REDUCING ESTABLISHMENT RATES OF NON-INDIGENOUS ZOOPLANKTON IN CONSTRUCTED WATERS

A thesis

submitted in partial fulfillment

of the requirements for the degree

of

Master of Science

in Biological Sciences

at

The University of Waikato

by

CLAIRE MAREE TAYLOR

THE UNIVERSITY OF
WAIKATO
Te Whare Wānanga o Waikato

2010

Abstract

Understanding the mechanisms that facilitate the establishment of non-indigenous species is imperative for devising techniques that will assist in reducing the establishment rates of non-indigenous species. The establishment non-indigenous species can have negative ecological, economic, and human health effects. Non-indigenous passively dispersing organisms such as zooplankton, have been reported to invade constructed lakes (e.g., dams, water supply reservoirs and ornamental ponds) at much faster rates than natural lakes. For example, in New Zealand, a high proportion constructed waters, including dams for hydroelectricity generation, ornamental ponds and disused mine pits, have been invaded by non-indigenous zooplankton, including a number of calanoid copepods that are seemingly currently confined to these habitats. This has lead to a number of theories that have attempted to explain what makes constructed water bodies more vulnerable to invasion than natural lakes. One common attribute of these water bodies is their relatively young age, leading to the assertion that low biotic resistance leads to higher vulnerability of zooplankton communities in early stages of development.

The aim of this study was to determine if seeding water bodies with sediments containing native zooplankton eggs in early stages of their development will accelerate community colonisation, leading to greater biotic resistance to subsequent establishment of new zooplankton species. Twenty outdoor tanks were filled with tap water, and nutrients added to provide eutrophic conditions. Sediments were added to all tanks. Ten treatment tanks contained sediments and associated diapausing zooplankton eggs, sourced from local water bodies. The sediments were autoclaved in the remaining ten, which acted as controls, and thus received zooplankton colonised via natural means only. Tanks were left to colonise for 12 months and community composition and environmental variables were regularly monitored. During the 12 month colonisation period, species richness increased to a mean of 4.6 species in the treatment tanks and 2.6 in control tanks. Community composition also rapidly diverged between control and treatment tanks. Treatment tanks acquired a greater proportion of species adapted to pelagic conditions, such as cladocerans and copepods, with control tanks

generally acquiring a high proportion of small, littoral dwelling rotifers. New species were added at 12 months, comprising of two copepods, four rotifers, and one cladoceran species, which were not established in the tanks already. After the introduction of these species, the unseeded control tanks had a much higher proportion of establishment of the new species during the three month post-introduction period. For example, the non-indigenous calanoid copepod *Skistodiaptomus pallidus* established exclusively in tanks that were void of any other calanoid copepod species. These were primarily control tanks, suggesting that native calanoid copepods play a key role in reducing establishment rates of this taxon. At 12 months, when the new species were added, none of the environmental variables measured (temperature, chlorophyll *a*, conductivity, specific conductance, DO concentration, DO saturation and pH) were statistically different between treatment and control tanks. This infers that at the time the new species were introduced to the tanks, they experienced similar abiotic conditions, and environmental variability was therefore not responsible for the differing establishment rates.

This study proves that biotic resistance plays an important role in reducing the establishment rate of non-indigenous zooplankton. It also provides strong evidence that seeding constructed water bodies with sediments containing diapausing eggs from locally sourced communities can be used as an effective management tool to reduce establishment rates of non-indigenous zooplankton.

Acknowledgements

My biggest thanks goes to my supervisor, Ian Duggan. Your patience, attention to detail, and positive attitude have assisted me greatly throughout this process. Thank you for your tireless effort in both the practical and theoretical components of this thesis, and for all the help with zooplankton identification, which I found quite challenging! Thank you for giving me this opportunity. I am now officially a lover of zooplankton!

Thank you to my dad, for your perpetual support and encouragement, and for providing me with the means (i.e. roof over my head) to complete five years of study. I hope my nocturnal sleeping patterns didn't interfere with your day to day life too much! To my mum, thank you so much for your never-ending encouragement and for always being proud of me. The continual support from you both has made this all possible.

Special mention has to go out to Jeroen Brijs. Thank you for your help as my monitoring 'buddy', especially on that cold winter morning when the only way of fixing a leaky tank was to have you dive in it! Thank you for putting up with my long-winded conservations about zooplankton and providing me with much laughter and happy times. I'm also very grateful for your proof-reading efforts.

A big thanks to my fellow masters student Samantha Parkes. We had many fun times collecting zooplankton, and many times when we thought we would be stranded in the middle of no-where forever.....I'm glad we made it out of Tasmania alive! Thanks for being my mate throughout this whole process, we did it!

Thank you to the staff and students of R block for providing a fun work environment whilst I spent many months counting zooplankton. And to Warrick, thank you for providing me with access to the big lolly jar in your office, I'll replace them one day!

Funding was provided by a RSNZ Marsden Grant, Contract: UOW0702. Thank you for your generous financial support throughout this project.

Table of Contents

List of Figures

List of Tables

Chapter One: Introduction

1.1 Biological invasions and their effects

Non-indigenous species are defined as organisms that establish, with the intentional or unintentional aid of humans, in areas beyond their ranges of natural dispersal. Natural biogeographical boundaries are disestablished as the new species interact with different biotic and abiotic conditions in their new habitat (Richardson and Pysek, 2008). The negative effects of non-indigenous species are plentiful, but generally fall into three categories: ecological, economic, and those affecting human health. The realisation that the widespread movement of non-indigenous organisms is one of the leading causes of global biodiversity decline has highlighted the true extent of this problem and has lead to greater research attention (Ricciardi and Rasmussen, 1998; Stohlgren and Schnase, 2006).

1.1.1 Ecological

The transportation of organisms around the planet as a consequence of human activity has long been identified as an agent of biotic homogenisation (Chester, 1999; Olden and Poff, 2004). Charles Lyell, one of the foremost geographers of his time, recognised in the early 1800s that human's actions, such as hunting and land use change, were responsible for the extinction of many species. He also noted that, since the discovery of America by Europeans, the 'diffusion' of humans to a new continent was coupled with accompanying flora and fauna. Lyell did not view homogenisation, and the associated extinctions of native species as a negative consequence. In fact, he viewed human-mediated eradication of existing species as a natural and necessary part of human geographical expansion. If new species are to prosper in a new habitat, others must forfeit (Wilkinson, 2004).

As continents became more accessible due to technological advancements (Colautti et al., 2006), organisms continued to mingle in places that were once not available to them. Homogenisation and subsequent loss of biodiversity were now perpetual. Gordon Orians coined this era the 'homogocene', whilst Harold Mooney calls this overly accessible planet the 'New Pangaea'. Harold's predictions of decreased genetic variation in the 'New Pangaea' renders it a place

of diminishing biodiversity (Rosenzweig, 2001). It was not until the late 1950s, when invasion ecologist Charles Elton published the book *The Ecology of Invasions by Animals and Plants* (Elton, 1958), that the true damage of species invasions, along with attempts to understand the mechanisms of the invasion process, rendered this facet of science mainstream (Levine and D'Antonio, 1999; Ricciardi and MacIsaac, 2008; Richardson and Pysek, 2008).

Separate from homogenisation, species invasions have the potential to induce major ecological harm in many habitats. The most noted effect of non-indigenous species spread is loss of native biodiversity (Manchester and Bullock, 2000; MacIsaac et al., 2004; Loo, 2009). Decline in biodiversity can occur when non-indigenous species are competitively superior to, or prey upon, native species in the recipient community. If similar resources, such as food and space, are required by both the non-indigenous and native species, the non-indigenous species may gradually deprive the native species of those resources, causing eventual decline or even extinction (Manchester and Bullock, 2000). The fishhook waterflea (*Cercopagis pengoi*), one of many Ponto-Caspian invaders of the Laurentian Great Lakes introduced by ballast water, is a predatory species. *Cercoagis pengoi* preys aggressively on smaller zooplankton communities and is able to respond faster to changes in zooplankton prey faster than small fish populations, making it competitively superior (Benoit et al., 2002).

In extreme cases, non-indigenous species that are able to out-compete native species may lead to the loss of a keystone species (Loo, 2009), affecting entire ecosystems. This has happened extensively throughout many North American forests as non-indigenous fungal pathogens such as the chestnut blight (*Cryphonectria parasitica*), Port-Orford cedar root (*Phytophthora lateralis*) and butternut canker (*Sirococcus clavigignentijuglandacearum*), have lead to the decline of many keystone tree species. This decline has been attributed to changes in hydrology, loss of food source for wildlife and changes to energy fluxes (Ellison et al., 2005; Loo, 2009). The Rhine River, a major river that runs through Europe, has also been affected by non-indigenous species of Ponto-Caspian origin. In this highly invaded ecosystem, the Ponto-Caspian amphipod (*Dikerogammarus villosus*), a predatory omnivore (Dick et al., 2002), has engineered major shifts in

the local macroinvertebrate community, whilst another Ponto-Caspian invader (*Chelicorophium curvispinum*), has worked to modify the natural substrate with muddy tubes. Together, these two non-indigenous species now embody 80-90% of the macroinvertebrate community in the Rhine River (van Riel et al., 2006).

Rosenzweig (2001) argued that the spread of non-indigenous species will have no effect on global biodiversity, and will lead to an increase in biodiversity on a local scale. However, local studies like the ones above have shown that invasion of non-indigenous species can cause continual decline of local biodiversity.

1.1.2 Economic

Economically, the negative effects that non-indigenous species inflict on host environments are easier to quantify than that of the ecological impacts. The fact that large amounts of money are being spent in areas that host many non-indigenous organisms has acted as a major incentive for many government policies to be overhauled (Lodge et al., 2006). Long term effects may be more difficult to predict, but prevention and eradication attempts, environmental degradation, loss of agricultural productivity and health problems cost the United States taxpayer hundreds of billions of dollars per year (Mack et al., 2000). In the United States, Pimentel et al. (2000) conducted a major study estimating the amount of economic loss generated by harmful non-indigenous species. Without even taking into account the total number of non-indigenous species in the United States, or all the possible environmental damages these species could incur, it was estimated that economic loss surpasses US$120 billion per year. In Canada, Colautti et al. (2006) identified 16 non-indigenous species that had accrued known costs in agricultural, forestry, aquatic and marine resources. These effects included loss of yield, research/management expenditure and loss of export income. The 21 negative effects that were associated with the chosen 16 non-indigenous species were estimated to cost the taxpayer somewhere between CDN$13 and $34.5 billion annually.

Aquatic related industries, such as sport and commercial fishing, aquaculture, and those associated with tourism lose approximately CDN$300-780 billion per year. These loses are predominantly in the commercial and sport fishing industries,

which are of major economic value to Canada (Colautti et al., 2006). The zebra mussel (*Dreissena polymorpha*), first discovered in 1988 and believed to have originated from the Baltic Sea (Ricciardi and MacIsaac, 2000), has invaded widely in waterways in the U.S. and Canada despite large economic efforts to contain its spread. *Dreissena polymorpha* costs many power plants US$3 million per year (Leung et al., 2002) due to pipe blockage, which can lead to reduced power production. Although the Great Lakes Fishery Commission exclusively spends US$22 million per year on research and eradication of the predatory sea lamprey (*Petromyzon marinus*), the cost to commercial and sport industries, not calculated in that survey, would have proven to be much greater (Colautti et al., 2006). If inaction occurs, allowing establishment of further species, the cost of non-indigenous species will only escalate. Lack of technology in developing feasible methods that only eradicate certain selected species adds further costs to the dilemma (Lodge et al., 2006).

1.1.3 Human Health

Non-indigenous species are often known to be carriers of parasites and disease (Rodriguez, 2006). Whilst outlining the ecological and economical impacts of non-indigenous species, human health can also suffer. The Asian tiger mosquito, *Aedes albopictus*, was introduced to the United States as larvae on imported car tires (Vitousek et al., 1997). This species is a known carrier of dengue fever and is also responsible for the introduction of eastern equine encephalitis, which has proved fatal to both horses and humans (Vitousek et al., 1997). In East Africa, an increase in the tsetse fly (*Glossina* spp), a carrier of the fatal Human African trypanosomiasis (or sleeping sickness), was attributed to the invasion of a small flowering plant, lantana *(Lantana camara*). *Lantana camara* provides an ideal habitat for *Glossina* to breed. In South Africa, many introduced woody plants have been used for building and forestry practices, but higher water requirements of these plants have affected natural hydrological systems. This has led to less available freshwater and a decline in the native heathland plant populations, which are often used in medicine (Pejchar and Mooney, 2009).

The dire consequences of these, often unintentional, introductions have proved costly not only in monetary terms. Ecologically, many habitats suffer from

lowered local biodiversity and native species decline (Yan et al. 2002: Ellison et al. 2005) and at a global level, the spread of non-indigenous species acts to homogenise organisms (Wilkinson, 2004), rendering habitats less distinctive. Humans have also suffered from death and hardship at the hand of introduced species (Pejchar and Mooney, 2009). Although this facet of science is relatively recent (Ricciardi and MacIsaac, 2008), our knowledge of the ecology of invasions must be utilised if we are to attempt successful management practices to reduce introduction rates, establishment rates, or to control them.

1.2 The invasion process

The process by which species invade new environments in which they are not native is a multistage progression (Mack et al., 2000). It involves the uptake of potential invaders by a transport vector, transportation to a new environment, release and subsequent successful establishment (Sakai et al., 2001; Kolar and Lodge, 2001; Bailey et al., 2005; Lockwood et al., 2005). Understanding this process is important for identifying trends and associations in invasions that may provide information on factors such as the important attributes of species that can establish and spread and where invasions are likely to occur. In turn, this information can be used to assist in the formulation of more effective management plans to reduce species establishment and spread.

1.2.1 Uptake, transport and introduction

With accessibility to different parts of the world at an all-time high, there is now a seemingly endless array of vectors by which organisms can travel over land and sea (Mills et al., 1993; Ricciardi and MacIsaac, 2000). At the initial stages of any invasion, the number or types of organisms entrained into any vector (uptake) commonly does not provide an adequate indication of future establishment success at the site of release, as the fortune of all organisms varies extremely between species (Mack et al., 2000). Many organisms perish along the way, depending on the environmental conditions within the vector. For example, *Driessena polymorpha* can only survive for up to two weeks out of water. Thus, if they become attached to a recreational boat, they will only survive to establish in a new habitat if transport time between two water bodies is less than two weeks

(Padilla et al., 1996). Some may become damaged, rendering them unable to reproduce. Along with the frequency of introduction events, the number of species that arrive viable at the new destination, called propagule pressure or introduction effort, has been found to correlate with successful establishment (Mack et al., 2000; Bailey et al., 2005). On occasions, a fraction of these species are able to survive the transportation stage (Mack et al., 2000).

1.2.2 Establishment

If the invader survives the journey, it must then contend with new biological and physical conditions in the recipient region, which may prove to be either favourable or detrimental to its survival. The recipient region may include native species and/or non-indigenous species from past invasion events (Mack et al., 2000). Only if the conditions are favourable, and there are enough individuals to reproduce, will a self-sustaining population establish (Kolar and Lodge, 2001). Ecological or economic harm can occur if the self-sustaining population then extends its range beyond that of the original site of colonisation (Lockwood et al., 2005). Knowledge of the invasion process has not only resulted in a greater appreciation of the different steps required for a species to establish, it has also challenged invasion ecologists to predict the likelihood of possible future invasions.

1.3 Predicting establishment

Despite many attempts to determine what makes an ideal invader, or the characteristics of an invasion resistant community, the exact mechanisms of invasion ecology remain somewhat elusive. Older literature focused on the above characteristics separately, whilst modern approaches, such as modeling, imply it is the interaction between the potential invader with host community assemblages, the vector of invasion (Buchan and Padilla, 1999), abiotic factors and history that collectively determine whether a successful invasion will take place or not (Lodge, 1993). Ricciardi and Rasmussen (1998) attempted to provide a criterion that would help identify where potential invasions would likely occur. They concluded the most significant attribute for identifying non-indigenous aquatic species is their commensalism with human activity, as this increases the likelihood of them

reaching a host community. This attribute was ranked more important than biological attributes such as short generation times, high genetic variability and wide environmental tolerances.

Most non-indigenous species use human activity as a dispersal mechanism. This knowledge, along with information on the history of past invasions, is likely the only method of invasion prediction that has been deemed successful (Ricciardi and Rasmussen, 1998). Invasion history was used by Mills et al. (1993b) to predict the invasion of the snail *Potamopyrgus antipodarum* into the Great Lakes. However, it is worth noting that the knowledge of this forecasted invasion did not prevent it from occurring (Ricciardi and Rasmussen, 1998).

Attempts by Marchetti et al. (2004) to form a profile of potential non-indigenous fish species were also far from conclusive when biological variables were compared across successful and failed introductions of freshwater fish. The association with humans was again, in their study, the best variable that explained successful invaders. Although invasion ecologists have found no clear cut method for predicting aquatic invasions, it is certain that humans are associated with the determination of invasion success (Padilla et al., 1996).

1.4 Factors affecting establishment

Along with the recognition that humans play a major role in assisting the establishment of non-indigenous species, three major factors have persisted in invasion ecology that seek to explain why non-indigenous species establish more readily in some habitats than others. As well as the relevance of their roles in establishment, factors such as disturbance, biotic resistance and propagule pressure, have been persistently contested, as invasion ecologists attempt to find a more universal explanation as to what determines the ability to invade (Havel et al., 2002; Von Holle and Simberloff , 2005).

1.4.1 Disturbance

In North American studies (Havel et al., 2005; Johnson et al., 2008) a strong argument has been made that the greater disturbance rates in dams contribute

significantly to the increased colonisation potential and subsequent spread of non-indigenous species in constructed waters. The general argument for disturbance contends that a disturbance prior to the arrival of non-indigenous species causes abrupt environmental changes that may prove too difficult for native species to adapt to. If non-indigenous species arrive after the disturbance and conditions are favourable, establishment may occur and native populations may decline (Mack et al., 2000). This theory has been entertained in terrestrial, marine and freshwater environments (Altman and Whitlatch, 2007).

In hard substrate marine communities (i.e. rocky shore), disturbance events act to free up space for potential invaders. Space is usually a limiting factor in hard substrate communities and disturbance regimes act to expand the range of potential colonisers for establishment and subsequent growth, providing the disturbance events are of a moderate frequency (Altman and Whitlatch, 2007).

Whilst the disturbance theory holds true for sessile organisms in hard substrate communities, in freshwater ecosystems, Havel et al. (2005) argue that disturbance in dams facilitates the invasion of non-indigenous passively dispersing organisms, such as zooplankton. It is argued that the high rate of hydrological disturbances in dams compared to natural lakes leads to greater spatial heterogeneity, providing a multitude of possible niches for potential invaders to establish.

In a study of the biogeography of Australian freshwater fishes, Olden et al. (2008) found that drains that were more frequently disturbed by human settlement exhibited higher rates of introduced species. Olden et al. (2008) argued that the establishment of introduced species into habitats with high disturbance rates due to out-competing native species cannot be attributed to disturbance alone. In this study concerning river basins of north-eastern Australia, major drainage systems along with accompanying higher disturbance rates are associated with human settlement. In these areas the direct proximity to human activity is more likely to explain increased non-indigenous fish invasion than disturbance alone, as introductions of aquarium pets and unintentional release of ornamental pond fauna have been recorded in these areas.

1.4.2 Biotic Resistance

Elton (1958) recognised the importance of biotic resistance in the invasion process 50 years ago (Reichard and White, 2003; Ricciardi and MacIsaac, 2008; Richardson and Pysek, 2008). Elton's works were largely concerned with the examination of ecosystems at the community level. His notion that communities with greater species richness were more resistant to invasion than those with fewer species was extrapolated to both terrestrial and aquatic settings. If a potential invader is to successfully establish it must overcome potentially detrimental interactions from the recipient community, such as predation, disease and competition for food and other resources (Alonso and Castro-Díez, 2008). Biotic resistance has gained interest due to its potential application in the conservation of native species, as understanding its mechanisms may lead to predications of where introductions of non-indigenous species may occur (Levine et al., 2004). The idea of biotic resistance was further supported by Lodge and MacArthur (Levine and D'Antonio, 1999). Studies by Dzialowski et al. (2007) show that in North America, *Daphnia lumholtzi* is only able to establish in late summer, when native zooplankton communities experience a decline in abundance. This infers that biotic resistance may be seasonal in some communities, only acting to repel invasion when diversity and abundance is suffice on a temporal scale.

1.4.3 Propagule pressure

Propagule pressure - the number and/or the frequency of propagules released at introduction – has more recently been recognized as an important factor contributing to the establishment success of introduced species. It is presumed that if there are a high number of individuals of a non-indigenous species introduced to a recipient habitat, colonization is more likely to occur than if propagule supply or 'introduction effort' is low. Larger propagule sizes are also advantageous in the face of stochastic events as it increases the likelihood of survival amongst the invading population. Forsyth and Duncan (2001) stated that this conjecture, tested on 14 species of exotic ungulate birds in New Zealand, holds true for all taxa.

The role of propagule pressure has emerged as the singular factor governing the success of plant invasions in an experimental study conducted by Von Holle and

Simberloff (2005). This study, at the time, was the first to experimentally test the roles of disturbance and ecological resistance against propagule pressure. Whilst propagule pressure was concluded as the foremost determinant of invasion success, seedlings and plants were used as opposed to seeds, which questions the experiments extrapolarity into a realistic invasion setting. If there are high propagule numbers, or release events, chances of establishment will also increase. The rainbow trout (*Oncorhynchus mykiss*) has been intentionally introduced repetitively in many countries for purposes of sport fishing. This aquatic species provides a good example of how high propagule pressure, along with a high number of release events, has lead to successful invasion of many freshwater ecosystems (Lockwood et al., 2005).

