Invasive non-native species and the management and exploitation of freshwater ecosystems

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Preface

This thesis is the result of my own work and includes nothing which is the outcome of work done in collaboration except as declared in the preface and specified in the text. This thesis is not substantially the same as any work that has already been submitted before for any degree or other qualification except as declared in the preface and specified in the text.

This thesis does not exceed the prescribed word limit of 60,000 words for the Degree Committee for Biology.

Summary

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The effects of climate warming, hydrological intensification, and eutrophication are driving unfavourable shifts in phytoplankton communities. In particular, harmful algal blooms (HABs), dominated by toxic cyanobacteria, are becoming increasingly common and often lead to ecosystem collapse and unsafe drinking water. Freshwater ecosystems are also vulnerable to the establishment of invasive non-native species (INNS), whose prevalence has rapidly accelerated through the expansion of global travel, trade, and transportation. INNS can drive significant changes in habitat suitability and native community composition. I investigated how INNS affect the tipping points of freshwater ecosystems, between clear water and phytoplankton-dominated states, and influence phytoplankton community structure and cyanobacteria abundance.

Through a meta-analysis of the global impacts of INNS on freshwater lake ecosystems, I identified that molluscs were potential drivers of a clear water state in lake ecosystems, encouraging system recovery and delaying ecosystem collapse. Invasive fish and crustaceans, however, were identified as potentially pushing systems towards a turbid state and resisting recovery.

I further assessed the effects of invasive molluscs by quantifying the impacts of quagga mussels (*Dreissena rostriformis bugensis*) in UK reservoirs. I identified that impacted sites demonstrated: an increase in overall reactive silica and soluble reactive phosphorus concentrations; reductions in overall diatom, cyanobacteria and *Aphanizomenon* sp. abundance; and an increase in chlorophyll a concentration. However, no significant changes were seen in overall green algae, *Microcystis* sp., *Anabaena* sp., or *Euglena* sp. abundance, ammonium concentration or turbidity.

The selective grazing behaviour of quagga mussels has been shown to vary between studies, an effect hypothesised to be driven by phenotypic plasticity or genotype selection between mussel populations. I explored putative population level differences in the discriminatory grazing behaviour of quagga mussels by observing mussels from both lentic and lotic environments, as hydrology is a known driver of phenotypic plasticity in mussels. I show that different populations of quagga mussels have the potential to drive different outcomes in phytoplankton communities. While both populations expressed comparable clearance rates and cleared green algae (*Chlorella vulgaris*) at a higher rate than cyanobacteria (*Synechococcus elongatus*), mussels from lotic environments ejected significantly greater quantities of cyanobacteria in pseudofaeces. Rejection of cyanobacteria by invasive mussels may drive HABs.

Beyond invasive mussels, I also assessed the allelopathic effect of invasive plants *Hydrocotyle ranunculoides* and *Crassula helmsii* on phytoplankton communities. The allelopathic effect of each plant was tested in both a whole, and crushed state, to test for natural excretion of allelochemicals. In its whole state, *H. ranunculoides* supressed the growth of green algae (*C. vulgaris*), whereas crushed *C. helmsii* supressed the growth rate of both green algae and cyanobacteria (*Synechocystis* sp.). The species-specific nature of these allelopathic interactions suggests that the use of allelopathy for controlling phytoplankton abundance may have to be assessed on a case-by-case basis. Although, a sophisticated understanding of this differential sensitivity may also allow for some targeted management of phytoplankton populations towards specific desirable end points.

Finally, I explored the potential role of invasive zebra mussels (*Dreissena polymorpha*) in the novel concept of Synthetic Ecology. I investigated how zebra mussel grazing behaviour could be harnessed to maintain favourable conditions for the cultivation of commercially valuable algae species in outdoor raceways by supressing the growth of competing cyanobacteria. Their potential was confirmed through the consistent preference for cyanobacteria (*Synechocystis* sp.) over green algae (*C. vulgaris*) observed across all treatment conditions.

The results of this thesis suggest, in certain contexts and where risks are appropriately managed, there may be opportunities to harness INNS in order to drive favourable changes in the alternative equilibria of freshwaters, reducing both overall phytoplankton and cyanobacteria abundance in biologically depleted ecosystems.

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So much has happened during the formation of this thesis. I have broken limbs, moved to a new house, got married, forged new friendships, and endured a global pandemic. Throughout these chaotic and turbulent times, there are many people whose constant encouragement and support have guided this project to its completion. I would like to take this rare opportunity to thank and acknowledge the people who have made this work possible.

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The Aquatic Ecology group has changed drastically through my PhD, beginning with only four members, and now totalling ten. There is a small part of them all in this thesis. To the 'founding members' I owe a particular debt of gratitude. David Willer has been the shining example, an unstoppable force (both academically and physically). I enjoyed the workouts and the chats and, probably unknowingly, he has helped keep me on course. Feng Tang has provided me with endless entertainment; we have been co-workers, co-founders, and co-conspirators. Regarding statistics, our relationship is significantly one-sided. Feng has continuously provided me with mathematical guidance, and for that, and everything else, I am forever grateful. Beyond the lab group and the scope of academe, our office in the David Attenborough Building provided many welcome distractions. I would like to thank Annette Green, Clare Bissel, and Lucy Goodman for providing the majority of these.

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Finally, I would like to thank my family. To my sister, Becky, for her encouragement and frequent visits. To my parents, for always allowing me to pursue my interests and for providing me with opportunities to extract myself temporarily from the Cambridge bubble. Mum and Dad, this thesis, like everything I do, is for you.

Table of Contents

List of Figures

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

List of Tables

Chapter 3

Chapter 6

List of Abbreviations

AICc	Akaike Information Criterion corrected
ANOVA	Analysis of Variance
BACIPS	Before-After Control-Impact Paired Series
BBM	Bold Basal Media
CCAP	The Culture Collection of Algae and Protozoa
Chla	Chlorophyll a
CI	Confidence Interval
DI	Deionised
FTU	Formazin Turbidity Units
GLM	Generalised Linear Model
GLMM	Generalised Linear Mixed Model
HABs	Harmful Algal Blooms
INNS	Invasive Non-Native Species
LED	Light Emitting Diode
NAC	Nucleic Acid Content
PAR	Photosynthetically Active Radiation
PUFAs	Polyunsaturated Long-Chain Fatty Acids
ROV	Remotely Operated Vehicle
SMD	Standardised Mean Difference
SRP	Soluble Reactive Phosphorus
TO	Transgressive Overyielding
UV	Ultraviolet

Chapter 1

Introduction

The global spread of human society has fundamentally altered the function and character of the biological world, as increased numbers of species have been relocated from their native environments and introduced, either intentionally or unintentionally, into the ranges of other species (Seebens et al., 2017, 2018). In recent decades, as a result of expanding global connectivity through mass transportation, travel, and trade, the spread of species to novel environments has accelerated dramatically (Hulme, 2009). Many of these non-native species have been introduced deliberately as food plants, cash crops, domesticated pets, livestock animals, or ornamental plants (Boy and Witt, 2013). Other non-native species have been transported unwittingly from one environment to another as a side effect of the mass movement of people, food, and goods around the planet (McNeely, 2006). Some of these introduced species possess characteristics which cause them to become '*invasive*' and are labelled as invasive non-native species (INNS). INNS is a term reserved to describe species of non-native plants, animals, pathogens, or other organisms, that on introduction to a novel environment, can have repercussions for ecosystem functioning (Simberloff et al., 2013), human health (Pyšek and Richardson, 2010) and economies (Kettunen et al., 2009; Vilà et al., 2010). Interest in INNS has grown significantly, energised by the increasing prevalence of INNS across the world, the significant impacts some INNS have on recipient ecosystems, a desire to predict species with invasive potential, and a growing need to identify efficient management strategies (David et al., 2017). This interest led to the creation of the independent field of invasion biology in the 1980s.

Current key indicators which are hypothesised to predict the potential successful establishment of INNS centre on addressing either the invasiveness of a species (i.e. traits which aid their transport, establishment, and dominance) or the invasibility of a recipient system (i.e. the vulnerability of communities or ecosystems to invasions) (Hui et al., 2016). Common explanations for species invasiveness often hinge on components of their evolutionary history, such as prey naiveté (Papacostas and Freestone, 2019), possession of novel weapons (Callaway and Ridenour, 2004), the evolution of increased competitive ability (Moles et al., 2012), and Darwin's naturalisation hypothesis (David et al., 2017). Explanations centred on the invasibility of recipient systems as indications of INNS success are often related to community structure, including: resource competition (González et al., 2010); recipient community diversity vs. biotic resistance-invasibility (Levine et al., 2004; Guo et al., 2015); phylogenetic, functional or ecological originality (David et al., 2017); environmental disturbance and 'invasional meltdown' (Simberloff and Von Holle, 1999); and propagule pressure (Lockwood et al., 2005).

It is worth acknowledging that despite the identification of traits and ecosystem characteristics which appear central to INNS success, in recent years, there have been 'intellectually dynamic' debates regarding the biology of invasions (Richardson and Ricciardi, 2013; Russell and Blackburn, 2017; Cassini, 2020). Some researchers question whether the common explanations of species invasiveness are exclusive to INNS, claiming that the same traits are seen in successful native species in disturbed or fertile habitats (Thompson et al., 1995; Leishman et al., 2010). However, despite a lack of definitive traits and characteristics which are universal across all successful INNS in all biomes, very few, if any, scholars deny that some INNS cause significant impacts in recipient ecosystems (Boltovskoy et al., 2020). INNS have been shown to alter the underlying structure and functioning of numerous ecosystems, altering baseline abiotic conditions (e.g. nutrient levels, light availability, transportation of materials and habitat complexity) and biotic characteristics (e.g. the density, distribution, diversity and biomass of other species) (Grosholz, 2002; Byrnes et al., 2007). One key set of ecosystems which are particularly prone to the establishment of INNS are aquatic ecosystems, particularly freshwater systems (Kolar and Lodge, 2002; Pollen-Bankhead et al., 2009).

It is thought that there are two central explanations why freshwater ecosystems are particularly likely to contain INNS. Firstly, many freshwater ecosystems experience significant environmental fluctuations, from regularly drying out, to flooding, or freezing over (Emery-Butcher et al., 2020). Species which inhabit these systems have long-evolved strategies which enable them to survive significant disturbances (Barrat-Segretain et al., 1998; Hengherr and Schill, 2011; Strachan et al., 2015). These adaptations often include the ability to tolerate broad ranges of environmental variables (e.g. temperature), possess high fecundity, undergo asexual reproduction, maintain generalist diets and display phenotypic plasticity (Emery-Butcher et al., 2020). Although these traits are not exclusive or universal between all INNS, these traits are common in many successful invaders (Strayer, 2010), and indicate that many freshwater species are pre-adapted to potentially become successful invaders (Emery-Butcher et al., 2020). Secondly, intense human reliance and use of rivers, lakes and coastlines fuels the movement of these species between freshwater systems, either undertaken intentionally or occurring through accidental introduction. Human endeavours such as aquaculture, canal construction, recreational activities (e.g. sailing), ballast water discharge, angling, and tourism, maintain a consistent propagule pressure and contribute to the unprecedented rate of species introductions in aquatic ecosystems (Carlton and Geller, 1993; Cohen and Carlton, 1998; Ruiz et al., 2000).

Freshwater ecosystems represent an ideal model system to understand the impacts of INNS as the ecology of aquatic systems is characterised by strong trophic links that can be highly disturbed by the loss, displacement or introduction of certain species (Carpenter et al., 1985). The impacts of INNS on an invaded freshwater system can vary greatly depending upon the species of the invader and its position within the food web (Thomsen et al., 2014). If an INNS enters a system at the upper trophic levels, then it may be expected to exert “top-down” changes in the abundance and biomass of species in the lower trophic levels. For example, the introduction of zooplanktivorous fish species in Spanish reservoirs resulted in a significant decline in both the abundance and species richness of large-bodied zooplankton, therefore reducing existing top-down grazing pressures on phytoplankton species, leading to increases in phytoplankton abundance (Ordóñez et al., 2010). Whereas an INNS which establishes within the lower levels of the trophic structure, may exert “bottom-up” controls, limiting nutrients and resources to higher levels of the food web. For example, prolific filter feeders like the zebra mussel (*Dreissena polymorpha*) can significantly affect phytoplankton community structure and reduce overall phytoplankton abundance within the system, as well as removing small zooplankton species (Ward and Ricciardi, 2007). This can promote the growth of bottom rooting macrophytes (by reducing competition for light and nutrient resources) and reduce food resource availability for small zooplanktivorous fishes, demonstrating that changes in phytoplankton abundance has significant implications for species occupying higher trophic levels.

The effects of some INNS on the recipient habitat and native community composition are further exacerbated by their role as "ecosystem engineers". Ecosystem engineers (a term initially posited by Jones et al., 1994) "directly or indirectly modulate the availability of resources to other species by causing physical state changes in biotic or abiotic materials" (Jones et al., 1994). This concept has sometimes been criticised for being too broad and flexible, in that, in some respect all species can be defined as engineers of their environment (Reichman and Seabloom, 2002a, 2002b). However, the concept explicitly excludes any INNS impacts which affect an ecosystem solely through the predation of other species, or by providing a novel food source for other species. Ecosystem effects driven by ecosystem engineering species include; sediment resuspension, caused by the introduction of the common carp (*Cyprinus carpio*) (Haynes et al., 2012); reduced light levels in the water column, caused by floating macrophytes such as floating pennywort (*Hydrocotyle ranunculoides*) (Cabrera Walsh et al., 2013); or creation of an increasingly structurally complex benthic habitat, reduced turbidity and alterations to nutrient cycling, resultant from quagga mussel (*Dreissena rostriformis bugensis*) establishment (Sousa et al., 2009). The ecological engineering impacts of INNS do not necessarily complement the impacts produced through their direct biotic interactions, and therefore the overall impact of the establishment of INNS largely depends on the intensity of both processes (Pollen-Bankhead et al., 2009). This additional layer of complexity may further confound the identification of a unified theoretical framework to explain and predict ecological impacts from INNS.

The impacts of INNS are often framed as negative, however, as with native species, many INNS may also impart beneficial impacts on biota, the environment, and the economy, which should be considered in assessments of their overall impact. For example, whether native or alien, the roots of macrophytes stabilise sediments during flooding to help prevent bank collapses (Pollen-Bankhead et al., 2009), while also providing complex habitat and predation refuges which can benefit native fish diversity (Bickel and Closs, 2008; Anderson et al., 2014). Similarly, invasive bivalves can improve water clarity and increase nutrient availability for other organisms (Sousa et al., 2014), while also providing complex refuges and colonisable habitat structures for native species, on an otherwise bare substrate, through the formation of mussel beds (Devanna et al., 2011). For example, mussel beds created by non-native dreissenid mussels in the North American Great Lakes have been implicated in the return and support of the native mayfly *Hexagenia limbata* and *H. rigida*, which are essential food sources for many economically important fish species (Hayward and Margraf, 1987; Schaeffer et al., 2000). These *Hexagenia* species became rare during eutrophic conditions in the Great Lakes during the 1950s, and their return has been consistently found to be associated with the presence of live dreissenid mussels, presumably due to the additional nutrients and protection they provide (Devanna et al., 2011). Even if such relationships are highly unusual, identifying them where they occur could be locally important for preventing loss of native taxa.

More broadly encompassing approaches for assessing the impact of INNS, which acknowledge both positive and negative impacts, will allow for a better understanding of their effects on ecosystem services, potential management strategies, and prospective commercial use cases. With regard to the management of water resources, a research area of particular interest is concerned with how the establishment of INNS may affect ecosystem shifts between alternative stable states. The theory of alternative equilibria demonstrates that shallow lakes primarily exist in one of two alternative stable states: clear with a high abundance of submerged vegetation, or turbid with high phytoplankton abundance and few submerged plants (Scheffer et al., 1993). The direct and ecological engineering effects of certain INNS, such as invasive bivalves, may assist in driving systems away from phytoplankton dominance and towards a clear water state (Figure 1.1). In addition to potential changes in overall phytoplankton abundance, changes in phytoplankton community structure are also crucially important to the functioning of freshwater ecosystems.

Freshwater phytoplankton occupy a pivotal trophic position as producers, providing energy and nutrients to the aquatic food web. Periodically, and with increasing frequency, certain phytoplankton species, particularly cyanobacteria, are promoted by environmental or ecological forces to rapidly accumulate biomass which dominate affected systems. Cyanobacteria are photosynthetic bacteria commonly referred to as ‘blue-green algae’. However, strictly speaking they are not algae, as this is a term reserved for eukaryotic phototrophs. Rapid accumulations of cyanobacteria biomass are often referred to as harmful algal blooms (HABs). Global changes, such as climate warming (Huisman et al., 2018), hydrological intensification (whereby arid areas become more arid and wet areas become wetter, accompanied by more frequent and intense precipitation events) (Huntington, 2006; Trenberth, 2011), eutrophication (Downing, 2014) and brownification (whereby freshwater systems contain high levels of dissolved organic matter) (Kritzberg and Ekström, 2012), have led to a recent upsurge in the prevalence of HABs (Reid et al., 2019). HABs pose significant threats to the ecology of an ecosystem by creating adverse physiological conditions for their competitors, dominating light resources, reducing dissolved oxygen concentrations, altering the flow of energy and nutrients, and producing allelopathic and toxic compounds which affect the growth, survival and reproduction of other organisms and contaminate food webs (Reid et al., 2019).

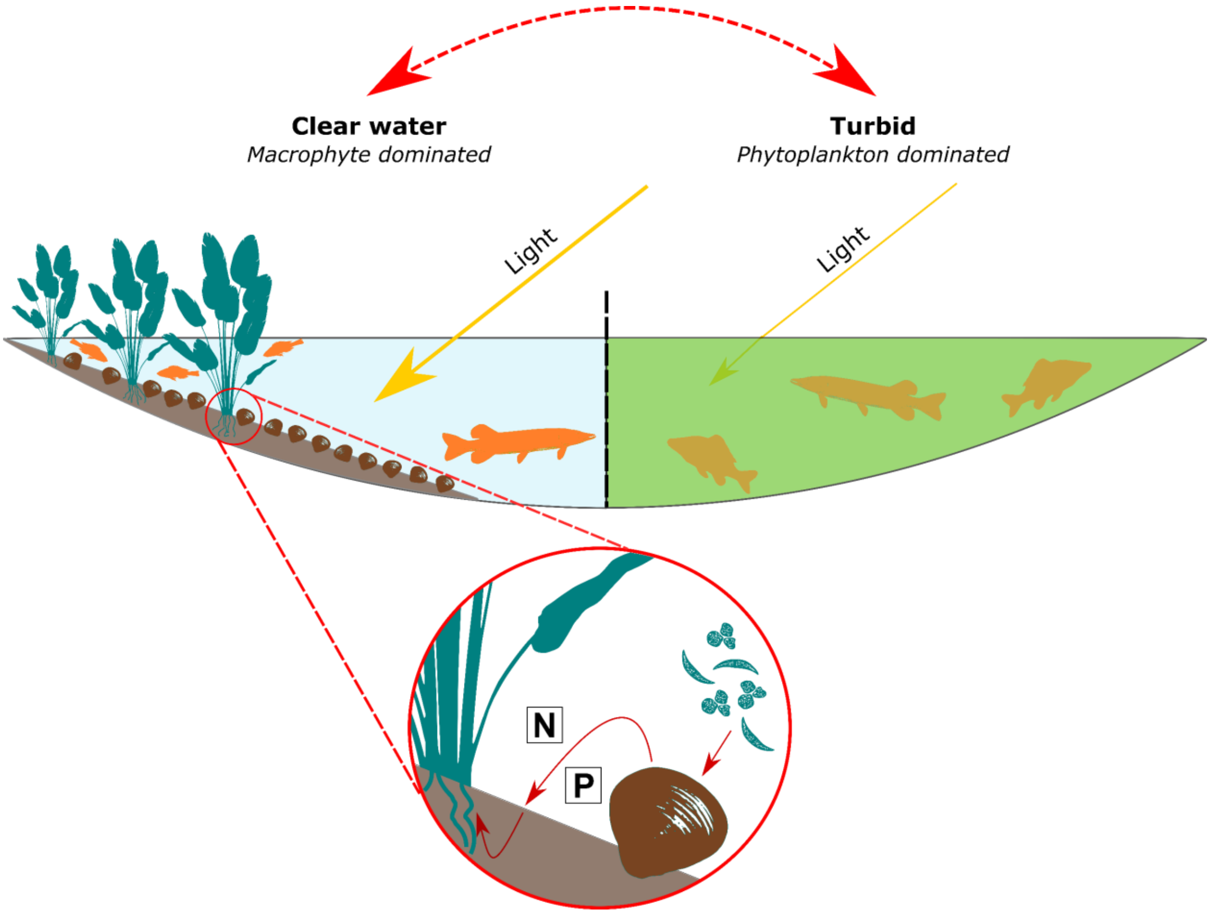


Figure 1.1 The potential effects of invasive ecosystem engineering bivalves on alternative stable states in shallow lake ecosystems. The diagram illustrates how the proliferation of invasive mussel beds could drive a system from a phytoplankton-dominated (right hand side) to a macrophyte-dominated (left hand side) stable state. The mussel beds add structure and predation refuges; allow for the accumulation of sediment; improve light penetration through the removal of phytoplankton biomass; encourage the establishment of macrophytes through sediment retention and phytoplankton removal (which, in turn, provide refuge for juvenile and small herbivorous fish species) and sequester nutrients to the sediment and benthic environment though faeces and pseudofaeces.

In addition to the impacts of HABs on the ecology of freshwater systems, they also threaten the health and water security of human populations. HABs are one of the most significant threats to the security of the Earth's surface freshwaters and are recurring events in many of the planet's most socio-economically important waterbodies which provide drinking water for millions of people (Davis et al., 2019). In 2007, a national lake survey conducted by the Environmental Protection Agency in the United States revealed that 76% of the lakes surveyed were dominated by cyanobacterial species, of which 95% contained toxin producing species (Loftin et al., 2016). Pressure on water resources means that many cities are forced to actively manage freshwater systems to reduce cyanobacteria abundance and attempt to neutralise toxins for many months of the year, increasing costs to water users while acting to undermine users' confidence in public water utilities. In recent years, drinking water advisories have been issued for major cities in China (Qin et al., 2010), the United States (Steffen et al., 2017) and Africa (Sitoki et al., 2012). For example, in 2018 elevated toxin levels produced by the cyanobacteria *Microcystis aeruginosa* led to a ‘do not drink’ advisory issued to Salem, the capital city of Oregon (The City of Salem, 2018). Management measures employed to prevent disruption to freshwater resources include: reducing nutrient loads (Paerl et al., 2011), aerating and chemically treating lake sediments (Prepas et al., 1997; Molot et al., 2014), applying algicides and flocculants (Shen et al., 2019), increasing water flows to reduce water residence time (Rastogi et al., 2015), and biological control through introducing organisms which consume HAB species (Reid et al., 2019). Chemical and physical management strategies are often expensive, ephemeral, and labour intensive, while chemical treatments can also be toxic to non-target species, including humans (Sun et al., 2018). Biological approaches have been suggested to offer more efficient, cost-effective, and sustainable control (Backer et al., 2015; Harke et al., 2016; Sun et al., 2018).

In this thesis, I explore how certain INNS may affect the alternative equilibria of freshwater ecosystems and influence phytoplankton community structure and cyanobacteria abundance, focusing on the direct impacts to phytoplankton and cyanobacteria communities driven by the nuanced grazing behaviour of dreissenid mussels (*Dreissena polymorpha* and *Dreissena rostriformis bugensis*) and the potential indirect impacts elicited by allelopathic chemicals secreted by invasive plants (*Hydrocotyle ranunculoides* and *Crassula helmsii*). These INNS, in their capacity as ecosystem engineers, have the power to drive ecosystem-level changes in invaded systems and therefore may provide interesting biological management opportunities. Not only may these organisms have the potential to aid water resource management by maintaining or reverting freshwater systems to a clear water state, or directly driving favourable changes in phytoplankton communities, through the removal of cyanobacterial species, but may also indirectly affect sustained ecosystem change to support the continued provision of these effects (Emery-Butcher et al., 2020).

Of course, the risks and benefits of introducing, maintaining, or encouraging the proliferation of INNS for management purposes, in either new or already impacted systems, need to be carefully assessed on a case-by-case basis. The risk profile of encouraging INNS varies vastly when comparing a closed system within an invaded range, to an open system with links to surrounding waterways, in an uninvaded range. I would never recommend introducing INNS into open water systems in an uninvaded range, however, there may be opportunities to leverage the desired effects of certain INNS within already invaded systems or closed and depleted systems within an invaded range.

The chapters within this thesis are written as discrete research papers. As such, each chapter has an introduction that sets the context of the specific study, and there are therefore occasions where common themes are unavoidably revisited between chapters. Chapter 2 explores, through meta-analysis, the potential impacts of INNS on the alternative equilibria of shallow lakes. Chapter 3 investigates the impacts of quagga mussel establishment on reservoir water quality. Chapter 4 contrasts the clearance rate and pseudofaeces production behaviours of quagga mussels from lentic and lotic environments when exposed to different phytoplankton communities. Chapter 5 examines the allelopathic effects of the invasive plants *Hydrocotyle ranunculoides* and *Crassula helmsii*, on the average population growth of certain phytoplankton species. This paper was published in *Frontiers in Environmental Science* (Reynolds and Aldridge, 2021). Chapter 6 considers how zebra mussels could be leveraged in the production of algal biofuels, and their potential role in the novel concept of Synthetic Ecology. This paper was published in *Scientific Reports* (Reynolds et al., 2019). Chapter 7 presents an overall synthesis of the findings outlined in each chapter, considering the role INNS could play in the management and exploitation of freshwater ecosystems.

Chapter 2

Global impacts of invasive species on the tipping points of shallow lakes

Abstract

There is growing acknowledgement that human-induced change can push ecosystems beyond tipping points, resulting in the dramatic and sudden loss of vital ecosystem services. Invasive non-native species (INNS) are spreading rapidly due to anthropogenic activities and climate change, and can drive changes to ecosystem functioning by altering abiotic conditions and restructuring native communities. Shallow lake ecosystems are especially vulnerable to perturbation from INNS as they can exist in two alternative stable states: either clear water with an abundance of vegetation, or turbid, unvegetated, and dominated by phytoplankton. Here we show that INNS can alter the trajectory of both the collapse and recovery of shallow lake ecosystems. Through a global meta-analysis of studies observing the effects of INNS on recipient lake ecosystems, we found that certain INNS drive significant changes in the abundance of key taxa and conditions that govern the balance of alternative equilibria in shallow lakes. Invasive fish and crustaceans demonstrated effects likely to lead to early ecosystem collapse to a turbid state, and delay ecosystem recovery. Invasive molluscs presented opposite effects, which are likely to delay ecosystem collapse and encourage ecosystem recovery. Our results demonstrate that INNS could significantly alter the tipping points of ecosystem collapse and recovery, but also that not all invasive species may initiate system collapse. Our results provide guidance for managers of invaded shallow lake ecosystems, which provide diverse services including sanitation, potable water supply, industrial cooling, aquaculture and recreational resources. Moreover, our approach could be applied to identify key potential drivers of change in other crucial ecosystems which demonstrate alternative equilibria, such as coral reefs and kelp forests.

