annals of Operations Research*, *271*(2), 357-403. https://doi.org/10.1007/s10479-017-2670-5

Cabral, J. A., Mieiro, C. L., & Marques, J. C. (1998). Environmental and biological factors influence the relationship between a predator fish, *Gambusia holbrooki*, and its main prey in rice fields of the Lower Mondego River Valley (Portugal). *Hydrobiologia*, *382*(1), 41-51. https://doi.org/10.1023/A:1003480920168

Caiola, N., & de Sostoa, A. (2005). Possible reasons for the decline of two native toothcarps in the Iberian Peninsula: evidence of competition with the introduced eastern mosquitofish. *Journal of Applied Ichthyology*, *21*(4), 358-363. https://doi.org/https://doi.org/10.1111/j.1439-0426.2005.00684.x

Callaghan, T. J., White, C. R., & Turschwell, M. P. (2021). Oxygen stress and reproduction do not impede aerobic performance in adult eastern mosquitofish (*Gambusia holbrooki*). *Environmental Biology of Fishes*, *104*(2), 143-154. https://doi.org/10.1007/s10641-021-01065-z

Campton, D. E., & Gall, G. A. E. (1988). Effect of individual and group rearing on age and size at maturity of male mosquitofish, *Gambusia affinis*. *Journal of Fish Biology*, *33*(2), 203-212. https://doi.org/https://doi.org/10.1111/j.1095-8649.1988.tb05463.x

Capps, K. A., Turner, C. B., Booth, M. T., Lombardozzi, D. L., McArt, S. H., Chai, D., & Hairston, N. G. (2009). Behavioral responses of the endemic shrimp *Halocardina rubra* (Malacostraca: Atyidae) to an introduced fish, *Gambusia affinis* (Actinopterygii: Poeciliidae) and implications for the trophic structure of Hawaiian anchialine ponds. *Pacific Science*, *63*(1), 27-37. https://doi.org/10.2984/1534-6188(2009)63[27:BROTES]2.0.CO;2

Cardona, L. (2006). Trophic cascades uncoupled in a coastal marsh ecosystem. *Biological Invasions, 8*(4), 835-842. https://doi.org/10.1007/s10530-005-0420-0

Carmona-Catot, G., Magellan, K., & García-Berthou, E. (2013). Temperature-specific competition between invasive mosquitofish and an endangered cyprinodontid fish. *PLoS One*, *8*(1). https://doi.org/10.1371/journal.pone.0054734

Case, T. J., & Cody, M. L. (1987). Testing theories of island biogeography. *American Scientist*, *75*(4), 402-411. http://www.jstor.org/stable/27854720

Casterlin, M. E., & Reynolds, W. W. (1977). Aspects of habitat selection in the mosquitofish *Gambusia affinis*. *Hydrobiologia*, *55*(2), 125-127. https://doi.org/10.1007/BF00021053

Castro, B. B., Marques, S. M., & Gonçalves, F. (2007). Habitat selection and diel distribution of the crustacean zooplankton from a shallow Mediterranean lake during the turbid and clear water phases. *Freshwater Biology*, *52*(3), 421-433. https://doi.org/https://doi.org/10.1111/j.1365-2427.2006.01717.x

Catford, J. A., Jansson, R., & Nilsson, C. (2009). Reducing redundancy in invasion ecology by integrating hypotheses into a single theoretical framework. *Diversity and Distributions*, *15*(1), 22-40. https://doi.org/https://doi.org/10.1111/j.1472-4642.2008.00521.x

Chadderton, L., Kelleher, S., Brow, A., Shaw, T., Studholme, B., & Barrier, R. (2001, 10-12 May). Testing the efficacy of rotenone as a piscicide for New Zealand pest fish species. In T. Dean, L. Chadderton, N. Grainger & R. Allibone (Eds.), *Managing invasive freshwater fish in New Zealand: Proceedings of a workshop hosted by Department of Conservation, Hamilton, New Zealand* (pp. 113–130).

Chan, F. T., Beatty, S. J., Gilles, A. S., Jr., Hill, J. E., Kozic, S., Luo, D., Morgan, D. L., Pavia, R. T. B., Jr., Therriault, T. W., Verreycken, H., Vilizzi, L., Wei, H., Yeo, D. C. J., Zeng, Y., Zięba, G., & Copp, G. H. (2019). Leaving the fish bowl: the ornamental trade as a global vector for freshwater fish invasions. *Aquatic Ecosystem Health & Management*, *22*(4), 417-439. https://doi.org/10.1080/14634988.2019.1685849

Chapman, A., Morgan, D. L., Beatty, S. J., & Gill, H. S. (2006). Variation in life history of land-locked lacustrine and riverine populations of *Galaxias maculatus* (Jenyns 1842) in Western Australia. *Environmental Biology of Fishes*, *77*(1), 21-37. https://doi.org/10.1007/s10641-006-9051-2

Chapman, M. A., & Green, J. (1999). Crustacean zooplankton communities in a New Zealand lake during four decades of trophic change. *New Zealand Journal of*

Marine and Freshwater Research, 33, 361-373. https://doi.org/10.1080/00288330.1999.9516883

Chapman, M. A., Green, J. D., & Jolly, V. H. (1985). Relationships between zooplankton abundance and trophic state in seven New Zealand lakes. *Hydrobiologia, 123*(2), 119-136. https://doi.org/10.1007/BF00018974

Chapman, M. A., Lewis, M. H., & Winterbourn, M. (2011). *Guide to the freshwater crustacea of New Zealand*. NZ Freshwater Sciences Society.

Chen, T., Beekman, M., & Ward, A. J. (2011). The role of female dominance hierarchies in the mating behaviour of mosquitofish. *Biology Letters, 7*(3), 343-345. https://doi.org/10.1098/rsbl.2010.1020

Chervinski, J. (1983). Salinity tolerance of the mosquito fish, *Gambusia affinis* (Baird and Girard). *Journal of Fish Biology, 22*(1), 9-11. https://doi.org/https://doi.org/10.1111/j.1095-8649.1983.tb04720.x

Chesson, L. A., Tipple, B. J., Howa, J. D., Bowen, G. J., Barnette, J. E., Cerling, T. E., & Ehleringer, J. R. (2014). Stable isotopes in forensics applications. In H. D. Holland & K. K. Turekian (Eds.), *Treatise on geochemistry (second edition)* (pp. 285-317). Elsevier. https://doi.org/https://doi.org/10.1016/B978-0-08-095975-7.01224-9

Clark, I. D., & Fritz, P. (1997). *Environmental isotopes in hydrogeology*. CRC Press.

Closs, G., Ludgate, B., & Goldsmith, R. (2001, 10–12 May). Controlling European perch (*Perca fluviatilis*): lessons from an experimental removal. In T. Dean, L. Chadderton, N. Grainger & R. Allibone (Eds.), *Managing invasive freshwater fish in New Zealand: Proceedings of a workshop hosted by Department of Conservation, Hamilton, New Zealand* (pp. 37-48).

Closs, G. P., Smith, M., Barry, B., & Markwitz, A. (2003). Non‐diadromous recruitment in coastal populations of common bully (*Gobiomorphus cotidianus*). *New Zealand Journal of Marine and Freshwater Research, 37*(2), 301-313. https://doi.org/10.1080/00288330.2003.9517168

Coffin, J. L., Kelley, J. L., Jeyasingh, P. D., & Tobler, M. (2022). Impacts of heavy metal pollution on the ionomes and transcriptomes of western mosquitofish (*Gambusia affinis*). *Molecular Ecology, 31*(5), 1527-1542. https://doi.org/https://doi.org/10.1111/mec.16342

Colborne, S. F., Fisk, A. T., & Johnson, T. B. (2017). Tissue-specific turnover and diet-tissue discrimination factors of carbon and nitrogen isotopes of a common forage fish held at two temperatures. *Rapid Communications in Mass Spectrometry*, *31*(17), 1405-1414. https://doi.org/https://doi.org/10.1002/rcm.7922

Cole, J. J., Carpenter, S. R., Kitchell, J., Pace, M. L., Solomon, C. T., & Weidel, B. (2011). Strong evidence for terrestrial support of zooplankton in small lakes based on stable isotopes of carbon, nitrogen, and hydrogen. *Proceedings of the National Academy of Sciences*, *108*(5), 1975-1980. https://doi.org/10.1073/pnas.1012807108

Collier, K. J., Garrett-Walker, J., Özkundakci, D., & Pingram, M. A. (2019). Characteristics of consumer trophic resources for Waikato shallow lake food webs. *New Zealand Journal of Marine and Freshwater Research*, *53*(4), 588-602. https://doi.org/10.1080/00288330.2018.1517098

Collier, K. J., Leathwick, J. R., & Rowe, D. K. (2017). Assessing vulnerability of New Zealand lakes to loss of conservation value from invasive fish impacts. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *27*(2), 534-546. https://doi.org/https://doi.org/10.1002/aqc.2705

Collier, K. J., Pingram, M. A., Francis, L., Garrett-Walker, J., & Melchior, M. (2018). Trophic overlap between non-native brown bullhead (*Ameiurus nebulosus*) and native shortfin eel (*Anguilla australis*) in shallow lakes. *Ecology of Freshwater Fish*, *27*(4), 888-897. https://doi.org/https://doi.org/10.1111/eff.12400

Collins, M., Vázquez, D., & Sanders, N. (2002). Species-area curves, homogenization and the loss of global diversity. *Evolutionary Ecology Research*, *4*.

Conde-Porcuna, J. M., & Declerck, S. (1998). Regulation of rotifer species by invertebrate predators in a hypertrophic lake: selective predation on egg-bearing females and induction of morphological defences. *Journal of Plankton Research*, *20*(4), 605-618. https://doi.org/10.1093/plankt/20.4.605

Connell, J. H. (1983). On the prevalence and relative importance of interspecific competition: evidence from field experiments. *The American Naturalist*, *122*(5), 661-696. https://doi.org/10.1086/284165

Connolly, R. M., Guest, M. A., Melville, A. J., & Oakes, J. M. (2004). Sulfur stable isotopes separate producers in marine food-web analysis. *Oecologia*, *138*(2), 161-167. https://doi.org/10.1007/s00442-003-1415-0

Cooper, R. A., & Millener, P. R. (1993). The New Zealand biota: historical background and new research. *Trends in Ecology & Evolution*, *8*(12), 429-433. https://doi.org/https://doi.org/10.1016/0169-5347(93)90004-9

Copp, G. H., Bianco, P. G., Bogutskaya, N. G., Erős, T., Falka, I., Ferreira, M. T., Fox, M. G., Freyhof, J., Gozlan, R. E., Grabowska, J., Kováč, V., Moreno-Amich, R., Naseka, A. M., Peňáz, M., Povž, M., Przybylski, M., Robillard, M., Russell, I. C., Stakėnas, S., Šumer, S., Vila-Gispert, A., & Wiesner, C. (2005). To be, or not to be, a non-native freshwater fish? *Journal of Applied Ichthyology*, *21*(4), 242-262. https://doi.org/https://doi.org/10.1111/j.1439-0426.2005.00690.x

Cote, J., Fogarty, S., Weinersmith, K., Brodin, T., & Sih, A. (2010). Personality traits and dispersal tendency in the invasive mosquitofish (*Gambusia affinis*). *Proceedings of the Royal Society B*, *277*(1687), 1571-1579. https://doi.org/10.1098/rspb.2009.2128

