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**IMPROVING THE SUCCESS OF A
TRANSLOCATION OF BLACK MUDFISH
(*Neochanna diversus*)**

A thesis submitted in partial fulfilment
of the requirements for the degree
of
Master of Science in Biological Sciences
at
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by
Amy Elizabeth M^cDonald



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Abstract

All of New Zealand's five endemic species of mudfish (*Neochanna*) are threatened, and translocation has been recommended as an option for conservation. This research undertakes a translocation of black mudfish (*Neochanna diversus*) into wetland margins of Lake Kaituna, in the Waikato region, and addresses research questions applicable to improving translocation success. Results from this research are intended to aid possible future translocations of the more threatened Northland mudfish (*Neochanna heleioides*) and other genetically distinct populations of black mudfish.

Captive rearing of juveniles collected from the wild is currently the most feasible option for sourcing translocation stock. Mudfish juveniles (>25 mm T.L.) had greater survival rates, compared to mudfish fry less than 25 mm T.L. Mudfish growth was far greater when fed on a combined diet of brine shrimp (*Artemia salina*) and white worms (*Enchytraeus albidus*) than when fed exclusively on brine shrimp. Temperature was found to have a small effect on mudfish growth, with a slightly greater growth in fish at 15°C than those at 10°C.

The introduced species *Gambusia affinis* has been the subject of concern for mudfish conservation and commonly found at wetland sites suitable for mudfish translocation. Found to prey on mudfish fry and eggs in aquaria, it was important to determine the effects of *Gambusia* density prior to undertaking a translocation to a location where *Gambusia* were present. Investigations were made into the effect of *Gambusia* density on black mudfish juveniles in 9 outdoor mesocosms. Increasing *Gambusia* density was found to have an inhibitory effect on black mudfish growth. This may be due to increased competition for food, a theory supported by analysis of zooplankton communities, where, in the presence of *Gambusia*, large zooplankton had been removed and smaller rotifers flourished.

Monitoring programmes are required to assess any impacts or improvements of mudfish populations, including those created by translocation. A Gee minnow trapping programme in outdoor mesocosms was conducted to test the reliability of traps, finding that water depth, mudfish density, mudfish memory and trap shyness had no effect on the trapability of mudfish. Trap position was found to have the most significant effect, with a greater number of mudfish caught when traps were set overnight at the surface than when set on the bottom of mesocosms.

Black mudfish adults and juveniles were translocated into 18 pools (~1 m diameter) on the wetland margins of Lake Kaituna in September 2006, followed by monthly monitoring. Water quality monitoring and an assessment of hydrology and vegetation was undertaken. Habitat characterisation was found to be a key factor, with correlations between water quality data and trapping results finding fewer fish remaining in pools with less suitable characteristics for mudfish (e.g. high turbidity and conductivity). Other species were found to have a large impact, with predation by shortfinned eels (*Anguilla australis*) thought to have eliminated mudfish from some pools. In addition fewer mudfish were caught in pools with *Gambusia*, possibly due to increased competition.

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1. Introduction

Worldwide many freshwater fish populations are facing severe threats, with many species in decline or facing extinction. Anthropogenic activities are the main cause, with threats to waterways including habitat destruction and degradation, fragmentation of habitats, altered hydrological regimes, pollution, increased sediment and nutrient loads from agricultural runoff, and competition from exotic species (Sheldon, 1988; Richter et al., 1997; Saunders et al., 2002). Freshwater habitats and species are proportionately more threatened than terrestrial habitats and fauna (Ricciardi and Rasmussen, 1999), with a large proportion of freshwater fauna seeming prone to extinction (Richter et al., 1997). However, awareness of threats to freshwater biota has increased all over the world, with major conservation efforts made since the 1970s (Maitland, 2004).

Threatened freshwater ecosystems include wetlands, which have experienced huge declines worldwide due to agricultural and commercial development (Gibbons et al., 2006). Over 90% of New Zealand's wetlands have been drained and converted into pasture or for other economic purposes (MfE, 1997). The degradation and loss of wetland areas is associated with a loss of species abundance and diversity and impacts on ecosystem functioning (Gibbons et al., 2006). Wetlands are essential habitat for many groups of organisms, and in New Zealand provide habitat for many threatened species. Figures from 1995 include 26 plants, 4 fish, 34 birds and 1 insect as species of conservation concern reliant on New Zealand wetlands (Cromarty and Scott, 1995).

Recent approaches to conservation biology suggest that efforts should be at a community level, with conservation of entire biological communities being the key to preserving biodiversity. However, conservation efforts have traditionally focused on individual species, as these are generally easier to monitor and model, and effort can

be concentrated on species under the greatest threat (Olden, 2003). Options for conservation of individual threatened freshwater species include *in-situ* and *ex situ* conservation (habitat restoration and captive breeding respectively) or species translocation (Singh and Sharma, 1998). *In-situ* conservation involves preserving a species by protecting the ecosystem in which it naturally occurs. Habitat restoration aims to return the degraded system to a state that is more valuable in a conservation sense. Processes leading to degradation need to be identified and methods developed to reverse or ameliorate the degradation (Hobbs and Norton, 1996). As with terrestrial systems, to thoroughly carry out habitat restoration in aquatic systems, the multiple scales of the environment need to be considered, from watershed-scale to pool-scale. Large-scale ecosystem processes like natural disturbance regimes create, maintain, and destroy suitable habitat patches and therefore should ideally be considered due to their influence on processes that operate at smaller scales (Labbe and Fausch, 2000). *Ex-situ* conservation, captive breeding and rearing of a population, is advantageous in the short-term for preserving species genetics and to rear stock in captivity for future translocation, but should not be viewed as a long-term solution to species conservation. It is not recommended to carry out captive breeding for more than one generation from small numbers of wild stock, due to the genetic difficulties that may arise (Maitland, 1995).

A translocation involves intentionally releasing wild individuals of a species into a new natural habitat in an attempt to establish, reestablish, or supplement a population (Griffith et al., 1989; Philippart, 1995). Historically, translocations have been extensively used to establish populations of non-native species around the world, and to restore populations of native species that have experienced excessive hunting or harvesting pressures. Translocation is now more commonly used in the conservation of threatened species, and is a well used technique for establishing new populations of threatened species, bolstering declining populations, or increasing the genetic diversity of small populations (Griffith et al., 1989). For many threatened fishes, the best scenario would be to protect the existing habitat, however, translocations often prove to be the cheaper and easier option for species conservation in the short term

than catchment management and restoration of the original native habitat (Maitland, 1995).

Species translocation is well established as a conservation tool, both in New Zealand and overseas, with numerous translocations of threatened species having been undertaken (Williams et al., 1988; Griffith et al., 1989; Wikramanayake, 1990; Minckley, 1995; Maitland, 1995; Stockwell and Weeks, 1999; Poly, 2003). Unfortunately most aquatic translocations have not been adequately monitored and reported, making it difficult to learn from past experiences (Minckley, 1995). Translocations of birds, mammals and fish generally have low success rates, with less than 50% of translocations establishing self-sustaining populations (Williams et al., 1988; Griffith et al., 1989). The quality of the habitat at the receiving site has been cited as one of the main factors influencing the success of translocations (Griffith et al., 1989; Sheller et al., 2006). Without high quality habitats there is a low chance of a successful translocation regardless of how many individuals are released (Griffith et al., 1989). Selection of a receiving site has become the most complex step in translocating a species (Minckley, 1995). The receiving site must be large enough to support a viable population and fulfill the life history requirements of the species (Williams et al., 1988). Translocations should be restricted to within the native or historic habitat wherever possible, with success rates found to be greater if the receiving site is located in the core of the historic range of the species (Griffith et al., 1989).

The number of individuals released is also a key consideration, with numbers required to establish a self-sustaining population specific to particular taxa (Harig and Fausch, 2002). Fish numbers need to be large enough to reflect the genetic composition of the source population (Williams et al., 1988). Some successful captive breeding programmes in the USA have begun with small founder populations of 10, 34, 40, 75 or 80 individuals, producing sometimes hundreds of thousands of fish to release into the wild (Philippart, 1995). Translocations, however, need to consider greater mortality rates, dispersal of fish, and resources required for

successful reproduction when in the wild. Sourcing fish for translocation from the wild has been found to have a much higher success rate than when using fish reared in captivity (Griffith et al., 1989).

Freshwater fish populations such as those of black mudfish are often confined to a single body of water, thereby making the whole population vulnerable to environmental disturbance or disease. Furthermore, fish populations in ephemeral wetlands may be susceptible to extirpation by fire during dry seasons, with many black mudfish populations impacted by wetland fires in recent years (Eldon, 1992). This means that when assessing the conservation status of the species, the number of separate populations can be of more importance than the number of individuals (Maitland, 2005). Establishing a new population through translocation increases the probability of the species avoiding catastrophic events, and can be another step towards preventing extinction (Minckley, 1995; Philippart, 1995).

In New Zealand, the Department of Conservation has a statutory role to preserve as far as practicable all indigenous fisheries and to protect freshwater habitats. Protection measures include conservation and restoration of fish habitats, pest fish control, ensuring adequate fish passage and water flows, and increasing public awareness. One tool widely used in conservation efforts is species translocation. Few translocations of fish species have been undertaken in New Zealand, but it has been suggested by the Department of Conservation's Mudfish Recovery Group that translocation is a key way of aiding conservation of the species (DoC, 2003). Several attempts have been made in the past to translocate Canterbury mudfish (*Neochanna burrowsius*) into new habitats with success (Eldon, 1992; Charteris, pers com), and once to translocate black mudfish (*Neochanna diversus*). The black mudfish translocation failed to establish self-sustaining populations as few individuals were used. These low initial numbers, combined with the resulting high rates of mortality once in the introduction site, meant that the numbers of surviving fish were low and no signs of reproduction were observed (Thompson, 1987). This research investigates aspects of translocation, aiming to improve the success of a translocation

of black mudfish. Black mudfish were translocated into a series of wetland pools, and environmental parameters were investigated with mudfish survival rates to determine parameters with the greatest influence on survival. Aspects such as rearing of fry in captivity prior to translocation, the effect of *Gambusia affinis* density on mudfish growth, and the reliability of Gee minnow traps for catching mudfish have all been investigated to enhance knowledge on translocation of black mudfish.

1.1 Mudfish (*Neochanna*)

New Zealand currently has 35 officially recognised and described native species of freshwater fish (McDowall, 2000). The majority of our native fish belong to the Galaxiidae family, within which are two genera – *Galaxias* and *Neochanna*. There are six species in the genus *Neochanna*, all referred to as mudfish, five of which are endemic to New Zealand and one occurs only in southern Australia. New Zealand mudfish have distinctive geographical ranges consisting of black mudfish (*N. diversus* Stokell, 1949), located in Waikato and Northland, Northland mudfish (*N. heleioides* Ling & Gleeson, 2001), located in Northland, brown mudfish (*N. apoda* Günther, 1867), located in Taranaki, Wairarapa and the West Coast of the South Island, Canterbury mudfish (*N. burrowsius* (Phillips, 1926)) located in Canterbury and north Otago, and Chatham Island mudfish (*N. rekohua* (Mitchell, 1995)) found only on the Chatham Islands. Despite their name, mudfish typically do not live in muddy conditions, but inhabit clean, non-turbid water. Habitat preferences of each species vary, with black mudfish and Northland mudfish living in infertile or oligotrophic bogs on acidic peaty soils and apparently the most specific about habitat type. Brown mudfish tolerate a wider range of habitats from peaty pakihi bogs to eutrophic raupo swamps, swampy lake margins, forest pools, and even spring-fed swampy streams. The Canterbury mudfish lives in weedy ephemeral streams, often fed by seasonal springs (Ling, 2001). The Chatham Island mudfish has only been found in peat lakes on the Chatham Islands (Mitchell, 1995, McDowall, 2004). Most of these preferred habitats for mudfish have been dramatically modified, reduced, or eliminated as a result of human development. This forces mudfish into the few

remaining habitats, many of which are still threatened by nutrient and sediment runoff from neighbouring land, reductions in water levels, grazing by stock, and competition by introduced fish species (Ling, 2001). All five New Zealand mudfish species are included in the national list of threatened species, with Canterbury mudfish and Northland mudfish classified by the Department of Conservation as Nationally Endangered, black mudfish and brown mudfish classed as in Gradual Decline, and Chatham Island mudfish classed as Range Restricted (DoC, 2007).

Mudfish have the unusual ability of being able to aestivate. When wetland habitats dry up over the summer months, mudfish will burrow into the soil, and remain there inactive until water reappears with autumn floods. During this period, mudfish need to be able to cope with hypoxia, desiccation, gas exchange out of water, toxic waste accumulation, and food deprivation (McPhail, 1999). To reduce desiccation mudfish retreat to damp refuges and produce a layer of mucus that covers their body aiding in retaining moisture. During aestivation, mudfish respire through their skin and gills, so it is imperative that fish can access air from their refuge (McPhail, 1999). Refuges tend to be located in the substrate, often protected from desiccation by overhanging vegetation, logs or other litter. The metabolic rate of the black mudfish is reduced by approximately 80% during aestivation, but a torpid state is not entered into, enabling fish to become active as soon as re-immersed in water (Barrier et al., 1996; Hicks and Barrier, 1996). McPhail (1999) suggested that mudfish <60 mm (young-of-the-year) can survive approximately 11 weeks of drought and most adults (> 90mm) could survive for 20 weeks based on weight loss when out of water. This ability to live in wetland habitats that dry out over the summer months enables mudfish to inhabit environments that limit the presence of other fish species, therefore reducing competition and predation (Hicks and Barrier, 1996; Ling, 2001).

The re-introduction of mudfish to suitable habitat has been identified as an action for all mudfish species in the Department of Conservation's New Zealand mudfish (*Neochanna* spp.) recovery plan (DoC, 2003). As part of this research, a translocation of black mudfish from the Whangamarino wetland to Lake Kaituna was

undertaken. Prior to undertaking the translocation, permission under section 26ZM of the Conservation Act 1987 was obtained. The permit process involved an application to the Department of Conservation accompanied by an operational plan for the translocation. Public notification was made in the classified section of the Waikato Times on two consecutive weekends in April 2006. Consultation was carried out with the Auckland/Waikato region of Fish and Game New Zealand, the Ministry of Fisheries, Nga Muku Trust and Tainui iwi. No enquiries were received from the public notification, and no concerns were expressed from consulted parties. The permit was granted in July 2006.

1.2 Black mudfish, *Neochanna diversus* Stokell, 1949

The black mudfish, *Neochanna diversus* Stokell, was recognised in the 1940s. Black mudfish are a cryptic species with nocturnal activity as adults and a secretive nature, meaning populations were initially underestimated (Ling, 2001). *Neochanna* species are tubular and elongate in shape, and unlike most other galaxiids they have reduced or no pelvic fins, giving them a more eel-like appearance (Figure 1.1). Mudfish have no scales, but have thick leathery skin with a mucus layer that protects against infection and reduces desiccation (Ling, 2001). Black mudfish are a dark smokey grey to black colour, with pale speckling on the back and sides, and a paler, smokey grey belly (McDowall, 1990). Adults are often 90 mm in length, but can reach a maximum size of around 160 mm, and are thought to live for up to ten years (Ling, 2001; McDowall, 2000). New Zealand mudfish are non-diadromous species, and black mudfish spend their entire life cycle in wetland habitats (Ling, 2001). Sexual maturity of black mudfish is reached at one year of age when fish are an average length of 70 mm. Spawning has been observed as an annual event, lasting for several months and relying on autumn rains (Thompson, 1987). Approximately 1000 small eggs are produced, and larvae are found from autumn to spring (McDowall, 2000).

Black mudfish are opportunistic carnivores, eating anything that fits in their mouths. Fry (5-7 mm total length) are active during the day, feeding in mid-waters on

zooplankton. By the time they are 2 months old (35 mm total length), fish have become nocturnal, eating small crustaceans (ostracods, copepods), earthworms, larvae of aquatic insects and terrestrial insects at the water surface (Ling, 2001; McDowall, 2000).



Figure 1.1 Black mudfish (*Neochanna diversus*) adult (Photo: N. Ling)

The preferred habitat of black mudfish is clean-water peat swamps on acidic soils that are dominated by reeds such as *Baumea* or *Schoenus* (Ling, 2001). They are located in the northern North Island, from north of Kaitaia to as far south as Piopio in the west, and the Hauraki Plains in the east as shown in Figure 1.2 (DoC, 2003). Despite being classed as a threatened species, black mudfish can be described as locally abundant in the Kaimaumau wetland, Whangamarino wetland and Kopouatai Peat Dome where populations may comprise millions of individuals (Ling, 2001). Apart from these stronghold populations, small populations occur in remnant wetlands, farm drains and swampy streams which, due to the small habitat area, are not as secure. Major threats to black mudfish are habitat destruction and fragmentation. Small wetlands experience larger and more frequent water level fluctuations, and can be greatly affected by runoff of nutrients and sediment from surrounding land. Edge effects increase substantially as wetland area decreases. Smaller habitats will support a smaller population size reducing the amount of genetic variation within the population (Ling, 2001). Further threats to mudfish include predation on fry by the introduced mosquitofish (*Gambusia affinis*), by eels on mudfish juveniles and adults, and by swamp bittern (Thompson, 1987; Hicks and Barrier 1996; Ling, 2006).

The genetic distribution of *Neochanna* has been addressed and Ling et al. (2001) identified three evolutionarily significant units (ESU) within the species range. These are northern Northland, southern Northland and Waikato. When considering a translocation of black mudfish sources need to be in the same ESU as the receiving site (Ling et al., 2001).

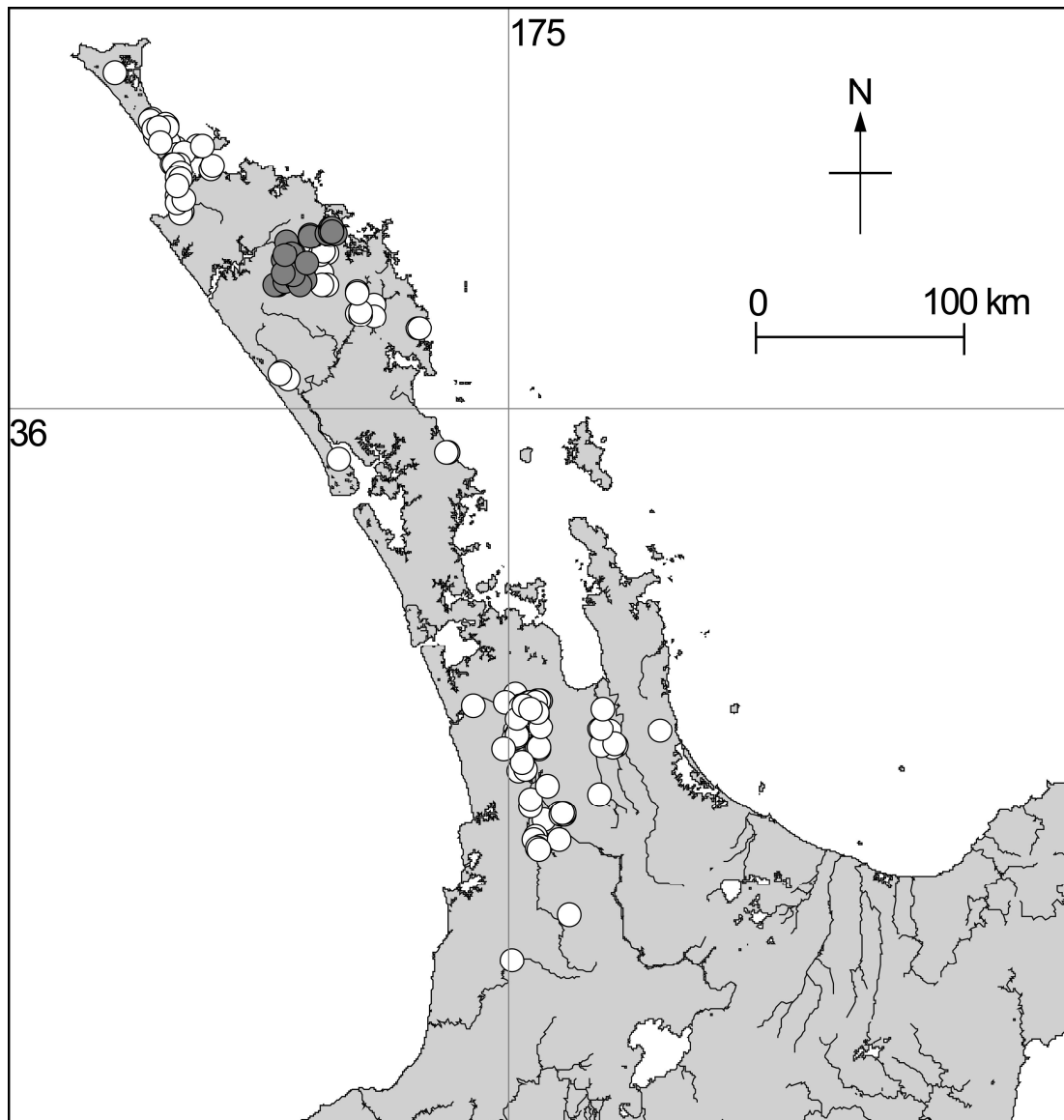


Figure 1.2 Distribution of black mudfish (white circles) and Northland mudfish (grey circles) in the North Island, New Zealand (NZ Freshwater Fish Database).

1.3 Northland mudfish, *Neochanna heleioides* Ling and Gleeson, 2001

The Northland mudfish has only recently been identified due to its inclusion within the geographical range of the black mudfish. The Northland mudfish is restricted to a few small wetland sites on the Kerikeri volcanic plateau and its associated coastal drainage. Its distribution is surrounded by that of the black mudfish causing the two species to be mistaken as one for a long time. The newly identified species can be distinguished from black mudfish because it has smaller eyes, a bulbous swelling behind the head, a mouth that extends to the posterior margin of the eye, larger dorsal and anal fins, a significantly reduced principal caudal fin ray count, and a reddish tinge to its otherwise grey to black colouring, in particular on the fins (Ling and Gleeson, 2001). In 2001, Ling and Gleeson described the distribution of the Northland mudfish as limited to only three sites on the Kerikeri volcanic plateau at elevations of around 200 m, all in small ephemeral wetlands on peaty soils. Since then, recorded sites for the Northland mudfish have increased to 23, all within the Kerikeri area as shown in Figure 1.2 (New Zealand freshwater fish database). As with all other mudfish species, the wetland habitats of the Northland mudfish continue to be degraded and destroyed by drainage for agricultural purposes and development. The scarcity of known populations of Northland mudfish has led to their classification as an acutely threatened species.

The habitat requirements of the Northland mudfish are very similar to those of the black mudfish (Ling, 2001) and therefore it is hoped that results from investigations into aspects of a translocation of black mudfish can be applied to the Northland mudfish. Black mudfish from the Waikato ESU, with the advantage of being locally abundant in a stronghold population like the Whangamarino wetland, are the preferable species for investigations due to their similarity in habitat preference to the Northland mudfish. It is also hoped that the knowledge gained from this translocation can be applied to translocations of the more threatened northern Northland and southern Northland ESUs of black mudfish.

1.4 Study sites

1.4.1 Whangamarino wetland – Translocation source

The Whangamarino wetland is a 4871 hectare Government Purpose (Wetland Management) Reserve administered by the Department of Conservation, and listed as a Ramsar site. The Ramsar Convention on Wetlands is an intergovernmental treaty which provides for the conservation and wise use of wetlands and their resources (Ramsar Convention Secretariat, 2006). The Whangamarino wetland is the second largest bog and swamp complex in the North Island, located 50 km north of Hamilton (Figure 1.3). The wetland is habitat for a number of threatened species, including the black mudfish. It consists of a mixture of peat bogs surrounded by more fertile swamps, with peat bog vegetation dominated by *Baumea* spp., *Empodisma minus* and *Leptospermum scoparium*. The surrounding swamp is covered with the introduced willows *Salix fragilis* and *S. cinerea*. Mean annual rainfall is approximately 1200 mm per year, and there is an annual mean air temperature of approximately 14°C (Barrier, 1993; Clarkson, 1997).

The Whangamarino wetland has four distinct areas of raised bog (Shearer, 1997), and mudfish for the translocation were collected from the bog area south of Monocable Road (Figure 1.3). Monocable Road sits on a causeway that was constructed in 1953 between the wetland and the Maramarua River, partially isolating the two features from each other, with the exception of one connecting culvert under the causeway. The levels of the Maramarua River along with the Whangamarino River control the hydrology of the bog area where mudfish were collected (Shearer, 1997). A weir installed on the Whangamarino River in 1999 has greatly improved minimum water levels over a large area that was previously affected by lowered water levels due to sand extraction in the Waikato River.