2.1 Introduction

Invasive non-native invasive species (INNS) can alter ecosystem functioning and structure, affecting native biota (e.g., density, distribution, species richness, abundance and biomass of other species) and abiotic conditions (e.g., nutrient availability, physical habitat structure, light levels and the flow of materials) (Charles and Dukes, 2007; Essl et al., 2020). Aquatic systems are particularly vulnerable to the establishment of INNS, due to a history of disturbance through intense anthropogenic exploitation of rivers, lakes and coastal areas for aquaculture, recreation, canal construction, discharge of ballast waters and sport fisheries (Ricciardi et al., 2000; Kolar and Lodge, 2002; Gallardo and Aldridge, 2013, 2018). As successful INNS often constitute new functional components in the recipient community, their ecological impacts propagate throughout the food web, often triggering trophic cascades (Strayer, 2010). The capabilities of INNS to propagate changes in invaded systems has led them to often be characterised as ecosystem engineers (Crooks, 2002; Sousa et al., 2009). The ability of INNS to influence the functionality of ecosystems suggests that their establishment could have implications for the alternative equilibria model for shallow lakes (Scheffer et al., 1993).

It has become increasingly clear that complex dynamical systems, from ecosystems to financial markets to the climate, have tipping points at which a sudden shift to a contrasting state may occur (Scheffer et al., 2009). The concept of alternative equilibria explains the phenomenon where a shallow lake can exist in one of two alternative stable states: clear with a high abundance of submerged vegetation, or turbid with few submerged plants (Scheffer et al., 1993) with the alternative equilibria stabilised by a number of reinforcing mechanisms (Scheffer et al., 1993) (Figure 2.1a). As a consequence of this reinforcement, once a system becomes turbid, it subsequently resists restoration efforts (Scheffer and Van Nes, 2007). For example, in turbid states with high phytoplankton biomass and suspended sediments, the buffering effects of bottom-rooting macrophytes are lost and restoration to the clear water state requires extensive reductions in nutrients or turbidity. This is a phenomena widely observed in shallow lake ecosystems (Hilt et al., 2017), and was originally brought into focus through the study of sites on the Norfolk Broads (Phillips et al., 1978), such as Hudson’s Bay and Hoveton Great Broad (Timms and Moss, 1984) and numerous Dutch lakes, such as Lake Bleiswijkse Zoom (Meijer et al., 1989).

In shallow lake ecosystems the level of the critical threshold, or tipping point, is thought to be driven by three basic assumptions: 1) Turbidity increases with the nutrient level as a result of phytoplankton growth; 2) Vegetation reduces turbidity; 3) Vegetation disappears when a critical turbidity is exceeded (Scheffer et al., 1993). This can be illustrated by a simple graphical model, initially presented by Scheffer et al. 1993 (Figure 2.1b). Equilibrium turbidity can be demonstrated by two different functions of the nutrient level in both macrophyte dominated and unvegetated states. Above the threshold of critical turbidity vegetation will be absent, and therefore the upper equilibria line applies.

Below the critical turbidity threshold, where vegetation can survive, the lower equilibria line applies. This simple model intuitively demonstrates the existence of alternative equilibria and how a system may demonstrate hysteresis and catastrophic transitions (tipping points). Steady nutrient enrichment of a system will cause it to progress along the lower limit line, until the critical threshold is reached, at which point vegetation disappears, and the system jumps a higher level of turbidity. For a system to revert to a clear water state, it must reach the alternative tipping point at which phytoplankton growth is limited enough to allow vegetation to establish and dominate.

The theory of alternative stable states remains a central concept for the study and understanding of shallow lake systems (Dakos et al., 2019; Carpenter et al., 2020; Schallenberg, 2020). While the effects of INNS on shallow lakes is widely documented, there has been no attempt to systematically appraise the likely outcome of multiple taxonomic or functional groups on the recipient ecosystem. The alternative equilibrium model provides an established and well-understood framework against which ecosystem-level outcomes can be mapped. We believe that due to the outsized effects INNS can have on an ecosystem, they may act to alter the rate at which tipping points are reached by directly or indirectly affecting interactions between the drivers of the alternative equilibria model illustrated in Figure 2.1a. This study presents the results of a comprehensive global meta-analysis of studies which report the effect of INNS on recipient ecosystems. This allowed us to calculate effect sizes of different taxonomic groups of INNS (fish, molluscs, plants and crustaceans) on key components of the alternative equilibria model in order to evaluate how INNS may affect tipping points in shallow lake ecosystems.

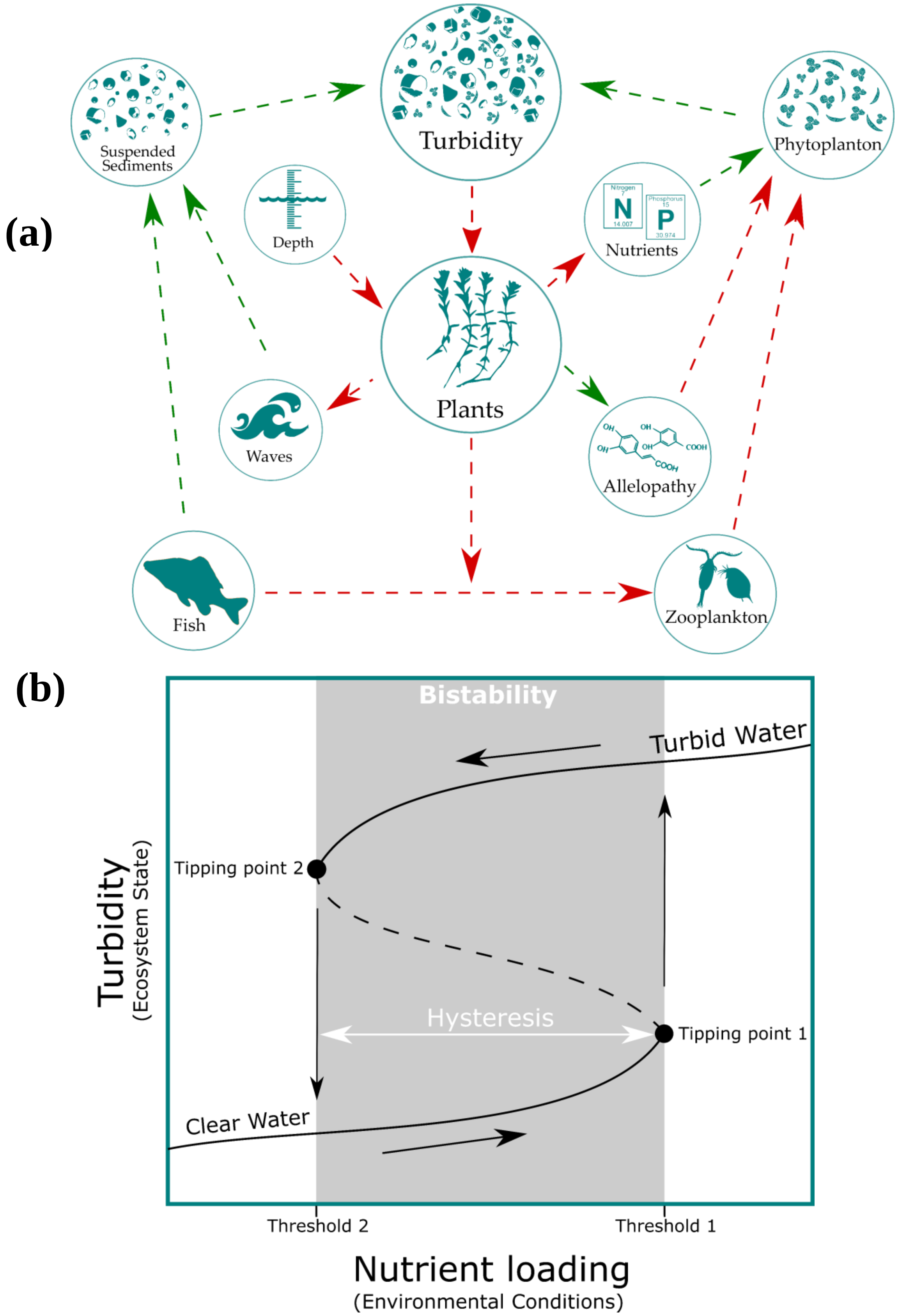


Figure 2.1 Interactions and tipping points which govern the alternative stable states of shallow lake ecosystems. (a) Main feedback loops thought to be responsible for alternative equilibria in shallow lakes. Green arrows indicate positive effects, red arrows negative effects. (b) Tipping points

mark the discontinuous changes in ecosystem state. The black solid lines represent the stable equilibria. The dashed line represents the border between the basins of attraction between the two stable states. The lower solid line indicates a clear water state. Plants can only exist on the lower line, until Threshold 1 is reached. Threshold 1 represents the critical turbidity level of the system. As nutrient loads increase, turbidity follows the lower solid line until the line crosses Threshold 1. At this point (Tipping point 1) the equilibrium disappears, and the system's turbidity moves abruptly to the upper, unvegetated and phytoplankton dominated, stable state. In order for a system to return to the lower line, nutrients must be reduced to a much lower level, where the system crosses Threshold 2 (Tipping point 2), or turbidity must be substantially reduced. The difference between the tipping points marks the hysteresis in the system.

2.2 Methods

2.2.1 Data acquisition

Data were collated from a publicly available dataset regarding the global ecological impacts of INNS on freshwater environments (Gallardo et al., 2016), and brought up to date through the collation of additional data gathered by Tang et al., (2020 [*PhD Thesis*]) (Tang, 2020), such that all available published data were collated up until 30 December 2019. In total, Tang et al., (2020 [*PhD Thesis*]), identified 51 additional studies published since Gallardo et al., (2016). Both studies implemented identical literature search methodologies, inclusion criteria and data collation (See Appendix A - Supplementary Methods).

The combined database was filtered to contain only data related to freshwater lakes and reservoirs (Table A1). Taxonomic level data was used to categorise the invasive species recorded (Table A2). For the final analysis, the biotic response variables were filtered to contain only abundance related data (comprising density, biomass and coverage metrics), as opposed to species richness data, and grouped by taxonomic level. Only abiotic variables categorised as "nutrients" were included in this study. This resulted in a dataset of 418 observations across 101 studies for 54 unique INNS.

2.2.2 Data Analysis

The Hedges' g effect size, also known as Standardised Mean Difference (SMD), and variance was calculated for each observation (418 observations) to estimate the differences in the response variable between the experimental and control treatments. The SMD weighs cases by their sample size and the inverse of their variance and was calculated as follows:

$$SMD = \frac{(X_E - X_C)}{J} S$$

Where X denotes the mean value of the response variable being abundance or nutrients in treatment (X_E) and control (X_C) groups; S is the pooled standard deviation of the two groups; and J is a weighting factor based on the number of replicates in the treatment and control groups. *J* was calculated as:

$$J = \left(1 - \frac{3}{4(X_E + X_C) - 9}\right)$$

SMD is unitless and ranges from -∞ to +∞. The magnitude of SMD can be interpreted as follows: ≤0.2 is considered a small effect, 0.2 ≤ |SMD| ≥ 0.5 is a medium effect; 0.5 ≤ |SMD| ≥ 0.8 a large effect; and ≥ 1.0 is a very large effect(Anton et al., 2019).

Meta-analysis was conducted using the metaphor package for R (Viechtbauer, 2010). We assessed the effect size of each taxonomic group of INNS (crustaceans, plants, fish and molluscs) on each environmental response variable (benthic invertebrates, plants, fish, phytoplankton, zooplankton and nutrients) by building a separate mixed-effect model for each combination of response variable and taxonomic group of INNS. Mixed-effects models used the study reference and invasive species name as random factors to account for the effect of multiple observations of an exotic species within a study and across multiple studies. Model results were considered significant if their 95% CI interval did not overlap with zero, and *P* values were ≤0.05. All models used the rma.mv function, which calculates statistical significance based upon a Wald-type test. Effect sizes were only calculated where there were ≥5 cases capturing the effect of an invasive taxa on a single response variable (Gallardo et al., 2016). We did not calculate effect sizes for interactions where the taxonomic group of INNS was the same as the taxonomic group of the response variable, for example, the effect of invasive plant species on existing plant species' abundance. This was done as, for the purposes of this study, we focused on the effect of INNS on the overall abundances of certain taxa. Studies which reported changes in the abundance of existing taxonomic groups did not provide data for the subsequent total abundance of that taxonomic group in the system. For example, although an invasive plant may lead to a decline in native plant abundance, the overall abundance of plants in the system may have increased.

2.2.3 Publication bias

Publication bias refers to the selective publication of articles finding significant effects over those which find non-significant effects. Publication bias could distort the results of this meta-analysis as it could lead to an overestimate of the effects of invasive species on the aquatic environment. As noted by Anton et al., 2019, regtest and trimfill functions are not implemented in the metafor package for mixed-effects models (Viechtbauer, 2010). Publication bias was therefore evaluated using Egger's regression test (Egger et al., 1997), running models which included the standard error of the effect sizes (as the square root of the variance) as a moderator (Viechtbauer and Cheung, 2010). Potential publication bias was determined when the intercept of the model was significantly different from zero (*P*≤0.05). The data was then examined for potential outliers, which were defined as effect sizes with standardised residual values exceeding the absolute value of three (Viechtbauer and Cheung, 2010) using the rstandard function in R. Potential outliers were removed to adjust for potential publication bias. Adjusting for publication bias did not change the outcome of the analyses. The need to adjust for publication bias would have been confirmed when the significance of the random-effects model, with the study reference as a random factor, changed before and after the removal of potential outliers (Table A3). The sensitivity analysis illustrated that the findings are robust against publication bias.

The focused scope of this study, robust calculation of effect sizes, implementation of appropriate mixed-effects modelling, assessment of publication bias using Egger's regression test, and transparency regarding methodology, raw data and analytical code allow this study to avoid the majority of literature review pitfalls outlined by Haddaway et al., (2020) (Haddaway et al., 2020).

2.3 Results

Data were systematically compiled from 418 observations across 101 studies for 54 unique INNS. The results of the overall analyses indicate that the arrival of invasive fish, crustaceans and molluscs are likely to have a significant negative effect on the abundance of certain taxa and nutrients which are central to the maintenance of states within alternative equilibria (Figure 2.2 and 2.3). However, invasive plants did not have a significant impact on the abundance of any taxa or nutrients (Figure 2.2 and 2.3).

Invasive crustaceans (Table A2) had a significant negative effect on zooplankton abundance (SMD=-0.74±0.69 (mean effect size ±95% CI), *P*=0.035) and plant abundance (SMD= -1.4±0.83, *P*<0.001). Non-significant effects of invasive crustaceans were observed for phytoplankton (SMD=0.36±0.63, *P*=0.254), nutrients abundance (SMD=-0.54±0.95, *P*=0.264) and on benthic invertebrate abundance (SMD=-0.34±0.54, *P*=0.222). There were insufficient data to calculate effect sizes for invasive crustacean impacts on fish abundances.

Invasive fish (Table A2) had a significant positive effect on nutrient abundance (SMD=0.73±0.48, *P*=0.003) and a negative impact on plant abundance (SMD=-1.01±0.66, *P*=0.003). Non-significant effects were found for abundances of benthic invertebrate (SMD=-0.39±0.59, *P*=0.196), phytoplankton (SMD=-0.25±0.47, *P*=0.297), and zooplankton (SMD=-0.15±0.47, *P*=0.517).

Invasive molluscs (Table A2) had a significant positive effect on the abundance of benthic invertebrates (SMD=0.89±0.72, *P*=0.015) and a negative effect on phytoplankton abundance (SMD=-0.98±0.92, *P*=0.037). Non-significant effects were calculated for abundance of zooplankton (SMD=-0.79±1.08, *P*=0.150) and nutrients (SMD=0.05±1.12, *P*=0.935). There were insufficient data to calculate the effects of invasive molluscs on fish or plant abundances.

Invasive plants (Table A2) had non-significant negative effects on abundance of benthic invertebrates (SMD=-0.74±1.18, *P*=0.222) and nutrients (SMD=0.59±0.68, *P*=0.088). There were insufficient data to calculate effect sizes of invasive plants on phytoplankton, zooplankton, and fish abundances.

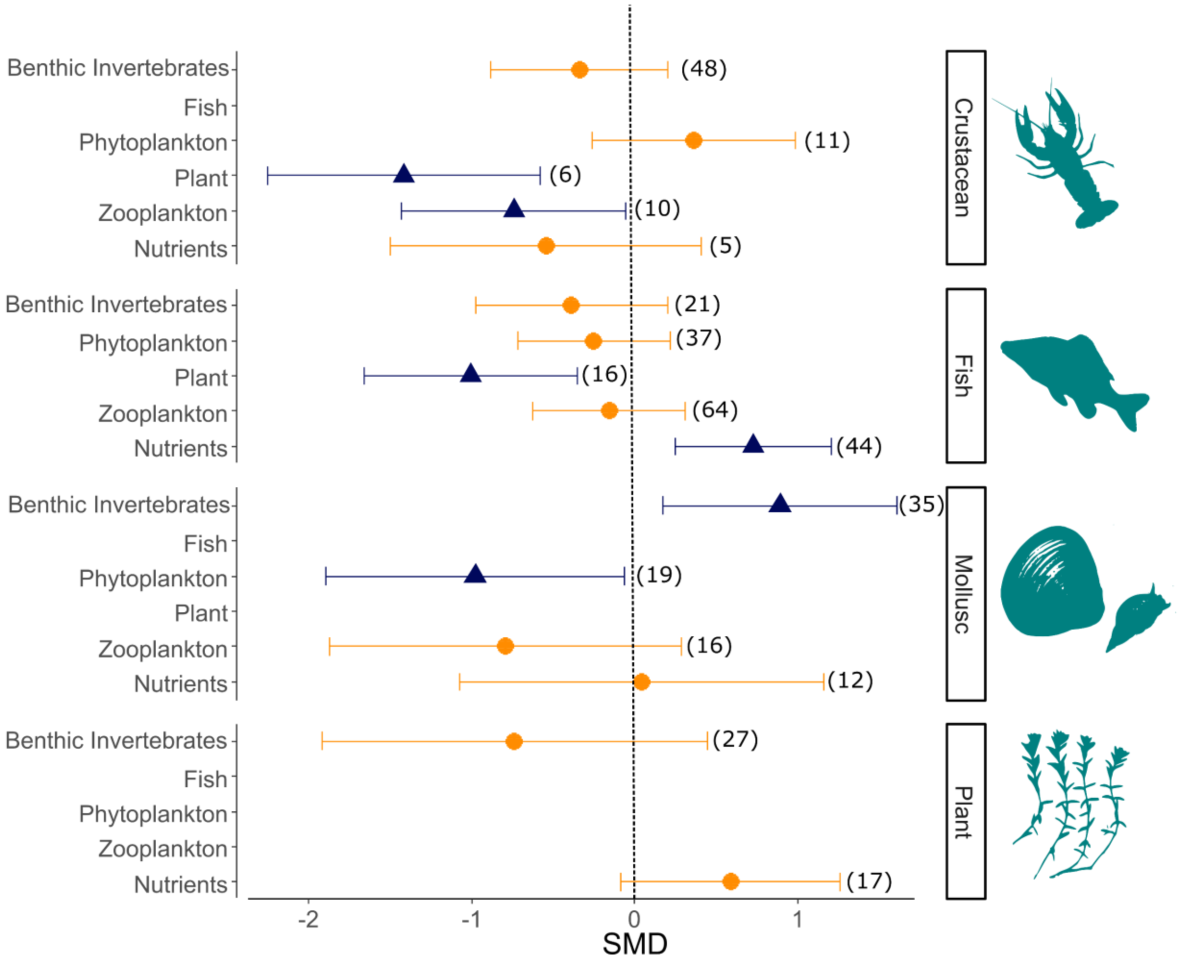


Figure 2.2 Mean effect size (Standard Mean Deviation ±95% CI) of invasive species on response variables that drive alternative stable states in shallow lakes. Effect size (SMD) indicates an increase (SMD > 0) or a decrease (SMD < 0) in abundance or the response variable. The dotted vertical line indicates where SMD = 0. The bars represent the 95% CI, significant effects (blue bars, triangle points) are observed when they do not overlap zero. Non-significant effects are denoted by yellow bars and circular points. Numbers in brackets denote the number of observations used to calculate the SMD. Blank interactions are a result of < 5 observations being available to calculate the SMD.

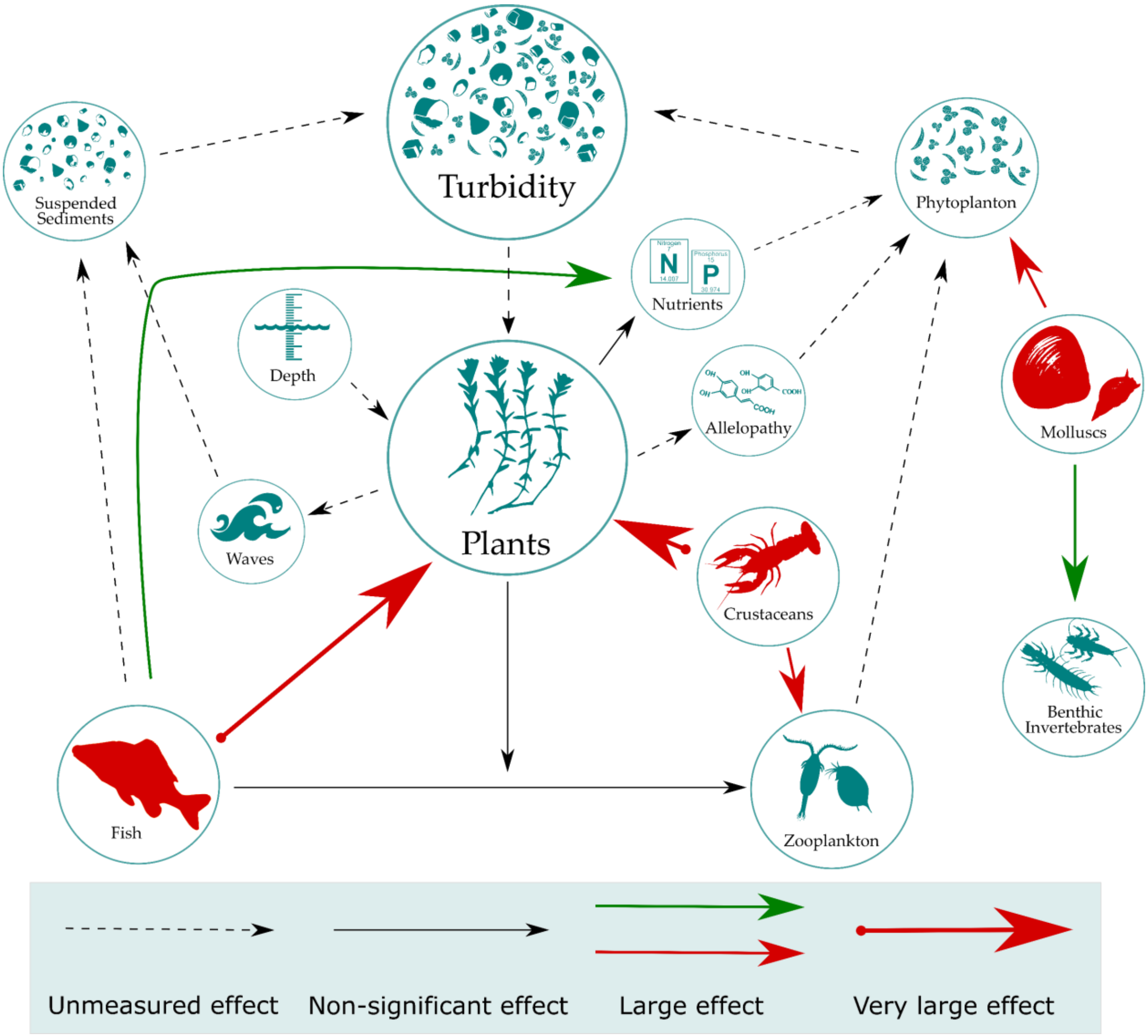


Figure 2.3 Interactions between significant invasive species effects on the dynamics of alternative equilibria in shallow lakes. The size and shape of the arrows indicate the effect size (SMD) of the invasive taxa on the abundance of relevant reponse variables. Red arrows indicate negative effects (SMD < 0), and green arrows indicate positive effects (SMD > 0). The size of the effect is determined by the value of the SMD, and can be interpreted as follows: ≤0.2 is considered a small effect, 0.2 ≤ |SMD| ≥ 0.5 is a medium effect; 0.5 ≤ |SMD| ≥ 0.8 a large effect; and ≥ 1.0 is a very large effect (Anton et al., 2019). Unmeasured effects capture the interactions within the system which were not directly measured in the studies available for meta analysis. Non-significant effect arrows indicate where there the SMD±95% CI overlaps with zero.

2.4 Discussion

2.4.1 The effects of invasive species on the dynamics of alternative equilibria

Figure 2.3 illustrates how the effects revealed through the meta-analysis map onto the dynamic system which governs the alternative equilibria of shallow lakes. Invasive fish had two large and synergistic effects on recipient ecosystems, with a large positive effect on nutrient abundance and a very large negative effect on plant abundance. Both of these effects have the consequence of pushing a system towards maintaining a turbid state. Reduced plant abundance may lead to increased wave action and suspension of sediments, while simultaneously increasing nutrient availability for phytoplankton and relieving potential allelopathic pressures on phytoplankton growth. The positive effect on nutrient abundance associated with invasive fish could be associated directly with factors such as disturbance-related release of benthic phosphorus (Adámek and Maršálek, 2013), or indirectly such as through foraging-related reductions in plant abundance (Miller and Provenza, 2007).

Crustaceans had a similarly synergistic effect on their invaded systems, with a large negative effect on zooplankton abundance and a very large negative effect on plant abundance, both of which can push a vegetated clear water system towards the critical turbidity threshold. The ecosystem-level effects of increased turbidity resulting from reduced plant abundance are exacerbated through simultaneous reductions in the grazing effects of zooplankton, further enabling proliferation of phytoplankton.

Systems invaded by molluscs experienced a large decline in phytoplankton abundance, and a large increase in benthic invertebrate abundance. The ability of bivalve molluscs to directly remove phytoplankton through their filter feeding activities may act to maintain a clear water state at higher nutrient levels and has the potential to push a turbid unvegetated system towards the tipping point for a clear water system. Indeed, studies of invasive zebra mussels (*Dreissena polymorpha*) in the tidal Hudson River revealed a 17-fold decrease in phytoplankton and proliferation of bottom-rooting macrophytes (Smith et al., 1998). Additionally, gastropods reduce algae abundance and encourage macrophyte growth through actively grazing periphytic algae which compete with macrophytes for light and nutrients (Lodge, 1986; Brönmark, 1989). Increased benthic invertebrate abundance resulting from mollusc invasions is likely tied to the often-noted effects of bivalve beds which in shallow systems provide substrate in the form of shells and increased macrophyte growth, shelter from predation and food for benthic invertebrates through the enrichment of sediments with organic matter from the bivalves' faeces and pseudofaeces (Strayer et al., 1998; Zhang et al., 2011; Duchini et al., 2018). This transfer of particulate matter and nutrients from suspension in the pelagic environment to deposition into the benthic sediment, and subsequent assimilation into benthic invertebrate biomass, can push systems towards a clear water state.