Crivelli, A. J., & Boy, V. (1987). The diet of the mosquitofish *Gambusia affinis* (Baird and Girard) (Poecilliidae) in Mediterranean France. *Revue d'écologie - la Terre et la Vie*, *42*(4), 421-435. https://hal.archives-ouvertes.fr/hal-03529439

Cucherousset, J., Aymes, J. C., Santoul, F., & Céréghino, R. (2007). Stable isotope evidence of trophic interactions between introduced brook trout *Salvelinus fontinalis* and native brown trout *Salmo trutta* in a mountain stream of south-west France. *Journal of Fish Biology*, *71*(sd), 210-223. https://doi.org/https://doi.org/10.1111/j.1095-8649.2007.01675.x

Cucherousset, J., & Olden, J. D. (2011). Ecological impacts of nonnative freshwater fishes. *Fisheries*, *36*(5), 215-230. https://doi.org/10.1080/03632415.2011.574578

Daigneault, A., Greenhalgh, S., & Samarasinghe, O. (2018). Economic impacts of multiple agro-environmental policies on New Zealand land use. *Environmental and Resource Economics*, *69*(4), 763-785. https://doi.org/10.1007/s10640-016-0103-6

Daniel, A. J., & Morgan, D. K. (2011). *Lake Ohinewai pest fish removal* (CBER Contract Report 120). Hamilton, New Zealand

Daugherty, C. H., Patterson, G. B., & Hitchmough, R. A. (1994). Taxonomic and conservation review of the New Zealand herpetofauna. *New Zealand Journal of Zoology*, *21*(4), 317-323. https://doi.org/10.1080/03014223.1994.9518002

Davis, A. M., Blanchette, M. L., Pusey, B. J., Jardine, T. D., & Pearson, R. G. (2012). Gut content and stable isotope analyses provide complementary understanding of ontogenetic dietary shifts and trophic relationships among fishes in a tropical river. *Freshwater Biology*, *57*(10), 2156-2172. https://doi.org/https://doi.org/10.1111/j.1365-2427.2012.02858.x

Davis, M. A. (2003). Biotic globalization: does competition from introduced species threaten biodiversity? *BioScience*, *53*(5), 481-489. https://doi.org/10.1641/0006-3568(2003)053[0481:Bgdcfi]2.0.Co;2

Dean-Speirs, T., Neilson, K., & Reeves, P. (2014). *Waikato region shallow lakes management plan: Volume 2*. Waikato Regional Council, Hamilton, New Zealand. https://www.waikatoregion.govt.nz/assets/WRC/WRC-2019/TR201459.pdf

Deniro, M. J., & Epstein, S. (1981). Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica et Cosmochimica Acta*, *45*(3), 341-351. https://doi.org/https://doi.org/10.1016/0016-7037(81)90244-1

Dionne, M. (1985). Cannibalism, food availability, and reproduction in the mosquito fish (*Gambusia affinis*): a laboratory experiment. *The American Naturalist*, *126*(1), 16-23. https://doi.org/10.1086/284392

Dodson, S. I. (1974). Zooplankton competition and predation: an experimental test of the size-efficiency hypothesis. *Ecology*, *55*(3), 605-613. https://doi.org/10.2307/1935150

Drenner, R. W., & Smith, J. D. (1991). Biomass-dependent effects of mosquitofish on zooplankton, chlorophyll and the size distribution of particulate phosphorus. *SIL Proceedings, 1922-2010*, *24*(4), 2382-2386. https://doi.org/10.1080/03680770.1989.11899970

Duggan, I. C. (2008). *Zooplankton composition and a water quality assessment of seventeen Waikato lakes using rotifer community composition* (CBER Contract Report 80). Waikato Regional Council, Hamilton, New Zealand. https://www.waikatoregion.govt.nz/assets/WRC/WRC-2019/TR0826.pdf

Dunn, N. R., Allibone, R. M., Closs, G. P., Crow, S. K., David, B. O., Goodman, J. M., Griffiths, M., Jack, D. C., Ling, N., Waters, J. M., & Rolfe, J. R. (2018). *Conservation status of New Zealand freshwater fishes, 2017* (New Zealand Threat Classification Series). Department of Conservation, Wellington, New Zealand. https://www.doc.govt.nz/Documents/science-and-technical/nztcs24entire.pdf

Edgar, N. B., & Green, J. D. (1994). Calanoid copepod grazing on phytoplankton: seasonal experiments on natural communities. *Hydrobiologia*, *273*(3), 147-161. https://doi.org/10.1007/BF00005638

Edwards, T., Clayton, J., & de Winton, M. (2005). *The condition of lakes in the Waikato Region using LakeSPI* (Technical Report HAM2010-065). Environment Waikato, Hamilton, New Zealand. https://www.waikatoregion.govt.nz/assets/WRC/WRC-2019/tr06-13.pdf

Edwards, T., de Winton, M., & Clayton, J. (2010). *Assessment of the ecological condition of lakes in the Waikato region using LakeSPI* (Client Report HAM2010-065). Waikato Regional Council, Hamilton, New Zealand. https://www.waikatoregion.govt.nz/assets/WRC/WRC-2019/TR-2010-24.pdf

Ehleringer, J. R., & Rundel, P. W. (1988). Stable isotopes: history, units, and instrumentation. In J. R. Ehleringer & P. W. Rundel (Eds.), *Stable Isotopes in Ecological Research* (pp. 544). Springer-Verlag.

Eivers, R. S., Duggan, I. C., Hamilton, D. P., & Quinn, J. M. (2018). Constructed treatment wetlands provide habitat for zooplankton communities in agricultural peat lake catchments. *Wetlands*, *38*(1), 95-108. https://doi.org/10.1007/s13157-017-0959-4

Fadda, A., Palmas, F., Camin, F., Ziller, L., Padedda, B. M., Luglié, A., Manca, M., & Sabatini, A. (2016). Analysis of δ^{13}C and δ^{15}N isotopic signatures to shed light on the hydrological cycle’s influence on the trophic behavior of fish in a Mediterranean reservoir. *Biologia*, *71*(12), 1395-1403. https://doi.org/10.1515/biolog-2016-0160

Feuchtmayr, H., & Grey, J. (2003). Effect of preparation and preservation procedures on carbon and nitrogen stable isotope determinations from zooplankton. *Rapid Communications in Mass Spectrometry*, *17*(23), 2605-2610. https://doi.org/10.1002/rcm.1227

Forsyth, D. J., & James, M. R. (1988). The Lake Okaro ecosystem 2. Production of the chironomid *Polypedilum pavidus* and its role as food for two fish species. *New Zealand Journal of Marine and Freshwater Research*, *22*(3), 327-335. https://doi.org/10.1080/00288330.1988.9516305

Forsyth, D. J., & James, M. R. (1991). Population dynamics and production. *Archiv für Hydrobiologie*, *120*(3), 287-314. https://doi.org/10.1127/archiv-hydrobiol/120/1991/287

France, R. L. (1995). Differentiation between littoral and pelagic food webs in lakes using stable carbon isotopes. *Limnology and Oceanography*, *40*(7), 1310-1313. https://doi.org/https://doi.org/10.4319/lo.1995.40.7.1310

France, R. L., & Peters, R. H. (1997). Ecosystem differences in the trophic enrichment of ^{13}C in aquatic food webs. *Canadian Journal of Fisheries and Aquatic Sciences*, *54*(6), 1255-1258. https://doi.org/10.1139/f97-044

Fry, B. (2006). *Stable isotope ecology* (1 ed.). Springer New York. https://doi.org/10.1007/0-387-33745-8

Fry, B., & Sherr, E. B. (1989). δ^{13}C measurements as indicators of carbon flow in marine and freshwater ecosystems. In: Rundel P.W., Ehleringer J. R. , Nagy K. A. (Eds.). *Stable Isotopes in Ecological Research*. New York: Springer, 1989, 196-229.

Fryxell, D. C., Arnett, H. A., Apgar, T. M., Kinnison, M. T., & Palkovacs, E. P. (2015). Sex ratio variation shapes the ecological effects of a globally introduced freshwater fish. *Proceedings of the Royal Society B*, *282*(1817). https://doi.org/10.1098/rspb.2015.1970

Fuex, A. N. (1977). The use of stable carbon isotopes in hydrocarbon exploration. *Journal of Geochemical Exploration*, *7*, 155-188. https://doi.org/https://doi.org/10.1016/0375-6742(77)90080-2

Galat, D. L., & Robertson, B. P. (1992). Response of endangered *Poeciliopsis occidentalis sonoriensis* in the Río Yaqui drainage, Arizona, to introduced *Gambusia affinis*. *Environmental Biology of Fishes*, *33*(3), 249-264. https://doi.org/10.1007/BF00005869

Gallardo, B., Clavero, M., Sánchez, M. I., & Vilà, M. (2016). Global ecological impacts of invasive species in aquatic ecosystems. *Global Change Biology*, *22*(1), 151-163. https://doi.org/https://doi.org/10.1111/gcb.13004

García-Berthou, E. (2007). The characteristics of invasive fishes: what has been learned so far? *Journal of Fish Biology*, *71*(sd), 33-55. https://doi.org/https://doi.org/10.1111/j.1095-8649.2007.01668.x

García-Berthou, E., Alcaraz, C., Pou-Rovira, Q., Zamora, L., Coenders, G., & Feo, C. (2005). Introduction pathways and establishment rates of invasive aquatic species in Europe. *Canadian Journal of Fisheries and Aquatic Sciences*, *62*(2), 453-463. https://doi.org/10.1139/f05-017

GBIF.org. (2022). *Occurrence Download* The Global Biodiversity Information Facility. https://doi.org/10.15468/DL.KCWJWF

Geist, J. A., Mancuso, J. L., Morin, M. M., Bommarito, K. P., Bovee, E. N., Wendell, D., Burroughs, B., Luttenton, M. R., Strayer, D. L., & Tiegs, S. D. (2022). The New Zealand mud snail (*Potamopyrgus antipodarum*): autecology and management of a global invader. *Biological Invasions*, *24*(4), 905-938. https://doi.org/10.1007/s10530-021-02681-7

Geyer, R. L., Smith, G. R., & Rettig, J. E. (2016). Effects of Roundup formulations, nutrient addition, and western mosquitofish (*Gambusia affinis*) on aquatic communities. *Environmental Science and Pollution Research International, 23*(12), 11729-11739. https://doi.org/10.1007/s11356-016-6381-2

Gherardi, F. (2007). *Biological invaders in inland waters: Profiles, distribution, and threats*. Springer Dordrecht. https://doi.org/10.1007/978-1-4020-6029-8

Gilbert, J. J., & Slemberger, R. S. (1985). Control of *Keratella* populations by interference competition from *Daphnia*. *Limnology and Oceanography, 30*(1), 180-188. https://doi.org/https://doi.org/10.4319/lo.1985.30.1.0180

Gkenas, C., Oikonomou, A., Economou, A., Kiosse, F., & Leonardos, I. (2012). Life history pattern and feeding habits of the invasive mosquitofish, *Gambusia holbrooki,* in Lake Pamvotis (NW Greece). *Journal of Biological Research, 17*, 121-136.