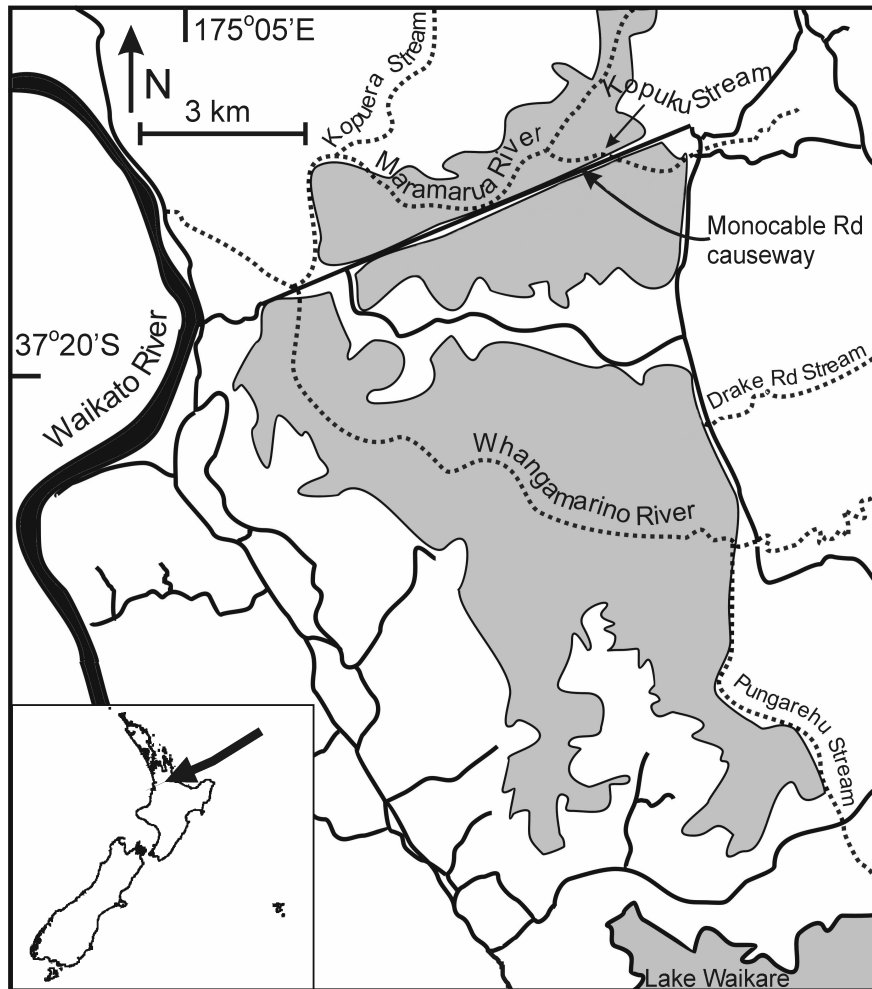


Figure 1.3 Location of the Whangamarino wetland. Mudfish were collected south of Monocable Road causeway.

1.4.2 Lake Kaituna (B) – Translocation destination

Lake Kaituna (also known as Lake B) is one of eight small peat lakes in the Horsham Downs Lakes complex located in Horsham Downs, just north of Hamilton (Figure 1.4). The lakes are situated within the historic Kainui peatland, which has been drained and used for farmland. Conversion to pasture has resulted in all of the Horsham Downs Lakes being highly modified, experiencing nutrient runoff, sedimentation and lowered water tables. This has affected their ecology and reduced their value as wildlife habitats (Fergie, 2003).

Lake Kaituna is a shallow, eutrophic peat lake with an area of 15 ha. It is located within the Horsham Downs Wildlife Management Reserve and is managed by the Department of Conservation. The lake receives inflows from Lake Kainui and several inlet drains from the surrounding farmland, and is linked to Lake Komakorau via its outlet drain. A rock weir is located at the head of the outlet drain of Lake Komakorau and this influences water levels in Lake Kaituna (Fergie, 2003).

In response to the habitat loss and deterioration witnessed at Lake Kaituna, the Lake Kaituna Care Group was formed in 1999 to restore the environment. In 7 years the Care Group has fenced the lake, removed large quantities of grey willow (*Salix cinerea*), carried out restorative planting, weed and animal pest control, and developed wader scrapes (Fergie, 2003). The once willow-dominated lake has seen improvements in water quality, and on lake margins a succession of regenerating wetland plants is occurring with an abundance of cabbage trees (*Cordyline australis*), some *Polygonum* spp., *Carex* spp., *Muehlenbeckia australis*, *Baumea* spp., and *Juncus* spp. (Fergie, 2003). Fish surveys by Fergie (2003) found short-finned eel (*Anguilla australis*), catfish (*Ameiurus nebulosus*), goldfish (*Carassius auratus*), common bully (*Gobiomorphus cotidianus*) and *Gambusi*. Rudd (*Scardinius erythrophthalmus*) and koi carp (*Cyprinus carpio*) are also known to be present in Lake Kaituna. A survey by Barrier (1993) found black mudfish in a drain in close proximity to Lake Kaituna. Translocation of black mudfish to wetland pools surrounding Lake Kaituna would be a step towards restoring the biodiversity of the area to its previous state.

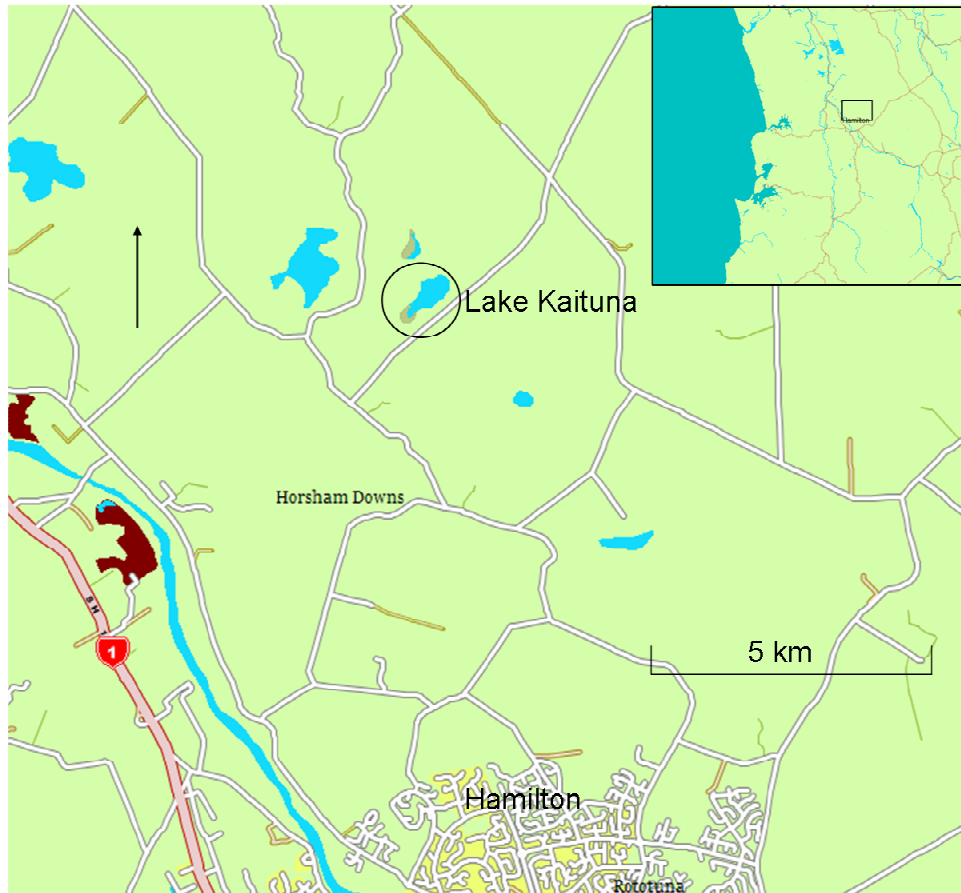


Figure 1.4 Location of Lake Kaituna

1.5 Habitat characterisation

The receiving site for translocated mudfish was a series of 18 small pools (approx 1-2 metre diameter) on the western margins of Lake Kaituna. Pools are all located in the wetland margins of the lake, with vegetation varying from true wetland species to rough pasture due to the staggering of restoration work through the area. An assessment was undertaken of the habitat addressing hydrology, water quality, vegetation and the presence of predators.

Black mudfish have been described as having specialised habitat requirements, largely inhabiting infertile or oligotrophic swamps, however, they have also been found living in farm drains in areas with peat soils (Ling, 2001). They can occupy

shallow, seasonally dry or deoxygenated habitats, where fish are most likely required to aestivate or breathe air.

1.6 Captive rearing of black mudfish

A key component of the conservation of mudfish species is the ability to successfully rear fish in captivity. This may be for purposes of translocation, for captive breeding, or for maintaining stock in a secure environment.

While it has been found that adult mudfish can readily be kept in captivity, mudfish fry suffer high mortality rates both in the wild and in captivity. High water quality, open areas of water for feeding, and appropriate foods such as micro-crustaceans are important for survival (Ling, 2001). Their small size and naivety decreases their chances of survival. This research will look at the optimum conditions for rearing juvenile mudfish in captivity, addressing aspects of the temperature and feeding regime required.

1.7 Impacts of *Gambusia* density on black mudfish

Gambusia affinis (mosquitofish) were introduced into New Zealand to control mosquitoes in the 1930s, and are now considered a pest fish. They are generalist predators, consuming zooplankton, surface aquatic invertebrates, some terrestrial fauna, as well as mudfish fry and eggs if available. They disrupt ecosystems by eating zooplankton grazers, which can result in increased phytoplankton growth and a subsequent decline in water quality (Mansfield and McArdle, 1998; Ling, 2004). *Gambusia* are aggressive fish, often exhibiting aggressive behaviour towards much larger adult fish, including juvenile mudfish in captivity (Barrier and Hicks, 1994; Ling, 2004). Due to their aggression and ferocious feeding behaviour it is thought they may pose a threat to black mudfish. To assess the effect *Gambusia* have on

mudfish in a wetland environment, wetland mesocosms were constructed to test the effect of *Gambusia* density on juvenile mudfish survival, growth and condition

Because *Gambusia affinis* is classified as an unwanted organism in New Zealand, regulatory authority was obtained from the Department of Conservation Biosecurity Unit under section 53 of the Biosecurity Act 1993 to keep and breed *Gambusia*.

1.8 Reliability of Gee minnow trapping

All of New Zealand's five threatened endemic mudfish species require monitoring programmes to ensure the viability of natural populations, and to monitor any impacts or improvements caused by anthropogenic or natural changes in habitat quality. Monitoring is also necessary to assess the success or failure of translocations. A key aspect of any monitoring programme is an ability to sample mudfish numbers effectively and reliably, therefore allowing an assessment of population health and impacts. Gee minnow traps are the most commonly used fishing technique for capturing mudfish, however, there is a lack of knowledge about the reliability of the technique with respect to factors such as fish density and water depth. To test the reliability of Gee minnow traps for catching black mudfish, mudfish were established in outdoor tanks and trapping over consecutive nights was carried out to test the effect of fish density, trap position, water depth, and the short-term and long-term memory of fish.

1.9 Study objectives

The objectives of this study were to:

1. Determine optimum feeding regimes and temperatures for maximum growth of mudfish juveniles in captive conditions.
2. Determine the most suitable habitat for receiving translocated fish.
3. To establish the effect of *Gambusia* density on mudfish survival, growth and condition.
4. Determine the reliability of Gee minnow traps for catching black mudfish, and the influence of fish density, water depth, trap position, and fish behaviour.
5. Develop knowledge to assist the restoration of biodiversity of rehabilitated freshwater ecosystems.

2. Captive Rearing of Black Mudfish

2.1 Introduction

Captive rearing is often an integral requirement for translocations of threatened fishes. Options for sourcing fish for translocations include collecting adult fish from a source population for direct translocation, collection of fry to be reared in captivity to sexual maturity or until large enough for release, or captive breeding from a select few adult fish. All options have their advantages and disadvantages with respect to effort required, chance of success, and impact on the source population. While direct translocation of adult fish does not require captive rearing, the removal of large numbers of adult fish may negatively impact on the source population. The removal of a large percentage of a species' breeding stock in one season could have serious adverse impacts on the health of the population. Fry have a high mortality rate in the wild, so it is recommended that fry are collected and captively reared (O'Brien and Dunn, 2005). The production of fry is normally greater than the habitat's capacity to support them, and high fry deaths result from competition for resources along with high rates of predation (O'Brien and Dunn, 2005). An advantage for translocation is that larger numbers of fry can be collected, increasing the genetic diversity of the founder stock.

A greater genetic diversity means stock will be better equipped for the different environment and natural selection processes they will experience at the translocation site. Stock with a high genetic diversity have also been shown to be more successful in terms of growth, survival, fecundity and developmental stability (Philippart, 1995; Yamamoto et al., 2006). Artificial rearing conditions pose the risk of unintentional selection of genotypes which may not be ideal for the receiving environment. Rearing cool water fish in warm laboratory conditions and feeding of artificial food could select for fish ill-adapted to a wetland habitat (Philippart, 1995).

Captive breeding is a difficult task requiring a great amount of time and effort, with uncertain success rates. Eggs can be collected from the wild or stripped from fish, or produced by spawning events in captivity by adults or fry that have been captively reared to sexual maturity (Philippart, 1995). However, captive breeding requires a detailed knowledge of the species and its breeding traits, provision of the required conditions, awareness of genetic constraints, care of juveniles, maintenance and disease prevention. Captive spawning of black mudfish has been successfully carried out twice by Gay (1999; in O'Brien and Dunn, 2005) and Perrie (2004), however, it was difficult to get individuald to progress beyond the fry stage. Perrie (2004) stripped eggs and milt from gravid females and mature males respectively, and succeeded in rearing some of these artificially fertilised eggs to a juvenile stage.

Therefore, captive breeding and spawning is not deemed to be a feasible option to provide source fish for translocation and collection and rearing of fry is currently a more suitable solution. Suitable conditions for captive rearing will be investigated, addressing temperature and feeding requirements. This information is also useful for management of stock in captivity and any potential breeding programmes. While it is known that mudfish can tolerate temperatures up to 22°C (Ling, 2001), optimum temperatures for growth are not known. High temperatures increase metabolic demands and food requirements and reduce dissolved oxygen levels (O'Brien and Dunn, 2005), whereas low temperatures may result in reduced growth. To test the effect of temperature on mudfish growth, juvenile mudfish will be reared in a constant temperature of either 10°C or 15°C for a two month period. The lower temperature is more indicative of natural conditions in black mudfish habitat at the time of the year when fry are present, but it was expected that the higher temperature would accelerate growth of captively-reared fry. Mudfish lengths and weights will be monitored to measure condition.

Mudfish in captivity have been fed a variety of foods including live tubifex worms (Tubificidae), whiteworms (*Enchytraeus albidus*), freshly hatched brine shrimp larvae (*Artemia* spp.), earthworms (*Lumbricus terrestris*) and chironomid larvae

(bloodworms) (Barrier, 1993; Ling, 2001; Perrie, 2004). To test the growth rates of mudfish fry on different food sources, mudfish were fed either brine shrimp (*Artemia salina*) alone, or a combination of whiteworms and brine shrimp. The crustaceans (brine shrimp) are easy to source and culture, and are used extensively for the culture of larval fish in commercial aquaculture. Their disadvantage is that, particularly when less than 72 hours old, they offer little nutritional value. Whiteworms cannot be cultured at the same high rates or with the same ease as brine shrimp, but they have the advantage of a high lipid content and consequently greater nutritional value.

2.2 Methods

Early-stage black mudfish fry were collected from the Whangamarino wetland in October and November 2005 by scooping fine-mesh (1 mm) nets across the water surface, and later-stage fry and juveniles in December 2005 with fine-mesh Gee minnow traps (3 mm x 3 mm wire mesh) that were set overnight. Gee minnow traps were set at the water surface with entrances submerged, providing an air space at the top of the trap to allow captured fish to breathe; in low oxygen conditions mudfish need to surface to breathe air. Fish were transported to the laboratory and reared in a series of 25 L aquaria containing 12 L of dechlorinated Hamilton City tap water (pH 7.0 – 7.5). Opaque plastic pipes were placed in the tanks to provide cover for fish. General conditions while in captivity were closely monitored, with care taken to prevent parasites and disease from the wild being introduced to hatchery stock (Philippart, 1995). Fish were regularly inspected for disease, with white spot (*Ichthyophthirius*) being the most common and persistent infection.

Stages of mudfish maturity were classified according to Thompson (1987) who described the transition from fry to juvenile occurring at 22-24 mm in length. Care was taken whenever transporting or handling fry due to their small size and delicate structure. The duration of time fish were out of water was kept to a minimum. Early-stage fry were moved using an ice cream container with a fine-mesh netting fitted at

its base to allow fish to remain in a pool of water in the corner of the container. Later-stage fry and juveniles were moved using soft, fine-mesh hand nets.

Fish total length was measured from the tip of the snout to the end of the tail. Early-stage fry were measured on a measuring board filled with water, and it was ensured the measuring board was moist when measuring late-stage fry. Early-stage fry were not weighed due to their sensitivity and the difficulty in removing excess water and attaining precise measurements. Juvenile fish with a total length of 28 mm and greater were weighed. Prior to weighing, fish were placed on a moist tissue to absorb any excess water on the fish. Weight of the whole fish was recorded to ± 0.01 g.

2.2.1 Initial Rearing

Sixty-five mudfish fry were caught on 31 October 2005. Subsequent trips on 4 November and 16 November caught approximately 100 and 70 fry, respectively. On arrival at the laboratory, fish were placed in aquaria filled with water collected from the Whangamarino wetland during each fishing trip. Due to prior experience with fungal infection on fish, aquaria were placed in a 10°C temperature-controlled room to curb fungus growth. Aquarium water was changed gradually, with one-fifth of the water removed every couple of days and replaced with dechlorinated Hamilton city tap water that had cooled to 10°C. This ensured all fish had a minimum of 2 weeks to adjust to the change from wetland water to dechlorinated tap water.

Brine shrimp were reared from eggs in the laboratory in aerated 35‰ salt water. Once hatched, 48-72 hours after being placed in water, the phototactic brine shrimp were attracted to the water surface with a light, and extracted with a pipette. This resulted in a concentrated solution of brine shrimp nauplii that was then fed to mudfish. New brine shrimp cultures were established every 2-3 days to ensure a continuous food supply for mudfish. Brine shrimp diets were supplemented with neonates of *Daphnia carinata* filtered from cultures established from a commercial source. Once large enough (~ 25 mm T.L.), mudfish were also fed white that were cultured on moist, washed potting mix and fed wholegrain bread. Worms

congregated around the bread and clumps of worms could be picked off with tweezers and fed to fish.

Mudfish were fed to satiation, which was measured by allowing fish to feed for 15 minutes. Initially fish were monitored for 15 minutes to ensure the correct quantities of food were given. If fish consumed all the food within the 15 minutes, more food was added. Any food remaining at the end of the 15 minute period was siphoned out of the tank. With experience the accuracy of measuring food quantities improved. Mudfish were initially fed 3 times a week, with feeding increasing to at least 5 times a week when fish mortalities persisted. Regular tank cleaning was required to avoid deterioration of water quality. Any fish with visible signs of disease were separated from healthy fish.

2.2.2 Feeding and Temperature Treatments

On 22 December 2005, minnow traps were set in the Whangamarino wetland overnight. Traps were retrieved the next day, recovering approximately 150 late-stage fry and small juveniles, larger than 25 mm in length, and 15 adult mudfish. Fish were taken back to the laboratory and placed in 3 tanks containing wetland water at 10°C, with water progressively changed to dechlorinated tap water as above.

Parameters of feeding regime and temperature were varied and the effects on mudfish growth assessed. Temperature and feeding treatments were commenced in February 2006, allowing all fish ample time to acclimatise to laboratory conditions prior to treatments commencing. Black mudfish were fed with two different food regimes; crustaceans only (brine shrimp), or white worms and crustaceans (brine shrimp) together. Fish were fed to satiation five times a week, with satiation judged by observation over a 15 minute period as described above.

Twelve aquaria were set up in two temperature controlled rooms, set at either 10°C or 15°C ($\pm 1^\circ\text{C}$). Both rooms had a 14 h light and 10 h dark cycle. The aquaria were evenly split between the rooms and, within a room, fish in three tanks were fed brine

shrimp only and fish in the other three tanks fed a combination of brine shrimp and white worms. Ten fish were placed in each tank and, because individual fish were unidentifiable, fish condition was averaged for each tank giving three replicates of each treatment. Prior rearing of mudfish had revealed that juvenile mudfish will readily cannibalise mudfish smaller than themselves by only several millimeters. Fish were therefore separated into size classes. Furthermore, due to the variability of size on capture there were insufficient numbers of fish of one uniform size. Fish were separated into 3 mm size classes and a small, medium and large size class was used for each treatment. Prior to being placed in tanks, fish were anaesthetised with MS-222 (0.1g/L) and their condition measured using total length to the nearest mm and weight to the nearest 0.01g as described above. Fish were maintained at the respective treatment for 2 months, and condition measured every month.

Statistical analysis

Two-way ANOVA was performed to compare diet and temperature within each time period. Due to the inability to identify individual fish, averages of lengths and weights of the ten fish in each tank were used. This resulted in three replicates (tanks) for each treatment. Two-way ANOVA also compared fish length and weight between the two temperatures and feeding regimes to ensure no difference between initial lengths and weights. Calculations were then made for the length increment and weight increment, comparing growth after 1 or 2 months with initial values. Post-hoc Tukey tests were performed on all significant data sets.

Mean lengths from each tank after one month and two months were plotted against the mean lengths at the beginning of treatments. Similarly the standard deviation of growth in each tank was plotted in the same way. This was repeated for weight data.

2.3 Results

2.3.1 Initial Rearing

Early-stage mudfish fry from collections in October and November 2005 suffered high rates of mortality over a 2 month period. Approximately half the fish suffered from white spot. Proprietary white spot cures were used unsuccessfully for one week, however, following a one off treatment of 2 drops/L of 5% formalin and weekly applications of 2 drops/L white spot cure for 3 weeks, fish began to improve. However, fish not showing any signs of disease also had high mortality rates.

Survival rates improved with the length of the fish, with fish greater than 24 mm suffering only 9% mortality (Figure 2.1). Small fish (<16 mm) suffered complete mortality.

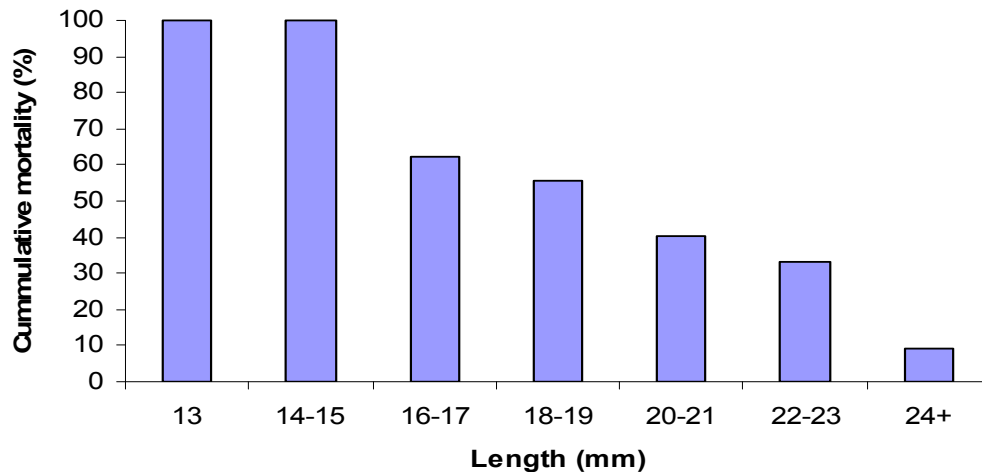


Figure 2.1 Cumulative mortality of black mudfish fry by initial size class following two months in captivity.

2.3.2 Feeding and Temperature Treatments

No diseases or mortality were observed in juvenile mudfish (>25 mm). Statistical analysis of fish lengths and weights at the beginning of treatments showed there was no significant difference in length or weight between the treatments. Treatments ran

for two months, and after both one month and two months, diet was found to significantly affect the rate of mudfish growth. Fish fed a combination of white worms and brine shrimp grew faster than fish fed only brine shrimp. Mudfish length increased significantly after both one month ($p < 0.001$, $F = 107.5$), and two months ($p < 0.001$, $F = 340.8$) when fed white worms and brine shrimp when compared to those fed just brine shrimp (Figure 2.2). This increase in growth is also reflected by significant differences between the weight of fish after one month ($p < 0.001$, $F = 168.3$) and after two months ($p < 0.001$, $F = 91.8$) (Figure 2.3). Differences in length increases became more pronounced over time. Fish fed only brine shrimp showed almost no growth and generally lost weight over time indicating that this diet was at best maintenance only.

Temperature was found to have a significant effect on length only after two months ($p = 0.0498$, $F = 5.3$). By this time, fish fed white worms and brine shrimp at 15° were significantly larger than fish fed white worms and brine shrimp at 10°C .

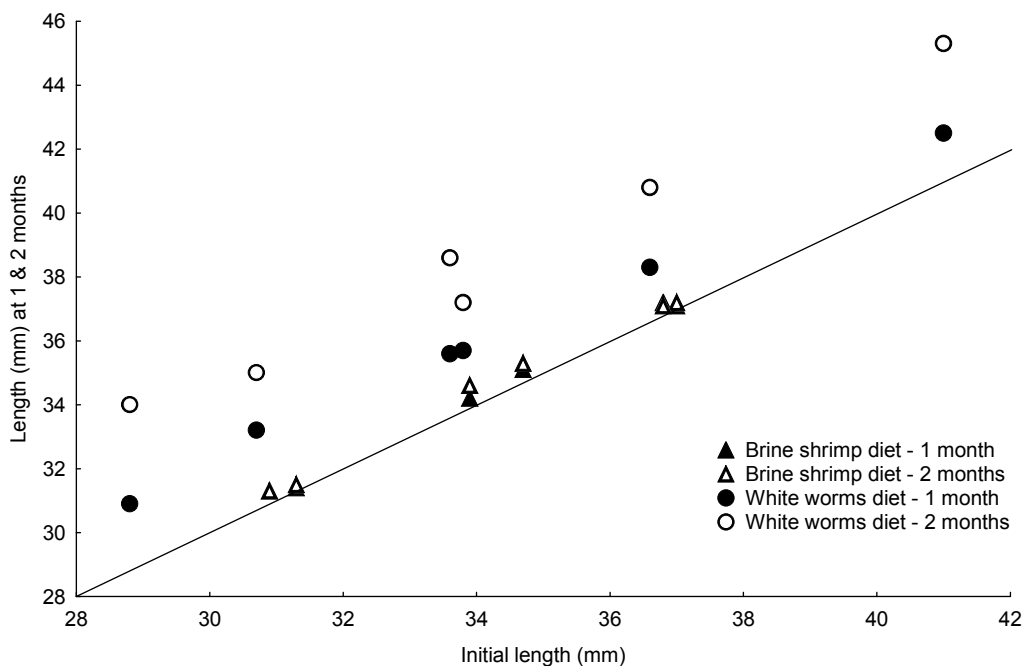


Figure 2.2 Mudfish length (mm) at the beginning of feeding treatments versus length (mm) after one month and two months.

