Grass carp and incidental invaders from aquaculture: a study of impacts on zooplankton communities and invader origins

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Abstract

The movement of fish from aquaculture facilities has led to invasions of non-native species globally, including of ‘hitch-hiking’ species, resulting in impacts on the composition of communities in recipient ecosystems. This thesis employs both community scale analysis and sensitive molecular techniques to assess the effects of grass carp translocations on zooplankton communities and to determine the origins of an incidental non-native invader.

In the first component, I tested the effects of grass carp translocations from aquaculture facilities on ponds in the Auckland region, New Zealand. Zooplankton community composition was quantified in 34 ponds that had been subject to grass carp release and 31 which had no grass carp introductions. A significant difference in zooplankton community composition was observed between ponds that had received grass carp translocations and those that had not. Differences in zooplankton community composition between ponds with and without carp releases were attributable to: 1) the establishment of zooplankton originating from aquaculture ponds, including non-native species; and 2) the effects of grass carp activity, through habitat modification. Effective measures to curb the proliferation of non-native taxa within aquaculture facilities, and to mitigate the unintentional movement of non-native taxa with translocations from these facilities, are required to reduce future introductions.

In the second component of this thesis I used a fragment of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene to more precisely identify the origins of invasions of freshwater calanoid copepod *Skistodiaptomus pallidus* (Herrick 1879). This was applied to populations sampled from within New Zealand, which have been linked to the release of fish from aquaculture facilities, and to populations found in Germany, which have possible links to a shipping vector. The *S. pallidus* COI sequences placed both the New Zealand and German specimens with those from the most easterly regions of the USA (e.g., New York, Virginia and Georgia). However, several haplotypes were found to be divergent between the New Zealand and German populations, indicating the exact sources of the introductions were likely different for each country. German sequences had greater haplotype diversity than those from New Zealand, supporting the suggestions of a shipping related vector of introduction to Germany. Both German and New Zealand populations contained haplotypes that were closely related to North American sequence records. However, further sampling of the native range will be required to determine the exact origin of the non-indigenous *S. pallidus* populations. With this additional information it may also be possible to determine more precisely the vectors of introduction.

Collectively, the two research chapters provide a broader understanding of invasion processes and the effects of grass carp translocations on the zooplankton communities within recipient ponds in New Zealand. My research has immediate application in the re-evaluation of fish translocation management practises and impacts.

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Thesis Introduction

Biological invasions

Human activities have greatly increased the spatial and temporal scales across which species spread through introduction outside of their native ranges (Vitousek et al., 1997; Kolar & Lodge, 2001; Wilson et al., 2009). Species that survive introduction may spread widely, becoming invasive in new environments (Kolar & Lodge, 2001). Biological invasions have the potential to cause adverse effects on ecosystems and vital infrastructure, resulting in economic and social impacts (Vitousek et al., 1997; Dudgeon et al., 2006). Effects on biodiversity can occur where species proliferate and act, for example, as competitors with, or predators on, native biota (Ruiz & Carlton, 2003; Riccardi & Giussani, 2007). Further, introduced taxa may alter the conditions within recipient environments, or act as carriers of parasites or disease, producing adverse outcomes for resident taxa (Fuller et al., 1999; Grosholz et al., 2015). Detrimental long-term outcomes, such as damage to ecosystems and infrastructure, may lead to a requirement for management programmes, which are often costly to address (Vitousek et al., 1997; Hofstra, 2014).

Invasions into aquatic environments have received increasing attention as the ecological impacts of both deliberate and unintentional introductions of non-native taxa are realised (Strayer, 2010; Grosholz et al., 2015). Introduced aquatic taxa consist of a non-random subset of species that have been translocated to recipient environments via vectors such as shipping, the aquarium trade, recreational boating and release or escape from aquaculture (Ruiz & Carlton, 2003; Copp et al., 2005; Strayer, 2010; Kelly et al., 2013). The process through which non-native taxa are introduced into new ecosystems occurs through a series of consecutive stages, beginning with entrainment (uptake and capture) into the vector, followed by transportation from a donor region, introduction into a recipient environment and establishment of a self-sustaining population within the new biogeographic region (Kolar & Lodge, 2001). Contributing to the non-random selection of species that successfully establish, is the likelihood of a given species being entrained into a vector, as well as environmental and species-related attributes that regulate survival at each stage of the invasion process (Kolar & Lodge, 2001; Colautti et al., 2006). For example, studies indicate that small zooplankton taxa are readily entrained into ballast tanks of ships, and that diapausing eggs provide a means for the long-distance survival in ship's sediments during the transport phase (Kolar & Lodge, 2001; Briski et al., 2011).

Identifying and quantifying the importance of vectors of invasion is essential to formulating management strategies, with vector-based intervention arguably the most effective means of curbing invasion rates (Strayer, 2010; Briski et al., 2012). For example, in North America, recognition of the relationship between transoceanic shipping, ballast exchange and the introduction of numerous species into the Laurentian Great Lakes resulted in changes to shipping practises and ongoing research into shipping-related vectors (Holeck et al., 2004; Briski et al., 2012). However, modes of introduction are not always clearly evident, and vectors such as aquaculture are subject to increasing speed of transport and interlinking of transport networks which may provide increased opportunity for establishment by non-native biota (Minchin, 2007).

Aquaculture

Aquaculture is described as the farming of aquatic organisms for purposes including food production, fish stocking, sport, ornamental culture and as biological control agents (Bartley, 2011). Aquaculture is a rapidly developing sector, undertaken in fresh, marine or estuarine waters and is the principal basis for the intentional transportation of aquatic organisms outside of their native range (Minchin, 2007; Bartley, 2011). Practises range from the accumulation and protection of wild stocks prior to distribution through to intensive cultivation, including hatchery production for stocking in the wild and the contained culturing of aquatic species over their full life-history (Minchin, 2007). In freshwater environments, the farming of aquatic organisms may involve utilisation of constructed ponds, or pens in natural lakes; however, taxa may later be translocated to other waters; for example, for use as biological control agents (Fuller et al., 1999; Bartley, 2011; Pipalova, 2006). The species most commonly utilised in aquaculture are typically fast growing with tolerances of a range of environmental conditions, and of these, fish are regularly farmed and relocated, including species of tilapia and carp (Pipalova, 2006; Bartley, 2011).

Aquaculture as a vector for invasion

Aquatic organisms that have been transported in large numbers for aquaculture across biogeographic barriers include fish, crustaceans, molluscs and algae (Reid & Pinto-Coelho, 1994; Grosholz et al., 2015). Unintentional introductions of ‘hitch-hiking’ species, carried incidentally, have occurred in concert with deliberate releases, resulting in the establishment of non-native taxa in recipient regions (Ruiz & Carlton, 2003; Strayer, 2010). For example, importations for aquaculture of oysters from the Atlantic and Pacific regions into California have resulted in the unintentional co-introduction of contaminant species, bivalve predators and pathogens (Grosholtz et al., 2015). Zooplankton species are regular hitch-hikers in association with aquaculture as they can be passively entrained and translocated at dormant or active life-stages (Strayer, 2010; Papa et al., 2012). For example, the spread of the cladoceran *Daphnia lumholtzi* in North America likely resulted from initial introduction into a Texas reservoir during stocking with Nile perch (*Lates niloticus*) and blue tilapia (*Oreochromis aureis*) imported from Africa (Havel & Hebert, 1993). In the Philippines, the calanoid copepod *Arctodiaptomus dorsalis* was detected in 1991 in Laguna de Bay, a site which had been subject to introductions of tilapia for aquaculture (Papa et al., 2012). The subsequent spread of *A. dorsalis* was associated with an increase in the intensity of aquaculture activities and has seemingly resulted in the displacement of native copepod species in lakes where *A. dorsalis* has established (Papa et al., 2012). While, in New Zealand, the recent establishment of populations of the North American calanoid copepod *Skistodiaptomus pallidus* has been linked to the intentional release of grass carp (*Ctenopharyngodon idella*) from aquaculture farms, highlighting the role of grass carp translocation as a vector for the introduction of incidental taxa (Duggan et al., 2014).

Grass carp

Grass carp is a herbivorous cyprinid, native to Asia, that has become an important aquatic species and is one of the ten most commonly introduced species for use in freshwater aquaculture worldwide (Pipalova, 2006; Bartley, 2011). Since the 1960s there has been increased importation and expanding utilisation of grass carp in Europe, North America, South America, Australasia and regions of Asia, primarily for the control of aquatic weed, but also for human consumption in warmer regions (Pipalova, 2006). In New Zealand, grass carp were introduced in 1966 and domestically cultured individuals have been increasingly released into ponds and lakes for the control of non-native macrophytes since the 1980s (Hofstra, 2014). The efficacy of utilising grass carp for the biological control of aquatic macrophytes is well documented, with stocking rates of 20-100 kg/ha resulting in 30-100% reductions in macrophyte biomass over a single year (Richard et al., 1985; Pipalova et al., 2009). However, a number of effects on recipient freshwater ecosystems have been reported in longitudinal studies following grass carp introductions. These effects are typically indirect and related to macrophyte consumption and the loss of plant-related habitat (Pipalova, 2006). Reported effects include increases in nutrient concentrations (Mitchell et al., 1984), changes to phytoplankton and fish communities (Richard et al., 1985; Maceina et al., 1992) and changes to zooplankton communities (Mitchell et al., 1984). However, effects on zooplankton are variable and not well studied on a regional scale (Mitchell et al., 1984; Maceina et al., 1992; Pipalova, 2006).

Translocations of grass carp are recognised as a vector for the co-introduction of hitch-hiker species (Deacon, 1988; Duggan et al., 2014). For example, the arrival of the Asian tapeworm (*Bothriocephalus acheilognathi*) in the USA is attributable to importation with infected grass carp (Deacon, 1988). The subsequent infection of baitfish cultured in ponds where grass carp were held, and the release of the infected baitfish into natural waters, resulted in parasite transfer to vulnerable native fish species (Deacon, 1988; Fuller et al., 1999).

In a longitudinal study of zooplankton communities in Lake Kereta, New Zealand, Duggan et al. (2014) found that *S. pallidus* had established following the intentional introduction of 15000 grass carp over four releases in 2008-2009 (de Winton, 2012; Duggan et al., 2014). While not found in Lake Kereta prior to grass carp introduction, *S. pallidus* was in high relative abundance after the releases. A decline in the relative abundance of the native calanoid copepod *Calamoecia lucasi* followed the establishment, indicating that *S. pallidus* had the potential to displace native species. While the earliest detection in New Zealand occurred in two constructed ponds with no known authorised grass carp introductions, further populations of *S. pallidus* were found in lakes that had been subject to grass carp releases (e.g., Lakes Rotomanu and Omapere in the North Island and Lake Hood in the South Island), indicating that grass carp translocations from aquaculture facilities were the probable vector for the movement of *S. pallidus* in New Zealand; however, the extent of the introductions and effects are not known and require investigation (Duggan et al., 2006; Banks & Duggan, 2009). In 2015, a systematic survey of New Zealand aquaculture facilities confirmed that *S. pallidus* was in ponds used to hold grass carp, strengthening the link between the invasion of this copepod and grass carp introductions (Duggan & Pullan, 2016, in review).

Since its discovery in New Zealand, non-indigenous populations of *S. pallidus* have been recorded in Germany and Mexico with the species now considered an emerging intercontinental invader (Duggan et al., 2006; Brandorff, 2011; Suarez-Morales & Arroyo-Bustos, 2012). The invasion by *S. pallidus*, into sites adjoining the Weser River in Bremen, Germany, was first recorded in 2010 (Brandorff, 2011). There, introduction through a shipping related vector was considered most likely as there had been considerable shipping traffic into Bremen during the twentieth century (Brandorff, 2011). However, as the vector of introduction is not certain, further investigation is required in order to better determine the mode of introduction and any further risk.

Use of molecular diagnostics to examine invasions

Genetic techniques provide a molecular diagnostic tool to investigate possible vectors of introduction by identifying the geographic origins of non-native populations (Hebert et al., 2003; Comtet et al., 2015). Comparison of barcodes (haplotypes) from native ranges and invaded sites can be used to form hypotheses relating to invasion vectors and histories (Makino et al., 2010; Duggan et al., 2012; Kotov & Taylor, 2014). For example, nucleotide sequencing of the mitochondrial gene cytochrome *c* oxidase subunit I (COI) has successfully been utilised to distinguish the comparative intra-specific diversity among native *Sinodiaptomus valkanovi* calanoid copepod populations in Japan and non-native populations in New Zealand, revealing the likely invasion pathway (Makino et al., 2010). Barcoding methodology was used to identify the native North American region of origin of *Daphnia pulex*, which had become widely distributed in South Island lakes of New Zealand since detection there in 2005 (Duggan et al., 2012).

Studies examining the genetic diversity of *S. pallidus* within its native distribution in North America indicate that there are four major divergent clades distributed across its native range (Thum & Derry, 2008; Thum & Harrison, 2009). As the data indicate clear genetic structuring, this provides a suitable basis for comparison with non-native populations in New Zealand and Germany to better determine if those populations have originated from a single source.

Thesis outline

In this thesis I: 1) utilise community scale analyses to assess the effects of grass carp translocations on zooplankton communities at a regional scale; and 2) use molecular techniques to determine if the intercontinental invasion of the North American calanoid copepod *Skistodiaptomus pallidus* in New Zealand and Germany, originated from a single source.

My first research chapter tests the effects of grass carp translocations from aquaculture facilities on ponds in the Auckland region, New Zealand. The aims of this chapter are to determine if there are effects on zooplankton community composition due to: 1) introductions of non-native zooplankton species in conjunction with fish releases; and 2) habitat modification through the action of grass carp.

In my second research chapter, I compare sequences of a portion of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene from non-native populations of *S. pallidus* in New Zealand and Germany with sequences from the native North American range. The aim of this chapter is to determine if the intercontinental invasion of the calanoid copepod *S. pallidus* in New Zealand—which has spread in association with aquaculture activities—and Germany, originated from a single source in North America.