Gladyshev, I. (2009). Stable isotope analyses in aquatic ecology (a review). *Journal of Siberian Federal University. Biology, 2,* 381-402. https://doi.org/10.17516/1997-1389-0220

Glibert, P. M., Middelburg, J. J., McClelland, J. W., & Jake Vander Zanden, M. (2019). Stable isotope tracers: enriching our perspectives and questions on sources, fates, rates, and pathways of major elements in aquatic systems. *Limnology and Oceanography, 64*(3), 950-981. https://doi.org/https://doi.org/10.1002/lno.11087

Glova, G. J. (1999). A test for competition between two *Gobiomorphus* species (Teleostei: Eleotridae) in artificial channels. *Ecology of Freshwater Fish, 8*(2), 70-77. https://doi.org/https://doi.org/10.1111/j.1600-0633.1999.tb00056.x

Gozlan, R. E. (2008). Introduction of non-native freshwater fish: is it all bad? *Fish and Fisheries, 9*(1), 106-115. https://doi.org/https://doi.org/10.1111/j.1467-2979.2007.00267.x

Gozlan, R. E., Britton, J. R., Cowx, I., & Copp, G. H. (2010). Current knowledge on non-native freshwater fish introductions. *Journal of Fish Biology, 76*(4), 751-786. https://doi.org/https://doi.org/10.1111/j.1095-8649.2010.02566.x

Graham, A. A., & Burns, C. W. (1983). Production and ecology of benthic chironomid larvae (Diptera) in Lake Hayes, New Zealand, a warm-monomictic eutrophic lake. *Internationale Revue der gesamten Hydrobiologie und Hydrographie, 68*(3), 351-377. https://doi.org/https://doi.org/10.1002/iroh.19830680307

Grey, J. (2006). The use of stable isotope analyses in freshwater ecology: current awareness. *Polish Journal of Ecology, 54,* 563-584.

Grey, J., Jones, R. I., & Sleep, D. (2000). Stable isotope analysis of the origins of zooplankton carbon in lakes of differing trophic state. *Oecologia*, *123*(2), 232-240. https://doi.org/10.1007/s004420051010

Grey, J., Jones, R. I., & Sleep, D. (2001). Seasonal changes in the importance of the source of organic matter to the diet of zooplankton in Loch Ness, as indicated by stable isotope analysis. *Limnology and Oceanography*, *46*(3), 505-513. https://doi.org/https://doi.org/10.4319/lo.2001.46.3.0505

Grey, J., Kelly, A., Ward, S., Sommerwerk, N., & Jones, R. I. (2004). Seasonal changes in the stable isotope values of lake-dwelling chironomid larvae in relation to feeding and life cycle variability. *Freshwater Biology*, *49*(6), 681-689. https://doi.org/https://doi.org/10.1111/j.1365-2427.2004.01217.x

Gu, B. (2009). Variations and controls of nitrogen stable isotopes in particulate organic matter of lakes. *Oecologia*, *160*(3), 421-431. https://doi.org/10.1007/s00442-009-1323-z

Gu, B., Schelske, C. L., & Waters, M. N. (2011). Patterns and controls of seasonal variability of carbon stable isotopes of particulate organic matter in lakes. *Oecologia*, *165*(4), 1083-1094. https://doi.org/10.1007/s00442-010-1888-6

Gurevitch, J., & Padilla, D. K. (2004). Are invasive species a major cause of extinctions? *Trends in Ecology & Evolution*, *19*(9), 470-474. https://doi.org/https://doi.org/10.1016/j.tree.2004.07.005

Gustafson, L., Showers, W., Kwak, T., Levine, J., & Stoskopf, M. (2007). Temporal and spatial variability in stable isotope compositions of a freshwater mussel: implications for biomonitoring and ecological studies. *Oecologia*, *152*(1), 140-150. https://doi.org/10.1007/s00442-006-0633-7

Haiahem, D., Touati, L., Baaziz, N., Samraoui, F., Alfarhan, A. H., & Samraoui, B. (2017). Impact of eastern mosquitofish, *Gambusia holbrooki*, on temporary ponds: insights on how predation may structure zooplankton communities. *Zoology and Ecology*, *27*, 124-132.

Harmon, J. J., & Smith, G. R. (2020). Invasive fish (*Gambusia affinis*) as an ecological filter for macroinvertebrate colonization of experimental ponds. *Freshwater Science*, *40*(1), 151-161. https://doi.org/10.1086/713006

Harmon, T. S. (2009). Methods for reducing stressors and maintaining water quality associated with live fish transport in tanks: a review of the basics. *Reviews in Aquaculture*, *1*(1), 58-66. https://doi.org/https://doi.org/10.1111/j.1753-5131.2008.01003.x

Hayes, J. W., & Rutledge, M. J. (1991). Relationship between turbidity and fish diets in Lakes Waahi and Whangape, New Zealand. *New Zealand Journal of Marine and Freshwater Research*, *25*(3), 297-304. https://doi.org/10.1080/00288330.1991.9516482

Haynes, J. L. (1993). Annual reestablishment of mosquitofish populations in Nebraska. *Copeia*, *1993*(1), 232-235. https://doi.org/10.2307/1446318

Haynes, J. L., & Cashner, R. C. (1995). Life history and population dynamics of the western mosquitofish: a comparison of natural and introduced populations. *Journal of Fish Biology*, *46*(6), 1026-1041. https://doi.org/https://doi.org/10.1111/j.1095-8649.1995.tb01407.x

Hebert, P. D. N., & Crease, T. J. (1980). Clonal coexistence in *Daphnia pulex* (Leydig): another planktonic paradox. *Science*, *207*(4437), 1363-1365. https://doi.org/doi:10.1126/science.207.4437.1363

Hicks, A. S., Jarvis, M. G., David, B. O., Waters, J. M., Norman, M. D., & Closs, G. P. (2017). Lake and species specific patterns of non-diadromous recruitment in amphidromous fish: the importance of local recruitment and habitat requirements. *Marine and Freshwater Research*, *68*(12), 2315-2323. https://doi.org/10.1071/MF16387

Hicks, B. J. (1997). Food webs in forest and pasture streams in the Waikato region, New Zealand: A study based on analyses of stable isotopes of carbon and nitrogen, and fish gut contents. *New Zealand Journal of Marine and Freshwater Research*, *31*(5), 651-664. https://doi.org/10.1080/00288330.1997.9516796

Hicks, B. J., Brijs, J., Daniel, A. J., Morgan, D. K. J., & Ling, N. (2015). Biomass estimation of invasive fish. In K. J. Collier & N. P. J. Grainger (Eds.), *New Zealand Invasive Fish Management Handbook* (pp. 116-122). Lake Ecosystem Restoration New Zealand (LERNZ) & Department of Conservation.

Hicks, B. J., & Ling, N. (2015). Carp as an invasive species In C. Pietsch & P. Hirsch (Eds.), *Biology and Ecology of Carp*. CRC Press. https://doi.org/https://doi.org/10.1201/b18547

Hinchliffe, C., Atwood, T., Ollivier, Q., & Hammill, E. (2017). Presence of invasive *Gambusia* alters ecological communities and the functions they perform in lentic ecosystems. *Marine and Freshwater Research*, *68*. https://doi.org/10.1071/MF16301

Hladyz, S., Nielsen, D. L., Suter, P. J., & Krull, E. S. (2012). Temporal variations in organic carbon utilization by consumers in a lowland river. *River Research and Applications*, *28*(5), 513-528. https://doi.org/https://doi.org/10.1002/rra.1467

Ho, S. S., Bond, N. R., & Lake, P. S. (2011). Comparing food-web impacts of a native invertebrate and an invasive fish as predators in small floodplain wetlands. *Marine and Freshwater Research*, *62*(4), 372-382. https://doi.org/10.1071/MF10222

Hoch, J. M., Spadafore, S., & Olesen, D. (2020). *Effects of predator cues on migration-related behaviors of eastern mosquitofish (Gambusia holbrooki) in temporary wetlands of the Everglades.* Nova Southeastern University Florida.

Holomuzki, J. R., & Biggs, B. J. F. (2006). Habitat-specific variation and performance trade-offs in shell armature of New Zealand mudsnails. *Ecology*, *87*(4), 1038-1047. https://doi.org/https://doi.org/10.1890/0012-9658(2006)87[1038:HVAPTI]2.0.CO;2

Howe, E., Howe, C., Lim, R., & Burchett, M. (1997). Impact of the introduced poeciliid *Gambusia holbrooki* (Girard, 1859) on the growth and reproduction of *Pseudomugil signifer* (Kner, 1865) in Australia. *Marine and Freshwater Research*, *48*(5), 425-434. https://doi.org/10.1071/MF96114

Hughes, A. L. (1985). Seasonal changes in fecundity and size at first reproduction in an Indiana population of the mosquitofish *Gambusia affinis*. *The American Midland Naturalist*, *114*(1), 30-36. https://doi.org/10.2307/2425237

Hulata, G. (1995). A review of genetic improvement of the common carp (*Cyprinus carpio L.*) and other cyprinids by crossbreeding, hybridization and selection. *Aquaculture*, *129*(1), 143-155. https://doi.org/https://doi.org/10.1016/0044-8486(94)00244-I

Hurlbert, S. H., & Mulla, M. S. (1981). Impacts of mosquitofish (*Gambusia affinis*) predation on plankton communities. *Hydrobiologia*, *83*(1), 125-151. https://doi.org/10.1007/BF02187157

Hurlbert, S. H., Zedler, J., & Fairbanks, D. (1972). Ecosystem alteration by mosquitofish (*Gambusia affinis*) predation. *Science*, *175*(4022), 639-641. https://doi.org/doi:10.1126/science.175.4022.639

Ingram, T., Dutoit, L., Mikheev, P., Khan, S., & Schallenberg, M. (2020). Phenotypic, ecological, and genomic variation in common bully (*Gobiomorphus cotidianus*) populations along depth gradients in New Zealand’s southern Great Lakes. *Canadian Journal of Fisheries and Aquatic Sciences*, *77*(10), 1678-1687. https://doi.org/10.1139/cjfas-2020-0015

Irons, K. S., Sass, G. G., McClelland, M. A., & Stafford, J. D. (2007). Reduced condition factor of two native fish species coincident with invasion of non-native Asian carps in the Illinois River, U.S.A. Is this evidence for competition and reduced fitness? *Journal of Fish Biology*, *71*(sd), 258-273. https://doi.org/https://doi.org/10.1111/j.1095-8649.2007.01670.x

Itzkowitz, M. (1971). Preliminary study of the social behavior of male *Gambusia affinis* (Baird and Girard) (Pisces: Poeciliidae) in aquaria. *Chesapeake Science*, *12*(4), 219-224. https://doi.org/10.2307/1350908

Jackson, M. C., Ruiz-Navarro, A., & Britton, J. R. (2015). Population density modifies the ecological impacts of invasive species. *Oikos*, *124*(7), 880-887. https://doi.org/https://doi.org/10.1111/oik.01661

Jackson, M. C., Woodford, D. J., Bellingan, T. A., Weyl, O. L. F., Potgieter, M. J., Rivers-Moore, N. A., Ellender, B. R., Fourie, H. E., & Chimimba, C. T. (2016). Trophic overlap between fish and riparian spiders: potential impacts of an invasive fish on terrestrial consumers. *Ecology and Evolution*, *6*(6), 1745-1752. https://doi.org/https://doi.org/10.1002/ece3.1893

James, K. E. S., & Poulin, R. (1998). The effects of perceived competition and parasitism on the foraging behaviour of the upland bully (Eleotridae). *Journal of Fish Biology*, *53*(4), 827-834. https://doi.org/https://doi.org/10.1111/j.1095-8649.1998.tb01835.x

James, M. R., Hawes, I., Weatherhead, M., Stanger, C., & Gibbs, M. (2000). Carbon flow in the littoral food web of an oligotrophic lake. *Hydrobiologia*, *441*(1), 93-106. https://doi.org/10.1023/A:1017504820168

Jellyman, D. J., Crow, S. K., & Robinson, P. (2012). Impacts of longfin eels (*Anguilla dieffenbachii*) on the behaviour of common bullies (*Gobiomorphus cotidianus*) held in captivity. *Ecology of Freshwater Fish*, *21*(1), 57-63. https://doi.org/https://doi.org/10.1111/j.1600-0633.2011.00523.x

Jeppesen, E., Lauridsen, T., Mitchell, S. F., & Burns, C. W. (1997). Do planktivorous fish structure the zooplankton communities in New Zealand lakes? *New Zealand Journal of Marine and Freshwater Research*, *31*(2), 163-173. https://doi.org/10.1080/00288330.1997.9516755

Jeppesen, E., Lauridsen, T. L., Mitchell, S. F., Christoffersen, K., & Burns, C. W. (2000). Trophic structure in the pelagial of 25 shallow New Zealand lakes: changes along nutrient and fish gradients. *Journal of Plankton Research*, *22*(5), 951-968. https://doi.org/10.1093/plankt/22.5.951

Jolly, V. H., & Brown, J. M. A. (1975). *New Zealand lakes* (1 ed.). Auckland University Press, Oxford University Press.