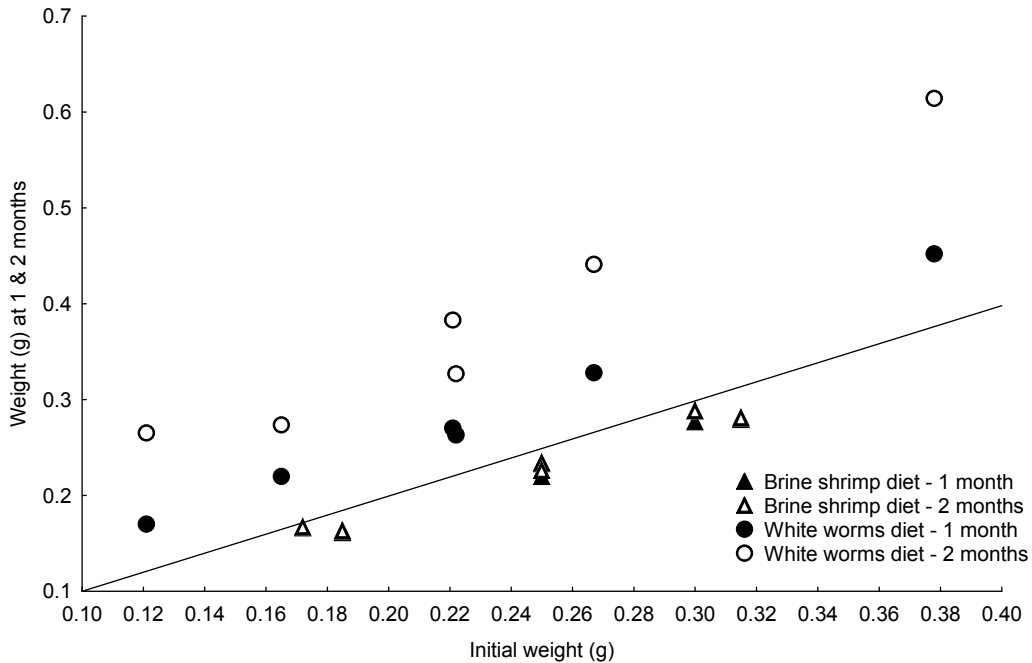


Figure 2.3 Mudfish weight (g) at the beginning of feeding treatments versus weight (g) after one month and two months.

Fish in tanks that were fed a combination of white worms and brine shrimp had much greater variance in length and weight increases, than did fish in tanks that were fed only brine shrimp (Figure 2.4, Figure 2.5) indicating that the white worm and brine shrimp diet resulted in much greater variation in growth between individual fish in each tank, presumably due to some dominant individuals monopolizing the food resource.

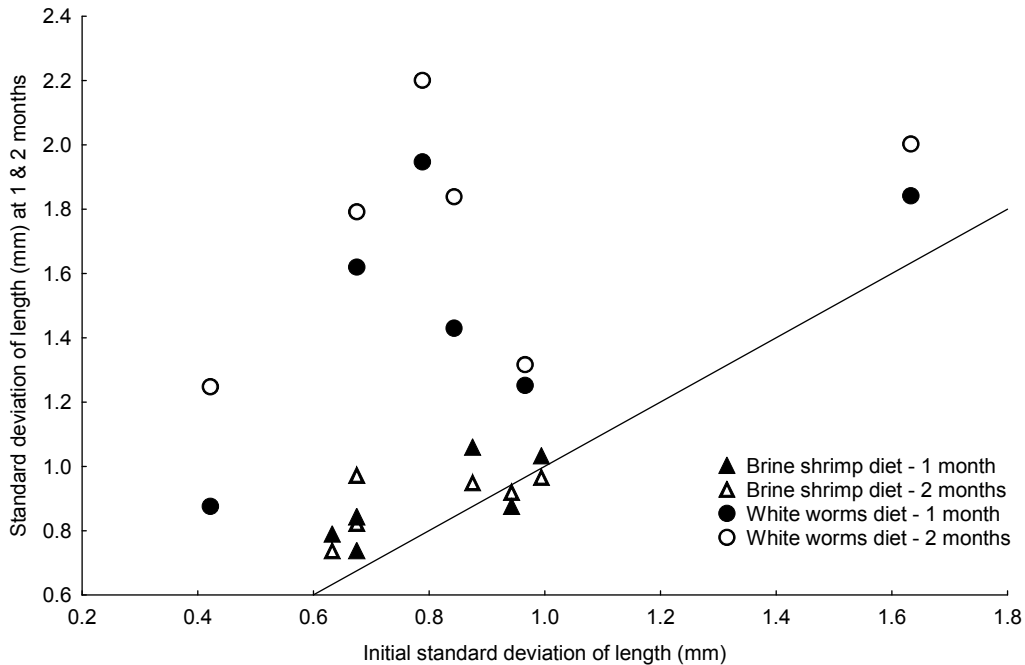


Figure 2.4 Standard deviation of mudfish length (mm) at beginning of treatments versus length (mm) after one month and two months.

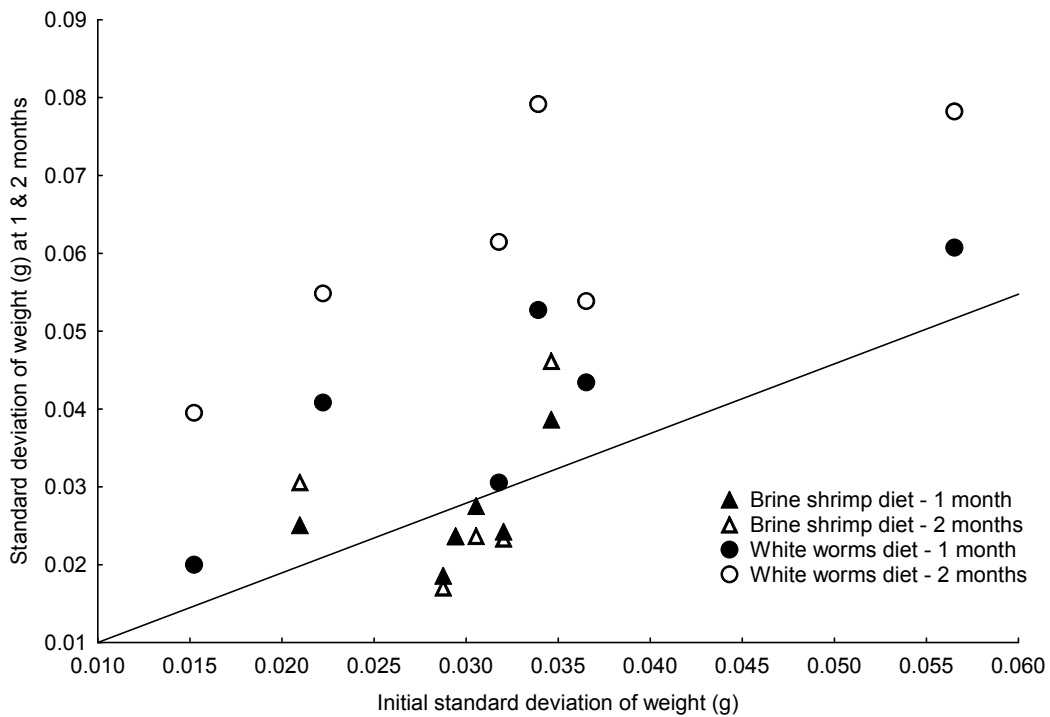


Figure 2.5 Standard deviation of mudfish weight (g) at beginning of treatments versus weight (g) after one month and two months.

2.4 Discussion

Mudfish fry less than 25 mm were found to have high death rates, while those caught at sizes larger than 25 mm suffered little mortality. Possible reasons for high larval death rates could include a greater sensitivity to changes in pH, water quality and diet. Greater sensitivity to handling is unlikely since almost all fish survived for at least one week after capture. Water in the Whangamarino wetland is high in organic matter and humic concentrations with a pH of around 5.5, whereas dechlorinated Hamilton City tapwater is free of particulate and dissolved organic matter and has a pH of around 7.5. Black mudfish fry in the wild have been found to eat copepods, copepod nauplii, *Ceriodaphnia* spp., *Bosmina* spp., rotifers and chironomid larvae (Perrie, 2004). It is likely that brine shrimp nauplii and *Daphnia* neonates represent large prey for the smallest mudfish fry, increasing the energy costs of prey capture and slowing rates of digestion. Perrie (2004) also found high mortality rates of black mudfish fry caught in the wild and raised under laboratory conditions.

Experiments on rearing conditions of juvenile black mudfish found that diet had a significant effect on growth. Brine shrimp were relatively easy and quick to feed to mudfish, with simple preparation of cultures and, due to their phototaxic nature, ease of harvest. Fry were able to catch large numbers of brine shrimp quickly and became visibly swollen in the belly with the pink shrimp nauplii. However due to the limited occurrence of meals per week, mudfish did not experience a large growth. In contrast, white worms required greater human effort to establish and maintain the cultures. While stocks were relatively easy to maintain, requiring only addition of moist bread twice a week, they can be susceptible to population crashes. Harvesting white worms required more care than brine shrimp and it was important to remove the soil attached to worms to minimise the impacts on water quality in the tanks. White worms were suitable for mudfish in this experiment, but are unsuitable for mudfish smaller than 25 mm as the worms were too large for the mudfish to consume. Results of the experiment found mudfish growth was greater for fish fed the white worm and brine shrimp diet compared to fish only fed brine shrimp. This

experiment was designed to mimic conditions that would be used to raise mudfish in captivity, and was not a highly controlled experiment accounting for nutritional resource allocation. Differences in dry weights between food types or the nutritional content of each food type was not quantified. In the 15 minute period used to judge fish were fed to satiation, mudfish were able to consume a greater nutritional content from white worms than brine shrimp. If, in a much more time consuming experiment, fish were fed at much more regular intervals, it is likely fish fed only brine shrimp would have experienced greater growth rates. However with the levels of effort used in this experiment it was found that while brine shrimp are the easier of the two foods to culture and feed to mudfish, mudfish cannot be reared on brine shrimp alone, and the white worm and brine shrimp diet is preferable.

Many experiments have been conducted on the effect of temperature on growth rates of fish, with the growth of most fish species increasing with increased temperatures (Wandsvik and Jobling, 1982; Dwyer and Piper, 1987). However, temperature was found to have a less significant effect on mudfish growth than diet. Mudfish kept at the higher temperature of 15°C for long durations of time may result in a larger growth increase when compared to mudfish kept at 10°C. These increases in growth at the higher temperature are so small though that it is suggested temperature should not be a major consideration. However, other considerations may be important, such as the advantage of lower temperatures inhibiting fungal growth on mudfish.

When feeding white worms to mudfish, the worms fall immediately to the bottom of tanks and remain in same location, allowing aggressive mudfish to dominate when feeding. This difference in nutritional intake led to a large variation in individual growth rates of mudfish. As cannibalism has been observed by mudfish only a few millimetres larger than their victim (pers. obs.), it is suggested for long-term captive rearing programmes, re-grading of mudfish into size categories may prevent cannibalism.

Captive rearing was found to be a very time-consuming task. As fish were reared in numerous separate tanks to isolate any outbreaks of disease, and to separate mudfish into size classes to reduce cannibalism, this increased the amount of time required for regular feeding and cleaning of tanks. For further experiments, mudfish were moved into outdoor mesocosms, as described in chapters 3 and 4. Mudfish fed on naturally established zooplankton populations in the mesocosms, with occasional supplementary feeding of chironomid larvae. After two months in mesocosms for a trapping experiment (Chapter 4), mudfish growth was greater than that experienced during rearing experiments. In the semi-natural environment of mesocosms, food was continuously available to mudfish rather than restricted to set feeding periods 5 times a week. Mesocosms were replenished with rainfall, and no maintenance was required. Rearing of mudfish fry has not been tried in outdoor mesocosms, however the presence of small zooplankton may be more suitable to their dietary requirements. Therefore, a less time-consuming and more cost-effective long-term captive rearing programme, it is suggested that outdoor mesocosms are established for juvenile mudfish rearing.

3. Impacts of *Gambusia* Density on Black Mudfish

3.1 Introduction

Two species of the family Poeciliidae, *Gambusia affinis* (Baird & Girard) and *Gambusia holbrooki* Girard, are commonly called mosquitofish. Both species are native to coastal drainages of the south-eastern United States with *G. affinis* referred to as the western mosquitofish and *G. holbrooki* the eastern mosquitofish. The two species are commonly mistaken for each other, and are found to be similar in habitat preference and tolerance, diet and behaviour (Rehage et al., 2005). Both species have been introduced worldwide as a means of biological control for mosquito larvae. This has resulted in these fishes being among the most widespread in the world, now found on every continent except Antarctica (Dawes, 1991). *Gambusia affinis* (Figure 3.1) was introduced to New Zealand in 1930 at Auckland (McDowall 1990) and populations have now established through much of the North Island (in particular northern North Island) (Ling, 2004).

Fish introductions to New Zealand have been common, with at least 21 fish species being introduced and, of these, 11 have established widespread self-sustaining populations (McDowall, 2000). The majority of established species have caused adverse effects ranging from the extinction of an endemic species to deterioration of habitat quality (McDowall, 1990). *Gambusia* have been listed as an unwanted organism under the Biosecurity Act 1993, under which their possession and distribution of the species is illegal. Worldwide, *Gambusia* have been involved in the displacement, decline, or elimination of numerous native fish and amphibian species (Goodsell & Kats, 1999; Economidis et al., 2000; Rincon et al., 2002; Ling, 2004).

Gambusia can tolerate a wide range of environmental conditions, and are found in shallow, still, or gently flowing waters in warmer regions. They are very tolerant of changing environments, surviving in a wide range of chemical conditions, brackish

water, and temperatures from near freezing to over 30°C (Dawes, 1991). They are more abundant when temperatures are greater than 20°C, making northern New Zealand wetlands ideal habitat (Baker et al., 2004). The microhabitat of mosquitofish is closely linked to the water surface for feeding purposes, and they are most often found near vegetation (García-Berthou, 1999). Maximum sizes for adult males ranges from 3-4 cm, and adult females from 5-7 cm. *Gambusia* are livebearers that reproduce rapidly. Reproduction can occur every 5-8 weeks with broods consisting of 10 – 80 fry, enabling them to establish populations quickly. Populations can build up during the summer even in the absence of males due to internal fertilisation which enables females to retain viable sperm over winter (Dawes, 1991; Baker et al., 2004).

Gambusia are aggressive, non-selective, predators eating mosquito larvae and pupae along with anything else they can swallow including fish fry and eggs. In most habitats their diet largely consists of zooplankton, in particular cladocerans, ostracods and copepods (García-Berthou, 1999). In Lake Waahi, New Zealand, their diet was found to predominantly consist of cladocerans, followed by surface fauna such as adult chironomids and Collembola, and also to consist of copepods, benthic fauna and rotifers (Wakelin, 1986). This may pose a great threat to native fish species, with the presence of *Gambusia* potentially creating a dramatic decrease in available resources. *Gambusia* are a very aggressive fish, known to nip the fins of fish larger than themselves, weakening and harassing fish, leaving them susceptible to infection and possibly death (Dawes, 1991).

Gambusia largely affect native fish in two ways; by predation, or by increased stress on native species due to constant aggression or competition for food. Predation by *Gambusia* of eggs or juvenile fish is common, and can cause recruitment failure and therefore reduced population growth rates for targeted species (Rincon et al., 2002; Rogowski et al., 2006). For some species, *Gambusia* presence can result in a lack of reproduction due to a stress-induced failure to produce eggs, rather than predation upon eggs and fry (Howe et al., 1997). Stress from agonistic behaviour by *Gambusia* will also result in a lack of growth by some species. In experiments by Howe et al.

(1997), supplementary feeding did not result in any further growth of *Pseudomugil signifer*, which suggested that in this experiment, nutritional conditions were adequate for growth, but that growth was inhibited by the presence of *Gambusia*. It was suggested that greater aggression occurs when fish are crowded, as in drought situations with shrinking pools.

As a result of direct predation or effects on fish condition and growth, concerns have been raised over *Gambusia* displacing native species. However, it has been suggested that, particularly in a country like New Zealand with a depauperate fish fauna, native species currently enjoy comparatively wide niche opportunities and no native species occupy the niche of an obligate top-feeding minnow (Ling, 2004). It is therefore thought that *Gambusia* may use unoccupied or under-utilised niches. This means that *Gambusia* may co-exist with native species, and both will have sufficient resources (Wakelin, 1986; Ling, 2004). Becker et al. (2005) also discusses a shift of niche in terms of distribution and abundance in the presence of *Gambusia*. He found that *G. holbrooki* was unable to out-compete landlocked populations of *Galaxias maculatus*, and experiments only found a very slight increase in the distance of *G. maculatus* from food sources and cover when *G. holbrooki* numbers dominated.

New Zealand's fish fauna includes several small-bodied native fish species that inhabit wetlands, lakes or slow flowing waters, giving the potential for *Gambusia* to have detrimental impacts. *Gambusia* have been implicated in mass mortalities of dwarf inanga (*Galaxias gracilis*) (Rowe, 1998), a species presumed to have evolved from landlocked populations of *G. maculatus*, and is now located in several Northland dune lakes. A rapid increase in *Gambusia* numbers in Lake Waikere coincided with a decline in dwarf inanga and aggression or predation was implied by Rowe (1998). When looking at other landlocked populations of *G. maculatus*, Ling (2004) found this species coexisting in several lakes with abundant *Gambusia* populations, and in Lake Ngatu this situation has persisted for 70 years. It is thought that aggressive competition or predation by *Gambusia* may be lake-specific, with lake morphology, nutrient levels and vegetation playing a role in fish interactions.

Laboratory studies in Australia by Becker et al. (2005) found no displacement of *Galaxias maculatus* by *G. holbrooki*, indicating that *Gambusia* could not out-compete the native species for cover and food resources. However, in laboratory trials in New Zealand, Baker et al. (2004) found *Gambusia* caused death of *G. maculatus*, primarily due to fin nipping, causing the loss of, or damage to the caudal fin and peduncle. Experiments found that damage and death of *G. maculatus* increased with temperature, indicating that *Gambusia* became more aggressive at increased temperatures (Baker et al., 2004).

Gambusia sometimes co-occurs in wetlands with the Northland mudfish and black mudfish in northern New Zealand wetlands. Laboratory experiments found that *Gambusia* will eat mudfish fry and that large *Gambusia* (males >24 mm TL, females >30 mm TL) showed aggressive behaviour towards juvenile mudfish (30-50 mm TL) by chasing and attempting to nip mudfish (Barrier, 1993). The presence of large *Gambusia* in a tank resulted in juvenile mudfish becoming more bottom-orientated as an escape mechanism, allowing *Gambusia* greater prey capture rates in surface and midwater positions.

Barrier (1993) found mudfish and *Gambusia* co-occurring at seven out of sixteen wetland sites where *Gambusia* were present. He reasoned that even though *Gambusia* prey on mudfish fry and can alter the feeding behaviour of mudfish, the ability of mudfish to aestivate and inhabit seasonally dry environments that exclude *Gambusia* gives them a reprieve. *Gambusia* breed and are most abundant over the summer months. During these periods water levels are low and wetland habitats can become reduced to isolated shallow pools, therefore restricting the movement of *Gambusia* into mudfish habitats. Conversely, black mudfish breed over winter when *Gambusia* numbers are low, therefore limiting predation on mudfish eggs and fry (Barrier, 1993). Wetlands therefore provide a refuge for mudfish as hypoxic and low water conditions during summer exclude *Gambusia*, and can also provide physical barriers to the dispersal of these predators (Rosenberger et al., 1999).

Experiments in aquaria reveal *Gambusia* to be cannibalistic, significantly aggressive towards each other and to other species, often attacking fish much larger than themselves (Barrier, 1993; Benoît et al., 2000). However, it is uncertain if this behaviour is exhibited in natural habitats. Ling and Willis (2005) conducted experiments in constructed wetland mesocosms to test *Gambusia* impacts in a more semi-natural habitat than aquaria, investigating the effect of the presence or absence of *Gambusia* on black mudfish, and concluded that *Gambusia* significantly reduced growth rates of juvenile mudfish. They also found that fewer mudfish fry were present in natural locations close to permanent water where *Gambusia* was most abundant, compared to ephemeral wetlands that provided unsuitable habitat for *Gambusia*. *Gambusia* were found to eat black mudfish fry in the absence of cover but this predation declined if alternative food resources were provided.

Little is known of the effect of *Gambusia* population density on black mudfish, although work by Rogowski et al. (2006) found that *Gambusia* density had no significant effect on the condition of White Sands pupfish (*Cyprinodon tularosa*) in mesocosm experiments. Because early New Zealand investigations have demonstrated an impact of *Gambusia* on juvenile mudfish growth and condition it is important to determine whether *Gambusia* density has an influence on the degree of impacts. As black mudfish are classed as in ‘gradual decline’ it is particularly important to determine the impacts that varying densities of *Gambusia* may have on black mudfish especially when considering further translocations of mudfish species into new habitats containing *Gambusia* populations.

3.2 Methods

Constructed wetland mesocosms were created to investigate the effect of *Gambusia* density on mudfish survival and condition. A series of nine circular concrete tanks (150 cm diameter x 55 cm deep) were set up in an outdoor area shaded from the north to reduce solar heating (Figure 3.2). Tanks were established in December 2005 and flushed three times with water over a period of three weeks to leach lime from the

concrete. Stand pipes, made of 20 mm internal diameter PVC pipe covered with 1 mm mesh allowed the water level of tanks to be adjusted, with water passing through the mesh before discharging to waste on an asphalt compound. This ensured that tanks could not overflow allowing fish to escape. Each tank was stocked with four plastic crates (36 x 36 x 28 cm) containing peat and *Carex virgata* plants collected from Torehape peat mine. For security and to prevent predation by birds, 25 mm wire netting was draped over the plants and the tanks and secured at ground level. Tanks were filled with water a month prior to the introduction of fish, and crates of plants were placed in tanks a week prior to fish introduction to stimulate development of pond-life (ie., zooplankton and plant life). The initial water level of each tank was set at 400 mm, and levels were maintained by natural rainfall.

Mudfish juveniles were anaesthetised with MS-222 (0.1g/L) and measured for total length (± 1 mm) and weight (± 0.01 g). *Gambusia* were collected over three days from the University of Waikato campus lakes. Dip nets were skimmed across the surface of the lake to catch fish, and *Gambusia* were transferred into buckets. In the laboratory, fish were anaesthetised with MS-222 (0.1g/L), measured, and were sexed with the aid of a microscope.

Experiments were conducted with juvenile mudfish of 43-48 mm total length (TL), *Gambusia* males greater than 20 mm TL, and *Gambusia* females greater than 25 mm TL. Fish were assigned to mesocosms in rotation until the required numbers were obtained. Five mudfish were randomly assigned to each of the mesocosms on 6 September 2006 and given two weeks to acclimatise. Varying densities of *Gambusia* were added to the tanks on 21 September 2006. Three mesocosms contained 40 *Gambusia* each (20 female and 20 male), three mesocosms contained 10 *Gambusia* each (5 female and 5 male), and three mesocosms contained only mudfish. *Gambusia* densities were assigned to mesocosms randomly.

Approximately 500 cultured *Daphnia carinata* were added to each mesocosm on 21 September and 6 October 2006, and a further 300 added on 10 November, to

supplement natural cultures of zooplankton. Samples were taken on 16 November 2006 and 4 December 2006 to assess the effect of *Gambusia* on mesocosm zooplankton populations. Ten litres of water was siphoned from each tank from a depth of approximately 200 mm, using a 20 mm diameter plastic hosepipe and filtered through a 37 µm mesh filter. Zooplankton were then preserved in 60% ethanol and analysed for species composition and abundance. Samples were diluted to a known volume and counted under a dissection microscope in 5 mL aliquots in a gridded Perspex counting tray at approximately 30x magnification. Repeated subsamples were counted until at least 400 individuals were counted. Species were identified using standard taxonomic guides by Chapman and Lewis (1976) and Shiel (1995).

From 4 December 2006, three fine-mesh Gee minnow traps were set overnight (approximately 16 hours) in each of the mesocosms for three consecutive nights and all captured fish were weighed and measured. The crates of plants were then removed and checked for fish and all water was then siphoned out of tanks, and passed through a 1 mm sieve to catch fish. All remaining debris on the bottom of the mesocosms was carefully removed and sorted in the laboratory to check for any remaining fish. All *Gambusia* recovered were sexed, and then euthanised using clove oil.

Statistical Analysis

As mudfish were held in captivity their condition factor was not comparable to wild populations. Therefore relative condition factor (K_n) was calculated based on the condition of mudfish in the zero *Gambusia* mesocosms. Relative condition was calculated with the equation:

$$K_n = (W/W') \quad (\text{Murphy and Willis, 1996})$$

where W is weight of the individual and W' is the length-specific mean weight for a fish in the population (standard weight). The standard weight (W') was calculated by

the line of best fit when plotting log₁₀ weight and log₁₀ length of mudfish in mesocosms without *Gambusia* (Murphy and Willis, 1996). One way ANOVAs were performed to test for significant differences in mudfish length, weight and condition followed by post-hoc Tukey's tests. Data are presented as mean values ± standard error of the mean (SEM).

Gambusia condition (CF) was calculated using the equation:

$$CF = 100 \times \frac{BW}{TL^3} \text{ (Koya et al., 2000).}$$

where BW is the fish bodyweight and TL is the total length of fish. A one way ANOVA was performed to test the significance of *Gambusia* density on condition. Weight data was log₁₀ transformed to fulfil assumptions of normality and homogeneity of variance. Tests were considered significant if $p < 0.05$. Data are presented as mean values ± SEM.

A principal component analysis was carried out in CANOCO version 4.51 on log-transformed zooplankton densities to create an ordination biplot to determine the effects of *Gambusia* densities on zooplankton community composition. Analysis was carried out on the two individual sampling periods.