Von Holle and Simberloff (2005) state that it is possible that the roles of biotic resistance and the supply of propagules interact to determine whether invasion is successful or not. Highly complex communities, although less susceptible to the establishment of non-indigenous species, may still be invaded if propagule supply is sufficient. While it is likely that a combination of invasion mechanisms and abiotic features all factor in determining whether invasions take place, the tendency for non-indigenous zooplankton to infiltrate constructed waters more readily provides key information on the ecological processes behind these introductions.

1.5 Vulnerability of freshwater ecosystems

Freshwater ecosystems, in particular, are experiencing the greatest decline in biodiversity when compared to terrestrial or marine ecosystems (Malmqvist and Rundle, 2002; Dudgeon et al., 2006). This is due, in part, to the large number of introductions of non-indigenous species, including those associated with constructed waters such as dams used for hydroelectricity, water supply and recreational purposes (Johnson et al., 2008). Introductions of this nature will be the focus of the remaining pages.

Globally, the correlation between habitat alteration and introduction of non-indigenous species is at its most obvious in limnetic systems, where species invasions are rampant (Ricciardi and Rasmussen, 1998; Dudgeon et al., 2006).

The freshwater environment has been interwoven with human activity for centuries. As a central part of our societal organisation, we have long altered and exploited freshwater resources for many uses such as electricity, sustenance, recreation, waste assimilation and transport. As our population increases exponentially, alterations to these vulnerable habitats will increase also, producing more examples that link habitat alteration to the spread of non-indigenous organisms in freshwater ecosystems (Bronmark and Hansson, 2002; Naiman and Turner, 2000). Many invasion ecologists now consider that no significant freshwater ecosystems exist that have not been permanently modified (Naiman and Turner, 2000).

Freshwater ecosystems also bear disproportionate species richness when compared to other ecosystems. Only 0.1% of the earth's total water surface contains freshwater, but almost one third of all vertebrates reside here at some stage, if not all, of their lifecycle. With such a high concentration of species in a globally minute area, the detrimental effects of human activity are likely to be echoed throughout surrounding habitats (Dudgeon et al., 2006).

Adding to the apparent vulnerability of freshwater systems, it is the estimated that the volume of water currently impounded in dams and reservoirs is as great as five times the amount of water in all the world's rivers (Rosenberg et al., 2000). This facilitates an extremely large potential habitat area for non-indigenous organisms.

1.6 Vectors

Freshwater ecosystems have had to contend with a number of invasions from non-indigenous species. For example, since European settlement, the Laurentian Great Lakes have received hundreds of non-indigenous species via a number of different human mediated vectors (Grigorovich et al., 2002). Many introductions have been intentional, but the majority are unintentional consequences of anthropogenic activity (Mills et al., 1993).

Since the 1970s, ballast water release has become the number one cause of unintentionally introduced species into aquatic environments worldwide (Ruiz et al., 1997). This vector is thought to be responsible for the majority of introduced

species into the North American Great Lakes (Mills et al., 1993). The most notable feature of invasions into this particular area is that they are predominantly comprised of freshwater and brackish species of the Black, Caspian, and Azov Seas, an area collectively known as the Ponto-Caspian region. These invaders, both benthic and pelagic dwelling, are now dominating local communities, causing extinctions of some native species (Ricciardi and MacIsaac, 2000). The release of unwanted pet fish or escape from culture facilities has lead to an increase of introductions related to the aquarium industry. This vector has not gained as much attention as that of ballast water, even though it could be more effectively contained (Rixon et al., 2005).

Other human mediated vectors that transfer non-indigenous species between water bodies can include recreational boats, bait buckets, water skiing/SCUBA gear, anchor lines, bilge water, float plane pontoons, fishing gear and on the boots of people (i.e. anglers) traveling to adjoining lakes (MacIsaac et al., 2004). Regional surveys conducted by Dzialowski et al. (2000) in eastern Kansas reservoirs indicated that the cladoceran, *Daphnia lumholtzi*, known for its broad limnological tolerances, was absent from all ponds inaccessible to boats. Nearby reservoirs within the same watershed that are accessible to recreational boats hosted *D.lumholtzi*, solidifying the importance of human mediated vectors in the dispersal of this exotic species.

Examples of human-mediated vectors such as the ones above are plentiful and it is reasonable to deduce that aquatic environments are vulnerable to biological invasions at the hand of man. It is clear that the homogocene is not a temporary era, and may only gain momentum as original assemblages of native and exotic communities merge in to one.

Colonisation of zooplankton communities by natural means is a slow process and dispersal can be infrequent. Natural vectors including insects, wind, rain and the bodies and/or faecal matter of waterfowl, are often stochastic and, on a temporal scale, are relatively sluggish (Jenkins and Underwood, 1998; Bilton et al., 2001).

For many freshwater invertebrates, such as zooplankton, resting eggs are used to assist in dispersal and establishment of new populations. Resting eggs are often resistant to desiccation and freezing, allowing long periods of dormancy by which transportation to a new location can occur. Investigations by Proctor and Malone (1965) showed that resting eggs can pass through the entire gut of some fish and bird species, without any detrimental effects to the invertebrate (Havel and Medley, 2006). However, studies by Jenkins and Underwood (1998) and Bilton et al. (2001) show that even with the advantage of resting eggs as a survival mechanism, the rate of movement in natural zooplankton dispersal is extremely slow. Human vectors, such as the ones discussed previously, provide a more rapid means by which zooplankton are able to disperse and subsequently establish.

1.7 Constructed waters

An emerging trend that non-indigenous passively dispersing organisms such as zooplankton, plants and algae, often invade constructed lakes at a much faster rate than natural lakes, has been observed on a global scale (Johnson et al., 2008; Banks and Duggan, 2009). One of the first, and most cited, examples of the invasion of non-indigenous zooplankton into constructed waterways is that of the cladoceran, *Daphnia lumholtzi*, which has spread through at least 125 lakes in the United States within one decade of its introduction into a Texas reservoir in 1990 (Havel et al., 1995). More recent surveys estimate *D. lumholtzi* to occupy most of the south-eastern U.S.A, with northward bound advancements eminent (Lemke et al., 2003). This cladoceran, native to the old world tropics, invaded constructed reservoirs at a much higher rate than natural lakes (Havel et al., 2005).

An extensive survey conducted by Johnson et al. (2008) which included the sampling of 1080 water bodies, showed that *Bythoytrephes longimanus* is significantly more likely to invade constructed lakes than natural lakes. This conclusion also reined true for four other non-indigenous species, including zebra mussel (*D. polymorpha*), rainbow smelt (*Osmerus mordax*), rusty crayfish (*Orconectes rusticus*) and the Eurasian watermilfoil (*Myriophyllum spicatum*). Recent findings of *D. lumholtzi* in the Upper Parana River floodplain in South America have been attributed to the favourable biotic features in upstream retention by dams. Here, decreased nutrients, increased water transparency and

high temperatures have provided ideal conditions for this rapidly spreading global invader (Simoes et al., 2009). These findings highlight the association between human activity, constructed water bodies, and the spread of non-indigenous propagules to new habitats. The acknowledgment that non-indigenous zooplankton invade constructed waters at a faster rate than natural lakes (Johnson et al., 2008; Banks and Duggan, 2009) suggest that invasion dynamics may also situate importance on the host habitat, rather than the amount of propagates *per se*.

Havel et al. (2005) argues that reservoirs are more susceptible to invasion from non-indigenous species due to their relative lack of biotic resistance, greater connectivity to other water bodies, and higher rates of disturbance than those found in natural lakes. The sequential placement of dams on river systems means that colonisation and spread by non-indigenous species will occur at a greater pace than in natural lakes, which are not as tightly connected to other water bodies. The physical location of dams and the downstream flow means passively dispersing non-indigenous organisms, such as zooplankton, will be given greater establishment opportunities along the modified river system. Havel et al. (2005) also argue that greater disturbance rates exhibited in dams allows greater opportunity for the establishment of non-indigenous organisms. This is attributed to disturbance events, prior to the arrival of non-indigenous species, causing abrupt environmental changes that native species may not be able to adapt to, leaving free 'resource gaps' for potential invaders (as discussed in previous sections).

Havel et al. (2005) also argue that reservoirs are more susceptible to invasion due to their comparatively low species richness, leading to low biotic resistance. This is due to the young age of reservoirs (or dams) and the fact that they are in comparatively earlier stages of community succession than older natural lakes. It is assumed that natural lakes have accumulated more species because of their relatively old age. Although this assumption has not been formally tested, pond experiments by Shurin (2000) concluded that native community complexity is negatively correlated to invasion success of non-indigenous zooplankton species (i.e. biotic resistance). Due to zooplankton being very slow to colonise new lakes by natural means (Jenkins and Underwood, 1998), the biotic resistance during this

time can be so low that propagule supply may not need to be very high for successful establishment to occur. Human mediated introductions, such as dumping of aquaria and sediment transfer between lakes, may prove to be significant vectors for establishing non-indigenous communities in this early, vulnerable stage.

Results obtained from Banks and Duggan (2009), in a survey conducted in the North Island, New Zealand, contest the role of disturbance and connectivity in zooplankton invasions in constructed waters, as non-indigenous species were found in ornamental ponds, a disused mine pit and a disused quarry. These constructed water bodies do not have high rates of disturbance and connectivity to other water bodies.

1.8 The New Zealand context

In New Zealand, much of the focus on non-indigenous species has been reserved for vertebrates and plants, although Emberson (2000) states that there are at least 2000 known non-indigenous invertebrate species that have established here. The impacts of many vertebrate and plant introductions are well documented, owing to the obvious deleterious effects these species have inflicted. In the case of zooplankton, a comparative lack of research has lead to less of an understanding about the impacts these species may inflict in New Zealand (Brockerhoff et al., 2010), especially when compared to that of North American studies (Yan et al., 2002). Unintentional introductions of zooplankton into New Zealand have included the jellyfish, *Craspedacusta sowerbii*, presumed to have originated from China, and the North American cladoceran *Daphnia dentifera* (Duggan et al., 2006).

Calanoid copepods, a zooplankton group that are easily detectable due to their morphological distinctiveness, have had a long history of intercontinental invasions and New Zealand now hosts some of these. In New Zealand inter-continental invasions of *Boeckella* into constructed water bodies are also evident. The Australian species, *B. minuta* and *B. symmetrica*, are considered non-indigenous and are both seemingly restricted to constructed waters in the North Island (Banks and Duggan, 2009). *B. minuta*, to date, has been identified in the

Karori Reservoir in Wellington, Turtle Lake, an ornamental pond in Hamilton, and several of the Waikato hydroelectricity reservoirs. *B. symmetrica* has been located in the Wiri Quarry reservoir (a retired quarry pit that has been infilled) and a pond in Puhinui, Auckland (although the origin of this pond remains uncertain; Banks and Duggan, 2009). The geographically restricted distribution of these species and the fact that they are seemingly confined to constructed water bodies, indicate that these invasions have occurred recently

Surveys by Duggan et al. (2006) found the North American *Skistodiaptomus palllidus* and Japanese *Sinodiaptomus valkanovi*. Again their distribution was restricted to constructed water bodies. *Skistodiaptomus pallidus* is widespread in eutrophic reservoirs and ponds in central North America. In their survey it was located in two ponds at the Auckland Botanical Gardens and another in Albany, North Auckland. This species was also found being sold, amongst other zooplankton species as live fish food from an aquarium shop in Hamilton. The Japanese, *Sinodiaptomus valkanovi*, was first observed in New Zealand in the Auckland Domain in an indoor ornamental pond (Duggan et al., 2006). Further surveys conducted by Banks and Duggan (2009), investigating whether non-indigenous species were found more frequently in constructed waters, found this species to be more widespread than that of the previous Auckland records, as illustrated in Figure 1. Their surveys found *S. valkanovi* to be also located in an infilled mine pit in Waihi and *S. pallidus* was found in Lake Rotomanu, a former quarry in New Plymouth. Establishment of these species indicates the possibility of separate introduction events or spread from the original point of invasion. Genetic analysis by Makino et al. (2010) found that all population of *S. valkanovi* were of one specific haplotype, indicating population establishment took place from one introduction event, with subsequent spread.

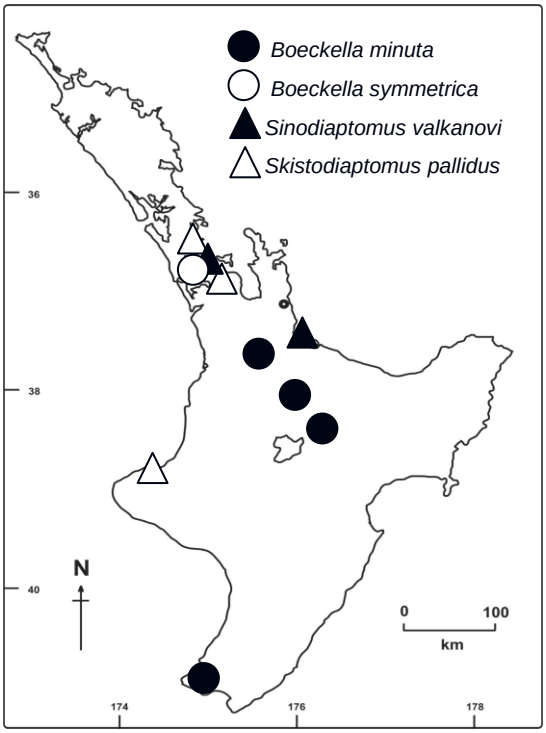

Figure 1: Distribution of *Boeckella minuta*, *B. symmetrica*, *Sinodiaptomus valanovi* and *Skistodiaptomus pallidus* in constructed water bodies in the North Island (modified from Banks and Duggan, 2009).

Both *Boeckella minuta* and *B. symmetrica* are believed to have originated from Australian populations (Banks and Duggan, 2009). Surveys by Maly (1984) revealed that these species were widespread along the eastern states of Queensland, New South Wales, Victoria and Tasmania. More recently, *B. minuta* has spread from the eastern states into ten constructed farm ponds in Western Australia (Maly and Maly, 1997). It has not been confirmed whether the dispersal of both *B. minuta* and *B. symmetrica* into New Zealand has occurred via natural or anthropogenic means. However, due to their relatively rapid spread, and the fact they are seemingly confined to constructed water bodies in New Zealand, leads to the assertion that human mediated vectors were involved (Banks and Duggan, 2009).

In New Zealand, most constructed lakes are less than 20 years old, whilst natural lakes are generally at least 500 years old (Chapman and Lewis, 1976). Globally, dam construction is of a similar timeframe, attaining its maximum in the 1970s when over 5000 dams were built worldwide. On average, most natural lakes are significantly older than constructed waters (Malmqvist and Rundle, 2002). In New Zealand, the construction of new standing waters for hydroelectricity

generation, water supply and infilling of retired mine pits, has been extensive. Twenty percent of all North Island lakes are constructed by humans (Lowe and Green, 1987). This percentage will only increase as more constructed reservoirs are planned. In 1999, the New Zealand Dam Inventory listed 400 active dams (McDowall, 2000).

The combination of high percentages of constructed water bodies (McDowall, 2000), increasing rates of non-indigenous zooplankton establishment in constructed waters (Banks and Duggan, 2009), and deficiencies in research on the effects that these introductions may have on freshwater ecosystems, signifies that urgent attention is needed in this field.

New Zealand surveys (Banks and Duggan, 2009), found that the high frequency of non-indigenous zooplankton in constructed waters is not only confined to dams, as in the Havel et al. (2005) study, but extends to ornamental ponds, disused mine pit, quarry and farm dams. These constructed water bodies have poor connectivity to other water bodies and low rates of disturbance, weakening the arguments of Havel et al. (2005) that disturbance and connectivity facilitate invasions into constructed waters. The comparatively young age of dams and other constructed waters such as the ornamental pond and disused mine pit in the Banks and Duggan (2009) survey, lead to the assertion that biotic resistance is the most compelling factor explaining the introduction of non-indigenous zooplankton into constructed waters. It is the only proposed factor affecting establishment that is common with all invaded water bodies, not just dams alone. This argument suggests that if biotic resistance can be accelerated, invasions into constructed waters can be reduced.

Past invasions have taught us that once in a new habitat, detection and subsequent eradication of non-indigenous zooplankton is impractical and unfeasible, deeming prevention the only means of obstructing the spread of these organisms.

1.9 Aim

Although the concept of biotic resistance has been largely accepted, it has not been formally tested with the aim of harnessing it to prevent future invasions in a natural setting. Invasion of constructed reservoirs may be accelerated due to the relatively low species diversity and low array of biotic interactions, both of which contribute to poor biotic resistance and increased vulnerability to invasions. Therefore, the introduction of native propagules into newly constructed reservoirs at an early stage of community development may provide biotic resistance as community development will be accelerated during a potentially vulnerable time. If propagule supplies of non-indigenous species are not overwhelming, the 'seeding' of new aquatic habitats with native zooplankton species could provide a means of reducing the establishment of non-indigenous zooplankton. If the introduction of native propagules increases biotic resistance during a potentially vulnerable time, seeding of reservoirs could be implemented on an international scale, thus maintaining the biodiversity of local ecosystems.

The overall aim of this study is to determine experimentally whether seeding water bodies with sediments containing native zooplankton eggs in the early stages of their development will increase the rate of community development (e.g. diversity) leading to greater biotic resistance to invasion by species introduced at later times. If successful, this can be applied to newly constructed water bodies to accelerate biotic resistance and reduce establishment rates of non-indigenous zooplankton, thus creating a valuable tool for the preservation of biodiversity.

2.1 Experimental Tank Set-Up

To examine if seeding water bodies with native propagules accelerates zooplankton community development, leading to greater biotic resistance against the subsequent introduction of non-indigenous species, 20 experimental tanks were set up outside the University of Waikato Aquatic Research Centre for a total period of 15 months. The tanks, purchased from Rotational plastics in Hamilton, were molded from UV stabilised food grade low density polyethylene (LDPE) (Figure 3).

Figure 2: Photo of tanks showing dimensions

The tanks held approximately 1800 liters of water, with dimensions of (~L=1.5 m × W=1.0 m × H=1.2 m). Each tank was three-quarters filled (90cms) with tap water, making a total volume of 1400 L per tank. This amount of water allowed 30 cm between the top of the tank and the surface, minimizing the risk of cross-contamination between the tanks due to splashing during monitoring periods and/or rainfall. Major nutrients, nitrogen and phosphorus (7.46 g of NH_3Cl and 6.41 g of K_2PO4) were added to attain eutrophic conditions similar to local water bodies. Additionally, other micronutrients were provided using the synthetic pond water formulation from Herbert & Crease (1980). One hundred and nineteen grams of $NaHCO_3$, 94.24 g of $CaSO_4$ and 74.4 g of $MgSO_4$ were dissolved into

each tank (Table 1). The supplementation of these nutrients ensured that nutrients would not become a limiting factor in community development.

Table 1: Final concentration of nutrients added to each tank.

Nutrient Added	Concentration (mg/L)
NH_3Cl	5.3
K_2PO4	4.6
$NaHCO_3$	85.0
$CaSO_4$	67.3
$MgSO_4$	53.1

The tanks were randomly separated into 10 controls and 10 treatments (Figure 4). Ample space was left to allow movement between each tank whilst monitoring (65 cm between the two rows of tanks and 40 cm between each tank within a row). After eight months of monitoring, Tank 9 obtained a leak in the bottom of the tank that proved unfixable and was excluded from the remaining experiment. There were 10 treatment and nine control replicates for the rest of the monitoring period.

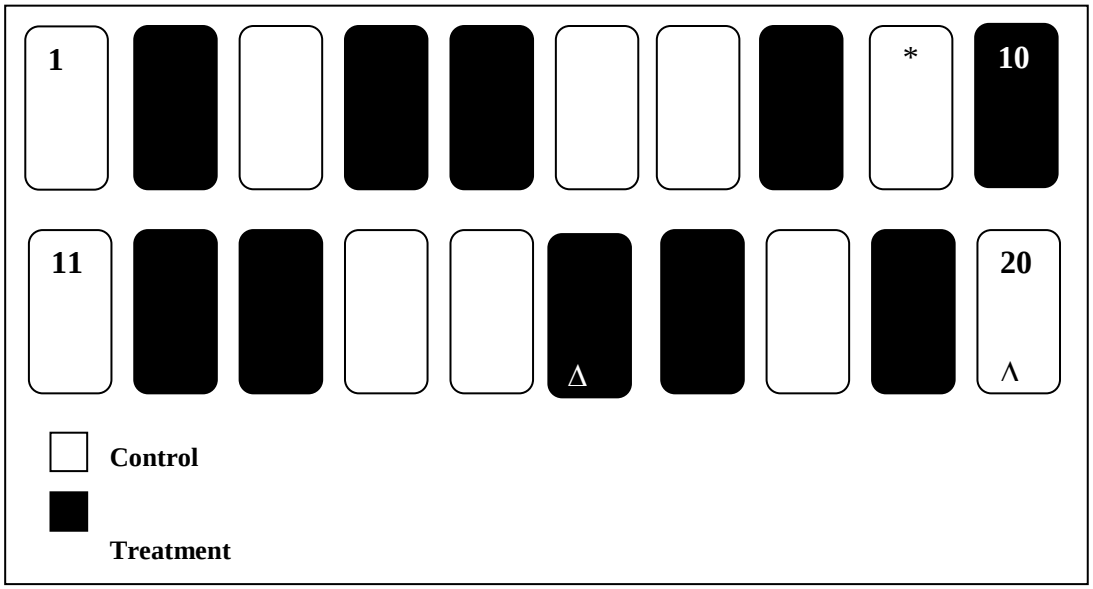

Figure 3: Schematic diagram of tank layout.