Jones, P., & Closs, G. (2017). The introduction of brown trout to New Zealand and their impact on native fish communities: biology, ecology and management. In J. Lobón-Cerviá & N. Sanz (Eds.), *Brown Trout: Biology, Ecology and Management* (pp. 545-567). John Wiley & Sons Ltd. https://doi.org/10.1002/9781119268352.ch21

Jones, R. I., Carter, C. E., Kelly, A., Ward, S., Kelly, D. J., & Grey, J. (2008). Widespread contribution of methane-cycle bacteria to the diets of lake profundal chironomid larvae. *Ecology*, *89*(3), 857-864. https://doi.org/https://doi.org/10.1890/06-2010.1

Jones, R. I., & Grey, J. (2004). Stable isotope analysis of chironomid larvae from some Finnish forest lakes indicates dietary contribution from biogenic methane. *Boreal Environment Research*, *9*, 17-23.

Jourdan, J., Riesch, R., & Cunze, S. (2021). Off to new shores: climate niche expansion in invasive mosquitofish (*Gambusia* spp.). *Ecology and Evolution*, *11*(24), 18369-18400. https://doi.org/https://doi.org/10.1002/ece3.8427

Jowett, I., & Richardson, J. (1996). Distribution and abundance of freshwater fish in New Zealand rivers. *New Zealand Journal of Marine and Freshwater Research*, *30*, 239-255. https://doi.org/10.1080/00288330.1996.9516712

Jowett, I. G., & Richardson, J. (2003). Fish communities in New Zealand rivers and their relationship to environmental variables. *New Zealand Journal of Marine and Freshwater Research*, *37*(2), 347-366. https://doi.org/10.1080/00288330.2003.9517172

Joy, M. K., Foote, K. J., McNie, P., & Piria, M. (2019). Decline in New Zealand's freshwater fish fauna: effect of land use. *Marine and Freshwater Research*, *70*(1), 114-124. https://doi.org/10.1071/MF18028

Kalogianni, E., Giakoumi, S., Andriopoulou, A., & Chatzinikolaou, Y. (2014). Prey utilisation and trophic overlap between the non native mosquitofish and a native fish in two Mediterranean rivers. *Mediterranean Marine Science*, *15*. https://doi.org/10.12681/mms.609

Kerr, N. R. (2017). *Links between personality and individual niche in the freshwater fish Gobiomorphus cotidianus* [Master of Science, Otago University].

Kjeldgaard, M. K., Hewlett, J. A., & Eubanks, M. D. (2021). Widespread variation in stable isotope trophic position estimates: patterns, causes, and potential consequences. *Ecological Monographs*, *91*(3). https://doi.org/https://doi.org/10.1002/ecm.1451

Kolar, C. (2004). Risk assessment and screening for potentially invasive fishes. *New Zealand Journal of Marine and Freshwater Research*, *38*(3), 391-397. https://doi.org/10.1080/00288330.2004.9517247

Kolar, C. S., & Lodge, D. M. (2001). Progress in invasion biology: predicting invaders. *Trends in Ecology & Evolution*, *16*(4), 199-204. https://doi.org/https://doi.org/10.1016/S0169-5347(01)02101-2

Komak, S., & Crossland, M. R. (2000). An assessment of the introduced mosquitofish (*Gambusia affinis holbrooki*) as a predator of eggs, hatchlings and tadpoles of native and non-native anurans. *Wildlife Research*, *27*(2), 185-189. https://doi.org/https://doi.org/10.1071/WR99028

Kristensen, E. A., & Closs, G. P. (2004). Anti-predator response of naïve and experienced common bully to chemical alarm cues. *Journal of Fish Biology*, *64*(3), 643-652. https://doi.org/https://doi.org/10.1111/j.1095-8649.2004.00328.x

Krumholz, L. A. (1944). Northward acclimatization of the western mosquitofish, *Gambusia affinis affinis*. *Copeia*, *1944*(2), 82-85. https://doi.org/10.2307/1438757

Lacerot, G., Kruk, C., Lürling, M., & Scheffer, M. (2013). The role of subtropical zooplankton as grazers of phytoplankton under different predation levels. *Freshwater Biology*, *58*(3), 494-503. https://doi.org/https://doi.org/10.1111/fwb.12075

Laha, M., & Mattingly, H. T. (2007). Ex situ evaluation of impacts of invasive mosquitofish on the imperiled Barrens topminnow. *Environmental Biology of Fishes*, *78*(1), 1-11. https://doi.org/10.1007/s10641-006-9040-5

Landman, M. J., Van Den Heuvel, M. R., & Ling, N. (2005). Relative sensitivities of common freshwater fish and invertebrates to acute hypoxia. *New Zealand Journal of Marine and Freshwater Research*, *39*(5), 1061-1067. https://doi.org/10.1080/00288330.2005.9517375

Lau, D. C. P., Leung, K. M. Y., & Dudgeon, D. (2009). What does stable isotope analysis reveal about trophic relationships and the relative importance of allochthonous and autochthonous resources in tropical streams? A synthetic study from Hong Kong. *Freshwater Biology*, *54*(1), 127-141. https://doi.org/https://doi.org/10.1111/j.1365-2427.2008.02099.x

Lee, F., Simon, K. S., & Perry, G. L. W. (2017). Increasing agricultural land use is associated with the spread of an invasive fish (*Gambusia affinis*). *Science of the Total Environment*, *586*, 1113-1123. https://doi.org/10.1016/j.scitotenv.2017.02.101

Lee, F., Simon, K. S., & Perry, G. L. W. (2018). Prey selectivity and ontogenetic diet shift of the globally invasive western mosquitofish (*Gambusia affinis*) in agriculturally impacted streams. *Ecology of Freshwater Fish*, *27*(3), 822-833. https://doi.org/https://doi.org/10.1111/eff.12395

Lee, J.-Y., Kim, J.-K., Owen, J. S., Choi, Y., Shin, K., Jung, S., & Kim, B. (2013). Variation in carbon and nitrogen stable isotopes in POM and zooplankton in a deep reservoir and relationship to hydrological characteristics. *Journal of Freshwater Ecology*, *28*(1), 47-62. https://doi.org/10.1080/02705060.2012.689999

Lehmann, M. F., Bernasconi, S. M., McKenzie, J. A., Barbieri, A., Simona, M., & Veronesi, M. (2004). Seasonal variation of the δC and δN of particulate and dissolved carbon and nitrogen in Lake Lugano: Constraints on biogeochemical cycling in a eutrophic lake. *Limnology and Oceanography*, *49*(2), 415-429. https://doi.org/https://doi.org/10.4319/lo.2004.49.2.0415

Lehmann, M. K., Hamilton, D. P., Muraoka, K., Tempero, G. W., Collier, K. J., & Hicks, B. J. (2017). *Waikato shallow lakes modelling* (ERI Report 94). Environmental Research Institute & University of Waikato, Hamilton, New Zealand

Leprieur, F., Brosse, S., García-Berthou, E., Oberdorff, T., Olden, J. D., & Townsend, C. R. (2009). Scientific uncertainty and the assessment of risks posed by non-native freshwater fishes. *Fish and Fisheries*, *10*(1), 88-97. https://doi.org/https://doi.org/10.1111/j.1467-2979.2008.00314.x

Linden, A. L., & Cech, J. J., Jr. (1990). Prey selection by mosquitofish (*Gambusia affinis*) in California rice fields: effect of vegetation and prey species. *Journal of the American Mosquito Control Association*, *6*(1), 115-120.

Ling, N. (2001). *New Zealand mudfishes: a guide*. Department of Conservation, Wellington, New Zealand.

Ling, N. (2004). *Gambusia* in New Zealand: really bad or just misunderstood? *New Zealand Journal of Marine and Freshwater Research*, *38*(3), 473-480. https://doi.org/10.1080/00288330.2004.9517254

Ling, N. (2010). Socio-economic drivers of freshwater fish declines in a changing climate: a New Zealand perspective. *Journal of Fish Biology*, *77*(8), 1983-1992. https://doi.org/10.1111/j.1095-8649.2010.02776.x

Ling, N., & Willis, K. (2005). Impacts of mosquitofish, *Gambusia affinis*, on black mudfish, *Neochanna diversus*. *New Zealand Journal of Marine and Freshwater Research*, *39*(6), 1215-1223. https://doi.org/10.1080/00288330.2005.9517387

Logan, J. M., Jardine, T. D., Miller, T. J., Bunn, S. E., Cunjak, R. A., & Lutcavage, M. E. (2008). Lipid corrections in carbon and nitrogen stable isotope analyses: comparison of chemical extraction and modelling methods. *Journal of Animal Ecology*, *77*(4), 838-846. https://doi.org/https://doi.org/10.1111/j.1365-2656.2008.01394.x

Lopez van Oosterom, M. V., Ocón, C., Ferreira, A. C., & Rodrigues Capitulo, A. (2016). The diet of *Pomacea canaliculata* (Gastropoda: Ampullariidae) in its native habitat based on gut content and stable isotope analysis. *INTROPICA*, *11*(1), 73-83.

Lowe, D. (2014). How Lake Rotoroa [Hamilton Lake] was formed: influence of the ancestral Waikato River. In J. Taylor (Ed.), *Hamilton Lake - City Playground* (pp. 6-13). Jeff Taylor, Hamilton, New Zealand.

Lowe, S. R., Woodford, D. J., Impson, D. N., & Day, J. A. (2008). The impact of invasive fish and invasive riparian plants on the invertebrate fauna of the Rondegat River, Cape Floristic Region, South Africa. *African Journal of Aquatic Science*, *33*(1), 51-62. https://doi.org/10.2989/AJAS.2007.33.1.6.390

Ludgate, B. G., & Closs, G. P. (2003). Responses of fish communities to sustained removals of perch (*Perca fluviatilis*). *Science for Conservation*(210), 38.