In Chapter IV, I summarise the findings and management implications of the two research chapters. I provide suggestions for future study ongoing from the results of my research.

Chapters II and III have been written as independent manuscripts for submission to international journals, and, as such, they have been formatted in the style required for publication in the journals: *Biological Invasions* and *Aquatic Invasions*, respectively. There is, consequently, some repetition of key information relating to the distribution of *S. pallidus* between chapters.

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Chapter II

Grass carp (*Ctenopharyngodon idella*) translocations from aquaculture ponds alter zooplankton communities in New Zealand ponds*

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Abstract

The movement of fish from aquaculture facilities has led to invasions of non-native species globally, including of ‘hitch-hiking’ species, resulting in impacts on the composition of communities in recipient ecosystems. We tested the effects of grass carp translocations from aquaculture facilities to ponds in the Auckland region, New Zealand. Zooplankton community composition was quantified in 34 ponds that had been subject to grass carp release and 31 which had no grass carp introductions. A significant difference in zooplankton community composition was observed between ponds that had received grass carp translocations and those that had not. Differences in zooplankton community composition between ponds with and without carp releases were attributable to: 1) the establishment of zooplankton originating from aquaculture ponds, including non-native species; and 2) the effects of activity of grass carp through habitat modification. Effective measures to curb the proliferation of non-native taxa within aquaculture facilities, and to mitigate the accidental movement of non-native taxa with translocations from these facilities, are required to reduce future introductions.

Introduction

Translocation of aquatic organisms across biogeographic barriers as a result of aquaculture activities has resulted in the establishment of numerous non-native species, both deliberately and unintentionally transported, into novel recipient ecosystems (Rahel 2007; Strayer 2010). The range of taxa intentionally transported and introduced by this vector, into both marine and freshwater environments, is extensive, and has included fish, molluscs, crustaceans and algae (Reid and Pinto-Coelho 1994; Grosholz et al. 2015). Intentional releases have frequently been accompanied by 'hitch-hiking' species carried incidentally. For example, importations for aquaculture of abalone from South Africa and oysters from the Atlantic and Pacific regions into California have resulted in the unintentional introduction of bivalve predators, pathogens and parasitic polychaete worms (Grosholtz et al. 2015). Translocations of freshwater taxa for utilisation in aquaculture have also been implicated in the coincidental introduction of zooplankton into new regions (Ferrari et al. 1991; Havel and Hebert 1993). In northern Italy, for example, the Australasian calanoid copepod *Boeckella triarticulata* was first detected in aquaculture ponds stocked with channel catfish (*Ictalurus punctatus*) in 1985 (Ferrari et al. 1991). Subsequent spread of *B. triarticulata* has occurred into the south of Italy, initially into constructed ponds, and with later detection in the natural waters of the River Po (Ferrari and Rossetti 2006; Alfonso and Belmonte 2008). Following the establishment of *B. triarticulata*, shifts were observed in zooplankton communities in invaded water bodies, including reductions in microfilter feeders (cyclopoid nauplii and rotifers) and reduced phytoplankton biomass (Ferrari et al. 1991).

The establishment of non-native zooplankton can alter pre-existing species assemblages through a number of mechanisms, including competition for food, direct predation and transference of parasites or pathogens as well as changes induced by exploitation of habitat, with potential displacement of native species and global homogenisation of assemblages (Strayer 2010; Briski et al. 2012). The invasion of *Daphnia lumholtzi* into North America is an example of the importation of fish stocks for aquaculture being the likely source of the coincidental introduction of a species with the potential to affect native communities (Havel and Hebert 1993). *Daphnia lumholtzi* was first detected in a reservoir in Texas following introductions of blue tilapia (*Oreochromis aureis*) and Nile perch (*Lates niloticus*) (Havel and Hebert 1993). The perch were imported from Lake Victoria, Africa, where *D. lumholtzi* was known to be abundant (Sorensen and Sterner 1992; Havel and Hebert 1993). This competitive cladoceran has since colonised much of North America, and recent genetic analyses indicate subsequent spread from North America into South America as far south as Argentina (Havel and Hebert 1993; Engel et al. 2014; Kotov and Taylor 2014).

In New Zealand, the spread of the North American calanoid copepod *Skistodiaptomus pallidus* has been linked to translocations of domestically bred grass carp (*Ctenopharyngodon idella*) (Duggan et al. 2014). Based on regular sampling of Lake Kereta in Auckland, *S. pallidus* was first detected in 2008 following grass carp introductions for the control of non-native macrophytes (Duggan et al. 2014). Following the establishment of *S. pallidus* in Lake Kereta, a decline was recorded in the relative abundance of the native calanoid copepod *Calamoecia lucasi* (Duggan et al. 2014). This was the first recorded occurrence of *S. pallidus* in a natural lake in New Zealand, following earlier detections among live food for fish and in a number of constructed water bodies of disjunct distribution (Duggan et al. 2006; Banks and Duggan 2009). Further populations of *S. pallidus* were subsequently found in lakes that had been subject to grass carp introductions (Lakes Omapere and Rotomanu, North Island, and Lake Hood, South Island), indicating that grass carp translocations were likely an active vector for the movement of *S. pallidus*, and potentially other zooplankton species (Duggan et al. 2014). A systematic survey of New Zealand aquaculture facilities in 2015 found *S. pallidus* to be present in ponds on several farms holding grass carp (Duggan and Pullan 2016, in review).

Grass carp are native to Asia and are one of the ten most commonly utilised species in aquaculture globally, primarily for the control of unwanted aquatic macrophytes (Pipalova 2006; Bartley 2011). The importation of grass carp into New Zealand first occurred in 1966 and following assessment of the species' suitability for aquatic weed control during the 1980s, domestic translocations have been regularly made for that purpose (Mitchell et al. 1984; Hofstra et al. 2014). The effectiveness of grass carp in the management of aquatic plants is well documented, with reported macrophyte reduction rates between 30-100% over a single year for grass carp stocking rates of 20-100 kg/ha (Mitchell et al. 1984; Richard et al. 1985; Pipalova et al. 2009). Research on the impacts of grass carp introductions on zooplankton communities have generally been through longitudinal studies, which have found that effects on abundance and composition have been of little or no significance (e.g., Maceina et al. 1992; Pipalova et al. 2009). Mitchell et al. (1984), however, found that abundances of cladoceran and rotifer species increased, which was likely due to increases in the rates of nutrient cycling and densities of phytoplankton following rapid macrophyte consumption by grass carp. Decreases in rotifer and cladoceran abundances in association with macrophyte reduction have also been reported, indicating a range of responses across studies (Maceina et al. 1992). The greatest reported impacts on zooplankton communities have been those resulting from the reduction or elimination in macrophyte cover by grass carp, which have produced shifts from plant associated taxa to those associated with planktonic conditions (Richard et al. 1985).

Regional scale investigations into the extent and effects of the coincidental movement of zooplankton species with non-native fish translocations, and the effects of macrophyte reduction by grass carp on zooplankton communities, is limited. Here, we test the effects of grass carp translocations from aquaculture facilities on zooplankton communities in two sets of ponds in the Auckland region, New Zealand, which have either been subject to grass carp releases, or have not. The aims of this study were to determine if there are effects on zooplankton community composition due to: 1) introductions of non-native zooplankton species in conjunction with fish releases; and 2) habitat modification through the action of grass carp.

Methods

Pond selection

Two groups of constructed ponds from the Auckland region were selected. The first group comprised 34 ponds that had been subject to one or more translocations of grass carp (hereafter: ‘carp ponds’), while the second group of 31 ponds had no known introductions of carp (hereafter: ‘non-carp ponds’). Ponds already known to have *S. pallidus* were excluded from selection. Carp ponds were identified based on release information provided by Auckland Council, Hofstra (2014) and S. Pullan (Ministry for Primary Industries, pers. comm.). Several carp ponds had also received translocations of silver carp (*Hypophthalmichthys molitrix*) stocked at low densities, similarly originating from aquaculture facilities. Initial selection of ponds for the non-carp dataset was made from a database of water-bodies in the Auckland region provided by Auckland Council, and from a systematic search of the Freshwater Ecosystems of New Zealand (FENZ) geo-database (Department of Conservation). Measures were employed to ensure that the two groups of ponds had similar environmental characteristics in order to minimise bias and to allow for comparisons between them. As such, a stratified random selection process was used because, although a random choice of ponds was important, the study required the final datasets (carp and non-carp ponds) to represent a similar range of surface areas, latitudes and ages. For example, using GIS tools, the surface area of each pond was measured. Ponds with areas greater than 0.05 ha were sorted into two categories: those less than 0.5 ha, or those greater than or equal to 0.5 ha. Ponds from each category were then selected. A similar process was employed for ponds from three latitudinal sub-regions from north to south Auckland. Ponds constructed less than eight years prior to sampling were excluded, commensurate with the age of the most recently constructed carp pond. To ensure independence of all sites, ponds with connectivity within one kilometre of any other carp or non-carp pond were excluded. To determine the probability of establishment by zooplankton by natural dispersal, the number and total surface area of freshwater bodies within a one kilometre radius of each selected carp and non-carp pond were calculated.

Sampling

A total of 69 ponds were sampled, with the final groups comprising 34 carp and 31 non-carp ponds (4 selected ponds were excluded due to higher levels of salinity). Sampling at each site took place during January and February 2015 (austral summer). Samples from shallow ponds were collected by wading, and where steep banks prevented wading, samples were collected from structures such as jetties, or the water's edge. Quantitative zooplankton samples were collected by ten replicated fillings of a 2 L container and subsequent filtering with a plankton net with a mesh size 40 µm, resulting in a total of 20 L filtered per sample. In order to more precisely determine the identification of calanoid copepods, which are common invaders in New Zealand, sufficient numbers of adult male specimens (which may be in low abundance) were required. Accordingly, additional samples were collected using horizontal net hauls from the pond edge with a plankton net (75 µm mesh-size, at a haul speed of approximately 1 $m.s^{-1}$) until a concentrated zooplankton sample was obtained. Immediately following collection, each sample was transferred to a container and preserved with ethanol (final concentration 70%). A visual estimate was made of the percentage of macrophyte cover within each pond. For analysis of total phosphorus (TP) and total nitrogen (TN), a water sample was collected from each pond in a 50 mL tube. Nutrient samples were stored frozen until analysed within one week of collection. For chlorophyll *a* analysis, a known volume of water (50 mL for ponds with lower algal content and 20 mL for ponds with higher algal content) was collected from each pond in a 60 mL syringe. This sample was immediately filtered through a 0.2 µm Whatman GF/C glass microfiber filter at low vacuum. The microfiber filter was then folded in half with the upper side inner-most. Each sample was wrapped in foil to prevent light exposure and placed into a plastic container on ice prior to being transferred to a laboratory freezer, where it was stored until analysis of chlorophyll *a*. Dissolved oxygen was measured in situ using a Yellow Springs Instruments (YSI) Model 55 handheld meter. Temperature and specific conductivity were measured using an YSI Model 30 meter. For each pond with adequate depth, a measurement of water clarity was made using a 25 cm Secchi disk.

Laboratory analyses

In the laboratory, taxa in the 20 L zooplankton samples were enumerated in 5 mL aliquots until a minimum of 300 individuals or the entire sample was counted. For identification, individuals were examined using a stereo dissecting microscope and compound microscope at up to 400x magnification and identified wherever possible to species level using available taxonomic keys (e.g., Koste 1978; Shiel 1995; Bayly 1992; Chapman et al. 2011). Due to the difficulty in ascertaining identifications of some taxa, immature copepods were not included in analyses. To better determine the distribution of *S. pallidus* in carp and non-carp ponds, calanoid copepods were identified from presence/absence samples. Nutrient concentrations were obtained using the persulfate method for simultaneous determination of TN and TP using a Flow Injection Analyser 8500 series II. Chlorophyll *a* was extracted by grinding each filter in a buffered acetone solution. Samples were then shaken for three seconds prior to being stored in darkness for approximately 20 hours, during which they were shaken a second time. Following the extraction period, samples were again shaken, centrifuged for 10 minutes at 1522 G using a Beckman GS-6R centrifuge, and left to stand for 30 minutes at room temperature. Readings were taken for supernatant using a Turner Designs 10 – AU fluorometer, then, to correct for phaeophytin degradation, 0.1 N hydrochloric acid solution was added and readings were taken for the acidified supernatant. Chlorophyll *a* was calculated in a pre-programmed spreadsheet based on calibration curves derived from samples of known concentrations. Trophic Level Index (TLI) values were calculated for each pond incorporating TN, TP, Secchi depth and chlorophyll *a* measurements using the equation from Burns et al. (1999). Where the shallow depth of ponds prevented a Secchi disk measurement being recorded, TLI was calculated based only on the other three variables.

Statistical analyses

Environmental variables for carp and non-carp ponds were compared using *t*-tests in Statistica (Statsoft Inc., Tulsa USA). Where data were non-normally distributed, these were transformed using log10, log(x+1) or x^2 transformations. Differences in the zooplankton community composition between carp and non-carp ponds in the Auckland region were assessed using non-metric Multidimensional Scaling (nMDS) and analysis of similarities (ANOSIM) (PRIMER v.6.1.13; Primer-E Ltd 2009). Only taxa comprising not less than 0.5%

of the total abundance in 2 or more samples were included in analyses, so as to reduce the influence of species sampled by chance. Abundance data were log(x+1) transformed to reduce any undue contribution of species with high abundance. Using PRIMER, nMDS was conducted on a similarity matrix based on the Bray-Curtis similarity coefficient calculated on the transformed data, producing a 2-dimensional representational plot of all samples based on their similarity to each other. The goodness of the fit of the plot in relation to the distance matrix is indicated by the stress value, with a stress value of zero indicating a perfect fit. To test for statistically significant differences in the zooplankton communities between carp and non-carp samples, ANOSIM was conducted (999 permutations executed) on the Bray-Curtis similarity matrix. This method produces a *P*-value, as well as an R-statistic (typically between 0 and 1), with an R-value approaching 1 indicating greater dissimilarity between zooplankton community composition than a value near 0. To determine the taxa most responsible for differences in community composition between datasets, for each taxon, the percentage occurrences in carp ponds and non-carp ponds was calculated, along with the difference between the two percentages.