*Denotes the control tank that obtained leak in month eight and was removed from experiment. Δ Indicates tanks that were left without the addition of new species at 12 months.

Each tank had a total of 300 g of sediments added to it, sourced from three separate local habitats known to contain diapausing eggs representative of local zooplankton communities. These habitats: Lake Ngaroto, an unnamed farm pond in Gordonton and Chapel Lake, located on the University of Waikato grounds, all contributed 100 g of sediment each. All these water bodies were of eutrophic status and shallow. Lake Ngaroto has a surface area of 108 hectares, Chapel Lake and the Gordonton farm pond were much smaller in size, with surface areas of 0.44 and 0.20 hectares respectively. Sediments were obtained at a depth of approximately one meter. One hundred grams of sediments from the three separate water bodies were weighed out and put in 20 separate glass flasks. The 10 flasks to be released into the control tanks were then autoclaved at 121^{o}C for fifteen minutes, so that all diapausing eggs contained within these sediments were killed. Aluminium foil was placed over each flask to prevent possible spillage from sediments boiling over due to high temperatures. The control tanks experimentally represent new water bodies allowed to be colonised by zooplankton via natural vectors. It was hypothesisised these would develop a low species richness and therefore exhibit low biotic resistance. Treatment tanks represent new water bodies seeded with natural lake sediments. The same volume and origin of sediments were used for both the control and treatment ponds to negate any potential differences in community composition due to, for example. 1) habitat variability, such as chemicals (including nutrients) arising from the sediments, 2) contrasting habitats for potential benthic species that prey on zooplankton eggs, or 3) habitat variability caused by sediments providing a refuge for zooplankton species in treatment tanks that is not available in control tanks. All sediments in the flasks were then transferred into the filled tanks. Communities were left to colonise by natural means during a 12 month period. During this period no other zooplankton or algal (food) propagules were intentionally added.

2.1.1 Monitoring

Initial monitoring began one day after sediments were added. Subsequent monitoring took place one week after sediment addition and twice monthly thereafter for a total of 15 months. Zooplankton monitoring was carried out using a PVC integrated plankton cylinder with a total height of 1.12 m and a diameter of

9 cm. The cylinder was vertically submerged into the water to a depth of 74.5 cm, approximately 15cm above the bottom of the tank. This isolated 4.74 L of water. Two cylinder samples were obtained per tank on each monitoring day and combined into a single sample of 9.48 L. The sample was then filtered through a 40µm sieve and the zooplankton retained on the filter was washed into a sample container and preserved in 75% ethanol (at a concentration of 50:50) for subsequent identification to the species level in the laboratory. Two microscopes were used to identify the zooplankton. For zooplankton counting, an Olympus SZ-ST stereo microscope was used at a magnification of 30x. For species identification, an Olympus BH-2 binocular microscope for magnification between 100x and 400x. Each sample was rinsed through a 40µm sieve and poured into a 7.5 cm × 4 cm counting tray. This tray was separated into twelve columns and five rows. If there were more than 200 individual zooplankton counted at the end of one single row, this sample was then multiplied by five (the number of rows in the counting tray) to attain the final abundance for that species. The Olympus BH-2 binocular microscope was used when higher magnification was needed, including identification of rotifer teeth and the fifth leg of calanoid species. Species abundance was also recorded. Environmental variables temperature, conductivity and specific conductivity were measured using a YSI 30 meter, oxygen concentration and oxygen saturation were recorded using a YSI 550A meter. A reading was taken approximately 15cm below the surface and 15cm above the bottom of each tank, so that two readings were obtained per tank for each of the environmental variables. Chlorophyll *a* samples were also obtained twice monthly. On each sampling day, a 200 ml sample was taken from the surface of each tank. All equipment was washed thoroughly with tap water between tanks to avoid cross contamination during sampling.

2.1.2 Chlorophyll *a*

To determine chlorophyll *a* concentrations, 20ml of tank water was extracted from each 200 ml sample through 25mm glass fibre filter paper using a Carbon 14 Centralen carousel attached to a GAST Manufacturing rotary valve lubricated laboratory vacuum pump at low pressure. The algae retained on the filter paper was placed in aluminium foil and put in a freezer until further processing.

To process the chlorophyll *a* samples, filter papers were removed from the freezer and ground in dim light using a mechanical tissue grinder and mixed with a solution of 90% $MgCO_3$-buffered acetone. This slurry was then left for 24 hours. After a 24 hour steeping period the filter slurry was shaken and centrifuged at 3300rpm for 10 minutes, this correlates to a G-force of 1,461 G. A 10-AU Turner Designs fluorometer, calibrated with standards of known chlorophyll concentrations, was used to measure the fluorescence of the supernatant before and after acidification, where, phaeophytin degradation products were corrected by using a 0.1 N HCI solution (Arar and Collins, 1997).

2.1.3 pH

pH levels were processed within 30 minutes of collection of the sample. A MeterLab PHM210 standard pH meter, calibrated with known standards before each sample lot, was used to determine pH levels on each monitoring day.

2.2 Addition of new zooplankton species

After a 12 month colonisation period from 7 September 2008 to 7 September 2009, new species of zooplankton from three different major taxa (rotifer, copepods and cladocerans) were gradually introduced into the tanks over a period of one week from separate test tubes. Care was taken to ensure new species were alive at the time of introduction. These species were not found in any of the tanks in the initial 12 month monitoring period. Table 1 shows the species and number of individuals introduced into the tanks after 12 months.

Table 2: New species introduced after 12 months.

Taxon	Species	No. Introduced
Copepoda	*Mesocyclops leuckarti*	25
	Skistodiaptomus pallidus	16
Rotifera	*Synchaeta pectinata*/*S.oblonga* combination*	40
	Trichocerca similes	3
	Ascomorpha ovalis	4
Cladocerans	*Chydorus sphaericus*	5

* *S.pectinata and S.oblonga* could not be distinguished among live samples without killing and identifying teeth. A combination of the two species were collected and placed into the tanks.

The new species were sourced locally from various water bodies that were of similar trophic state to the tanks and small in size. This was to ensure only a small change in habitat variability occurred between the source and tank environments, thus enabling the new species a better chance of colonisation. These water bodies were different from those used in initial sourcing of zooplankton eggs. *S.pectinata*, *T.similis* and *C.sphaericus* were sourced from the Lake Rotoroa, *M.leuckarti* and *A.ovalis* were sourced from a small pond in the Taitua Arboretum, Whatawhata and the North American invader *S.pallidus* was obtained from mixed zooplankton cultures sold as fish food at a local pet shop.

The introduction of multiple new species was so determine whether their establishment depended upon the existing species richness within the tanks, as well as clarifying the interacting roles of propagule pressure and biotic resistance. One treatment and one control tank (tanks 16 and 20) did not receive any new species after 12 months (Table 1). These were intentionally left so that species composition without new colonists could be monitored and compared to the 'seeded' tanks. As one control tank was lost from the experiment due to leakage, this left a total of eight control tanks and nine treatment tanks with additional species added at twelve months.

A subsequent three month period was used to monitor the colonisation success of the new species. Community data and environmental variables in each tank were monitored fortnightly, as had occurred in the previous twelve months. The composition of the communities was compared between the initial twelve month monitoring period and the subsequent three month 'post-invasion' period.

2.3 Statistical Analysis

The PRIMER v6 statistical package (Plymouth Marine Laboratory) was used to produce non-metric multidimentional scaling (MDS) analysis of similarities (ANOSIM) and similarity percentage analysis (SIMPER). These analyses were used to follow changes in community composition between treatment and control groups and calculate the role of individual species within a group and how those species contribute to the differences between groups (Clarke & Gorley, 2006).

MDS is a multivariate ordination technique that allows simple interpretation of changes in community composition based on differences in community composition, defined by a metric distance. It uses a resemblance (or similarity) matrix, such as that based on the Bray-Curtis similarity co-efficient, to form ordination points that represent how community composition differs between and within sample groups. If communities are similar in composition, they will be in a similar position in the 2-dimensional plot; communities that differ in composition between each other will be placed further apart on the plot, depending on their differences in community composition (Clark & Gorley, 2006).

To find the relative differences in composition between treatment and control groups, the Bray-Curtis similarity co-efficient was used to form a log (x+1) transformed resemblance matrix. Samples were omitted if they contained less than two species on a particular sample date. This was done to ensure that species irrelevant to community composition were not included in the analysis. This transformation was chosen to increase the importance of the less abundant species and downweight the contributions of the most abundant species. MDS ordination was used to display the differences in community composition between treatment and control groups throughout the initial 12 month monitoring period, and how they changed during the time of new species introductions.

ANOSIM was used to test if the differences in community composition were statistically significant over the fifteen month period. ANOSIM provides a global *R*-statistic between 0 and 1, based on the Bray-Curtis dissimilarity matrix. *R* values closer to zero indicate groups that are similar, while values approaching one indicate groups are very dissimilar. 999 permutations were performed on treatment and control samples that exhibited two or more species on each sampling date.

SIMPER analysis show the contribution of each species to the Bray-Curtis dissimilarity between treatment and control groups. It is set out so that the species responsible for divergence between the groups are listed as a percentage contribution. A species that is highly abundant in one group only, therefore responsible for a proportion of divergence between the groups, will exhibit a high

percentage contribution of dissimilarity. Each species is listed in descending order of its percentage contribution to the dissimilarity between the groups (Clarke & Gorley, 2006).

To compare relative contributions of each species between treatment and control tanks a SIMPER analysis was applied to log transformed Bray-Curtis dissimilarity data. Again only samples that contained two or more species were included. The top 95% of contributing species were included in this analysis.

Statistica version 7.1 (Statsoft Inc., Tulsa. USA) was used when ***t***-tests were performed to compare the statistical significance of mean values between treatment and control tanks for the environmental variable data at twelve and fifteen months. All tests were considered significant at the 0.05 level.

The Shannon-Wiener diversity index was also used to measure diversity between the two groups during the total monitoring period. This index, has long been used by ecologists to compare richness between communities (Magurran, 2004).
The Shannon-Wiener index is given as:

$$\mathbf{H_s = -\sum (Pi)\ (ln\ Pi)}$$

Where:

$\mathbf{H_s}$ = Shannon-Wiener diversity in a sample

Pi = the proportion of individuals found in the i^{th} species, $=n_i/N$

N = total number of individuals

$\mathbf{n_i}$ = number of individuals of i^{th} species

ln = natural log

The product of this calculation is an index value between 1-4, with a higher number representing a more diverse community.

Chapter Three: Results

3.1 Environmental variables

3.1.1 Temperature

Temperatures in the tanks at the initial time of filling in September 2008 were around 15.5 °C, rising to a maximum of 25.3 °C in early January 2009, corresponding to the austral summer. The minimum temperature recorded was 4.6 °C in early June 2009. After June 2009, temperatures rose steadily to 22 °C at the end of the monitoring period in November 2009 (Figure 4).

Throughout the entire 15 month monitoring period, temperatures remained similar between treatment and control tanks. There were only minor divergences in average temperatures between groups from early September 2009 to mid September 2009, equating to 0.6 °C and 0.5 °C respectively. Differences between top and bottom readings were generally minor with the exception of mid-July 2009, where there was a 2.7 °C temperature difference in the top and bottom of treatment tanks, and during the summer months of November 2008 to January 2009, where average differences up to a maximum of 1.75 °C were attained, as warmer temperatures were observed in the surface waters of the tanks.

When the new zooplankton species were added during the first week of September 2009, the average temperatures in the treatment and control tanks were 16.3 °C and 15.7 °C respectively.

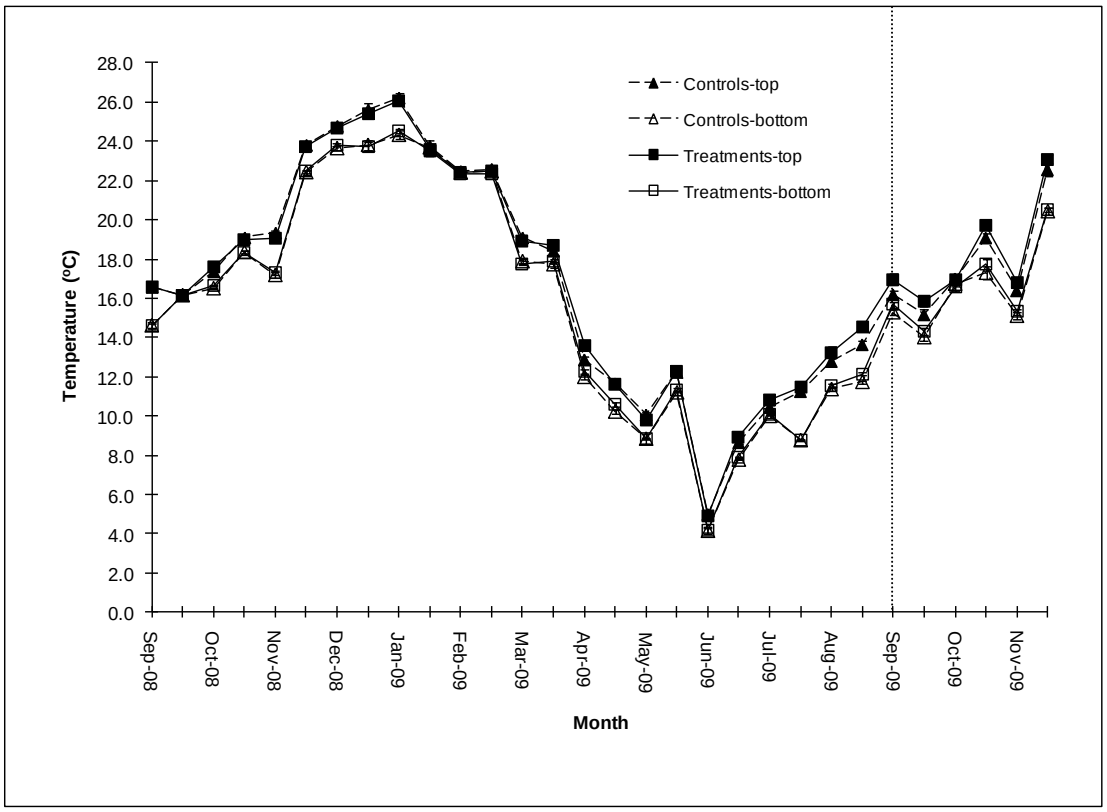

Figure 4: Average temperatures in treatment and control tanks. Dashed vertical line indicates time of species introduction. Error bars indicate +/- 1 standard error of the mean (SEM).

3.1.2 Dissolved Oxygen Concentration

Dissolved oxygen (DO) concentrations did not exhibit major differences between treatment and control group averages (Figure 5). The largest divergence between the two groups was recorded in early November 2008 when average control DO concentration was 1.5 mg L^{-1} higher than average treatment DO concentration. From the first reading in September 2008, DO concentration increased in October, before a subsequent sharp decline in the treatment tanks in early November. The control tanks then declined sharply two weeks later in mid November. From here average DO concentrations vary slightly until early May, with the exception of a noticeable increase in mid-January 2009 in the treatments. After May 2009, average DO concentrations increased before leveling out between August and September, before decreasing slightly in the final two months of monitoring. Average DO concentrations reached their maximum in October 2008, with 14.9 mg L^{-1} in the treatment tanks and 13.4 mg L^{-1} in control tanks. The minimum DO concentration

was recorded in December, two months after the highest reading. In both groups, the mean DO concentration declined to 6.7 mg L^{-1}. DO concentrations were more variable in the first six months of the experiment.

When the new zooplankton species were added to the tanks after 12 months, DO concentrations in each group were very similar. Treatment and control groups were 13.5 mg L^{-1} and 13.4 mg L^{-1} respectively.

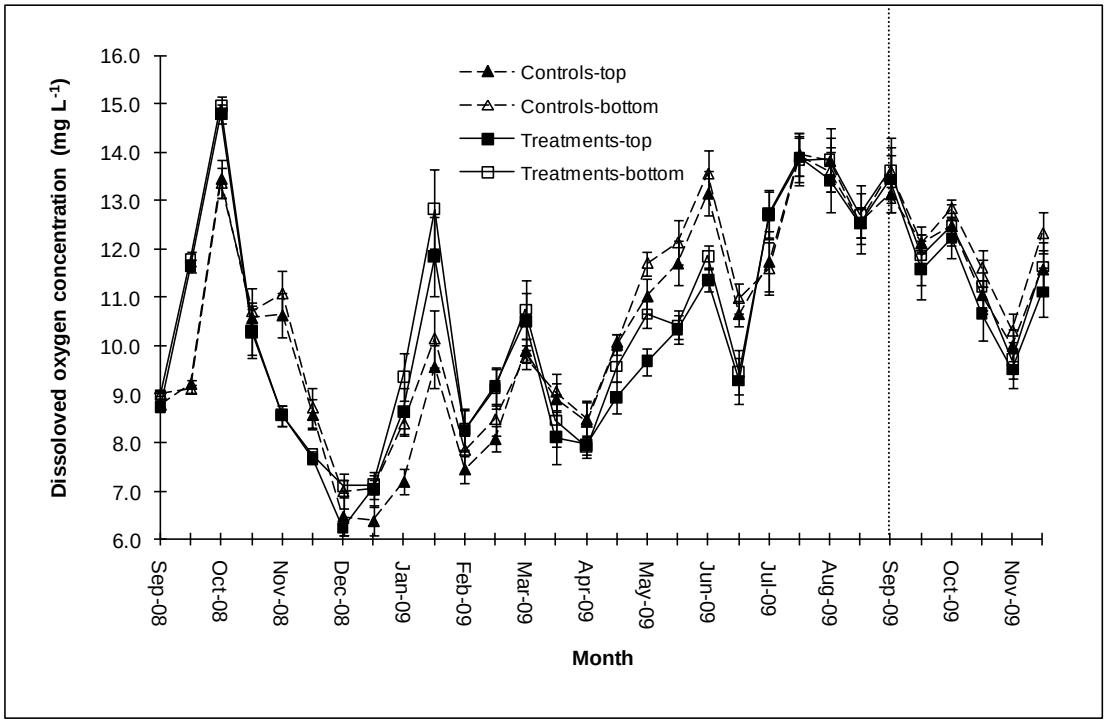

Figure 5: Dissolved oxygen concentration in treatment and control tanks. Dashed vertical line indicates time of species introduction. Error bars indicate +/- 1 SEM.

3.1.3 Dissolved Oxygen Saturation

DO saturation trends exhibited a similar pattern to that of DO concentration throughout the 15 month monitoring period (Figure 6). After reaching the maximum one month after monitoring began, average DO saturation readings declined steeply from October 2008 through to December 2008. In mid-January 2009, average treatment DO saturation rapidly increased to 141.6%, before dropping down to around 90.0% in February 2009. DO saturation remains relatively stable until mid-

June 2009, when it increases before attaining another relatively stable pattern after September 2009.

Average DO saturation was highest in October 2008 when treatment and control tanks were 154.2% and 139.1% respectively. The lowest overall average DO saturation reading was obtained in April 2009, when DO saturation in treatment and control tanks were 75.1% and 79.5% respectively. The greatest difference in DO saturation was recorded in November 2008, when the average treatment saturation was 90.8% and the average control tank saturation was 115.1%, a total difference of 24.3%. Differences in DO saturation were not considerable between the top and bottom of the tanks. The greatest difference was observed in early December 2008, where DO saturation had a difference of 9.5% between the top and bottom of the treatments, with higher DO saturation observed in the bottom section of the tanks.

At the time of addition of the new zooplankton species in September 2009, DO saturation levels were 137.0% and 135.2% in control and treatment tanks respectively, a total difference of only 1.8%.

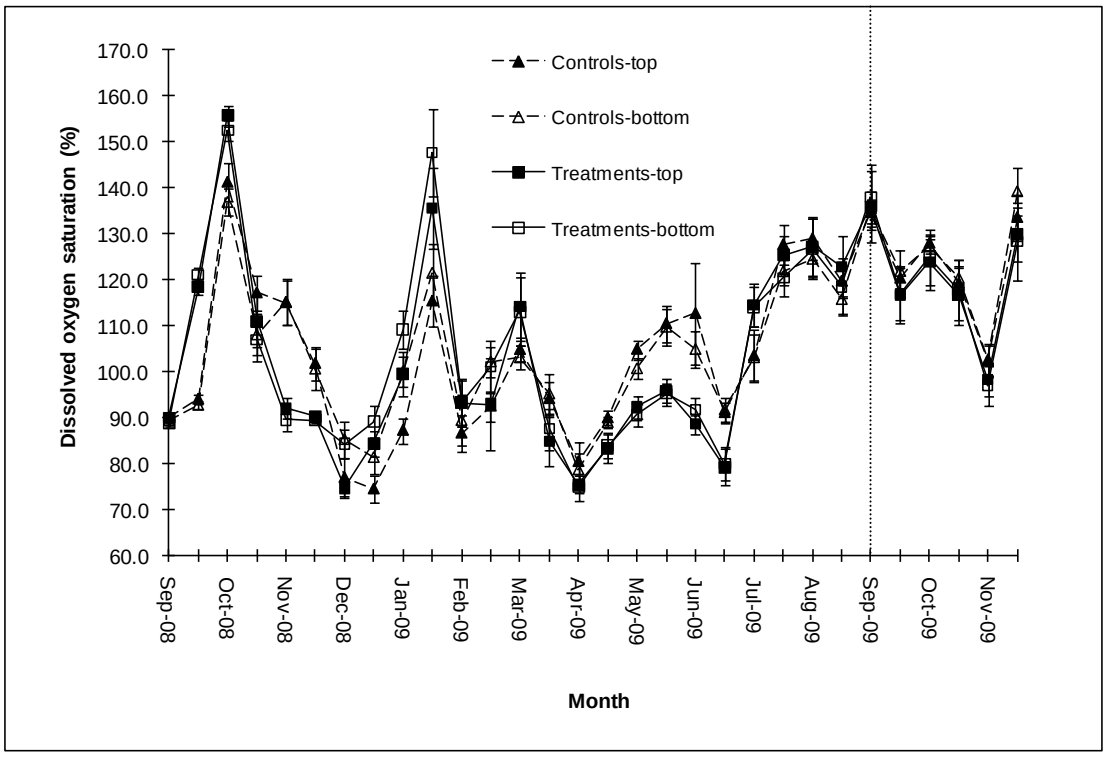

Figure 6: Average dissolved oxygen saturation in treatment and control tanks. Dashed vertical line indicates time of species introduction. Error bars indicate +/- 1 SEM.