Lydeard, C., & Belk, M. C. (1993). Management of indigenous fish species impacted by introduced mosquitofish: an experimental approach. *The Southwestern Naturalist*, *38*(4), 370-373. https://doi.org/10.2307/3671617

MacCrimmon, H. R., & Marshall, T. L. (1968). World distribution of brown trout, *Salmo trutta*. *Journal of the Fisheries Research Board of Canada*, *25*(12), 2527-2548. https://doi.org/10.1139/f68-225

Macdonald, J., & Tonkin, Z. (2008). *A review of the impact of eastern gambusia on native fishes of the Murray-Darling Basin*. Murray-Darling Basin Authority, Canberra, Australia.

Macdonald, J., Tonkin, Z., Ramsey, D., Kaus, A., King, A., & Crook, D. (2012). Do invasive eastern gambusia (*Gambusia holbrooki*) shape wetland fish assemblage structure in south-eastern Australia? *Marine and Freshwater Research*, *63*. https://doi.org/10.1071/MF12019

Macinnis-Ng, C., McIntosh, A. R., Monks, J. M., Waipara, N., White, R. S. A., Boudjelas, S., Clark, C. D., Clearwater, M. J., Curran, T. J., Dickinson, K. J. M., Nelson, N., Perry, G. L. W., Richardson, S. J., Stanley, M. C., & Peltzer, D. A. (2021). Climate-change impacts exacerbate conservation threats in island systems: New Zealand as a case study. *Frontiers in Ecology and the Environment*, *19*(4), 216-224. https://doi.org/https://doi.org/10.1002/fee.2285

Mack, H. R., Conroy, J. D., Blocksom, K. A., Stein, R. A., & Ludsin, S. A. (2012). A comparative analysis of zooplankton field collection and sample enumeration methods. *Limnology and Oceanography: Methods*, *10*(1), 41-53. https://doi.org/https://doi.org/10.4319/lom.2012.10.41

Mansfield, S., & McArdle, B. H. (1998). Dietary composition of *Gambusia affinis* (family Poeciliidae) populations in the northern Waikato region of New Zealand. *New Zealand Journal of Marine and Freshwater Research*, *32*(3), 375-383. https://doi.org/10.1080/00288330.1998.9516832

Margaritora, F., Ferrara, O., & Vagaggini, D. (2001). Predatory impact of the mosquitofish (*Gambusia holbrooki* Girard) on zooplanktonic populations in a pond at Tenuta di Castelporziano (Rome, Central Italy). *Journal of Limnology*, *60*, 189-193. https://doi.org/10.4081/jlimnol.2001.1.189

Marshall, J. C., Blessing, J. J., Clifford, S. E., Hodges, K. M., Negus, P. M., & Steward, A. L. (2019). Ecological impacts of invasive carp in Australian dryland rivers. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *29*(11), 1870-1889. https://doi.org/https://doi.org/10.1002/aqc.3206

Marshall, J. D., Brooks, J. R., & Lajtha, K. (2007). Sources of variation in the stable isotopic composition of plants. In R. Michener & K. Lajtha (Eds.), *Stable Isotopes in Ecology and Environmental Science, Second Edition* (pp. 22-60). Blackwell Publishing Ltd. https://doi.org/10.1002/9780470691854.ch2

Martin, R. G. (1975). Sexual and aggressive behavior, density and social structure in a natural population of mosquitofish, *Gambusia affinis holbrooki*. *Copeia*, *1975*(3), 445-454. https://doi.org/10.2307/1443641

Matthews, B., & Mazumder, A. (2003). Compositional and interlake variability of zooplankton affect baseline stable isotope signatures. *Limnology and Oceanography*, *48*(5), 1977-1987. https://doi.org/https://doi.org/10.4319/lo.2003.48.5.1977

Matthews, B., & Mazumder, A. (2007). Distinguishing trophic variation from seasonal and size-based isotopic ($\delta^{15}N$) variation of zooplankton. *Canadian Journal of Fisheries and Aquatic Sciences*, *64*(1), 74-83. https://doi.org/10.1139/f06-168

Matveev, V., Matveeva, L., & Jones, G. (2002). Relative impacts of *Daphnia* grazing and direct stimulation by fish on phytoplankton abundance in mesocosm communities. *Freshwater Biology*, *44*, 375-385. https://doi.org/10.1046/j.1365-2427.2000.t01-1-00574.x

Mazumder, D., Williams, R. J., Reid, D., Saintilan, N., & Szymczak, R. (2008). Variability of stable isotope ratios of glassfish (*Ambassis jacksoniensis*) from mangrove/saltmarsh environments in southeast Australia and implications for choosing sample size. *Environmental Bioindicators*, *3*(2), 114-123. https://doi.org/10.1080/15555270802266003

McCarter, N. H. (1986). Food and energy in the diet of brown and rainbow trout from Lake Benmore, New Zealand. *New Zealand Journal of Marine and Freshwater Research*, *20*(4), 551-559. https://doi.org/10.1080/00288330.1986.9516175

McCarthy, T. M., & Fisher, W. A. (2000). Multiple predator-avoidance behaviours of the freshwater snail *Physella heterostropha pomila*: responses vary with risk. *Freshwater Biology*, *44*(3), 387-397. https://doi.org/https://doi.org/10.1046/j.1365-2427.2000.00576.x

McDonald, A. E. (2007). *Improving the success of a translocation of black mudfish (Neochanna diversus)* [Master of Science, University of Waikato].

McDowall, R. M. (1968). Interactions of the native and alien faunas of New Zealand and the problem of fish introductions. *Transactions of the American Fisheries Society*, *97*(1), 1-11. https://doi.org/https://doi.org/10.1577/1548-8659(1968)97[1:IOTNAA]2.0.CO;2

McDowall, R. M. (2000). *The Reed field guide to New Zealand freshwater fishes*. Raupo Publishing (NZ) Ltd.

McDowall, R. M. (2007). On amphidromy, a distinct form of diadromy in aquatic organisms. *Fish and Fisheries*, *8*(1), 1-13. https://doi.org/https://doi.org/10.1111/j.1467-2979.2007.00232.x

McDowall, R. M. (2010). *New Zealand freshwater fishes: an historical and ecological biogeography* (Vol. 32). Springer Dordrecht.

McDowall, R. M., & Whitaker, A. H. (1975). The freshwater fishes. In G. Kuschel (Ed.), *Biogeography and Ecology in New Zealand* (pp. 689). Springer Dordrecht. https://doi.org/10.1007/978-94-010-1941-5

McHugh, P. A., McIntosh, A. R., & Jellyman, P. G. (2010). Dual influences of ecosystem size and disturbance on food chain length in streams. *Ecology Letters*, *13*(7), 881-890. https://doi.org/https://doi.org/10.1111/j.1461-0248.2010.01484.x

McIntosh, A., McHugh, P., Dunn, N., Goodman, J., Howard, S., Jellyman, P., O'Brien, L., Nyström, P., & Woodford, D. (2010). The impact of trout on galaxiid fishes in New Zealand. *New Zealand Journal of Ecology*, *34*, 195-206.

McPeek, M. A. (1992). Mechanisms of sexual selection operating on body size in the mosquitofish (*Gambusia holbrooki*). *Behavioral Ecology*, *3*(1), 1-12. https://doi.org/10.1093/beheco/3.1.1

Meffe, G. K. (1992). Plasticity of life-history characters in eastern mosquitofish (*Gambusia holbrooki*: Poeciliidae) in response to thermal stress. *Copeia*, *1992*(1), 94-102. https://doi.org/10.2307/1446539

Meffe, G. K., & Snelson, F. F. J. (1989). An ecological overview of Poeciliid fishes. In F. F. J. Snelson (Ed.), *Ecology and Evolution of Livebearing Fishes (Poeciliidae)* (pp. 13-31). Prentice Hall Engelwood Cliffs.

Meffe, G. K., Weeks, S. C., Mulvey, M., & Kandl, K. L. (1995). Genetic differences in thermal tolerance of eastern mosquitofish (*Gambusia holbrooki*; Poeciliidae) from ambient and thermal ponds. *Canadian Journal of Fisheries and Aquatic Sciences*, *52*(12), 2704-2711. https://doi.org/10.1139/f95-259

Merkley, S. S., Rader, R. B., & Schaalje, G. B. (2015). Introduced western mosquitofish (*Gambusia affinis*) reduce the emergence of aquatic insects in a desert spring. *Freshwater Science*, *34*(2), 564-573. https://doi.org/10.1086/680381

Metzke, B. A., & Pederson, C. L. (2006). Utilization of the exotic cladoceran *Daphnia lumholtzi* by *Gambusia affinis*. *Transactions of the Illinois State Academy of Science*, *99*, 7. https://link.gale.com/apps/doc/A189833486/AONE?u=anon~6df1b36&sid=googleScholar&xid=84b32a7f

Mills, M. D., Rader, R. B., & Belk, M. C. (2004). Complex interactions between native and invasive fish: the simultaneous effects of multiple negative interactions. *Oecologia*, *141*(4), 713-721. https://doi.org/10.1007/s00442-004-1695-z

Mitchell, A. J., & Kelly, A. M. (2006). The public sector role in the establishment of grass carp in the United States. *Fisheries*, *31*(3), 113-121. https://doi.org/10.1577/1548-8446(2006)31[113:TPSRIT]2.0.CO;2

Mitchell, C. P. (1986). Effects of introduced grass carp on populations of two species of small native fishes in a small lake. *New Zealand Journal of Marine and*

Freshwater Research, *20*(2), 219-230. https://doi.org/10.1080/00288330.1986.9516146

Mittelbach, G. G., & Schemske, D. W. (2015). Ecological and evolutionary perspectives on community assembly. *Trends in Ecology & Evolution*, *30*(5), 241-247. https://doi.org/https://doi.org/10.1016/j.tree.2015.02.008

Napolitano, G. E. (1999). Fatty acids as trophic and chemical markers in freshwater ecosystems. In M. T. Arts & B. C. Wainman (Eds.), *Lipids in Freshwater Ecosystems* (pp. 21-44). Springer New York. https://doi.org/10.1007/978-1-4612-0547-0_3

Nawrocki, B., McLeod, A. M., Hussey, N. E., Colborne, S. F., Papa, J. D., & Fisk, A. T. (2020). Assessing trophic position quantification methods for three piscivorous freshwater fish using stable isotopes and stomach contents. *Journal of Great Lakes Research*, *46*(3), 578-588. https://doi.org/https://doi.org/10.1016/j.jglr.2020.03.017

Nesbit, D. H., & Meffe, G. K. (1993). Cannibalism frequencies in wild populations of the eastern mosquitofish (*Gambusia holbrooki*: Poeciliidae) in South Carolina. *Copeia*, *1993*(3), 867-870. https://doi.org/10.2307/1447254

Newton, J. (2016). Stable isotopes as tools in ecological research. In *eLS* (pp. 1-8). John Wiley & Sons, Ltd https://doi.org/https://doi.org/10.1002/9780470015902.a0021231.pub2

Ning, N., Nielsen, D., Hillman, T., & Suter, P. (2009). The influence of planktivorous fish on zooplankton communities in riverine slackwaters. *Freshwater Biology*, *55*, 360-374. https://doi.org/10.1111/j.1365-2427.2009.02283.x

Norazmi-Lokman, N. H., Purser, G. J., & Patil, J. G. (2016). Gravid spot predicts developmental progress and reproductive output in a livebearing fish, *Gambusia holbrooki*. *PLoS One*, *11*(1), e0147711. https://doi.org/10.1371/journal.pone.0147711

Nygard, B. (2007). *Population ecology of Gambusia: the effect of habitat quality and interactions with inanga* [Master of Science, University of Auckland].