Results

Environmental variables

The percentage of macrophyte cover and dissolved oxygen concentrations were significantly higher in the non-carp ponds (Table 1). No significant differences between carp and non-carp ponds were found for chlorophyll *a*, specific conductivity, temperature, pond area and age, indicating that the carp and non-carp pond groups were comparable in these characteristics. The concentrations of TN and TP were significantly higher in the ponds subject to grass carp releases. However, the TLI did not differ significantly between carp and non-carp ponds. All carp ponds were in the range of eutrophic TLI or higher (>4), as were most of the non-carp ponds, with the exception of three, which were mesotrophic (3-4). Further, there was no significant difference between the groups in relation to the number of ponds or surface area of water within one kilometre, indicating all ponds have a similar probability of zooplankton establishment by natural dispersal (Table 1).

Table 1. Mean values and standard deviations of environmental variables in Auckland ponds that have had no grass carp introductions (Non-Carp) and those which have been subject to grass carp introductions (Carp). Bolded t-test *P*-values indicate a significant difference between groups.

Environmental variable	**Non-carp mean**	**Std dev**	**Carp mean**	**Std dev**	***t*-test *P*-value**
Macrophyte cover (%)	39.84	38.57	13.53	22.75	**0.005**
Total phosphorus (mg/L^{-1})	0.08	0.07	0.13	0.11	**0.006**
Total nitrogen (mg/L^{-1})	0.77	0.42	1.03	0.63	**0.034**
Chlorophyll *a* (mg/L^{-1})	18.73	23.41	14.26	12.73	0.959
Trophic Level Index (TLI)	5.05	0.84	5.38	0.61	0.069
Dissolved oxygen (mg/L^{-1})	9.57	3.63	7.26	2.11	**0.005**
Specific Conductivity (µS/cm@ 25°C)	228.47	94.86	245.96	67.22	0.214
Temperature (^{o}C)	25.69	1.70	25.77	1.91	0.848
Water body age (yr)	21.74	15.12	26.88	27.11	0.429
Water body area (Ha)	0.50	0.51	0.76	1.15	0.215
Water bodies within 1 km	3.67	2.50	3.76	2.52	0.841
Water area within 1 km (Ha)	6.45	20.22	1.30	1.29	0.497

Bold *P*-values indicate statistical significance (*a*=0.05), N=65

Community composition

The nMDS plot indicated that there was a difference in zooplankton community composition between the carp and non-carp ponds, with carp ponds distributed primarily to the top-left of the ordination and non-carp ponds distributed towards the bottom-right. Some overlap is evident between the two groups (Figure 1). The ANOSIM results indicate there was a significant difference in zooplankton community composition between carp and non-carp ponds (Global *R*-statistic = 0.111, *P* = 0.001). The 2-dimensional stress of 0.25 indicates that the nMDS provides an informative depiction of the data when the large sample size is considered (Clarke and Warwick 1994).

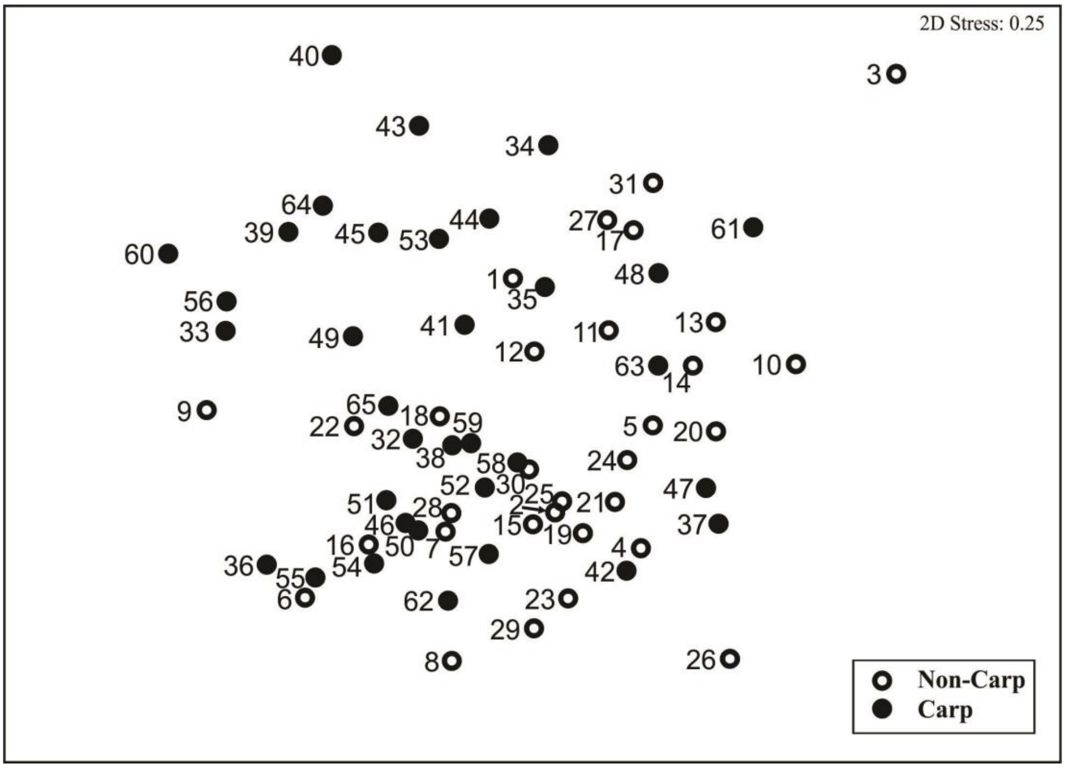

Figure 1. Non-metric Multidimensional scaling (nMDS) plot representing comparative zooplankton communities within Auckland ponds which had received no grass carp introductions (Non-Carp) or were subject to grass carp introductions (Carp).

Table 2. Key to numbers on the non-metric Multidimensional Scaling plot and geo-references for Auckland ponds that have received no grass carp introductions (Non-Carp; numbers 1-31) and ponds that have been subject to grass carp introductions (Carp; 32-65).

No.	**Non-Carp Ponds**	**Latitude**	**Longitude**
1	Alfriston	37° 01’ 08.82”S	174° 54’ 36.37”E
2	Arkles Bay	36° 38’ 35.64”S	174° 44’ 33.15”E
3	Bruce Pulman	37° 02’ 44.00”S	174° 56’ 32.07”E
4	Burr Verissimo	36° 57’ 39.11”S	174° 55’ 42.48”E
5	Burswood	36° 55’ 16.83”S	174° 53’ 53.47”E
6	Ernies Reserve	37° 11’ 31.57”S	174° 54’ 36.43”E
7	Glen Innes	36° 52’ 51.07”S	174° 52’ 01.92”E
8	Henderson Park	36° 52’ 37.65”S	174° 37’ 35.81”E
9	Lake Panorama	36° 53’ 00.23”S	174° 36’ 16.54”E
10	Lough Bourne	37° 11’ 08.98”S	174° 53’ 47.91”E
11	Lucas Creek	36 ° 43’13.47”S	174° 43’ 04.77”E
12	Mary Poynton	36° 47’ 06.39”S	174° 45’ 28.11”E
13	Maungarei Springs	36° 53’ 23.28”S	174° 50’ 07.70”E
14	McLennan Park	37° 03’ 17.38”S	174° 56’ 39.11”E
15	Murphys	36° 57’ 55.97”S	174° 55’ 41.29”E
16	Nassipour Way	36° 56’ 00.25”S	174° 52’ 45.52”E
17	Ormiston	36° 57’ 53.30”S	174° 53’ 58.19”E
18	Oteha Valley	36° 43’ 57.49”S	174° 43’ 33.58”E
19	Paremuka Park	36° 52’ 12.71”S	174° 36’ 45.22”E
20	Pavillion	36° 58’ 52.91”S	174° 46’ 37.54”E
21	Peninsula Park	36° 58’ 47.51”S	174° 48’ 04.39”E
22	Quarry lake	36° 47’ 06.36”S	174° 45’ 28.52”E
23	Ranui Park	36° 51’ 54.98”S	174° 35’ 54.92”E
24	Riverstone Road	36° 50’ 32.48”S	174° 39’ 30.24”E
25	Rosedale Detention	36° 44’ 19.08”S	174° 43’ 23.97”E
26	Spartan Road	37° 02’ 09.58”S	174° 54’ 56.51”E
27	Tom Pearce	36° 59’ 56.91”S	174° 47’ 36.92”E
28	Toplands	36° 57’ 39.79”S	174° 55’ 20.51”E
29	Unitech	36° 52’ 43.48”S	174° 42’ 31.83”E
30	Waikumete	36° 54’ 05.33”S	174° 39’ 14.06”E
31	Westney	36° 59’ 07.67”S	174° 47’ 50.04”E

Table 2 (continued).

No.	Carp Ponds	Latitude	Longitude
32	Bayside	36° 43’ 23.65”S	174° 44’ 21.38”E
33	Chelsea Pond 4	36° 49’ 12.00”S	174° 43’ 29.00”E
34	Cyril French	36° 57’ 16.09”S	174° 54’ 37.92”E
35	De Havilland	36° 59’ 45.70”S	174° 53’ 31.48”E
36	Hayman	36° 59’ 34.59”S	174° 52’ 30.73”E
37	Kellaway	36° 56’ 12.56”S	174° 54’ 25.67”E
38	Link Drive	36° 45’ 57.27”S	174° 44’ 20.91”E
39	Lloyd Elsmore	36° 54’ 10.76”S	174° 54’ 13.30”E
40	Lochview	37° 11’ 13.41”S	174° 54’ 09.86”E
41	Logan Carr	36° 55’ 53.48”S	174° 54’ 58.46”E
42	Maygrove	36° 35’ 21.97”S	174° 41’ 04.11”E
43	Montgomerie North	36° 59’ 05.93”S	174° 46’ 46.03”E
44	Montgomerie South	36° 59’ 07.38”S	174° 46’ 46.35”E
45	North Shore Golf Club	36° 44’ 43.06”S	174° 41’ 19.49”E
46	Preston	36° 58’ 03.76”S	174° 53’ 27.09”E
47	Puhinui Domain	36° 59’ 25.01”S	174° 51’ 52.59”E
48	Puhinui Reserve	37° 00’ 38.10”S	174° 50’ 13.35”E
49	Regency Park	36° 36’ 35.77”S	174° 47’ 22.36”E
50	Rosedale East	36° 44’ 33.95”S	174° 41’ 55.71”E
51	Rosedale West	36° 44’ 36.10”S	174° 41’ 43.69”E
52	Sancta Maria Long	36° 57’ 26.67”S	174° 54’ 26.14”E
53	Sancta Maria Triangle	36° 57’ 23.63”S	174° 54’ 23.07”E
54	Sherwood	36° 43’ 03.75”S	174° 44’ 11.98”E
55	Stredwick lower	36° 42’ 02.47”S	174° 43’ 58.06”E
56	Tahuna Torea	36° 52’ 20.49”S	174° 52’ 55.41”E
57	Totara Park Long	37° 00’ 09.86”S	174° 55’ 01.37”E
58	Totara Park Square	37° 00’ 09.71”S	174° 54’ 54.28”E
59	Trias Road	36° 45’ 36.77”S	174° 43’ 53.49”E
60	Unsworth Lower	36° 45’ 33.39”S	174° 43’ 01.80”E
61	Waiatarua Reserve	36° 52’ 55.40”S	174° 49’ 34.00”E
62	Wattle Farm West	37° 02’ 25.88”S	174° 53’ 29.71”E
63	Western Springs	36° 51’ 59.07”S	174° 43’ 28.28”E
64	Whakamaumahara	36° 55’ 55.16”S	174° 54’ 34.77”E
65	Wiri Lower	36° 59’ 54.26”S	174° 52’ 53.14”E

Taxa in carp or non-carp ponds

A total of 101 zooplankton taxa were identified in this study, with a mean of 14 taxa in carp samples and 17 in non-carp samples. Twenty-five taxa were present in single ponds only. With the exception of *Microcodides robustus*, present in McLennan Pond, all have been previously recorded in New Zealand (Shiel et al. 2009). *Microcodides robustus* has an almost cosmopolitan distribution globally, with the exclusion of Africa to date, but it is rarely encountered (Segers 2007). It is described by Shiel (1995) as rare in Australia, with two records of occurrence there.

Differences in community composition between carp and non-carp datasets were evident. Notably, there was a greater proportion of three non-native species in carp ponds than in non-carp ponds: *Daphnia pulex*, *D. galeata* and *S. pallidus*. *Daphnia pulex* was present only in ponds that had grass carp releases, present in 35% of all carp ponds (Table 3). Those ponds were distributed broadly across the study area (Figure 2). As *D. pulex* has not previously been recorded in the North Island, these 12 records are new for this species. *Skistodiaptomus pallidus* was widespread in the study area and more commonly found in ponds that had been subject to grass carp releases, being present in 29% of the carp ponds, and in 10% of non-carp ponds; the non-carp ponds with *S. pallidus* were all situated in close proximity to one another in Albany, North Auckland (Figure 2). All of these sites are new records for the distribution of *S. pallidus* in New Zealand. The second species of North American *Daphnia* found, *D. galeata*, was recorded at seven sites (Figure 3) distributed across the study area, with a greater proportion of them (five) being carp ponds. The distribution of indigenous *Brachionus lyratus*, recorded in seven carp ponds and three non-carp, was also notable as it showed a similar pattern to the distribution of the non-native *S. pallidus.*

Taxa that were present in higher percentages of carp ponds were primarily species associated with open-water conditions. In addition to the aforementioned non-native crustaceans, planktonic taxa in higher proportions of carp ponds included the rotifer species *Keratella slacki*, *K. tropica*, *B. lyratus*, *B. calyciflorus* and *Filinia novaezealandiae*, as well as the predatory *Asplanchna brightwelli.* Taxa in high percentages of non-carp ponds were primarily benthic and plant associated, and included three species of *Lecane: L. bulla*, *L. closterocerca* and *L.*

hamata. The smaller cladocerans, *Chydorus sphaericus* and *Alona* sp., were more highly represented in ponds with no grass carp releases, as was the larger *Simocephalus vetulus*, although this was less common. The rotifer species *Cephalodella gibba*, *Lepadella ovalis* and *Anuraeopsis fissa* were also found in a greater percentage of the non-carp than carp ponds.