In this analysis, species were merged into appropriate taxonomic groups as to best reflect the taxonomic categories presented in the model of alternative equilibria. By doing so, it is recognised that both species level data and information pertaining to the trophic level of certain organisms, would be omitted from the analysis. By following this approach, we lose the ability to identify certain native species which may be particularly affected by the arrival of INNS, and equally, we cannot identify specific INNS which may have an outsized effect on the abundance of certain native species. However, as this analysis is focused at assessing the impact of INNS on the established model of alternative equilibria at the ecosystem level, it was appropriate to group INNS based upon the categories outlined in the model. Gallardo et al. (2016) use this dataset to extensively explore the effect of the trophic position of an INNS on certain biotic and abiotic characteristics of freshwater ecosystems, however, in the context of a further study, there may be value in exploring some of the species-specific interactions in the data which are yet to be quantified.

2.4.2 Invasive molluscs could delay a tipping point to a turbid state

In the context of a system in a clear water state, invasive molluscs could delay a tipping point to a turbid system. The ability of molluscs to supress phytoplankton biomass masks the effect of increasing eutrophication. This masking effect has been noted in studies of the invasive bivalve, *Dreissena polymorpha* (Dzialowski and Jessie, 2009). The removal of phytoplankton results in a potential delay in a system reaching the catastrophic tipping point, shifting the threshold of stress at which the system collapse occurs to a higher stress level (Figure 2.4a).

2.4.3 Invasive fish and crustaceans could lead to an earlier tipping point to a turbid state

Opposite to the effects of molluscs, invasive fish and crustaceans may lead to a tipping point occurring at a lower level of environmental stress, increasing the risk of ecosystem collapse. The detrimental effects of fish and crustaceans on zooplankton and plant abundance may reduce the capacity of plants to outgrow and outcompete phytoplankton for nutrients and light. This reduction in the suppressive power of plants and zooplankton on phytoplankton growth, accelerates the rate at which a system reaches its tipping point and supresses its ability to maintain a clear water state (Figure 2.4b).

2.4.4 Invasive species could affect system recovery to a clear water state

In the case of a recovering system, the effects of INNS on tipping points are reversed, with molluscs leading to an earlier recovery tipping point (Figure 2.4c), and fish and crustaceans resulting in a delayed tipping point (Figure 2.4d). What this demonstrates, especially in the case of invasive molluscs, is that under a high abundance of certain INNS a smaller decrease in environmental stress, in this case nutrients, is necessary to reach a tipping point. In the case of fish and crustaceans, due to the slower growing nature of bottom-rooting vegetation compared to phytoplankton growth, nutrients would need to be reduced to a much lower level to reach a tipping point. It is even possible that heavily affected systems, invaded by high abundances of crustaceans or fish, could reach a point at which nutrients would need to be reduced to a biologically impossible level to reach the tipping point. In this scenario a system may lose its bistability once in a turbid state, while the invasive crustaceans and fish remain.

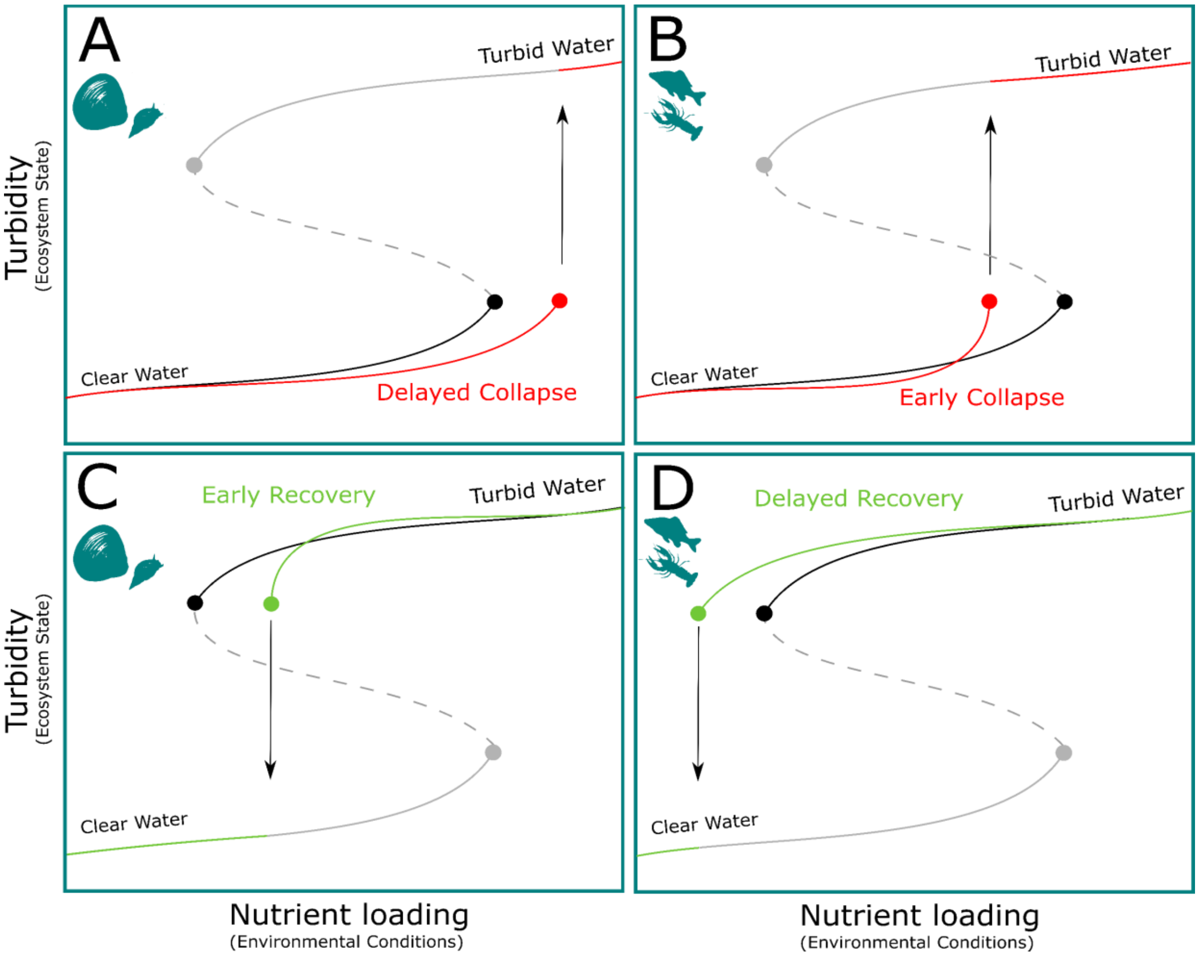


Figure 2.4 Predicted alterations of trajectories of ecosystem collapse (red solid lines) and recovery (green solid lines) as a consequence of invasive species establishment. The black and grey lines represent the two alternative stable states of a reference ecosystem containing no invasive species. The grey dashed lines indicate the unstable boundary between the two states, with circles denoting the tipping points. The coloured lines indicate the predicted trajectories of invaded systems as revealed by the meta-analysis, red lines signify collapse trajectories and green lines denote recovery trajectories. Panels **A** and **C** indicate systems with established populations of invasive molluscs. Panels **B** and **D** illustrate systems with established populations of invasive fish and/or crustaceans.

2.4.5 Implications for management of invasive species in shallow lakes

With the global rise in the spread of INNS (Essl et al., 2020), our study highlights some of the effects that may arise as a result of their establishment in shallow lake systems. Understanding how particular INNS can drive driving tipping points in shallow lakes could allow water resource managers to make better informed decisions. For example, the establishment of invasive molluscs in turbid systems could be leveraged as an opportunity (McLaughlan and Aldridge, 2013), triggering a management focus on reducing nutrient loads, to revert a system to a clear water state at an increased rate. Furthermore, if nutrient loads were reduced sufficiently before eradication of an invasive mollusc, the hysteresis effect could prevent a system from rebounding to a turbid state and would aid the reinstatement of native flora. In the case of invasive fish or crustacean establishment their removal should be targeted at the earliest opportunity, as they both have the potential to accelerate the decline of a clear water state towards a turbid condition that is difficult to revert. As such, lake managers may wish to employ early detection and rapid response plans for high risk invasive fish and crayfish.

Alternative equilibrium models in natural ecosystems focus on the key drivers behind tipping points. In reality, there are likely multiple stable states that shallow lakes can adopt (Scheffer and Van Nes, 2007). Additionally, there are nuances regarding the specific effects of a particular INNS and the potential ephemeral nature of an INNS' impact. These aspects are governed by factors such as predator-prey dynamics and dynamic shifts in prey communities. Such ecological relationships may allow species unaffected by INNS to flourish within affected trophic communities and therefore sustain an ecological trait which is critical for driving the dynamics of alternative equilibria. For example, it is unlikely that an invasion of *D. polymorpha* could indefinitely prevent a system from reverting to a turbid state. This is because the grazing pressure of *D. polymorpha* can favour phytoplankton with faster growth rates (Lucas et al., 2016), or for buoyant species which can occupy areas of the water column that do not mix with mussel beds (Smith et al., 1998; Lucas et al., 2016), or towards inedible toxic species (Vanderploeg et al., 2001). Over time, predator-prey dynamics could result in mussel mortality and reduced phytoplankton grazing pressure (Wilson, 2003). These ecosystem-level responses in community structure may explain why early stage invasions can cause dramatic, but sometimes short-lived changes of state in shallow lakes (Wilson, 2003; Barbiero and Tuchman, 2004; Strayer et al., 2011).

Although INNS are recognised as one of the greatest threats to global biodiversity, this study demonstrates that their effects on ecosystem services are not uniformly negative. While it would be unacceptable to promote selective introductions of INNS into shallow lakes, embracing already established INNS that provide favourable ecosystem engineering could be of great value.

2.4.6 Invasive species establishment, management and changing tipping points

The importance of tipping points in explaining ecosystem change and informing ecosystem management is becoming increasingly recognised. Drivers such as changes in species traits within or among populations, through mechanisms of phenotypic plasticity, species sorting, or evolutionary trait change, may affect an ecosystem's response to stress and therefore influence the occurrence of catastrophic tipping points (Dakos et al., 2019). We suggest that the establishment of INNS has the potential to rapidly alter the functional traits which exist in many ecosystems and may predictably alter the likelihood of a catastrophic tipping point being met. For example, invasive macrophytes have been shown to facilitate the early collapse of coral-dominated reefs, contributing to the establishment of an alternative macroalgae-dominated state (Neilson et al., 2018). Establishment of invasive sea urchins (*Centrostephanus rodgersii*) have precipitated the early collapse of Tasmanian kelp beds through overgrazing, maintaining an alternative barrens habitat (Ling and Keane, 2018). Invasion of stable desert shrublands by non-native grass species increase fire frequency, shrub mortality and soil loss, accelerating the shift to a desertified state (Ravi et al., 2009). The invasive black locust (*Robinia pseudoacacia),* a prominent and "non-flammable" invasive deciduous tree in American pitch pine forests, increases the ecosystem's critical threshold resistance to fire and disrupts natural cycling between alternative stable states (Dibble and Rees, 2005). We propose that employing a meta-analysis framework to quantify the effect size of INNS in ecosystems with established feedback mechanisms which drive alternative stable states, will help identify how INNS may alter critical ecosystem tipping points. Outcomes can help managers prioritise resources towards high-risk INNS and can allow informed management of ecosystems towards a desirable equilibrium.

Chapter 3

Impacts of invasive quagga mussels (*Dreissena rostriformis bugensis*) on reservoir water quality, as revealed by Progressive-Change BACIPS analysis

Abstract

Invasive quagga mussels (*Dreissena rostriformis bugensis)* are an emerging threat to the functioning and management of freshwater ecosystems. The quagga mussel's fast growth rate, high population densities, and ability to filter large volumes of water is thought to have led to quagga mussels displacing zebra mussels in much of North America. Compared to the zebra mussel, the ecological impacts of quagga mussels, such as reducing phytoplankton biomass, altering nutrient cycling and biofouling, are suggested to be more severe and pervasive in scope. Quagga mussels were first recorded in the UK in 2014 and have subsequently established at high densities in a number of major reservoirs. We show that a Progressive-Change BACIPS (Before-After-Control-Impact Paired Series) analysis can be used to interrogate industry standard, long-term datasets on abiotic and biotic parameters to quantify the impacts of quagga mussels, when the timeframe of species arrival is known. We found that, following quagga mussel establishment, the following trends were observed: reduced diatom and cyanobacteria abundances; increased soluble reactive phosphorus and reactive silica concentrations; and reduced abundances of toxic cyanobacteria *Aphanizomenon* sp. Interestingly, we also found quagga mussel establishment led to slightly increased overall chlorophyll a measurements and no changes in *Microcystis* sp. abundance or turbidity, which are common indicators of dreissenid invasion. Our results show that quagga mussels may have had significant effects on the reservoir ecosystem which, through their impacts on phytoplankton communities, are likely to affect reservoir management. Quagga mussels may reduce harmful bloom events which place acute pressures on fish stocks, release potentially lethal toxins into the water supply and block filters during water treatment. However, they may also displace native biota which serve as food for native fish and bird species, as well as aid the establishment of further invasive species. This may result in further displacement and reduce the ability of reservoirs to support angling and waterfowl. Our study also demonstrates the power of Progressive-Change BACIPS to use already existing data to inform quantitatively on any perturbation that can be attributed a time point, such as, new treatment processes, pollution events or establishment of other invasive species. Understanding the effects of past perturbations will help guide the most effective management strategies.

3.1 Introduction

Reservoirs are essential for meeting the continuous, and increasing, anthropogenic demand for water resources from the non-continuous natural water supply from the environment. Reservoirs are not only important for supporting growing human populations, expanding economies and agriculture, they also uphold the fundamental human right for access to clean water, they are integral for protecting against flooding, represent an important recreational resource and conserve biodiversity. However, it is important to note that the construction of new reservoirs, as well as requiring large areas of land and significant funding, if not appropriately mitigated, they can have deleterious downstream impacts. These downstream effects may inhibit the movements of migratory fish, fragment river connectivity, affect navigation and transport, cause microclimatic changes, affect crop yields and alter downstream biodiversity (Fu et al., 2010; Abd-El Monsef et al., 2015; Grill et al., 2019).

The introduction of invasive non-native species (INNS) can have pronounced effects on the ecosystem services provided by reservoirs (Gallardo and Aldridge, 2020). For example, the predatory fishhook water flea (*Cercopagis pengoi*) native to the Ponto-Caspian region of eastern Europe has established in lakes and reservoirs in North America, leading to declines in native zooplankton and knock-on increases in algal blooms through reduced grazing pressure (Vanderploeg et al., 2002). Invasive non-native bivalve molluscs, such as zebra mussels (*Dreissena polymorpha*) can reach densities of over 700,000 m^{-2} (Pathy, 1994), and individually filter large volumes of water (Elliott et al., 2008). These characteristics enable zebra mussels to drive especially dramatic change in algal and cyanobacterial dynamics (Sousa et al., 2009).

In a meta-analysis of dreissenid impacts on freshwater systems, it was shown that dreissenids significantly reduce overall phytoplankton abundances by 35 to 78% after invasion due to their filter feeding behaviour (Higgins and Vander Zanden, 2010). This included significant reductions in cyanobacterial abundance of up to 58%, which has also been noted in reservoir specific studies (Kirsch and Dzialowski, 2012). Despite these overall reductions in cyanobacterial biomass, there is a body of evidence that suggests, in the presence of dreissenid mussels, certain individual cyanobacterial species increase in abundance, in some, but not all, ecosystems (Higgins and Vander Zanden, 2010). Particularly in North American lakes, a pattern of increases in nuisance cyanobacteria such as *Microcystis aeruginosa*, have been observed (Raikow et al., 2004; Knoll et al., 2008). It is thought that dreissenids may increase levels of toxic cyanobacteria through the mechanism of ‘selective rejection’, whereby toxic or unpalatable phytoplankton are ejected by mussels in pseudofaeces where it can then be resuspended in the water column (Vanderploeg et al., 2013; Tang et al., 2014).

The quagga mussel (*Dreissena rostriformis bugensis*) is an emerging INNS, which is rapidly spreading around the world as a result of its prolific biofouling characteristics (Karatayev et al., 2007). In recent decades, quagga mussels have displaced existing invasive populations of zebra mussels, often leading to increased overall mussel densities, which has significant environmental and economic implications (Strayer et al., 2019). Originally native to the Dnieper River system in the northern region of the Black Sea (Son, 2007), less is known of the life history, biology and impacts of quagga mussels compared to zebra mussels. The quagga mussel was first discovered in the Great Lakes region in North America in 1989 and has since spread to all five lakes. Although its spread was slower than the previous zebra mussel invasion, once established it replaced zebra mussels in nearshore regions and colonised deeper regions where zebra mussels were never found (Nalepa, 2010). The quagga mussel's ability to displace zebra mussels is attributed to its lower respiration rate, accelerated shell growth and mass, faster filtration rate and superior assimilation efficiency (Diggins, 2001; Baldwin et al., 2003; Stoeckmann, 2003). Although ecological impacts are thought to be similar to the zebra mussel, it is suggested that changes may be more severe and pervasive in scope (Nalepa, 2010). Studies in North America have documented numerous ecosystem-level shifts following quagga mussel invasion including reductions in overall abundances of green algae, cyanobacteria and diatoms as a result of mussel grazing activity (Higgins and Vander Zanden, 2010); increases in Soluble Reactive Phosphorus (SRP) and ammonium (NH_4), attributed to mussels excreting a high level of dissolved nutrients (Karatayev et al., 2015; Ozersky et al., 2015); increases in reactive silica as SiO_2 as a result of reduced assimilation into diatom biomass as mussel grazing reduces diatom populations (Rockwell et al., 2005); decreases in turbidity and chlorophyll a concentrations from reduced phytoplankton abundances (Higgins and Vander Zanden, 2010; Kirsch and Dzialowski, 2012); and increases in abundance of toxin forming cyanobacteria, as a result of the selective rejection of these species in mussel pseudofaeces (Raikow et al., 2004; Vanderploeg et al., 2013; Tang et al., 2014).

Based on the widespread ecological change that has been observed following quagga mussel invasions, the species was identified as the largest risk to Britain's biodiversity in a multi-taxon, multi-ecosystem horizon scan of INNS (Roy et al., 2014). Subsequently, in 2014 the quagga mussel was discovered for the first time in the UK, in the Wraysbury River, Surrey (51˚26.55'N, 00˚31.25'W) (Aldridge et al., 2014) and has since established large populations in a number of reservoirs in southwest London (Aldridge, 2015). With quagga mussels becoming a more pressing threat to the UK's freshwater ecosystems and infrastructure, there is a growing need for research focussed on this species. Specifically, there is a dearth of research of dreissenid impacts on man-made reservoirs, relative to other aquatic habitats, which needs to be addressed (Kirsch and Dzialowski, 2012).

Thames Water Utilities (Thames Water) is responsible for the management of the reservoir network across southwest London, and has collected standardised data on water quality parameters, including abundance of algae and cyanobacteria, for over three decades. As the arrival and rapid population increase of quagga mussels can be attributed to a short window in time, this provides an opportunity to employ a Progressive-Change BACIPS (Before-After-Control-Impact Paired Series) design to interrogate this dataset to identify any potential effects of the recent quagga mussel invasion on key abiotic and biotic parameters.

BACIPS analysis is recognised as an effective and valuable method for evaluating both natural and anthropogenic perturbations on ecological variables when the treatment site cannot be randomly chosen (Stewart-Oaten et al., 1986; Downes et al., 2002; Popescu et al., 2012; Russell et al., 2015; Conner et al., 2016; Wauchope et al., 2021). BACIPS has been applied to a range of studies, including the effects of rainforest logging on birds and small mammals (Crome et al., 1996), nuclear power plants on kelp (Bence et al., 2007), invasive catfish on invertebrates (Kadye and Booth, 2012), and the diversion of the Mississippi river on nekton community structure (de Mutsert and Cowan, 2012). The BACIPS methodology allows the evaluation of both the before *versus* after measurements at impacted sites, and the control sites *versus* impacted sites. This is why BACIPS analysis is preferential for ecological systems as it incorporates not only time, but controls (Conger and Portier, 2006). Progressive-Change BACIPS is a new approach which generalises and expands the scope of BACIPS analysis (Thiault et al., 2017; Wauchope et al., 2021). Whereas traditional BACIPS analyses assume that effects are sudden, long-lasting and constant, i.e. exhibit a step-change, Progressive-Change BACIPS allows for the identification of step, linear, asymptotic or sigmoid responses to an impact (Thiault et al., 2017, 2019).

The aim of this study was to apply the Progressive-Change BACIPS methodology to routinely collected reservoir water quality measurements in order to identify the potential abiotic and biotic impacts of quagga mussels in invaded Thames Water reservoirs.

3.2 Methods

3.2.1 The reservoirs

Data were collated from ten reservoirs located in southwest London and situated within a 9 km radius (Figure 3.1): Queen Mother Reservoir (51°28'57.06"N, 0°33'1.57"W); Wraysbury Reservoir (51°27'41.26"N, 0°31'31.40"W); King George VI Reservoir (51°26'59.44"N, 0°30'9.63"W); Staines North Reservoir (51°27'1.28"N, 0°29'23.92"W); Staines South Reservoir (51°26'38.95"N, 0°29'9.96"W); Queen Mary Reservoir (51°24'56.18"N, 0°27'31.79"W); Knight Reservoir (51°23'56.31"N, 0°23'45.85"W); Bessborough Reservoir (51°24'4.04"N, 0°23'21.71"W); Queen Elizabeth II Reservoir (51°23'31.04"N, 0°23'34.90"W); and the Island Barn Reservoir (51°23'27.26"N, 0°21'51.89"W).

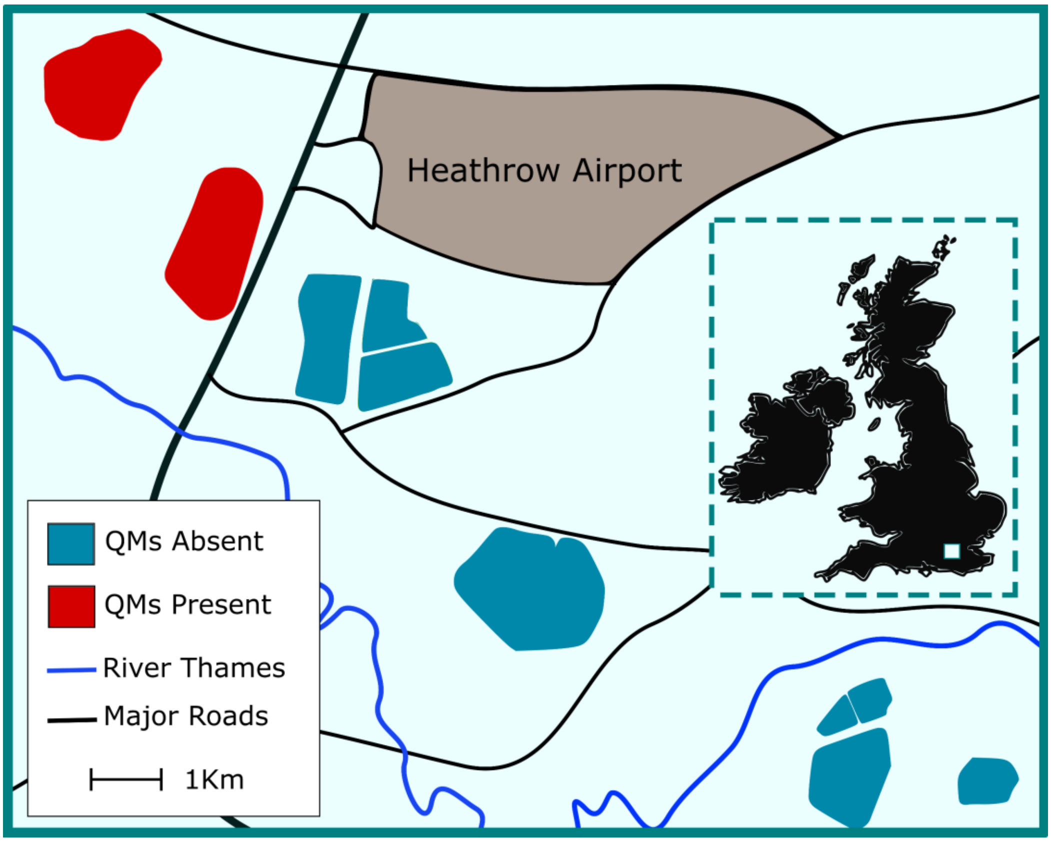


Figure 3.1 Map illustrating the location of reservoirs included in this study. The insert map of the UK indicates the approximate location of the reservoir location map. The reservoirs, from top left to bottom right, are: The Queen Mother Reservoir, Wrasbury Reservoir, King George VI Reservoir, Staines North Reservoir, Staines South Reservoir, Queen Mary Reservoir, Knight Reservoir, Bessborough Reservoir, Queen Elizabeth II Reservoir and Island Barn Reservoir.

Surveys to assess the presence and abundance of quagga mussels and zebra mussels were conducted at all reservoirs during 2015 (Aldridge, 2015). Surveys included inspection of marginal walls of reservoirs, boat-based dredging, sampling on undersides of pontoons and marker buoys, inspection of accessible underdrains and culverts, and use of an ROV camera. Data were complemented by data held by the UK Environment Agency (Aldridge, 2015). Queen Mother and Wraysbury Reservoirs contained a high abundance of quagga mussels, characterised by a continuous ‘crust’ of mussels at least 2cm thick across all submerged hard surfaces at >1m depth. The remaining reservoirs were either uninvaded or had very low abundances of zebra and/or quagga mussels (occasional individuals encountered, but hard surfaces were >95% clear of mussels). Therefore, the Wraysbury and Queen Mother Reservoirs were considered impacted sites, and the remainder of the reservoirs were considered controls.

3.2.2 Selection of variables

The following abiotic water quality metrics were selected for analysis in this study: Ammonium as NH_4 (mg/l); Turbidity in Formazin Turbidity Units (FTU); Reactive Silica as SiO_2 (mg/l); and Soluble Reactive Phosphorus (SRP) as PO_4 (mg/l). The biotic water quality metrics selected were: Chlorophyll a (ug/l); Average cyanobacteria abundance (cells/ml); Average green algae abundance (cells/ml); and Average diatom abundance (cells/ml). Common nuisance phytoplankton abundances (cells/ml) of species known to cause toxic blooms under favourable conditions were also considered, *Microcystis* sp., *Anabaena* sp., *Aphanizomenon* sp., and *Euglena* sp. (Zimba et al., 2017; Huisman et al., 2018).