Odum, H. T., & Caldwell, D. K. (1955). Fish respiration in the natural oxygen gradient of an anaerobic spring in Florida. *Copeia*, *1955*(2), 104-106. https://doi.org/10.2307/1439312

Pagnucco, K., Remmal, Y., & Ricciardi, A. (2016). An invasive benthic fish magnifies trophic cascades and alters pelagic communities in an experimental freshwater system. *Freshwater Science*, *35*. https://doi.org/10.1086/685285

Parkyn, S. M., Davies-Colley, R. J., Halliday, N. J., Costley, K. J., & Croker, G. F. (2003). Planted riparian buffer zones in New Zealand: do they live up to expectations? *Restoration Ecology*, *11*(4), 436-447. https://doi.org/https://doi.org/10.1046/j.1526-100X.2003.rec0260.x

Pelicice, F. M., & Agostinho, A. A. (2009). Fish fauna destruction after the introduction of a non-native predator (*Cichla kelberi*) in a Neotropical reservoir. *Biological Invasions*, *11*(8), 1789-1801. https://doi.org/10.1007/s10530-008-9358-3

Pen, L. J., & Potter, I. C. (1991). Reproduction, growth and diet of *Gambusia holbrooki* (Girard) in a temperate Australian river. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *1*(2), 159-172. https://doi.org/https://doi.org/10.1002/aqc.3270010205

Pen, L. J., Potter, I. C., & Calver, M. C. (1993). Comparisons of the food niches of three native and two introduced fish species in an Australian river. *Environmental Biology of Fishes*, *36*(2), 167-182. https://doi.org/10.1007/BF00002797

Pennuto, C. M., Cudney, K. A., & Janik, C. E. (2018). Fish invasion alters ecosystem function in a small heterotrophic stream. *Biological Invasions*, *20*(4), 1033-1047. https://doi.org/10.1007/s10530-017-1609-8

Peterson, B. J., & Fry, B. (1987). Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics*, *18*(1), 293-320. https://doi.org/10.1146/annurev.es.18.110187.001453

Phillips, D. L., Inger, R., Bearhop, S., Jackson, A. L., Moore, J. W., Parnell, A. C., Semmens, B. X., & Ward, E. J. (2014). Best practices for use of stable isotope mixing models in food-web studies. *Canadian Journal of Zoology*, *92*(10), 823-835. https://doi.org/10.1139/cjz-2014-0127

Pilastro, A., Benetton, S., & Bisazza, A. (2003). Female aggregation and male competition reduce costs of sexual harassment in the mosquitofish *Gambusia holbrooki*. *Animal Behaviour*, *65*(6), 1161-1167. https://doi.org/https://doi.org/10.1006/anbe.2003.2118

Pingram, M. A. (2005). *Ecology of freshwater fish in the littoral zone of Lake Waikere, Kai Iwi Lakes, Northland, New Zealand: for the conservation of the dune lakes galaxias (Galaxias sp.)* [Master of Science, University of Otago].

Pingram, M. A., Collier, K. J., Özkundakci, D., & Garrett-Walker, J. (2020). Food web characteristics of fish communities across degraded lakes provide insights for management in multi-stressor environments. *Aquatic Ecology*, *54*, 1-19. https://doi.org/10.1007/s10452-020-09750-4

Post, D. M. (2002). Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology*, *83*(3), 703-718. https://doi.org/https://doi.org/10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2

Preston, D. L., Hedman, H. D., Esfahani, E. R., Pena, E. M., Boland, C. E., Lunde, K. B., & Johnson, P. T. J. (2017). Responses of a wetland ecosystem to the controlled introduction of invasive fish. *Freshwater Biology*, *62*(4), 767-778. https://doi.org/https://doi.org/10.1111/fwb.12900

Preston, D. L., Hedman, H. D., & Johnson, P. T. J. (2018). Nutrient availability and invasive fish jointly drive community dynamics in an experimental aquatic system. *Ecosphere*, *9*(3). https://doi.org/https://doi.org/10.1002/ecs2.2153

Pyke, G. H. (2005). A review of the biology of *Gambusia affinis* and *G. holbrooki*. *Reviews in Fish Biology and Fisheries*, *15*(4), 339-365. https://doi.org/10.1007/s11160-006-6394-x

Pyke, G. H. (2008). Plague minnow or mosquito fish? A review of the biology and impacts of introduced *Gambusia* species. *Annual Review of Ecology, Evolution, and Systematics*, *39*, 171-191. http://www.jstor.org/stable/30245159

Quinn, J. M., & Stroud, M. J. (2002). Water quality and sediment and nutrient export from New Zealand hill‐land catchments of contrasting land use. *New Zealand Journal of Marine and Freshwater Research*, *36*(2), 409-429. https://doi.org/10.1080/00288330.2002.9517097

Rauchenberger, M. (1988). *Systematics and biogeography of the genus Gambusia (Cyprinodontiformes: Poeciliidae)* [Doctor of Philosophy, City University of New York].

Rautio, M., Mariash, H., & Forsström, L. (2011). Seasonal shifts between autochthonous and allochthonous carbon contributions to zooplankton diets in a subarctic lake. *Limnology and Oceanography*, *56*(4), 1513-1524. https://doi.org/https://doi.org/10.4319/lo.2011.56.4.1513

Raven, J. A., Johnston, A. M., Kübler, J. E., Korb, R., McInroy, S. G., Handley, L. L., Scrimgeour, C. M., Walker, D. I., Beardall, J., Vanderklift, M., Fredriksen, S., & Dunton, K. H. (2002). Mechanistic interpretation of carbon isotope discrimination by marine macroalgae and seagrasses. *Functional Plant Biology*, *29*(3), 355-378. https://doi.org/10.1071/pp01201

Rees, A. B. H., Holt, K. A., Hinojosa, J. L., Newnham, R. M., Eaves, S., Vandergoes, M. J., Sessions, A. L., & Wilmshurst, J. M. (2021). Duelling narratives of chironomids and pollen explain climate enigmas during the last glacial-interglacial transition in North Island New Zealand. *Quaternary Science Reviews*, *263*(106997). https://doi.org/https://doi.org/10.1016/j.quascirev.2021.106997

Rehage, J. S., Barnett, B. K., & Sih, A. (2005). Behavioral responses to a novel predator and competitor of invasive mosquitofish and their non-invasive relatives (*Gambusia* sp.). *Behavioral Ecology and Sociobiology*, *57*(3), 256-266. https://doi.org/10.1007/s00265-004-0850-1

Rehage, J. S., Lopez, L. K., & Sih, A. (2020). A comparison of the establishment success, response to competition, and community impact of invasive and non-invasive *Gambusia* species. *Biological Invasions, 22*(2), 509-522. https://doi.org/10.1007/s10530-019-02113-7

Rehage, J. S., & Sih, A. (2004). Dispersal behavior, boldness, and the link to invasiveness: a comparison of four *Gambusia* species. *Biological Invasions*, *6*(3), 379-391. https://doi.org/10.1023/B:BINV.0000034618.93140.a5

Reid, A. J., Carlson, A. K., Creed, I. F., Eliason, E. J., Gell, P. A., Johnson, P. T. J., Kidd, K. A., MacCormack, T. J., Olden, J. D., Ormerod, S. J., Smol, J. P., Taylor, W. W., Tockner, K., Vermaire, J. C., Dudgeon, D., & Cooke, S. J. (2019). Emerging threats and persistent conservation challenges for freshwater biodiversity. *Biological Reviews*, *94*(3), 849-873. https://doi.org/https://doi.org/10.1111/brv.12480

Remon, J., Bower, D. S., Gaston, T. F., Clulow, J., & Mahony, M. J. (2016). Stable isotope analyses reveal predation on amphibians by a globally invasive fish (*Gambusia holbrooki*). *Aquatic Conservation: Marine and Freshwater Ecosystems, 26*(4), 724-735. https://doi.org/https://doi.org/10.1002/aqc.2631

Rettig, J. E., & Smith, G. R. (2021). Relative strength of top-down effects of an invasive fish and bottom-up effects of nutrient addition in a simple aquatic food web. *Environmental Science and Pollution Research, 28*(5), 5845-5853. https://doi.org/10.1007/s11356-020-10933-7

Reznick, D. N., & Braun, B. (1987). Fat cycling in the mosquitofish (*Gambusia affinis*): fat storage as a reproductive adaptation. *Oecologia*, *73*(3), 401-413. https://doi.org/10.1007/BF00385257

Ricciardi, A., & Kipp, R. (2008). Predicting the number of ecologically harmful exotic species in an aquatic system. *Diversity and Distributions*, *14*(2), 374-380. https://doi.org/https://doi.org/10.1111/j.1472-4642.2007.00451.x

Ricciardi, A., & Rasmussen, J. B. (1999). Extinction rates of North American freshwater fauna. *Conservation Biology*, *13*(5), 1220-1222.

Richardson, J., Boubée, J. A. T., & West, D. W. (1994). Thermal tolerance and preference of some native New Zealand freshwater fish. *New Zealand Journal of Marine and Freshwater Research*, *28*(4), 399-407. https://doi.org/10.1080/00288330.1994.9516630

Rincón, P. A., Correas, A. M., Morcillo, F., Risueño, P., & Lobón-Cerviá, J. (2002). Interaction between the introduced eastern mosquitofish and two autochthonous Spanish toothcarps. *Journal of Fish Biology*, *61*(6), 1560-1585. https://doi.org/https://doi.org/10.1111/j.1095-8649.2002.tb02498.x

Rivas, L. R. (1963). Subgenera and species groups in the Poeciliid fish genus *Gambusia* Poey. *Copeia*, *1963*(2), 331-347. https://doi.org/10.2307/1441352

Rosen, D. E., & Bailey, R. M. (1963). The poeciliid fishes (Cyprinodontiformes) : their structure, zoogeography, and systematics. Bulletin of the AMNH ; v. 126, article 1.