Figure 3.1 Male *Gambusia affinis*



Figure 3.2 Mesocosms used to study *Gambusia* density on black mudfish

3.3 Results

All mudfish were recovered from the mesocosms, and no evidence of aggressive *Gambusia* behaviour (fin-nipping) was observed. Significant differences were found between the three *Gambusia* density treatments for mean mudfish length, weight and condition at the end of the experiment (Figure 3.3, Figure 3.4, Figure 3.5). Mudfish in the control tanks all had a greater increase in mean length, weight and condition than mudfish in treatments with low and high *Gambusia* density. Mudfish in tanks with a high density of *Gambusia* experienced the smallest increase in length, weight and condition. Mudfish mean length data was found to be significantly lower in high density *Gambusia* mesocosms than low density and zero *Gambusia* mesocosms ($p < 0.001$, $F = 35.5$, $d.f. = 2$). Post-hoc Tukey tests on length data found significant differences between the control and high density *Gambusia* tanks and between low density and high density *Gambusia* tanks, but not between the control and low density tanks (Figure 3.3). There were significant differences between all three treatments for weight ($p < 0.001$, $F = 51.9$, $d.f. = 2$) and condition factor ($p < 0.001$, $F = 38.1$, $d.f. = 2$) (Figure 3.4, Figure 3.5).

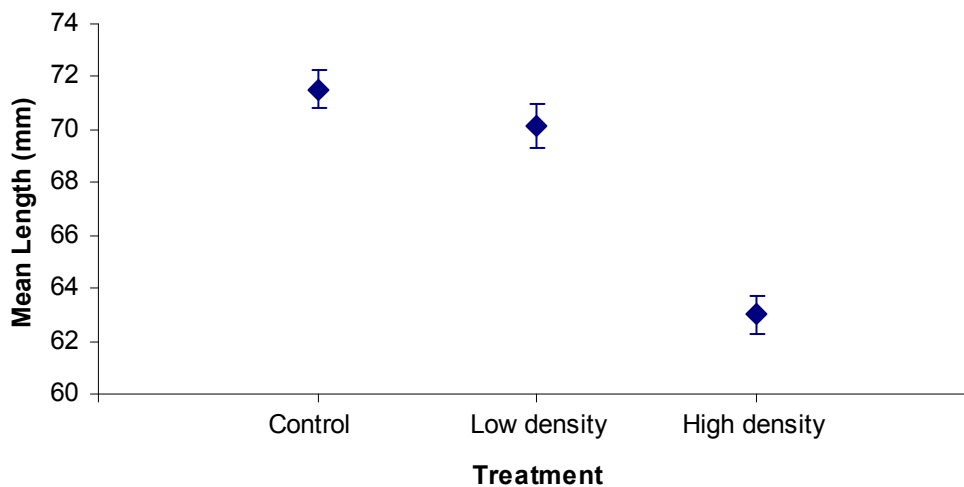


Figure 3.3 Mean mudfish total length (mm) \pm SEM ($n = 15$) when reared for 3 months with different densities of *Gambusia*: control (no *Gambusia*), low density (2 x *Gambusia*) or high density (8 x *Gambusia*).

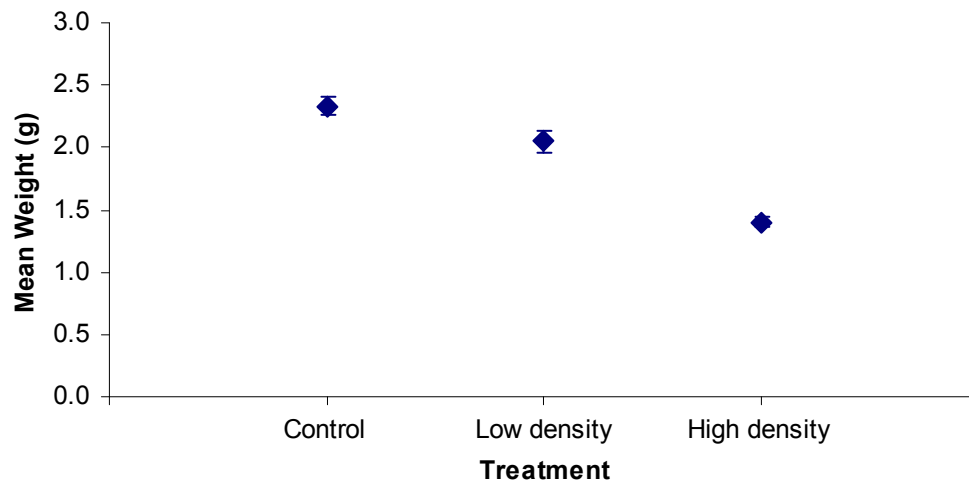


Figure 3.4 Mean mudfish weight (g) \pm SEM (n = 15) when reared for 3 months with different densities of *Gambusia*: control (no *Gambusia*), low density (2 x *Gambusia*) or high density (8 x *Gambusia*) for 3 months.

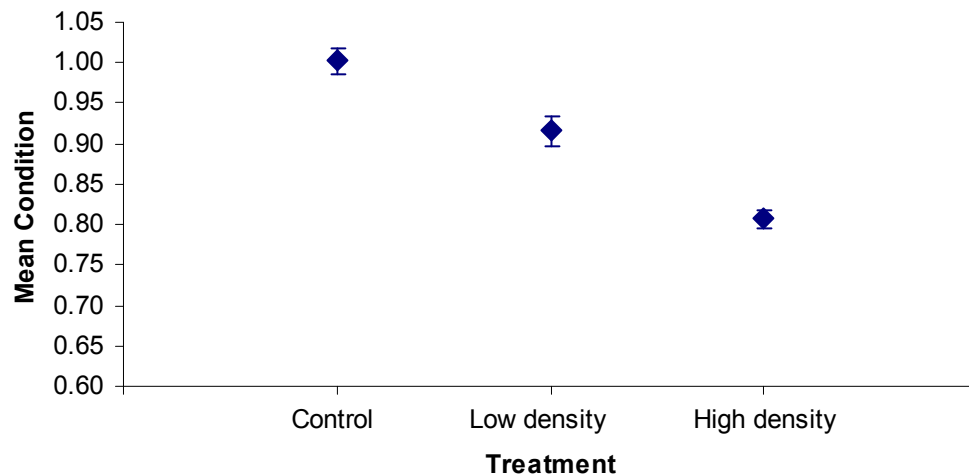


Figure 3.5 Mean mudfish condition \pm SEM (n = 15) when reared for 3 months with different densities of *Gambusia*: control (no *Gambusia*), low density (2 x *Gambusia*) or high density (8 x *Gambusia*).

Not all *Gambusia* were recovered from mesocosms, with 8, 9 and 10 of 10 released *Gambusia* collected from the three low density mesocosms, and 32, 32 and 37 of 40 released *Gambusia* collected from the three high density mesocosms. The fate of

unrecovered *Gambusia* is unknown, possibly dying of natural causes, as a result of cannibalism, aggressive behaviour from other fish (*Gambusia* or mudfish), or that they were well hidden among mesocosm plants. No *Gambusia* fry or juveniles were found in mesocosms, either a result of temperatures not being high enough for reproduction, or due to predation from larger fish.

Gambusia growth was affected by their stocking density. Analysis of length data found significant differences between the two densities ($p < 0.01$, $F = 7.52$, d.f. = 1) with length increases being greater for fish stocked at low density (Figure 3.6). Weight also showed a greater increase for fish stocked at low density ($p < 0.01$, $F = 8.21$, d.f. = 1) (Figure 3.7). However, stocking density was found to have no significant effect on *Gambusia* condition. Figure 3.8 shows a higher condition value for fish stocked at low densities, but this difference was not significant due to high individual variation in condition.

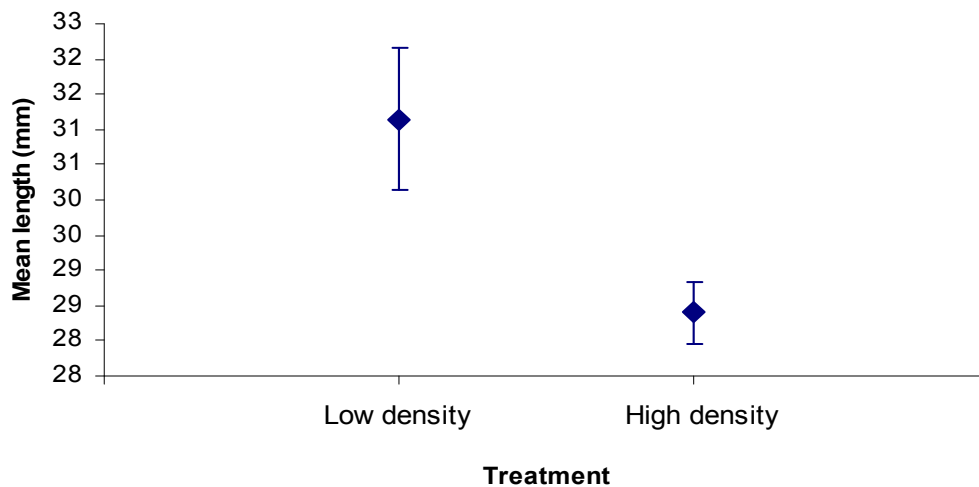


Figure 3.6 Mean *Gambusia* length (mm) \pm SEM when reared for 3 months stocked at either a low density ($n = 27 + 5$ mudfish) or high density ($n = 101 + 5$ mudfish).

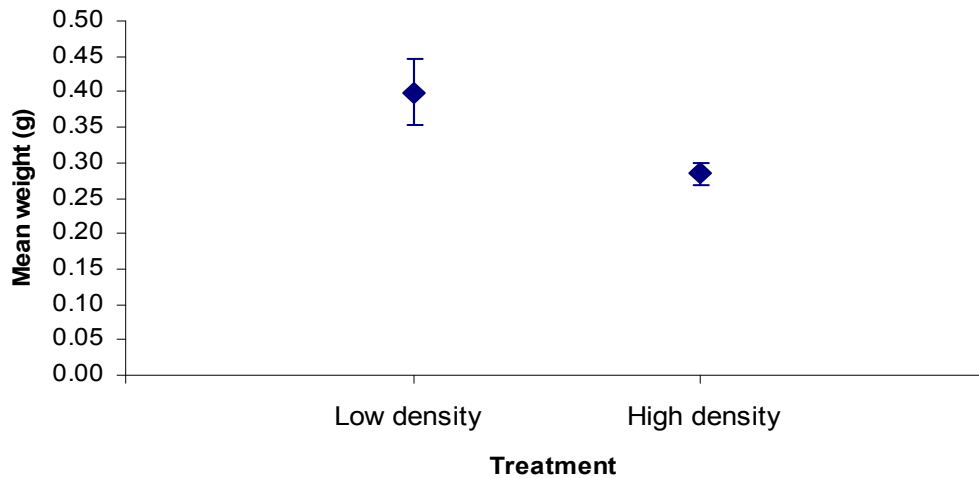


Figure 3.7 Mean *Gambusia* weight (g) \pm SEM when reared for 3 months stocked at either a low density (n = 27 + 5 mudfish) or high density (n = 101 + 5 mudfish).

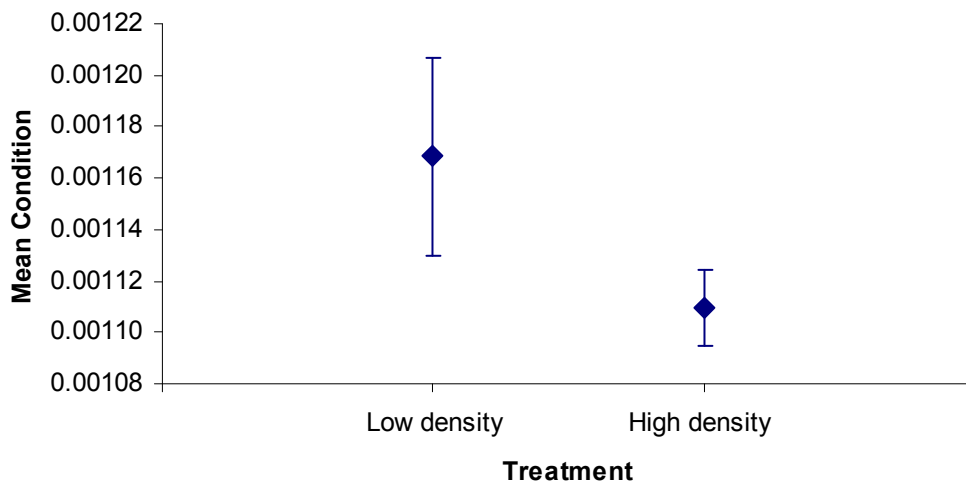


Figure 3.8 Mean *Gambusia* condition \pm SEM when reared for 3 months stocked at either a low density (n = 27 + 5 mudfish) or high density (n = 101 + 5 mudfish).

Analysis of mesocosm zooplankton samples taken on 16 November using principal component analysis revealed several trends between the three different treatments (Figure 3.9A). The arrangement of mesocosms in the biplot (circles) indicates how closely the zooplankton community composition is related for each treatment. The length of biplot arrows indicates the importance that each species has on the distribution of samples (mesocosms) on the axes, and the direction of the arrows

indicates the association between species and samples. Axis 1 (x-axis) was found to account for 51% of the variation, while axis 2 (y-axis) accounted for a further 17%. Community composition in the three mesocosms with zero *Gambusia* were all found to be very similar, with their position in the biplot (negatively associated with axis 1, on the left of the plot) influenced by a greater abundance of large crustacean zooplankton such as Calanoid copepods, *Chydorus sphaericus*, *Simocephalus vetulus* and abundant copepod nauplii in these mesocosms. Zooplankton populations in mesocosms with *Gambusia* (both low density and high density) were more varied in their composition. However, all were substantially different to mesocosms without *Gambusia*, having distributions on the biplot either strongly positively associated with axis 1, or only weakly negatively associated with this axis (i.e., distributed in the centre and right of the ordination). The majority of mesocosms with *Gambusia* showed very significant depletion of larger zooplankton, with communities dominated by greater abundance and diversity of rotifers (primarily small *Lecane* species). Interestingly, no *Daphnia carinata*, which were added to all mesocosms on three occasions, were found in samples from any of the mesocosms. This indicates *D. carinata* was readily consumed by mudfish, and most likely by *Gambusia* as well.

Mesocosm zooplankton samples taken on 4 December showed a similar trend, with zooplankton populations in mesocosms with no *Gambusia* dominated by larger, crustacean, zooplankton (Figure 3.9B). Again, *Simocephalus vetulus*, copepod nauplii and Calanoid copepods were a predominant part of the community, along with Harpacticoid copepods and *Bosmina*. By the three month stage in the treatments, zooplankton populations in mesocosms with high densities of *Gambusia* were more similar to one another as shown by their close association in the ordination. In high density *Gambusia* mesocosms the rotifers *Lepadella*, *Keratella* and *Lecane* species were the predominant members of the zooplankton communities. Zooplankton communities in low density *Gambusia* mesocosms were highly varied, but were also dominated by rotifer species. Axis 1 was found to account for 39% of the variation, while axis 2 accounted for 16%.

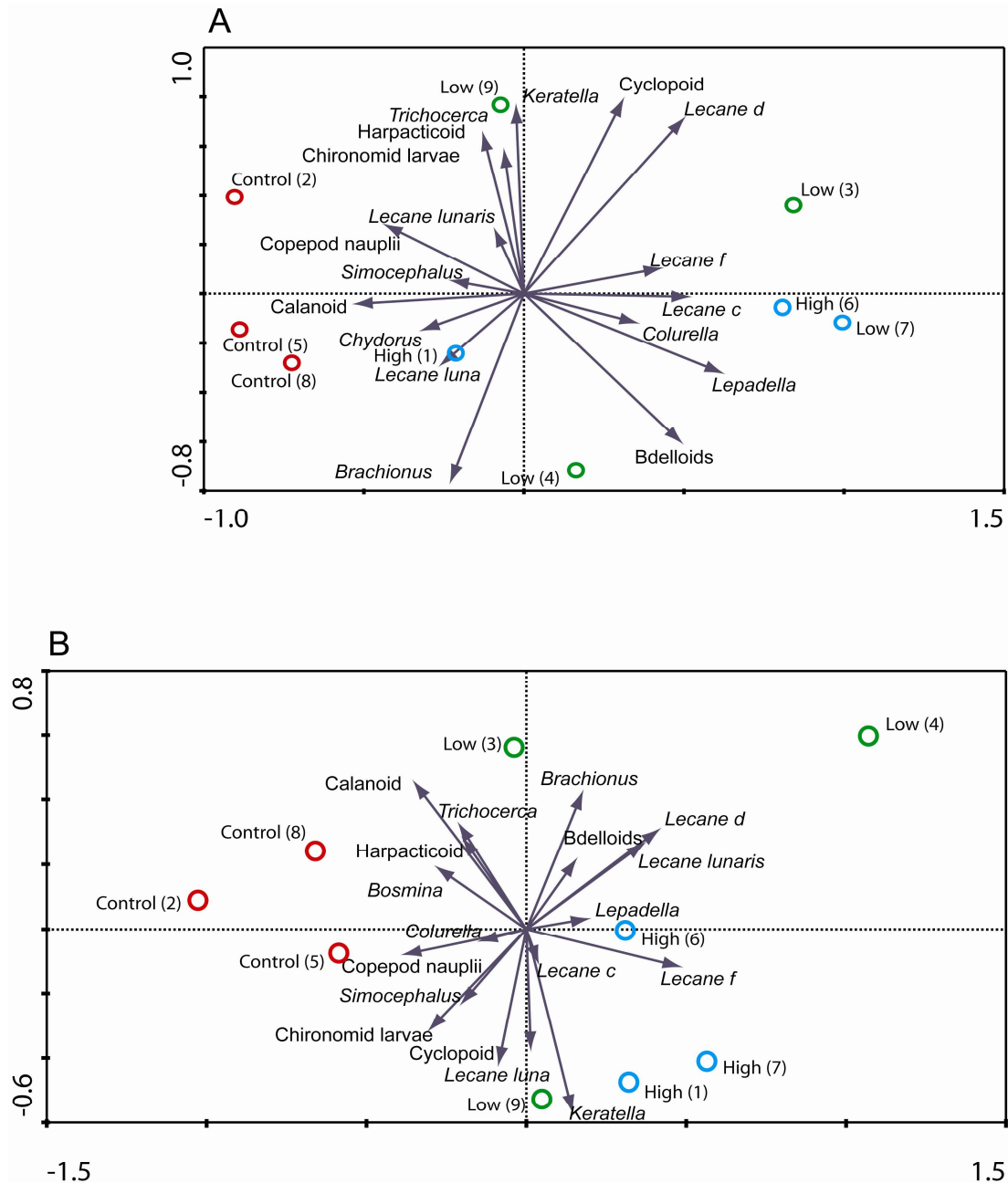


Figure 3.9 Principal component analysis on mesocosm zooplankton populations when stocked with 5 mudfish each and different *Gambusia* densities: control (no *Gambusia*), low density (2 x *Gambusia*) or high density (8 x *Gambusia*) (*Simocephalus* = *S. vetulus*, *Chydorus* = *C. sphaericus*, *Brachionus* = *B. quadridentatus*, *Harpacticoid* = *Phyllognathopus viguieri*, *Calanoid* = *Skistodiaptomus pallidus*, *Lecane c* = *L. closterocerca*, *Lecane f* = *L. flexilis*, *Lecane d* = *L. decipiens*, *Lepadella* = *L. accuminata*, *Keratella* = *K. slacki*, *Trichocerca* = *T. similis*, *Colurella* = *C. uncinata*).

A After 10 weeks; **B** After 13 weeks

3.4 Discussion

An increasing inhibition of black mudfish growth with increasing *Gambusia* density demonstrates a negative effect by *Gambusia* on mudfish. This finding is similar to experiments by Howe et al. (1997), Rincon et al. (2002) and Ling and Willis (2005) on interactions between native fish species and *Gambusia*, all of whom found native species were negatively affected by *Gambusia*, resulting in a decreased growth rate of native fishes. Ling and Willis (2005) found the presence of *G. affinis* in mesocosms with black mudfish caused a decrease in mudfish condition, and suggested this was due to interspecific competition. Similarly Rincon et al. (2002) found the growth of young of the year Spanish toothcarp (*Aphanuius iberus*) in mesocosms with *G. holbrooki* to be reduced. They also suggested this was a result of an overall increased density of fish, including *Gambusia*. However, Howe et al. (1997) suggested that nutritional conditions in all tanks were adequate for growth, and reduced growth rates may be due to stress after finding that supplementary feeding provided no additional growth in *Pseudomugil signifer* in the presence of *G. holbrooki*.

Gambusia have been found to chase juvenile mudfish in laboratory experiments (Barrier and Hicks, 1994), however, no evidence of aggressive behaviour (damage from fin-nipping) was seen in the mesocosm experiments and all juvenile mudfish survived the 3 month exposure to *Gambusia*. A number of factors have been found to decrease *Gambusia* aggression to other fish species including the relative body size of both species, with less aggression exhibited towards fish larger than themselves (Barrier & Hicks, 1994); lower temperatures (Rincon et al., 2002); increased shelter and refuges (Benoît et al., 2000; Ling and Willis, 2005); and the presence of alternative live prey (Rincon et al., 2002; Ling and Willis, 2005). All of these factors were present in this experiment, and are also present in most black mudfish habitats.

It was also found that *Gambusia* stocked at high densities experienced less growth than *Gambusia* stocked at lower densities. *Gambusia* are known to be cannibalistic and aggressive towards conspecifics (Benoît et al., 2000) and this could possibly have

negatively affected their own condition when stocked at high densities via intraspecific aggression. Furthermore, not all *Gambusia* were recovered from the mesocosms possibly due to cannibalism, predation by mudfish, or natural mortality.

With no physical evidence of *Gambusia* aggression on either fish species, it is thought that the differences in both mudfish and *Gambusia* condition are most likely due to competition for food resources based on fish biomass per tank, and that decreased mudfish growth in the presence of *Gambusia* is not the result of *Gambusia* aggression towards mudfish. Furthermore, the nocturnal habits of mudfish reduces opportunities for aggression by *Gambusia*, a largely diurnal species (Ling and Willis, 2005).

Analysis of the zooplankton community found the presence of *Gambusia* had altered the community composition. Large zooplankton were less prevalent in mesocosms with *Gambusia* indicating *Gambusia* had consumed the large prey items. Presumably mudfish also consumed these same large prey species, however, due to the lower overall density of fish in the zero *Gambusia* mesocosms, reproduction of large zooplankton can still occur allowing persistence of these species. *Gambusia* preferentially consumed large zooplankton species, such as cladocerans, and progressively fed on smaller prey items as the larger ones were removed. Mudfish, therefore, also had to prey on progressively smaller food items and with the increased competition for food resources, both species experienced less growth. The absence of large grazing zooplankton in mesocosms with *Gambusia* has seen an increase in the availability of phytoplankton resources, resulting in a large increase in rotifer numbers. Large zooplankton such as cladocerans are known to be more efficient exploiters of food resources and therefore suppress small rotifers (Gilbert, 1985; Fradkin, 1995).

Hurlbert et al. (1972) also found *Gambusia* to have a preference for larger prey items, resulting in changes in the composition of the aquatic ecosystem. He found changes were progressive, with *Gambusia* first consuming *Daphnia* and insects, allowing

increases in planktonic rotifers. *Gambusia* then fed on rotifer populations, allowing phytoplankton blooms to occur. Angeler et al. (2002) found the same pattern occurring, with *Gambusia* removing large cladocerans first, resulting in rotifer and copepod dominated communities.

Both *Gambusia* and black mudfish will eat a variety of food, largely dictated by what is available and their respective gape size. Investigations into *Gambusia* diet by Mansfield and McArdle (1998) found macroinvertebrates such as cladocerans, copepods and chironomid larvae and pupae comprised >65% of their diet, with rotifers and terrestrial fauna only comprising a further 18%. Black mudfish diets are known to be similar, consisting of chironomids, copepods, cladocerans, ostracods and oligochaetes (McLea, 1986; McDowall, 2000).

Conservation Implications

Previous studies by Barrier (1993), Barrier and Hicks (1994) and Ling and Willis (2005) have established that, due to several environmental and behavioural traits, there is a reduced chance of aggressive *Gambusia* behaviour adversely affecting juvenile and adult mudfish, although mudfish recruitment may be threatened by the presence of *Gambusia*. However, population peaks and breeding periods occur in different seasons diminishing the risks of *Gambusia* predation on mudfish fry. During summer dry periods mudfish have the ability to aestivate and therefore inhabit ephemeral habitats where *Gambusia* will perish. In addition, the nocturnal habits of mudfish, while *Gambusia* are largely diurnal, result in reduced conflict between the species, and the habitat complexity typical in mudfish habitats provides refuges for mudfish.

Although direct predation or aggression by *Gambusia* on mudfish does not seem to be significant, this research indicates that when *Gambusia* are present in natural mudfish habitats they will increase competition for food resources, with competition increasing as *Gambusia* density increases. The intensity of the competition may be influenced by seasonal variables with warm summers resulting in increased

Gambusia numbers and therefore greater competition. Increased interspecific competition and reduced growth of juvenile mudfish is of concern as the body size of mudfish prior to summer droughts and aestivation is correlated with their ability to survive extended periods of aestivation (McPhail, 1999). A reduced growing season for adult mudfish as a result of competition with *Gambusia* may also have a negative impact on mudfish populations. If adult mudfish growth is greatly inhibited, the reduction in energy reserves may have a negative impact on reproduction (Hislop et al., 1978), particularly because adult mudfish need to establish reproductive condition during autumn, following the summer aestivation, at a time when *Gambusia* numbers remain high. Although *Gambusia* are excluded from aestivation habitats during summer, reinvasion of ephemeral wetlands from adjacent permanent pools is likely to affect mudfish adult conditioning. This hypothesis could be tested in natural habitats by comparing different mudfish habitats with varying *Gambusia* densities or by using exclusion barriers to prevent *Gambusia* incursions.