3.1.4 pH

After some initial variability, pH levels were fairly consistent throughout the entire 15 month period (Figure 7). There were no significant differences in pH between treatment and control tanks throughout the monitoring period, with the greatest difference of 0.60 recorded in June 2009. From the first reading in September 2008 to October 2008, pH levels increased rapidly; from here there was another rapid fluctuation as levels decreased until December 2008. From December, average pH of control tanks remained relatively stable for the rest of the monitoring period, ranging between 8.09 and 8.62. The pH of treatment tanks wavered slightly, but remained between 7.56 and 8.86. In the summer months of 2009, the treatment pH was higher than control levels until March, where the levels remained lower than that of the controls for the remainder of the monitoring period.

Average pH reached the maximum level in October 2008 at 9.11 and 8.71 in treatment and control groups, respectively. Lowest levels were recorded two months later in December 2008, at 7.65 and 7.74 in treatment and control groups respectively.

At the time the new zooplankton species were added to the tanks in September 2009, pH stood at 8.20 in the treatment tanks and 8.39 in the control tanks.

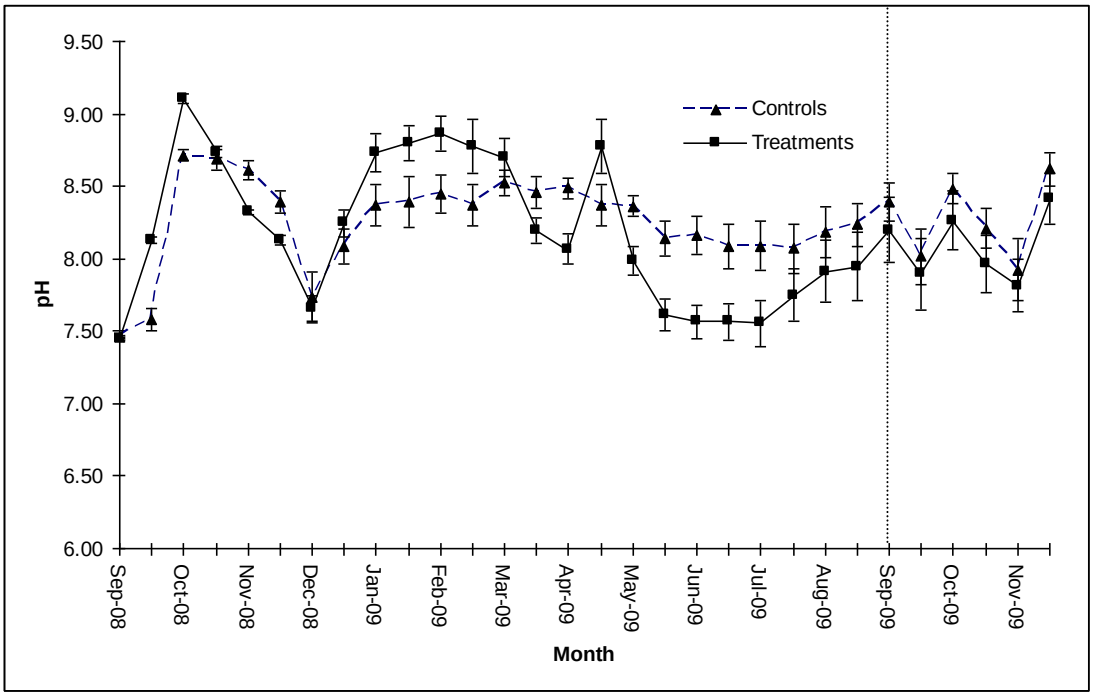

Figure 7: Average pH levels in treatment and control tanks. Dashed vertical line indicates time of species introduction. Error bars indicate +/- 1 SEM.

3.1.5 Conductivity

Conductivity (not corrected for temperature) did not differ greatly between treatment and control tanks during the 15 month monitoring period. In February and March 2009, the averages diverged slightly, with the greatest difference between the two groups during this time being 17.0 µS cm^{-1}. There were no major differences in conductivity between the top and bottom areas of the tanks (Figure 8).

When initial monitoring began, the mean conductivity was 351.9 µS cm^{-1} in the treatment tanks and 355.5 µS cm^{-1} in the control tanks. Conductivity dropped slightly

in October 2008, and then rose to its highest point in January 2009, where conductivity was 388.4 µS cm^{-1} and 394.3 µS cm^{-1} in treatment and control tanks respectively. From January, conductivity steadily declined until June 2009 to 204.6 µS cm^{-1} and 198.7 µS cm^{-1} in control and treatment tanks respectively. From June until the end of the monitoring period in November 2009, conductivity remained relatively stable. After June, conductivity stabilised and the majority of the readings fell between 180 µS cm^{-1} and 210 µS cm^{-1}.

When the new zooplankton species were added in September 2009, the difference in conductivity between treatment and control tanks was 6.1 µS cm^{-1}.

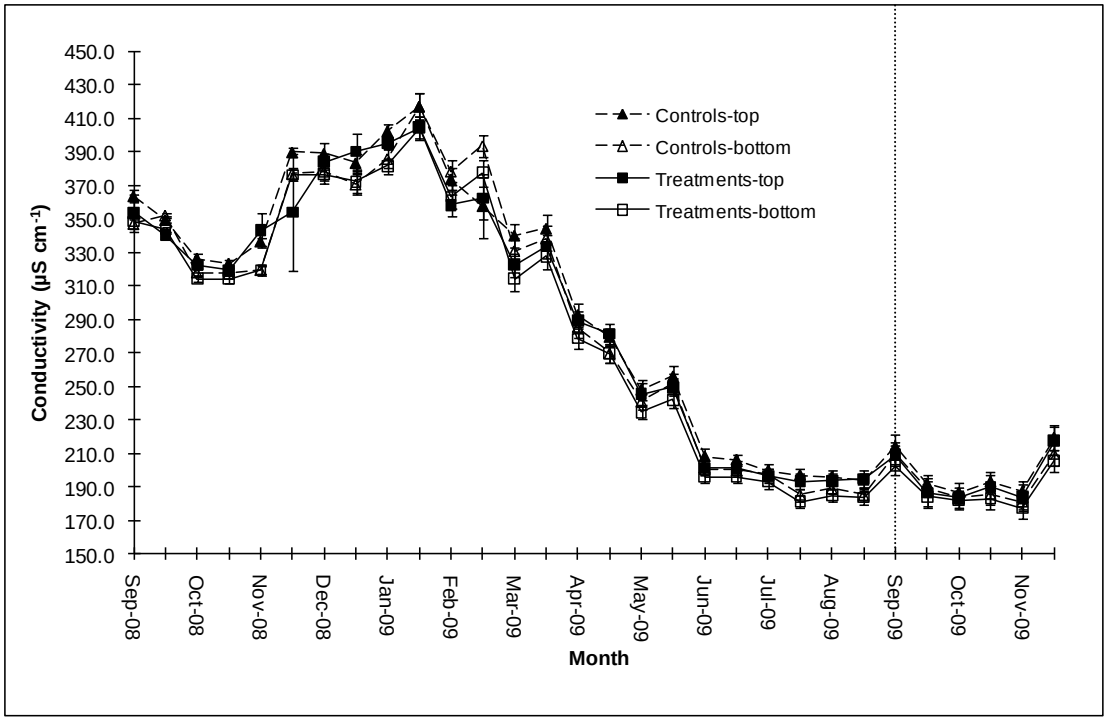

Figure 8: Average conductivity in treatment and control tanks. Dashed vertical line indicates time of species introduction. Error bars indicate +/- 1 SEM.

3.1.6 Specific Conductance

Throughout the monitoring period, specific conductance (at 25°C) remained similar between the treatment and control groups (Figure 9). The two groups diverged slightly from January until April 2009. The greatest difference between treatment and

control tanks was 16.5 µS cm^{-1}, in February 2009. After April, specific conductance between the two groups gradually became more similar. It is also from this time that specific conductance declined steadily until October 2009. The difference between average specific conductance in the top and bottom of the tanks was minor, with a maximum difference attained in early February, when the average specific conductance was 7.65 µS cm^{-1} higher in the bottom section of the tanks than the top section.

Average specific conductance attained a maximum of 434.9 µS cm^{-1} at the beginning of the monitoring period in September 2008. Conductance declined slightly before reaching its second highest peak of 405.1 µS cm^{-1} in mid-February 2009. The decline after this month fell to a minimum of 212.1 µS cm^{-1} in October 2009.

When the new zooplankton species were added in September 2009, specific conductance was at its most similar between treatment and control tanks, at 246.6 µS cm^{-1} and 246.2 µS cm^{-1} respectively.

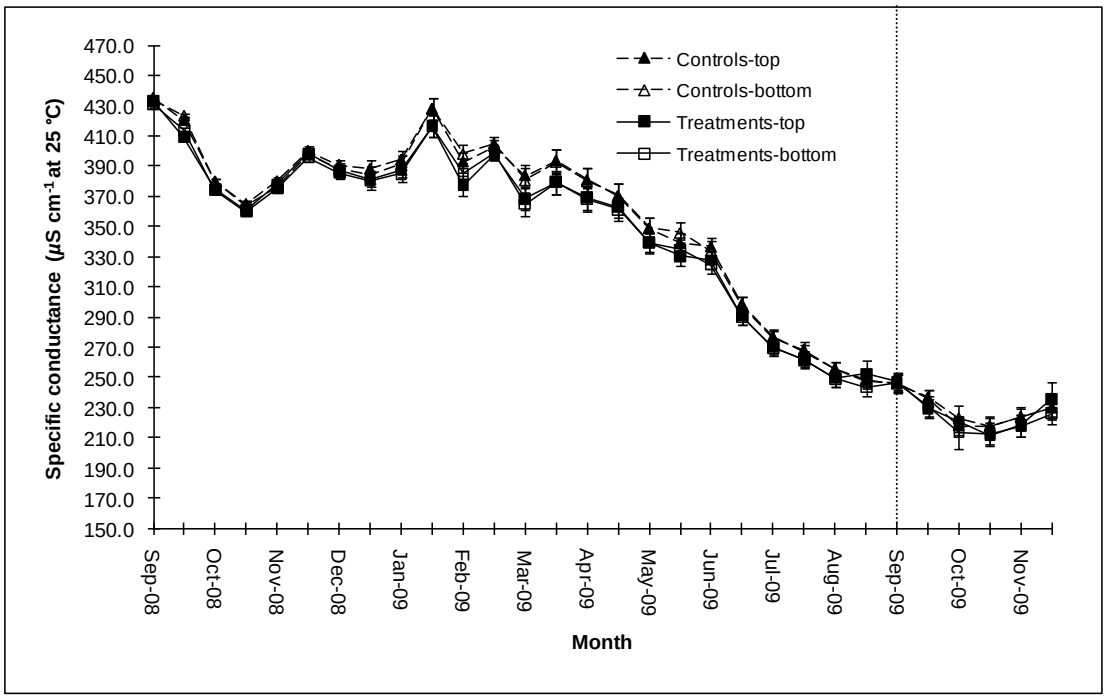

Figure 9: Specific conductivity in treatment and control tanks. Dashed vertical line indicates time of species introduction. Error bars indicate +/- 1 SEM.

3.1.7 Total Chlorophyll *a*

The chlorophyll *a* levels (and therefore algal concentrations) varied between treatment and control tanks throughout the 15 month monitoring period (Figure 10). Chlorophyll *a* in the control tanks remained fairly stable with a minimum reading of 4.15 mg L^{-1} in December 2008 and a maximum of 20.85 mg L^{-1} in November 2009. General trends show that chlorophyll *a* levels began relatively low during the first spring months and then increased steadily from December 2008 until August 2009. From August there was a slight decline before the maximum reading in November 2009.

Treatment tanks exhibit greater variation and chlorophyll *a* levels were generally higher than that of the controls. The lowest chlorophyll *a* recorded was 1.6 mg L^{-1} in early November 2008, with a maximum of 52.6 mg L^{-1} reached in early February 2009. The general pattern saw chlorophyll *a* at a relatively high initial level before a subsequent three month period of low levels. A rapid increase was observed between December 2008 to February 2009, followed by a sharp decline until May 2009. After May, chlorophyll *a* remains relatively stable, fluctuating only 10.6 mg L^{-1} during the last six months of monitoring. The largest variation between treatment and control groups was observed in February 2008, with a difference of 24.1 mg L^{-1}.

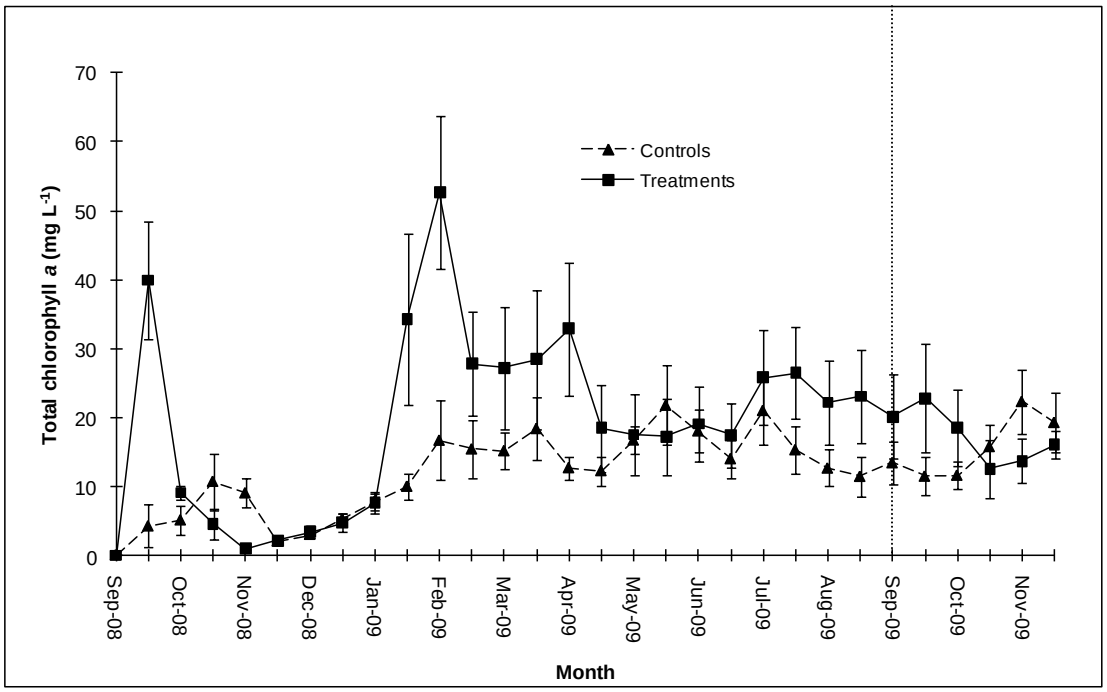

Figure 10: Average total chlorophyll *a* concentrations in treatment and control tanks. Dashed vertical line indicates time of species introduction. Error bars indicate +/- 1 SEM.

3.2 Environmental variables at addition of new species

Using STATISTICA (Statsoft Inc., Tulsa. USA), *t*-tests were performed that compared the average mean values for treatment and control groups at the time the new species were added. All results show that when the new species were introduced, there were no statistically significant differences between the control and treatment groups for all environmental variables (Table 3). Significance levels were set at 5% ($p < 0.05$). These results infer that at the time the new zooplankton species were introduced to tanks, they experienced similar abiotic conditions in the treatments and controls.

Table 3: Mean values of environmental variables at 12 months and P value indicating statistical significance ($p<0.05$).

Environmental Variable Monitored	Mean - Treatment	Mean - Control	P value
Temperature (°C) - top	13.24	12.80	0.092
Temperature (°C) - bottom	11.54	11.37	0.113
Conductivity (µS cm^{-1}) - top	193.67	194.07	0.946
Conductivity (µS cm^{-1}) - bottom	185.19	189.22	0.464
Specific Conductance (µS cm^{-1}) - top	249.33	255.44	0.378
Specific Conductance (µS cm^{-1}) - bottom	249.22	255.65	0.364
DO Concentration (mg L^{-1}) - top	12.52	12.55	0.972
DO Concentration (mg L^{-1}) - bottom	12.71	12.54	0.821
DO Saturation (%) - top	127.23	128.97	0.831
DO Saturation (%) - bottom	126.67	124.57	0.79
pH	8.19	8.38	0.457
Total Chlorophyll *a*	23.11	11.48	0.145

3.3 Zooplankton

3.3.1 Mean species richness

Species richness increased gradually over the entire 15 month monitoring period (Figure 11). Even in the initial sampling, species richness in the treatment tanks was higher than that of the controls, as was apparent for the whole monitoring period. In the control tanks, species richness did not begin to markedly increase until approximately five months after the tanks were set up. After five months, mean diversity increased to an average of 1.9 species present per tank at 8.5 months. This was followed by a slight decrease, and then a subsequent steady increase to the 12 month point. At 12 months, before the addition of the new zooplankton species, mean diversity in the control tanks stood at 2.6 species. This is the highest species richness reached in the controls before the addition of the new zooplankton species. Species richness in the treatment tanks began increasing steadily after two weeks. This general trend continued for the remaining 15 month monitoring period with the exception of a slight decrease in richness five to seven months after monitoring was initiated. Within one month, mean diversity in the treatment tanks had doubled that of

the control tanks diversity. By month three, the mean treatment species richness was 2.9. This average species richness was not reached in the control tanks until 12 months after initial tank set up. Before new zooplankton additions, mean richness reached 5.0 and 4.9 during months ten and eleven respectively. The last monitoring before the addition of the new zooplankton species saw mean species richness at 4.6 in the treatment tanks.

After the introduction of new species at 12 months, the diversity in the control tanks increased rapidly. Within 1.5 months of introductions, mean diversity in controls rose from 2.6 to 4.6, as the new species established. After the initial increase at 12 months, diversity fluctuated between 3.6 and 4.7 for the remaining months. The acquisition of the new zooplankton species in control tanks between 12.5 and 13.5 months depicted the most rapid increase in mean species diversity within a one month time bracket during the total 15 month period.

After the introduction of new species at 12 months, little change was observed in the overall species richness of the treatment tanks. On average, a total of just 0.6 species were acquired in the subsequent three months after the new species were added. This minor increase in species richness was small when compared to the initial 12 month trend of the treatment tanks.

Overall, during the first 12 months, species richness diverged rapidly between the treatment and control tanks. The treatment tanks exhibited greater species richness almost immediately, whilst the control tanks took one year to acquire the diversity reached within just three months in the treatment tanks. In the three months after new zooplankton species were introduced, mean diversity increased considerably in the control tanks, whilst only a minor increase was observed in the treatment tanks.

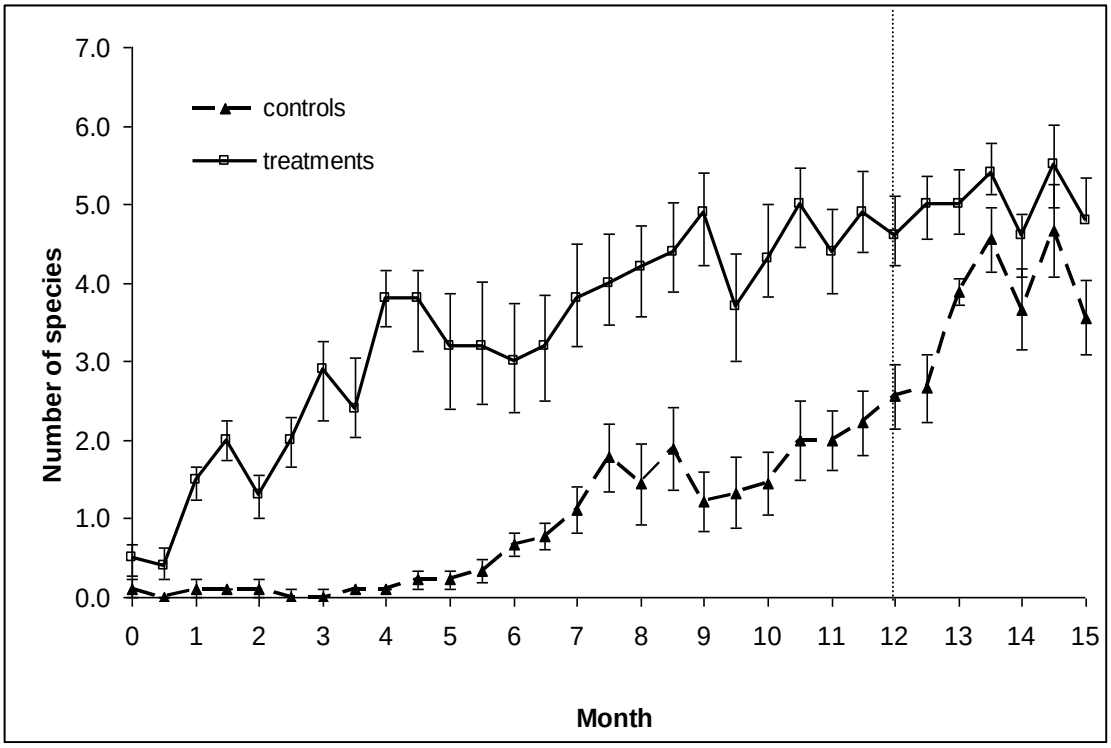

Figure 11: Standing mean species richness in treatment and control tanks. Dashed vertical line indicates time of species introduction. Error bars indicate +/- 1 SEM.