Rowe, D. K. (1999). Factors influencing the abundance of the common bully, *Gobiomorphus cotidianus* McDowall, in small, North Island, New Zealand, lakes. *Fisheries Management and Ecology*, *6*(5), 377-386. https://doi.org/https://doi.org/10.1046/j.1365-2400.1999.00162.x

Rowe, D. K., & Chisnall, B. L. (1996). Size-related differences in diel feeding activity, prey selection and nocturnal migration strategy for the planktonic larvae of *Gobiomorphus cotidianus* in Lake Rotoiti (NI), New Zealand. *Archiv für Hydrobiologie*, *135*(4), 485-497. https://doi.org/10.1127/archiv-hydrobiol/135/1996/485

Rowe, D. K., Nichols, S., & Kelly, G. R. (2001). Depth distribution and abundance of the common bully, *Gobiomorphus cotidianus* (Eleotridae), in three oligotrophic New Zealand Lakes, one of which is turbid. *Environmental Biology of Fishes*, *61*(4), 407-418. https://doi.org/10.1023/A:1011675602774

Rowe, D. K., Smith, J. P., & Baker, C. (2007). Agonistic interactions between *Gambusia affinis* and *Galaxias maculatus*: implications for whitebait fisheries in New Zealand rivers. *Journal of Applied Ichthyology*, *23*(6), 668-674. https://doi.org/https://doi.org/10.1111/j.1439-0426.2007.00912.x

Rowe, D. K., & Wilding, T. (2012). Risk assessment model for the introduction of non-native freshwater fish into New Zealand. *Journal of Applied Ichthyology*, *28*(4), 582-589. https://doi.org/https://doi.org/10.1111/j.1439-0426.2012.01966.x

Ruesink, J. (2005). Global analysis of factors affecting the outcome of freshwater fish introductions. *Conservation Biology*, *19*, 1883-1893. https://doi.org/10.1111/j.1523-1739.2005.00267.x-i1

Ruiz-Navarro, A., Cubedo, D., Torralva, M., & Oliva-Paterna, F. (2013a). Removal control of the highly invasive fish *Gambusia holbrooki* and effects on its population biology: Learning by doing. *CSIRO Wildlife Research*, *40*, 82-89. https://doi.org/10.1071/WR12172

Ruiz-Navarro, A., Moreno-Valcárcel, R., Torralva, M., & Oliva-Paterna, F. J. (2011). Life-history traits of the invasive fish *Gambusia -holbrooki* in saline streams (SE Iberian Peninsula): Does salinity limit its invasive success? *Aquatic Biology*, *13*(2), 149-161. https://www.int-res.com/abstracts/ab/v13/n2/p149-161/

Ruiz-Navarro, A., Torralva, M., & Oliva-Paterna, F. J. (2013b). Trophic overlap between cohabiting populations of invasive mosquitofish and an endangered toothcarp at changing salinity conditions. *Aquatic Biology*, *19*(1), 1-11. https://www.int-res.com/abstracts/ab/v19/n1/p1-11/

Rupp, H. R. (1996). Adverse assessments of *Gambusia affinis*: an alternate view for mosquito control practitioners. *Journal of the American Mosquito Control Association*, *12*(2), 155-166.

Sagar, P., & Glova, G. (1994). Food partitioning by small fish in a coastal New Zealand stream. *New Zealand Journal of Marine and Freshwater Research*, *28*, 429-436. https://doi.org/10.1080/00288330.1994.9516633

Sagar, P. M., & Eldon, G. A. (1983). Food and feeding of small fish in the Rakaia River, New Zealand. *New Zealand Journal of Marine and Freshwater Research*, *17*(3), 213-226. https://doi.org/10.1080/00288330.1983.9515999

Sagrario, G., Ángeles, M. d. l., & Balseiro, E. (2010). The role of macroinvertebrates and fish in regulating the provision by macrophytes of refugia for zooplankton in a warm temperate shallow lake. *Freshwater Biology*, *55*(10), 2153-2166. https://doi.org/https://doi.org/10.1111/j.1365-2427.2010.02475.x

Sagrario, G., Ángeles, M. d. l., Balseiro, E., Ituarte, R., & Spivak, E. (2009). Macrophytes as refuge or risky area for zooplankton: a balance set by littoral predacious macroinvertebrates. *Freshwater Biology*, *54*(5), 1042-1053. https://doi.org/https://doi.org/10.1111/j.1365-2427.2008.02152.x

Sakano, H., & Iguchi, K. i. (2009). Food web structure composed of alien fishes in Okinawa Island, Japan: a stable isotope approach. *Journal of Freshwater Ecology*, *24*(3), 357-365. https://doi.org/10.1080/02705060.2009.9664307

Sampson, S. J., Chick, J. H., & Pegg, M. A. (2009). Diet overlap among two Asian carp and three native fishes in backwater lakes on the Illinois and Mississippi rivers. *Biological Invasions*, *11*(3), 483-496. https://doi.org/10.1007/s10530-008-9265-7

Sass, G. G., Hinz, C., Erickson, A. C., McClelland, N. N., McClelland, M. A., & Epifanio, J. M. (2014). Invasive bighead and silver carp effects on zooplankton communities in the Illinois River, Illinois, USA. *Journal of Great Lakes Research*, *40*(4), 911-921. https://doi.org/https://doi.org/10.1016/j.jglr.2014.08.010

Sayer, C. D., Copp, G. H., Emson, D., Godard, M. J., Zięba, G., & Wesley, K. J. (2011). Towards the conservation of crucian carp *Carassius carassius*: understanding the extent and causes of decline within part of its native English range. *Journal of Fish Biology*, *79*(6), 1608-1624. https://doi.org/https://doi.org/10.1111/j.1095-8649.2011.03059.x

Schaefer, J. F., Heulett, S. T., & Farrell, T. M. (1994). Interactions between two poeciliid fishes (*Gambusia holbrooki* and *Heterandria formosa*) and their prey in a Florida marsh. *Copeia*, *1994*(2), 516-520. https://doi.org/10.2307/1447002

Schrank, S. J., Guy, C. S., & Fairchild, J. F. (2003). Competitive interactions between age-0 bighead carp and paddlefish. *Transactions of the American Fisheries Society*, *132*(6), 1222-1228. https://doi.org/10.1577/T02-071

Sharpe, D. M. T., De León, L. F., González, R., & Torchin, M. E. (2017). Tropical fish community does not recover 45 years after predator introduction. *Ecology*, *98*(2), 412-424. https://doi.org/https://doi.org/10.1002/ecy.1648

Shiel, R. (1995). *A guide to identification of rotifers, cladocerans and copepods from Australian inland waters.* Co-operative Research Centre for Freshwater Ecology, Murray-Darling Freshwater Research Centre.

Simberloff, D., Parker, I. M., & Windle, P. N. (2005). Introduced species policy, management, and future research needs. *Frontiers in Ecology and the Environment*, *3*(1), 12-20. https://doi.org/https://doi.org/10.1890/1540-9295(2005)003[0012:ISPMAF]2.0.CO;2

Singh, N., & Gupta, P. (2010). Food and feeding habits of an introduced mosquitofish, *Gambusia holbrooki* (Girard) (Poeciliidae) in a subtropical lake, Lake Nainital, India. *Asian Fisheries Science*, *23*, 355-366355. https://doi.org/10.33997/j.afs.2010.23.3.007

Skinner, M. M., Cross, B. K., & Moore, B. C. (2017). Estimating in situ isotopic turnover in rainbow trout (*Oncorhynchus mykiss*) muscle and liver tissue. *Journal of*

Freshwater Ecology, *32*(1), 209-217. https://doi.org/10.1080/02705060.2016.1259127

Sloterdijk, H., James, N. C., Smith, M. K. S., Ekau, W., & Weyl, O. L. F. (2015). Population dynamics and biology of an invasive population of mosquitofish *Gambusia affinis* in a temperate estuarine lake system. *African Zoology, 50*(1), 31-40. https://doi.org/10.1080/15627020.2015.1021169

Smith, B. N., & Epstein, S. (1971). Two categories of $^{13}C/^{12}C$ ratios for higher plants 1. *Plant Physiology, 47*(3), 380-384. https://doi.org/10.1104/pp.47.3.380

Smith, G., & Smith, L. (2015). Effects of western mosquitofish (*Gambusia affinis*) on tadpole production of gray treefrogs (*Hyla versicolor*). *Herpetological Conservation and Biology*, *10*, 723-727.

Specziár, A. (2004). Life history pattern and feeding ecology of the introduced eastern mosquitofish, *Gambusia holbrooki,* in a thermal spa under temperate climate, of Lake Hévíz, Hungary. *Hydrobiologia*, *522*, 249-260.

Špoljar, M., Dražina, T., Kahriman, K., Medić, N., & Cvetnić, M. (2019). The impact of invasive eastern mosquitofish (*Gambusia holbrooki* Girard, 1859) on zooplankton in small Mediterranean ponds. In B. Mioč & I. Širić (Eds.), *54th Croatian & 14th International Symposium on Agriculture* (pp. 411-417). University of Zagreb.

Srean, P., Almeida, D., Rubio-Gracia, F., Luo, Y., & García-Berthou, E. (2017). Effects of size and sex on swimming performance and metabolism of invasive mosquitofish *Gambusia holbrooki*. *Ecology of Freshwater Fish*, *26*(3), 424-433. https://doi.org/https://doi.org/10.1111/eff.12286

Staples, D. (1975). Production biology of the upland bully *Philypnodon breviceps* Stokell in a small New Zealand lake. *Journal of Fish Biology*, *7*, 1-24. https://doi.org/10.1111/j.1095-8649.1975.tb04573.x

Stephens, R. T. T. (1978). *The biology of Gobiomorphus cotidianus (Pisces: Eleotridae) in Lake Waahi* [Master of Science, University of Waikato].

Stephens, R. T. T. (1982). Reproduction, growth and mortality of the common bully, *Gobiomorphus cotidianus* McDowall, in a eutrophic New Zealand lake. *Journal of Fish Biology*, *20*(3), 259-270. https://doi.org/https://doi.org/10.1111/j.1095-8649.1982.tb04707.x

Stevens, M., & Hicks, B. (2009). Mitochondrial DNA reveals monophyly of New Zealand's *Gobiomorphus* (Teleostei: Eleotridae) amongst a morphological complex. *Evolutionary Ecology Research*, *11*, 109-123.

Stewart, S. D., Hamilton, D. P., Baisden, W. T., Dedual, M., Verburg, P., Duggan, I. C., Hicks, B. J., & Graham, B. S. (2017). Variable littoral-pelagic coupling as a food-web response to seasonal changes in pelagic primary production. *Freshwater Biology*, *62*(12), 2008-2025. https://doi.org/https://doi.org/10.1111/fwb.13046

Sulieman, Y., Ahmed, A., Pengsakul, T., & Afifi, A. (2016). Laboratory evaluation of the biological control of the snail, *Biomphalaria pfeifferi*, the intermediate host of *Schistosoma mansoni*, using the fish, *Gambusia affinis*. *Egyptian Journal of Biological Pest Control*, *25(2)*, 351-354.

Swales, S., & West, D. W. (1991). Distribution, abundance and conservation status of native fish in some Waikato streams in the North Island of New Zealand. *Journal of the Royal Society of New Zealand*, *21*(4), 281-296. https://doi.org/10.1080/03036758.1991.10420827

Swanson, C., Cech Jr, J., & Piedrahita, R. H. (1996). *Mosquitofish: biology, culture, and use in mosquito control*. Mosquito and Vector Control Association of California and the University of California Mosquito Research Program, Davis, California, USA.