4. Reliability of Gee Minnow Trapping

4.1 Introduction

A crucial aspect of a successful species monitoring programme is the ability to capture the species, and therefore confidently report on the population health and impacts on the species. Therefore it is important to understand the reliability of the trapping techniques used. The reliability of trapping techniques is also an important consideration when measuring the success of a translocation. It is necessary to understand how representative trapping results are of actual species numbers in a natural environment, and whether the reliability of results is limited to presence or absence of a species, or if they are reliable enough to establish population densities.

Several methods have been used to capture black mudfish, including electric fishing, seine netting, mini fyke nets, dip nets, and Gee minnow traps (Thompson, 1978; Eldon, 1992). The success of each method varies greatly, and it has been found that Gee minnow traps set overnight are the most reliable passive capture technique for sampling all *Neochanna* species (Ling, pers. com.). Gee minnow traps require little labour, and can be used in water with many obstructions and vegetation present, as is typical of the majority of mudfish habitat. Gee minnow traps also have the advantage that they cause little disruption to the habitat. Traps are set overnight, achieving a prolonged fishing effort during periods when mudfish are active and feeding. Various factors will affect the success of passive fishing gear, including mesh size, with catches enhanced by the use of fine-mesh traps (2 mm x 2 mm) as opposed to larger mesh sizes such as 6 mm x 6 mm (O'Brien, 2005). Weather conditions and habitat characteristics will also affect capture rates, along with levels of fish foraging, which is influenced by the abundance of food and the presence of competitors or predators (O'Brien, 2005). Trapping has been identified as being selective for size and species (Laarman and Ryckman 1982; Beamederfer and Rieman, 1988) however,

in the case of mudfish, only the very young fry (<25 mm TL) are not caught as they can swim through the mesh.

O'Brien (2005) found that the presence of other fish species such as shortfinned eels and upland bullies (*Gobiomorphus breviceps*) reduced the numbers of Canterbury mudfish captured in Gee minnow traps, with shortfinned eels having the largest influence on catch rates. The presence of predators can cause changes in behaviour, such as reduced activity (Fuiman and Magurran, 1994), therefore mudfish activity could be reduced in the presence of predators such as eels, resulting in a lower probability of capture in minnow traps. O'Brien (2005) suggested that when trapping for mudfish, it should also be checked whether predators such as *A. australis* are present before ruling out the presence of mudfish.

Most trapping gear decreases in efficiency from the time traps are set (Ricker, 1975). The presence of fish in the trap may deter other mudfish from entering, or conversely, trapped fish may attract fish. For example, trapping studies of sunfishes have found that a trapped fish will attract others to it, sometimes resulting in one small trap containing a dozen fish, while ones nearby are nearly empty (Ricker, 1975).

Problems may arise when trapping with Gee minnow traps in wetlands if mudfish use vegetation as refuges. Also, mudfish do not appear to move large distances within a wetland (Perrie, 2004), which means their capture may be largely dependent on the proximity of the trap. Catchability may also vary with seasonal changes in water levels and temperatures, and physiological and behavioural changes of the fish.

To investigate a number of parameters that may influence the efficiency of Gee minnow traps a sampling programme in a constructed habitat was undertaken to determine the effects of the short-term and long-term memory of mudfish, trap shyness, the presence of fish already in traps, trap location (surface vs bottom) and different mudfish densities and water depth.

4.2 Methods

A series of tanks, consisting of nine concrete cattle troughs (550 mm deep x 1500 mm diameter), was established in an area shaded from the north as described in Chapter 3 (Figure 4.1). Tanks were filled with Hamilton city tap water that was allowed to age for one week, and kept free of any plants or debris. Tanks were stocked with three different densities of mudfish, replicated three times i.e. 3 x 5 mudfish, 3 x 10 mudfish and 3 x 20 mudfish per tank. Mudfish were assigned to tanks randomly until the required numbers were reached in each tank. Mudfish length ranged from 32 – 48 mm. Water depth was initially set at 400 mm.

Fish were left to acclimatise to tanks for one week prior to trapping. Fish were fed commercially prepared frozen bloodworms (chironomid larvae) approximately 3-4 times a week. Four Gee minnow traps per tank were set overnight in tanks for three consecutive nights. Traps were set beginning at 4:30 pm with one trap set every minute thereafter. Traps were collected the following morning beginning at 9:00 am with one trap removed every minute. Captured mudfish were collected in a bucket and the number of fish in each trap counted. Fish were released back into the tanks once all traps had been collected.

The first series of trapping was carried out with water depth set at 400 mm and traps set at the surface. After the initial trapping for three consecutive nights, fish were left undisturbed for one week before commencing trapping for another three consecutive nights with traps set at the surface. Fish were always left undisturbed for one week between consecutive trapping. With water depth still set at 400 mm, traps were set at the bottom of the tanks for two lots of three consecutive nights. The water depth was then dropped to 200 mm and traps set for two lots of three consecutive nights. At 200 mm and traps resting on the bottom, water did not quite reach the top of traps. At the end of the trapping program, all tanks were drained and fish collected, measured and weighed.



Figure 4.1 Gee minnow traps set in tanks at 400 mm water depth

Statistical analysis

Unpaired t-tests were performed in Microsoft Excel to determine if there were differences in the mean number of fish caught between the first and third nights.

F-tests were performed in Microsoft Excel to test whether significant variance between catches of individual traps on the first and third nights occurred. Data was grouped separately to account for different fish densities, water levels, trap positions and replicates resulting in 18 groups of data.

To analyse the effect of trap position, total catch per trap night was converted into a percentage of fish present, and data grouped depending on trap position for each trap night. Data was analysed by trap position and fish density, and then analysed for trap

position with the three different fish densities combined. An ANOVA was performed in Statistica, with post-hoc Tukey tests carried out on both data sets.

Density data was log₁₀ transformed so that assumptions of normality and homogeneity of variance were fulfilled, and an ANOVA performed in Statistica to test for an effect of density.

4.3 Results

No significant difference was found between the mean number of fish caught between the first and third nights for any of the treatments (Figure 4.2, Figure 4.3 and Figure 4.4). Therefore, trapping events were considered independent for analysis of trap position.

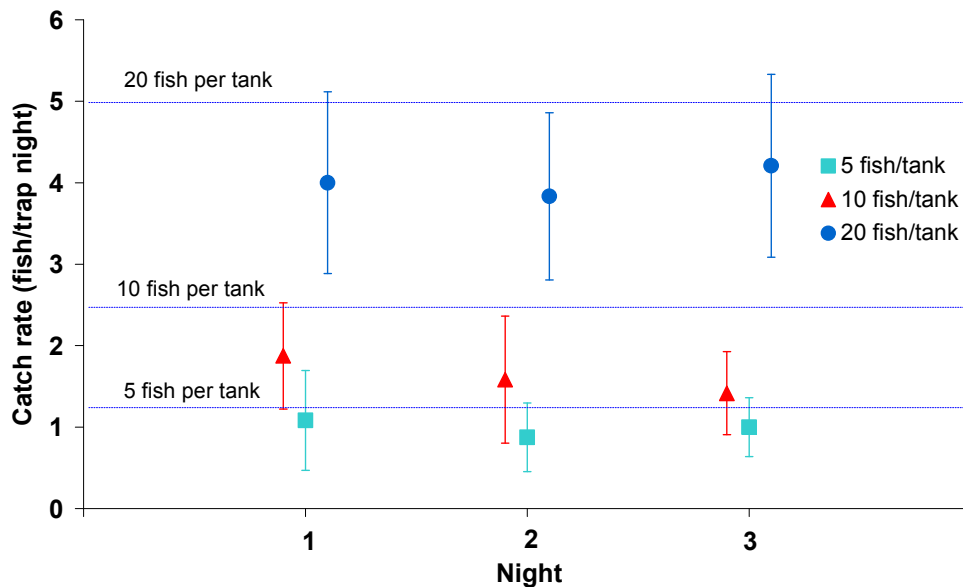


Figure 4.2 Mean catch rate (\pm SEM) of differing mudfish densities over three consecutive nights when water depth was 400mm and traps were set on the surface. Dotted lines indicate the maximum mean catch possible for each fish density.

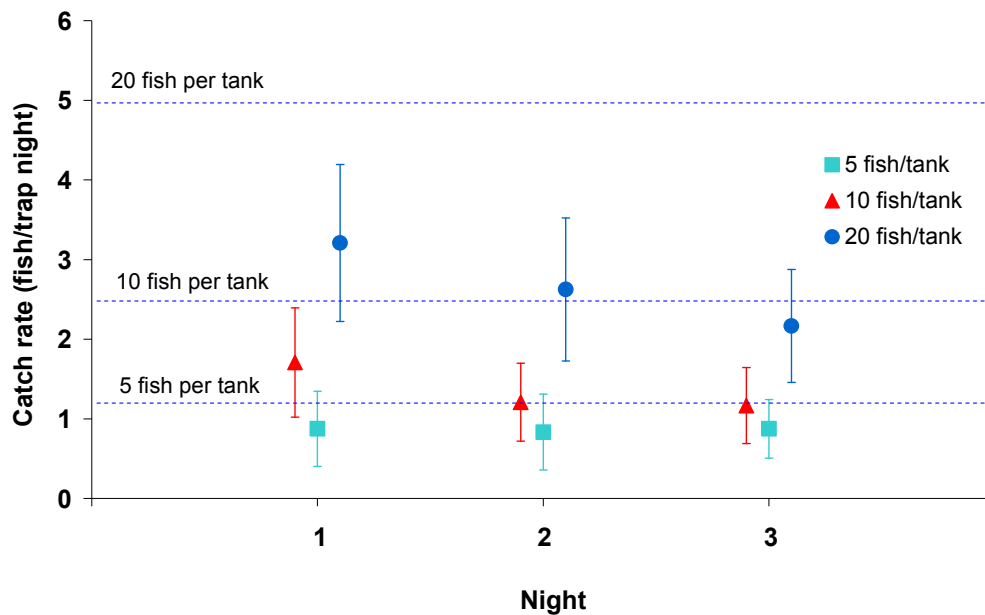


Figure 4.3 Mean catch rate (\pm SEM) of differing mudfish densities over three consecutive nights when water depth was 400mm and traps were set on the bottom. Dotted lines indicate the maximum mean catch possible for each fish density.

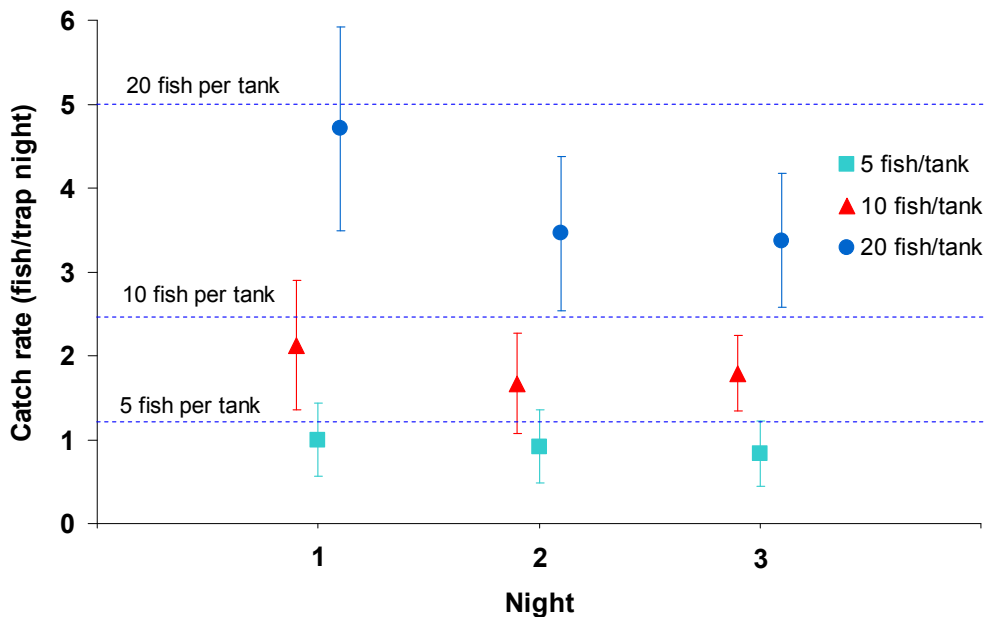


Figure 4.4 Mean catch rate (\pm SEM) of differing mudfish densities over three consecutive nights when water depth was 200mm. Traps were approximately 200 mm deep so caught fish on the bottom and surface. Dotted lines indicate the maximum mean catch possible for each fish density.

Tests of variance of the number of fish caught in traps between the first and third nights found significant differences in 7 out of 18 treatments. Of these seven, six treatments had a decrease in variance over time, indicating fish may avoid traps where numerous fish have already been trapped. However, for the majority of tanks no significant difference in variance between nights was found.

It was found that the position of traps influenced how many fish were caught (Figure 4.5), with a greater number of fish caught when traps were set on the surface or in 200 mm of water, than when they were set on the bottom in deeper water. Tukey post-hoc tests found significant differences between traps set on the surface (densities of 20 and 5 fish/tank) and traps set on the bottom (densities of 20 and 10 fish/tank) ($p < 0.05$). Traps set in 200 mm of water (density of 20 fish/tank) were also significantly different from bottom-set traps in deeper water (densities of 20 and 10 fish/tank) ($p < 0.05$).

When the different densities of fish were combined (Figure 4.6) it was again found that traps set on the bottom caught significantly fewer fish than traps that were set on the surface ($p < 0.001$, $F = 15.0$, d.f. = 1) or in shallower water ($p < 0.001$, $F = 14.6$, d.f. = 1).

Analysis of variance found there was no significant effect of fish density on the percentage of fish caught per tank. The mean percentage of fish caught at each density was 70% for 20 fish/tank, 65% for 10 fish/tank and 74% for 5 fish/tank.

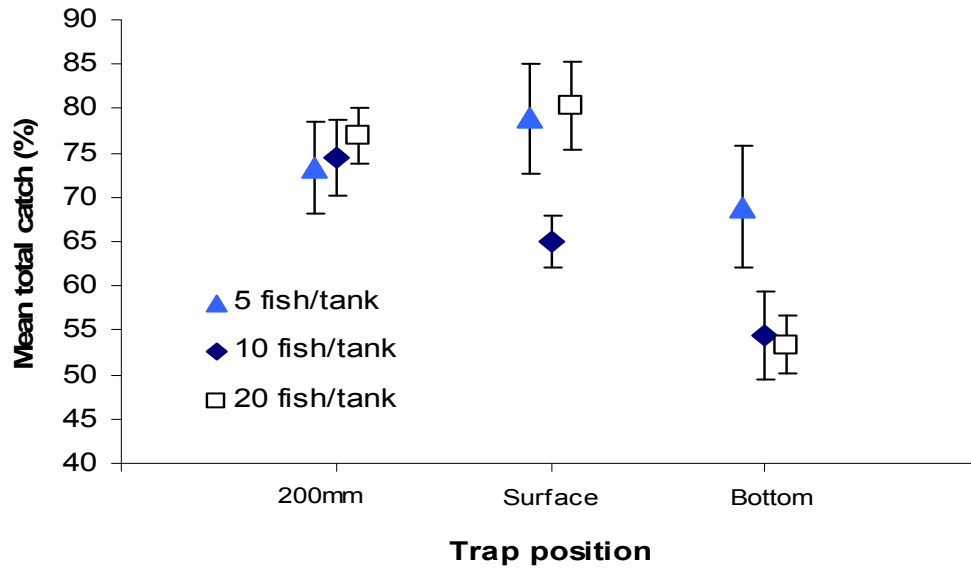


Figure 4.5 Mean total catch (%) \pm SEM (n = 18) of mudfish when at different densities, and varying trap position. Trap positions were: in 200 mm of water where trap touches the bottom and the surface; in 400 mm of water where trap is set at the water surface; or in 400 mm of water where trap is set on the bottom of tank.

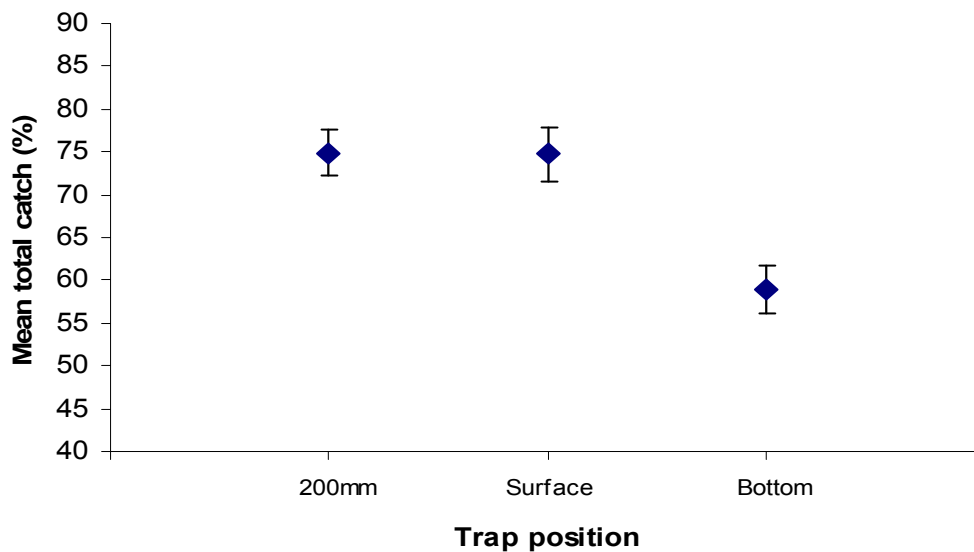


Figure 4.6 Mean total catch (%) \pm SEM (n = 54) of mudfish when varying trap position. Trap positions were: in 200 mm of water where trap touches the bottom and the surface; in 400 mm of water where trap is set at the water surface; or in 400 mm of water where trap is set on the bottom of tank.

4.4 Discussion

Results from this trapping programme help to establish the reliability of Gee minnow traps for trapping black mudfish. A key finding to aid in the assessment of Gee minnow trap reliability was that mudfish were not trap shy over the short-term or long-term. In the short-term, mean numbers of fish caught between the first and third nights of consecutive trapping for all treatments did not differ from each other. When trapping was resumed after a period of one week, high numbers of fish were again caught, with means for the same treatments not varying. Stott (1970) also found that perch (*Perca fluviatilis*) were not trap-shy, and that having been caught before did not decrease the subsequent vulnerability of perch from being trapped again. However, when trapping over consecutive nights the catch rate of perch tended to be related to water temperature, with catch rates positively correlated with temperature up to about 10.5°C. Temperature was not a factor investigated in this study, but could be an area for further investigation in the future.

It was previously unknown whether mudfish were attracted to, or avoided, traps that already contained mudfish. Other fish species, such as the sunfish, are known to be attracted to traps already containing fish, with sunfish in traps near spawning beds appearing to attract others to them resulting in many fish in one trap, with nearby traps remaining empty (Ricker, 1975). Tests showed that for most of the mudfish trapping treatments, there was no difference in variance over time, with only one-third of the treatments having a decrease in variance over time. The variance between numbers of fish caught per trap remained high throughout the trapping programme, possibly indicating mudfish are attracted to traps already containing fish, resulting in many mudfish in one trap while others have few. However, the likelihood of mudfish being caught in Gee minnow traps is also likely to be a stochastic event as a result of behaviours such as foraging for food.

Trap position was found to have a significant effect on the number of mudfish trapped, with a greater number of fish caught when traps were placed just below the

water surface. Fish were still caught in traps positioned at the bottom of tanks, however, catch rates were reduced. The greater catch rates when traps were set at the surface were most likely due to the feeding behaviour of mudfish, with mudfish diets including insects trapped at the water surface (Ling, 2001). Current recommendations are to set Gee minnow traps just below the water surface when trapping mudfish (Ling, 2001) because mudfish feed at the water surface at night and to provide an air space for trapped fish. It is not unusual for natural mudfish habitats to have low dissolved oxygen levels, and an air space in traps ensures mudfish can gulp air at the surface. Results from this experiment reinforce recommendations to set traps just below the water surface, as in addition to providing air for trapped fish in waters with low oxygen levels, traps in this position will also catch a greater number of mudfish. No mudfish mortality ensued from setting traps on the bottom in this experiment because dissolved oxygen levels in the tanks were always close to saturation.

Catch rates were the same when traps were set at the surface of deep water, as when traps were set in shallow water. Therefore trap position seems to be of far greater importance in this experiment than water level.

No effect was found of fish density on the percentage of fish trapped, with the mean percentage of fish caught in each density ranging from 65% to 74%. Variance within densities was high, however the absence of any overall effect of density is advantageous when considering calculations for population estimates. In this experiment relative efficiency did not vary meaning catch per unit effort (CPUE) is a good indication of fish density. This is in accordance with Ricker (1940; in Ricker 1975) who stated “it is well established that CPUE is proportional to the mean stock present during the time fishing takes place”. However, CPUE should be used with caution as a measure of relative abundance (Stott, 1970; He and Lodge, 1990) as the efficiency of traps may vary with environmental factors. It is possible that catch rates in this experiment may be unusually high as minnow traps provided structure within an otherwise bare tank (Petrik and Levin, 2000). Mudfish may have been attracted to

the potential cover offered by the structure, and catch rates were potentially higher than what would be expected in a natural environment with plentiful refuges. Perrie (2004) observed recapture rates of tagged mudfish in natural habitats to vary from 5 to 58%. However, the density of traps in my experiment, at 2.3 traps per m², was substantially higher than would typically be deployed in any natural habitat, but was used to reflect the intended trap density deployed in the wetland pools of the Lake Kaituna translocation site. Furthermore, behavioural interactions between mudfish and species such as shortfin eels resulting in reduced mudfish activity may decrease the vulnerability of mudfish to capture (O'Brien, 2005). He and Lodge (1990) also found difficulties with trapping species in the presence of other species, suggesting that catch rates of mudminnows (*Umbra limi*) were decreased due to behavioural interactions between mudminnows and redbelly and finescale dace (*Phoxinus eos* and *Phoxinus neogaeus*).

It can be concluded that catch rate is independent of mudfish density or repetitive trapping, as mudfish are not trap shy or deterred by traps already containing mudfish. Trap position was the most important variable when considering Gee minnow trapping of black mudfish, with catch rates increased when traps are set just below the water surface.

5. Habitat Characterisation and Translocation

5.1 Introduction

5.1.1 Habitat characterisation

Often only a few key environmental factors are significant in determining the habitat distribution of a species. These may be physical factors such as temperature and oxygen, or biotic factors like predation or competition (Holomuzki, 1991). When attempting to determine these influential factors, the concept of scale is a crucial consideration (Bergin, 1992). If the scale is too large, important details may be omitted, and if too small a scale, abundant data may result in a high level of variability (O'Brien, 2005). O'Brien (2005) addressed the concept of scale when characterising habitats of Canterbury mudfish. Vegetation surveys conducted at a coarse-scale (percentage cover of macrophytes, branches etc) and a fine-scale (where each plant was identified) found that fine-scale analysis of macrophyte taxa proved to be the most reliable method for characterising the habitat of Canterbury mudfish. However, it was suggested that the association between Canterbury mudfish and macrophyte species could be indirect, with other factors such as hydrology and water quality being of greater importance (O'Brien, 2005).

The presence of aquatic plants in pools influences the environment in many ways, adding structural complexity, influencing the abundance and composition of macro-invertebrate communities, and also altering the physical and chemical properties of the water. Greater structural complexity of wetlands and pools may reduce hunting efficiency by predators, such as eels and mosquitofish (Chapman et al., 1996). Plants can cause variability in oxygen and temperature over spatial and temporal scales, which along with structural complexity, creates refugia for mudfish which have evolved to withstand the hypoxic water often present in wetlands and still pools (Miranda et al., 2000). The presence of predators may influence microhabitat

selection by mudfish, making those with an abundance and complexity of plants preferable (O'Brien, 2005).

Work by Hicks and Barrier (1996) addressed habitat selection by black mudfish, and attempted to define their preferred habitat based on several variables. It was found that one of the most influential factors was the absence of water during summer. Eighty-seven percent of sites with mudfish were found to be dry at some point over summer. Vegetation was recorded at a coarse scale (emergent, submerged and overhanging vegetation, submerged woody debris, and tree roots). Mudfish were present more frequently where emergent vegetation, overhanging vegetation and tree roots were present. This vegetation coincided with a rating of low to moderate disturbance to the area. Another factor found to be significant was the soil type, with more fish present on semi-mineralised or peat bog substrate types. Black mudfish were not common in highly turbid water and other water quality variables were recorded but not correlated with mudfish distribution. It was also found that where species other than juvenile eels and *Gambusia* were present (eg. common bullies or inanga), black mudfish were absent.

Identifying a site for translocation is a key step that requires a thorough understanding of the life history attributes of the species to be translocated, and the required habitat for its complete life-cycle (O'Brien and Dunn, 2005; Sheller, 2006). Long term, in-depth knowledge of the translocation site is also essential (O'Brien and Dunn, 2005). Lake Kaituna was recommended as a suitable site for the translocation of black mudfish by DoC's mudfish recovery group, however, hydrological and habitat data were not available. Therefore, fortnightly habitat monitoring of pools on wetland margins of Lake Kaituna was undertaken as part of this project to assess the suitability of the site for black mudfish. These parameters were then correlated with mudfish survival rates after the translocation. Ideally this monitoring would occur prior to translocation, however, due to restricted time available for conducting research, habitat monitoring was undertaken concurrently with the translocation.

5.1.2 Translocation

Several large populations of black mudfish exist in secure locations, however, to increase chances of survival, the creation of an additional population would be advantageous, and also provide translocation data which may be applied to other mudfish species.