A second pattern of distribution was evident in the geographic range of the populations of the non-indigenous calanoid copepod *Boeckella symmetrica,* native to Australia, which had no strong relationship to the release of grass carp. We found *B. symmetrica* at eight sites where they had not previously been recorded – in ponds in the southeast of the study area, as well as their continued presence in the Puhinui Reserve pond (Figure 3). Four sites with *B. symmetrica* were non-carp ponds and the other five had been subject to carp translocations. The Japanese *Sinodiaptomus valkanovi* was recorded in Logan Carr pond (a carp pond), which constitutes a new location for the species within New Zealand. While non-native calanoid copepods were widespread, the only native calanoid copepod species recorded was *Calamoecia lucasi*, which was present in only three ponds: Lake Panorama, Quarry Lake and Mary Poynton. None of these ponds have been subject to grass carp introductions.

Table 3. Percentage of Auckland ponds in which zooplankton taxa were present for Carp (grass carp released) and Non-Carp (no grass carp released) groups, and the percentage difference between the groups. Taxa with the most positive and most negative percentages are shown; species were included if present in > 10% of ponds in either group.

Taxa	**% of Carp ponds with taxa**	**% of Non-Carp ponds with taxa**	**% difference of Carp from Non-Carp**
			Positive
Daphnia pulex	35.29	0.00	35.29
Asplanchna brightwelli	44.12	19.35	24.76
Skistodiaptomus pallidus	29.41	9.68	19.73
Keratella slacki	55.88	38.71	17.17
Brachionus lyratus	20.59	9.68	10.91
Brachionus calyciflorus	55.88	45.16	10.72
Keratella tropica	29.41	19.35	10.06
Filinia novaezealandiae	38.24	29.03	9.20
Daphnia galeata	14.71	6.45	8.25
			Negative
Lecane hamata	11.76	25.81	-14.04
Simocephalus vetulus	5.88	22.58	-16.70
Anuraeopsis fissa	44.12	61.29	-17.17
Ostracods	52.94	70.97	-18.03
Cephalodella gibba	5.88	25.81	-19.92
Chydorus sphaericus	35.29	58.06	-22.77
Lepadella ovalis	29.41	54.84	-25.43
Alona sp.	20.59	48.39	-27.80
Lecane closterocerca	14.71	48.39	-33.68
Lecane bulla	55.88	90.32	-34.44

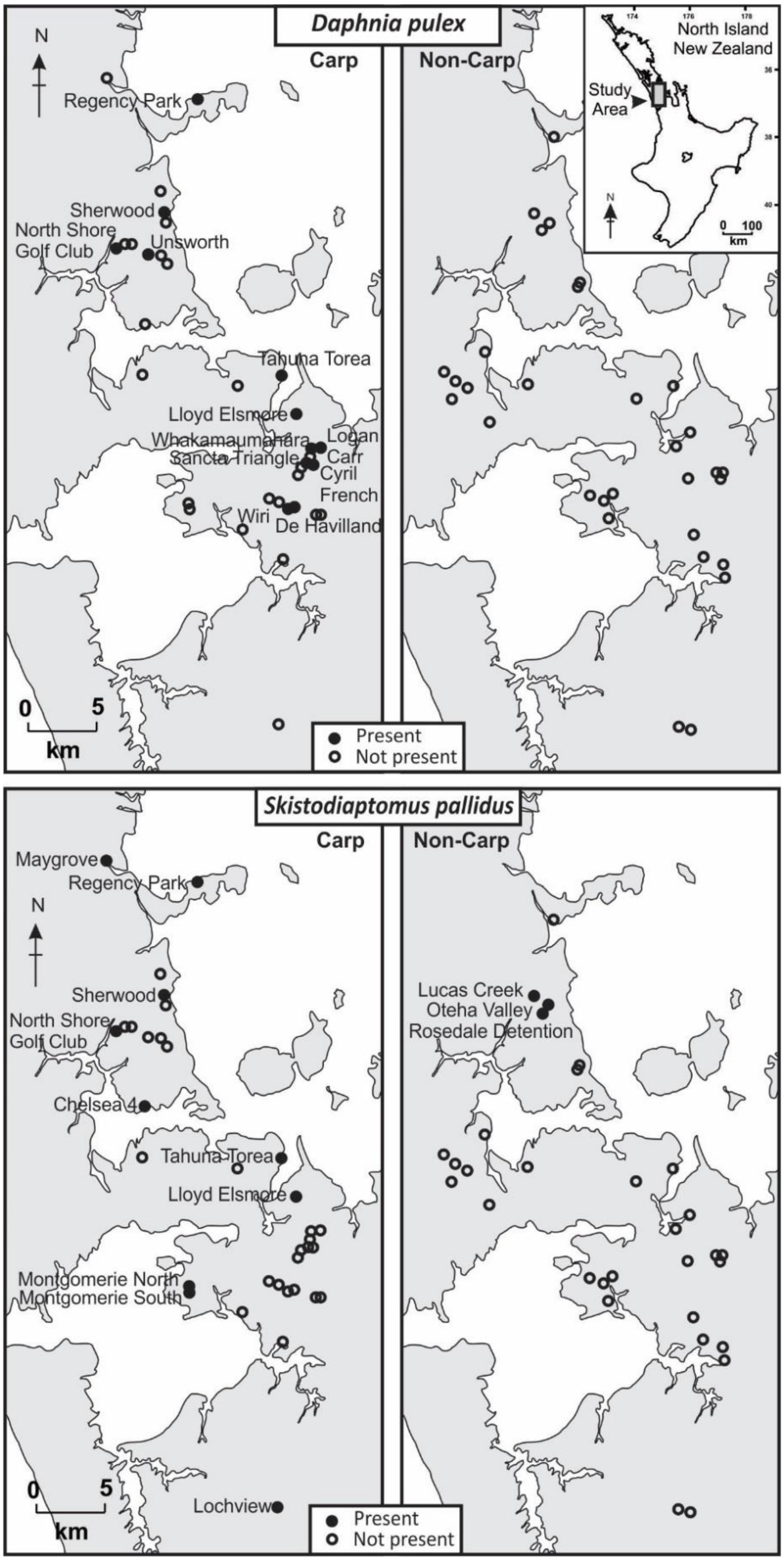

Figure 2. Distributions of the non-native crustaceans *Daphnia pulex* (top) and *Skistodiaptomus pallidus* (bottom) in Auckland ponds, 31 of which had received no grass carp introductions (Non-Carp) and 34 which had been subject to grass carp translocations (Carp). Ponds are named and marked with filled circles if the species was present at that site.

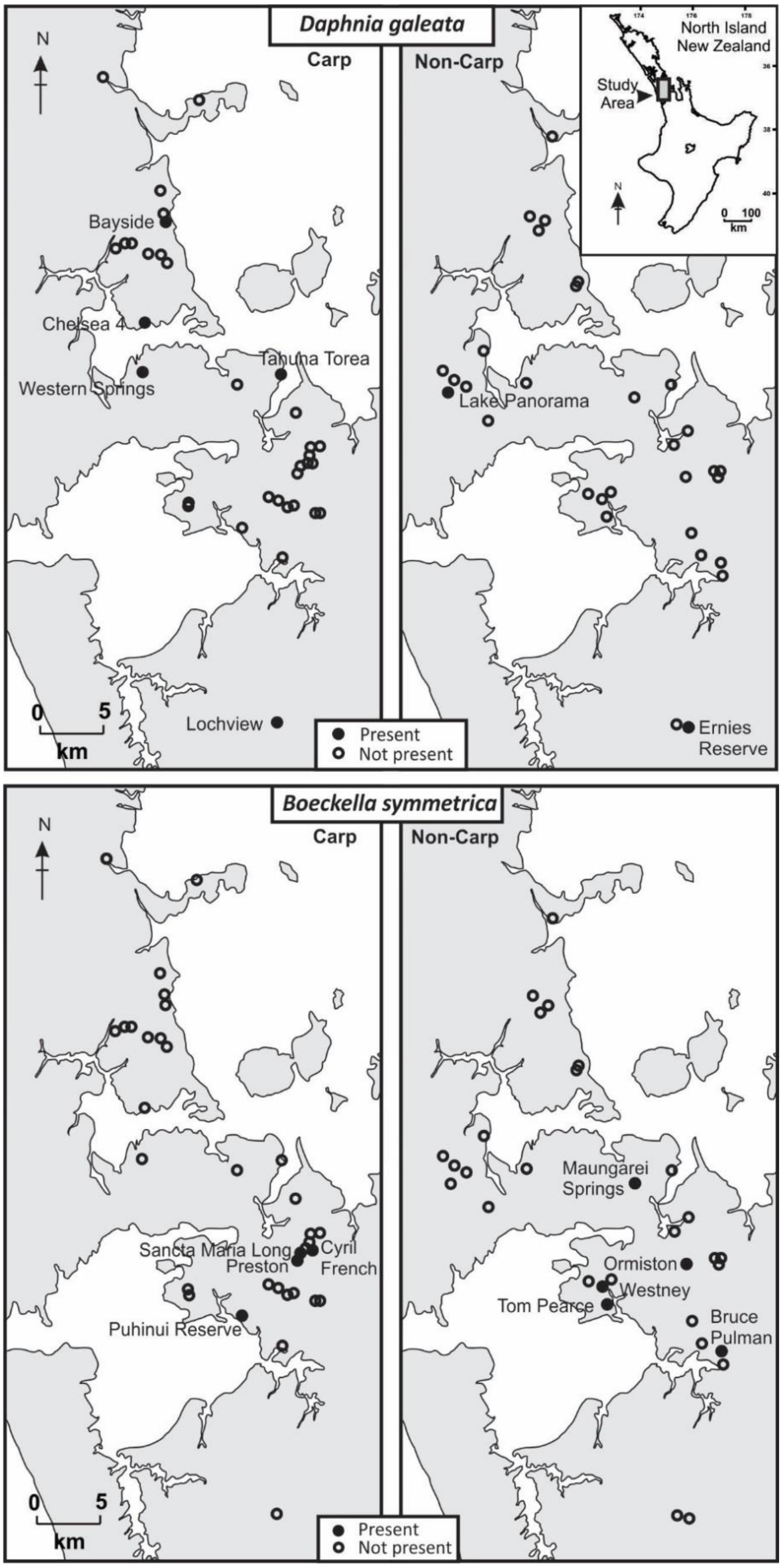

Figure 3. Distributions of the non-native crustaceans *Daphnia galeata* (top) and *Boeckella symmetrica* (bottom) in Auckland ponds, 31 of which had received no grass carp introductions (Non-Carp) and 34 which had been subject to grass carp translocations (Carp). Ponds are named and marked with filled circles if the species was present at that site.

Discussion

We observed a significant difference in zooplankton community composition between ponds that had, and those that had not, received grass carp translocations from aquaculture facilities. As other key physical characteristics (e.g. pond size and age) of the two groups of ponds were comparable, there are two likely explanations for the differences in community composition: 1) direct effects related to the introduction and establishment of zooplankton from farm ponds; and 2) indirect effects caused by the activity of grass carp through habitat modification.

Direct effects of species introductions

The presence of non-native species in a higher proportion of carp ponds compared to non-carp ponds indicated that these species were likely to have been derived from aquaculture facilities and moved with water in which grass carp were transported. Example taxa included two non-native planktonic cladocerans, *D. pulex* and *D. galeata*, and the calanoid copepod *S. pallidus*. *Daphnia pulex* was present in 35% of ponds where carp were released and was not recorded in any of the ponds without carp. Previously, the recorded New Zealand distribution for *D. pulex* was limited to the South Island, with the species expanding its range there from 2005 onwards (Duggan et al. 2012).

Established populations of *S. pallidus* in ten ponds, comprising 29% of all sites with carp releases, and establishment in three (10%) non-carp ponds, indicates a strong relationship between grass carp releases and the introduction of *S. pallidus* in Auckland. The establishment of *S. pallidus* in lakes following the release of grass carp had previously been observed in Lakes Kereta, Omapere and Rotomanu in the North Island, as well as in Lake Hood in the South Island (Duggan et al. 2006; Duggan et al. 2014). The link between grass carp translocations and the coincidental introduction of non-native species became evident when *S. pallidus* was detected in these lakes which, although geographically disjunct, shared a common factor of having received grass carp for macrophyte control (Duggan et al. 2014). Furthermore, *S. pallidus* was one of a number of zooplankton species established in ponds used to hold grass carp, reported in a 2015 systematic survey of aquaculture facilities (Duggan and Pullan 2016, in review). The earliest records of *S. pallidus* in New Zealand occurred in sites that have had no authorised grass carp introductions and include the

Auckland Regional Botanic Gardens and a pond in Albany. However, as grass carp releases have been occurring in Auckland since the 1980s, dispersal of *S. pallidus* by other means such as birds could have occurred from lakes in which *S. pallidus* had previously established (Green and Figuerola 2005; Duggan et al. 2006; Hofstra 2014). The Albany pond, for example, is situated within 2 km of a 3.2 ha sewage detention pond into which 3500 grass carp were released in 2008 (S. Pullan, Ministry for Primary Industries, pers. comm.) and large numbers of birds have been surveyed using the sewage detention site (Don 2015). The three non-carp ponds in which we detected *S. pallidus* were also situated within a three kilometre radius of the sewage and Albany ponds. The sewage detention pond may have acted as a dispersal hub, and for this reason, it was sampled subsequent to the detection of *S. pallidus* in non-carp ponds we surveyed, and was found to have *S. pallidus*.