3.3.2 Accumulated species richness

The most notable features of the accumulated mean data is the rapid divergence between species richness in treatment and control tanks and the fast increase in control species richness after 12 months. When the accumulated mean is compared between the two groups after 12 months, treatment tanks only acquired 0.7 new species, whilst the control tanks acquired 3.1 species (Figure 12).

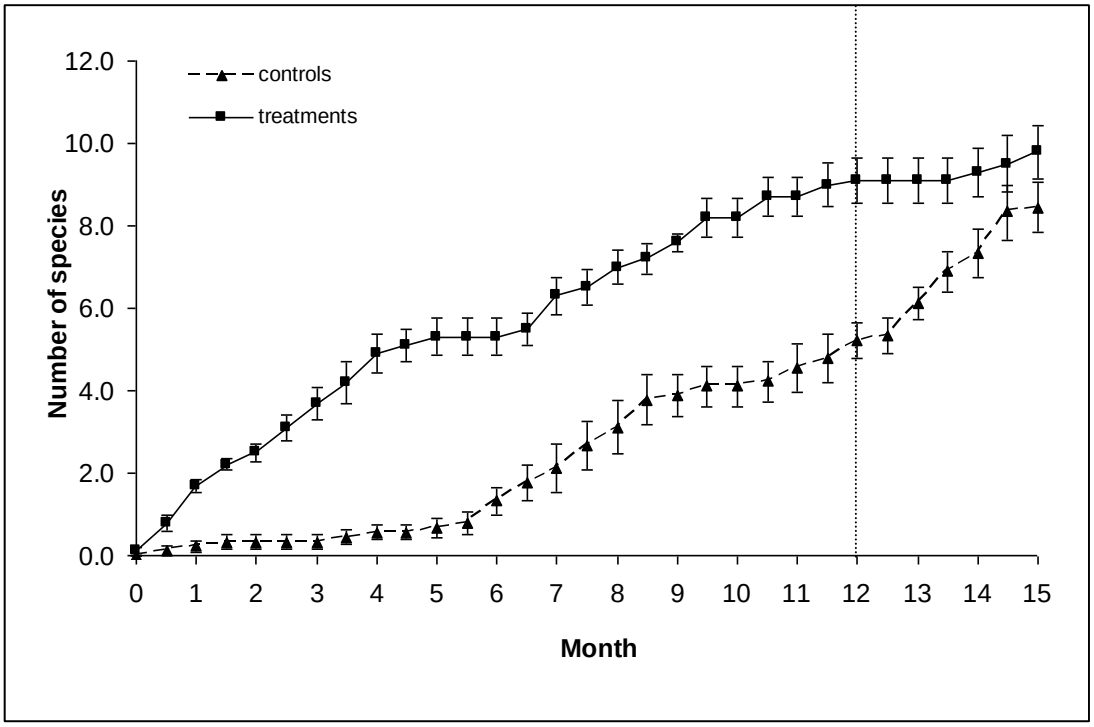

Figure 12: Accumulated mean species richness in treatment and control tanks. Dashed vertical line indicates time of species introduction. Error bars indicate +/- 1 SEM.

T-tests performed in STATISTICA display the difference in mean diversity between the treatment and control tanks at twelve and fifteen months (Table 4). At twelve months, before the addition of new species, the standing mean richness in treatment and control tanks was significantly different ($p=0.0017$), as was accumulated mean diversity ($p=0.0001$). Three months after the addition of new species, both the standing and accumulated mean richness between treatment and control tanks converged and were no longer significantly different ($p > 0.05$). This is due to the rapid establishment of new zooplankton in the previously unseeded control tanks and the relatively low establishment rate of new species in the treatment tanks, where species richness had remained higher during the previous twelve month period.

Table 4: T-test results comparing species richness between treatment and control tanks at 12 and 15 months

	At 12 months	At 15 months
Standing Mean	p = 0.0017*	p = 0.124
Accumulated Mean	p = 0.0001*	p = 0.146

* indicates a significant difference ($p < 0.05$)

3.3.3 Shannon-Wiener Diversity

The Shannon-Wiener diversity index was also used to measure diversity between the two groups during the total monitoring period. The product of this calculation is an index value between 1 and 4, with a higher number representing a more diverse community.

This index illustrated the relative diversity exhibited in the treatment and control. In the control tanks, diversity remains relatively low compared to treatment diversity, until the introduction of new species in month 12 (Figure 13). After month 12 diversity rose rapidly, reaching a peak of 0.89 in month 14. After 12.5 months, all index values for control diversity remained above 0.65.

Treatment diversity increased at a much faster rate and remained higher than that of the controls until month 13. The maximum diversity value for treatments was reached in month 10, at a value of 1.04. At month 13, Shannon-Wiener values were very similar between the two groups (both at around 0.7). Treatment diversity increased during months 13-14.5, but not as considerably as that exhibited by the control tanks from months 12.5-14. This index indicates that the species introduced at month 12 had much more of an effect on species richness in the unseeded control tanks than they did in the seeded treatment tanks. This index exhibited similar patterns when compared to the standing and accumulated mean diversity shown in the previous two graphs. Collectively, they exhibit rapid divergence in diversity between treatments and controls, followed by a high acquisition of new species after twelve months in the control tanks. T-tests on the Shannon-Wiener diversity values showed that there were no significant differences in diversity between treatment and control tanks at 12 and

15 months. The application of this diversity index will be discussed in the subsequent chapter.

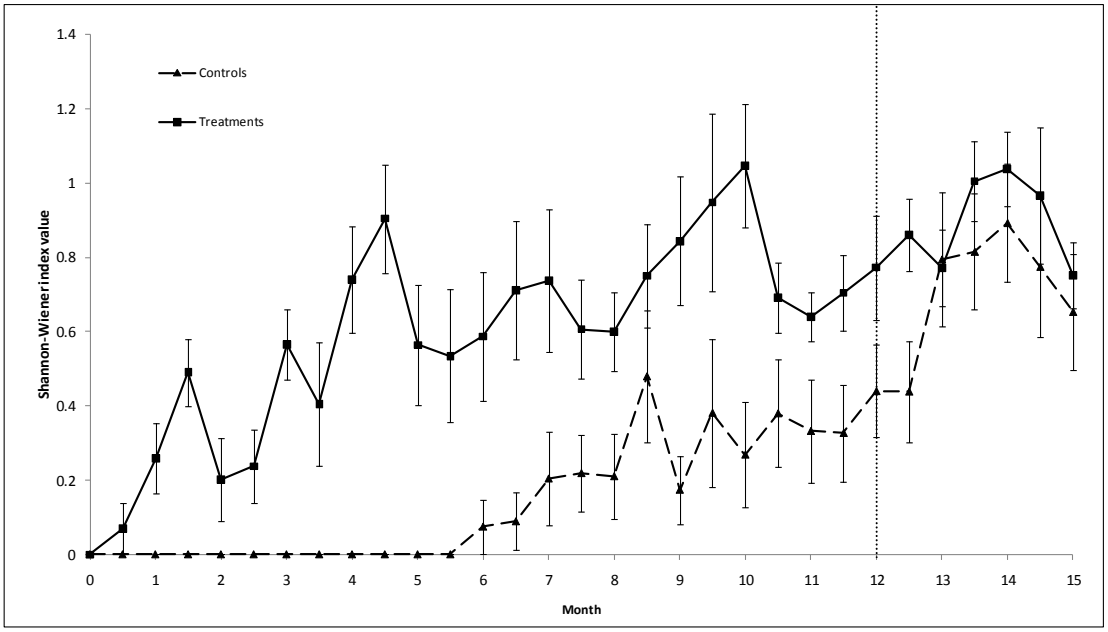

Figure 13: Shannon-Wiener diversity in treatment and control tanks. Dashed vertical line indicates time of new species introductions. Error bars indicate +/- 1 SEM.

3.3.4 Species presence tables

The number of individual species and the community composition at 12 months in the treatment tanks was much different to that of the control tanks, prior to the introduction of new species (Table 5). Treatments contained greater species richness than controls. At the time the new zooplankton species were added, eleven species (*Acanthocyclops robustus*, *Lepadella ovalis*, *Squatinella mutica*, *Boeckella delicata*, *Calamoecia lucasi*, *Eucypris virens*, *Filinia longiseta*, bdelliods, *Cephalodella catellina*, *Moina tenuicornis* and *Ceriodaphnia dubia*), were already present within the treatment tanks. Five of these species were truly planktonic copepods or cladocerans, another five were rotifers, and one was an ostracod. Seven species (*A. robustus*, *L. ovalis*, *Lecane flexilis*, *S. mutica*, bdelloids, *C. catellina* and *C. dubia*) were present within the unseeded control tanks. All of these species, except *A. robustus* and *C. dubia*, were rotifers.

At 15 months, species composition in treatment and control tanks differed greatly from those present at 12 months. Treatments still attained more different species overall, but the control tanks acquired more of the new species that were introduced at 12 months (Table 6). Control tanks attained five new species: *Skistodiaptomus pallidus*, *Synchaeta oblonga*, *Mesocyclops leuckarti*, *Chydorus sphaericus* and *Synchaeta pectinata*, while treatment tanks attained only two new species *S. pallidus* and *C. sphaericus*. These two species were only present in four out of the nine treatment tanks that new species were released into. Within these four tanks, only one single species established per tank. The new species in the control tanks were present, at a minimum, in two of the tanks, whilst two of the new species (*S. pallidus* and *M. leuckarti*) were present in seven out of eight control tanks. Out of the control tanks that received new species, half of them gained a minimum of three species, whilst 38% gained four new species.

Table 5: Species present after 12 months in treatment and control tanks.

† indicates tanks that did not receive new species at 12 months

	Controls									Treatments									
Zooplankton species:	**1**	**3**	**6**	**7**	**11**	**14**	**15**	**18**	**20†**	**2**	**4**	**5**	**8**	**10**	**12**	**13**	**16†**	**17**	**19**
Acanthocyclops robustus					*									*			*	*	
Lepadella ovalis	*	*	*	*	*	*	*	*	*	*	*				*	*		*	
Trichocerca pusilla																			
Lecane flexilis	*																		
Squatinella mutica	*					*									*	*			
Boeckella delicata										*	*	*	*	*			*		*
Calamoecia lucasi										*	*		*				*		*
Eucypris virens										*	*		*	*					*
Filinia longiseta														*					*
Bdelliods		*		*		*							*	*					
Cephalodella catellina		*					*								*	*			
Moina tenuicornis													*						
Ceriodaphnia dubia			*	*			*	*									*	*	*
Pleuroxus hastirostris																			
Bosmina meridionalis																			
Daphnia carinata																			
Species added at 12 months:																			
Skistodiaptomus pallidus																			
Synchaeta oblonga																			
Mesocyclops leuckarti																			
Chydorus sphaericus																			
Synchaeta pectinata																			
Trichocerca similis																			
Ascomorpha ovalis																			

Table 6: Species present after 15 months in treatment and control tanks.

† indicates tanks that did not receive new species at 12 months

	Controls									Treatment									
Zooplankton Species:	**1**	**3**	**6**	**7**	**11**	**14**	**15**	**18**	**20†**	**2**	**4**	**5**	**8**	**10**	**12**	**13**	**16†**	**17**	**19**
Acanthocyclops robustus												*						*	
Lepadella ovalis	*			*				*	*							*			*
Trichocerca pusilla																			
Lecane flexilis																			
Squatinella mutica																			
Boeckella delicata										*	*		*	*			*	*	*
Calamoecia lucasi										*	*			*			*	*	*
Eucypris virens											*			*	*				*
Filinia longiseta																		*	*
Bdelliods				*					*							*	*		
Cephalodella catellina																			
Moina tenuicornis													*						
Ceriodaphnia dubia			*	*			*	*					*			*	*	*	*
Pleuroxus hastirostris																	*		
Bosmina meridionalis																*			
Daphnia carinata																		*	
Species added after 12 months:																			
Skistodiaptomus pallidus	*	*		*	*	*	*	*				*			*				
Synchaeta oblonga		*		*				*											
Mesocyclops leuckarti		*	*	*	*	*	*	*											
Chydorus sphaericus		*	*			*					*		*						
Synchaeta pectinata				*				*											
Trichocerca similis																			
Ascomorpha ovalis																			

3.3.5 Accumulation of species

In the treatments tanks the copepod *Acanthocyclops robustus* established after one month, with *Lepadella ovalis*, a rotifer, present after 1.5 months (Table 7). After 2.5 months, three cladocerans *Moina tenuicornis*, *Ceriodaphnia dubia* and *Pleuroxus hastirostris* were also present, along with the copepod *Boeckella delicata* and ostracod *Eucypris virens*. The majority of the rotifer species: *Trichocerca pusilla*, *Squatinella mutica*, bdelloids and *Cephalodella catellina*, did not establish until at least five months after the experiment was initiated. Out of the new species introduced at 12 months *Skistodiaptomus pallidus* and *Chydorus sphaericus* (the only two species to establish from the introductions) did not establish until after at least 14.5 months.

The unseeded control tanks took substantially longer to naturally accumulate species over the initial twelve month monitoring period (Table 8). Out of the eleven species established during the first 12 months, seven of these (*Lepadella ovalis*, *Trichocerca pusilla*, *Lecane flexilis*, *Squatinella mutica*, *Filinia longeseta*, Bdelloids and *Cephalodella catellina*) were small rotifers. The other four species, two cladocerans (*C. dubia* and *P. hastirostris*), one cyclopoid (*A. robustus*), and one ostracod (*E. virens*), were also found in the treatment tanks at an earlier stage. These four species were also located in nearby fish ponds. Following the introduction of the new species at twelve months, it only took one month before *S. pallidus*, *S. oblonga* and *S. pectinata* had established. This increased up to five species in the final two weeks of monitoring as *M. leuckarti* and *C. sphaericus* established in the unseeded tanks

Table 7: Species establishment throughout the 15 month monitoring period in the treatment tanks.

	Month																														
Species	0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7	7.5	8	8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15
Acanthocyclops robustus			*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		*	*	*	*
Lepadella ovalis				*												*	*			*	*	*	*	*	*	*	*	*	*	*	*
Trichocerca pusilla														*	*	*	*	*	*	*	*			*				*	*	*	
Lecane flexilis																															
Squatinella mutica																			*	*	*		*	*	*	*	*	*	*	*	
Boeckella delicata						*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Calamoecia lucasi								*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Eucypris virens						*	*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Filinia longiseta							*	*	*	*		*			*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*
Bdelliods											*	*			*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*
Cephalodella catellina																				*	*	*	*	*	*	*	*	*	*	*	
Moina tenuicornis					*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Ceriodaphnia dubia					*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Pleuroxus hastirostris						*	*		*	*	*	*	*	*	*					*						*				*	*
Bosmina meridionalis																								*							*
Daphnia carinata																														*	*
Species added at 12 months:																															
Skistodiaptomus pallidus																															*
Synchaeta oblonga																															
Mesocyclops leuckarti																															
Chydorus sphaericus																														*	*
Synchaeta pectinata																															
Trichocerca similis																															
Ascomorpha ovalis																															

Table 8: Species establishment throughout the 15 month monitoring period in the control tanks.

	Month																														
Species	0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7	7.5	8	8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15
Acanthocyclops robustus			*	*																											
Lepadella ovalis												*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		*		*
Trichocerca pusilla													*	*		*	*		*	*	*				*		*				
Lecane flexilis																			*	*	*	*	*	*	*	*	*		*	*	
Squatinella mutica											*					*							*	*	*	*	*		*		
Boeckella delicata																															
Calamoecia lucasi																															
Eucypris virens							*																								
Filinia longiseta																						*									
Bdelliods												*	*	*	*	*	*	*	*	*	*		*	*	*	*	*		*	*	*
Cephalodella catellina																					*		*	*	*	*					
Moina tenuicornis																															
Ceriodaphnia dubia								*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		*	*	*
Pleuroxus hastirostris															*	*	*	*	*	*	*	*	*		*		*			*	
Bosmina meridionalis																															
Daphnia carinata																															
Species added at 12 months:																															
Skistodiaptomus pallidus																										*	*		*	*	*
Synchaeta oblonga																										*	*		*	*	*
Mesocyclops leuckarti																													*	*	*
Chydorus sphaericus																														*	*
Synchaeta pectinata																										*	*		*	*	*
Trichocerca similis																															
Ascomorpha ovalis																															

3.3.6 Community composition from 0-12 months

The multidimentional scaling (MDS) plots show that differences in community composition for the initial 12 monitoring period can be seen between the treatment (closed triangles) and control (open triangles) groups (Figure 14). Markers that are spaced close together indicate that species within the communities are very similar in composition. This plot illustrates that community composition in the treatment and control communities were generally different. The stress value of 0.16 denotes that the MDS model represented the sample relationships well (a perfect fit would be zero).

The most noticeable feature is the separate clustering of the treatment and control groups. In general, treatments are clustered together on the left of the ordination, with controls to the right. The exceptions to this are the treatment samples located on the right of the plot with the controls. These represent tanks 12 and 13, treatments that exhibited exceptionally low species richness during the sample period and did not contain any of the calanoid species that all of the other treatment tanks had acquired.

The general tendency for the two groups to occupy different sections of the plot illustrates the unseeded control tanks had different communities compared to the seeded treatment tanks, whose diversity built up considerably faster. It is clear that, in general, communities in treatment and control tanks were considerably different throughout initial 12 month monitoring period.

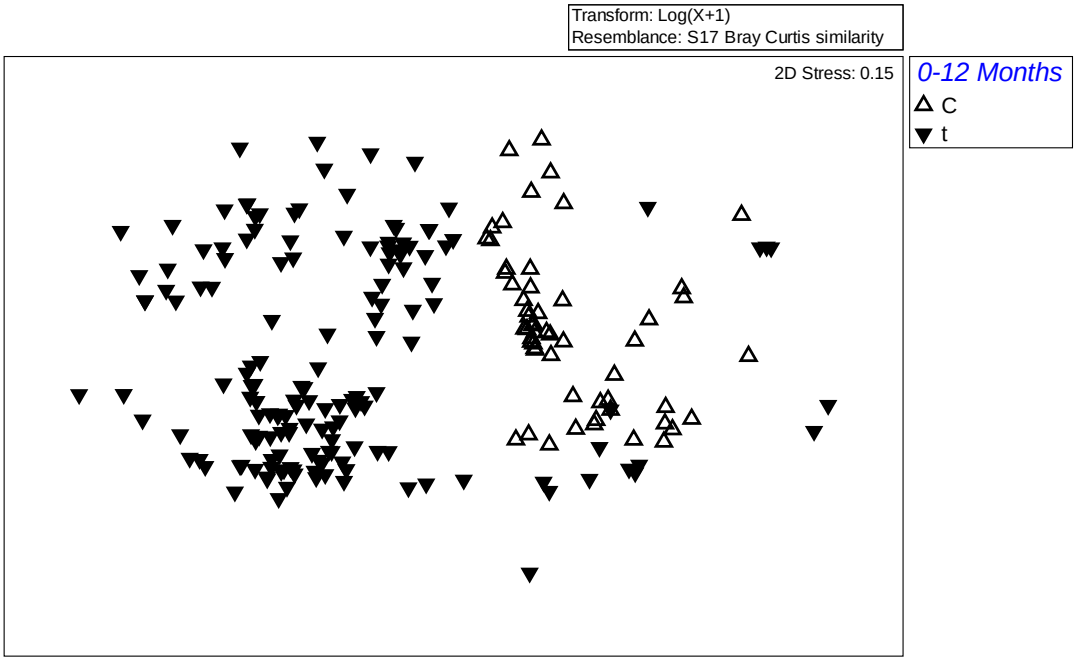

Figure 14: MDS comparing community composition between treatment and control tanks from 0-12 months.

3.3.7 Community composition at 12 months

An MDS plot of community composition at 12 months illustrates the differences in community composition between treatment and control tanks, just before the addition of the new species (Figure 15). The plot shows that, at this time, composition between the treatment and control tanks was generally very different from one another. The large space in the plot separating the two groups is indicative of the differing community compositions. After 12 months, the seeded treatment tanks were near their peak of diversity, whilst the controls were significantly lower. The two exceptions in this plot are the treatments located near the control points on the lower right side of the plot. These two samples represent tanks 12 and 13 at 12 months, whose diversity was relatively low (see Table 5). These tanks were not only low in species richness, but they also exhibited similar species composition to many of the control tanks. They did not contain any of the calanoids (*Acanthocyclops robustus*,*Calamoecia lucasi* and *Boeckella delicata*), but the rotifers (*Lepadella ovalis*, *Squatinella mutica* and *Cephalodella catellina*) were present. These rotifers were present in some of the controls at 12 months, but in no other treatments.