A number of factors need to be considered prior to a translocation in regards to both the receiving site, and the fish to be translocated. Important considerations include whether the receiving site is in the species' natural or historic range, if it is hydrologically, physicochemically, and biologically similar to where the fish originate from, and whether it will provide adequate food, spawning and rearing habitat for all life stages. Consideration needs to be made of predators that are present, and the effects the introduction will have on existing community structure and the subsequent increased competition for food and habitat. Caution should be taken where introductions could endanger or adversely affect other rare or endemic taxa. When locating a suitable receiving site, it is important to ensure the site is secure from any future threats of habitat destruction (Williams et al., 1988; Maitland, 1995). The number of fish used to establish a translocated population needs to be considered, along with the threat and control of disease and fungal growth on fish, how the new population's sex ratios relate to those of natural populations and the timing of the translocation in relation to reproduction and fecundity (Minckley, 1995). Options for translocation sites include either restored natural wetlands or newly constructed wetlands.

The genetic diversity of the translocated population is a key factor, with sufficient individuals required to prevent serious inbreeding or loss of genetic variation by random drift or bottlenecks (Philippart, 1995). Populations established with small numbers of individuals generally have a reduced genetic variability and lower population growth rates (Stockwell & Leberg, 2002). High genetic diversity is crucial for populations to withstand demographic and environmental stochasticity and interspecific competition and predation (Wikramanayake, 1990). It is suggested that

an effective population size of 200 is sufficient in hatchery stocks of salmonids, however, in the wild, greater numbers may be required to compensate for unbalanced sex ratios, age structure, etc (Williams et al., 1988).

It is important to attempt to match the receiving habitat to the source habitat as closely as possible, as local adaptation to disturbances such as drying out over summer are likely (O'Brien, 2005). If the two habitats are not well matched, failure of the new population or rapid phenotypic shifts in response to the different habitats may result (Williams et al., 1988; Stockwell & Leberg, 2002).

Historically, black mudfish are thought to have been present at Lake Kaituna as the area once included extensive peat lands and black mudfish have been recorded locally (Barrier, 1993). Lake Kaituna and the Whangamarino wetland are both within the same ESU, so the translocation will not disrupt the current genetic structure within the species. Initial investigations into the two habitats have shown them to be not too dissimilar, and habitat characterisation work at Lake Kaituna will reveal any differences. Fish surveys have shown the fish community is largely made up of introduced species, with the main threats to mudfish being predation by eels and *Gambusia*. The receiving site is managed by the Department of Conservation, and surrounded by farmland with a supportive landowner. It is crucial that good management of Lake Kaituna habitat is continued to ensure a suitable environment for mudfish.

Individual traits of each species will help determine their translocation success. Wikramanayake (1990) found in a translocation of four fish species in Sri Lanka that *Barbus nigrofasciatus*, the more generalised fish in terms of habitat and ecological requirements, was more successful than the other three species, which only succeeded in habitat matching that from which they originated. It was thought that the habitat generalist may have utilised underexploited habitat types and food resources in the translocated site, experiencing less competition from other species than in their original habitat. This emphasises the importance of habitat characterisation for

species such as the black and Northland mudfish that have specialised habitat preferences, as opposed to the brown mudfish which occupies a much greater range of habitats.

A successful long term self-sufficient population is desirable and monitoring is required. Indicators of success include rapid initiation of breeding, resuming a normal period of breeding, the presence of juveniles and adults in subsequent years, reproduction in successive seasons, and increases in spawning sites (Minckley, 1995).

5.2 Methods

5.2.1 Habitat Characterisation

Water quality – Fortnightly measurements were made of water quality in all pools and the adjacent large wader pool at Lake Kaituna, commencing on 14 April 2006. The large pool was used to represent Lake Kaituna which was too difficult to reach regularly for sampling. Field measurements were taken of water temperature, conductivity, dissolved oxygen and water levels. A YSI Model 30 handheld salinity, conductivity and temperature meter was used to record both temperature and conductivity values, and a YSI Model 55 handheld dissolved oxygen meter was used to record dissolved oxygen. Water level gauges were installed in each pool, and readings taken on each visit (± 1 mm). A water sample was collected from each pool, kept cool during transit to the laboratory, and analysed within 7 hours of collection for pH, and within 48 hours of collection for turbidity and humic concentration. pH was measured with a Radiometer MeterLab standard pH meter (PHM210). Samples were shaken to resuspend particles, placed in a 55 mm x 20 mm x 20 mm cuvette and measured in a Drott Trübungsmesser TRM-1R turbidity meter. To measure humic concentration, samples were filtered through GC-50 glass fibre filter papers, then measured using a spectrophotometer. The light absorbance of the filtered water samples in 10 mm path length cuvettes was measured by a spectrophotometer (Shimadzu UV1601), with adsorbance measured at 440 nm (A_{440}) against a distilled

water blank. Humic concentration (A_{440}/m) was calculated as $230.3 \times A_{440}$ (Vant & Davies-Colley, 1984).

All water quality variables were plotted over time. Using CANOCO version 4.51, principal component analysis of all pool variables was conducted to create an ordination biplot that described intersample distances between pools.

Hydrology – In addition to manual water levels taken during fortnightly sampling of each pool, four water-level dataloggers were installed at the lake. One was set up on the lake margins to record levels of Lake Kaituna, and three were set up in pools. One datalogger was located in each of the three groups of pools; in pools 5, 8 and 16. Odyssey pressure and temperature dataloggers were installed in the lake and pool 5 in September 2006, while TruTrack GPSE 101 400 water level dataloggers were installed in pools 8 and 16 in October 2006. A survey with a dumpy level was carried out to assess whether there were any differences in water levels between pools and therefore calibrate datalogger and manual water level recorders.

Vegetation – A vegetation survey of the in-pool species and vegetation surrounding pools was undertaken. Within pools, species present were listed, and a percentage cover rating given. A list of species and description of habitat surrounding the pools was recorded.

5.2.2 Translocation

On 1 March 2006, 18 pools were constructed on the edges of Lake Kaituna (Figure 5.1). Pool construction made use of previously existing pools and depressions, largely present due to willow removal during initial restoration activities. Pools or depressions were dug to be ideally at least 1 m in diameter and approx 500 mm depth at the current water level. Resulting pools varied from 1 m diameter to 2.8 m maximum axis width (Figure 5.2). Pool construction was carried out six months prior to the mudfish translocation to ensure sediment settling and vegetation growth. Pools

were divided into three groups of six pools (referred to as Group A (pools 1-6), B (pools 7-12) and C (pools 13-18)), with each group varying in stage of restoration and its vegetation. The groups also varied in their proximity to the lake and a large pool constructed for wildfowl habitat. Both the lake and large pool were a source of other fish species such as eels and *Gambusia* which may pose a threat to mudfish.



Figure 5.1 Pool locations on wetland margins of Lake Kaituna

The translocation plan proposed the transfer of five adult fish into each of nine pools and ten juveniles into each of the remaining nine pools (3 adult and 3 juvenile introductions in each of the 3 groups of 6 pools). It was initially anticipated that fish from the captive rearing treatments would be of sufficient size to tag and translocate, and that these along with freshly caught mudfish fry would be used in the translocation. Unfortunately growth rates were not as great as expected and captively reared fish were instead translocated as juveniles, and adult fish were caught from the Whangamarino wetland in August 2006. Fish were kept in captivity for at least 2 weeks to acclimatise and monitor for disease. Forty-seven healthy adult fish were tagged with numbered visible implant silicon elastomer tags as described by Perrie (2004) (Figure 5.3). Tagging involved deep anaesthesia with MS-222 (0.1g/L). Fish were then placed on a moist tissue under a stereomicroscope, and a scalpel used to make an incision on the belly, mid-way between the gut and anus and to the side of a large ventral abdominal blood vessel. The tag was loaded into the tagging device which was slid under the surface layer of transparent skin. Utensils were sterilised with 95% ethanol between fish to reduce infections and spread of disease. Fish were kept in tanks for at least one week after surgery and monitored for tag acceptance. Length (mm) and weight (g) measurements were recorded for each fish. On the day of releasing fish into Lake Kaituna, it was found that three fish had rejected their tags, resulting in only 44 tagged adult fish being released. An extra fish was tagged and again left for over a week to ensure recovery from tagging. Lengths and weights for juvenile fish were also measured, but were monitored as average values for each pool due to the inability to identify individuals.

Mortality is often greater among tagged fish, resulting either directly from the tag or indirectly due to capture, handling and physiological stress to the fish from tagging procedures (Ricker, 1975). Tags are small, under the skin, and only visible from beneath, so are thought to have no impact on mudfish behaviour. Some tagging or marking methods have been found to affect fish's behaviour, altering their chances of recapture or rates of growth (Ricker, 1975).



Figure 5.2 A mudfish pool at Lake Kaituna

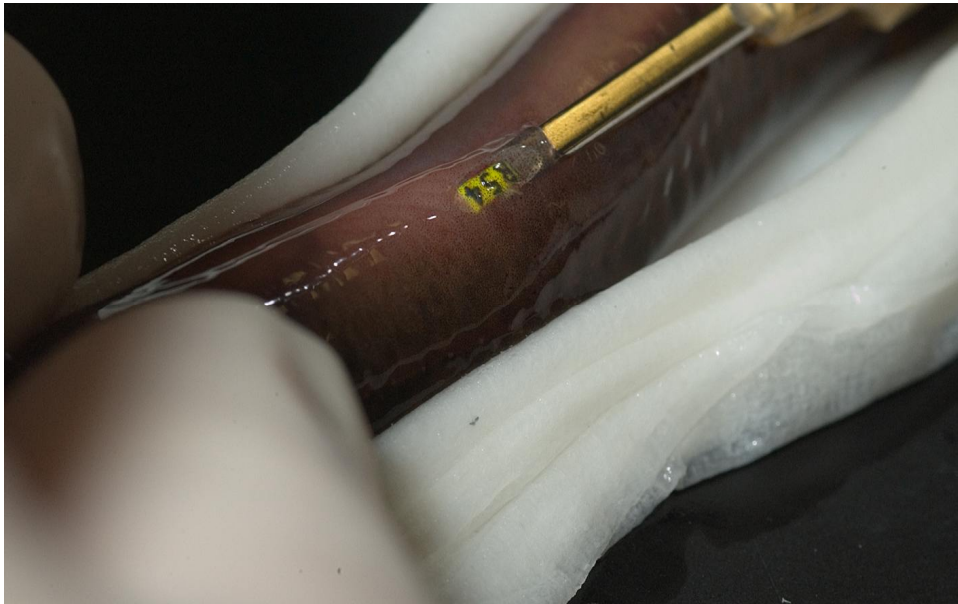


Figure 5.3 Tagging a mudfish with a numbered visible implant silicon elastomer tag
(Photo: Barry O'Brien)

As the sex of black mudfish is indistinguishable externally (Hicks & Barrier, 1996), fish were not sexed but randomly selected. Random selection increases the likelihood of a near equal mix of males to females, however, the low numbers involved (5 adults per pool) mean there was a good chance of uneven sex ratios.

Visual inspections were made for any indications of disease, and those with any sign of illness were excluded from the translocation. All fish were held for a minimum of two weeks, a sufficient time to enable parasites to complete their life cycle or for their densities to become great enough to be observed (Williams et al., 1988).

Prior to release, water was collected from the pools on the margins of Lake Kaituna and on two occasions, two days apart, mudfish tanks were given a 50% water change with the replacement water from Lake Kaituna pools. Water changes were carried out in the three days prior to release to aid the fish with acclimating to their new habitat. pH levels at the lake varied from 4.5 – 5.5, while that of the dechlorinated tap water used for fish in captivity has a pH of 7.5. The temperature mudfish were kept in had been 16°C prior to release. This meant fish will have experienced a degree of thermal shock when released into pools of about 9°C. Fortunately a reduction in temperature is easier for fish to cope with, as it will slow down their metabolism and therefore reduce stress (Guppy and Withers, 1999).

Mudfish were released on 8 September 2006, once the translocation permit was granted, and the worst of the winter floods, which could lead to flooding of pools and loss of fish, were over. Mudfish were selected randomly from tanks and placed into consecutive numbered buckets in order to randomise fish going into each pool. Buckets were numbered 1 –18 to match pools, with adult and juvenile mudfish going into alternate buckets.

Mudfish pools were fished monthly for three months after the release (October, November and December). Gee minnow traps were placed in each pool overnight (approximately 15 hours). Traps were set at the water surface, aiming to catch fish

when feeding, and ensuring there was sufficient air for trapped fish. Traps were set at the same density in each pool so, depending on the pool size, trap numbers varied from 2-5. All mudfish caught were anaesthetised (0.1g/L MS-222), weighed, measured and returned to the pool they were trapped in. Any eels caught were anaesthetised in MS-222, measured and returned to the pool they were trapped in, and captured *Gambusia* were measured and euthanised in MS-222 because their status as unwanted organisms means it is illegal to release *Gambusia*.

5.3 Results

5.3.1 Habitat Characterisation

Temperatures in all pools responded as would be expected with a decrease in June, followed by a steady, gradual increase from September until monitoring ceased in January (Figure 5.4). Temperatures varied between pools by approximately 2-4°C with the lower temperatures generally seen in the deeper pools with greater shading, and warmer temperatures seen in those with smaller surface areas and shallower depths. pH readings were fairly stable for pools over the duration of monitoring with fluctuations coinciding with variations in water levels (Figure 5.5 and Figure 5.6). pH values ranged from 3.5 (pool 3) to 6 (pools 14, 16 and big pool). The lowest pH values were in pools in Group A, particularly pools 3, 5 and 6. Conductivity varied by approximately 50-100 $\mu\text{S}/\text{cm}$ between pools and was found to be continually high in some pools (pool 3, 17, 18 and the big pool), while low in others (pools 4, 8, and 11) (Figure 5.7). Dissolved oxygen readings were found to be very erratic, and few trends could be seen (Figure 5.8). Many pools predominantly displayed low dissolved oxygen values for the duration of monitoring, most of these in group A pools. Low oxygen values are likely to exclude many possible predatory or competing fish species. Turbidity levels remained low for winter months (most < 10 NTU), however, increases in turbidity were seen from October onwards as pools began to dry out (Figure 5.9). Large increases in turbidity were seen in several pools, most of which were located in close proximity to pasture (pools 3, 16, 17, 18).

Humic concentrations were seen to increase slowly after pool creation in December 2005 (Figure 5.10). Humic concentrations varied greatly by January 2007 with most pools having a concentration greater than 20, however, pools 3, 5 and 6 stand out as having very low humic concentrations.

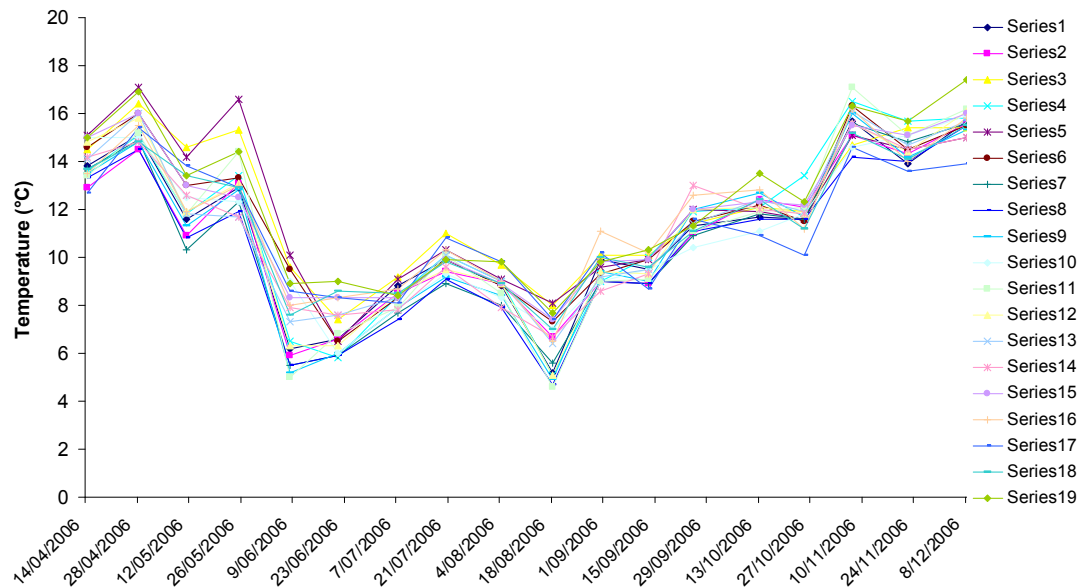


Figure 5.4 Fortnightly temperature readings from April 2006 to January 2007 from pools on margins of Lake Kaituna.

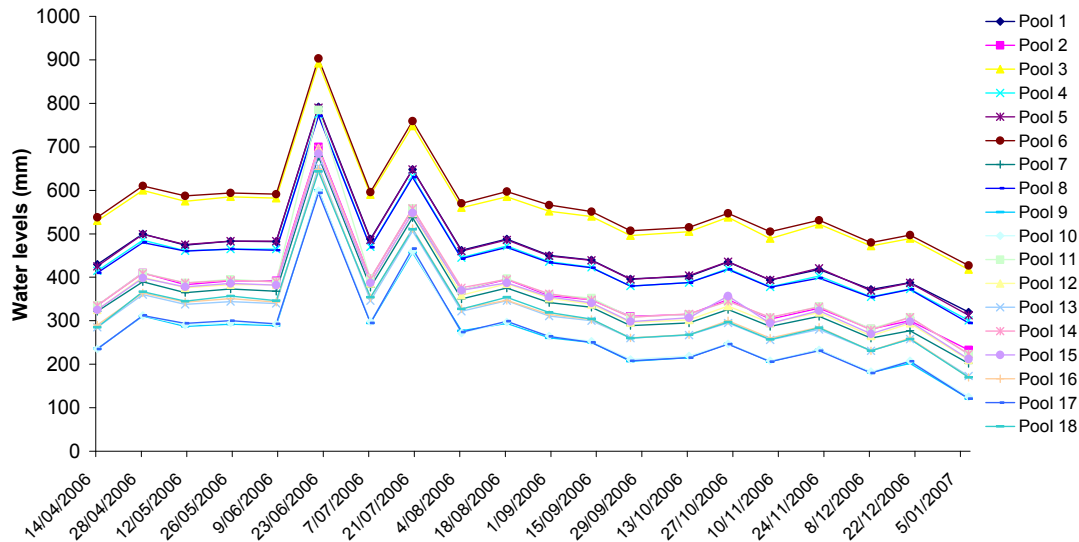


Figure 5.5 Fortnightly water levels from April 2006 to January 2007 from pools on margins of Lake Kaituna.

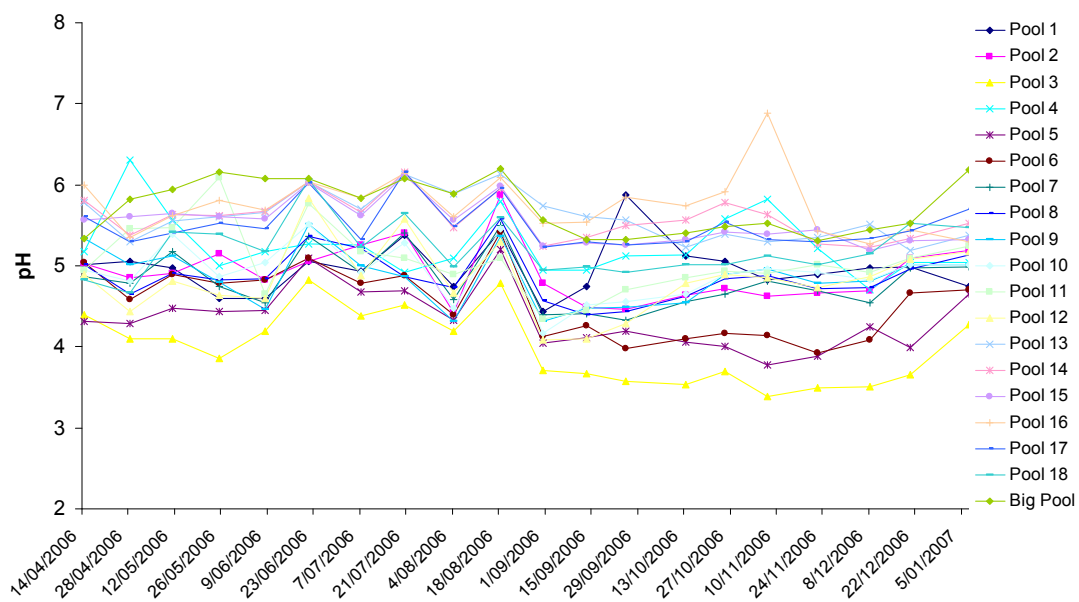


Figure 5.6 Fortnightly pH measurements from April 2006 to January 2007 from pools on margins of Lake Kaituna. Note a different pH meter was used on 18/8/06 than on other dates.

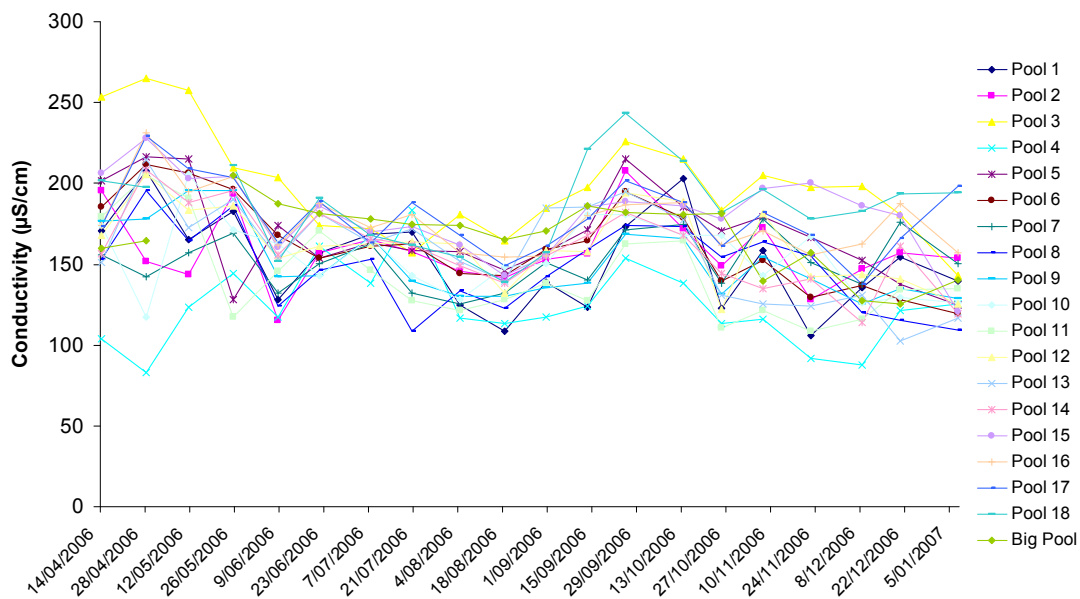


Figure 5.7 Fortnightly conductivity measurements from April 2006 to January 2007 from pools on the margins of Lake Kaituna.

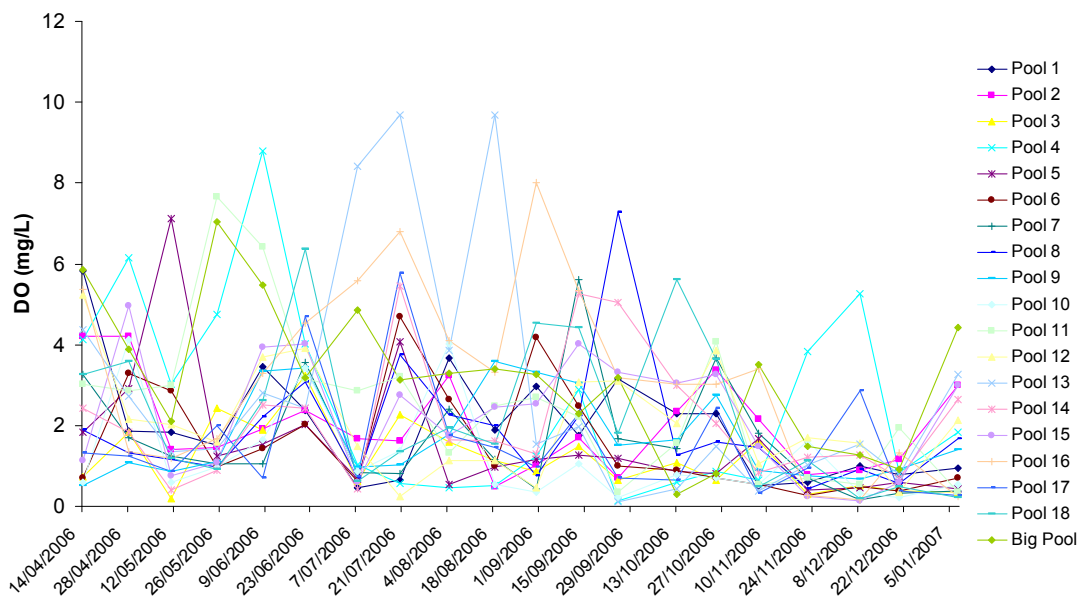


Figure 5.8 Fortnightly dissolved oxygen (mg/L) measurements from April 2006 to January 2007 from pools on the margins of Lake Kaituna.

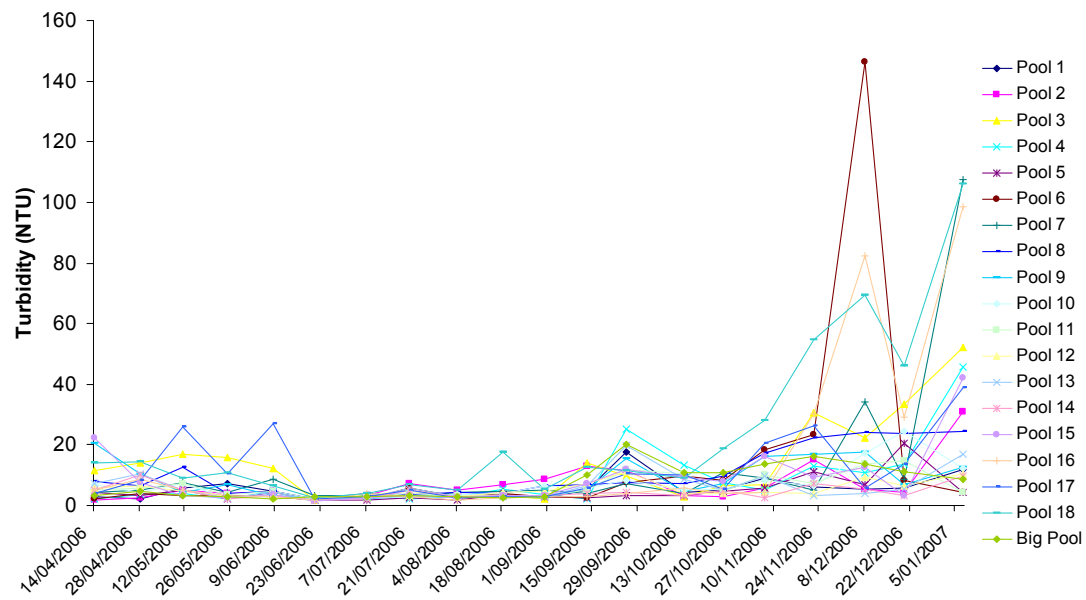


Figure 5.9 Fortnightly turbidity measurements from April 2006 to January 2007 from pools on the margins of Lake Kaituna.