Syväranta, J., Tiirola, M., & Jones, R. (2008). Seasonality in lake pelagic δ^{15}N values: Patterns, possible explanations, and implications for food web baselines. *Fundamental and Applied Limnology / Archiv für Hydrobiologie*, *172*, 255-262. https://doi.org/10.1127/1863-9135/2008/0172-0255

Taipale, S., Kankaala, P., Tiirola, M., & Jones, R. I. (2008). Whole-lake dissolved inorganic ^{13}C additions reveal seasonal shifts in zooplankton diet. *Ecology*, *89*(2), 463-474. http://www.jstor.org/stable/27651558

Taylor, M. J., Graynoth, E., & James, G. D. (2000). Abundance and daytime vertical distribution of planktonic fish larvae in an oligotrophic South Island lake. *Hydrobiologia*, *421*(1), 41-46. https://doi.org/10.1023/A:1003965116777

Tempero, G., & Hicks, B. J. (2017). *Responses of the fish community and biomass in Lake Ohinewai to fish removal and the koi carp exclusion barrier* (Technical Report 2017/10). Waikato Regional Council, Hamilton, New Zealand. https://www.waikatoregion.govt.nz/assets/WRC/WRC-2019/tr201710.pdf

Tempero, G. W., Ling, N., Hicks, B. J., & Osborne, M. W. (2006). Age composition, growth, and reproduction of koi carp (*Cyprinus carpio*) in the lower Waikato region, New Zealand. *New Zealand Journal of Marine and Freshwater Research*, *40*(4), 571-583. https://doi.org/10.1080/00288330.2006.9517446

Thacker, C., Geiger, D. L., & Shelley, J. J. (2021a). Two new cryptic species of the freshwater fish genus *Gobiomorphus* (Gobiiformes: Gobioidei: Eleotridae) in New Zealand. *New Zealand Journal of Marine and Freshwater Research*, 1-17. https://doi.org/10.1080/00288330.2021.2007959

Thacker, C., Shelley, J., McCraney, W., Unmack, P., & McGee, M. (2021b). Delayed adaptive radiation among New Zealand stream fishes: joint estimation of divergence time and trait evolution in a newly delineated island species flock. *Systematic Biology*, *71*. https://doi.org/10.1093/sysbio/syab014

Thibault, R. E., & Schultz, R. J. (1978). Reproductive adaptations among viviparous fishes (Cyprinodontiformes: Poeciliidae). *Evolution, 32*(2), 320-333. https://doi.org/10.2307/2407600

Thompson, K., & Champion, P. (1993). *Esplenade reserve recommendations for lakes Serpentine, Mangahia, Rotomanuka, Ruatuna, and Cameron (Waipa District)* (Conservation Advisory Science Notes). Department of Conservation, Wellington, New Zealand. https://www.doc.govt.nz/globalassets/documents/science-and-technical/casn47.pdf

Thomsen, M., Olden, J., Wernberg, T., Griffin, J., & Silliman, B. (2011). A broad framework to organize and compare ecological invasion impacts. *Environmental Research, 111*, 899-908. https://doi.org/10.1016/j.envres.2011.05.024

Tonkin, Z., Ramsey, D. S. L., Macdonald, J., Crook, D., King, A. J., & Kaus, A. (2014). Does localized control of invasive eastern gambusia (Poeciliidae: *Gambusia holbrooki*) increase population growth of generalist wetland fishes? *Austral Ecology, 39*(3), 355-366. https://doi.org/https://doi.org/10.1111/aec.12088

Townsend, C. R. (1996). Invasion biology and ecological impacts of brown trout *Salmo trutta* in New Zealand. *Biological Conservation, 78*(1), 13-22. https://doi.org/https://doi.org/10.1016/0006-3207(96)00014-6

Townsend, C. R., & Winfield, I. J. (1985). The application of Optimal Foraging Theory to feeding behaviour in fish. In P. Tytler & P. Calow (Eds.), *Fish Energetics New Perspectives* (pp. 67-99). Croom Helm.

Trochine, C., Guerrieri, M., Liboriussen, L., Willems, P., Lauridsen, T., Søndergaard, M., & Jeppesen, E. (2017). Factors controlling the stable isotope composition and C:N ratio of seston and periphyton in shallow lake mesocosms with contrasting nutrient loadings and temperatures. *Freshwater Biology, 62*. https://doi.org/10.1111/fwb.12971

Tsang, A. H. F., & Dudgeon, D. (2021). Do exotic poeciliids affect the distribution or trophic niche of native fishes? Absence of evidence from Hong Kong streams. *Freshwater Biology*, *66*(9), 1751-1764. https://doi.org/https://doi.org/10.1111/fwb.13789

Valéry, L., Fritz, H., Lefeuvre, J. C., & Simberloff, D. (2007). In search of a real definition of the biological invasion phenomenon itself. *Biological Invasions*, *10*, 1345-1351.

Vander Zanden, H. B., Soto, D. X., Bowen, G. J., & Hobson, K. A. (2016). Expanding the isotopic toolbox: applications of hydrogen and oxygen stable isotope ratios to food web studies. *Frontiers in Ecology and Evolution*, *4*.

Vander Zanden, M. J., Casselman, J. M., & Rasmussen, J. B. (1999). Stable isotope evidence for the food web consequences of species invasions in lakes. *Nature*, *401*(6752), 464-467. https://doi.org/10.1038/46762

Vander Zanden, M. J., Hulshof, M., Ridgway, M. S., & Rasmussen, J. B. (1998). Application of stable isotope techniques to trophic studies of age-0 smallmouth bass. *Transactions of the American Fisheries Society*, *127*(5), 729-739. https://doi.org/10.1577/1548-8659(1998)127<0729:AOSITT>2.0.CO;2

Vander Zanden, M. J., & Rasmussen, J. B. (1999). Primary consumer δ^{13}C and δ^{15}N and the trophic position of aquatic consumers. *Ecology*, *80*(4), 1395-1404. https://doi.org/https://doi.org/10.1890/0012-9658(1999)080[1395:PCCANA]2.0.CO;2

Vander Zanden, M. J., & Rasmussen, J. B. (2001). Variation in δ^{15}N and δ^{13}C trophic fractionation: Implications for aquatic food web studies. *Limnology and Oceanography*, *46*(8), 2061-2066. https://doi.org/10.4319/lo.2001.46.8.2061

Vargas, M. J., & de Sostoa, A. (1996). Life history of *Gambusia holbrooki* (Pisces, Poeciliidae) in the Ebro delta (NE Iberian peninsula). *Hydrobiologia*, *341*(3), 215-224. https://doi.org/10.1007/BF00014686

Vila-Gispert, A., Alcaraz, C., & García-Berthou, E. (2005). Life-history traits of invasive fish in small Mediterranean streams. *Biological Invasions*, *7*(1), 107-116. https://doi.org/10.1007/s10530-004-9640-y

Vondracek, B., Wurtsbaugh, W. A., & Cech, J. J. (1988). Growth and reproduction of the mosquitofish, *Gambusia affinis*, in relation to temperature and ration level: consequences for life history. *Environmental Biology of Fishes*, *21*(1), 45-57. https://doi.org/10.1007/BF02984442

Walton, W. E. (2007). Larvivorous fish including *Gambusia*. *Journal of the American Mosquito Control Association, 23*, 184-220, 137. https://doi.org/10.2987/8756-971X(2007)23[184:LFIG]2.0.CO;2

Wang, S., Wang, T.-T., Xia, W.-T., Chen, Z.-B., Stewart, S. D., Yang, F.-J., Cheng, G., Wang, X.-D., Wang, D.-Y., & Xie, S.-G. (2021). Longitudinal pattern of resource utilization by aquatic consumers along a disturbed subtropical urban river: Estimating the relative contribution of resources with stable isotope analysis. *Ecology and Evolution*, *11*(23), 16763-16775. https://doi.org/https://doi.org/10.1002/ece3.8304

Ward, A. J. W., & Mehner, T. (2010). Multimodal mixed messages: the use of multiple cues allows greater accuracy in social recognition and predator detection decisions in the mosquitofish, *Gambusia holbrooki*. *Behavioral Ecology, 21*(6), 1315-1320. https://doi.org/10.1093/beheco/arq152

Weidel, B. C., Carpenter, S. R., Kitchell, J. F., & Vander Zanden, M. J. (2011). Rates and components of carbon turnover in fish muscle: insights from bioenergetics models and a whole-lake ^{13}C addition. *Canadian Journal of Fisheries and Aquatic Sciences*, *68*(3), 387-399. https://doi.org/10.1139/F10-158

Welker, M. T., Pierce, C. L., & Wahl, D. H. (1994). Growth and survival of larval fishes: roles of competition and zooplankton abundance. *Transactions of the American Fisheries Society*, *123*(5), 703-717. https://doi.org/https://doi.org/10.1577/1548-8659(1994)123<0703:GASOLF>2.3.CO;2

Wen, Z., Xie, P., & Xu, J. (2010). Mussel isotope signature as indicator of nutrient pollution in a freshwater eutrophic lake: species, spatial, and seasonal variability. *Environmental Monitoring and Assessment*, *163*(1), 139-147. https://doi.org/10.1007/s10661-009-0823-y

Werner, E. E., & Hall, D. J. (1974). Optimal foraging and the size selection of prey by the bluegill sunfish (*Lepomis Macrochirus*). *Ecology*, *55*(5), 1042-1052. https://doi.org/10.2307/1940354

Wilhelm, F. M., Closs, G. P., & Burns, C. W. (2007). Seasonal diet and amphipod size selection of juvenile common bully, *Gobiomorphus cotidianus*, in a coastal New Zealand lake. *Hydrobiologia*, *586*(1), 303-312. https://doi.org/10.1007/s10750-007-0703-9

Wilson, K.-J. (1997). Extinct and introduced vertebrate species in New Zealand: a loss of biodistinctiveness and gain in biodiversity. *Pacific Conservation Biology, 3*(3), 301-305. https://doi.org/10.1071/PC970301

Winkler, P. (1979). Thermal preference of *Gambusia affinis affinis* as determined under field and laboratory conditions. *Copeia*, *1979*(1), 60-64. https://doi.org/10.2307/1443729

Winterbourn, M. J. (1990). Interactions among nutrients, algae and invertebrates in a New Zealand mountain stream. *Freshwater Biology*, *23*(3), 463-474. https://doi.org/https://doi.org/10.1111/j.1365-2427.1990.tb00288.x

Wood, Z. T., Shepard, I. D., Hurley, S. T., Paisker, M. R., Hensley, V. R., & Kinnison, M. T. (2020). Sex-dependent cold tolerance at the northern invasive range limit of *Gambusia affinis* on Cape Cod, Massachusetts. *Copeia*, *108*(3), 670-678. https://doi.org/10.1643/CE-19-332

Woodward, C. A., & Shulmeister, J. (2006). New Zealand chironomids as proxies for human-induced and natural environmental change: transfer functions for temperature and lake production (chlorophyll *a*). *Journal of Paleolimnology*, *36*(4), 407-429. https://doi.org/10.1007/s10933-006-9009-6

Wu, N., Daniel, A., & Tempero, G. (2013a). *Fish biomass and gonad development in the Rotopiko (Serpentine) lakes* (Environmental Research Institute Report 20). University of Waikato, Hamilton, New Zealand. https://www.waikato.ac.nz/__data/assets/pdf_file/0007/161827/ERI-Rept-20-Rotopiko-Lakes.pdf

Wu, N., Górski, K., & Daniel, A. J. (2013b). Abundance of larval native and nonnative fishes in floodplain habitats of the lower Waikato River, New Zealand. *Inland Waters*, *3*(3), 359-368. https://doi.org/10.5268/IW-3.3.550

Wurtsbaugh, W., Cech Jr, J. J., & Compton, J. (1980). Effects of fish size on prey size selection in *Gambusia affinis. Proceedings and papers of the forty-eighth annual conference of the California Mosquito and Vector Control Association* (pp. 48-51).

Yuma, M., Hosoya, K., & Nagata, Y. (1998). Distribution of the freshwater fishes of Japan: an historical overview. *Environmental Biology of Fishes*, *52*(1), 97-124. https://doi.org/10.1023/A:1007451417556

Zeiber, R. A., Sutton, T. M., & Fisher, B. E. (2008). Western mosquitofish predation on native amphibian eggs and larvae. *Journal of Freshwater Ecology*, *23*(4), 663-671. https://doi.org/10.1080/02705060.2008.9664254

Zulian, E., Bisazza, A., & Marin, G. (1995). Variations in male body size in natural populations of *Gambusia holbrooki*. *Ethology Ecology & Evolution*, *7*(1), 1-10. https://doi.org/10.1080/08927014.1995.9522966