Another recent colonist, *D. galeata*, was present in both groups of ponds, but was found in 15% of carp relative to only 6% of non-carp ponds, ranging across the Auckland region. *Daphnia galeata* was first recorded in New Zealand in 1993 and its distribution has expanded through the North Island and into the South Island since that time. It has been recorded in facilities used to hold grass carp; further indicating grass carp releases have facilitated its spread (Hofstra 2014; Duggan and Pullan 2016, in review).

The distribution of the rotifer *Brachionus lyratus*, although considered native, was also notable as it showed a similar pattern to the distribution of *S. pallidus*. This was unexpected as *B. lyratus* has a very limited known distribution, being uncommon in lakes in the North Island but occurring in shallow water bodies in the Waikato (Duggan 2007). The presence of *B. lyratus* in aquaculture facilities could provide an explanation for the Auckland distribution (Duggan and Pullan 2016, in review).

The coincidental introduction of non-native species with grass carp translocations is likely to be an important factor in the significant differences in zooplankton communities found between carp and non-carp ponds due to their presence and the induced effects on the community. Of the taxa predominantly associated with grass carp ponds, the introduced crustaceans *D. pulex*, *D. galeata* and *S. pallidus* are species that, following their establishment, are likely to have induced compositional changes to recipient zooplankton communities (Byron and Saunders 1981; Gilbert 1989). One mechanism resulting in effects on communities occurs through exploitative competition as a result of the greater feeding efficiency of comparatively large crustaceans, such as *Daphnia* and *Skistodiaptomus*, than that of smaller competitors, such as rotifers (Hall et al. 1976). *Daphnia* can also have marked effects on assemblages through interference-competition, negatively affecting smaller species such as rotifers in physical encounters (Gilbert 1989). For example, *D. pulex* is a competitive consumer of phytoplankton and has been shown to reduce phytoplankton abundance and affect community structure markedly in enclosure experiments in the USA, particularly in eutrophic conditions where *D. pulex* populations reach high densities (Vanni 1986). Vanni (1986) found that the introduction of *D. pulex* into enclosures within a small lake resulted in a significant reduction in the abundance of smaller species, including cladocerans and rotifer taxa. Balvert et al. (2009) found that the establishment of *D. galeata* in Lake Puketirini, New Zealand, coincided with a reduction in the abundance (or complete absence) of the previously dominant rotifers, while calanoid copepod and *Asplanchna* abundance were not affected. Further, *Daphnia* have the ability within small constructed ponds to utilise unoccupied thermal and trophic niches, and to exploit these through reproductive timing and dietary selectivity, gaining a competitive advantage over native species (Vanni 1986; Engel et al. 2014). For example, Burns (2013) found *D. pulex* could achieve higher densities than the New Zealand native *D. thomsoni* in conditions of higher warmth and food supply. The establishment of *D. pulex* in Lake Hayes, South Island, was followed by the subsequent partial displacement of the native *D. thomsoni*, particularly during summer months (Burns 2013). Despite *D. pulex* being found commonly in carp ponds, it was not found in the survey of New Zealand aquaculture facilities (Duggan and Pullan 2016, in review). Late summer peaks in population densities of *D. pulex* may explain the non-detection of the species, as the surveys of the primary sources of grass carp for release to the Auckland ponds was undertaken in early spring 2015.

Skistodiaptomus pallidus has also been found to have effects on zooplankton communities through competition, with reductions in native species observed following its establishment. For example, Duggan et al. (2014) examined the effects of invasion in a longitudinal study of zooplankton communities in Lake Kereta, Auckland, after *S. pallidus* established there in August 2008 coincident with releases of grass carp. Samples collected over a five year period showed that following establishment of *S. pallidus* there was a significant change in zooplankton community composition. In particular, the native calanoid copepod *Calamoecia lucasi* declined in relative abundance and did not return to abundances recorded prior to *S. pallidus* establishment, even following a subsequent decline in the abundance of the invader (Duggan et al. 2014). Byron and Saunders (1981) found similar impacts on zooplankton communities following the establishment of *S. pallidus* in a eutrophic, semi-enclosed, constructed section of Lake Tahoe Keys in 1979, an invasion well outside of the species' known native range. Following detection of *S. pallidus* there, populations of the calanoid copepods *Leptodiaptomus tyrrelli* and *Epischura nevadensis* were partially displaced as *S. pallidus* increased in abundance (Byron and Saunders 1981). Examination of competitive interactions between similar sized calanoid copepods that have substantial dietary overlap typically indicate co-existence rarely occurs (Hutchinson 1967; Maly and Maly 1997). The overlap in size and consequent competition between *S. pallidus* and *C. lucasi* in New Zealand (and potentially other native copepods) may have negative implications due to a low diversity of native crustacean zooplankton (Chapman and Green 1987). A competitive species such as *S. pallidus* is likely to thrive in the environmental conditions present in Auckland ponds where adequate propagules are introduced, as it is an adaptable omnivore suited to eutrophic conditions (Williamson and Vanderploeg 1988; Van Egeren et al. 2011). Further, perennial breeding, large egg clutch size and the ability to produce diapausing eggs, enhance potential establishment success following dispersal opportunities (Chapman et al. 1985; Dowell 1997; Torke 2001).

In concert with the introduction of species, constructed water bodies may be facilitating the spread of non-native taxa (Havel et al. 2005; Banks and Duggan 2009). The mean age of the carp ponds was relatively young at 26 years, and constructed ponds in New Zealand typically have varied zooplankton assemblages and would consequently be expected to have potentially vacant niches and low biotic resistance against invaders (Parkes and Duggan 2012). Taylor and Duggan (2012) demonstrated that *S. pallidus* could readily establish in experimental ponds in New Zealand with low propagule numbers under conditions where another calanoid copepod was not present. Grass carp releases in the Auckland region ranged from 5 to approximately 2000 individuals (with stocking rates being dependent upon water body area), potentially resulting in the concomitant introduction of large numbers of zooplankton (Hofstra 2014). Establishment success may be regulated to some extent by the biotic resistance exerted by recipient communities. For example, Shurin (2000) found that under experimental conditions, the interactions with occupant species strongly affected establishment by invaders. Nevertheless, sufficient propagule pressure may overcome biotic barriers (Von Holle and Simberloff 2005; Taylor and Duggan 2012). As such, effects on zooplankton communities, like the partial displacement observed in Lake Kereta, may have already occurred at other sites. For example, *S. pallidus* was the only calanoid copepod sampled in Chelsea 4 pond, while previous records show that *S. pallidus* was not recorded at that site in a survey in 2006 (Duggan et al. 2006). Notably, the native copepods *C. lucasi* and *Boeckella propinqua* have been recorded there at earlier dates, but we recorded neither of the native species, suggesting displacement may have occurred (Jolly 1955; Jolly 1957).

Indirect effects of grass carp

Accompanying the effects of non-native zooplankton introductions on recipient communities are effects induced by grass carp activity. These effects will occur indirectly on zooplankton, through the reduction or elimination of macrophyte biomass by grass carp and through subsequent increases in nutrient cycling rates, or directly, through predation (Mitchell et al. 1984; Pipalova 2006). Auckland ponds where grass carp were released had significantly lower macrophyte cover than ponds with no carp introductions, which is consistent with carp-associated macrophyte reductions found in other studies (Maceina et al. 1992; Pipalova et al. 2009; Hofstra et al. 2014). There were also significant differences in zooplankton composition between carp and non-carp ponds, with planktonic taxa, such as the rotifers *Keratella slacki*, *Filinia novaezealandiae* and *Brachionus calyciflorus*, present in a higher proportion of carp ponds. Loss of macrophyte habitat is likely to have favoured species adapted to planktonic conditions and may explain lower percentages of plant-associated species including the rotifer *Lecane bulla*, cladoceran *Chydorus sphaericus* and *Alona* sp. in carp ponds. To date, studies examining the effects of grass carp releases on zooplankton abundance and composition have commonly found that effects are minimal (Fry and Osborne 1980; Pipalova et al. 2009). Nevertheless, other studies have reported increased zooplankton abundance and shifts to species-poor, rotifer-dominated communities (Kirkagac and Demir 2004). In New Zealand, Duggan et al. (2014) found that following the introduction of carp into Lake Kereta, the relative abundance of taxa associated with planktonic conditions (including *D. galeata* and *Bosmina meridionalis*) increased, while taxa associated with littoral conditions (e.g., *Mesocyclops* sp. and *Chydorus* sp.) decreased. That study also found, rotifer abundances did not change greatly. Similar to our findings, Richard et al. (1985) reported shifts in zooplankton composition following grass carp introduction into ponds in Florida, where greater macrophyte cover was associated with a higher abundance of littoral taxa such as *Lecane*, whereas planktonic taxa such as *Brachionus*, *Keratella* and *Bosmina* became more abundant in conditions of reduced macrophyte cover.

In addition to habitat change, other biotic factors affect communities. In Florida ponds, overall zooplankton density increased after weed removal by carp and a shift occurred from cladoceran and/or copepod dominated assemblages to rotifer and small suspension-feeder dominated communities, likely due to the loss of refugia from size-selective predation by planktivorous fish (Richard et al. 1985). Maceina et al. (1992) conducted a study over a seven year period in a Texas reservoir and found similar shifts in community composition, except that zooplankton abundance decreased throughout the study. In those two studies, zooplankton community responses were attributed to increased rates of nutrient availability, changes to the food base (phytoplankton) and increases in planktivorous fish abundance that exerted size-selective predation pressure on larger crustaceans (Richard et al. 1985; Maceina et al. 1992). Nevertheless, we found that large crustaceans were evident in high percentages of carp-ponds and this may indicate that predation pressure by fish in these habitats is low, as observed in previous New Zealand studies (Chapman and Green 1987).

Changes in nutrient conditions induced by grass carp may also contribute to some community compositional changes. We found that nutrient availability was typically higher in carp ponds than non-carp, as indicated by significantly higher concentrations of TN and TP (Table 1). Rotifer species prevalent in the carp group (*Asplanchna brightwelli, B. calyciflorus, Keratella slacki*, and *K. tropica*), are common in small eutrophic lakes, which may reflect a preference for the higher nutrient levels in the carp ponds (Duggan et al. 2002). Grass carp typically have low efficiency in utilising ingested plant matter, producing egested food and excrements which are nutrient rich (Lembi et al. 1978). This, in turn, generates increased pond nutrient levels as macrophytes are consumed (Lembi et al. 1978). Rapid weed removal can be followed by increased cycling of nutrients and associated effects on the abundance and species composition of phytoplankton and, consequently, species of zooplankton (Maceina et al. 1992; Pipalova 2006). Mitchell et al. (1984) found both an increase in nutrient concentrations and an increase in zooplankton abundance in Parkinson’s Lake, New Zealand, following rapid macrophyte removal by grass carp. The rate of plant removal is a key factor in nutrient cycling and is dependent upon macrophyte biomass, pond size, water residence time and carp stocking density (Pipalova 2006). In the Auckland region, initial stocking rates into ponds were made at a mean density of around 140 fish per vegetated hectare, which is classed as a high stocking rate for New Zealand conditions and the effects of this would be sufficient to influence zooplankton communities following macrophyte loss (Hofstra 2014).

The size of the grass carp released into the Auckland study sites ranged from 65 mm to 600 mm in length (measured from snout to tail-fin fork), with the majority of carp released being >250 mm in length (Hofstra et al. 2014). Direct predatory effects of introduced grass carp on zooplankton are likely negligible however, due to a switch in the diet of grass carp fry from an insect/zooplankton dominated diet to an herbivorous diet when fry attain length of approximately 55 mm (Watkins et al. 1981; He et al. 2013). Additionally, the static conditions in ponds are not suitable for breeding by this species, effectively precluding zooplankton predation by grass carp in Auckland ponds studied (Hofstra et al. 2014).

Other localised effects

The distribution of the Australian calanoid copepod *B. symmetrica* showed a different pattern to other non-native crustaceans discussed, in that there was no strong relationship with the release of grass carp. Five sites with *B. symmetrica* had received carp translocations, four had not, suggesting that the vector of coincidental translocation with carp is less likely than other means of localised dispersal. Dispersal is likely to have occurred as *B. symmetrica* has been present in Puhinui Reserve for some time, being misidentified as *B. triarticulata* at that site in 1924, and not identified as *B. symmetrica* until 1967 (Henry 1924; Bayly 1967). It was also recorded by Banks and Duggan (2009) in Wiri Quarry, a lake that was in close proximity to Puhinui Reserve, but which has since been infilled.

It is not known how long the populations have been at the sites in proximity to Puhinui Reserve and the former Wiri Quarry, as most have never been surveyed previously, but dispersal from Puhinui Reserve is highly probable.

Conclusion

Overall, the habitat modification associated with the influences of grass carp on environmental conditions is likely to have influenced zooplankton community composition. However, neither direct or indirect influences of the grass carp themselves fully account for the prevalence of the taxa associated most strongly with carp ponds: the non-indigenous species *D. pulex* and *S. pallidus*. The significant difference in zooplankton community composition between ponds that were, or were not, subject to the introduction of grass carp from aquaculture facilities, has likely been influenced by those species. Notably, prior to this study, *D. pulex* was known in New Zealand only in the South Island and has now been found in the North Island only in ponds subject to the introduction of grass carp, while *S. pallidus* has already been linked to grass carp introductions and to carp holding ponds, strongly supporting the link between the spread of these taxa and grass carp translocations. Consequently, risk exists in the further coincidental translocation or natural dispersal of these non-native species to natural lakes within New Zealand. The translocation of non-native zooplankton species that have successfully populated ponds following their introduction from fish farms suggests that the existing procedures to prevent their movement with grass carp have been inadequate. Eradication of those species from pond-based farming facilities would reduce the risk of translocation, escape or dispersal into other waterways but is unlikely to be achieved. As such, focus on the management of fish transportation is the most likely measure to reduce the rate of future introductions through translocations.