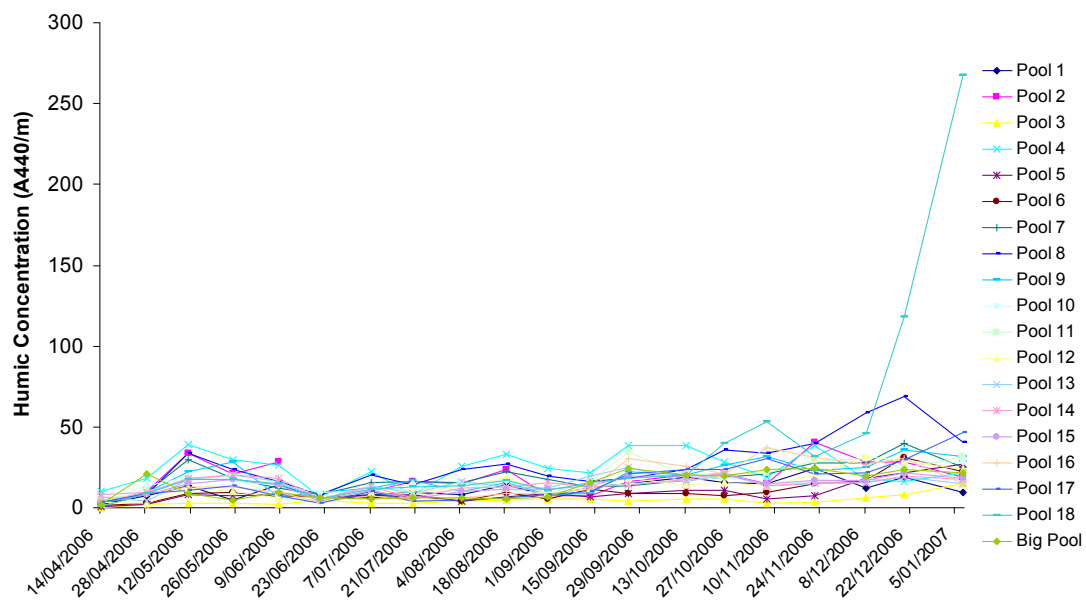


Figure 5.10 Fortnightly humic concentration measurements from April 2006 to January 2007 from pools on the margins of Lake Kaituna.

Ordination biplots showed pool parameters changed considerably from when monitoring commenced in April, to measurements made in December (Figure 5.11A and 5.8B). The April ordination biplot shows pools fairly closely clustered together indicating similar values. Ninety-nine percent of the variance is explained by axis 1 and 2 (90% and 9% respectively), however, no parameters were seen to have a dominant effect at this time period. The ordination biplot for December shows a different scenario, with turbidity and conductivity having a large effect on the separation of pools. Again the majority of the variance is explained by the first 2 axes (67% and 31%, respectively). Pools 3, 6, 15, 16 and 18 are all shown to have high turbidity or conductivity, neither of which are preferable for good quality mudfish habitat.

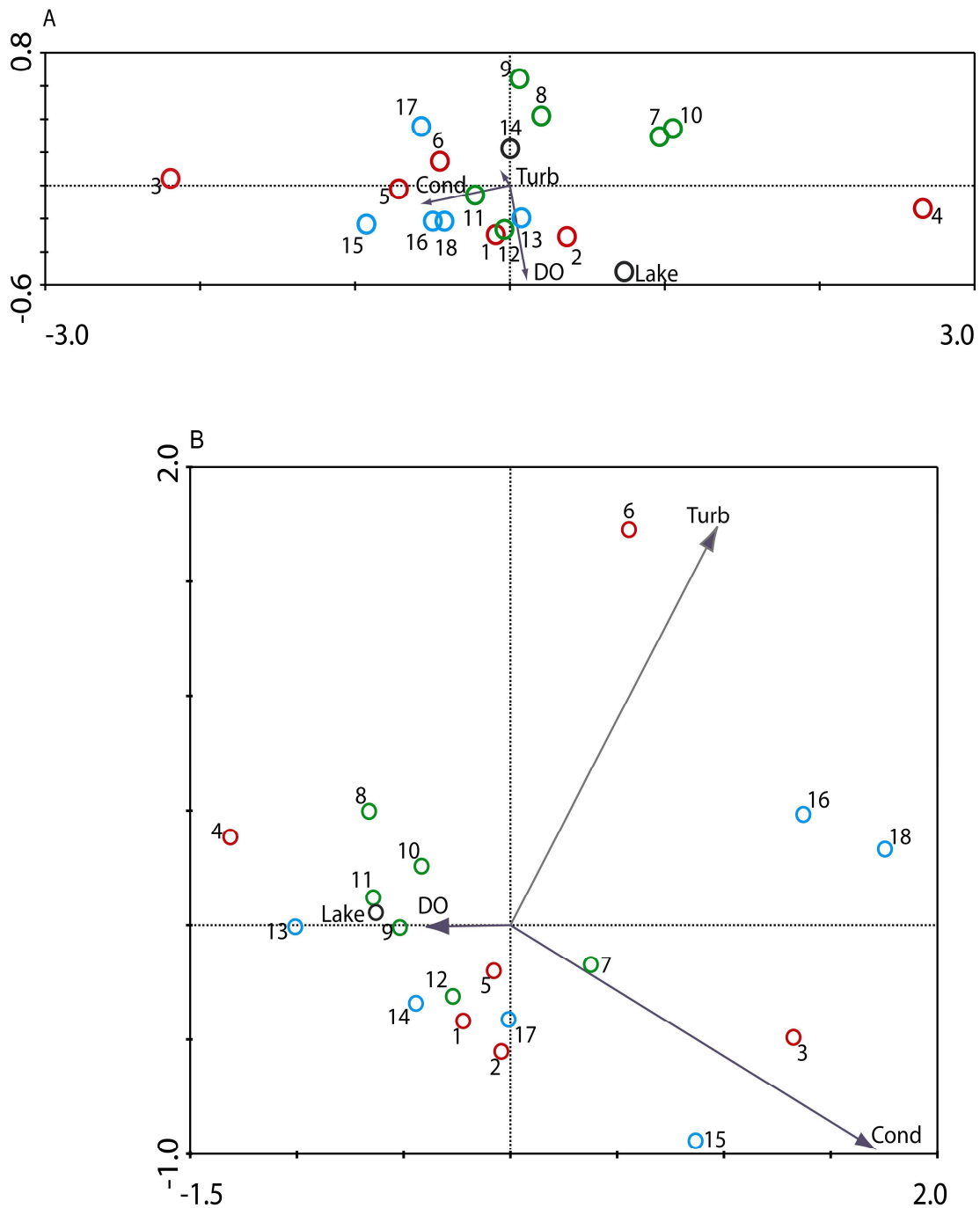


Figure 5.11 Principal component analysis of pool water quality characteristics

A. April 2006 (2 sampling periods combined)

B. December 2006 (2 sampling periods combined)

Manual water level measurements beginning in April 2006 show that all pools experience relatively similar water level fluctuations (Figure 5.5). Peak water level measurements were seen in June and July followed by a gradual continuous decrease in water levels approaching summer. A survey in December 2006 found differences in water levels between pools of only 1-2 mm, which was most likely due to human error. Therefore when comparing water levels, all pools should be considered to be at the same relative level. Plotted levels have been vertically off-set on Figures 5.12 and 5.13 for ease of comparison. Water-level dataloggers also showed a decline in water levels until the end of December. Odyssey dataloggers installed in Lake Kaituna and pool 5 show a very similar pattern in water level variation (Figure 5.12). Pool 5 is one of the larger pools (approximately 3 m²) and while more sensitive to water level fluctuations than the lake, indicates that pools follow similar hydrological fluctuations as the lake. Trutrack dataloggers installed in pools 8 and 16 show that responses to rain events and droughts differ depending on the pool morphology (Figure 5.13). It can be seen that pool 8 (approximately 1.4 m² with an initial depth of 370 mm) which is larger than pool 16 (approximately 0.9 m² with an initial depth of 250 mm) can buffer some of these water fluxes. Pool 8 water levels take longer to respond to water inputs and decrease at a slower rate than the smaller pool 16 that has many small water level fluctuations.

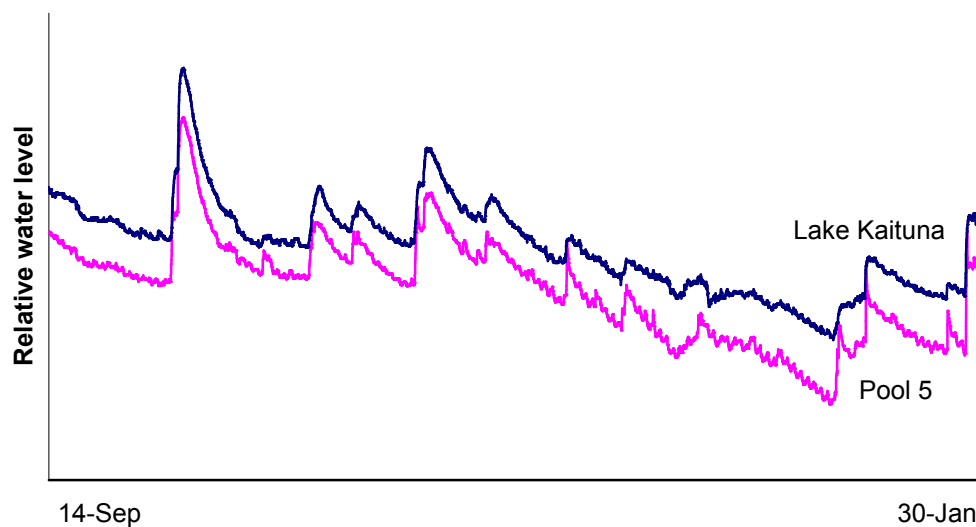


Figure 5.12 Relative water levels of Lake Kaituna and pool 5. Plots off-set from each other to aid viewing

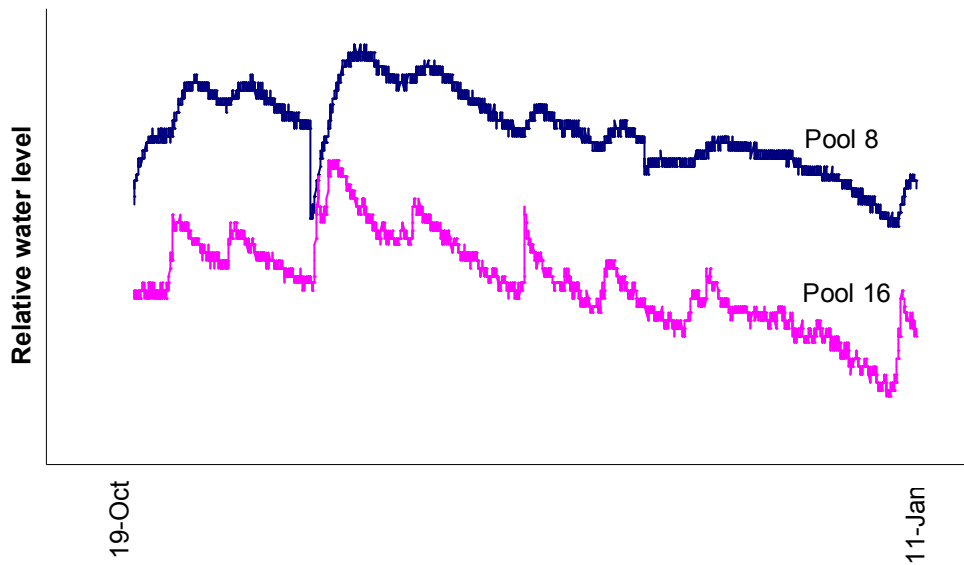


Figure 5.13 Relative water levels of pool 8 and pool 16. Plots off-set from each other to aid viewing

A vegetation survey conducted in December 2006 found only 6 of the 18 pools had greater than 75% of the pool surface clear from vegetation. An algal cover was common on pools, in particular pools in group A. Other common in-pool species included *Agrostis stolonifera*, *Juncus articulatus*, *Ludwigia palustris*, *Ranunculus flammula*, *Holcus lanatus* and *Lotus pedunculatus*. When describing vegetation surrounding pools, a distinct difference was observed between group A compared with groups B and C, highlighting the progress of restoration efforts. Willow removal from the area with group B and C pools occurred earlier than restoration work in the group A area. Group A pools are surrounded by vegetation that can be described as rough pasture dominated by introduced species such as those species found in pools. A few wetland species such as *Juncus* spp., *Carex* spp. and small *Cordyline australis* were present, and the average height of vegetation was 0.5 m. Vegetation surrounding pools in groups B and C was predominantly sedgeland, dominated by *Carex* spp, with some *Baumea rubiginosa* and *C. australis* with an average height of 1.5 m. Introduced pasture species while still present, were not as common.

5.3.2 Translocation

Overall recapture rates of black mudfish were not high, with the first recapture in October 2006 recovering 38% of adults and 23% of juveniles. Numbers decreased to 29% of adults and 7% of juveniles by December 2006. Catch rates varied between the three groups of 6 pools, with the mean catch of adult mudfish being greatest from pools in group B followed by pools in group A (Figure 5.14). Catches from group C were low for all months. Catch rates in group A declined over time, while catch rates in the other two groups peaked in the second month and the smallest catch for all groups was in the third month.

A higher proportion of juvenile mudfish were caught from pools in group A than were caught from either of the other two groups (Figure 5.15). There was a decrease in catch rate for all groups over time, with trapping on the third month only catching a mean of 1 fish or less per pool.

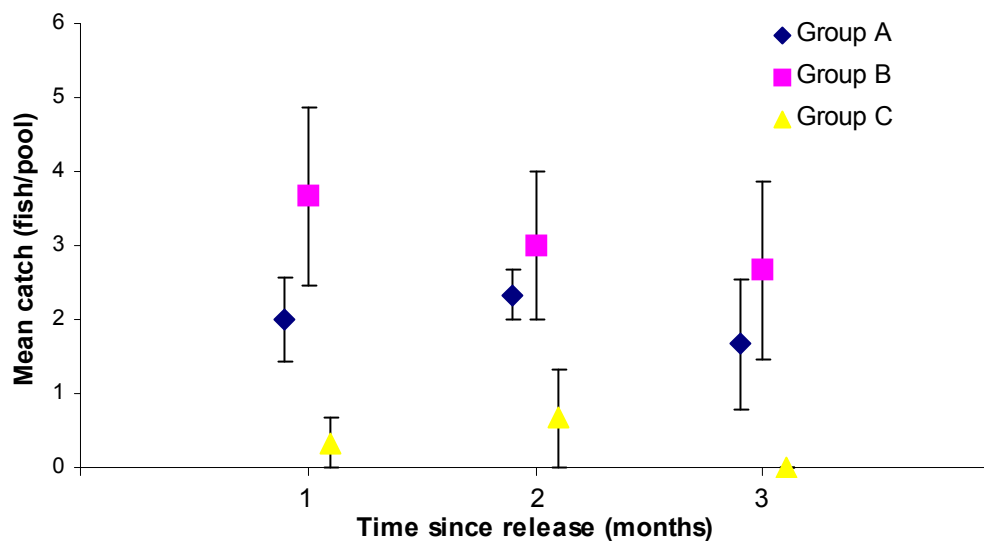


Figure 5.14 Mean adult mudfish catch for each group of pools \pm SEM. Group A = pools 1, 3 and 5; Group B = pools 7, 9 and 11; Group C = pools 13, 15 and 17. Mudfish were released in September (5 fish per pool) and sampling was carried out monthly after the release.

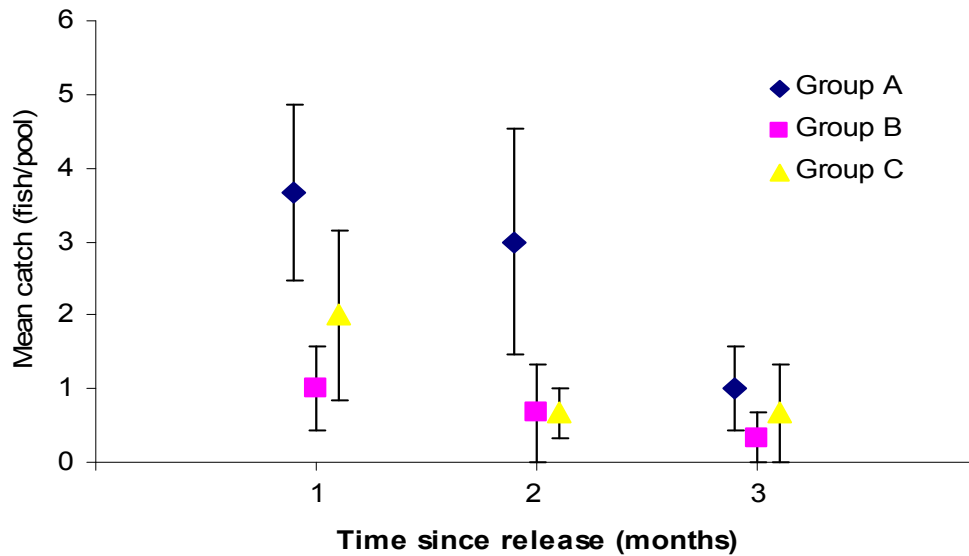


Figure 5.15 Mean juvenile mudfish catch for each group of pools \pm SEM. Group A = pools 2, 4 and 6; Group B = pools 8, 10 and 12; Group C = pools 14, 16 and 18. Mudfish were released in September (10 fish per pool) and sampling was carried out monthly after the release.

Gambusia were caught in five out of the six pools in group C, with 141 caught between the five pools on the first recapture date. The number of *Gambusia* caught reduced to five and six on the second and third trappings, respectively, largely due to captured fish being euthanised. *Gambusia* had not been observed in pools prior to the mudfish release, but it is quite likely they invaded pools prior to the mudfish release during periods of flooding. The location of these five pools meant that during flooding events, *Gambusia* could invade from the lake or the adjacent large pool, both of which had abundant *Gambusia* populations. Pools in group C had low mean catches of both adult and juvenile mudfish, possibly influenced by the presence of *Gambusia*.

Shortfin eels were caught twice, the first time being in pool 12 in the October trapping. Pool 12 contained juvenile mudfish, and after October no further fish were caught from this pool. The second eel was captured from pool 9 during the December trapping. One adult fish was caught from the same pool at this time,

however, this was the last trapping prior to summer droughts so the effects of the eel are unknown.

Unfortunately during the November trapping, three adults were found dead in traps set in pools 3 and 5. Traps had been set the previous evening during heavy rain, and while an air space was present at the time of trapping, this had been reduced by the morning following water level increases. Both pools 3 and 5 also had a thick layer of algae covering the pool surface.

Tagging of adult mudfish enabled the growth of individual fish to be monitored. From adult mudfish growth data (Figure 5.16) it can be seen that the majority of recaptured mudfish were present from group B. These fish all showed a steady increase in length. While fewer fish were recaptured from pools in group A, these fish appear to have a slightly higher growth rate than fish in other pools, indicating preferential habitat characteristics. Only three fish were recaptured from pools in group C, and these all showed minimal or no growth.

When released, individual juvenile mudfish lengths ranged from 37-51 mm, and were randomly mixed between pools. It can be seen from Figure 5.17 that juvenile mudfish have grown rapidly in the pool environments with two fish reaching 81 and 82 mm in length after three months. Growth rates were not found to differ greatly between pools and by the final trapping in particular, catch rates were commonly down to 1 or 2 fish per pool, therefore means for pools were possibly skewed by 1 or 2 individuals.

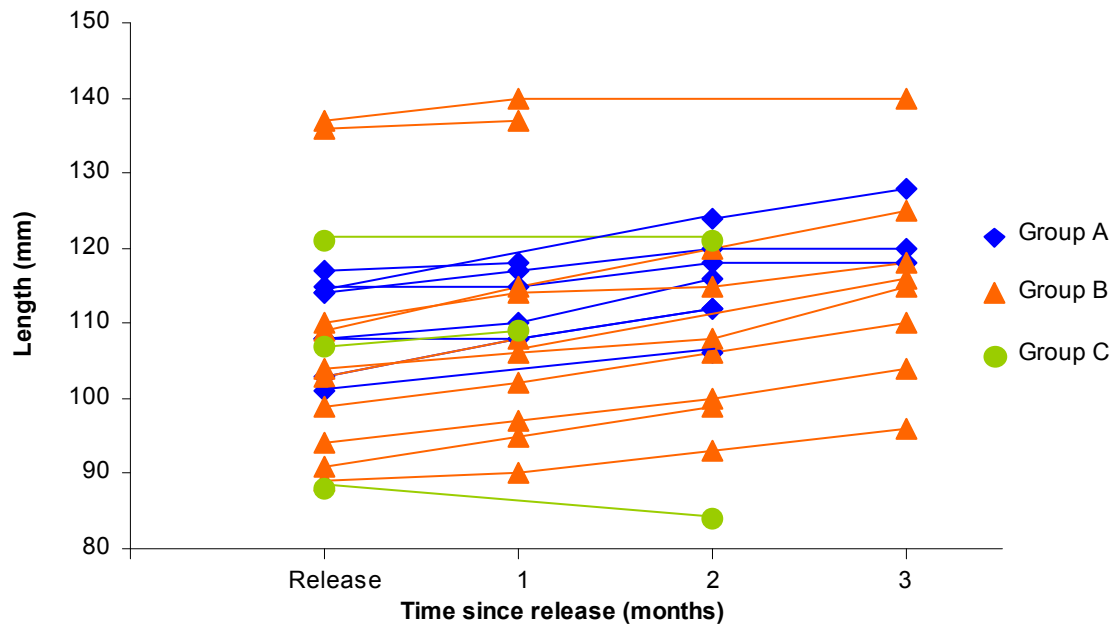


Figure 5.16 Adult mudfish growth since release into pools. Each line represents the length (mm) of an individual fish. Group A = pools 1, 3 and 5; Group B = pools 7, 9 and 11; Group C = pools 13, 15 and 17. Mudfish were released in September and sampling was carried out monthly after the release.

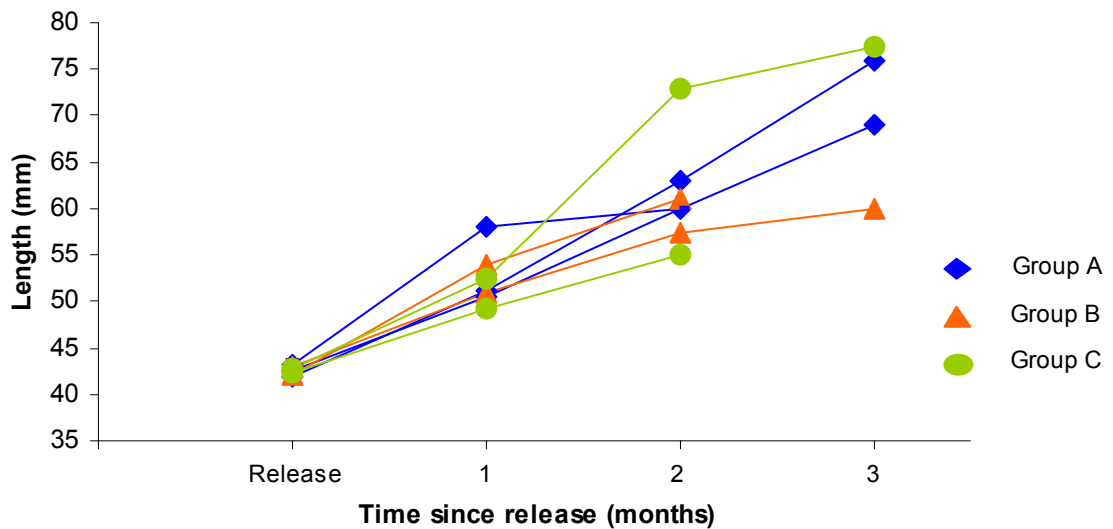


Figure 5.17 Mean juvenile mudfish growth per pool since release. Each line represents the average length (mm) of mudfish captured from one pool. Group A = pools 2, 4 and 6; Group B = pools 8, 10 and 12; Group C = pools 14, 16 and 18. Mudfish were released in September and sampling was carried out monthly after the release.

5.4 Discussion

Monitoring of pool water quality parameters revealed there was a considerable variability between pools, however, this variability was not necessarily consistent within the three groups of pools as had been expected. Individual pool morphology largely dictated the temperature and response to water influxes of each pool, and no distinct trends between other parameters were seen along the gradient of pools. The analysis of data using ordination biplots, however, revealed attributes of individual pools, that when combined with translocation data explained some of the translocation results.