3.2.3 Estimation of algal cell abundance

Phytoplankton abundances were measured by Thames Water using a cell counting chamber and recorded by ascribing a rating on an ACFOR scale where: Abundant (>20 cells/mm^2); Common (<20 cells/mm^2); Frequent (21-100 cells per slide); Occasional (3-20 cells per slide); and Rare (1-2 cells per slide). In order to estimate the abundance of algal cells denoted by the ACFOR scale ratings, each ranking was normalised to represent the lowest bound of cells per ml which would be required to achieve each rating, based on the use of a standard cell counting chamber of 0.1um depth. Therefore, all phytoplankton abundance estimates represent the lowest possible cell numbers present.

3.2.4 Selection of study timeframe

Data from January 2001 until November 2018 were used for all variables. This timeframe was selected because, as of January 2001, weekly algae abundance data were collected from each reservoir.

To satisfy the paired sample criteria of the Progressive-Change BACIPS methodology, for each reservoir, a monthly average value was calculated for each water quality metric. In months for which only invaded or uninvaded reservoirs had data for a specific metric, the data for that month were omitted. The monthly averaged data were then averaged between impact sites (Queen Mother and Wraysbury reservoirs) and control sites (King George VI reservoir; Staines North and South reservoirs; Queen Mary Reservoir; Knight Reservoir; Bessborough Reservoir; Queen Elizabeth II Reservoir; and the Island Barn Reservoir) to create a paired dataset of monthly measurements for impact and control sites.

3.2.5 Assigning the impact date for Progressive-Change BACIPS analysis

The date of January 2014 was used in the analysis as the likely date of invasion. Aldridge et al., (2014) estimate that the mussels arrived in the waterways surrounding the invaded reservoirs in late 2013 or early 2014. These dates of establishment are reinforced from observations in Aldridge (2015) of populations dominated by year 1 and 2 quagga mussels and the absence of any dead shells in the

Wraysbury and Queen Mother Reservoirs. Therefore, measurements before January 2014 are considered to be before the impact and measurements from and including January 2014 are considered to be after the impact for the purposes of the Progressive-Change BACIPS analysis.

3.2.6 Progressive-Change BACIPS analysis

The data were analysed in order to identify both overall changes, and seasonal changes which may arise as the result of quagga mussel invasion. Spring was designated as March to May; Summer was June to August; Autumn was September to November; and Winter was December to February.

The Progressive-Change BACIPS was implemented following the methodology outlined by Thiault et al., (2017) (Figure B1). For each variable, the difference, Δ, was determined between the impact and paired control sites for each month. The analysis assesses whether there is a significant change in Δ for each variable from before and after the establishment of quagga mussels in 2014. Unlike traditional BACIPS analysis, which assumes and tests for a step-change in Δ, this methodology compares how the data conforms to four competing models: step-change (Eq 3.1), linear (Eq 3.2), asymptotic (Eq 3.3) and sigmoid models (Eq 3.4). As for all BACIPS analysis, the assumption is made that in the Before period, Δ was stationary through time (i.e. there was no consistent temporal trend in the difference between impact and control sites prior to the establishment of quagga mussels), however after mussel establishment, Δ changed with time, with an increase indicating a positive effect of mussel establishment and decreases indicating a negative effect. The differences between an impact site and its control after the arrival of mussels (Δ_{After}) can be described as:

$$\text{Step change response: } \Delta_{After,i} = M + \Delta_{Before} + \varepsilon_i$$

(Eq 3.1)

$$\text{Linear response: } \Delta_{After,i} = rt_i + \Delta_{Before} + \varepsilon_i$$

(Eq 3.2)

$$\text{Asymptotic response: } \Delta_{After,i} = \frac{Mt}{L+t} + \Delta_{Before} + \varepsilon_i$$

(Eq 3.3)

$$\text{Sigmoid response: } \Delta_{After,i} = \frac{M(\frac{t}{L})^K}{1 + (\frac{t}{L})^K} + \Delta_{Before} + \varepsilon_i$$

(Eq 3.4)

Where *t* is time (in years) since 1 January 2001 associated with the i^{th} sampling survey; Δ_{Before} is the underlying spatial variation between the impact and control sites in the absence of quagga mussels (estimated during the Before period); *r* and *M* are the rate of divergence and magnitude of change between the impact and control sites, respectively; *L* is the time required to reach half of the long-term effect; *K* is a scaling parameter in the sigmoid model that affects the steepness of the curve; and ε is the error associated with the i^{th} survey. Each candidate model was fitted setting the before data to have $t = 0$, and the magnitude of the response to mussel establishment (effect size) was then measured based on the predictions of the best-fit model (highest AICc score). After identifying the most appropriate model, a GLM was implemented with the prescribed interaction in order to estimate the effect size. For step-change interactions the effect size indicates the change in Δ from before to after the impact, for linear interactions the effect size indicates the average change in Δ per year (Figure B1).

As only step-change and linear models were identified for the variables in this study, the GLM used a Gaussian distribution and identity error distribution. For seasonal trends, the same methodology was followed, however the data was partitioned so that only the data for the desired season was fitted to the models and subsequently analysed using GLM. The 95% confidence intervals for all significant effect sizes were calculated to ensure that they did not overlap zero, therefore confirming that the effect size is significantly different from zero. All analysis was performed in R studio (v.1.1.442) (R Core Team, 2018).

3.3 Results

The pattern of change in response to quagga mussel establishment for all variables demonstrating significant effect sizes supported either a linear change (in which the difference between impact and control sites increased linearly with time since mussel establishment) or a step-change (where an immediate shift was noted in the impact relative to the control site). In no cases were asymptotic or sigmoidal models supported by the data. For two variables, winter average diatom abundance and overall SRP concentration, the likelihood (ω) of the second-best model was comparable to the best-fit model (within 10%). For consistency, only the results of the best-fit model are presented and are summarised in Table 3.1.

3.3.1 Changes in abiotic parameters

Reactive silica concentrations demonstrated a positive step-change in Δ after the impact, with an overall average increase of 2.06 mg/L (ω Step = 99.9%, 95% CI: 1.39 to 2.73, P<0.0001) (Figure 3.2a), this was consistent across all seasons (Table 3.1) with the largest increase, of 3.61 ug/l, occurring in the autumn (ω_{step} = 99%, 95% CI: 2.18 to 5.03, P<0.0001) (Figure B2c). Overall SRP trends exhibited a positive linear change at impacted sites of 0.005 mg/L/year (ω_{linear} = 52.5%, 95% CI: 0.001 to 0.009, P=0.0094) (Figure 3.2b). The greatest change in SRP was observed in autumn where it has increased linearly at 0.012 mg/L/year (ω_{linear} = 94%, 95% CI: 0.005 to 0.019, P= 0.0011) since the impact (Figure B3).

There were no significant changes in Δ before and after the arrival of quagga mussels for either ammonium concentrations or turbidity.

3.3.2 Changes in biotic parameters

Total diatom abundance exhibited a linear decrease at impacted sites, with an average decline of 1204.90 cells/ml/year (ω_{linear} = 90.9%, 95% CI: -1668.63 to -741.20, $P<0.0001$) (Figure 3.3a). The linear trend was consistent across spring, autumn and winter (Table 3.1), however, in summer, a step-change interaction was observed indicating an average decline of -2973.83 cells/ml (ω_{step} = 66.3%, 95% CI: -5777.21 to -170.45, P=0.0426) after the impact (Figure B4b). Total cyanobacteria abundance declined linearly at impacted sites on average 604.20 cells/ml/year (ω_{linear} = 75.9%, 95% CI: -1192.63 to -15.83, P=0.0454) (Figure 3.3b). The decline was greatest in autumn decreasing on average 1645.60 cells/ml/year (ω_{linear} = 69.1%, 95% CI: -3004.55 to -304.68, P=0.0199) (Figure B5). The nuisance cyanobacteria *Aphanizomenon* sp. demonstrated a linear decline with an average reduction of 1817.20 cells/ml/year at impacted sites (ω linear = 79.1%, 95% CI: -3425.82 to -208.57, P=0.0279) (Figure 3.3c). The impact on *Aphanizomenon* sp. was greatest in the autumn, declining on average 4406 cells/ml/year (ω_{linear} = 77.9%, 95% CI: -7968.63 to -842.99, P= 0.0189) (Figure B6). Total chlorophyll a concentration indicated a positive step-change of 2.77 ug/L (ω_{step} = 83%, 95% CI: 1.16 to 4.39, P=0.0009) at impacted sites (Figure 3.3d). The change was greatest in the winter, with an increase of 5.47 ug/L (ω_{Step} = 67.6%, 95% CI: 1.56 to 9.39, P=0.0084) (Figure B7).

There were no significant changes in Δ for average abundances of green algae, *Microcystis* sp., *Anabaena* sp., or *Euglena* sp.

Table 3.1 Best-fit model results for all significant effects. The effect size, 95% confidence intervals and P-values were calculated using a generalised linear model with a Gaussian distribution and identity error distribution. Effect size for linear change is expressed as change per year post impact, for step-change effect size is expressed as the difference before and after mussel invasion. The best-fit model and likelihood of fit was calculated using the methodology outlined by Thiault et al., (2017).

Abiotic					
Water Quality Metric	**Overall and Seasonal Changes**	**Best-fit model and likelihood of fit**	**Estimated effect size**	**95% confidence intervals**	**P value**
Reactive Silica SiO2 (mg/l)	Overall	ω step = 99.9%	2.06	1.39 – 2.73	<0.0001
	Spring	ω step = 93.1%	1.68	0.64 – 2.71	0.0025
	Summer	ω step = 80.4%,	1.35	0.10 – 2.59	0.0390
	Autumn	ω step = 99.0%	3.61	2.18 – 5.03	0.0001
	Winter	ω step = 61.5%	1.52	0.59 –2.46	0.0024
Soluble Reactive Phosphorus PO_4 (mg/l)	Overall	ω linear = 52.5%,	0.005	0.001 – 0.009	0.0094
	Autumn	ω linear = 94.0%	0.012	0.005 – 0.019	0.0011
Biotic					
Cyanobacteria (cells/ml)	Overall	ω linear = 75.9%	-604.20	-1192.63 – -15.83	0.0454
	Autumn	ω linear = 69.1%	-1654.60	-3004.55 – -304.68	0.0199
Diatoms (cells/ml)	Overall	ω linear = 90.9%	-1204.90	-1668.63 – -741.20	<0.0001
	Spring	ω linear = 75.9%	-989.50	-1687.90 – -291.12	0.0076
	Summer	ω step = 66.3%	-2973.83	-5777.21– -170.45	0.0426
	Autumn	ω linear = 84.4%	-1231.60	-2240.79 – -222.40	0.0204
	Winter	ω linear = 52.4%	-1962.80	-2993.77 – -931.92	0.0005
Aphanizomenon (cells/ml)	Overall	ω linear = 79.1%	-1817.20	-3425.82 – -208.57	0.0279
	Autumn	ω linear = 77.9%	-4406.00	-7968.63 – -842.99	0.0189
Chlorophyll a (ug/l)	Overall	ω step = 83.0%	2.77	1.16 – 4.39	0.0009
	Winter	ω step = 67.6%	5.47	1.56 – 9.39	0.0084

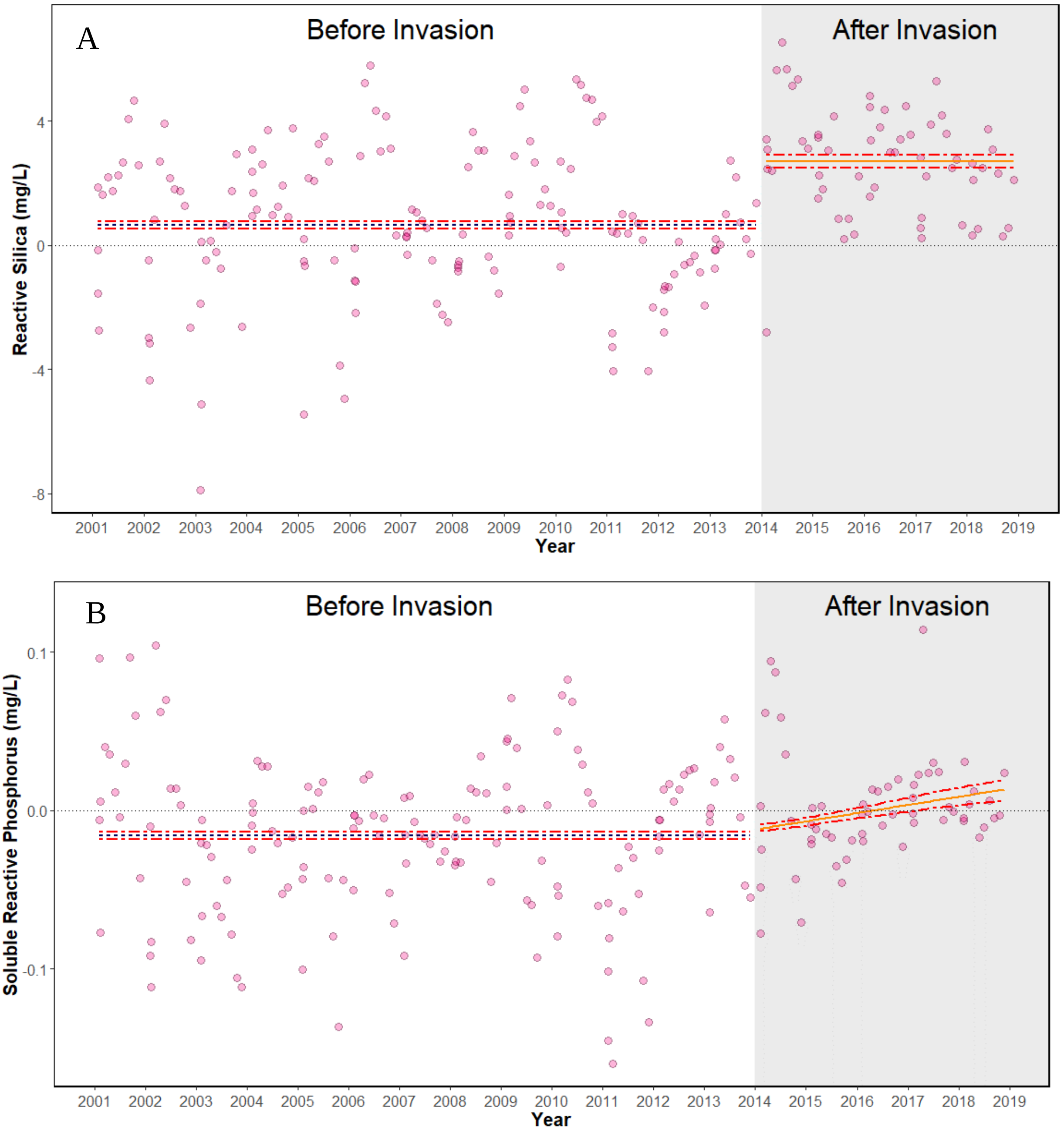
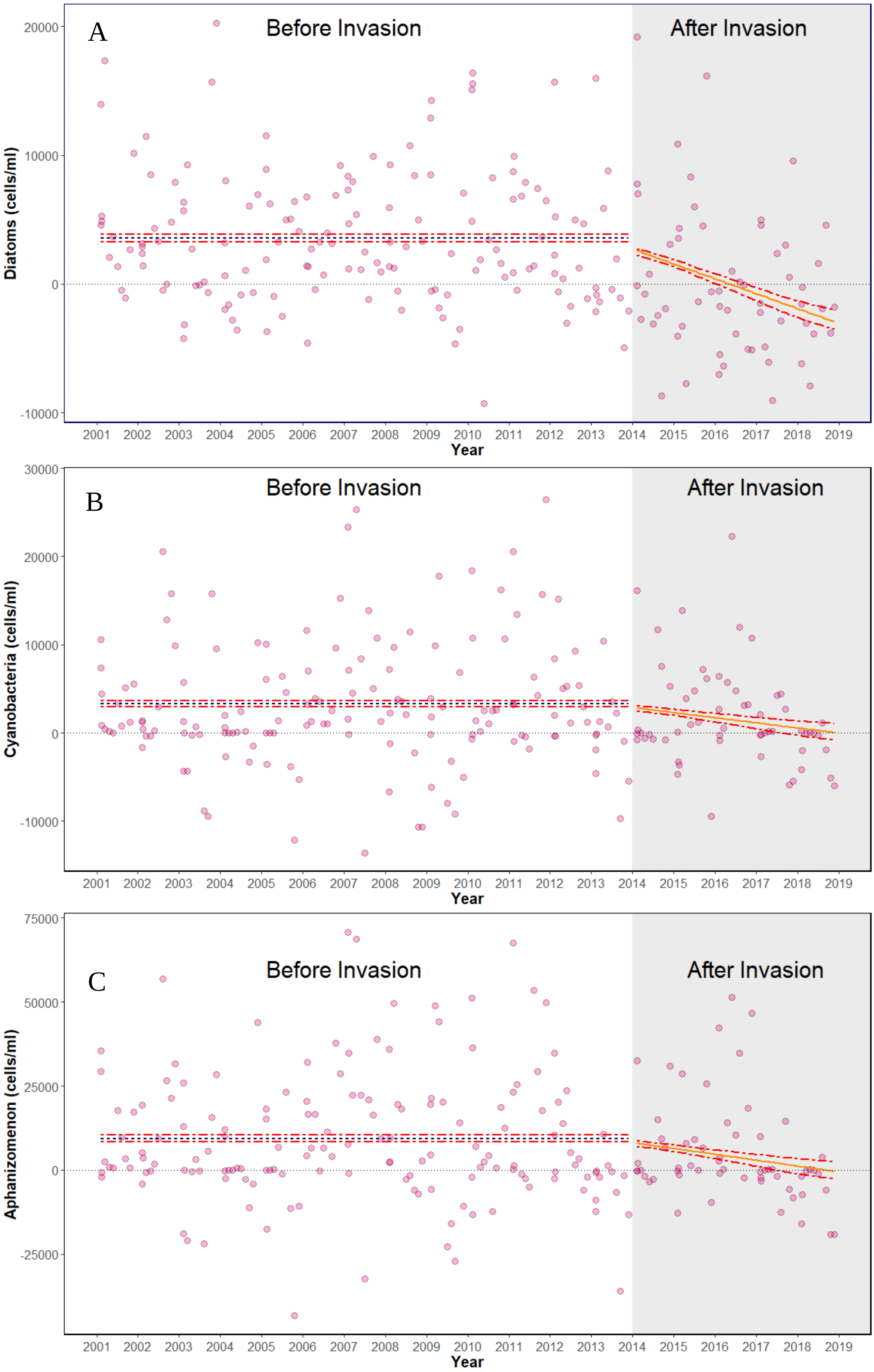


Figure 3.2 The changes in abiotic variables before (white background) and after (grey background) the arrival of quagga mussels. (A) Reactive Silica (mg/L); (B) Soluble Reactive Phosphorus (mg/L). The points demonstrate the monthly differences between the impact and control sites (impact-control), values above or below the horizontal dotted line at zero indicate where the value of the variable is higher or lower at impacted sites relative to control sites, respectively. The blue dashed line and the orange solid line indicate the GLM predicted values for the response variable before and after the arrival of quagga mussels, respectively. The red two-dashed line illustrates the 95% confidence interval of the GLM predicted values.


A
Before Invasion
After Invasion
Diatoms (cells/ml)
20000
10000
0
-10000
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018
2019
Year
B
Before Invasion
After Invasion
Cyanobacteria (cells/ml)
30000
20000
10000
0
-10000
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018
2019
Year
C
Before Invasion
After Invasion
Aphanizomenon (cells/ml)
75000
50000
25000
0
-25000
2001
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2003
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2005
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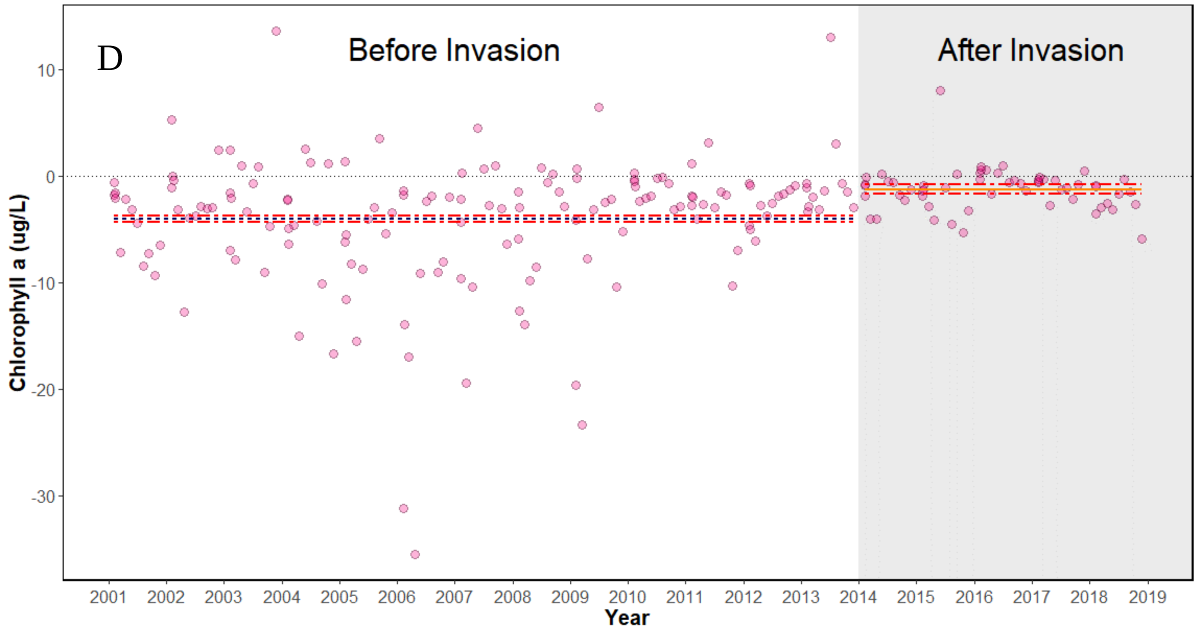


Figure 3.3 The changes in biotic variables before (white background) and after (grey background) the arrival of quagga mussels. (A) Cyanobacterial abundance (cells/ml); (B) Diatom abundance (cells/ml); (C) Aphanizomenon abundance (cells/ml); (D) Chlorophyll a concentration (ug/L) The points demonstrate the differences between the impact and control sites (impact-control), values above or below the horizontal dotted line at zero indicate where the value of the variable is higher or lower at impacted sites relative to control sites, respectively. The blue dashed line and the orange solid line indicate the GLM predicted values for the response variable before and after the arrival of quagga mussels, respectively. The red two-dashed line illustrates the 95% confidence interval of the GLM predicted values.

3.4 Discussion

The use of Progressive-Change BACIPS analysis demonstrated that the establishment of quagga mussels led to substantial changes in the abiotic and biotic character of invaded reservoirs. In terms of abiotic parameters: impacted sites demonstrated an increase in overall reactive silica and SRP concentrations. In terms of biotic parameters: overall diatom, cyanobacteria and *Aphanizomenon* sp. abundances decreased and chlorophyll a concentration increased at impacted sites. No significant changes were seen in overall green algae, *Microcystis* sp., *Anabaena* sp., or *Euglena* sp. abundances, ammonium concentrations or turbidity.

It may also be of interest to note that for chlorophyll a, SRP and reactive silica concentrations, there was a noticeable decrease in the variance of these parameters following quagga mussel establishment. These reductions in variance may further validate the ecological engineering capabilities of quagga mussels (Sousa et al., 2009). The potential ability of quagga mussels to alter and stabilise the long-term consistency of certain variables may have implications for driving and maintaining shifts between alternative equilibria (Scheffer et al., 1993). The extent to which the stabilisation of certain environmental parameters may contribute to shifts between alternative stable states is beyond the scope of this chapter, but certainly may warrant further investigation.

3.4.1 Impacts of quagga mussel establishment

3.4.1.1 Diatom abundance and reactive silica

An overall decline in diatom abundance by 1205 cells/ml/year was observed, declining consistently in all seasons. Despite limited research on the specific impact of quagga mussels on diatom communities, large quagga mussels (>16mm) have been shown to demonstrate a grazing preference for colonial diatoms in mesocosm experiments (Tang et al., 2014). In natural systems, declines in diatom abundance have been linked to the establishment of zebra mussels in short-term experiments in Saginaw Bay, Lake Huron (Heath et al., 1995; Lavrentyev et al., 1995). The increase in reactive silica concentrations in impacted reservoirs can likely be linked to the grazing activities of quagga mussels and the removal of diatoms, which incorporate silica into their cell walls (frustules) (De Tommasi et al., 2017). Reduced diatom abundances at impacted reservoirs, may have reduced the usual levels of silica uptake from the environment for frustule formation, leading to the observed positive step change in reactive silica concentrations noted in our study. The same effect and seasonal changes were noted in Lake Erie, following the invasion of zebra mussels, with the greatest changes occurring in the spring and autumn (Nicholls et al., 1997; Rockwell et al., 2005), which are the seasons when diatom blooms typically occur (Sigee, 2005).

3.4.1.2 Cyanobacteria and *Aphanizomenon* sp. abundance

Overall average cyanobacterial abundance decreased by 604 cells/ml/year, with the greatest decrease of 1655 cells/ml/year occurring in the autumn. These results suggest that quagga mussel establishment acted to supress cyanobacterial abundance in invaded reservoirs. In European studies, quagga mussels have been suggested as management tools for harmful algal blooms in urban ponds, where they have been shown to supress cyanobacterial abundance (Waajen et al., 2016). Similarly, in both UK mesocosm experiments (Reynolds et al., 2019) and in Dutch lakes (Reeders and Bij de Vaate, 1990; Dionisio Pires et al., 2005a), zebra mussels have been shown to supress overall cyanobacteria abundance. Equally, they have been shown to remove a broad size range of cyanobacteria, regardless of toxicity (Dionisio Pires et al., 2005a). However, particularly in the North American Great Lakes, dreissenids have been linked to increases in cyanobacterial biomass, particularly of the toxic, bloom forming, cyanobacterium *Microcystis aeruginosa* (Vanderploeg et al., 2001; Nicholls et al., 2002; Pillsbury et al., 2002; Bierman et al., 2005; Conroy and Culver, 2005; Naddafi et al., 2007).

Only *Aphanizomenon* sp., of all the nuisance phytoplankton species included in our study, demonstrated any significant changes in abundance following the invasion of quagga mussels, decreasing by 1817 cells/ml/year on average, with the greatest effect in the autumn. In the UK, *Aphanizomenon* sp. is often at its peak during the autumn months (Henderson et al., 2008), which may explain why the greatest decrease relative to control reservoirs was during this season. In recent mesocosm experiments, the presence of *Aphanizomenon* sp. at bloom-level concentrations was shown to negatively affect sperm motility, veliger survival and fully inhibit spawning events in quagga mussels (Boegehold et al., 2018, 2019a, 2019b). Active removal of *Aphanizomenon* sp. may serve to reduce overall exposure of the mussels to harmful cyanotoxins.