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Chapter III

Mitochondrial DNA indicates an east coast North American origin for New Zealand and German invasions of *Skistodiaptomus pallidus**

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Abstract

The freshwater calanoid copepod *Skistodiaptomus pallidus* (Herrick 1879), native to the Mississippi basin of North America, has recently established non-indigenous populations in New Zealand, Germany and Mexico. Here, we used the mitochondrial cytochrome *c* oxidase subunit I (COI) gene to more precisely identify the origins of *S. pallidus* populations within New Zealand and Germany. The *S. pallidus* COI sequences suggested that both the New Zealand and German specimens were most similar to those from the most easterly regions of the USA (e.g., New York, Virginia and Georgia). However, several haplotypes were found to be divergent between the New Zealand and German populations, indicating the exact sources of the introductions were likely different for each country. German sequences had greater haplotype diversity than those from New Zealand, supporting suggestions of a shipping related vector of introduction to Germany. Although both German and New Zealand populations contained haplotypes that were closely related to North American sequence records, an identical haplotype match was not found. Further sampling of the native range will be required to determine the exact origin of the non-indigenous *S. pallidus* populations and may also help to identify more precisely the vectors of introduction.

Introduction

Long-distance translocations of aquatic invertebrate taxa, as a result of human-related activities, are well documented globally (Ricciardi and MacIsaac 2000; Havel and Hebert 1993). Freshwater invertebrates can be transported as active and dormant life-stages by a variety of vectors, including in association with species utilised in aquaculture or the aquarium trade and in the ballast of shipping vessels (Duggan et al. 2005; Ferrari and Rossetti 2006; Briski et al. 2012). The consequent transportation of non-native freshwater taxa across biogeographical barriers is resulting in the global homogenisation of species with potential consequences to diversity and ecosystem function (Olden et al. 2004; Briski et al. 2012). Accurate and early identification of non-native taxa, their vector for movement and invasion history are key steps in developing management practises to reduce potential impacts on recipient aquatic systems (Holeck et al. 2004; Comtet et al. 2015).

DNA sequences are increasingly co-utilised with morphological identifications as an accurate means of identifying taxa and are applicable to all life-cycle stages. Further, slight variations in DNA sequences can aid in the discrimination of the biogeographical origins of invasive species (Havel et al. 2000; Hebert et al. 2003; Briski et al. 2011). For example, Makino et al. (2010) used mitochondrial cytochrome *c* oxidase subunit I (COI) gene sequences to identify regional haplotypes of the Japanese calanoid copepod *Sinodiaptomus valkanovi* (Kiefer 1938). This allowed an assessment of the invasion corridor through which the species made a ‘jump’ from its native range surrounding the Seto Inland Sea to sites in north-east Japan and a subsequent and more recent long-distance invasion into New Zealand (Duggan et al. 2006; Makino et al. 2010).

The calanoid copepod *Skistodiaptomus pallidus* (Herrick 1879), native to North America, is emerging as an intercontinental invader, having recently been found to have established populations in the geographically disjunct locations of New Zealand, Germany and Mexico (Duggan et al. 2006; Brandorff 2011; Suarez-Morales and Arroyo-Bustos 2012). First recorded outside of its North American range in New Zealand in 2000, *S. pallidus* was initially found in constructed ponds at the Auckland Regional Botanic Gardens, although how long the species had been there is not known (Duggan et al. 2006). This was followed by detection among live food for fish sold from an aquarium store in 2004, raising the possibility that *S. pallidus* was being dispersed via the aquarium trade (Duggan et al. 2006). Records were further expanded during a systematic survey by Banks and Duggan (2009), who showed *S. pallidus* had successfully colonised constructed ponds elsewhere in New Zealand. The first record of *S. pallidus* establishing in a natural lake was observed coincident with an intentional translocation of grass carp (*Ctenopharyngodon idella*, Valenciennes 1844) into Lake Kereta, Auckland (Duggan et al. 2014; Hofstra 2014). Further populations were subsequently found in other lakes that had been subject to grass carp releases (Duggan et al. 2014; Hofstra 2014). The link between aquaculture and the spread of *S. pallidus* in New Zealand has been further reinforced by the detection of *S. pallidus* and other non-native taxa within aquaculture facilities used in the cultivation of grass carp stocks; these ponds were also determined to be the source of the individuals sold as live food in aquarium stores (Duggan and Pullan 2016, in review). A systematic survey of Auckland ponds showed that *S. pallidus* was present in 35% of ponds that had been subject to grass carp translocations, relative to 10% of ponds which had no known grass carp releases (Chapter II). While grass carp translocations have been identified as the major vector of spread within New Zealand, it is not clear whether the known populations were derived from a single importation event, or whether any vectors of introduction into New Zealand are still active.

The first discovery of *S. pallidus* in Europe was made in 2010, in samples collected from the moat of Bremen (Stadtgraben) adjoining the Weser River, northern Germany (Brandorff 2011). A second population was found a few months later, in a pond in the area of the Juliusplate on the floodplain of the Weser River, although it is unknown how long the populations had been established at either of those sites (Brandorff 2011). Characteristics of the sites were similar in that they were both shallow eutrophic constructed water bodies, indirectly linked with the Weser River - a waterway that had been subject to considerable shipping activity from diverse international locations during the twentieth century (Brandorff 2011). While the introduction of *S. pallidus* via migrating birds or aquarium products cannot be ruled out, introduction by transportation in a shipping related vector such as ballast water or residual sediments was considered more likely (Green and Figuerola 2005; Brandorff 2011).

Populations of *S. pallidus* have established in an expanding range within North America, including into the Lake Tahoe Keys, California, well outside of the north-central and Mississippi basin where it is considered native (Byron and Saunders 1981; Torke 2001; Reid and Hudson 2008). Additionally, a more recent intracontinental range expansion has occurred with *S. pallidus* recorded in the Jose Lopez Portillo Reservoir in Sinaloa, Mexico, possibly in association with aquaculture within the reservoir or via bird migration from the north (Suarez-Morales and Arroyo-Bustos 2012).

The genetic diversity of *S. pallidus* has received some attention, with mitochondrial genes utilised in the study of populations across the species' North American range (Thum and Harrison 2009). Thum and Derry (2008) studied 13 regional *S. pallidus* populations in order to determine whether geographically isolated copepod populations had colonised different regions of North America from glacial refugia following the end of the last glaciation (Stemberger 1995). Data indicated that there were four major divergent clades from different geographic regions and that dispersal from those regions had been limited by natural drainage boundaries (Thum and Derry 2008).

Here, our aim was to determine the origins of the two intercontinental invasions by *S. pallidus*, into New Zealand and Germany. As an increasing number of *S. pallidus* populations in New Zealand have occurred in lakes and ponds that have been subject to grass carp translocations, it is likely that they were introduced into those lakes from the same New Zealand source. In order to determine the sources of *S. pallidus* from New Zealand and German populations, we sequenced individuals from these countries at the COI gene locus and compared these sequences with those available from native populations in North America. We hypothesized that: 1) specimens collected from New Zealand would have strong genetic similarity based on the expectation that the propagule size introduced into New Zealand in association with fish farming was likely to be small compared to vectors such as shipping; and 2) there would be greater diversity in the specimens sampled from Germany than New Zealand, based on the expectation that an introduction via a shipping related vector would potentially introduce a greater number of propagules or result in repeated introductions.

Methods

Sampling

In order to estimate haplotype diversity within New Zealand populations, ten sites were selected over a broad geographic range from the known distribution of *S. pallidus* within the North Island (Figure 1). Sampling was carried out at six New Zealand sites and sequences from an additional four sites for which records already existed on the Barcode of Life Data Systems (BOLD; www.boldsystems.org) were included in analyses but not resampled. The BOLD records included one store-bought individual purchased in Hamilton, which originated from a site which had been subject to grass carp release (Figure 1). The Auckland Botanic Gardens and Albany ponds had received no authorised grass carp releases. Collection of additional New Zealand samples took place during November and December 2015, with the exception of samples sourced from an aquaculture facility in Te Aroha, which were obtained in May 2015 (Table 1). Zooplankton were sampled by making multiple horizontal net hauls from the water body shoreline with a plankton net (40 µm mesh-size, at a haul speed of approximately 1 $m.s^{-1}$) until a concentrated zooplankton sample was obtained. Samples were transferred to containers and preserved with ethanol (90% final concentration) immediately following collection and cool-stored in a lidded bin to minimise light exposure. The German samples were collected from the moat of Bremen in early November 2015 (Figure 1) using the method described above, and individuals sent to New Zealand for genetic analysis. Once in the laboratory, all samples were transferred to dark-storage at 4° C. Specimens were identified morphologically using standard taxonomic keys (e.g. Dussart and Defaye 1995) prior to genetic analyses.

DNA extraction and amplification

Extraction of total genomic DNA was undertaken for each whole individual specimen using the Red Extract n Amp (Sigma-Aldrich) kit following the manufacturer’s instructions using: 10 µL extract solution (ex) and 2.5 µL tissue preparation solution (TP) per reaction tube. Tubes where then placed into a darkened area to incubate at room temperature for three hours. Following incubation, tubes were heated to 95° C for 3 minutes in an Eppendorf thermocycler before 10 µL of neutralising solution was added to each tube. Tubes were then vortexed before being stored at 4° C. The mitochondrial cytochrome *c* oxidase subunit I (COI) gene locus was selected for this study as comparative sequences were available from GenBank (Benson et al. 2000) and analysis of other genes supported the COI haplotypes in previous studies (Thum and Derry 2008). A 658 bp fragment of the COI gene was PCR amplified from each extraction at the University of Waikato using either the universal primers HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') and LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') (Folmer et al. 1994) or the paired primers Lep F1 (5'-ATTCAACCAATCATAAAGATATTGG-3') and Lep R1 (5'-TAAACTTCTGGATGTCCAAAAAATCA-3') (Hebert et al. 2004). The PCR reactions of 15 µL were made up of 7.5 µL of PCR Master Mix solution (iNtRON Biotechnology Inc., Korea), 0.4 µL of each primer (forward and reverse), 5.7 µL of deionised (Milli-Q) water and 1 µL of sample extract. Thermo-cycling consisted of an initial denaturing period of 1 minute at 94° C followed by 5 cycles of 94° C for 1 minute, 45° C for 1.5 minutes and 72° C for 1 minute. This was followed by denaturation and polymerase amplification through 35 cycles of 94° C for 1 minute, 51° C for 1.5 minutes and 72° C for 1 minute. The final extension period occurred at 72° C for 5 minutes. A subsample (3 µL) of each PCR product was visualised under UV light in a MultiImage light cabinet (Alpha Innotech, Protein Simple, CA USA) following electrophoresis at 44 volts for 45 minutes in 1% agarose gel containing 5 µL/100mL RedSafe Nucleic Acid Staining Solution (20,000x) (iNtRON Biotechnology Inc., Korea). Successfully amplified products were cleaned using a master mix containing 0.1 µL Exonuclease I (EXO) (10 U/µL) and 0.1 µL Shrimp Alkaline Phosphate (SAP) (1 U/µL) solution (Illustra Global Science) and 5 µL PCR product at 37° C for 15 minutes to degrade remaining primers and nucleotides, then 80° C for 15 minutes to de-activate the reagent. Purified products were sequenced in both directions using the same primers as per PCR on an ABI3130xL sequencer at the University of Waikato DNA Sequencing Facility.

Genetic analyses

Taxonomic verification of *S. pallidus* sequences produced in this study was carried out using the GenBank nucleotide BLASTn algorithm with default parameters (Benson et al. 2000). Three *S. pallidus* individuals were sequenced from each of the New Zealand sites, which provided 17 COI sequences >640 nucleotide positions. Ten individuals were sequenced from the German site producing seven COI sequences of >640 nucleotide positions. Four additional sequences from New Zealand sites were obtained from BOLD (Table 1). Primer regions were trimmed and sequences were aligned using multiple sequence comparison by log-expectation (MUSCLE) as implemented in Geneious v6.1.2 (Drummond et al. 2010). The confirmed absence of stop codons indicated that mitochondrial DNA had been amplified. There were no insertions or deletions, and the alignment was straightforward. Haplotypes were verified by visual inspection of the trace files. Sequences were aligned with a further 27 North American *S. pallidus* COI sequences downloaded from GenBank: accession numbers EU825105.1-EU825131.1 (Thum and Derry 2008). The alignment was further trimmed to 631 bp to be compatible with the sequence lengths of the USA populations from GenBank. Chi-square (X^2) tests using PAUP*4.0 (Swofford 2001) were conducted to ascertain base pair frequency among sites and substitution at codon positions. *Skistodiaptomus oregonensis* and *S. mississippiensis* were used as outgroups (accession numbers EU582590.1 and EU582579.1; Thum and Harrison 2009). Neighbour Joining (NJ), Maximum Parsimony (MP) and Maximum Likelihood (ML) trees were produced using Molecular Evolutionary Genetic Analysis (MEGA) v5.2.2 (Tamura et al. 2011). Bootstrap values of 75% and above support for branches (1000 replicates) were included in the final tree (Felsenstein 1985). The optimal model of nucleotide substitution for the ML tree was the General Reversible Model (GTR+I+G) as determined using jModelTest v2.1.1 (Darriba et al. 2012). For NJ and MP tree construction the Jules-Cantor model of nucleotide substitution was used with gamma distribution. To assess intraspecific divergence, a pairwise genetic distance matrix of evolutionary divergence was produced in MEGA. To visualise haplotype relationships, a haplotype network was produced using TCS v1.21 (Clement et al. 2000). All sequences and supporting information were deposited in the BOLD database under the project NZPLC (Freshwater Calanoids of New Zealand and Australia).