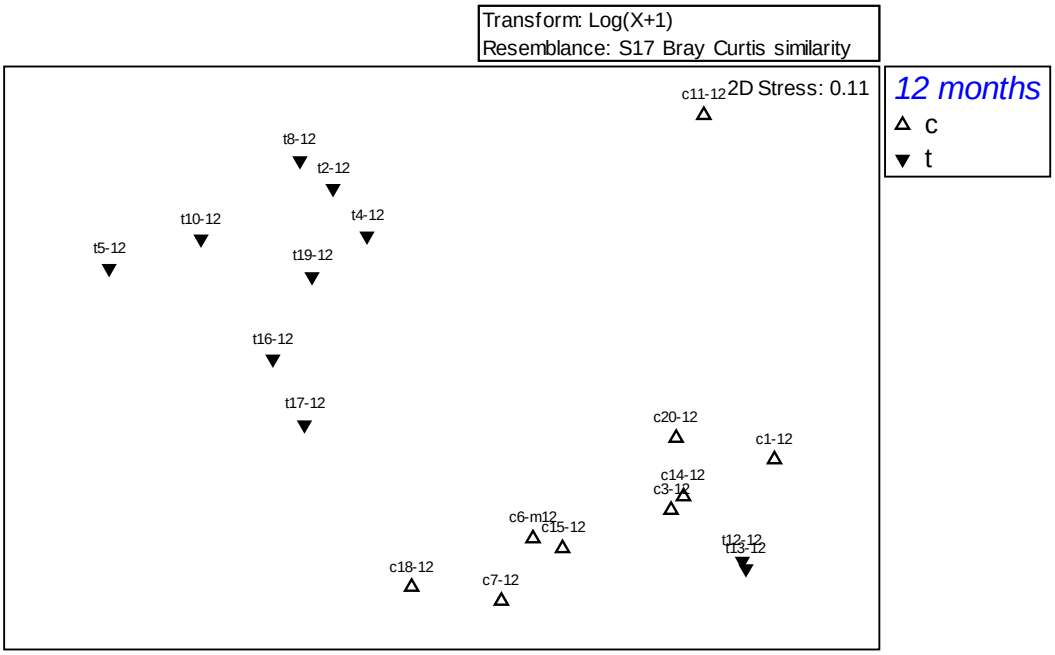

Figure 15: MDS comparing community composition between treatment and control tanks at 12 months. Data labels display tank number (e.g. c18) and month (12) .

3.3.8 Community composition at 15 months

An MDS plot of community composition at 15 months illustrates the differences in community composition between treatment and control tanks (Figure 16). In general, the treatment and control groups are still spaced apart to form two clusters, on the left and right hand sides of the plot, but when compared to the previous MDS plot, minor changes can be observed. The two treatment samples that are observed on the right side of the plot represent tanks 5 and 12, the only two treatment tanks that *S. pallidus* established in throughout the final three month period (see Table 6).

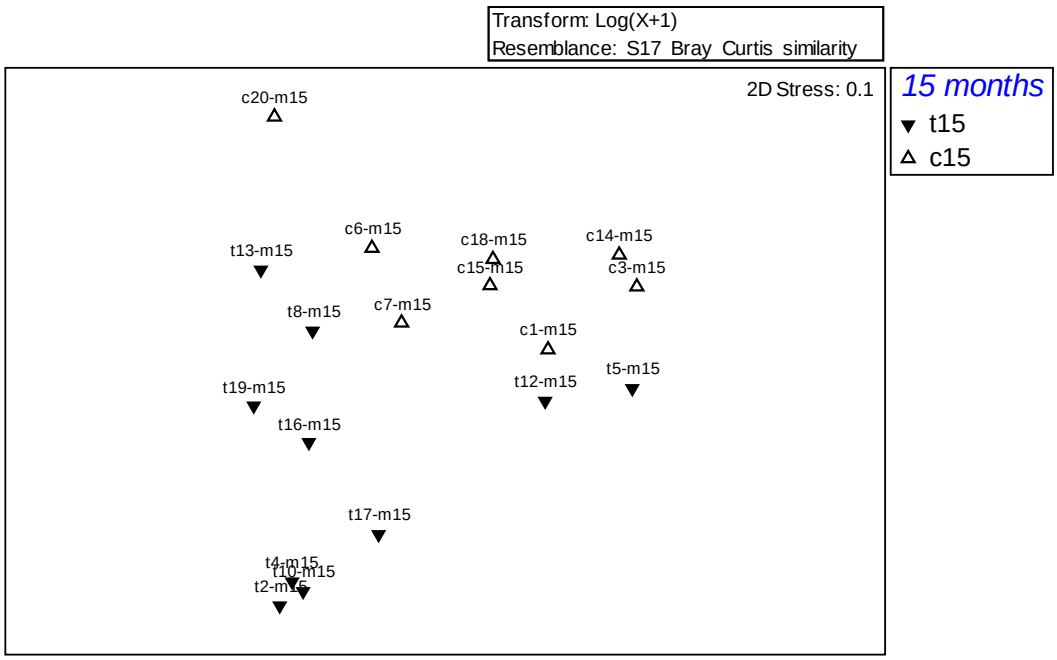

Figure 16: MDS comparing community composition between treatment and control tanks at 15 months. Data labels display tank number (e.g. c18) and month (15) .

3.3.9 Relative invasion success

The relative establishment success of each new species in treatment and control tanks shows successful establishment was much greater in the control tanks (Figure 17). Of the five species that established in the control tanks, the copepods *Skistodiaptomus pallidus* and *Mesocyclops leuckarti* established populations in 88% of the control tanks that received new species at 12 months. *S. pallidus* only established in 22% of the treatment tanks that received new species at 12 months, while *M. leuckarti* did not establish in any treatment tanks. The rotifers *Synchaeta pectinata* and *Synchaeta oblonga* established in 25% and 38% of control tanks respectively, while neither established in any of the treatment tanks. *Chydorus sphaercus*, a cladoceran, was the only other new species present in the treatments. It established in 22% of the treatments and 38% of the controls. The rotifers *Ascomorpha ovalis* and *Trichocerca similis* did not establish in any of the tanks during the three month period.

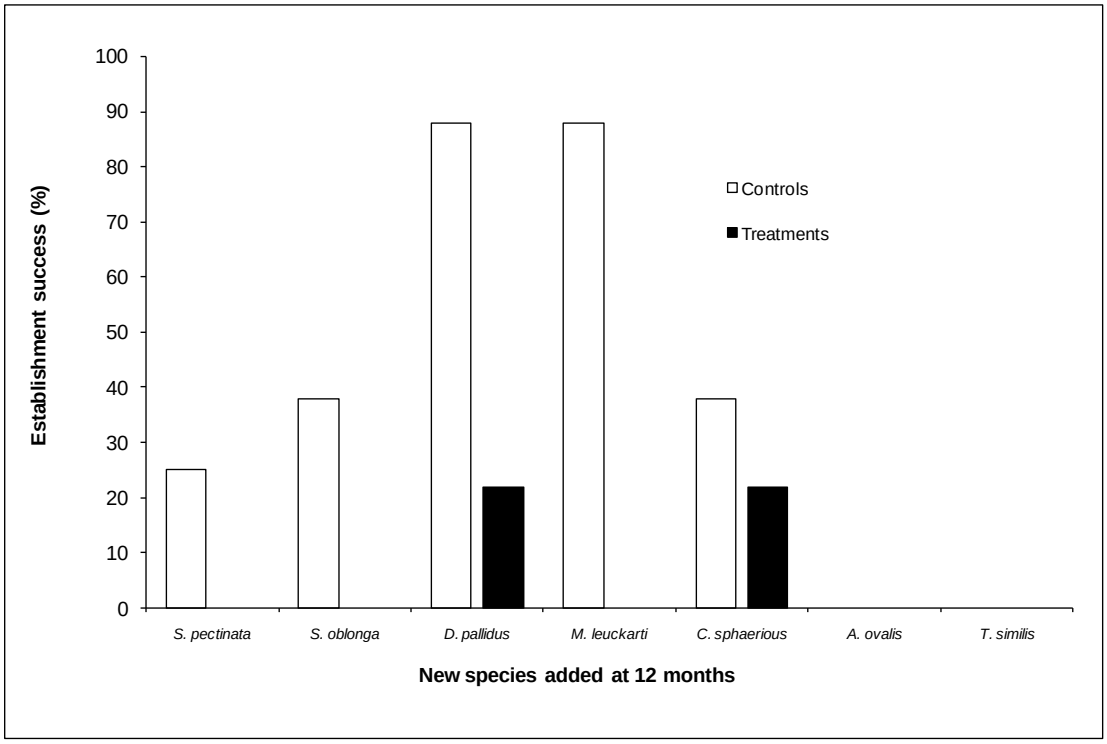

Figure 17: Relative establishment success of new species in treatment and control tanks

3.3.10 Effect of new species on community composition

Both treatment and control communities changed between the initial introduction of the new species after 12 months and the end of the 15 month monitoring period (Figure 18). But the control tanks were most affected by the introduced species during this time (represented by dashed arrows). The change in community composition between pre- and post-introduction samples in the control tanks is represented by a generally unvarying pattern, from right to left in the top half of the plot. The difference in community composition between 12 and 15 months is generally much greater than that of the treatments. Tank 20, which intentionally did not receive any new species, exhibited the least amount of change between 12 and 15 months, as shown in the right side of the plot at the top.

Changes to treatment community composition were generally less pronounced and exhibited a less obvious pattern. Distances between the 12 and 15 month samples were smaller than for the control samples at these times. The exception to this comes from tanks 12 and 13. At 12 months, their markers are located on the upper right side of the plot, at a close distance to many control markers of this time. This can be attributed to their low species richness in the initial 12 month

period and the fact they were both void of the two calanoid species throughout the twelve month colonization period, rendering them different from other treatment communities at twelve months. They were also the only treatment tanks to be invaded by *S. pallidus*. Overall, the data in this plot is consistent with previous data in this section that proves considerably more new species established in the unseeded control tanks than the seeded treatment tanks.

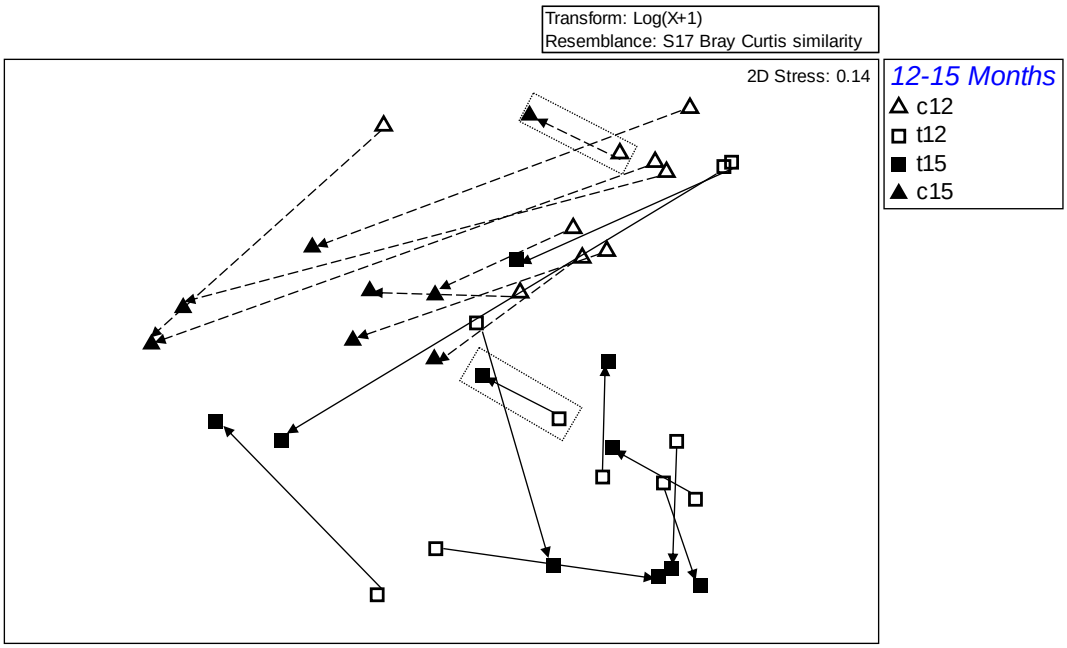

Figure 18: MDS comparing community composition in treatments and controls at 12 and 15 months**.** Arrows indicate change in composition between 12 and 15 months. Dashed boxes represent tanks void of species introductions at 12 months (treatment tank 16 and control tank 20).

3.4 ANOSIM

Results from an ANOSIM performed on samples from zero to 12 months, 12 to 15 months and at 12 and 15 months (independently), showed that community composition differed significantly between treatment and control groups at all of these four stages of community development ($P < 0.01$) (Table 10). *R*-statistic values (attained from the Bray-Curtis dissimilarity matrix used in the previous MDS plots) closer to zero imply two groups are very similar in composition, whilst *R* values approaching one imply that the groups are less similar. From zero to 12 months, an *R* value of 0.523 was attained, but from 12 to 15 months, this value declined to 0.499, exhibiting that the differences in community composition (although still statistically significant) became less pronounced during this time. At 12 months, the *R* value was 0.503, which indicates the communities were more

dissimilar between the treatment and control tanks than they were at 15 months, when the *R* value dropped to 0.332. The lower *R* value obtained at fifteen months indicates the effect on community composition that occurred as a result of the introduced species establishing most of the control tanks.

Table 9: ANOSIM of community composition between treatment and control tanks.

Time period	*R* value	Level of significance
0-12 Months	0.523	p = 0.001*
12-15 Months	0.499	p = 0.001*
12 Months	0.503	p = 0.002*
15 Months	0.332	p = 0.008*

* indicates a significant difference ($p < 0.05$)

3.5 Similarity percentage analysis (SIMPER)

A SIMPER analysis was run on all samples ranging from zero to 12 months to show the contribution of each species to the Bray-Curtis dissimilarity between treatment and control groups. The average Bray-Curtis dissimilarity between control and treatment groups was 89.38%, illustrating how distinct the two groups were. When this dissimilarity is broken down to the species level, the rotifer *Lepadella ovalis* was responsible for 19.29% of the total 89.38% of dissimilarity between the groups.

The cladocercan *C. dubia* was the second most discriminating species explaining 16.51% of the total 89.38% dissimilarity between the groups. The three copepods that were present in the tanks during the first 12 months: *B. delicata*, *C. lucasi* and *A. robustus*, were the next most discriminating species, responsible for 13.13%, 8.86%, and 8.62% of the total dissimilarity respectively.

Because species richness is higher in the treatment tanks throughout the monitoring period, the relatively high occurrence of *L. ovalis* and *C. dubia* in the control tanks renders them the most discriminating species between the two groups. *Lepadella ovalis* has an average abundance of 2.55 ind L^{-1} in control tanks and just 0.30 ind L^{-1} in the treatment tanks, whilst *C. dubia* has an average abundance of 2.07 ind L^{-1} and 0.84 ind L^{-1} in control and treatment tanks respectively. The three copepods, *B. delicata*, *C. lucasi* and *A. robustus*,

contribute less to the total dissimilarity between the two groups than *L. ovalis* and *C. dubia*, but have a much higher average abundance the treatment tanks than in the control tanks.

Table 10: SIMPER analysis of dissimilarity between treatment and control tanks

	Controls	Treatments		
Species	Av.Abundance (ind L^{-1})	Av.Abundance (ind L^{-1})	Av.Dissimilarity (%)	Contribution to Dissimilarity (%)
L. ovalis	2.55	0.30	17.24	19.29
C. dubia	2.07	0.84	14.76	16.51
B. delicata	0.04	1.66	11.74	13.13
C. lucasi	0.00	1.17	8.01	8.96
A. robustus	0.02	0.93	7.70	8.62

The results obtained from this study support the hypothesis that seeding habitats with sediments, containing resting eggs of zooplankton species from native pond communities, during early stages of development will increase biotic resistance and reduce establishment rates of non-indigenous zooplankton. In seeded treatment tanks, where species diversity was relatively high, community composition developed along a different trajectory than that of the control tanks, and significantly fewer tanks were invaded after new species were introduced at twelve months. Every unseeded control tank had at least one species establish during this period. This suggests that the seeding of new water bodies with native zooplankton may provide an effective managerial tool for reducing establishment rates of non-indigenous species in constructed water bodies. The following discussion will explain the results obtained in this study and how they support this hypothesis. These results will then be used to help explain the roles of biotic resistance and propagule pressure in the establishment of non-indigenous zooplankton.

The probability of establishment of new zooplankton species in this experiment was dependent on the species richness and/or composition of the community into which it was introduced, as these were the major variants between treatment and control groups. No environmental variables were significantly different between treatment and control tanks when the new species were introduced. This suggests that biological processes, such as biotic resistance, are important in determining the likelihood of zooplankton establishment in constructed water bodies. It can be stated that constructed water bodies, such as dams, ornamental ponds and infilled mine pits, are more readily invaded by non-indigenous zooplankton because of their low biotic resistance.

4.1 Zooplankton colonisation

My results showed that the seeding of habitats with sediments containing native zooplankton species significantly increased the rate of community development within the treatment tanks. Initial species richness in the seeded tanks increased rapidly and community composition began to diverge almost immediately

following tank set-up. Rapidly following experimental setup, the copepod *Acanthocyclops robustus* and rotifer *Lepadella ovalis* were present in the treatment tanks, whilst only *A. robustus* was present in the control tanks. The presence of *A. robustus* in the unseeded tanks at such an early time might be due to its transfer from other treatment tanks by, for example, birds, or other natural vectors (*A. robustus* was present in all treatment tanks at this time). Colonisation studies on the natural arrival sequence of zooplankton in temporary experimental ponds by Frisch and Green (2007), found that new ponds were colonised naturally by rotifers and copepods within the first two weeks of the initiation of experiments, with copepods the most dominant. The majority of the subsequent species to colonise the control tanks were small benthic and littoral rotifers including *Trichocerca pusilla*, *Lecane flexilis*, *Squatinella mutica* and bdelliods. The comparatively early establishment of rotifers in these experiments is consistent with colonisation studies by Jenkins and Buikema (1998), Frisch and Green (2007) and Caceres and Soluk (2002), who all found that early colonisation was dominated by rotifer species. These studies, and the results obtained in my study, infer that natural colonisation of species other than rotifers is slow, and communities are of low species richness in initial stages of development.

In the seeded treatment tanks, early colonisation was likely to be largely from hatching and subsequent establishment of species from diapausing eggs or other resting stages in the sediments. As a result, community composition was dominated by different species than the control tanks. By three months, three cladocerans (*Moina tenuicornis, Ceriodaphnia dubia* and *Pleuroxus hastirostris*), two copepods (*A. robustus* and *Boeckella delicata*), one ostracod (*Eucypris virens*) and one rotifer species (*Filinia longiseta*) had established. Although seasonal temperature changes, for example, can determine emergence timing of diapausing eggs (Hairston et al., 2000), cladocerans are often the first taxon to hatch in studies conducted on diapause emergence (Gyllstrom and Hansson, 2004). With the exception of *A. robustus*, the remaining species did not appear in any control tanks in the first four months, indicating it is likely that all these emerged from eggs or other diapausing stages in the sediments. The relatively lower richness of rotifer species in the treatment tanks may be explained by the large number of cladocerans present in the treatment tanks. In a study conducted by Balvert et al.

(2009) examining the effects of non-indigenous *Daphnia* in an infilled mine pit on community composition, the rotifer species present in the early development of the lake were gradually outcompeted by the larger, more efficient filter feeding cladocerans. This common occurrence is due to the greater competitive abilities of this taxon, which has been shown in a number of experimental studies, including Gilbert (1988) and Nandini et al. (2002).

4.2 Biotic resistance

The concept of biotic resistance, introduced by Charles Elton in the 1950s (Elton, 1958), has until now not been formally tested to determine its role in zooplankton invasions in constructed water bodies. At 12 months, species richness in the seeded tanks was almost double than that observed in the unseeded tanks. The greatest accumulation rate of species in the seeded tanks occurred within the first four months of the experiment. This rapid acquisition of diversity in the seeded tanks therefore suggests sediments containing diapausing zooplankton eggs are effective for accelerating community development. This may in turn reduce establishment rates of non-indigenous species as, due to the slow colonisation rates by natural means, as shown by my results and others (e.g. Jenkins & Underwood, 1998; Bilton et al., 2001), communities are most likely vulnerable in these early stages of low biotic resistance.

The first recorded findings of the non-indigenous calanoid copepods *Sinodiaptomus valkanovi* (Japanese) and *Skistodiaptomus pallidus* (North American) by Duggan et al. (2006) were confined to constructed ponds. More recent surveys by Banks and Duggan (2009) have also found that these species are confined to constructed water bodies. These findings concur with the hypothesis that low biotic resistance increases the likelihood of invasion. In the present study, *S. pallidus* was used as one of the species introduced at 12 months, and had an 88% establishment rate in the unseeded control tanks from the 16 individuals that were introduced to each tank. This was far greater than in the seeded treatment tanks, as *S.pallidus* established in only 22% of the tanks (two of nine tanks) after the three month 'post-introduction' period. The only two seeded treatment tanks (tanks 5 and 12) that *S. pallidus* was able to establish in were also the only two

treatment tanks which did not have established populations of *Boeckella delicata* and *Calamoecia lucasi* after the fifteen month monitoring period.