3.4.1.3 Soluble reactive phosphorus

The overall SRP increase of 5 ug/L/year at impacted reservoirs can likely be tied to the grazing activities of quagga mussels as dreissenids play an important role in nutrient cycling (Vanderploeg et al., 2002). The phosphorus associated with phytoplankton biomass is ingested by the mussels, and although some phosphorus is retained as mussel biomass, excess is remineralised and is excreted as PO_4 (Ozersky et al., 2015). Ozersky et al., (2015) noted that quagga mussel SRP excretion rates peaked in October at ~16ug/gram of shell-free dry mass/hour. This previously observed seasonality of quagga mussel excretion rates may explain the 12 ug/L/year increase observed in invaded reservoirs in our study during the autumn. SRP excretion in zebra mussels is thought to be weight dependant, with larger mussels excreting higher levels of SRP. This may help to explain the linear nature of the relationship as increasing densities and biomass of older, larger mussels enhance nutrient cycling (Fishman et al., 2009).

3.4.1.4 Chlorophyll a

Despite declines in diatom and cyanobacterial abundance, no significant decline in chlorophyll a concentration was observed. In fact, overall average chlorophyll a concentration increased by an estimated 2.77 ug/L, with the biggest increase occurring in the winter. Research suggests this observation is not unusual, even in shallow well-mixed lakes, such as Lake Shawano, Wisconsin, where a 2.7 ug/L increase in chlorophyll a concentration was observed post dreissenid invasion (Cha et al., 2013). The winter increase in chlorophyll a concentration may be explained by seasonal declines in mussel clearance rates combined with the observed autumn rise in SRP; mesocosm studies have shown that winter clearance rates of quagga mussels are halved compared to the rest of the year (Diggins, 2001). This could have led to increased overwintering of phytoplankton after quagga mussel establishment.

3.4.2 Unchanged abiotic and biotic variables at invaded reservoirs

3.4.2.1 Ammonium and turbidity

It was hypothesised that ammonium concentrations may increase in invaded reservoirs as a result of the quagga mussels' feeding behaviour, returning nutrients stored in phytoplankton biomass to the water column through excretion of dissolved nutrients (Ozersky et al., 2015). Although ammonium concentrations have been shown to increase in some enclosure experiments containing quagga mussels, no consistent effects have yet been recorded in natural systems (Higgins and Vander Zanden, 2010; Kirsch and Dzialowski, 2012). In many studies of natural systems, and in our study, measurements were constrained to a single location at a shallow depth. This potentially makes it difficult to assess the degree of depth and spatial variation in nutrient cycling occurring within waterbodies (Ozersky et al., 2015). Dreissenids have been shown to redirect nutrients from offshore pelagic zones to nearshore benthic zones, known as "nearshore shunt" (Hecky et al., 2004). In our study, the occurrence of nearshore shunt may have been obscured by the method of data collection.

Declines in turbidity are widely reported as an impact of dreissenid invasion (Vanderploeg et al., 2002; Higgins and Vander Zanden, 2010; Karatayev et al., 2015), however this was not observed in our study. Although dreissenids have been shown to reduce turbidity caused by phytoplankton biomass, the impact of dreissenids on non-algal turbidity is less well understood (Kirsch and Dzialowski, 2012). Research suggests that zebra mussels are capable of removing some suspended solids from the water column, however quagga mussel mesocosm experiments found that they had no significant impact on total suspended solids (Higgins and Vander Zanden, 2010; Mei et al., 2016). Therefore, if the turbidity of a system is largely as a result of high abundances of suspended solids, opposed to phytoplankton biomass, quagga mussels may be unlikely to reduce the turbidity of the system. The reservoirs we studied are fed from the River Thames, which experiences frequent high flow events which can result in high suspended sediment loads. The results of our study suggest turbidity in the impacted reservoirs may have been driven by non-algal suspended solids, opposed to phytoplankton biomass.

3.4.2.2 Green algae and nuisance phytoplankton species

We hypothesised that green algae abundances would decline in impacted reservoirs due to the grazing activity of quagga mussels (Higgins and Vander Zanden, 2010). However, no effect was observed in our study. Impacts of zebra mussels on green algae are mixed, some studies have expressed that zebra mussels avoid green algae in preference for other phytoplankton taxa (Naddafi et al., 2007; Fahnenstiel et al., 2010; Reynolds et al., 2019), while others noted significant falls in green algae abundance (Heath et al., 1995; Kirsch and Dzialowski, 2012). Quagga mussel clearance rate experiments have shown that green algae, particularly species with thick cell walls, are cleared at low rates and often rejected in pseudofaeces (Tang et al., 2014). While they have demonstrated preferential removal of large colonial diatoms and cyanobacteria species (Tang et al., 2014; Waajen et al., 2016). In our study, impacted reservoirs prior to invasion, hosted abundances of cyanobacteria up to six times higher, and diatom abundances up to seven times higher than green algae. The high abundances of potentially preferential food sources in diatoms and cyanobacteria, may explain why grazing impacts on green algae were not observed in our study. Additionally, our dataset does not encompass all algal species present in each reservoir, as during routine data collection only the ten most abundant species were recorded. Therefore, the overall increase in chlorophyll a may be driven by overall increases in green algae species that were not specifically recorded.

Our study did not identify an effect of quagga mussel establishment on potentially toxic *Microcystis* sp., *Anabaena* sp., or *Euglena* sp. abundance; however, other studies have noted increases in toxic algae abundance following dreissenid establishment, potentially as a result of selective rejection of toxic cyanobacteria in mussel pseudofaeces (Raikow et al., 2004; Vanderploeg et al., 2013; Tang et al., 2014). An effect on *Microcystis* sp. may not have been observed in our study as nuanced nutrient and strain-dependent relationships have been noted between *Microcystis* sp. and dreissenids. Firstly, in systems with total phosphorus levels of >25 ug/L, as was observed in all reservoirs in our study, dreissenids were shown to have no effect on *Microcystis* sp. abundance (Raikow et al., 2004; Sarnelle et al., 2005). Secondly, Vanderploeg et al., (2013) demonstrated extreme variance in the grazing response of zebra and quagga mussels to different strains of *M. aeruginosa,* observing significant differences in the uptake and rejection of *M. aeruginosa* between laboratory strains and even strains from the same lake, which could not be explained by toxicity or colony size. In the case of *Anabaena* sp., Tang et al., (2014) observed that filamentous cyanobacteria such as *Anabaena* and *Aphanizomenon* sp. were readily ingested by quagga mussels in mesocosm experiments. In our study the filamentous cyanobacteria *Aphanizomenon* sp., which did experience a significant decline after quagga mussel establishment, occurred at twice the density of *Anabaena* sp. within all reservoirs prior to quagga mussel invasion. The lower abundance of *Anabaena* sp. in comparison with *Aphanizomenon* sp. could be responsible for the absence of an impact in our study. Finally, in the case of *Euglena* sp., which is the only known green algae to produce a toxin (Zimba et al., 2017), there are no studies which directly assess its relationship with dreissenids. It may be that the specific potent toxin, euglenophycin, prevents *Euglena* sp. from being ingested by quagga mussels or that the motile nature of *Euglena* sp. allows it to occupy areas of the water column unaffected by the substrate fixed quagga mussels.

3.4.3 Progressive Change BACIPS analysis for informing reservoir management

The Progressive-Change BACIPS methodology provides a powerful tool which can be applied to routinely collected data collated by water resource managers. This methodology can easily be applied to other reservoir systems hosting invasive dreissenid populations with a known arrival date to quantify their effects on the environment. Furthermore, if the overall and seasonal changes observed in this study are found to be consistent across other known invaded reservoir systems they could serve as potentially useful indicators of dreissenid invasions in reservoirs where benthic biodiversity monitoring or water drawdowns are not performed regularly. These potential indicators could include progressive declines in diatom and cyanobacteria abundances combined with an otherwise unexplained increase in SRP. Progressive-Change BACIPS is not only informative in the context of invasive dreissenid populations, but also allows investigation of other major perturbations which can be attributed to a time point. These perturbations could include installation of new treatment processes, pollution events, water drawdowns, fish introductions for angling, and establishment of other high impact INNS such as killer shrimps (Madgwick and Aldridge, 2011). Understanding which aspects of the reservoir environment are being impacted, and the magnitude of the effects, will provide useful guidance for developing effective and desirable management strategies.

3.4.4 Implications for reservoir management

The results of our study indicate that quagga mussels may have impacts on recipient ecosystems that can be of both positive and negative value to reservoir managers, and the ecosystem services provided.

There are several potentially beneficial effects of quagga mussel establishment for reservoir management. Firstly, although reductions in diatom abundances are unlikely to have any significant effects on water quality, as diatom blooms are rarely toxic, reduced abundances may act to reduce filter blockages at water treatment plants (Henderson 2008). Secondly, reduced cyanobacteria abundance may improve water quality by reducing the risk of cyanotoxins entering the water supply. Cyanotoxins can be hazardous to human and animal health, leading authorities to prevent access to waterbodies for recreational activities, and potentially poisoning native fish, birds and mammals (World Health Organization, 2003; Graham et al., 2010; Hilborn and Beasley, 2015; Huisman et al., 2018). Many cyanobacterial species, including *Aphanizomenon* sp., are known to produce cyanotoxins which are a concern for human health, deteriorate water taste and create unpleasant odours. The majority of cyanotoxins remain even after mechanical filtration and have taste thresholds of only a few nanograms per litre (Graham et al., 2010; World Health Organization, 2017). Thirdly, reductions in cyanobacterial abundance may reduce the likelihood of harmful bloom events which can create anoxic water conditions (Waajen et al., 2016). Large blooms threaten the survival of all oxygen-dependent species in the waterbody, potentially affecting biodiversity and recreational value from fishing (Wolf et al., 2017). Finally, when blooms die and are washed up on shorelines, the decay creates a foul smell which further limits the recreational use of the waterbody (Nalepa, 2010). Therefore, the observed declines in cyanobacterial biomass may reduce risks posed by cyanotoxins, reduce the frequency of bloom events and associated risks to native biota while reducing the frequency of reservoir closures.

While declines in phytoplankton can potentially reduce some management burdens, phytoplankton are key primary producers in aquatic ecosystems. Therefore, reductions in cyanobacteria and diatom abundances may have long-term implications for the reservoir's wider ecology and may result in fundamental changes to nutrient and energy cycling, shifting pelagic production to the benthos (Rowe et al., 2015). Quagga mussels compete directly with native species for phytoplankton resources. As a result, it has been observed that zooplankton biomass can decline by 40% after dreissenid invasion, although this varies largely between waterbodies (Higgins and Vander Zanden, 2010). Additionally, the arrival of dreissenid mussels in the North American Great Lakes led to declines in native fauna, particularly of the benthic amphipod *Diporeia* (McKenna et al., 2017). Both zooplankton and amphipods are highly nutritious food for most benthic and demersal fish (Mckenna 2019). Therefore, quagga mussel invasion could have consequences for the health, size and abundance of fish stocks in invaded systems. Any deleterious effects on fish stocks will negatively affect angling activities and may also have implications for piscivorous native and migratory waterfowl.

A further risk to native fish and bird species from the establishment of quagga mussels is the prospect of expediating invasional meltdown, whereby the establishment of one INNS can facilitate the establishment of an invader of common origin as they have co-evolved under similar environmental conditions (Simberloff and Von Holle, 1999). This is of particular concern for the sites identified in our study, which are located in the south-east of England, an area identified as the highest risk of invasion by multiple Ponto-Caspian species (Gallardo and Aldridge, 2015). Facilitating the establishment of additional INNS is likely to exacerbate any impacts of INNS on existing communities and reduce the ability of invaded waterbodies to conserve native species.

It may be that in species rich reservoirs of high ecological and conservation value or where angling is of high priority, quagga mussel reduction measures should be considered to limit their impact on the local biota and nutrient flows. Whereas, in reservoirs deemed as ecologically deficient, quagga mussels may bring beneficial changes through reductions in cyanobacteria and diatom abundance. While we do not advocate for intentional introductions of quagga mussels, with appropriate risk assessment and consultation with the relevant authorities, there may be scenarios where maintenance of a quagga mussel population could be beneficial (McLaughlan and Aldridge, 2013; Waajen et al., 2016).

Chapter 4

Population-level differences in selective grazing by invasive quagga mussels (*Dreissena rostriformis bugensis*) could explain harmful algal blooms

Abstract

Invasive non-native species are significant catalysts of environmental change, with the ability to challenge native species for essential resources and alter ecosystem processes which are central to the health and wellbeing of humans. A key resource under pressure from invasive non-native species is the provision of potable water. The invasive freshwater dreissenid mussel, *Dreissena rostriformis bugensis* (quagga mussel), is a prolific filter feeder and resilient ecological engineer which can attain high population densities. The filter feeding behaviour of dreissenids has been shown to be discriminatory, whereby certain unpalatable phytoplankton species are rejected by the mussels in pseudofaeces. The exact criteria for phytoplankton rejection are inconsistent across existing studies, and it has been hypothesised that it may ultimately be driven by genotypic or phenotypic differences between dreissenid populations. In this study we show that different populations of quagga mussels have the potential to drive different outcomes in phytoplankton communities, primarily through distinct phytoplankton ingestion and rejection behaviours. We demonstrate that quagga mussels from different hydrodynamic environments (lentic and lotic) expressed comparable clearance rates, both clearing green algae species at a higher rate than cyanobacteria species. However, mussels from lotic environments ejected significantly greater quantities of cyanobacteria in pseudofaeces. This nuanced feeding behaviour has considerable implications for phytoplankton community structure in invaded waterbodies, indicating that quagga mussel provenance may affect their hypothesised role in the proliferation of cyanobacteria through ‘selective rejection’ and the occurrence of harmful algal blooms. The hydrodynamic environment has been shown to drive phenotypic plasticity in the gill architecture of unionid mussels, which are key apparatus in filter feeding. We believe future studies should further scrutinise the putative effect of the hydrodynamic environment in driving phenotypic plasticity and genotypic selection of dreissenid mussels which may alter their feeding behaviour, so that we may better understand their role in promoting or suppressing harmful algal blooms.

4.1 Introduction

Invasive non-native species (INNS) are recognised as major drivers of global change (Pejchar and Mooney, 2009) posing the second greatest risk to species endangerment and extinction after habitat destruction (Wilcove et al., 1998). Through the alteration of habitat structure, INNS can have significant effects on ecosystem processes which are fundamental to the health and well-being of humans (Charles and Dukes, 2007; C. McLaughlan et al., 2014; Paini et al., 2016). The continued supply of clean and plentiful drinking water, the maintenance of freshwater ecosystems to support recreational activities, and species conservation, are three such services under pressure from INNS. The invasive freshwater bivalves *Dreissena polymorpha* (zebra mussel) and *Dreissena rostriformis bugensis* (quagga mussel), are native to the Ponto-Caspian region and have spread throughout Europe and North America. The ability of dreissenid mussels to reach high population densities (Pathy, 1994) and individually filter high volumes of water (Elliott et al., 2008), can lead to dramatic changes in resident phytoplankton communities (C. McLaughlan et al., 2014).

The grazing activity of dreissenids has been shown to affect phytoplankton communities directly and indirectly. Directly, through selective phytoplankton removal from the water column, selective digestion of phytoplankton species and, due to the high rate of filtration, removing all but the fastest growing phytoplankton species (Bastviken et al., 1998; Baker and Levinton, 2003; Fernald et al., 2007). Indirectly, filtration by mussels can increase light penetration and change the rate and stoichiometry of nutrient recycling, both of which can alter phytoplankton community structure (Edwards et al., 2016; Kleinteich et al., 2020). Understanding the role that dreissenids play in shaping phytoplankton community structure is important as, given their current global spread and status as ecosystem engineers (Sousa et al., 2009), they play a central role in the establishment of alternative equilibria (Scheffer et al., 1993; Sousa et al., 2009; Emery-Butcher et al., 2020) and occurrence of harmful algal blooms (HABs) (Vanderploeg et al., 2013).

The role of dreissenid mussels in increasing cyanobacterial biomass and promoting Harmful Algal Blooms (HABs) has been the focus of numerous studies (Vanderploeg et al., 2001, 2013; Burmester et al., 2012; White and Sarnelle, 2014). HABs often occur in systems with low flushing rates (Elliott, 2010) and are characterised by high cyanobacterial biomass (Huisman et al., 2018), low dissolved oxygen levels (Sun et al., 2018), high turbidity (Capuzzo et al., 2015), and the release of toxins which may be harmful to humans and aquatic life (Paerl et al., 2001; Huisman et al., 2018). HABs often lead to the death of significant numbers of aquatic organisms (sometimes including dreissenids), reducing biodiversity (Lund, 1965; Reynolds and Walsby, 1975; Nasri et al., 2008; Huisman et al., 2018), reducing the aesthetic value of the waterbody (Mitra and Flynn, 2006), preventing recreational activities (Otten and Paerl, 2015), and threatening human health directly through the release of toxins and indirectly through increases in necrotic material (Lam et al., 1995). The evidence of the impact of dreissenid mussels on driving HABs is conflicting. Some studies suggest dreissenids supress cyanobacterial biomass (Reeders et al., 1989; Caraco et al., 1997; Smith et al., 1998; Waajen et al., 2016; Reynolds et al., 2019), demonstrating that the mussels filter and remove a broad size range of cyanobacteria, regardless of toxicity (Dionisio Pires et al., 2005a). However, in some systems and especially in the North American Great Lakes, it has been found that the arrival of dreissenid mussels has led to increases in cyanobacterial biomass (MacIsaac, 1996; Vanderploeg et al., 2001; Nicholls et al., 2002; Pillsbury et al., 2002; Bierman et al., 2005; Conroy and Culver, 2005; Naddafi et al., 2007). A definitive explanation of the inconsistency of the relationship between dreissenids and cyanobacteria has not been uncovered. It has been suggested that unknown phenotypic adaptations or genotypic differences between mussel populations could be responsible (Vanderploeg et al., 2013; Huisman et al., 2018).

A key element shaping the relationship between dreissenids and phytoplankton community structure is the role of selective ingestion and rejection of algae and cyanobacteria and the production of pseudofaeces (Tang et al., 2014). Mussels actively draw water through their incurrent siphon into their mantle cavity, selecting only a portion of the total seston particles present for ingestion, while unpalatable particles or phytoplankton are bound in mucus and ejected as pseudofaeces (Baker et al., 2000; Vanderploeg et al., 2001; Ward and Shumway, 2004). Phytoplankton loosely bound in pseudofaeces can be dispersed back into the water column, and has been implicated in driving toxic algal blooms (Vanderploeg et al., 2001, 2013). However, selective ingestion and rejection of the cyanobacterium *Microcystis aeruginosa*, a widespread and key constituent of many HABs, has been shown to vary between populations of dreissenid mussels and even between different strains of *M. aeruginosa* (Vanderploeg et al., 2013).

The ingestion or rejection of particles drawn through the incurrent siphon is determined by the structure and function of the gills, which capture and direct food particles (Way et al., 1989; Silverman et al., 1996), and the labial palps, which further sort and select particles for ingestion or rejection (Tankersley, 1996). These structures have demonstrated structural plasticity between mussel populations (Drent et al., 2004; Galbraith et al., 2009). It has been shown in unionid mussels that the gill structure can be affected by the hydrodynamic environment, with clearance rates and gill structure complexity displaying variation between lentic and lotic mussel populations (Silverman et al., 1997). While in marine bivalves, gill-to-palp mass ratios have been shown to respond plastically to changes in ambient sediment characteristics, with large ratios in coarse sediment and small ratios in fine sediment environments (Drent et al., 2004).

As quagga mussels are fixed to their substrate, the hydrodynamic environment plays an important, yet understudied, role in their grazing behaviour (Hasler et al., 2019). Hydrodynamics are an important driver of phytoplankton community structure and thus food abundance, with cyanobacteria preferring environments with low flush rates, such as lakes and reservoirs (Paerl et al., 2001; Bakker and Hilt, 2016). Differences in sediment loads, with higher levels of suspended sediments likely in lotic environments such as rivers, will demand different particle sorting requirements. Plasticity in particle sorting and retention has been observed in the marine mussel *Mytilus edulis* in response to changes in ambient particle size distribution (Strohmeier et al., 2012). Hydrology has already been indicated as a driver of a plastic response in the shell morphology of quagga mussels (Peyer et al., 2010), and therefore, given the aforementioned impact of hydrology on food resources, could conceivably play a role in the architectural plasticity of the gills and labial palps.

In this study we aimed to quantify differences in clearance rates and pseudofaeces production and composition between populations of quagga mussels from lentic and lotic environments and by doing so, further our understanding of how these INNS may affect the occurrence of HABs and shifts between alternative equilibria. The quagga mussel was chosen for this study as the potential impacts of quagga mussels are less well understood than the more comprehensively researched zebra mussel, which is one of the best studied freshwater invertebrates (Nalepa and Schloesser, 2013; Mei et al., 2016). Understanding quagga mussels is of imminent importance as they are replacing zebra mussels in much of their invaded ranges (Ricciardi and Whoriskey, 2004; Wilson et al., 2006; Naddafi and Rudstam, 2014; Karatayev et al., 2015; Mei et al., 2016), and it is suggested their impacts may be more severe and pervasive in scope (Nalepa, 2010). Moreover, quagga mussels often proliferate in reservoir systems, where HABs are a particular concern for the management and supply of potable water (Karatayev et al., 2007; Gallardo and Aldridge, 2020). Comparisons of clearance rates and pseudofaeces composition were studied under different phytoplankton concentrations in both co-cultures and monocultures of green algae and cyanobacteria.

The main hypotheses guiding this study were: (1) Mussels from both environments will demonstrate a preference for green algae over cyanobacteria, demonstrated by a relatively higher clearance rate for green algae (Vanderploeg et al., 2001); (2) Mussels from both environments will reject a greater number of cyanobacteria compared to green algae in pseudofaeces (Vanderploeg et al., 2001; Juhel et al., 2006); (3) Mussels from lotic populations may have a greater clearance rate than those from lentic environments due to the putative expression of a more complex gill structure, in order to remove phytoplankton from fast-flowing water (Englund and Heino, 1996; Silverman et al., 1997; Zieritz et al., 2019); and (4) Mussels from lentic environments may more efficiently ingest and remove cyanobacterial species and have lower levels of cyanobacterial rejection in pseudofaeces due to past exposure (Elliott, 2010; Richardson et al., 2018).

4.2 Methods

4.2.1 Cultivating algae species

The cyanobacterium *Synechococcus elongatus* (Strain 7942 Wild Type, Pasteur Culture Collection of Cyanobacteria, Paris, France) and green alga *Chlorella vulgaris* (Strain 211/11B Culture Collection of Algae and Protozoa, Oban, UK) were selected for this experiment. *Synechococcus elongatus* is a rod shaped, single celled, cyanobacterium with a length of ~5 μm. *Chlorella vulgaris* is a spherical, single celled, green algae with a diameter of ~3 μm.

To maximise the growth rate of the algal stocks, *C. vulgaris* was cultured in 3N-BBM-V growth media and *S. elongatus* was cultured in BG11 growth media. Culture methodologies and media constituents for both 3N-BBM-V and BG11 followed CCAP protocols (www.ccap.ac.uk/pdfrecipes.htm). Both algae cultures were incubated at 25°C with a 16:8h light:dark cycle.

4.2.2 Collecting and processing the quagga mussels

Quagga mussels were collected from two separate sites which represent lotic and lentic conditions. The lentic environment quagga mussels were collected from the Queen Mother Reservoir, Slough, SE England (51.4809° N, -0.5514° W). The mussels were collected by hand from the underside of fixed buoys and associated chains. The lotic environment quagga mussels were collected from the Wrasbury River, Staines, SE England (51.4576° N, -0.5182° W). The mussels were collected from the riverbed using a net. Both sites were hydrologically connected within the River Thames catchment (Euclidian distance 6km). The lentic and lotic mussels were transported to the laboratory in sealed buckets immersed in water from their respective environments.

In the laboratory the aggregations of mussels were separated by cutting the byssus threads with a scalpel. The surfaces of the mussel shells were gently scrubbed with a plant fibre scrubbing pad to remove any attached debris and algae. The mussels were then placed in dechlorinated tap water for 48 hours leading up to the experiment. The dechlorinated water was changed several times to ensure that the mussels were flushed and had excreted any pseudofaeces and faeces prior to the experiment. The average shell length of the lentic and lotic mussels used in the experiment was similar: 26.6±0.3 mm and 27.2±0.4 mm, respectively ($t = -1.307$, $df=84.97$, $p= 0.195$).

4.2.3 Experimental design

The clearance rates and pseudofaeces production and composition for both lentic and lotic quagga mussels were measured across a range of phytoplankton concentrations under three different phytoplankton conditions: *C. vulgaris* monoculture, *S. elongatus* monoculture and a co-culture of *C. vulgaris* and *S. elongatus*. There was a total of 90 experiments, which were split by mussel origin and phytoplankton treatment. Therefore, there were 45 experiments per environment (lotic and lentic) and

15 experiments per phytoplankton condition. The experiments were run over the course of two hours and were undertaken in one litre capacity aquaria (129 mm x 133 mm x133 mm). Each experiment had a total volume of 200 ml, comprising dechlorinated water and the phytoplankton inoculum. Each aquarium was aerated throughout the experiment to prevent oxygen depletion and to aid the suspension of phytoplankton. The temperature of the room was maintained at 18°C throughout the experiments.

In the single phytoplankton species experiments, a series of phytoplankton concentrations between 20 µg/L and 190 µg/L of each phytoplankton was used. In the mixed phytoplankton treatments, the concentration of both phytoplankton species was targeted to be of equal value. For example, in the lowest concentration mixed treatment, there was ~20 µg/L of *C. vulgaris* and ~20 µg/L of *S. elongatus*. For comparison, in the lowest single phytoplankton species treatment there was 20 µg/L of the target species. The phytoplankton concentrations were determined using a fluoroprobe (bbe Moldaenke GmbH, Schwentinental, Germany), which estimated the concentration (µg/L) of each phytoplankton taxa in a given sample. Using the FluoroProbe, a standard curve for each phytoplankton taxa was calculated prior to each experiment. The phytoplankton inoculum of known concentration was added to each aquarium to achieve the desired concentration (µg/L) in the total volume of 200 ml. The initial phytoplankton concentration measurement was taken prior to the addition of the mussels to the aquaria. There were nine control experiments, three for each of the phytoplankton conditions, where no mussels were present. Each control had a target phytoplankton concentration of 80 µg/L for each species. The final concentration measurements were taken two hours after the mussel was added. After the final concentration measurement was taken, the mussels were dissected, and the soft tissue removed and dried to a constant mass (AB54-S Analytical Balance, Mettler Toledo, Columbus, Ohio).

All phytoplankton concentration measurements were made using the FluoroProbe (bbe Moldaenke GmbH, Schwentinental, Germany). The FluoroProbe was mounted in the ‘workstation 25 standard version’, in which it can analyse the content of a 25 ml cuvette. The bbe++ software package was used to create the measurement parameters, operate the FluoroProbe and take phytoplankton concentration readings.

4.2.4 Clearance Rates

Clearance rate was calculated using Equation 4.1, adapted from Riisgard (Riisgård, 2001), which incorporates the dry tissue mass of mussels to control for differences in size across the population samples, replicates, and treatments.

$$\mathrm{CR} = \frac{\mathrm{Vol}}{DW.t}\left(LN\left(\frac{C.initial}{C.final}\right)\right) \qquad \text{(Eq 4.1)}$$

Where Vol is the total volume of water in the experiment (ml), DW is the dry tissue mass of the mussel (g), t is time (hr) and *C.initial* and *C.final* are the phytoplankton concentrations measured at the beginning and end of the experiment (µg/L).