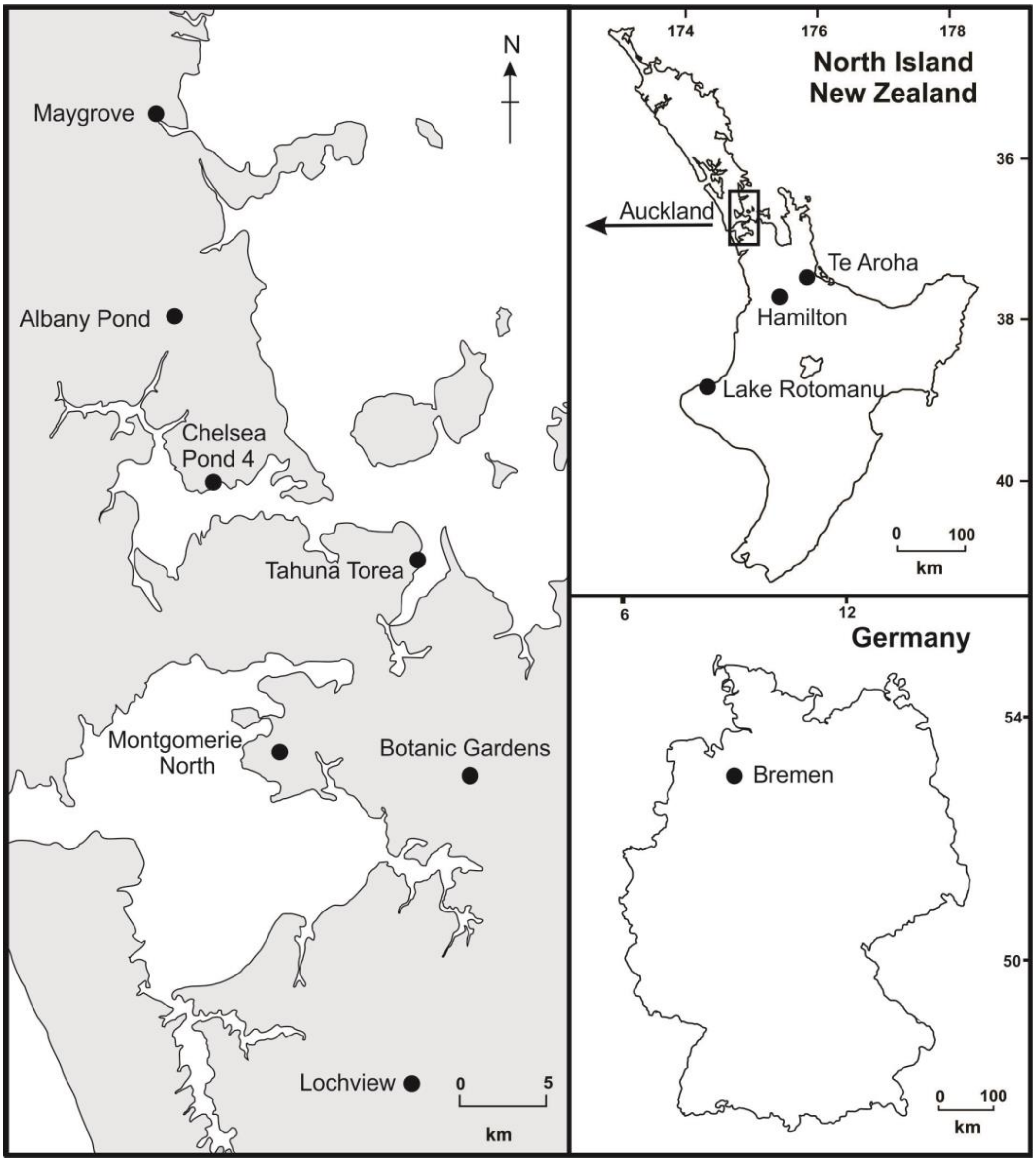

Figure 1. Locations of the New Zealand (left and top right) and German (bottom right) sites where *Skistodiaptomus pallidus* were collected in this study.

Table 1. Sources of *Skistodiaptomus pallidus* material examined in this study.

Locality	Specimen code	Geo-reference		Collection date
New Zealand				
Albany	NZPL711, 713	36°43’33.53”S	174°42’28.56”E	09 Aug 2011
Auckland Botanic Gardens	NZPL059	37°00’39.33”S	174°54’23.89”E	25 Apr 2007
Chelsea Pond 4	N12	36°49’12.00”S	174°43’29.00”E	17 Nov 2015
Aquarium Store, Hamilton	NZPL058	37°46’20.00”S	175°17’00.60”E	03 May 2007
Lochview	N01, N02, N03	37°11’13.41”S	174°54’09.86”E	17 Nov 2015
Maygrove	N13, N14, N16	36°35’21.97”S	174°41’04.11”E	17 Nov 2015
Montgomerie North	N05	36°59’05.93”S	174°46’46.03”E	17 Nov 2015
Lake Rotomanu	N27, N28, N29	39°02’32.00”S	174°06’54.20”E	01 Dec 2015
Tahuna Torea	N07, N08, N09	36°52’20.49”S	174°52’55.41”E	17 Nov 2015
Te Aroha	N30, N31, N32	37°31’45.00”S	175°42’40.20”E	21 May 2015
Germany				
Moats of Bremen	G18-G22, G25, G26	53°04’30.50” N	8°48’48.33”E	08 Nov 2015

Results

A total of 57 individuals were included in our final alignment of 631 nucleotide positions. Of the 631 nucleotide positions, 453 were constant, 176 were parsimony informative and 2 were variable but parsimony uninformative. Composition across all nucleotide positions were biased towards A-T (A = 25.76%; T = 36.58%; G = 21.58%; C = 16.08%). Base frequencies were homogeneous across sequences where all sites were analysed ($X^2_{162} = 23.28$, p = 1.000). Base frequencies across variable sites ($X^2_{162} = 99.82$, p = 0.999) and uninformative sites ($X^2_{162} = 103.11$, p = 1.000) were also homogeneous. Base frequencies were homogeneous for first ($X^2_{162} = 3.53$, p = 1.000), second ($X^2_{162} = 0.06$, p = 1.000) and third codon positions, with greatest variation in third codon positions ($X^2_{162} = 94.90$, p = 1.000). The ML analyses, as estimated using the GTR+I+G model (–lnL=2655.89), were concordant with NJ and MP results and indicated that the sequences from both New Zealand and Germany were positioned in Clade A, as defined by Thum and Harrison (2009) (Figure 2).

Fifteen unique *S. pallidus* haplotypes were identified. Of these, the New Zealand specimens consisted of one major haplotype (S1) present at all sites, with two additional haplotypes from ponds at Maygrove (S2) and Tahuna Torea (S3) (Figure 3, Appendix 1). New Zealand (S1) sequences formed a group with specimens sampled from eastern USA sites in Virginia, New York and Georgia (VA001, NY801, GA002). The German samples consisted of three haplotypes, the first (S1) shared with New Zealand, Virginia, New York and Georgia and the second (S4) shared with sequences obtained from sites in Virginia, New York, Georgia and Connecticut (VA002, NY517, GA001 and CT502) (Figure 3). The third haplotype (S7) was not recorded elsewhere.

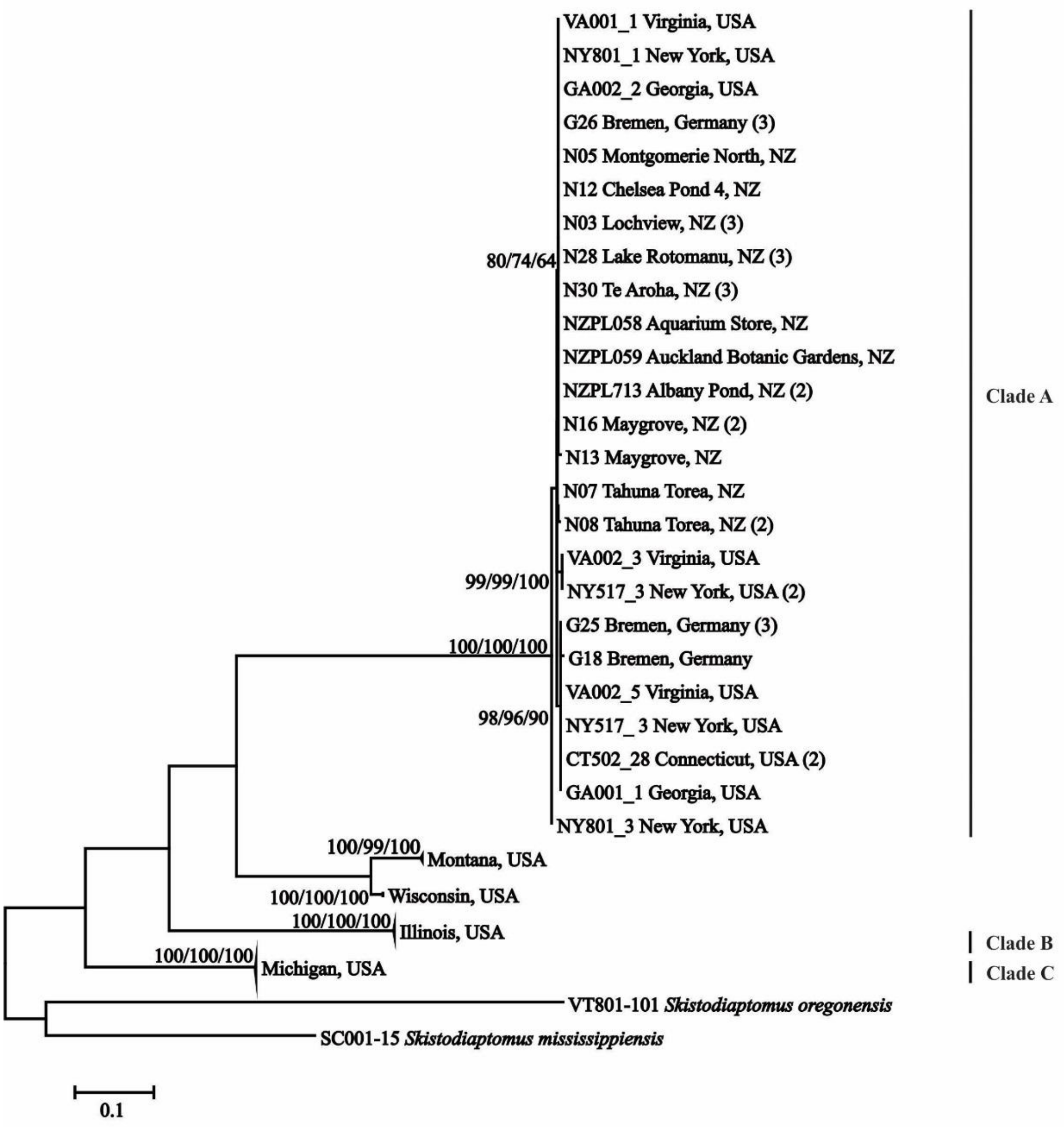

Figure 2. Maximum Likelihood tree estimated from mitochondrial COI gene sequences for *Skistodiaptomus pallidus* individuals collected in Germany, New Zealand (NZ) and the United States (USA). Bootstrap support values (1000 replicates) greater than 75% are shown at nodes for ML/MP/NJ analyses. The number of individuals with identical sequences is shown in parentheses for each collection location and triangles represent collapsed cluster diversity. Three divergent Clades (A-C) as defined by Thum and Harrison (2009) are shown.

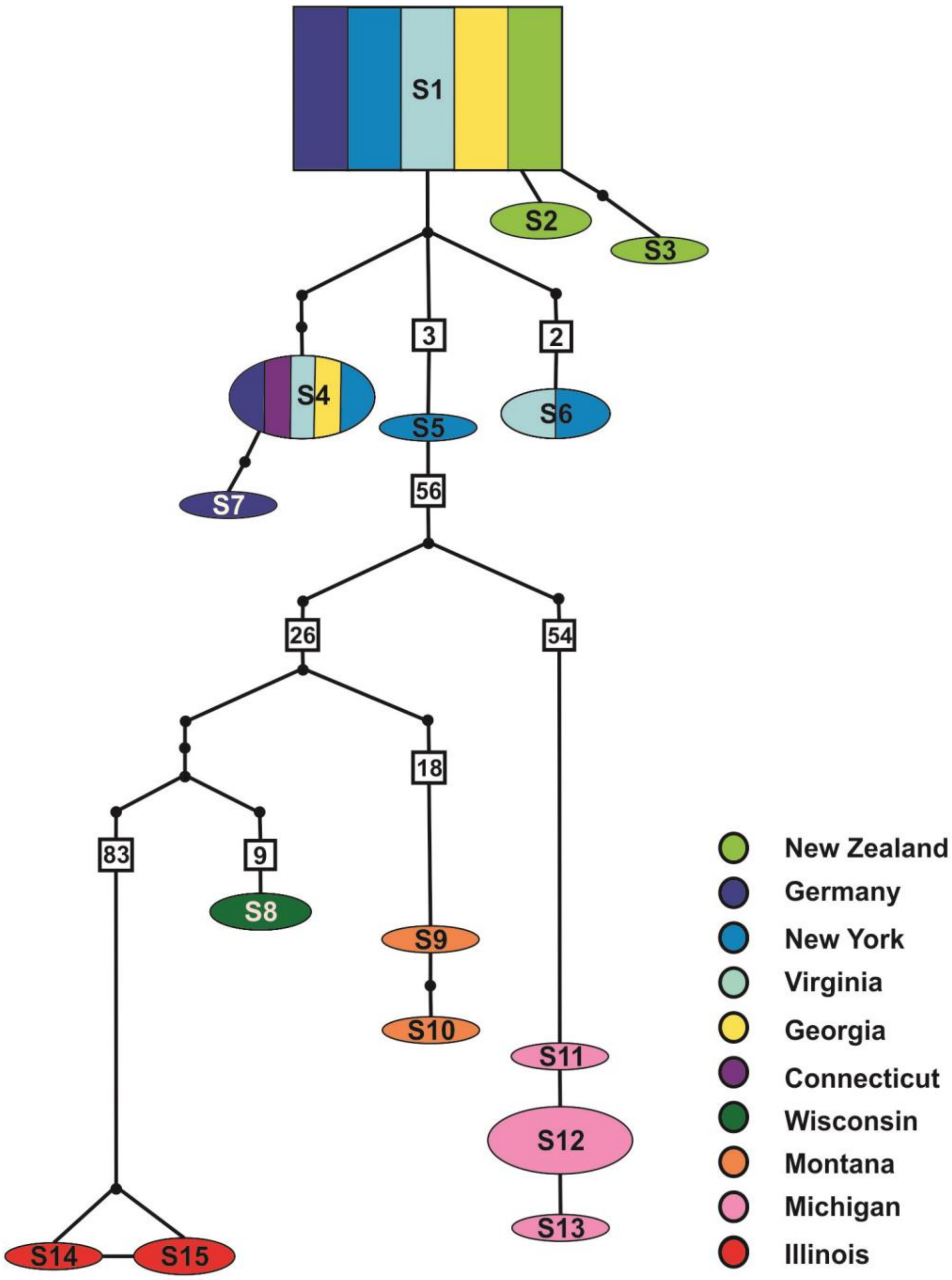

Figure 3. Network of 15 unique *Skistodiaptomus pallidus* haplotypes (labelled S1-S15) with colour coding indicating the collection location for each. The boxed numbers indicate the number of mutational steps which separate haplotypes and black dots represent single mutational steps with haplotype S1 being the most derived haplotype.