The fact that *S. pallidus* established in 88% of the control tanks, and only in the two treatment tanks that were void of any *B. delicata*, not only supports the findings by Hutchinson (1967) that calanoid copepods of similar size cannot co-exist, it also solidifies the importance of biotic resistance as an important factor in reducing the establishment of non-indigenous zooplankton in newly constructed waters. The surveys conducted by Banks and Duggan (2009) found the non-indigenous calanoid copepods *Skistodiaptomus pallidus*, *Sinodiaptomus valkanovi*, *Boeckella symmetrica* and *B. minuta* in constructed waters, such as a farm dam, disused mine pits and ornamental ponds. Although North American studies by Havel et al. (2005) and Johnson et al. (2008) suggests that establishment of non-indigenous species in constructed reservoirs may be due to the their high connectivity to other water bodies and regular disturbance regimes, many of the water bodies these non-indigenous species have been found in New Zealand, such as ornamental ponds, were not subject to disturbance or connected to other water bodies. The patterns observed by Banks and Duggan (2009), along with the findings in this study that *S. pallidus* did not establish in any tanks that contained calanoids of similar size class (i.e. *Boeckella delicata*), suggest that lack of biotic resistance is responsible for the establishment of non-indigenous zooplankton in New Zealand constructed waters. Regular disturbance and connectivity, which are more applicable to the North American dam studies by Havel et al. (2005) and Johnson et al. (2008), cannot be a factor explaining the establishment rates in these constructed water bodies.

Species of *Boeckella* and *Calamoecia* are often found to co-exist in Australia (Maly, 1984), but is apparently less common in New Zealand (Chapman and Green, 1987). Surveys by Maly and Maly (1997) suggest that species of *Boeckella* and *Calamoecia* are found to co-exist when they are of differing sizes (*Calamoecia* prosome length is generally smaller than *Boeckella* prosome length), which suggests differing dietary and habitat requirements. Kobayashi (1995) found that the *Boeckella* and *Calamoecia* species that co-exist in the Wallerawang Reservoir, N.S.W, Australia, exhibited significant differences in dietary overlap.

For example, gut examination in this study revealed that *Boeckella minuta* (prosome length of 0.98 mm) consumed phytoplankton of different species than that of *C. lucasi*, which has a mean prosome length of 0.63 mm (Kobayashi, 1995). These results suggest that species of differing body sizes will naturally select different sized food particles, promoting niche separation and allowing co-existence. The prosome length of many *Skistodiaptomus* species is similar to that of many *Boeckella* species (Vanderploeg et al., 1988). *Skistodiaptomus pallidus* prosome length has been measured at 0.84 mm, whilst the prosome length of *B. delicata* has been measured at 0.94 mm (Banks, 2006). Size and associated diet differences therefore likely explains the co-occurrence of *B. delicata* and *C. lucasi* in the treatment tanks, and also why no *S. pallidus* were able to establish in any of the treatment tanks in which *B. delicata* were present. Since *Boeckella* and *Skistodiaptomus* will have similar dietary requirements, as inferred by their similar sizes, they likely cannot co-exist. These results are also in accordance with the distribution of *S. pallidus* and *S. valkanovi*, confined to constructed water bodies in the North Island, with native calanoid species not recorded at these sites.

Due to the significantly higher proportion of successful establishment of introduced species in the unseeded tanks, it is clear that the sediments containing diapausing eggs worked effectively in increasing species richness, increasing the rate of community development, and therefore increasing the biotic resistance. The fact that two species (*S. pallidus* and *C. sphaericus*) were each able to establish in 22% of the seeded treatment tanks suggests that the resources available were adequate for their establishment and survival, while no significant differences were recorded in environmental variables. The establishment of these two species in the treatment tanks may be attributed to lack of species with similar ecological requirements at that particular period of introduction.

For the effective acceleration of biotic resistance in new water bodies with the application of sediments containing resting eggs of native zooplankton communities, the composition of the species within the sediments will be extremely important. They must contain community assemblages with high species richness, or key species, that renders all resources as limiting factors, so that non-indigenous species with potentially similar niche requirements have less

chance of establishing. This will require the inclusion of species that occupy benthic, littoral and pelagic zones of lentic habitats. In the control tanks, the majority of the species to colonise in the first 12 months were small benthic and littoral species, whilst those that hatched out of the treatment sediments were truly planktonic, such as the calanoid copepods. This was effective in repelling the potential invaders. This means that community composition, as well as species richness, will be more effective at reducing establishment rates. As there is a strong trend for non-indigenous calanoid copepods to establish in constructed waters, native calanoids should be considered as key species to be introduced to new or young water bodies. However, as the calanoid *B. delicata* has only been found to have a narrow native range in New Zealand (Banks and Duggan, 2009), it would be inappropriate to include this species in the sediments if this takes it out of its native range. This example highlights the importance of local sourcing or 'ecosourcing'. This consideration will ensure the integrity of local communities is maintained.

Out of the seven new species that were introduced, the rotifers were clearly the least successful at establishing populations. Four species were introduced, yet only two species, *Synchaeta pectinata* and *Synchaeta oblonga*, established in 25% and 38% of the control tanks respectively. *Ascomorpha ovalis* and *Trichocerca similis* did not establish in any tanks, and out of the four rotifer species, none established in the treatment tanks. Most of the species in the communities that naturally colonised in the control tanks in the first 12 months consisted of the small littoral rotifers *Lepadella ovalis*, *Trichocerca pusilla*, *Lecane flexilis*, *Squatinella mutica* and *Cephalodella catellina* (De Smet, 1998; Duggan, 2001; Castro et al., 2005). Differences in community composition between treatment and control tanks could explain why *S. pectinata* and *S. oblonga*, both limnetic species (Duggan, 2001), were the most successful rotifers to establish in the control tanks, as there were no other limnetic species to compete with. This may also be attributed to propagule pressure, which will be discussed below. SIMPER analysis revealed that the rotifer, *L. ovalis*, which occurred more frequently in control tanks, was the species that was responsible for the greatest amount of dissimilarity in treatment and control groups.

The Shannon-Wiener index did not show a significant difference between treatment and control tanks after 12 months of colonisation. Although it was used in my study to provide an estimate of diversity, independent of species richness, it is recognized that there are problems associated with this method. Gray (2000), for example, stated that even though the Shannon-Wiener index is one of the most commonly used diversity indexes, it is often insensitive when used for comparing species diversity as it is more affected by the middle ranked species within the community. However, the patterns in Shannon-Wiener diversity in tanks through time generally corresponded well to the standing and accumulated mean species richness results.

My results strongly infer that biotic resistance in zooplankton communities is strongest when there are species in the native community that have similar habitat requirements than those of the potential invaders. This leaves less available ecological space for the potential invaders, decreasing their chance of successful establishment. This is in accordance with colonisation studies by Shurin (2000) and Dzialowski et al. (2007). Because the dietary preferences between zooplankton families are generally different (DeMott, 1986; Barnett et al., 2007), sediments containing diapausing eggs with species that collectively exhibit a high diversity of dietary and habitat preferences will be most effective in accumulating biotic resistance.

Whilst the seeded treatment tanks accumulated species richness, which lead to higher biotic resistance, the low biotic resistance in the control tanks after 12 months highlights the cause for concern in aquatic environments that are highly modified. Low biotic resistance is apparent in areas that have a high proportion of constructed water bodies, such as in areas where dams are constructed for hydroelectricity generation. In New Zealand, the end of the Second World War initiated extensive construction of dams for purposes of hydroelectricity (Smale et al., 2001). In 1999, the New Zealand Dam Inventory listed 400 active dams (McDowall, 2000). Global dam construction peaked in the 1970s, meaning that a significant amount of constructed water bodies are less than 50 years old (Rosenberg, 2000). This infers that many water bodies, both in New Zealand and internationally, have low biotic resistance and are more likely to be invaded than

older natural lakes, as was revealed in surveys carried out by Banks and Duggan (2009).

4.3 Propagule pressure

In my study, the species that were introduced in the lowest numbers were generally the least successful in establishing populations in the tanks. *Trichocerca similis* and *Ascomorpha ovalis* were introduced to the tanks as three and four individuals respectively. They did not establish in any treatment or control tanks. *Chydorus sphaericus* had five individuals introduced into the tanks, and established in 38% of the control tanks and 22% of the treatment tanks, indicating that only a small number of individuals may be required for this species to establish. The copepods, *S. pallidus* and *M. leuckarti*, introduced in numbers of 16 and 25 respectively, had significantly higher success rates, both establishing in 88% of the controls. In the treatment tanks, *S. pallidus* established in 22% of the tanks, with *M. leuckarti* failing to establish at all. However, although *S. pectinata* and *S.oblonga* were introduced in numbers of approximately 40 individuals (mixed), they only established in 25% and 38% of the control tanks respectively. These results show, in general, higher propagule pressure increases the chances of successful establishment, although this will not always be the case.

My results are in accordance with Lockwood et al. (2009) that higher propagule pressure increases the likelihood of invasion success. In this experimental study, propagule pressure was easy to manipulate, leading to easy interpretation of its importance in the invasion process. The amount of individuals required to establish a self-sustaining population varies between species, meaning the importance of propagule pressure in species invasions has usually only been dealt with on a case by case basis, or limited to certain species (Godfray and Rees, 2002). This species specific variation was exhibited by *C. sphaericus*. This cladoceran established populations in three control tanks and two treatment tanks after 15 months, from only five individuals. The rotifers *A. ovalis* and *T. similis* were introduced in similar numbers (four and five individuals respectively), yet failed to establish in any tanks. However, the results of this study show that, in general, establishment rate is positively co-related to propagule pressure, therefore making it helpful to predict the likelihood of sites that are vulnerable to potential

establishment of non-indigenous species. This is in accordance with findings from Kolar and Lodge (2001) and Lockwood et al. (2005).

In my study, all experimental tanks were subject to the same propagule pressure for any one species, so it is impossible to determine the exact role propagule pressure played in whether a species succeeded or failed to establish. This could only be achieved if further colonisation studies were carried out, examining establishment rates with differing concentrations of propagules for individual species. Von Holle and Simberloff (2005) state that species establishment can result from the interaction of both propagule pressure and biotic resistance and that if propagule supply is high enough, even diverse communities may be invaded. This is in accordance with my study, that greater establishment success generally corresponds to greater propagule numbers. If higher propagule numbers were used in this study, it is likely that more species would have established.

4.4 Manipulation of Biotic Resistance

My results show that the most important biological mechanism explaining the success or failure to establish is biotic resistance. They have also shown that in an aquatic setting, biotic resistance is easy to manipulate to reduce invasion of new zooplankton species. Even though the propagule pressure applied in this study was the same throughout each experimental group, the differing levels of invasion success between the species suggest that this mechanism, although important, is species specific and harder to manipulate. In a natural setting, propagule pressure may be harder to reduce, especially in the case of zooplankton where most non-indigenous species introductions occur unnoticed. It is for these reasons that the reduction of establishment rates of non-indigenous zooplankton can be more easily managed by accelerating biotic resistance with sediments. If attempts to reduce propagule pressure are successful, the seeded waters will be even more likely to resist the establishment of non-indigenous zooplankton than unseeded waters.

4.5 Environmental variability in tanks

It is widely recognised in invasion literature that abiotic conditions can influence the establishment of non-indigenous species (Havel et al., 2005; Jerde and Lewis, 2007). In order to clearly analyse the role of biotic resistance, it is imperative that abiotic variation between comparison groups be kept as minimal as possible to avoid misleading interpretations. In this study, the lack of variation in physical conditions between the seeded and unseeded tanks allowed for accurate interpretation of the community data obtained.

The comprehensive fortnightly monitoring of temperature, conductivity, specific conductance, DO concentration, DO saturation, pH and chlorophyll *a* throughout the total experimental period was carried out to ensure that environmental variables did not play a role in the composition of zooplankton communities in relation to their position as either a treatment or control. Major differences between environmental variables in treatment and control tanks have the potential to favour or hinder the establishment of species, causing invalidity in the integrity of the experiment. In this investigation there is not one environmental variable that may have caused differences in the community data obtained, while those that did vary most greatly, such as chlorophyll *a* and DO concentration, were most likely a function of the communities present. Environmental variables recorded between the experimental groups at 12 months were so similar, that it can be justifiable to infer that biotic resistance in the treatment tanks – due to higher species richness or the presence of key species – was responsible for significantly less species establishing in them, and not the environmental variables themselves. For all seven environmental variables monitored, there were no significant statistical differences between treatment and control groups when the new species were added at twelve months.

Mean surface temperatures were between 13.2 °C and 12.8 °C, whilst bottom temperatures were recorded between 11.3 °C and 11.5 °C in the seeded and unseeded tanks at 12 months. When the new species were introduced at twelve months, they were kept at room temperature (18 °C) in the laboratory for up to three days, apart from the North American *S. pallidus*, which was stored in a chiller at 4 °C for up to four days. Collecting individuals before transfer into the

tanks was carried out in the laboratory at 18 °C. It is fair to assume that the transfer procedure from the extraction in the laboratory to the release into the tanks may have caused a degree of thermal stress to the individuals, possibly affecting their survival rate when introduced to the tanks. However, experiments conducted on a number of *Daphnia* species concluded that reduced reproduction and/or mortality rarely occurred in temperature changes less than 10 °C (Folt, 1999), while copepods are known to be more tolerant to temperature fluctuations (Jiang, 2008). If temperature-dependent mortality did occur in some individuals, the insignificant temperature differences between the treatment and control tanks would ensure that this potential mortality was spread evenly across both groups, and not partial to one group, thus ensuring minimal bias between them. This potential for mortality due to the changes the individuals experienced during the transfer procedure could apply to the other environmental variables, but the same principle applies in regards to this occurring without variation between the treatment and control tanks.

Both conductivity and specific conductance were very similar between both groups at the twelve month point. In the lead up to this time, these readings declined considerably from May 2009 until the end of the monitoring period. This coincided with winter and the onset of increased rainfall. This had a diluting effect, leading to decreased conductivity through time. All readings across the 15 month monitoring period were below 500 µS cm^{-1}, which according to Soto and De los Rios (2006) is a level thought to be more favourable to copepod survival. When the new species were added at twelve months, conductivity levels were near their lowest, at approximately 194 µS cm^{-1} and 252 µS cm^{-1} for conductivity and specific conductance, respectively. This may explain the relative success of establishment for the copepods in the control tanks when compared to the other taxa. Although it is clear that even though there may have been ideal physical conditions for *S. pallidus* and *M. leuckarti*, it is biotic resistance that will have prevented establishment into 88% of the treatment tanks.

Dissolved oxygen concentrations are known to exhibit effects on the growth, reproduction and filtering rates of zooplankton. Many freshwater species are unable to survive when DO concentrations fall below 2.0 mg L^{-1} (Davidson et al.,

1998). In both treatment and control tanks, DO concentrations rarely fell below 7.0 mg L^{-1}. At the time of new species introductions, the oxygen levels were saturated, with averages of 121.15% in the control tanks and 116.83% in the treatment tanks. DO concentration averages in treatment and control groups were 13.5 mg L^{-1} and 13.4 mg L^{-1} respectively. These levels provided sufficient oxygen for the biological functioning of the zooplankton throughout the total monitoring period as most of these species are able to tolerate a wide range of dissolved oxygen concentrations, along with temperature and pH levels (Garcia et al., 2009).

Tank pH levels were slightly alkaline, ranging between 7.65 and 9.11 throughout the monitoring period. Most natural water bodies have pH levels between six and nine due to the activity of photosynthesis and respiration of other aquatic organisms (Yin and Niu, 2008). At the time of the new species introductions at twelve months, average pH levels were 8.20 in the treatment tanks and 8.39 in the control tanks. These levels will have been sufficient for growth and reproduction of zooplankton species in the treatment and control tanks (Yin and Nui, 2008).

In general, chlorophyll *a* concentrations varied greatly in the treatment tanks, particularly in the first five months of the study. Chorophyll *a* levels were slightly lower across the unseeded control tanks, although the difference in concentrations between treatment and control groups were not statistically significant when the new species were added at twelve months. An initial peak in chlorophyll *a* concentrations in the treatment tanks may have coincided with algae present in the sediments upon release into the tanks. Although the interaction between phytoplankton and zooplankton are complex, and often species-specific (Bergquist and Carpenter, 1986), Tessier et al. (2001) state that chlorophyll *a* levels usually decrease considerably in the presence of grazing zooplankton, such as those of the *Ceriodaphnia* genus. When zooplankton abundance is considered, the lower chlorophyll *a* levels in control tanks can be explained. Although *Ceriodaphnia dubia* was present in both treatment and control tanks, SIMPER analysis revealed that *C. dubia* were much more abundant in control tanks, with an average of 2.55 ind L^{-1} and an average of just 0.84 ind L^{-1} in treatments. In several control tanks, *C. dubia* abundance reached very high numbers (>1000 ind L^{-1} per fortnightly sample). With such a high abundance of this efficient grazer,

the chlorophyll *a* levels remained lower than that of the treatment tanks for most of the 15 month period. The difference in chlorophyll *a* levels between treatment and control groups when the new species were introduced at twelve months was only 6.71 mg L^{-1}, with an average of 20.16 mg L^{-1} in the treatments and 13.45 mg L^{-1} in the controls. This statistically insignificant difference would be unlikely to favour the establishment of the new species in one group over the other.

4.6 Summary

All results collected from this investigation, both biological and physical, indicate that introducing native propagules into new habitats during the early stages of community development accelerates community development, for example resulting in greater numbers of planktonic species at a faster rate, and leads to greater species diversity in seeded tanks. When faced with new species after one year, these species established in significantly fewer seeded tanks, validating the role of biotic resistance in reducing establishment rates of non-indigenous zooplankton. The environmental variability and scientifically rigid experimental design encouraged equal opportunities for species establishment over both experimental groups. The sole discrepancy between diversity in treatment and control tanks could only be explained by the seeding of native propagules in the treatment tanks.

Chapter Five: Conclusions

The threat of biodiversity loss as a result of the invasion of non-indigenous organisms is arguably one of the top two environmental threats of this age (Mack et al, 2000; Ricciardi and Rasmussen, 1998; Vitousek et al, 1997). Although the link between humans and the introduction of non-indigenous species has long been recognised (Elton, 1958), the ability of many non-indigenous species to exploit human-altered habitats, such as reservoirs, has not been clarified until now. If the mechanisms that influence the seemingly high establishment rates of non-indigenous zooplankton in constructed waters can be identified, successful management in controlling these mechanisms may be attainable.

The results of this study demonstrate that biotic resistance plays an important role in reducing the establishment rates of non-indigenous zooplankton. The results also provide evidence that biotic resistance may be manipulated as a management tool to significantly reduce establishment rates. Because of the substantially lower establishment rate of zooplankton that were introduced into tanks seeded with resting eggs, it can be accurately said that these eggs acted to increase species diversity, increase community development rates, and increase biotic resistance towards subsequent invasions. The seeding of new lakes with appropriate species therefore provides an extremely viable and practical option for environmental managers as a tool for reducing establishment rates of non-indigenous zooplankton in constructed water bodies.

In New Zealand, and at a global level, the extensive amount of reservoir construction suggests that there are many water bodies that exhibit low biotic resistance and are therefore vulnerable to invasions. Although other explanations for higher establishment rates of non-indigenous zooplankton in constructed waters, such as disturbance (Havel et al. 2005; Johnson et al. 2008), may indeed hold true in some systems (i.e. dams), the discovery of non-indigenous calanoid copepod species in relatively undisturbed constructed water bodies in New Zealand infers that biotic resistance explains these introductions with greater accordance.

Although the effect of non-indigenous zooplankton have not yet been intensively studied in New Zealand, Balvert et al. (2009) found that the presence of the non-indigenous *Daphnia dentifera* in a recently filled mine pit drastically reduced the native rotifer community due to its greater competitive abilities. It is for reasons such as this, that the addition of native propagules to constructed water bodies to stimulate biotic resistance and repel potential future invaders should be implemented at a national (or international) scale. If non-indigenous species such as the predatory *Bythotrephes longimanus* establish in water bodies in New Zealand, it is likely their effects will be detrimental to the species diversity of native zooplankton communities, as has shown to occur in North American lakes (Yan et al., 2002). Effects such as these can only be managed before establishment takes place, and the probability that this might occur may be increased through the seeding of constructed waters with sediments containing native propagules.

Preserving the biodiversity of many aquatic species may be attainable if the results from this study are extrapolated into a natural setting. Due to the increasing amounts of human mediated non-indigenous species introductions, it is recommended that the addition of native propagules is implemented with a sense of urgency, as this will increase effectiveness of this biodiversity conserving tool.

The implications of this study are widespread. Further research will need to be undertaken to maximise the effectiveness of the sediments and field tests will need to be carried out in order to investigate the practicality of this potential managerial tool. Once this has taken place, this method has the potential to be utilised in aquatic environments on an international scale.

References

Alonso, A. and P. Castro-Diez. 2008. What explains the invading success of the aquatic snail *Potamopyrgus antipodarum* (Hydrobiidaem molluska)?. Hydrobiologica **614**:107-116.

Altman, S. and R. B. Whitlatch. 2007. Effects of small-scale disturbance on invasion success in marine communities. Journal of Experimental Marine Biology and Ecology **342**:15-29.

Arar, E. J. and G. B. Collins. 1997. *In vitro* determination of Chlorophyll *a* and pheophytin *a* in marine and freshwater algae by flurescence. Environmental Monitoring System Laboratory, Office of Research and Development, US Environmental Protection Agency , Cincinnati, OH.