4.2.5 Pseudofaeces collection and composition

Pseudofaeces samples were collected at the end of the experiments after the final phytoplankton concentration measurement was taken. Pseudofaeces were easily identified as they contrasted against the base of the aquaria. The identification criteria cited by Galimany et al., (2018) was used as a reference. In contrast to faeces, pseudofaeces were lightly-packed, cloud-like deposits which were easily resuspended (Galimany et al., 2018). The pseudofaeces were collected using a glass Pasteur pipette and deposited into an upright Eppendorf tube and allowed to settle and form a pellet. Once the pseudofaeces settled out of suspension, the supernatant was removed and 100 µl of dechlorinated water was added to the Eppendorf tube. A vortex mixer was used to resuspend the phytoplankton. For dense suspensions, samples were diluted further to aid counting.

The number of phytoplankton cells was quantified under a microscope with a 40X objective, using a haemocytometer counting chamber (Neubauer Improved Haemocytometer Counting Chamber, Hawksley, Lancing, England). Using a hand tally counter, cells were counted and averaged across five sets of 16 counting squares. The phytoplankton were easily discriminated from one another given their distinct morphologies.

4.2.6 Statistical analysis

Experimental replicates where mussels were assumed to have not been filter feeding during the experiment were removed from the analysis. These experiments were characterised as either those experimental replicates where the clearance rate was equal to zero, or where the clearance rate fell within the percentage change in phytoplankton concentrations observed in the control experiments.

All analysis was performed in R studio (v.1.1.442) (R Core Team, 2018). To compare the clearance rates of lentic and lotic quagga mussels under different phytoplankton conditions at various concentrations, a generalised linear model (GLM) with a gamma error distribution and log link was used, as the dependant variable is continuous and above zero. Phytoplankton species, treatment type, and the mussel's origin were used as categorical factors. Initial phytoplankton concentration was an independent continuous variable and clearance rate was the dependant continuous variable.

To compare the pseudofaeces production and composition between lentic and lotic quagga mussels under different phytoplankton conditions at various concentrations, the following method was used. The dataset contained the count data for the number of phytoplankton cells (*C. vulgaris* and/or *S. elongatus*) found in the pseudofaeces of mussels from both hydrodynamic environments (lentic or lotic) under each experimental treatment type (single or mixed phytoplankton species), under all initial phytoplankton concentrations. Therefore, despite there being only 90 experimental replicates, there are 120 potential data points, as each of the thirty mixed phytoplankton experiments produced a count for both *C. vulgaris* and *S. elongatus* from a single pseudofaeces sample. Using the DHARMa package for R (Hartig, 2020), the count data was confirmed to express overdispersion as well as excess zeros. Excess zeros likely occurred as a result of many mussels, especially at lower phytoplankton concentrations, producing no pseudofaeces. To correct for these characteristics of the data, a hurdle model (which corrected for excess zeros) with a negative binomial distribution (which corrected for overdispersion) was used (Zeileis et al., 2008). The hurdle model is a two-component model which has a truncated count component for positive counts and a hurdle component which models the zero counts likelihood of being a positive count. The hurdle model requires the use of the package ‘pscl’ (Jackman, 2020) for R. The model requires phytoplankton species as a categorical factor, initial algae concentration as an independent continuous variable, the mussel’s origin as a categorical factor, the phytoplankton treatment type as a categorical factor and the number of phytoplankton cells observed in the pseudofaeces as a dependant discrete count variable.

4.3 Results

4.3.1 Clearance Rates

Chlorella vulgaris had a higher clearance rate than *S. elongatus* when present in the same concentration (t=-3.609, df = 88, P<0.001) (Figure 4.1). As the concentration of phytoplankton increased, the clearance rate of both lentic and lotic mussels increased for each phytoplankton species (t=6.095, df=88, P<0.001) (Figure 4.1). There was no significant difference in clearance rates between mixed and single species phytoplankton treatments (t=-0.184, df=88, P=0.855), or between mussels from lentic or lotic environments (t=-1.172, df=88, P=0.244).

4.3.2 Pseudofaeces quantity and composition

The hurdle model identified that lotic mussels produced more pseudofaeces than lentic mussels at the same phytoplankton concentrations (Z= 3.238, df=101, P<0.01) (Figure 4.2). For both lentic and lotic mussels, the amount of pseudofaeces produced was greater in mixed phytoplankton treatments than single species treatments (Z=-2.778, df=101, P<0.01) (Figure 4.2). *Synechococcus elongatus* was found more frequently than *C. vulgaris* in the pseudofaeces of both mussel types (Z=6.494, df=101, P<0.01) (Figure 4.2). Equally, as the concentration of phytoplankton increased as did the total amount of pseudofaeces that was produced (Z=4.925, df=101, P<0.01) (Figure 4.2). To assess any effect of mussel body condition on pseudofaeces production, a ratio of shell length (mm) and dry weight (g) was used as a proxy variable. The hurdle model found that this factor was not significant in predicting pseudofaeces production or the number of cells found in the pseudofaeces, and so it was removed from the model and can be discounted as a factor influencing pseudofaeces quantity and composition.

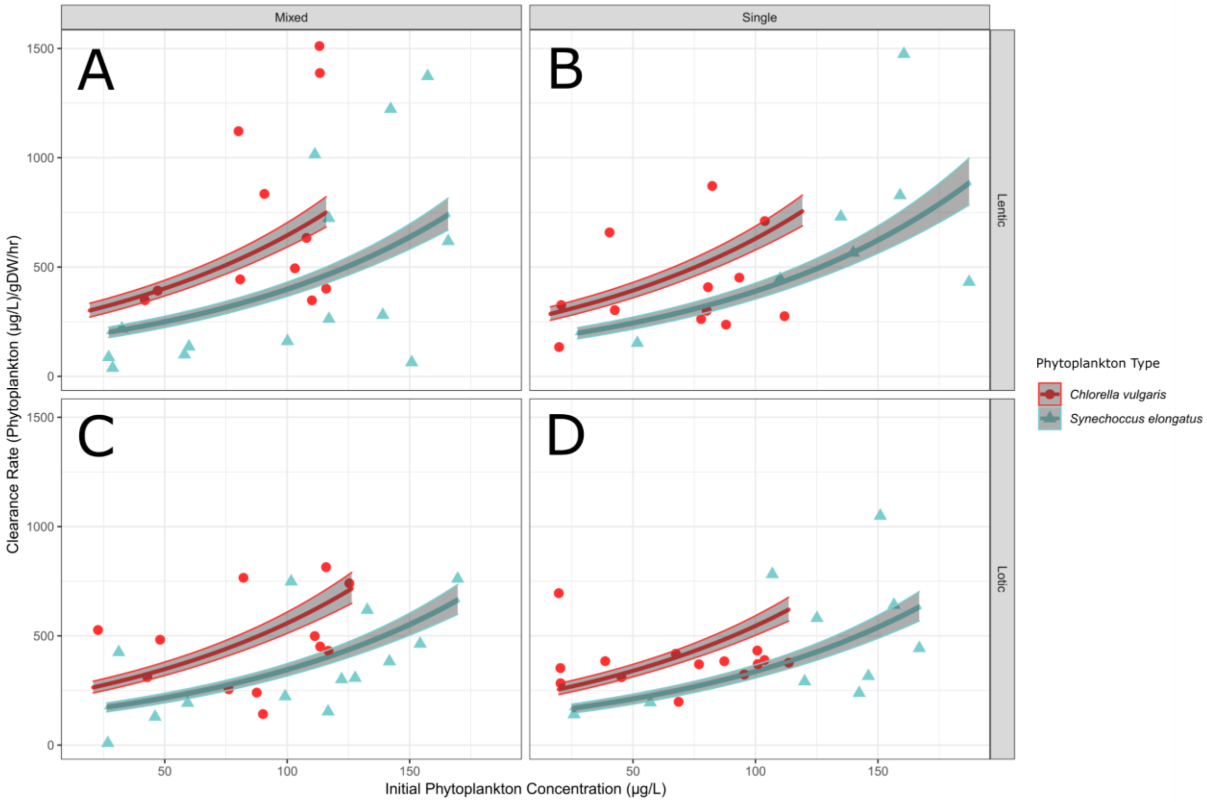


Figure 4.1 Average clearance rates of lentic and lotic quagga mussels across a range of phytoplankton conditions. Modelled average clearance rates (red and blue lines) for individual clearance rate experiments (circles and triangles) across a range of phytoplankton concentrations for quagga mussels from both lentic (panels A and B) and lotic (panels C and D) environments, in mixed species (panels A and C) and single species (panels B and D) treatments for both *C. vulgaris* (red line and circles) and *S. elongatus* (blue line and triangles). The shaded area represents the 95% confidence interval. DW denotes dry weight tissue mass.

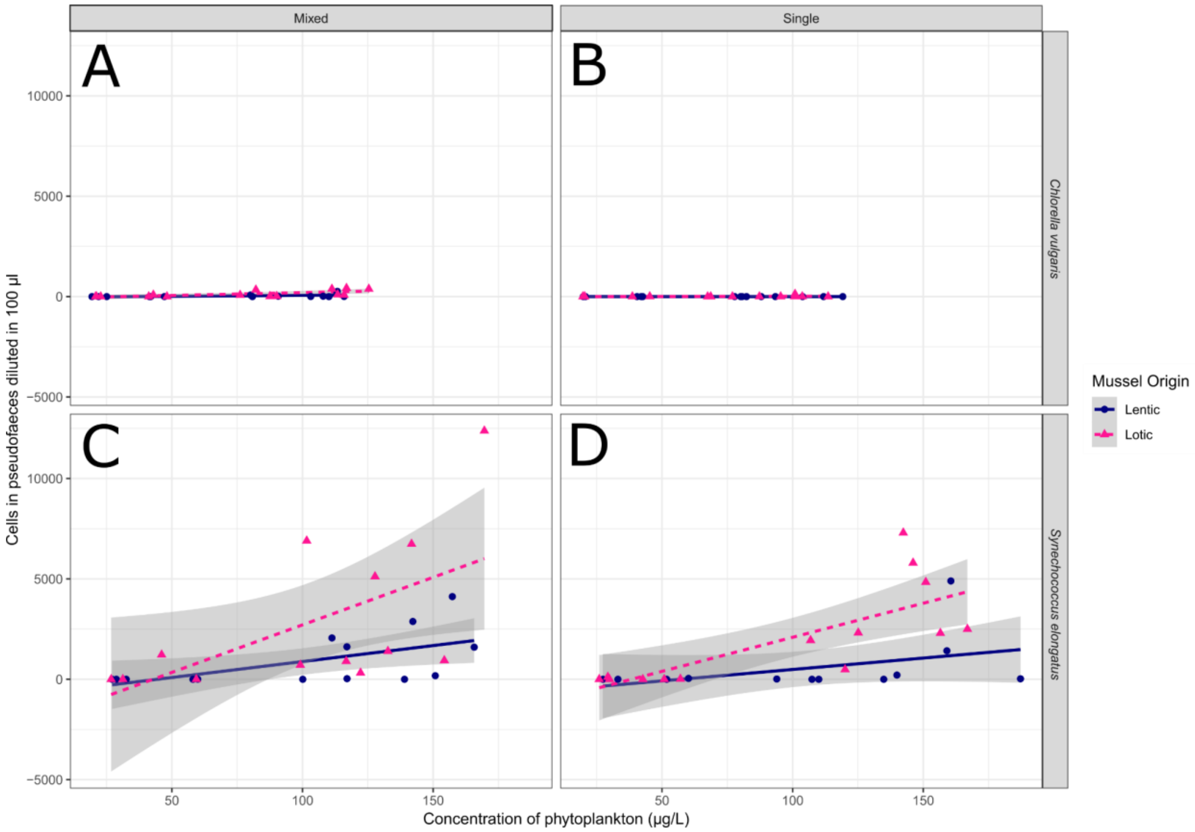


Figure 4.2 Pseudofaeces content of lentic and lotic quagga mussels across a range of phytoplankton conditions. The number of *C. vulgaris* (panels A and B) and *S. elongatus* (panels C and D) cells found in the pseudofaeces of both lentic (blue solid line and blue filled dots) and lotic (pink dashed line and pink filled triangles) quagga mussels, in both mixed (panels A and C) and single (panels B and D) phytoplankton treatments. The lines represent linear regression models, and the shaded areas represent the 95% confidence intervals. The following equations denote the intercept, coefficient, R^2 and P value of the linear regression models for each panel: (A) Lentic [y = 65.6 + 0.224 x R2 = 0.22 p = 0.078], Lotic [y = 54.3 + 0.159 x R2 = 0.45 p = 0.006]; (B) Lentic [y = 75.5 – 0.274 x R2 = 0.20 p = 0.108], Lotic [y = 65.7 + 0.284 x R2 = 0.08 p = 0.331]; (C) Lentic [y = 80.9 + 0.022 x R2 = 0.35 p = 0.026], Lotic [y = 81.8 + 0.007 x R2 = 0.34 p = 0.036]; (D) Lentic [y = 96.8 + 0.016 x R2 = 0.18 p = 0.170], Lotic [y = 63.3 + 0.017 x R2 = 0.57 p = 0.002].

4.4 Discussion

The key findings from this study are that both lentic and lotic populations of quagga mussels demonstrated a higher clearance rate for *C. vulgaris* than *S. elongatus*, in both single and mixed phytoplankton treatments; for lentic and lotic mussel populations, the clearance rate of both *C. vulgaris* and *S. elongatus* increased in relation to phytoplankton concentration; lotic mussels produced more pseudofaeces than lentic mussels at the same phytoplankton concentration; pseudofaeces production was higher in mixed phytoplankton treatments than single species treatments; *S. elongatus* was found more frequently in pseudofaeces than *C. vulgaris*; and the total amount of pseudofaeces produced increased in relation with the concentration of phytoplankton.

4.4.1 Effect of phytoplankton species on clearance rate

The first hypothesis posed by our study, and supported by the results, was that both populations of quagga mussels would demonstrate a higher clearance rate for *C. vulgaris* over the cyanobacteria *S. elongatus*. Many studies have suggested zebra mussels, a congeneric of quagga mussels, can alter their feeding behaviour to ingest the highest quality phytoplankton, where quality is defined by the concentration of polyunsaturated long-chain fatty acids (PUFAs) (Reeders and Bij de Vaate, 1990; Heath et al., 1995; Lavrentyev et al., 1995; Baker et al., 1998; Dionisio Pires and Van Donk, 2002; Dionisio Pires et al., 2005a; Naddafi et al., 2007). PUFAs are usually found in higher concentrations in green algae than cyanobacteria (Ahlgren et al., 1992; Vanderploeg et al., 1996). For quagga mussels, it has been noted that phytoplankton taxon, rather than size, was a more significant predictor of clearance rate (Tang et al., 2014). This behaviour appears to be replicated in our study, where the clearance rate of the green alga *C. vulgaris* was consistently higher across all concentrations and in both mixed and single phytoplankton treatments, than the clearance rate of the cyanobacterium *S. elongatus*, despite their similar size. A further predictor of selective grazing in quagga mussels is nucleic acid content (NAC), with quagga mussels prioritising taxa with a high NAC (Props et al., 2018). This may further explain the putative preference for green algae as, on average, *S. elongatus* has a genome size of ~2.7 Mb (Sugita et al., 2007) while *C. vulgaris* has a genome of ~40 Mb (Cecchin et al., 2019).

The relationship between phytoplankton concentration and clearance rate is comparable to the observations made by Kemp and Aldridge (Kemp and Aldridge, 2018), suggesting that, except at the most extreme algal concentrations, quagga mussels are likely to maintain a feeding rate which is proportional to the food resources available (Kemp and Aldridge, 2018). The quagga mussel's high population densities combined with their prolific and selective grazing behaviour may likely exert significant top-down control on phytoplankton community structure, with changes amplified at higher phytoplankton concentrations. The results of this study indicate that the selective grazing of quagga mussels could shift phytoplankton communities, towards cyanobacteria dominance. These shifts between phytoplankton taxa have been noted in natural phytoplankton communities in Lake Michigan, where the arrival of quagga mussels has been linked to the suppression of all spring blooming phytoplankton communities except for cyanobacteria and certain chlorophytes (Fahnenstiel et al., 2010; Vanderploeg et al., 2010).

4.4.2 Effect of phytoplankton species on pseudofaeces production

The second hypothesis of this study, predicting a greater number of *S. elongatus* cells in the pseudofaeces of both mussel populations than *C. vulgaris*, was also supported by the results.

Additionally, the number of cells in the pseudofaeces increased with higher phytoplankton concentrations, as noted with mussel clearance rates. Furthermore, an increase in the number of cells in the pseudofaeces from mixed phytoplankton treatments was observed. These results support the notion of selective rejection in quagga mussels, whereby mussels reject one phytoplankton species over another, a behaviour well documented in zebra mussels (Vanderploeg et al., 1996, 2001; Dionisio Pires and Van Donk, 2002).

The greater pseudofaeces production in mixed phytoplankton treatments, compared to single treatments, may be due to the elevated total concentration of phytoplankton in the aquaria. The elevated overall phytoplankton concentration is likely to have led to a greater total biomass of phytoplankton being captured by the gills and entering the labial palps. Although the clearance rates across single and mixed species treatments remained consistent, and the total individual biomass of *C. vulgaris* and *S. elongatus* was the same in single compared to mixed treatments, the mussels may have become increasingly selective in their sorting in mixed treatments due to the availability of a potentially more palatable option. The presence of *C. vulgaris* in the mixed treatments, may signal to the mussels that there is a reduced energetic cost to rejecting the less palatable cyanobacteria, whereas in single treatments the *S. elongatus* represents the sole source of nutrients.

Numerous studies have demonstrated that cyanobacterial species are selectively rejected by dreissenid mussels, with much of the focus on the potentially toxic *Microcystis aeruginosa* (Vanderploeg et al., 2001; Raikow et al., 2004; Bierman et al., 2005; Tang et al., 2014). However, this pattern is not universally observed, with some studies reporting the positive selective uptake of *M. aeruginosa* combined with only limited rejection in the pseudofaeces (Dionisio Pires et al., 2005a). The difference observed between studies in the selection and rejection of certain phytoplankton by dreissenids has often been attributed to discrimination against phytoplankton species which are small (Naddafi et al., 2007), toxic (Vanderploeg et al., 2001), possess a thick cell wall (Dionisio Pires and Van Donk, 2002) or have a gelatinous outer layer (Tang et al., 2014).

The size of *C. vulgaris* (~3 µm) and *S. elongatus* (~5 µm) places them on the border between picoplankton (<2 µm) and nanoplankton (2-20 µm). Phytoplankton species of this size are expected to be largely expelled by dreissenids, as it is thought that it would be energetically inefficient to ingest them (Naddafi et al., 2007). However, this study demonstrates that selective grazing behaviours are maintained by quagga mussels even for food resources at this small size range, which suggests that size is not a universal indicator of selective rejection. Similarly, Vanderploeg et al., (2013) demonstrated that neither colony size or toxicity may fully explain differences in selective rejection, as the authors noted significant differences in the uptake and rejection of *M. aeruginosa* between different laboratory strains, and even between strains from the same lake, which could not be explained by toxicity or colony size. Leading the authors to suggest that genotypic or phenotypic differences between mussel populations may be responsible.

4.4.3 Effect of mussel origin on clearance rates and pseudofaeces production

The third hypothesis, that mussels from lotic environments would exhibit an elevated clearance rate in comparison to mussels from lentic environments, is rejected by the results of this study. The reason this effect was not observed in this study may be due to the use of static, rather than flow through, mesocosms. Indeed, the greatest differences noted in the clearance rates of lotic and lentic unionid mussel species implemented flow-through mesocosm systems (Vanden Byllaardt and Ackerman, 2014) or made in-situ observations (Zieritz et al., 2019). Therefore, it may be that putative clearance rate adaptations made by mussels to the hydrodynamic environment are best observed under the environmental conditions which may have conferred the adaptation.

The fourth hypothesis, that lentic mussels may have a higher tolerance for cyanobacteria and produce less pseudofaeces than lotic mussels, is supported by the results. A literature review undertaken by Hasler et al., (2019) on the biological effects of flow hydrodynamics on dreissenid mussels, identified only four papers studying hydrodynamic effect on dreissenid suspension feeding behaviour (Ackerman, 1999; Tuchman et al., 2004; Edwards et al., 2005; Boegman et al., 2008; Hasler et al., 2019), none of which focus on how the hydrodynamics of the environment may drive long term adaptions in feeding behaviour between populations. Lentic populations of quagga mussels are more likely to have historical exposure to cyanobacteria, as cyanobacteria are more prevalent in systems with stable water columns (Elliott, 2010). Due to this historical exposure, it is possible that lentic mussels may ingest and retain more *S. elongatus,* and therefore eject fewer *S. elongatus* in pseudofaeces, as cyanobacteria may commonly account for a greater proportion of their regular diet, compared to mussels from lotic environments where cyanobacteria are less common. Mussels from lotic environments, which displayed a comparable clearance rate to lentic mussels in this experiment, would potentially be encountering atypical levels of cyanobacterial species, perhaps leading to the elevated rejection of *S. elongatus* observed in this experiment.

As the hydrodynamic environment can dictate the composition of algal communities and therefore the diet of the quagga mussels, the ability to ingest the prominent phytoplankton species available would confer an ecological fitness advantage to the mussel. It may be that hydrodynamics, in driving algal community structure spatially and biologically, may give rise to yet unknown putative genetic and phenotypic differences between quagga mussel populations as postulated by Vanderploeg et al., (2013) and Huisman et al., (2018). As observed by Galbraith et al., (2009) and Silverman et al., (1997), the gill morphology between lentic and lotic unionid mussel species was significantly different. Mussels use the cilia on their gills like a sieve to capture food particles and also to pump water through the inhalant siphon into the mantle cavity, drawing water across the filter feeding gill structures (Gardiner et al., 1991; McMahon and Bogan, 2001). It is hypothesised that different densities of cilia on the gill may allow mussels to specialise for different particle types or sizes (Silverman et al., 1997). Silverman et al., 1997 observed that lotic unionid mussels had more complex and denser cilia structure than lentic unionids and demonstrated a higher clearance rate for *Escherichia coli*. Although this difference in morphology has been observed between species from different environments, no research has been conducted to ascertain if this characteristic extends to the phenotypic plasticity which may occur within a species residing in different environments.

4.4.4 Potential impact of selective feeding on phytoplankton community structure and development of HABs.

The potentially selective nature of quagga mussel grazing, and the differences noted between mussels from different populations may have implications for the formation of HABs, which are often dominated by cyanobacterial species, such as *Synechococcus sp.* (Beardall, 2008). The differential grazing pressures exerted by quagga mussels on *S. elongatus* compared to *C. vulgaris* may act to reduce competition for light and nutrients faced by cyanobacteria. Reduced competition for resources could lead to cyanobacterial dominance and favourable conditions for HABs.

The significant presence of *S. elongatus* in pseudofaeces, compared to *C. vulgaris*, could act to further drive HABs. Selective rejection has been previously noted in quagga mussels (Tang et al., 2014), and has been suggested as a key mechanism behind bloom formation of *M. aeruginosa* in the presence of zebra mussels (Bierman et al., 2005; Fishman et al., 2009; Vanderploeg et al., 2013). Selective rejection may drive HABs as many cyanobacteria have been shown to remain viable in pseudofaeces, with the potential to be resuspended (Vanderploeg et al., 2001; White and Sarnelle, 2014). Elevated excretion of viable cyanobacterial cells in pseudofaeces acts to accelerate population disparities between cyanobacteria and other, non-HAB forming phytoplankton species, such as *C. vulgaris*, which are retained by the mussels.

Beyond differential clearance rates and selective rejection of phytoplankton species, the overall impact of mussels on natural phytoplankton communities will be highly dependent on the density of the established mussel population and also the mixing regime of the system (Reeders and Bij de Vaate, 1990; Mellina et al., 1995; Zhang et al., 2011). A system with low quagga mussel density, may be expected to experience little change to phytoplankton community structure due to negligible grazing pressure. Similarly, in a system with weak water mixing and thermal stratification, phytoplankton may only be removed around the mussel bed as mussels will re-filter the same water (Zhang et al., 2011; McLaughlan and Aldridge, 2013). Poor mixing may also limit the ability of viable phytoplankton cells to resuspend once trapped in the pseudofaeces, and therefore selective rejection may play a reduced role in driving HABs.

The results of this study demonstrate that there is significant evidence that different populations of quagga mussels have the potential to drive different outcomes in phytoplankton communities, primarily through distinct phytoplankton ingestion and rejection behaviours. However, further studies are required to identify whether the patterns observed in this study are consistently found between lotic and lentic systems, and whether these differences are driven by phenotypic plasticity or the selection of particular genotypes.

4.4.5 Future Directions

This study demonstrates that the clearance rate and selectivity of quagga mussels varies between phytoplankton taxa and mussel populations. To further understand the selective pressures exerted by quagga mussels on phytoplankton bloom dynamics, future research should focus on further clarifying the underlying drivers of these processes. Characterisation of the clearance rate and pseudofaeces production of quagga mussels from multiple lentic and lotic populations will help to confirm the role of the hydrodynamic environment in governing grazing behaviours. Further determining how phytoplankton selection varies with the cell's nutritional content and quality, as well as phytoplankton morphology (cell wall thickness, toxicity, mucilage, buoyancy), will aid understanding of grazing behaviours in respect to HAB dynamics. The consistency of these selective grazing pressures across lentic and lotic mussel populations should be confirmed with experiments where mussels are exposed to relevant phytoplankton communities and hydrodynamic environments found in nature. Potential genotypic and phenotypic differences between populations of quagga mussels from different hydrodynamic environments should also be recorded and quantified. Fruitful exploration of phenotypic differences between mussel populations may emerge through the study of gill structures and the labial palps, following the methodologies set out by Silverman et al., (1997) and Galbraith et al., (2009). If such differences in grazing exist between populations of the same species, especially INNS, this could have significant repercussions not only for understanding their potential impacts on invaded ranges, and HAB formation, but perhaps identify possible ways of exploiting particular populations of dreissenids for habitat management (McLaughlan and Aldridge, 2013).