Discussion

Analysis of the *S. pallidus* COI sequences placed the New Zealand and German specimens within the monophyletic group identified as Clade A by Thum and Harrison (2009), with strong bootstrap support. In the native range, Clade A is made up of specimens collected from the most easterly regions of the USA, which includes New York, Connecticut, Virginia and Georgia (Thum and Harrison 2009). Although Clade A haplotypes were common to waterbodies spread over the eastern USA, they were highly divergent from those collected in Michigan, Illinois (Clades B and C), Wisconsin and Montana. There were two widespread haplotypes within Clade A in the USA, and while New Zealand specimens included one of these (S1), German specimens included both (S1 and S4). The presence of both major haplotypes in the German population is an indication that the propagule supply may have been larger than that into New Zealand, and is consistent with large or multiple introductions from shipping in Germany. Additionally, with New Zealand and Germany each having unique minor haplotypes, it is likely these originated from different sources from within the eastern United States.

Brandorff (2011) indicated that the most likely vector for *S. pallidus* introduction into the German sites was via ballast-related transport from North America, as there had been a large volume of shipping into the port of Bremen during the 20th century. The transportation and introduction of non-native taxa is well documented in studies related to the Laurentian Great Lakes with numerous intercontinental invasions attributed to shipping related vectors, although introductions were typically into the lakes, not from them (Duggan et al. 2005; Drake and Lodge 2007). Movement of organisms between the Weser River and the partitioned moat could occur due to a pumping system which moves water into the moat from where it can flow back into the river (Brandorff 2011). However, while *S. pallidus* has been found in an increasing range adjoining the river, it has not been detected within it (Brandorff, unpubl. data). Despite the advent of ballast water exchange, shipping vectors are still potentially very active (e.g. Chain et al. 2016). In contrast, international shipping in New Zealand is limited to marine environments; hence, this mode of introduction is unlikely. Although the New York sequences used for comparison in this study were from sites over 100 km from major ports adjoining Lakes Erie and Ontario, earlier studies indicate the range of *S. pallidus* included natural and constructed water bodies of the Lake Ontario drainage, in close proximity to the lakes (Mills et al. 1993; Thum and Stemberger 2006). Stemberger (1995) found *S. pallidus* in constructed impoundments to the north of its native range and Reid and Hudson (2008) argued that *S. pallidus* should not be considered non-native in the Laurentian Great Lakes due to its distribution in surrounding systems. Further, *S. pallidus* specimens have been periodically sampled from Lakes Ontario, Erie and St Clair, and are likely washed in with drainage from surrounding areas. Their presence in the lakes suggests that transport by a shipping vector could occur from these locations (Mills et al. 1993). For example, between 1981 and 2000, Lakes Ontario and Erie accommodated the first port of call in the Laurentian Great Lakes for 72% of transoceanic vessels declaring no ballast on board, and after cargo was unloaded, ballast was typically taken on board (Holeck et al. 2004). Other vectors of introduction into Germany, such as the movement of aquarium or ornamental taxa are also possible, but less likely. For example, there is an earlier European record of *S. valkanovi* from a botanical garden in Bulgaria (with the species now considered extirpated from that site), suggesting that the introduction of copepods could unintentionally occur with the introduction of other non-native taxa such as aquatic plants (Ueda and Ohtsuka 1998).

The genetic similarity among a variety of New Zealand populations indicates *S. pallidus* may have been introduced there from a limited or single introduction event, with later spread to other New Zealand sites, including in association with translocations of grass carp. Studies indicate that a reduction in genetic diversity in introduced populations can be less frequent than anticipated due to multiple introductions or propagule sizes (Roman and Darling 2007). While there is no obvious vector of introduction for *S. pallidus* into New Zealand, within New Zealand a vector related to the transportation of fish is suggested by the strong association of *S. pallidus* with aquaculture facilities used in the domestic culturing of grass carp (Duggan et al. 2014).

The geographic range of Clade A haplotypes, from Georgia to New York, and their proximity to major population centres in the eastern United States, indicates the similarity between the German and New Zealand *S. pallidus* haplotypes is probably coincidental and likely reflects the association between human activities and invasions (Carlton 1996). Haplotype diversity for the COI locus within each clade is likely to be underestimated by the limited amount of data currently available from the native range; typically two to five specimens per site, and representing a single haplotype (Thum and Derry 2008). Consequently, these data cannot yet provide a specific location for the origin of haplotypes found in New Zealand and Germany, which might otherwise inform the probable transportation vectors. Narrowing this down to a single location may also be complicated by dispersal of *S. pallidus* in association with constructed water bodies and human activities, such as boating and aquaculture (Mills et al. 1993). A more intensive sampling of sites in the eastern United States would provide a more detailed indication of the distribution of haplotypes and intraspecific diversity in *S. pallidus*. In Germany, the continued sampling of sites adjacent to the River Weser would help to monitor the spread of *S. pallidus* there. In New Zealand, the haplotypes recorded to date are likely to be a subset of those present in the infected aquaculture facilities, making further sampling of those facilities a future step in determining the genetic diversity therein. Sampling of the Mexican site of *S. pallidus* introduction will allow for comparison of the characteristics of the intracontinental introduction with what is currently known.

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Chapter IV

Thesis Summary and Conclusions

Aquaculture is increasingly utilised globally in the cultivation of freshwater aquatic species, and the movement of fish from aquaculture facilities has been linked to introductions of non-native species, including of species ‘hitch-hiking’ with intentional releases (Bartley, 2011). The introduction of grass carp has been observed to result in effects on recipient ecosystems, including effects on the composition of zooplankton communities in individual ecosystems (Mitchell et al., 1984; Minchin, 2007; Duggan et al., 2014). In this thesis I: 1) utilised community scale analyses to assess the effects of grass carp translocations on zooplankton communities at a regional scale; and 2) used molecular techniques to determine if the invasion of the copepod *Skistodiaptomus pallidus* in New Zealand, which has spread in association with activities related to aquaculture, and Germany, originated from a single North American source.

In Chapter II, I tested the effects of grass carp translocations from aquaculture facilities on ponds in the Auckland region. I quantified zooplankton community composition in 34 ponds that had been subject to grass carp release and 31 which had no grass carp introductions. I found that there was a significant difference in the composition of zooplankton communities between ponds that had been subject to grass carp translocations and those that had not. As other key pond characteristics, including pond size and age, were comparable for the two groups of ponds, differences in zooplankton communities between ponds with and without grass carp introductions were attributable to two major factors: 1) the direct effects associated with the establishment of zooplankton originating from aquaculture ponds, including non-native species; and 2) the indirect effects of activity of grass carp through changes to habitat.

The significant difference in zooplankton community composition between ponds that were, or were not, subject to grass carp releases, was influenced by non-native species that were more prevalent in ponds with carp release: the North American cladoceran *Daphnia pulex* and copepod *S. pallidus*. While effects on recipient communities had been observed in previous longitudinal studies, my results indicate that there are significant impacts on a regional scale (Byron & Saunders, 1981; Burns, 2013; Duggan et al., 2014). Prior to this study, *D. pulex* was known only in South Island lakes and is now known in the North Island only from ponds subject to the introduction of grass carp from aquaculture farms. *Skistodiaptomus pallidus* introductions have already been linked to grass carp introductions, and are known from ponds in New Zealand grass carp farms, strongly supporting the link between the spread of these taxa and grass carp translocations (Duggan et al., 2012; Duggan et al., 2014; Duggan & Pullan, 2016, in review). Habitat modification occurring as a result of significant reductions in macrophyte cover is also likely to have influenced zooplankton community composition as the open-water conditions would be more favourable to planktonic taxa (Mitchell et al., 1984; Duggan et al., 2014). Planktonic taxa were associated with ponds where grass carp had been released and, conversely, taxa typically associated with macrophytes were found in a higher proportion of ponds with no grass carp introductions. Based on my research, I am able to conclude that the translocation of grass carp from aquaculture farms has effects on recipient zooplankton community composition at a regional scale, through the direct effects of the introduction of zooplankton and the indirect effects of grass carp activity. Furthermore, the translocation of grass carp presents an ongoing risk for the unintentional introduction of non-native zooplankton with likely adverse effects on recipient native communities in New Zealand freshwater ecosystems and is deserving of immediate management attention.

In Chapter III, I analysed variation in a portion of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene to more precisely identify the origins of invasions of the freshwater calanoid copepod *Skistodiaptomus pallidus*. This technique was applied to populations sampled from within New Zealand, which have been linked to the release of fish from aquaculture facilities, and to populations found in Germany, which have possible links to a shipping vector (Brandorff, 2011; Duggan et al, 2014). The *S. pallidus* COI sequences clearly placed both the New Zealand and German specimens with those from the most easterly regions of the USA (Thum & Derry, 2008; Thum & Harrison, 2009). Some haplotypes were divergent between the New Zealand and German populations, indicating that the exact origins of the introductions were likely different for each country. German sequences had greater haplotype diversity than those from New Zealand, supporting the suggestions of a larger introduction via a shipping related vector into Germany (Brandorff, 2011). A conclusion drawn from these results is that the intercontinental invasions into New Zealand and Germany likely originated from separate sources in the eastern regions of the USA.

Future research

The research conducted in this study provides crucial information about aquaculture as a vector for the movement of non-native crustaceans within New Zealand and highlights the requirement for effective management policies to curb the proliferation of non-native taxa within aquaculture facilities, and to mitigate the coincidental movement of non-native taxa with translocations from them. As the rate of invasions into freshwater ecosystems is increasing worldwide, the risk of the introduction of unwanted species known to cause damage to ecosystems, including of 'hitch-hikers,' is also increased (Minchin, 2007). My study identifies effects relating to the introduction of non-native zooplankton that will likely have ongoing impacts throughout freshwater ecosystems. Therefore, there is a clear requirement for the development of effective detection and response measures for the ongoing management of freshwater ecosystems. The development of methods to reduce the abundances of, or to eradicate, non-native zooplankton taxa from aquaculture ponds would be informative. However, a focus on research into the protocols of fish transportation is likely to be the most effective measure to reduce the rate of future introductions through translocations. As there is limited knowledge of the invasion risks and transport linkages from aquaculture facilities internationally, systematic sampling of the zooplankton communities present in these facilities elsewhere is required in order to determine risks at a global scale. This is a priority for facilities from which translocations of species are made into the wild.

My research indicates that the genetic diversity of *S. pallidus* is underestimated, particularly within the native North American range (Chapter III). More intensive sampling and genetic analyses in New Zealand and Germany, as well as in the native North American range, will improve knowledge of the genetic diversity of this emerging invader. Further, the use of more rapidly evolving genes in future research will enable an increase in the level of sensitivity in testing hypotheses relating to the invasion histories of this species (Makino et al., 2010).

As the management of transportation vectors is likely to be the most effective method of curbing further introductions of non-native species (Briski et al., 2012), greater resolution of sampling within the native range including, for example, shipping ports and aquaculture facilities, will enable a more exact determination of the origins, and more precise linking to vectors, of introductions of this species into New Zealand and Germany. Accordingly, this knowledge will inform decisions on the reduction or prevention of further invasions. Sampling of the non-indigenous *S. pallidus* population in Mexico will enable a more precise determination of the origin of that population and its relationship to those in North America, New Zealand and Germany. International collaboration to gain a better understanding of introduction vectors will contribute to the future management of biological invasions.

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Appendix

Appendix 1. Pairwise sequence divergence (uncorrected *P*-distance) for a 631bp mtDNA COI fragment for 15 haplotypes of *Skistodiaptomus pallidus* sampled from New Zealand, Germany and the United States.

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15
S1															
S2	0.002														
S3	0.003	0.005													
S4	0.006	0.008	0.010												
S5	0.008	0.010	0.011	0.011											
S6	0.008	0.010	0.011	0.011	0.013										
S7	0.010	0.011	0.013	0.003	0.014	0.014									
S8	0.160	0.162	0.160	0.160	0.154	0.162	0.163								
S9	0.162	0.163	0.158	0.165	0.162	0.163	0.168	0.054							
S10	0.160	0.162	0.157	0.163	0.160	0.162	0.166	0.057	0.003						
S11	0.177	0.179	0.181	0.177	0.177	0.177	0.181	0.154	0.158	0.157					
S12	0.176	0.177	0.179	0.176	0.176	0.176	0.179	0.155	0.160	0.158	0.002				
S13	0.177	0.179	0.181	0.177	0.177	0.177	0.181	0.157	0.162	0.160	0.003	0.002			
S14	0.177	0.179	0.179	0.173	0.177	0.182	0.176	0.155	0.174	0.174	0.160	0.162	0.162		
S15	0.177	0.179	0.179	0.173	0.177	0.182	0.176	0.155	0.174	0.174	0.160	0.162	0.160	0.002	