Bailey, S. A., I. C. Duggan, P. T. Jenkins, and H. J. MacIsaac. 2005. Invertebrate resting stages in residual ballast sediment of transoceanic ships. Canadian Journal of Fisheries and Aquatic Sciences **62**:1090-1103.

Balvert, S. F., I. C. Duggan, and I. D. Hogg. 2009. Zooplankton seasonal dynamics in a recently filled mine pit lake: the effect of non-indigenous *Daphnia* establishment. Aquatic Ecology **43**:403-413.

Banks, C. M. 2006. New Zealand calanoid copepod invasions: has artificial lake construction facilitated invasions, and are our coastal waters uninvaded. Unpublished MSc. Thesis, The University of Waikato, Hamilton.

Banks, C. M. and I. C. Duggan. 2009. Lake construction has facilitated calanoid copepod invasions in New Zealand. Diversity and Distributions **15**:80-87.

Barnett, A. J., K. Finlay, and B. Beisner. 2007. Functional diversity of crustacean zooplankton communities: towards a trait-based classification. Freshwater Biology **52**:796-813.

Benoit, H. P., D. M. Warner, W. G. Sprules, and L. G. Rudstam. 2002. Assessing the impact of a recent predatory invader: the population dynamics, vertical distribution, and potential prey of *Cercopagis pengoi* in Lake Ontario. Limnology and Oceanography **47**:626-635.

Bergquist, A. M. and S. R. Carpenter. 1986. Zooplankton effects on phytoplankton in lakes of contrasting trophic states. Limnology and Oceanography **36**:64-90.

Bilton, D. T., J. R. Freeland, and B. Okamura. 2001. Dispersal in freshwater invertebrates. Annual Review of Ecology and Systematics **32**:159-181.

Brockerhoff, E. G., B. I. Barratt, J. R. Beggs, L. L. Fagan, M. K. Kay, C. B. Phillips, and C. J. Vink. 2010. Impacts of exotic invertebrates on New Zealand's indigenous species and ecosystems. New Zealand Journal of Ecology **34**:158-174.

Bronmark, C. and L. Hansson. 2002. Environmental issues in lakes and ponds: current state and perspectives. Environmental Conservation **29**:290-306.

Buchan, L. A. and D. K. Padilla. 1999. Estimating the probability of long-distance overland dispersal of invading aquatic species. Ecological Applications **9**:254-265.

Caceres, C. E. and D. A. Soluk. 2002. Blowing in the wind: a field test of overland dispersal and colonization by aquatic invertebrates. Oecologica **131**:402-408.

Castro, B. B., R. P. Antunes, A. M. V. M. Soares, and F. Goncalves. 2005. Rotifer community structure in three shallow lakes: seasonal fluctuations and explanatory factors. Hydrobiologica **543**:221-232.

Chapman, M. A. and J. D. Green. 1987. Zooplankton ecology. Inland waters of New Zealand (ed by A. B. Viner), pp. 225-264.

Chapman, M. A. and M. A. Lewis. 1976. An introduction to the freshwater Crustacea of New Zealand. Collins, Auckland, New Zealand.

Chester, C. C. 1999. Review: the coming homogocene. Conservation Biology **13**:456-457.

Clarke, K. R. and R. N. Gorley. 2006. PRIMER v6: User Manual/Tutorial. PRIMER-E Ltd, Plymouth, UK.

Colautti, R. I., S. A. Bailey, K. A. van Overdijk, and H. J. MacIsaac. 2006. Characterised and predicted costs of nonindigenous species in Canada. Biological Invasions **8**:45-59.

Davidson, N. L., W. E. Kelso, and D. Rutherford. 1998. Relationships between environmental variables and the abundance of cladocerans and copepods in the Atchafalaya River Basin. Hydrobiologica **379**:175-181.

De Smet, W. H. 1998. *Cephalodella segersi* n.sp. (Notommatidae, Monogononta), a new rotifer from Belgium, with notes on *C. catellina* (O.F. Muller, 1786), *C. fluviatilis* (Zavadovsky, 1926) and *C. maior* Zavadovsky, 1926 stat. nov. Hydrobiologica **367**:1-13.

DeMott, W. R. 1986. The role of taste in food selection by freshwater zooplankton. Oecologica **69**:334-340.

Dick, J. T. A., D. Platvoet, and D. W. Kelly. 2002. Predatory impact of the freshwater invader *Dikerogammarus villosus* (Crustacea: Amphipoda). Canadian Journal of Fisheries and Aquatic Sciences **59**:1078-1084.

Dudgeon, D., A. H. Arthington, M. O. Gessner, Z. Kawabata, D. J. Knowler, C. Leveque, R. J. Naiman, A. Prieur-Richard, D. Soto, M. L. Stiassny, and C. A. Sullivan. 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biological Reviews **81**:163-182.

Duggan, I. C. 2001. The ecology of periphytic rotifers. Hydrobiologica **446/447**:139-148.

Duggan, I. C., J. D. Green, and D. F. Burger. 2006. First New Zealand records of three non-indigenous zooplankton species: *Skistodiaptomus palllidus, Sinodiaptomus valkanovi* and *Daphnia dentifera*. New Zealand Journal of Marine and Freshwater Research **40**:561-569.

Dzialowski, A. R., J. T. Lennon, and V. H. Smith. 2007. Food web structure provides biotic resistance against plankton invasion attempts. Biological Invasions **9**:257-267.

Dzialowski, A. R., W. J. O'Brien, and S. M. Swaffar. 2000. Range expansion and potential dispersal mechanisms of the exotic cladoceran *Daphnia lumholtzi*. Journal of Plankton Research **22**:2205-2223.

Ellison, A. M., B. D. Clinton, E. A. Colburn, K. Elliot, C. R. Ford, D. R. Foster, B. D. Kloeppel, D. J. Knoepp, G. M. Lovett, J. Mohan, D. A. Orwig, N. L. Rodenhouse, W. V. Sobczak, K. A. Stinson, J. K. Stone, C. M. Swan, J. N. Thompson, B. von Holle, and J. R. Webster. 2005. Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. Frontiers in Ecology and the Environment **3**:479-486.

Elton, C. S. 1958. The ecology of invasions by animals and plants. Methuen, London.

Emberson, R. M. 2000. Endemic biodiversity, natural enemies and the future of biological control. In: Spencer NR ed. Tenth International Symposium on Biological Control of Weeds:875-880.

Folt, C. L., C. Y. Chen, M. V. Moore, and J. Burnaford. 1999. Synergism and antagonism among multiple stressors. Limnology and Oceanography **44**:864-877.

Forsyth, D. M. and R. P. Duncan. 2001. Propagule size and the relative success of exotic ungulate and bird introductions to New Zealand. The American Naturalist **157**:583-595.

Frisch, D. and A. J. Green. 2007. Copepods come in first: rapid colonization of new temporary ponds. Fundamental and Applied Limnology **168**:289-297.

Garcia, C. E., S. Nandini, and S. S. Sarma. 2009. Seasonal dynamics of zooplankton in Lake Huetzalin, Xochimilco (Mexico City, Mexico). Limnologica **39**:283-291.

Gilbert, J. J. 1988. Suppression of rotifer populations by *Daphnia*: a review of the evidence, the mechanisms, and the effects on zooplankton community structure. Limnology and Oceanography **33**:1286-1303.

Godfrey, H. and M. Rees. 2002. Population growth rates: issues and an application. Philosophical Transactions of the Royal Society B: Biological Sciences **357**:1307-1319.

Gray, J. S. 2000. The measurement of marine species diversity, with an application to the benthic fauna of the Norwegian continental shelf. Journal of Experimental Marine Biology and Ecology **250**:23-49.

Grigorovich, I. A., H. J. MacIsaac, N. V. Shadrin, and E. L. Mills. 2002. Patterns and mechanisms of aquatic invertebrate introductions in the ponto-Caspian region. Canadian Journal of Fisheries and Aquatic Sciences **59**:1189-1208.

Gyllstrom, M. and L. Hansson. 2004. Dormancy in freshwater zooplankton: Induction, termination and the importance of benthic-pelagic coupling. Aquatic Sciences **66**:274-295.

Hairston, N. G., A. Hansen, and W. R. Schaffner. 2000. The effect of diapause emergence on the seasonal dynamics of a zooplanktron assemblage. Freshwater Biology **45**:133-145.

Havel, J. E., C. E. Lee, and J. vander Zanden. 2005. Do reservoirs facilitate invasions into landscapes? Bioscience **55**:518-525.

Havel, J. E., W. R. Mabee, and J. R. Jones. 1995. Invasion of the exotic cladoceran *Daphnia lumholzi* into North American reservoirs. Canadian Journal of Fisheries and Aquatic Sciences **52**:151-160.

Havel, J. E. and K. A. Medley. 2006. Biological invasions across spatial scales: intercontinental, regional, and local dispersal of cladoceran zooplankton. Biological Invasions **8**:459-473.

Havel, J. E. and J. B. Shurin. 2004. Mechanisms, effects, and scales of dispersal in freshwater zooplankton. Limnology and Oceanography **49**:1229-1238.

Havel, J. E., J. B. Shurin, and J. R. Jones. 2002. Estimating dispersal from patterns of spread: spatial and local control of lake invasions. Ecology **83**:3306-3318.

Hebert, P. D. and T. J. Crease. 1980. Clonal Coexistence in *Daphnia pulex* (Leydig): another planktonic paradox. Science **207**:1363-1365.

Holland, T. A. and D. G. Jenkins. 1998. Comparison of processes regulating zooplankton assemblages in new freshwater ponds. Hydrobiologica **387/388**:207-214.

Hutchinson, G. E. 1967. A treatise on limnology, Vol 11. John Wiley & Sons, New York, NY. .

Jenkins, D. G. and A. L. Buikema. 1998. Do similar communities develop in similar sites: a test with zooplankton structure and function. Ecological Monographs **68**:421-443.

Jenkins, D. G. and M. O. Underwood. 1998. Zooplankton may not disperse readily in wind, rain, or waterfowl. Hydrobiologica **387-388**:15-21.

Jerde, C. L. and M. A. Lewis. 2007. Waiting for invasions: a framework for the arrival of non-indigenous species. American Naturalist **170**:1-9.

Jiang, Z., J. Zeng, Q. Chen, Y. Huang, X. Xu, Y. Liao, L. Shou, and J. Liu. 2008. Tolerance of copepods to short-term thermal stress caused by coastal power stations. Journal of Thermal Biology **33**:419-423.

Johnson, P. T., J. D. Olden, and J. Van der Zanden. 2008. Dam invaders: impoundments facilitate biological invasions into freshwaters. Frontiers in Ecology and the Environment **6**:357-363.

Kobayashi, T. 1995. Different patterns of resource use between two coexisting freshwater calanoid species. Australian Journal of Marine and Freshwater Research **46**:481-484.

Kolar, C. S. and D. M. Lodge. 2001. Progress in invasion biology: predicting invaders. TRENDS in Ecology and Evolution **16**:199-204.

Lemke, A. M., J. A. Stoeckel, and M. A. Pegg. 2003. Utilization of the exotic cladoceran *Daphnia lumholtzi* by juvenile fishes in an Illinois river floodplain. Journal of Fish Biology **62**:938-954.

Leung, B., D. M. Lodge, D. Finnof, J. F. Shogren, M. A. Lewis, and G. Lamberti. 2002. An ounce of prevention or a pound of cure: bioeconomic risk analysis of invasive species. Proceedings of the Royal Society of Biological Sciences **269**:2407-2413.

Levine, J. M. and C. M. D'Antonio. 1999. Elton revisited: a review of evidence linking diversity and invasibility. OIKOS **87**:15-26.

Levine, J. M., P. B. Adler, and S. G. Yelenik. 2004. A meta-analysis of biotic resistance to exotic plant invasions. Ecology Letters. **7**: 975-989

Lockwood, J. L., P. Cassey, and T. M. Blackburn. 2005. The role of propagule pressure in explaining species invasions. TRENDS in Ecology and Evolution **20**:223-228.

Lockwood, J. L., P. Cassey, and T. M. Blackburn. 2009. The more you introduce the more you get: the role of colonization and propagule pressure in invasion ecology. Diversity and Distributions **15**:904-910.

Lodge, D. M. 1993. Biological invasions: lessons for ecology. TRENDS in Ecology and Evolution **8**:133.

Lodge, D. M., S. Williams, H. J. MacIsaac, K. R. Hayes, B. Leung, S. Reichard, R. N. Mack, P. B. Moyle, M. Smith, D. A. Andow, J. T. Carlton, and A. McMichael. 2006. Biological invasions: recommendations for U.S. policy and management. Ecological Applications **16**:2035-2054.

Loo, J. A. 2009. Ecological impacts of non-indigenous invasive fungi as forest pathogens. Biological Invasions **11**:81-96.

Lowe, D. and J. D. Green. 1987. Origins and development of lakes. Department of Scientific and Industrial Research, Wellington, New Zealand.

MacIsaac, H. J., J. V. Borbely, J. R. Muirhead, P. A. Graniero, and 2004. Backcasting and forecasting biological invasions of inland lakes. Ecological Applications **14**:773-783.

Mack, R. N., D. Simberloff, W. M. Lonsdale, H. Evans, M. Clout, and F. A. Bazzaz. 2000. Biotic invasions: causes, epodemiology, global consequences, and control. Ecological Applications **10**:689-710.

Magurran, A. E. 2004. Measuring biological diversity. Blackwell Publishing, Oxford, England.

Makino, W., M. A. Knox, and I. C. Duggan. 2010. Invasion, genetic variation and species identity of the calanoid copepod *Sinodiaptomus valkanovi*. Freshwater Biology **55**:375-386.

Malmqvist, B. and S. Rundle. 2002. Threats to the running water ecosystems of the world. Environmental Conservation **29**:134-153.

Maly, E. J. 1984. Interspecific copulation in and co-occurrence of similar-sized freshwater centropagid copepods. Australian Journal of Marine and Freshwater Research **35**:153-165.

Maly, E. J. and M. P. Maly. 1997. Predation, competition, and co-occurrences of *Boeckella* and *Calamoecia* (Copepoda: Calanoida) in Western Australia. Hydrobiologica **354**:41-50.

Manchester, S. J. and J. M. Bullock. 2000. The impacts of non-native species on UK biodiversity and the effectiveness of control. Journal of Applied Ecology **37**:845-864.

Marchetti, M. P., P. B. Moyle, and R. Levine. 2004. Invasive species profiling?. Exploring the characteristics of non-native fishes across invasion stages in California. Freshwater Biology **49**.

McDowall, R. M. 2000. Biogeography of the New Zealand torrentfish, *Cheimarrichthys fosteri* (Teleostei: Pinguipedidae): a distribution driven mostly by ecology and behaviour. Environmental Biology of Fishes **58**:119-131.

Mills, E. L., J. H. Leach, J. T. Carlton, and C. L. Secor. 1993. Exotic species in the Great Lakes: a history of biotic crises and anthropogenic introductions. Journal of Great Lakes Research **19**:1-54.

Mills, E. L., J. H. Leach, C. L. Secor, and J. T. Carlton. 1993(b). What's next? The prediction and management of exotic species in the Great Lakes. Great Lakes Fishery Commission, Ann Arbor, Michigan.

Naiman, R. J. and M. G. Turner. 2000. A future perspective on North America's freshwater ecosystems. Ecological Applications **10**:958-970.

Nandini, S., S. S. Sarma, and H. Bocanegra. 2002. Effect of four species of cladocerans (Crustacea) on the population growth of *Brachionus patulus* (Rotifera). Acta Hydrochimica et Hydrobiologica **30**:101-107.

Olden, J. D., M. J. Kennard, and B. Pusey. 2008. Species invasions and the changing biogeography of Australian freshwater fishes. Global Ecology and Biogeography **17**:25-37.

Olden, J. D. and N. L. Poff. 2004. Clarifying biotic homogenization. TRENDS in Ecology and Evolution **19**:282-283.

Padilla, D. K., M. A. Chotkowski, and L. A. Buchan. 1996. Predicting the spread of zebra mussels (*Dreissena polymorpha*) to inland waters using boater movement patterns. Global Ecology and Biogeography Letters **5**:353-359.

Pejchar, L. and H. A. Mooney. 2009. Invasive species, ecosystem services and human well-being. TRENDS in Ecology and Evolution **24**:497-504.

Pimentel, D., L. Lach, R. Zuniga, and D. Morrison. 2000. Environmental and economic costs of nonindigenous species in the United States. Bioscience **50**:53-65.

Proctor, V. W. and C. R. Malone. 1965. Further evidence of the passive dispersal of small aquatic organisms via the intestinal tract of birds. Ecology **46**:728-729.

Reichard, S. H. and P. S. White. 2003. Invasion biology: an emerging field of study. Annals of the Missouri Botanical Garden **90**:64-66.

Ricciardi, A. and H. J. MacIsaac. 2000. Recent mass invasion of the North American Great Lakes by Ponto-Caspian species. TRENDS in Ecology and Evolution **15**:62-65.

Ricciardi, A. and H. J. MacIsaac. 2008. The book that began invasion ecology. NATURE **452**:34.

Ricciardi, A. and J. B. Rasmussen. 1998. Predicting the identity and impact of future biological invaders: a priority for aquatic resource management. Canadian Journal of Fisheries and Aquatic Sciences **55**:1759-1765.

Richardson, D. M. and P. Pysek. 2008. Fifty years of invasion ecology - the legacy of Charles Elton. Diversity and Distributions **14**:161-168.

Rixon, C. A. M., I. C. Duggan, N. M. N. Bergeron, A. Ricciardi, and H. J. MacIsaac. 2005. Invasion risks posed by the aquarium trade and live fish markets on the Laurentian Great Lakes. Biodiversity and Conservation **14**:1365-1381.

Rodriguez, L. F. 2006. Can invasive species facilitate native species? Evidence of how, when, and why these impacts occur. Biological Invasions **8**:927-939.

Rosenberg, D. M., P. McCully, and C. M. Pringle. 2000. Global-scale environmental effects of hydrological alterations: Introduction. Bioscience **50**:746-751.

Rosenzweig, M. L. 2001. The four questions: what does introduction of exotic species do to diversity?. Evolutionary Ecology Research **3**:361-367.

Ruiz, G. M., J. T. Carlton, E. D. Grosholz, and A. H. Hines. 1997. Global invasions of marine and estuarine habitiats by non-indigenous species: mechanisms, extent, and consequences. American Zoologist **37**:621-632.

Sakai, A. K., F. W. Allendorf, J. S. Holt, D. M. Lodge, J. Molofsky, K. A. With, S. Baughman, R. J. Cabin, J. E. Cohen, N. C. Ellstrand, D. E. McCauley, P. O'Neil, I. M. Parker, J. N. Thompson, and S. G. Weller. 2001. The population biology of invasive species. Annual Review of Ecology and Systematics **32**:305-332.

Shurin, J. B. 2000. Dispersal limitation, invasion resistance, and the structure of pond zooplankton communities. Ecology **81**:3074-3086.

Simoes, N. R., B. A. Robertson, F. A. lansac-Toha, E. M. Takahashi, C. C. Bonecker, L. F. M. Velho, and C. Y. Joko. 2009. Exotic species of zooplankton in the Upper Parana River floodplain, *Daphnia lumholtzi* Sars, 1885 (Crustacea: Branchiopoda). Brazilian Journal of Biology **69**:551-558.

Smale, M. C., P. T. Whaley, and P. N. Smale. 2001. Ecological restoration of native forest at Aratiatia, North Island, New Zealand. Restoration Ecology **9**:28-37.

Soto, D. and P. De Los Rios. 2006. Influence of trophic status and conductivity on zooplankton composition in lakes and ponds of Torres del Paine National Park (Chile). Biologia **61**:541-546.

Stohlgren, T. J. and J. L. Schnase. 2006. Risk analysis for biological hazards: what we need to know about invasive species. Risk Analysis **26**:163-173.

Tessier, A. J., E. V. Bizina, and K. C. Geedy. 2001. Grazer-resource interactions in the plankton: are all daphniids alike? Limnology and Oceanography **46**:1585-1595.

van Riel, M. C., G. van der Veld, S. Rajagopal, S. Marguillier, F. Dehairs, and A. bij de Vaate. 2006. Trophic relationships in the Rhine food web during invasion and after establishment of the ponto-Caspian invader *Dikerogammarus villosus*. Hydrobiologica **565**:39-58.

Vanderploeg, H. A., G. Paffenhofer, and J. R. Liebig. 1988. *Diaptomus* vs. net phytoplankton: Effects of algal size and morphology on selectivity of a behaviorally flexible, omnivorus copepod. Bulletin of Marine Science **43**:377-394.

Vitousek, P. M., C. M. D'Antonio, L. L. Loope, M. Rejmanek, and R. Westbrooks. 1997. Introduced species: a significant component of human-caused global change. New Zealand Journal of Ecology **21**:1-16.

Von Holle, B. and D. Simberloff. 2005. Ecological resistance to biological invasion overwhelmed by propagule pressure. Ecology **86**:3212-3218.

Wilkinson, D. M. 2004. The long history of the biotic homogenization concept. TRENDS in Ecology and Evolution **19**:283-284.

Yan, N. D., R. Girard, and S. Boudreau. 2002. An introduced invertebrate predator (*Bythotrephes*) reduces zooplankton species richness. Ecology Letters **5**:481-485.

Yin, X. W. and C. J. Niu. 2008. Effect of pH on survival, reproduction, egg viability and growth rate of five closely related rotifer species. Aquatic Ecology **42**:607-616.