Chapter 5

Embracing the allelopathic potential of invasive aquatic plants to manipulate freshwater ecosystems

Abstract

Freshwater ecosystems provide essential resources and vital ecosystem services. These ecosystems exist in a delicate state of balance and are under increasing anthropogenic and climatic pressures. One of the major anthropogenic threats to freshwater ecosystems is eutrophication that often leads to algal blooms, some of which may be extremely harmful. Current chemical and physical interventions to prevent algal blooms can be expensive, ephemeral and disruptive to other aspects of the ecosystem. Therefore, there is interest in utilising biological methods of control. This study aimed to assess the viability of allelopathic repression of nuisance algae species by invasive aquatic plants. The allelopathic effect of *Hydrocotyle ranunculoides* (Floating Pennywort) and *Crassula helmsii* (Swamp Stonecrop) were tested in both whole plant and crushed plant states for their ability to affect the average population growth of monocultures and co-cultures of the green algae *Chlorella vulgaris* and the cyanobacterium *Synechocystis* sp. PCC6803. Methanol extracts from these species have been identified in the literature to have high allelopathic potential. The key findings of this study are that, for *Chlorella*: whole *H. ranunculoides* and crushed *C. helmsii* had a negative effect on the average population growth; whole *H. ranunculoides* had a greater negative effect than crushed *H. ranunculoides*; and crushed *C. helmsii* had a more negative effect than crushed *H. ranunculoides*. For *Synechocystis*: crushed *C. helmsii* had a greater negative effect on the average population growth than crushed *H. ranunculoides*; and the presence of *Chlorella* in co-culture experiments had a universally positive effect on its average population growth. The species-specific nature of these allelopathic interactions suggests that the use of allelopathy for algal bloom control may have to be assessed on a case-by-case basis and the use of combination treatments should be assessed. Moreover, the effects exerted by allelochemicals in open water systems is likely to be direct, indirect and context specific. Although this study explores the possibility of embracing the allelopathic potential of invasive aquatic plants, we do not encourage novel introductions of invasive species into open freshwater systems. However, potential allelopathic effects could be leveraged in already invaded systems, or in closed systems within an invaded range.

5.1 Introduction

Freshwater ecosystems deliver numerous essential ecosystem services, including the provision of drinking water, food resources, diverse habitat, irrigation, and recreation. However, anthropogenic pressures on freshwater environments through increased nutrient run-off from agriculture, pollution from industrial operations, increased abstraction and the effects of climate change are counteracting the favourable clear water state freshwater systems require to provide many of these services (Moss et al., 2011; Jeppesen et al., 2017).

Many freshwater ecosystems, especially shallow lakes, exist in a delicate state of balance between alternate equilibria, either occupying a favourable clear water state dominated by bottom-rooting aquatic vegetation, or a turbid state characterised by high microalgal biomass (Scheffer et al., 1993). The most prominent anthropogenic disruption to this balance is eutrophication (Michalak et al., 2013). Eutrophication refers to the pollution of a waterbody through the addition of excess nutrient elements, such as nitrogen (N), phosphorus (P) and potassium (K). Potential sources of additional nutrients include agricultural and aquaculture runoff, sewage, atmospheric deposition and groundwater flow. Elevated nutrient conditions are one of the key causes of Harmful Algal Blooms (HABs). These accumulations of harmful algae vastly reduce water quality and disrupt aquatic ecosystems through increased turbidity (Capuzzo et al., 2015), oxygen depletion (Sun et al., 2018), shading out other organisms in the water column (Hu and Hong, 2008) and the release of algal toxins which can poison ecosystems and water supplies (Paerl et al., 2001; Azevedo et al., 2002). These effects can result in the death of large numbers of aquatic organisms; reducing biodiversity (Lund, 1965; Reynolds and Walsby, 1975; Nasri et al., 2008); reducing the aesthetic value of affected waterbodies (Mitra and Flynn, 2006); and toxins and necrotic material threaten human health by entering drinking water supplies (Lam et al., 1995).

Current methods used to control HABs include chemical, physical and biological approaches. Chemical methods include use of metals (Magdaleno et al., 2014), herbicides (Nagai et al., 2016) and photosensitisers (Pohl et al., 2015). Physical methods include ultrasound disruption (Park et al., 2017), vertical destratification through mechanical mixing (Paerl et al., 2001) and membrane filtration (Zhao et al., 2017). Despite the efficacy of chemical and physical disruption of HABs, these methodologies can be expensive, ephemeral, labour intensive and disruptive to other aspects of the ecosystem while chemical interventions can be toxic to non-target species, including humans (Sun et al., 2018). Biological approaches offer the potential of a more efficient, cost-effective, and sustainable control through the use of aquatic animals, plants and algicidal microorganisms (McLaughlan and Aldridge, 2013; Backer et al., 2015; Harke et al., 2016; Sun et al., 2018).

This study seeks to assess the feasibility of masking the effects of eutrophication by leveraging the allelopathic effects of invasive aquatic plants in freshwater ecosystems. Allelopathy is the term that describes the inhibitory or stimulatory effects of one plant or phytoplankton on another plant or phytoplankton via the release of chemical compounds into the environment (Rice, 1983). These chemical compounds are secondary metabolites referred to as allelochemicals. The allelochemicals released by aquatic plants belong to different chemical classes including oxygenated fatty acids, sulphur compounds, polyacetylenes and polyphenols (Nakai et al., 2012). These allelochemicals can have a wide range of effects such as reducing the growth of competitors, repelling herbivores, resisting pathogens and interfering with decomposition (Grutters et al., 2017). Identifying aquatic plants which may have the ability to produce and release allelochemicals which suppress the growth of harmful phytoplankton species such as cyanobacteria, would provide a highly useful tool for the management and manipulation of eutrophic freshwater ecosystems. It has been shown in numerous studies that cyanobacteria are more sensitive than green algae and diatoms to allelopathic substances (Gross and Jüttner, 2003; Hilt and Gross, 2008; Jasser, 1995; Planas et al., 1981; and van Donk and van de Bund, 2002).

The majority of the research which has explored the ability of aquatic plants to affect the growth of cyanobacteria has been carried out using water soluble allelochemicals extracted from isolated plant material using methanol or through the use of exudate from target plant species (Table C1). A recent experiment by Grutters et al., (2017) illustrated that the phylogeny, growth strategy and stoichiometry of a plant can be used to determine its allelopathic potential. Grutters et al., (2017) used methanol extracts and agar diffusion assays, to measure the allelopathic potential of 34 plants. The authors concluded that eudicot plant species with an emergent growth strategy and a high plant carbon-to-phosphorus (C:P) ratio exhibited the highest allelopathic potential. It is important to highlight that there is a fundamental difference between allelopathic potential and a true allelopathic effect. Bioassays provide a broad view of allelopathic potential, reflecting the effect of all chemicals produced by a plant species. This standardised, effect-based, comparison of allelopathic potential allows plant species with differing chemistries to be compared on an equivalent basis (Meiners, 2014). However, plants identified as having high allelopathic potential using this methodology is not evidence of allelopathy, as there is no confirmation that the chemicals driving the observed effect would be naturally excreted by the plant (Gross et al., 2007).

In order for allelopathic plants to be used in the management of eutrophic waters allelochemicals must be naturally excreted. Therefore, we aimed to observe the allelopathic effect of two invasive plant species, *Hydrocotyle ranunculoides* (Floating pennywort) and *Crassula helmsii* (Swamp stonecrop) which, according to Grutters et al., (2017), meet the criteria for high allelopathic potential. Instead of using plant extractions, we focused on the effect of the whole plant, compared to the crushed plant, on their ability to affect axenic populations of the green algae *Chlorella vulgaris* (hereafter *Chlorella*) and the cyanobacteria *Synechocystis* sp. PCC6803 (hereafter *Synechocystis*), in both co-culture and in monoculture. Testing whole plants will provide evidence of whether the allelochemicals identified through methanol extraction are naturally excreted by plants into the environment in high enough concentrations to affect phytoplankton population growth under natural conditions.

Testing for allelopathic effects in phytoplankton co-culture as well as monoculture allows for observations of potential interactions between phytoplankton taxa which may occur in natural phytoplankton communities when exposed to allelopathic compounds. If allelopathic effects are observed to consistently vary between phytoplankton taxa, particularly in co-culture experiments, this could have implications for algal bloom dynamics. For example, it has been suggested that the allelopathic impact of plants on cyanobacteria can have opposite effects depending on the presence or absence of green algae, with green algae negating the suppressive effect of allelopathy on cyanobacterial growth, and actually resulting in increased cyanobacterial growth in co-culture experiments (Chang et al., 2012).

Synechocystis and *Chlorella* are both small unicellular phytoplankton of similar size (~2µm). They were selected for this study as they have been widely used in experiments which seek to investigate algal bloom dynamics (Schubert et al., 1995; Oudra et al., 2000; Martins et al., 2005; Murray et al., 2010; Qu et al., 2014; Dervaux et al., 2015; Chen and Bridgeman, 2017). The rise of cyanobacterial blooms has been reported across many freshwater ecosystems (Huisman et al., 2018). However, due partly to the small size of these cyanobacteria, single celled, non-colonial picoplankton are still a relatively poorly studied section of phytoplankton communities, despite their increasing impact on aquatic ecosystems and water quality (Chorus and Bartrum, 1999; Paerl and Otten, 2013).

Hydrocotyle ranunculoides and *C. helmsii* are introduced non-native species in the United Kingdom. *Hydrocotyle ranunculoides* originates from the Americas, forming dense mats of vegetation which float at the water surface. *Crassula helmsii* is native to Australia and New Zealand, occurring in free floating, submerged, emergent or terrestrial forms. Although Grutters et al. (2017), conclude that invasive species do not have significantly greater allelopathic effects compared to non-invasive plants which share the same key indicators of allelopathic potential, known invasive species were selected for this study due to their ability to become ecosystem engineers. Species which can successfully invade and establish themselves in new ecosystems have characteristics which make them attractive tools for the manipulation of eutrophic freshwaters. Firstly, invasive species have the potential to assume a dominant role as ecosystem engineers in their receptive environment, as established organisms in the introduced range will often be naïve to their competitive growth strategies (Hastings et al., 2006). This ability to act as ecosystem engineers can lead to the displacement of existing organisms from the system (Crooks, 2002). Additionally, invasive species are highly resilient, allowing them to survive and thrive in unfamiliar environments. These qualities are key attributes in the potential for harnessing invasive species to manipulate eutrophic freshwater systems. It is important to emphasise that we would not advocate the introduction of invasive plants to open water systems for water quality management, but rather propose that their potential allelopathic attributes could be embraced in already invaded systems or closed systems within an invaded range.

Our research had three main hypotheses. The first hypothesis was that both whole plants and crushed plants have a negative effect on the population growth of *Synechocystis* and *Chlorella*, as these plants were identified as having high allelopathic potential by Grutters et al. (2017). The second hypothesis was that crushed plants would have a greater effect on the population growth of *Synechocystis* and *Chlorella* compared to whole plants, as allelochemicals contained within the plant would be released into the environment without having to be naturally exudated. The final hypothesis was that the relationships between the plants and *Synechocystis* and *Chlorella*, may vary between phytoplankton monoculture and co-culture conditions, with green algae potentially promoting the population growth of cyanobacteria (Chang et al., 2012). The evaluation of these hypotheses will help inform how the potential allelopathic effect of these aquatic plants could be harnessed to favourably manipulate phytoplankton communities in freshwater systems.

5.2 Methods

5.2.1 Cultivating phytoplankton species

The cyanobacteria *Synechocystis* sp. PCC6803 (Strain 6803 Wild Type, Pasteur Culture Collection of Cyanobacteria, Paris, France) and green alga *Chlorella vulgaris* (Strain 211/11B Culture Collection of Algae and Protozoa, Oban, UK) were selected for this experiment. In order to produce sufficient quantities of stock culture these species were grown in their prescribed optimal growth media at stock concentrations prior to the experiment.

Chlorella was cultured in sterile 3N-BBM-V growth media (25g/L $NaNO_3$; 2.5g/L $CaCl_2.2H_2O$; 7.6g/L $MgSO_4.7H_2O$; 7.5g/L $K_2HPO_4.3.H_2O$; 17.5g/L KH_2PO_4; 2.5g/L NaCl) and trace element solution (Minerals were added to DI water in the following sequence: 97mg/L $FeCl_3.6H_2O$; 41mg/L $MnCl_2.4H_2O$; 5mg/L $ZnCl_2$; 2mg/L $CoCl_2.6H_2O$; 4mg/L $Na_2MoO_4.2H_2O$). Further details can be obtained at www.ccap.ac.uk. *Synechocystis* was grown in BG11 media (Sigma-Aldrich Cyanobacteria BG11 Freshwater Solution, Dorset, England).

5.2.2 Plant collection and processing

Hydrocotyle ranunculoides was collected from the River Cam at Fen Ditton, Cambridge, Cambridgeshire, UK (52°13'18.1"N 0°10'01.7"E). *Crassula helmsii* was collected from Bewl Water Reservoir, East Sussex, UK (51°04'22.5"N 0°23'38.6"E). One week prior to the experiment, plants were cleaned with dechlorinated water to remove any soil and debris. The plants were kept in dechlorinated water with saturated levels of nutrient solution (1.5 mg P/L as K2HPO4 and 12 mg N/L as NaNO3) (Vanderstukken et al., 2014) and 24 hour light.

The plants were prepared on 14 and 15 February 2018. Both *H. ranunculoides* and *C. helmsii* were tested for their allelopathic effects in a whole plant and crushed plant state, in both cases 40 g of plant wet weight was used. A dual blade food processor was used to prepare the crushed plants. The plant was loaded into the food processor and run on high setting for 3-5 minutes until the plant matter was sufficiently processed. In the case of whole plants, a sufficient number of stems and leaves were used in order to reach a wet weight of 40 g. For the control, *H. ranunculoides* and *C. helmsii* were replaced by 40 g of fake aquarium plant (Tall Aquarium Plant, Pistachio Pet Ltd, London, UK). Only whole state aquarium plant was used.

The whole, crushed and fake plants were placed in dialysis tubing with a molecular weight cut-off of 14,000 (Sigma-Aldrich Dialysis Tubing Cellulose Membrane D9402, Dorset, England). The tubing was cut into ninety sections of equal length and each section was tied closed at one end. After the addition of the relevant plant material to the tubing, the section of tubing was filled with nutrient saturated dechlorinated water and the open end tied closed. The use of dialysis tubing is an established methodology in testing for allelopathic interactions between aquatic plants and phytoplankton with the highest degree of realism (Gross et al., 2007). The membrane prevents direct contact between the plant and microalgae while still allowing the movement of nutrients and allelochemicals, and therefore any noted effects should be attributable to allelopathy (Körner and Nicklisch, 2002; Chang et al., 2012; Priyadarshani and Rath, 2012; Vanderstukken et al., 2014).

5.2.3 Experimental design

The experiment used a nested design with six replicates per treatment. Treatments monitored the population growth of *Chlorella* alone, *Synechocystis* alone, or *Chlorella* + *Synechocystis* in co-culture. Experiments were run in the presence and absence of intact and processed *H. ranunculoides;* intact and processed *C. helmsii*; and a control experiment was run in the presence of intact aquarium-safe plastic plants, such that a total of ninety experiments were undertaken.

Experiments were run in one litre capacity aquaria (129 mm x 133 mm x 133 mm). Each aquarium was filled to a total volume of 250 ml. The aquaria were first filled with the required volume of dechlorinated and nutrient saturated water (1.5 mg P/L as K2HPO4 and 12 mg N/L as NaNO3) (Vanderstukken et al., 2014); followed by the phytoplankton inoculum added from a stock culture using a syringe attached to a 4 mm diameter hose; and the allocated plant, inside the dialysis tubing, was added last.

A LED lighting unit (Mithril Technology Ltd., Surrey, UK) was fitted 20 cm above the aquaria, emitting light 24 hours per day at specific wavelengths (370 nm, 470 nm, 525 nm, 570 nm, 590 nm and 610 nm). The average Photosynthetically Active Radiation (PAR) level was 26μmol$\cdot$m$^{-2}\cdot$s^{-1}. The positioning of the aquaria was randomised to eliminate the effect of differential lighting on algal growth in different treatments. Each aquarium was supplied with constant air through a 4mm internal diameter silicone air line from a standard aquarium pump and permeated through an air stone. The temperature of the room was maintained between 18 ˚C and 20 ˚C.

A calibrated FluoroProbe (bbe Moldaenke GmbH, Schwentinental, Germany) was used to estimate the number of *Chlorella* and *Synechocystis* cells present in each sample. The bbe++ software package (bbe Moldaenke GmbH., 2013) was used to set the measurement parameters, operate the FluoroProbe and record the results of the experiments. The FluoroProbe uses spectral fluorescence to quantify phytoplankton biomass through selective excitation of accessory pigments which differ between the major taxonomic groups of phytoplankton (Catherine et al., 2012). The measurement of subsequent emissions of fluorescence by reaction centre chlorophyll ɑ (Chlɑ) allows the probe to estimate μgL^{-1} of Chlɑ which can be transformed into an estimate of phytoplankton cells/ml. The bbe++ software package applies conversion factors for calculating cell counts derived from group specific Chlɑ properties, these conversion factors are: 5.30×10^5 for green algae, and 1.0×10^6 for cyanobacteria (Hartmann et al., 2019). The FluoroProbe was mounted in the 'workstation 25 standard version' in which the FluoroProbe can analyse the contents of a 25 ml cuvette.

The measurements were carried out between the 15 and 26 of February 2018. Samples from each aquarium were taken at 12 intervals, once per day at approximately midday. After resuspending settled phytoplankton, a 25 ml sample was collected from each aquarium at each time point using a syringe then added to the FluoroProbe cuvette for measurement. After the measurement was recorded, the 25 ml sample was replaced in the aquarium to maintain an overall aquarium volume of 250 ml for the duration of the experiment. The cuvette was rinsed with deionised (DI) water and dried between each measurement. The aquaria were twice topped up, once per week, with additional saturating concentrations of nutrient stock solution, to maintain a nutrient saturated state.

5.2.4 Statistical analysis

To discern whether the growth of *Chlorella* or *Synechocystis* was affected by the type of plant present in the treatment (*H. ranunculoides, C. helmsii* or Fake), the state of the plant (crushed or whole) or co-culture conditions the following methodology was used. The average increase or decrease in the number of cells per hour for each of the 120 recorded timeseries (a single timeseries for each phytoplankton monoculture experiment, and two timeseries for each mixed phytoplankton experiment) was calculated by taking the value of the slope of a fitted linear model, which represents the average change in phytoplankton cell numbers per hour. Subsequently, a linear model for each treatment condition was fitted to the relevant slope values calculated from the timeseries, to examine how the average population growth can be predicted by the treatment variables (Figure C1). The linear models underwent a two-way Analysis of Variance (ANOVA) test and a post-hoc Tukey test, to discern the significance of the various treatment conditions on the average population growth of each species. Q-Q plots were assessed to confirm the data met the premisses for ANOVA. All analysis was performed in R Studio (v.1.0.136) (R Core Team, 2018).

5.3 Results

There was both a significant interaction between the effect of plant type on the population growth of *Chlorella* (F=4.0183, df=2,54, P=0.0236), and the effect of the state of the plant (F=12.4120, df=1,54, P=0.0008). As illustrated in Figure 5.1, and demonstrated through a post-hoc Tukey test, in both co-culture and single phytoplankton conditions the average population growth for *Chlorella* under whole *H. ranunculoides* treatments was significantly lower when compared to the fake plant control (P=0.0186). Additionally, whole *H. ranunculoides* had a significantly more negative effect on the average population growth of *Chlorella* than crushed *H. ranunculoides* (P=0.0255). The crushed form of *C. helmsii* had a greater negative effect on the population growth of *Chlorella* than crushed *H. ranunculoides*, while whole *C. helmsii* had no effect on the population growth of *Chlorella*. Finally, crushed *C. helmsii* had a significantly negative effect on the population growth of *Chlorella* when compared to the control (P=0.0183).

There was both a significant interaction between the effect of plant type on the average population growth of *Synechocystis* (F=7.6837, df=2,54, P=0.0012), and the effect of the treatment type i.e. whether *Synechocystis* was grown in a co-culture or in a monoculture (F=12.4120, df=1,54, P<0.0008). However, unlike *Chlorella*, *H. ranunculoides* had no significant effect on the population growth of *Synechocystis*. As illustrated in Figure 5.1, and demonstrated through a post-hoc Tukey test, the average population growth of *Synechocystis* was lower in treatments containing crushed *C. helmsii* when compared to treatments containing crushed *H. ranunculoides* (P=0.0029), however the population growth did not differ significantly from the fake plant control. A large influence on the population growth of *Synechocystis* was the presence of *Chlorella*, the average population growth of *Synechocystis* was significantly elevated across all treatments when grown in a co-culture (P<0.0001).

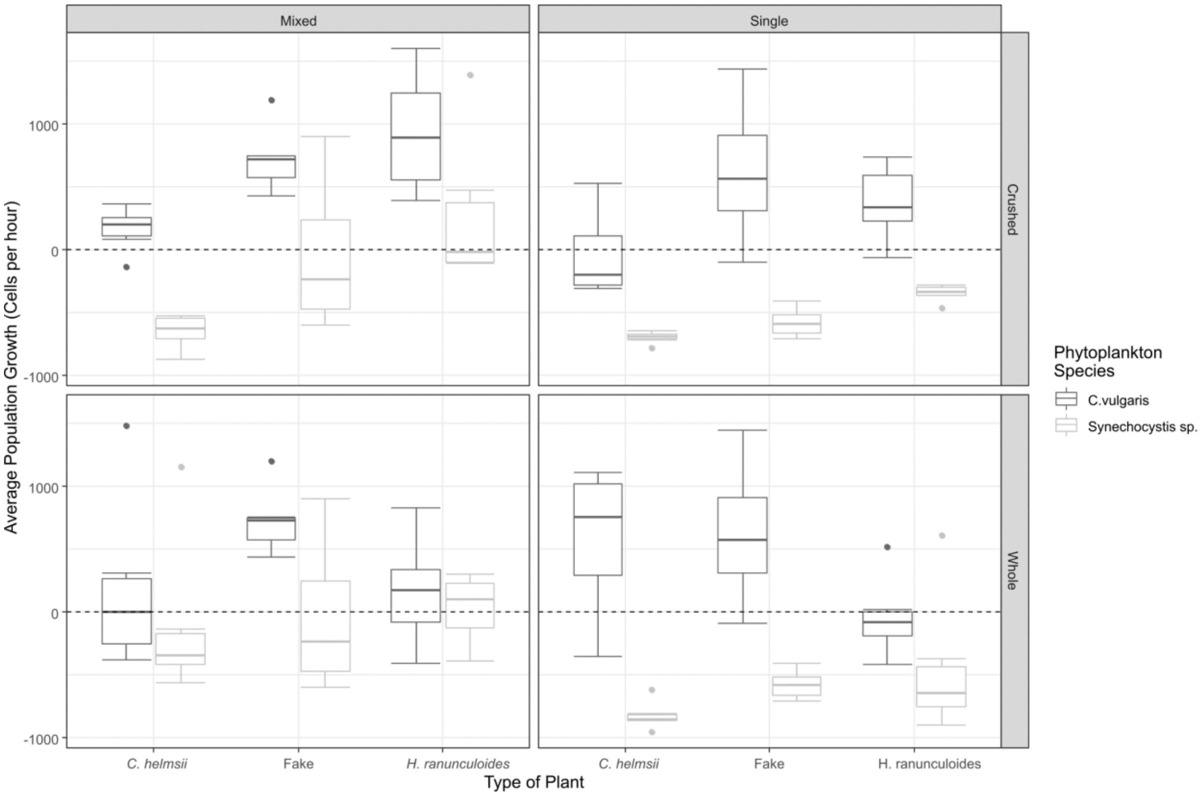


Figure 5.1 The average population growth (cells per hour) of phytoplankton under varying conditions. The boxplots indicate the average population growth of both *Chlorella* (Dark Grey) and *Synechocystis* (Light Grey) under each set of conditions. The dotted line across the centre of each graph represents an average population growth of 0. The mean value is represented by the dark central line, the upper and lower bounds of the box represent the 75% and 25% percentile respectively. The whiskers represent the highest and lowest value observed within 1.5 times the inner quartile range. The circles represent datapoints that lie beyond 1.5 times the inner quartile range. Mixed and Single refer to co-culture and monoculture phytoplankton conditions, respectively. Crushed and Whole refer to the state of the plant.

5.4 Discussion

The results present a mixed picture; whole *H. ranunculoides* supressed only the growth rate of *C. vulgaris*, while crushed *H. ranunculoides* had no effect on either phytoplankton species. Conversely, whole *C. helmsii* had no effect on either phytoplankton species, whereas crushed *C. helmsii* supressed the growth rate of both phytoplankton species. Additionally, the presence of *Chlorella* in co-culture experiments had a universally positive effect on *Synechocystis* population growth (Figure 5.2).

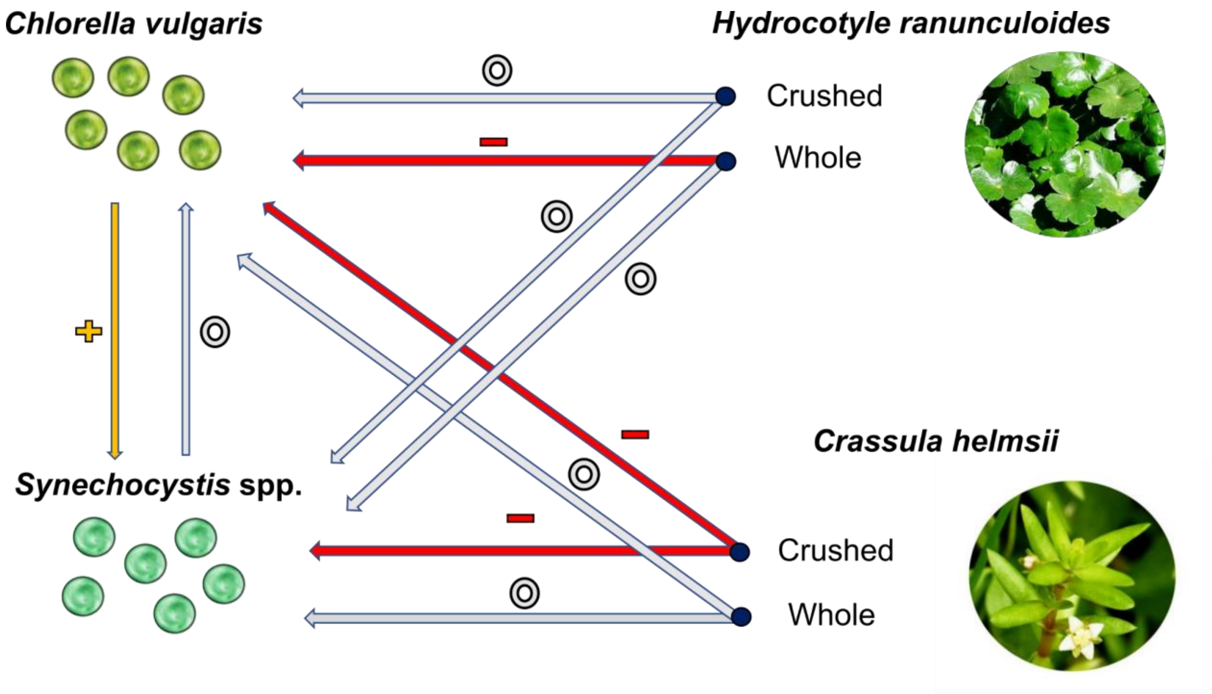


Figure 5.2 A simplified diagram of the interactions between the different plant species, plant states and phytoplankton species. The arrows indicate interactions between the plants and phytoplankton. All interactions relate to effects on the average growth rate for the indicated phytoplankton species. The red arrows accompanied by the (-) symbol indicate negative interactions; the grey arrows accompanied by the (o) symbol indicate neutral interactions; and the yellow arrows accompanied by the (+) symbol indicate positive interactions. The bold text refers to the species names, whereas the bullet points indicate the state of the plant species.