Biplots showed pools 3, 6, 15, 16 and 18 all to be greatly influenced by turbidity and conductivity. Black mudfish are known to prefer clear rather than turbid water, and generally inhabit infertile areas as opposed to eutrophic wetlands (Barrier, 1993; Ling, 2001). Catch rates were generally low from all of these pools. Interestingly, the conditions in pool 18 were seen to deteriorate over the sampling period, a deterioration that was reflected by the decrease in trapping success from 4 - 1 - 0 over the 3 sampling periods. However, the fate of these fish is unknown, with many fish having possibly begun aestivating by December sampling. No other water quality variables were found by Barrier (1993) to show a relationship with mudfish distribution. However, turbidity and conductivity are only two water quality parameters to consider among many habitat variables, and interpretation of suitable translocation sites may not be as simple as considering only these. Pool 7 is also shown to be influenced by high conductivity in December, however, this pool had the most successful trapping rates, with a 93% overall catch rate. With the benefit of hindsight, mudfish would possibly not have been released into pools with high turbidity or conductivity levels. This emphasises the importance of undertaking a detailed habitat characterisation prior to a translocation, ideally including sampling for a full year to ensure parameters are suitable in all seasons.

Unlike investigations into habitat preferences of black mudfish (Barrier 1993), mudfish were translocated into pools between which the habitat varied. Mudfish are

largely confined to the pools, with flooding events being their only opportunity to seek out suitable alternative refuges. No major floods occurred between releasing fish in August, and the last sampling event in December, however, minor floods allowed fish to move into adjacent pools, and 3 fish were found in pools different to those in which they were released.

Mudfish catch rates from pools decreased with time, a trend possibly due to mudfish death (due to predation, unsuitable habitat or other natural causes), mudfish commencing aestivation or mudfish simply not being recaptured. All mudfish grew after the translocation with the exception of two recaptured fish in group C pools. Both adult and juvenile mudfish had high rates of growth when in group A pools. Surprisingly these pools are located in a more recent stage of restoration, surrounded by rough pasture with many introduced plant species present. These findings differ to that of Barrier (1993) who, in investigations of black mudfish habitat, found that overhanging vegetation was a significant positive habitat variable. For long-term survival, overhanging vegetation provides protection from predation from birds such as bitterns and kingfishers. However, in the short-term, possibly other variables such as a greater abundance of prey items were of more importance to mudfish growth. Juvenile mudfish growth was much greater than that of adults, as was expected due to a common pattern in fish growth of a decrease in length increment as fish age (Ricker, 1975).

Interpretation of translocation data was complicated by the presence of other fish species, *Gambusia* and shortfinned eels (*A. australis*). *Gambusia* were found in 5 out of 6 pools in group C, the least successful pool group for recapturing adult mudfish. Furthermore, growth rates of adult mudfish were conspicuously lower in these pools compared with the other pool groups. *Gambusia* are known to negatively affect mudfish growth, although not to attack adult mudfish (as described in Chapter 3). It is unknown if the presence of *Gambusia* has affected recapture rates of mudfish. Possibly mudfish have altered their feeding behaviour in the presence of high densities of *Gambusia* such that they are not trapped, they may avoid traps with

Gambusia present, or possibly mudfish have left the pools or died. Only once were mudfish and *Gambusia* found in the same trap. O'Brien (2005) found that capture rates of Canterbury mudfish in Gee minnow traps was reduced in the presence of other fish species (*A. australis* and *Gobiomorphus breviceps*). Fish are known to exhibit avoidance behaviours to reduce the probability of encountering a predator (Fuiman and Magurran, 1994), and although *Gambusia* are not predators, possibly mudfish display such behaviours within pools with aggressive *Gambusia*, reducing the likelihood of mudfish being captured in traps. However on a larger scale, Barrier (1993) found that neither *Gambusia* or shortfinned eels affected the distribution of black mudfish.

Both juvenile and adult mudfish were caught in the same pools during the sampling period when shortfinned eels were caught. With mudfish confined in the small area of each pool, it is highly likely that eels, which can travel over land, will have devoured mudfish from the pools they were found in. No further mudfish were caught from pool 12 after a shortfinned eel was trapped there in October, and the other eel was trapped in December so the effects are yet unknown. Eels are known to prey on Canterbury mudfish (Eldon 1979), and it has been speculated that the same occurs to black mudfish (Barrier, 1993). The presence of mudfish in a trap would be an easy meal for an eel, and it is unknown if eels entered traps pursuing their prey. One black mudfish from the December sampling had a large bite out of the side of its tail, indicating some ability for mudfish escape from these predators. Future translocations should consider the devastating effects of eel predation when identifying a receiving site. Locating sites a distance from eel sources may reduce the chance of eels devouring their way through new and small mudfish populations.

Unfortunately during the November trapping, three fish were found dead in traps in the morning. Dissolved oxygen levels in many of the pools had decreased by mid-November to less than 2 mg/L, meaning that extra care was required when setting traps to ensure an airspace was left allowing mudfish to surface and gulp air. However, heavy rainfall overnight resulted in water level increases in pools,

minimising the air space available to trapped fish. In addition to minimal airspace, a dense layer of algae was present on the surface of both pools where deaths occurred. While dislodged during trap placement, the presence of algae and other plants in the pools will have altered the temperature and oxygen levels in the pools, with the lowest oxygen levels experienced near dawn (Miranda et al., 2000). O'Brien (2005) also experienced deaths of Canterbury mudfish in low dissolved oxygen levels, highlighting the need for extra care when trapping in hypoxic wetland habitats. A recommendation for all future trapping would be the inclusion of a float within each minnow trap allowing traps to rise or fall with changes in water level, rather than staking or tying the traps at a given depth.

Overall, indications are that the translocation of black mudfish to the Lake Kaituna wetland is only partially successful. Further monitoring will be necessary throughout the post-summer period once water levels are restored by autumn rains, and the presence of reproduction in the population during the ensuing winter will be crucial to the long-term success of the translocation. The operational plan for this translocation recommends continued monitoring for a period of five years following the mudfish release.

6. General Conclusion

All of New Zealand's five endemic species of mudfish are threatened, and translocation has been recommended as an option for conservation. An increase in the number of populations of each species will increase their chance of survival in the face of numerous threats. While black mudfish are classed as being in gradual decline, results from this research will aid any possible future translocations of black mudfish from other evolutionarily significant units, as well as the more threatened Northland mudfish.

Captive breeding of some mudfish species has been attempted in the past, and has proved to be extremely difficult with low success rates. Therefore, captive rearing of fry or juveniles collected from the wild has been suggested as a more feasible option for sourcing translocation stock. While much simpler than captive breeding, rearing fish in captivity is still a labour-intensive task. Research into the optimum temperature and diet for rearing juvenile black mudfish found mudfish do not grow on the easily cultured brine shrimp alone, but their diet should include a more nutritional food such as white worms. Surprisingly, mudfish growth was only slightly influenced by temperature, with only a small increase in growth rate at increased temperatures after 2 months. In captive rearing, once juvenile fish reached a size of >32 mm they were transferred into outdoor mesocosms. Growth in this outdoor situation was more rapid, required minimal effort and is more similar to natural environments as fish are required to pursue live prey. Therefore, for future captive rearing programmes, it is recommended that outdoor mesocosms may be a better option than indoor captive rearing.

The adverse effects of the aggressive, introduced species *Gambusia affinis* on black mudfish are of concern. Several studies have investigated the interactions between the two species, however, none had addressed the effect of *Gambusia* density on black

mudfish. This is of particular concern when establishing new mudfish populations via translocation to areas where *Gambusia* are present. Information on *Gambusia* density effects will help determine if receiving sites are suitable by taking into account the numbers of *Gambusia* present. Mesocosm experiments containing black mudfish juveniles and different densities of *Gambusia* found an effect of reduced fish growth with increasing *Gambusia* density. This was thought to be a result of increased interspecific competition for food resources. Analysis of zooplankton communities found that large-bodied zooplankton had been removed by the *Gambusia*. In mesocosms with few large-bodied zooplankton, the decrease in competition for phytoplankton resources resulted in rotifer population increases. The effects on mudfish growth and zooplankton communities was exacerbated as *Gambusia* density increased, with less fish growth in high density *Gambusia* mesocosms, along with a greater removal of cladocerans, and associated increase in rotifer numbers. Results clearly show that once mudfish have reached juvenile size, the greatest risk from *Gambusia* appears to be increased competition for food resources. Reduced growth in mudfish is of concern, as juvenile size prior to entering aestivation is important for survival, and for adults, reduced condition may reduce successful reproduction.

To effectively monitor a population, including those created by translocation, it is crucial to understand how reliable and reproducible trapping methods are. The most effective method for trapping mudfish has been identified as the use of Gee minnow traps set overnight. A trapping programme in mesocosms was conducted to investigate the reliability of Gee minnow traps for monitoring mudfish. The investigation found no effect of trap shyness, long-term or short-term mudfish memory, water level, or mudfish density. The factor found to be most significant was the position in which the traps were set. Traps set at the water surface at two different water levels caught significantly more fish than traps set on the bottom of mesocosms. It is hypothesised that mudfish foraging behaviour brings fish to the water surface during the night, making them prone to trapping at the surface. Additionally, traps set at the surface are advantageous as they allow provision of an

airspace in the traps, allowing fish caught in hypoxic waters to surface for air. To comprehensively understand the mechanisms involved in trapping mudfish in Gee minnow traps, future work could investigate processes of mudfish feeding and foraging, addressing mudfish position in the water column.

One of the key findings from the translocation of black mudfish is the importance of conducting a thorough habitat assessment prior to releasing fish. Correlation of water quality data with results from trapping of translocated mudfish found lower catch rates in pools with less suitable water quality. The presence of other fish species was also found to influence mudfish catch rates, with fewer mudfish caught in pools with *Gambusia*. It is unknown whether mudfish were still present in pools with *Gambusia* and simply not trapped due to behavioural changes, or if mudfish had perished possibly due to starvation. Shortfinned eels were found to have a severely negative impact on mudfish populations, with no mudfish subsequently caught from pools where eels were found. Future translocations of mudfish should attempt to locate receiving sites a substantial distance from any sources of *Gambusia* or eels. Mudfish catch rates declined over the monthly sampling periods after translocation. It is unknown whether this was a result of mudfish commencing aestivation, migration, death, or simply not being trapped. The success of this translocation will only be determined by further trapping after water levels increase with autumn rains to assess mudfish survival following the summer, and by the presence of fry in winter.

References

- Angeler DG, Álvarez-Cobelas M, Sánchez-Carrillo S, Rodrigo MA 2002. Assessment of exotic fish impacts on water quality and zooplankton in a degraded semi-arid floodplain wetland. *Aquatic Science* 64: 76-86.
- Baker C, Rowe D, Smith J 2004. *Gambusia* – a biodiversity threat? *Water and Atmosphere* 12: 24-25.
- Barrier RFG 1993. Investigation of habitat preferences of black mudfish in the Waikato region and their interaction with mosquitofish. Unpublished MSc thesis, University of Waikato. 86 p.
- Barrier RFG, Hicks BJ 1994. Behavioural interactions between black mudfish (*Neochanna diversus* Stokell, 1949: Galaxiidae) and mosquitofish (*Gambusia affinis* Baird & Girard, 1854). *Ecology of Freshwater Fish* 3: 93-99.
- Beamesderfer RC, Rieman BE 1988. Size selectivity and bias in estimates of population statistics of smallmouth bass, walleye, and northern squawfish in a Columbia River reservoir. *North American Journal of Fisheries Management* 8: 505-510.
- Becker A, Laurenson LJB, Jones PL, Mewman, DM 2005. Competitive interactions between the Australian native fish *Galaxias maculatus* and the exotic mosquitofish *Gambusia holbrooki*, in a series of laboratory experiments. *Hydrobiologia* 549: 187-196.

- Benoît HP, Post JR, Barbet AD 2000. Recruitment dynamics and size structure in experimental populations of the mosquitofish, *Gambusia affinis*. *Copeia* 1: 216-221.
- Bergin TM 1992. Habitat selection by the western kingbird in western Nebraska: A hierarchical analysis. *The Condor* 94: 903-911.
- Chapman LJ, Chapman CA 1996. Wetland ecotones as refugia for endangered fishes. *Biological Conservation* 78: 263-270.
- Chapman MA, Lewis MH 1976. An introduction to the freshwater crustacea of New Zealand. Collins, Auckland. 261 p.
- Clarkson BR 1997. Vegetation recovery following fire in two Waikato peatlands at Whangamarino and Moanatuatua, New Zealand. *New Zealand Journal of Botany* 35: 167-179.
- Cromarty P, Scott DA (eds) 1995. A directory of wetlands in New Zealand. Department of Conservation, Wellington, New Zealand. 395 p.
- Dawes J 1991. Livebearing Fishes, A guide to their aquarium care, biology and classification. Blandford, London. 240 p.
- Department of Conservation 2003. New Zealand mudfish (*Neochanna* spp.) recovery plan 2003-13. Threatened Species Recovery Plan 51. Wellington. 25 p.
- Department of Conservation 2007. New Zealand threat classification system list 2005. Science and Technical Publishing, Wellington. 194 p.
- Dwyer WP, Piper RG 1987. Atlantic salmon growth efficiency as affected by temperature. *The Progressive Fish-Culturist* 49: 57-59.

- Economidis PS, Dimitriou E, Pagoni R, Michaloudi E, Natsis L 2000. Introduced and translocated fish species in the inland waters of Greece. *Fisheries Management and Ecology* 7: 239-250.
- Eldon GA 1979. Habitat and interspecific relationships of the Canterbury mudfish, *Neochanna burrowsius* (Salmoniformes: Galaxiidae). *New Zealand Journal of Marine and Freshwater Research* 13: 111-119.
- Eldon GA 1992. The difficulties of capturing mudfish. *Freshwater Catch* 48: 16-17.
- Fergie S 2003. Horsham Downs peat lakes resource inventory. Environment Waikato Internal Series IS03/04. Environment Waikato and Department of Conservation, Hamilton.
- Fradkin SC 1995. Effects of interference and exploitative competition from large-bodied cladocerans on rotifer community structure. *Hydrobiologia* 313/314: 387-393.
- Fuiman LA, Magurran AE 1994. Development of predator defenses in fishes. *Reviews in Fish Biology and Fisheries* 4: 145-183.
- García-Berthou E 1999. Food of introduced mosquitofish: ontogenetic diet shift and prey selection. *Journal of Fish Biology* 55: 135-147.
- Gibbons JW, Winne CT, Scott DE, Willson JD, Glaudas X, Andrews KM, Todd BD, Fedewa LA, Wilkinson L, Tsaliagos RN, Harper SJ, Greene JL, Tuberville TD, Metts BS, Dorcas ME, Nestor JP, Young CA, Akre T, Reed RN, Buhlmann KA, Norman J, Croshaw DA, Hagen C, Rothermel BB 2006. Remarkable amphibian biomass and abundance in an isolated wetland: implications for wetland conservation. *Conservation Biology* 20: 1457-1465.

- Gilbert JJ 1985. Competition between rotifers and *Daphnia*. *Ecology* 66: 1943-1950.
- Goodsell JA, Kats LB 1999. Effect of introduced mosquitofish on pacific treefrogs and the role of alternative prey. *Conservation Biology* 13: 921-924.
- Griffith B, Scott JM, Carpenter JW, Reed C 1989. Translocation as a species conservation tool: status and strategy. *Science* 245: 477-480.
- Günther A 1867. On a new form of mudfish from New Zealand. *Annals and Magazine of Natural History* 3: 305-309.
- Guppy M, Withers P 1999. Metabolic depression in animals: physiological perspectives and biochemical generalizations. *Biological Review* 74: 1-40.
- Harig AL, Fausch KD 2002. Minimum habitat requirements for establishing translocated cutthroat trout populations. *Ecological Applications* 12: 535-551.
- He X, Lodge DM 1990. Using minnow traps to estimate fish population size: the importance of spatial distribution and relative species abundance. *Hydrobiologia* 190: 9-14.
- Hicks BJ, Barrier RFG 1996. Habitat requirements of black mudfish (*Neochanna diversus*) in the Waikato region, North Island, New Zealand. *New Zealand Journal of Marine and Freshwater Research* 30: 135-151.
- Hislop JRG, Robb AG, Gauld JA 1978. Observations on effects of feeding level on growth and reproduction in haddock, *Melanogrammus aeglefinus* (L.) in captivity. *Journal of Fish Biology* 13: 85-98.

- Hobbs RJ, Norton DA 1996. Towards a conceptual framework for restoration ecology. *Restoration Ecology* 4: 93-110.
- Holomuzki JR 1991. Macrohabitat effects on egg deposition and larval growth, survival, and instream dispersal in *Ambystoma barbouri*. *Copeia* 3: 687-694.
- 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: 425-34.
- Hurlbert SH, Zedler J, Fairbanks D 1972. Ecosystem alteration by mosquitofish (*Gambusia affinis*) predation. *Science, New Series* 175: 639-641.
- Koya Y, Inoue M, Naruse T, Sawaguchi S 2000. Dynamics of oocyte and embryonic development during ovarian cycle of the viviparous mosquitofish *Gambusia affinis*. *Fisheries Science* 66: 63-70.
- Labbe TR, Fausch KD 2000. Dynamics of intermittent stream habitat regulate persistence of a threatened fish at multiple scales. *Ecological Applications* 10: 1774-1791.
- Laarman PW, Ryckman JR 1982. Relative size selectivity of trap nets for eight species of fish. *North American Journal of Fisheries Management* 2: 33-37.
- Ling N 2001. New Zealand mudfishes: a guide. Department of Conservation, Wellington. 21 p.
- Ling N 2004. *Gambusia* in New Zealand: really bad or just misunderstood? *New Zealand Journal of Marine and Freshwater Research* 38: 473-480.

- Ling N, Gleeson DM 2001. A new species of mudfish, *Neochanna* (Teleostei: Galaxiidae), from northern New Zealand. *Journal of the Royal Society of New Zealand* 31: 385-392.
- Ling N, Gleeson DM, Willis KJ, Binzegger SU 2001. Creating and destroying species: the 'new' biodiversity and evolutionarily significant units among New Zealand's galaxiid fishes. *Journal of Fish Biology* 59A: 209-222.
- Ling N, Willis K 2005. Impacts of mosquitofish, *Gambusia affinis*, on black mudfish, *Neochanna diversus*. *New Zealand Journal of Marine and Freshwater Research* 39: 1215-1223.
- McDowall RM 1990. *New Zealand freshwater fishes: A natural history and guide*. Heinemann-Reed, Auckland, New Zealand. 553 p.
- McDowall RM 2000. *The Reed field guide to New Zealand freshwater fishes*. Reed, Auckland. 224 p.
- McDowall RM 2004. The Chatham Islands endemic galaxiid: a *Neochanna* mudfish (Teleostei: Galaxiidae). *Journal of the Royal Society of New Zealand* 34: 315-331.
- McLea MC 1986. *Ohinewai regional resource study: biology and water quality*. Technical publication 37. Waikato Valley Authority, Hamilton, New Zealand. 199 p.
- McPhail JD 1999. A fish out of water: observations on the ability of black mudfish, *Neochanna diversus*, to withstand hypoxic water and drought. *New Zealand Journal of Marine and Freshwater Research* 33: 417-424.

- Maitland PS 1995. The conservation of freshwater fish: past and present experience. *Biological Conservation* 72: 259-270.
- Maitland PS 2004. Ireland's most threatened and rare freshwater fish: an international perspective on fish conservation. *Biology and Environment: Proceedings of the Royal Irish Academy* 104B: 5-16.
- Mansfield S, McArdle BH 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: 375-383.
- Minckley WL 1995. Translocation as a tool for conserving imperilled fishes: experiences in Western United States. *Biological Conservation* 72: 297-309.
- Mitchell CP 1995. A new species of *Galaxias* (Pisces: Galaxiidae) from Chatham Island, New Zealand. *Journal of the Royal Society of New Zealand* 25: 89-93.
- Miranda LE, Driscoll MP, Allen MS 2000. Transient physicochemical microhabitats facilitate fish survival in inhospitable aquatic plant stands. *Freshwater Biology* 44: 617-628.
- Murphy BR, Willis DW (eds) 1996. *Fisheries Techniques*, 2nd edition. American Fisheries Society, Bethesda, Maryland. 732 p.
- O'Brien LK 2005. The conservation ecology of Canterbury mudfish (*Neochanna burrowsius*). Unpublished PhD thesis, University of Canterbury, Christchurch. 241 p.
- O'Brien L, Dunn N 2005. Captive management of mudfish *Neochanna* (Teleostei: Galaxiidae) spp. DOC Research & Development Series 205, Department of Conservation, Wellington. 29 p.

- Olden JD 2003. A species-specific approach to modelling biological communities and its potential for conservation. *Conservation Biology* 17: 854-863.
- Perrie AC 2004. Life history and ecophysiology of black mudfish (*Neochanna diversus* Stokell, 1949). Unpublished MSc thesis, University of Waikato. 78 p.
- Petrik R, Levin PS 2000. Estimating relative abundance of seagrass fishes: a quantitative comparison of three methods. *Environmental Biology of Fishes* 58: 461-466.
- Philippart JC 1995. Is captive breeding an effective solution for the preservation of endemic species? *Biological Conservation* 72: 281-295.
- Phillipps WJ 1926. New or rare fishes of New Zealand. *Transactions and Proceedings of the New Zealand Institute* 56: 529-537.
- Poly WJ 2003. Design and evaluation of a translocation strategy for the fringed darter (*Etheostoma crossopterum*) in Illinois. *Biological Conservation* 113: 13-22.
- Ramsar Convention Secretariat 2006. *The Ramsar Convention Manual: a guide to the Convention on Wetlands (Ramsar, Iran, 1971)*. 4th ed. Ramsar Convention Secretariat, Gland, Switzerland.
- Rehage JS, Barnett BK, Sih A 2005. Foraging behaviour and invasiveness: do invasive *Gambusia* exhibit higher feeding rates and broader diets than their non-invasive relatives? *Ecology of Freshwater Fish* 14: 352-360.
- Ricciardi A, Rasmussen JB 1999. Extinction rates of North American freshwater fauna. *Conservation Biology* 13: 1220-1222.

- Richter BD, Braun DP, Mendelson MA, Master LL 1997. Threats to imperilled freshwater fauna. *Conservation Biology* 11: 1081-1093.
- Ricker WE 1975. Computation and interpretation of biological statistics of fish populations. Department of the Environment, Fisheries and Marine Service, Ottawa. 382 p.
- Rincón PA, Correas AM, Morcillo F, Risueño R, Lobón-Cerviá J 2002. Interaction between the introduced eastern mosquitofish and two autochthonous Spanish toothcarps. *Journal of Fish Biology* 61: 1560-1585.
- Rogowski DL, Stockwell CA 2006. Assessment of potential impacts of exotic species on populations of a threatened species, White Sands pupfish, *Cyprinodon tularosa*. *Biological Invasions* 8: 79-87.
- Rosenberger AE, Chapman LJ 1999. Hypoxic wetland tributaries as faunal refugia from an introduced predator. *Ecology of Freshwater Fish* 8: 22-34.
- Rowe D 1998. Management trials to restore dwarf inanga show mosquitofish a threat to native fish. *Water & Atmosphere* 6: 10-12.
- Saunders DL, Meeuwig JJ, Vincent ACJ 2002. Freshwater protected areas: strategies for conservation. *Conservation Biology* 16: 30-41.
- Shearer JC 1997. Natural and anthropogenic influences on peat development in Waikato/Hauraki Plains restiad bogs. *Journal of the Royal Society of New Zealand* 27: 295-313.
- Sheldon AL 1988. Conservation of stream fishes: patterns of diversity, rarity, and risk. *Conservation Biology* 2: 149-156.

- Sheller FJ, Fagan WF, Unmack PJ 2006. Using survival analysis to study translocation success in the Gila topminnow (*Poeciliopsis occidentalis*). *Ecological Applications* 16: 1771-1784.
- Shiel RJ 1995. A guide to the identification of rotifers, cladocerans and copepods from Australian inland waters. Albury, N.S.W., Co-operative Research Centre for Freshwater Ecology, Murray-Darling Freshwater Research Centre. 144 p.
- Singh D, Sharma RC 1998. Biodiversity, ecological status and conservation priority of the fish of the River Alaknanda, a parent stream of the River Ganges (India). *Aquatic Conservation: Marine and Freshwater Ecosystems* 8: 761-772.
- Stockwell CA, Leberg PL 2002. Ecological genetics and the translocation of native fishes: emerging experimental approaches. *Western North American Naturalist* 62: 32-38.
- Stockwell CA, Weeks SC 1999. Translocations and rapid evolutionary responses in recently established populations of western mosquitofish (*Gambusia affinis*). *Animal Conservation* 2: 103-110.
- Stokell G 1949. The systematic arrangement of the New Zealand galaxiidae. Part II. Specific classification. *Transactions of the Royal Society of New Zealand* 77: 472-496.
- Stott B 1970. Some factors affecting the catching power of unbaited fish traps. *Journal of Fish Biology* 2: 15-22.
- The Ministry for the Environment 1997. *The State of New Zealand's Environment 1997*. The Ministry for the Environment, Wellington, New Zealand.

- Thompson FV 1987. Notes on the black mudfish. *Freshwater Catch* 32: 6-10.
- Vant WN, Davies-Colley RJ 1984. Factors affecting clarity of New Zealand lakes. *New Zealand Journal of Marine and Freshwater Research* 18: 367-377.
- Wandsvik A, Jobling A 1982. Observations on growth rates of Arctic charr, *Salvelinus alpinus* (L.), reared at low temperature. *Journal of Fish Biology* 20: 689-699.
- Wakelin R 1986. The biology of *Gambusia affinis* (Baird and Girard) in Lake Waahi, Huntly. Unpublished MSc thesis, University of Waikato. 93 p.
- Wikramanayake ED 1990. Conservation of endemic rain forest fishes of Sri Lanka: results of a translocation experiment. *Conservation Biology* 4: 32-37.
- Williams JE, Sada DW, Williams CD and other members of the Western Division Endangered Species Committee 1988. American Fisheries Society guidelines for introductions of threatened and endangered fishes. *Fisheries* 13: 5-11.
- Yamamoto S, Maekawa K, Tamate T, Koizumi I, Hasegawa K, Hitoshi K 2006. Genetic evaluation of translocation in artificially isolated populations of white-spotted charr (*Salvelinus leucomaenis*). *Fisheries Research* 78: 352-355.