5.4.1 The effect of plant species on phytoplankton population growth

The first hypothesis proposed by our study was that plants, regardless of their state, will have a negative effect on the average population growth of each phytoplankton species. This hypothesis can be rejected based on the results of our study. Both *H. ranunculoides* and *C. helmsii* were identified by Grutters et al., (2017) as meeting the criteria for high allelopathic potential, based upon their inhibition of the growth of the cyanobacterium *Dolichospermum flos-aquae* following methanol extraction. In our study, however, the allelopathic effect of both plant species varied in their effects. With *H. ranunculoides* only affecting the average growth of *Chlorella* negatively when in its whole state and having no effect on *Synechocystis* average growth. While *C. helmsii* had a negative effect on the average growth of both *Cholrella* and *Synechocystis* when in a crushed state. This highlights how species-specific effects might be important in determining the effect of allelopathic agents on different algal communities (Jasser, 1995; Körner and Nicklisch, 2002; Gross et al., 2003; Mulderij et al., 2005). In addition, whereas most studies show that the allelopathic effects of aquatic plants are inhibitory, there have been studies which revealed that certain macrophytes are ineffective or even stimulatory to certain phytoplankton (Hilt and Gross, 2008). For example, van Aller et al. (1985) observed that *Eleocharis microcarpa* could inhibit the growth of *Anabaena flos-aquae.* Whereas *Oscillatoria tenuis*, had a stimulatory effect on *Euglena gracilis* (Van Aller et al., 1985). While *Ceratophyllum demersum* has been shown to stimulate the growth of the green algae *Chlorella* and *Scenedesmus* (Kogan, 1972), it inhibits the growth of the cyanobacteria *Anabaena* (Van Vierssen and Prins, 1985).

5.4.2 The effect of plant state on phytoplankton population growth

The second hypothesis posed by our study was that crushed plants would have a greater effect on the average population growth of phytoplankton than whole, intact, plants. This hypothesis can also be rejected based on the results of our study. The design of our study allowed us to test whether the allelopathic potential of the plants persisted when the plant was in a whole or crushed state. The maintenance of saturating levels of nutrients was implemented to offset any positive effects on phytoplankton growth that may be brought about by nutrient release from crushed plants. In the experiment, the dialysis tubing acted to prevent the movement of bacteria and cell debris from coming into contact with the phytoplankton, while allowing potential allelochemicals to diffuse through the dialysis membrane. Therefore, if the plant contained water soluble allelopathic substances, the allelochemical will have been able to cross the membrane. It was hypothesised that the crushed plant experiments should contain a higher available concentration of the allelopathic substances due to the mechanical disruption of the plant cell walls and membranes.

The results of our study were mixed. The average population growth of *Synechocystis* was only affected by *C. helmsii* in its crushed form, and unaffected by *H. ranunculoides*, whereas the average population growth of *Chlorella* was only affected by the whole form of *H. ranunculoides* and the crushed form of *C. helmsii.* There are several potential explanations for the differing effects between the states of the plants on phytoplankton population growth. Firstly, it has been shown that certain environmental factors may influence the production and release of allelopathic compounds. For example, phosphorus and nitrogen limitation has been found to prompt the production and release of allelochemicals in certain macrophytes, such as *Myriophyllum spicatum* (Gross et al., 2003). The maintenance of a state of nutrient saturation employed in this experiment, while eliminating nutrient competition as a potential confounding factor, is likely to have prevented the plants from experiencing nutrient limitation and therefore may have resulted in the plants limiting the production of allelopathic compounds. Light has also been shown to affect allelochemical production (Cronin and Lodge, 2003), with sun exposed shoots of *M. spicatum* and *Myriophyllum verticilliatum*, containing higher levels of phenolic compounds than shade adapted plants (Choi et al., 2002). The light levels used in this experiment may have encouraged the whole plants to produce and release allelochemicals, whereas the crushed plants would have not been able to produce further allelochemicals.

Bacteria have also been shown to affect the efficacy of allelochemicals. Müller et al. (2007) demonstrated that certain epiphytic bacteria isolated from *M. spicatum* displayed polyphenol-degrading activity, which prevented its ability to inhibit cyanobacterial growth. While, in contrast, it has also been shown that photolytically and microbially degraded tannic acid from *M. verticilliatum* had an increased allelopathic effect, indicating that in some cases the degraded by-products of allelochemicals could be more harmful than the allelochemical itself (Bauer et al., 2012). In addition to these local effects, the season has also been shown to influence the allelopathic activity of certain macrophytes (Hilt, 2006). Therefore, environmental factors such as the light regime and seasonality of the plant material used in this experiment may have implications for the observed allelopathic effects. The potential impact of environmental factors may further indicate that experiments focused on isolated plant extracts and exudates to identify potentially allelopathic compounds, may overlook many important interactions which play a role in defining the allelopathic relationship between aquatic plants and phytoplankton in natural communities.

5.4.3 The effect of co-culture on cyanobacteria

The final objective of this study was to evaluate whether the allelopathic effect altered when the phytoplankton were grown in monoculture or co-culture. In this experiment co-culture had no significant effect on the average population growth of *Chlorella*, however it did have a significantly positive effect on the average population growth of *Synechocystis*. In all experiments, regardless of the plant type or state, *Synechocystis* had an elevated average population growth when grown in co-culture with *Chlorella*. This result may imply a commensal relationship, where *Synechocystis* receives a benefit from the presence of *Chlorella* without affecting *Chlorella's* growth, however, further work would be required to confirm this. There is a significant amount of information in the literature detailing symbiosis between algae and bacteria, as many micro-algae are reliant on exogenous supplies of vitamins to grow, such as cobalamin, thiamine and biotin (Kazamia et al., 2012b; Xie et al., 2013; Grant et al., 2014). However, there is a lack of literature detailing symbioses between green algae and cyanobacteria. The results of this study align with the findings of Chang, Eigemann and Hilt, 2012, which suggests this area may warrant further investigation, if cyanobacteria can derive a benefit from the presence of green algae there may be fundamental implications for the understanding of phytoplankton community and ecosystem dynamics.

5.4.4 Implications for allelopathy in ecological engineering of phytoplankton communities in freshwater systems

This study demonstrates that there may be opportunities to harness aquatic plants to manipulate algal communities towards a desired end point. Although much of the literature indicates that the allelopathic effects of aquatic plants on phytoplankton are of an equally inhibitory nature, this study is one of many emerging studies which indicate that some plants can simultaneously elicit no significant response from one species of phytoplankton, while inhibiting the growth of another (Mulderij et al., 2007; Mohamed and Al Shehri, 2010; Wang et al., 2013). Therefore, the harnessing of the allelopathic capacity of certain aquatic plants could not be used generically. To be truly effective, the plants used should be carefully selected based on their ability to supress the specific species of harmful algae present in the target waterbody. For example, this study would suggest that the presence of *H. ranunculoides,* may act to supress *Chlorella*, while *Synechocystis* is unaffected. Furthermore, the results of this study suggest that allelochemicals may be used to affect desirable outcomes through indirect pathways. For example, using allelopathy to supress *Chlorella* could have the benefit of reducing growth in harmful cyanobacteria such as *Synechocystis* which appears to benefit from the presence of *Chlorella*. This study may have implications for the management of invasive species in areas where cyanobacterial blooms are also of significant risk. For example, it is possible that breaking down *C. helmsii* either through mechanical disruption or grazing pressure may help to supress the growth of cyanobacteria.

Of course, as an invasive species, the risks and benefits of introducing or encouraging the proliferation of invasive aquatic plants, in either new or established systems, need to be carefully assessed on a site-by-site basis for any proposed application of allelopathy to be used responsibly and successfully. The risk profile varies vastly when comparing the use of invasive plants in a closed system within an invaded range, to an open system with links to surrounding waterways, in an uninvaded range. We would never recommend introducing invasive species into open water systems in an uninvaded range, however, there may be opportunities to leverage allelopathic effects within already invaded systems or closed systems within an invaded range. Any decision to encourage invasive plants should only be taken following discussion and appraisal with the relevant authorities at the regional, national or international level, depending on the perceived risks (McLaughlan and Aldridge, 2013).

Although inferring the ecological relevance of allelopathic effects observed in mesocosm experiments can be difficult, the methods employed in this study, through the use of dialysis tubing, allows for testing with the highest degree of realism (Gross et al., 2007). While mesocosms allow for control of nutrient competition and light availability, which aids the identification of allelopathic effects, in some natural systems these factors may overshadow the impact of a plant’s allelopathic properties (Hilt and Gross, 2008). To build on the findings of this study, further research should explore how allelopathic interactions may change across a range of plant biomass to phytoplankton ratios, in order to identify at which plant densities allelopathic impacts may be greatest or become insignificant. It is clear, however, that allelopathy offers considerable potential as part of a wider biomanipulation approach. Research into biological controls for manipulating and managing toxic or nuisance algae is of increasing importance, as biological solutions require minimal management and are free of the cost and supply chain issues associated with chemical or mechanical solutions.

Chapter 6

Harnessing Synthetic Ecology for commercial algae production

Abstract

Synthetic Ecology is a novel concept describing the design of *de novo* ecological communities for a designated purpose. This study is a proof of concept for harnessing Synthetic Ecology in expanding the scale of commercially relevant micro algae (*Chlorella vulgaris*) cultivation using stable Synthetic Ecologies in open environments as opposed to vulnerable monocultures. We focused on whether the grazing activity of zebra mussels (*Dreissena polymorpha*) would result in a consistent, and commercially favourable, dominance of *Chlorella* in cultures that were also inoculated with a competing and potentially invasive cyanobacteria (*Synechocystis sp. PCC6803*). The key result of this study was that in axenic mixed species co-cultures, zebra mussels had a significantly greater negative effect on *Synechocystis* cell numbers than *Chlorella* ($P<0.0001$). The zebra mussels' putative preference for *Synechocystis* over *Chlorella* suggests they could be used to maintain the dominance of *Chlorella* in outdoor cultivation systems prone to contamination by invasive cyanobacteria.

6.1 Introduction

The goal of synthetic biology is to extend or modify the behaviour of organisms and engineer them to perform new tasks (Andrianantoandro et al., 2006). Simplistically, this is achieved by deconstructing the biology of an organism to a hierarchy of its constituent parts and understanding how the context and interactions between these distinct parts dictates the organism's phenotype and behaviour. The organism can then be reprogrammed genetically to achieve a desired characteristic or behaviour. Synthetic Ecology aims to apply the same principles to the behaviour and design of artificial ecosystems that could be used to enhance biotechnological processes (Kazamia et al., 2012a). Through characterising the hierarchy of ecological interactions in a desired model system, a *de novo* system could theoretically be created through the artificial combination of nutrients and deliberately designed synthetic communities of organisms. This technique could be used to design systems which provide beneficial ecosystem services while behaving as self-regulating climax communities resilient to biological invasion.

The aim of our study was to investigate a proof of concept for the emerging field of Synthetic Ecology, studying the ability of filter feeding zebra mussels (*Dreissena polymorpha*) to drive and stabilise a shift in a *de novo* algal community to enable the proliferation of an algal species of commercial interest (*Chlorella vulgaris*). An alternative to popular high-cost small-scale closed production systems is growing algae in open raceways, which have lower associated construction costs and environmental burdens in terms of energy consumption (Stephenson et al., 2010). However, the current drawback to algae production in open raceways is maintaining the optimum conditions for algae growth, while limiting contamination from invasive organisms which introduce competition and reduce the yields of the target cultivated algae species.

The zebra mussel is a prolific invasive bivalve, originating from the Black, Caspian and Azov Sea regions of central and eastern Europe. Several studies have focused on assessing the impact of the zebra mussel in its invaded range on existing algal communities (Berg et al., 1996; Smith et al., 1998; Dzialowski and Jessie, 2009; Kirsch and Dzialowski, 2012). A single zebra mussel filters approximately two litres of water per day (Elliott et al., 2008) and significantly reduces the total biovolume of cyanobacteria in reservoir mesocosm experiments (Kirsch and Dzialowski, 2012). Additionally, Smith et al., (1998) measured a shift in dominance from cyanobacterial to diatom species when analysing changes in the cell density and taxonomic composition of phytoplankton assemblages in the freshwater portion of the Hudson River before and after the successful invasion of zebra mussels (Smith et al., 1998).

Increases in nutrients, especially phosphorus (P), will stimulate algal production (Elser et al., 2007) and algal biomass is tightly linked to phosphorus in lakes worldwide (Dillon and Rigler, 1974). However, Zebra mussels have been shown to negate or mask any positive growth effects of nutrient enrichment on algal biomass up to 150 µg of phosphorus per litre (Dzialowski and Jessie, 2009). It has also been suggested that the effect of zebra mussels on the cyanobacteria *Microcystis spp.* is nutrient dependant (Raikow et al., 2004; Sarnelle et al., 2005; Knoll et al., 2008). Raikow et al. (2004) noted a positive relationship between zebra mussels and *Microcystis* spp. in lakes with low Total P concentrations (<25 µg/L), but no relationship between zebra mussels and *Microcystis* spp. in lakes with high Total P concentrations (>25 µg/L).

This study aimed to distinguish whether zebra mussels under certain nutrient conditions can be utilised to drive a favourable shift in a *de novo* algal co-culture by suppressing the growth of a potentially invasive cyanobacterial species (*Synechocystis sp. PCC6803*) and so supporting the proliferation of a commercially valuable green algae species (*Chlorella vulgaris*). *Chlorella* is a widely cultivated microalgae species and has application as a health food and food supplement, alternative agricultural feedstock, production of biofuels, as well as having many uses in the pharmaceutical and cosmetics industry (Priyadarshani and Rath, 2012; Safi et al., 2014). By demonstrating the ability to achieve desirable end points in this model system this would open the opportunity for developing Synthetic Ecologies in more complex communities and for other applications. Therefore, the main aims of this study were twofold: (1) investigate the effect of zebra mussels on a simple axenic algal community structure and (2) to identify whether the relationship between zebra mussels and cyanobacteria is dependent on the levels of Total P.

6.2 Methods

6.2.1 Cultivating algae species

The cyanobacteria *Synechocystis sp.* PCC6803 (hereafter *Synechocystis*) (Strain 6803 Wild Type, Culture Collection of Algae and Protozoa, Oban, UK) and green alga *Chlorella vulgaris* (hereafter *Chlorella*) (Strain 211/11B Culture Collection of Algae and Protozoa, Oban, UK) were selected for this experiment. To maximise the growth rate of these algae species they were grown in their prescribed optimal growth media at stock concentrations prior to the experiment.

Chlorella was cultured in sterile 3N-BBM-V growth media (25g/L $NaNO_3$; 2.5g/L $CaCl_2.2H_2O$; 7.6g/L $MgSO_4.7H_2O$; 7.5g/L $K_2HPO_4.3.H_2O$; 17.5g/L KH_2PO_4; 2.5g/L NaCl) and trace element solution (The following minerals were added to 1L of DI water in the following sequence: 97mg $FeCl_3.6H_2O$; 41mg $MnCl_2.4H_2O$; 5mg $ZnCl_2$; 2mg $CoCl_2.6H_2O$; 4mg $Na_2MoO_4.2H_2O$). Details at (www.ccap.ac.uk). *Synechocystis* was grown in BG11 media (Sigma-Aldrich Cyanobacteria BG11 Freshwater Solution, Dorset, England).

6.2.2 Zebra mussels and water collection

Zebra mussels were collected from Roswell Pits Site of Special Scientific Interest, Ely, SE England (52.4013° N, 0.2852° E). This lake is a disused, flooded gravel pit disconnected from the adjacent gravel pits and the nearby river Great Ouse. The average length of the mussels used in the experiment was 32 mm ± 5 SE. Twenty-five litres of reservoir water was also collected from Roswell Pits. The water was filtered through a polyethersulfone filter vacuum with a 0.22 µm membrane to remove any algae or bacteria in the sample prior to use in the experiment.

6.2.3 Experimental design

Experiments were run in one litre capacity aquaria (129 mm x 133 mm x133 mm) inside a low light laboratory. Each aquarium was filled to a total volume of 250 ml, which included filtered reservoir water and the algae inoculum. The aquaria were first filled with the allocated volume of water (Table 3). The required volume of algae culture was added to the aquaria using a syringe attached to a 4mm hose. The algae inoculum was removed directly from the stock culture, during its growth phase, prior to being introduced to the aquaria. A LED lighting unit (Mithril Technology Ltd., Surrey, UK) was fitted 20 cm above the aquaria, emitting light 24 hours per day at specific wavelengths (370 nm, 470 nm, 525 nm, 570 nm, 590 nm and 610 nm) to optimise conditions for photosynthesis. The positioning of the aquaria was randomised to eliminate the effect of differential lighting on algal growth in different treatments. The average Photosynthetically Active Radiation (PAR) level was 26μmol$\cdot$m$^{-2}\cdot$s^{-1}. Each aquarium had a constant air supply supplied through a 4mm internal diameter silicone air line from a standard aquarium pump and permeated through an air stone. The temperature of the room was maintained between 18^{o}C and 20^{o}C.

Experiments used a nested design with six replicates per treatment. Treatments monitored the growth of *Chlorella* alone, *Synechocystis* alone, or *Chlorella* + *Synechocystis* in co-culture. Experiments were run in the presence and absence of mussels, and in low and high phosphorus conditions, such that a total of 72 experimental were undertaken (Table 6.3).

Mussel treatments each used two zebra mussels, which increases the chances of a constant grazing pressure on the algae. Zebra mussels of the size used in this experiment (35 mm ± 5SE) have been shown to have clearance rates of 247 ml$\cdot$mussel$^{-1}\cdot$hour^{-1} (Kryger and Riisgård, 1988) and even as high as 574 ml$\cdot$mussel$^{-1}\cdot$hour^{-1} (Elliott et al., 2008). Therefore, a stocking density of two mussels should result in the equivalent total volume of the experiment being filtered up to twice every hour, allowing for a shorter experimental run time. The mussels expressed a constant feeding pattern following the algae inoculation, with at least one of the mussels feeding at any one time.

Low phosphorus treatments used water from the collection site without enrichment. High phosphorus treatments were enriched with a phosphate stock solution containing 870µg/L K_2HPO_4 and 680µg/L KH_2PO_4.The concentration of orthophosphate (PO_4-P) in high P and low P treatments was calculated using a phosphate cuvette test (Hach Lange LCK349 Phosphate (Ortho/Total) cuvette test 0.05-1.5 mg/L PO4-P). The test indicated the low P treatments contained <3µg PO_4-P/L, whereas the high P treatments contained 412µg PO_4-P/L.

A calibrated FluoroProbe (bbe Moldaenke GmbH, Schwentinental, Germany) was used to estimate the number of *Chlorella* and *Synechocystis* cells present in each sample (cells/ml). The FluoroProbe discriminated between *Chlorella* and *Synechocystis* by measuring the presence of photosynthetic accessory pigments, which result in identifiable ‘fluorescence fingerprints’. *Chlorella* is rich in chlorophyll *a* and *b*, while *Synechocystis* contains phycocyanin. The FluoroProbe measured the fluorescence emitted by the sample following excitation of these different photosynthetic accessory pigments using six LEDs emitting at different wavelengths (370 nm, 470 nm, 525 nm, 570 nm, 590 nm and 610 nm) (Catherine et al., 2012). The FluoroProbe was mounted in the 'workstation 25 standard version' in which it can analyse the contents of a 25ml cuvette. The bbe++ software package (bbe Moldaenke GmbH., 2013) was used to set the measurement parameters, operate the FluoroProbe and log the results of the experiments.

The measurements were carried out between the 27 and 30 March 2018. Samples from each aquarium were taken at 10 intervals over 84 hours. A 25ml sample was collected from each aquarium, after resuspending settled algae, at each time point using a syringe then added to the FluoroProbe cuvette for measurement. After the measurement was taken the sample was replaced in the aquarium. The cuvette was rinsed with DI water and dried between each measurement.

6.2.4 Statistical analysis

Two generalised linear mixed models (GLMM), with a Poisson log link and error distribution, were used to identify relationships between the number of algae cells/ml and the set variations in conditions. The fixed effects for assessing changes in cells/ml for *Chlorella* and *Synechocystis* across all treatments were P level (high or low), mussels (presence or absence), treatment type (Single algae culture or mixed culture) and the interaction between the mussels and treatment type. The random effects were the number of hours and the individual experiments. This accounts for the modelling of potentially differing intercept values and curves between experiments. Analysis was performed in R Studio (v.1.0.136) (R Core Team, 2018) with the package lme4 (v1.1-12) (Bates et al., 2015).

6.3 Results

The presence of mussels resulted in a significant decline in the number of cells for both species in all treatment conditions when compared to treatments in the absence of mussels (*Chlorella* (Z Value -7.8, P<0.0001); *Synechocystis* (Z Value -7.1, P<0.0001)) (Table 6.1; Table 6.2; Figure 6.1).

In mixed species treatments, *Synechocystis* grew more successfully than when grown in monoculture (Z Value 4.5, P<0.0001). The growth of *Chlorella* in mixed species co-culture and in monoculture experimental conditions was similar (Table 6.1). The initial lag phase of *Chlorella* appears to be slightly elongated when grown in a mixed species co-culture treatment, but the stationary phase achieved similar total cell numbers (Figure 6.1). Although the presence of mussels did have a significant effect on *Chlorella* cell numbers, the effect was small in comparison to effect of mussels on the number of *Synechocystis* cells. The growth of *Synechocystis* was negatively affected to a much greater extent when grown in the presence of mussels, both in single species treatments and mixed species treatments (Z value -2.6, P<0.01). The initial level of P did not have a significant measurable effect on the number of cells for either species in any of the experimental treatments (Table 6.1; Table 6.2).

Table 6.1 GLMM results for Chlorella cell numbers. Random effect variance and standard deviation with fixed effect coefficient estimates, standard errors and significance tests for a GLMM (with a Poisson error distribution) of the number of *Chlorella* cells per ml as a function of phosphate (P) level, the presence or absence of mussels, mixed or single species growth treatments, and as a combination of mixed growth treatment and mussel presence.

Random Effects

Groups	*Name*	*Variance*	*Std. Dev.*
Treatment Number	(Intercept)	0.0181	0.1347
No Hours	(Intercept)	0.2758	0.5252
Number of obs: 480. Groups: Treatment.Number, 48; No.Hours, 10			

Fixed Effects

	Estimate	*Std. Error*	*Z value*	*Pr(>|z|)*
(Intercept)	12.2144	0.1738	70.2870	< 0.0001 ***
P: High P	-0.0333	0.0389	-0.8570	0.3920
Mussel: Mussel	-0.4297	0.0550	-7.8110	<0.0001 ***
Treatment.Type: Mixed	0.0050	0.0551	0.0910	0.9280
Mussel:Mussel*Treatment.Type:Mixed	0.0067	0.0778	0.0860	0.9310

Table 6.2 GLMM results for *Synechocystis* cell numbers. Random effect variance and standard deviation with fixed effect coefficient estimates, standard errors and significance tests for a GLMM (with a Poisson error distribution) of the number of *Synechocystis* cells per ml as a function of phosphate (P) level, the presence or absence of mussels, mixed or single species growth treatments, and as a combination of mixed growth treatment and mussel presence.

Random Effects

Groups	*Name*	*Variance*	*Std.Dev.*
Treatment Number	(Intercept)	0.0500	0.2236
No. Hours	(Intercept)	0.1287	0.3587
Number of obs: 480. Groups: Treatment.Number, 48; No.Hours, 10			

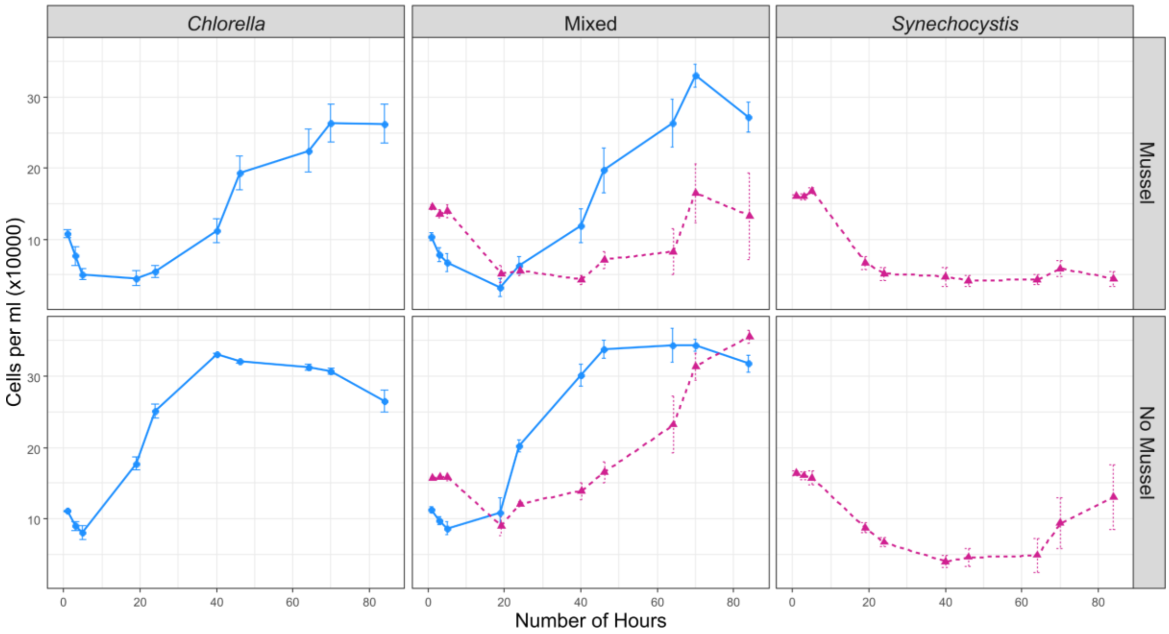
Fixed Effects

	Estimate	*Std. Error*	*Z value*	*Pr(>|z|)*
(Intercept)	11.6367	0.1352	86.0670	< 0.0001 ***
P: High P	-0.0642	0.0646	-0.9930	0.3206
Mussel: Mussel	-0.3078	0.0916	-3.3600	0.0008 ***
Treatment.Type: Mixed	0.4128	0.0916	4.5070	<0.0001 ***
Mussel:Mussel*Treatment.Type:Mixed	-0.3304	0.1292	-2.5570	0.0106 *

Table 6.3 Summary of nested experimental design. Each treatment had six replicates.

Treatment Number	*Reservoir Water (ml)*	*Chlorella Stock (ml)*	*Synechocystis Stock (ml)*	*Number of Mussels*	*Phosphate Stock (ml)*
1	225	25	0	2	0
2	225	25	0	0	0
3	225	0	25	2	0
4	225	0	25	0	0
5	200	25	25	2	0
6	200	25	25	0	0
7	225	25	0	2	2.5
8	225	25	0	0	2.5
9	225	0	25	2	2.5
10	225	0	25	0	2.5
11	200	25	25	2	2.5
12	200	25	25	0	2.5

(a) High P Conditions



(b) Low P Conditions

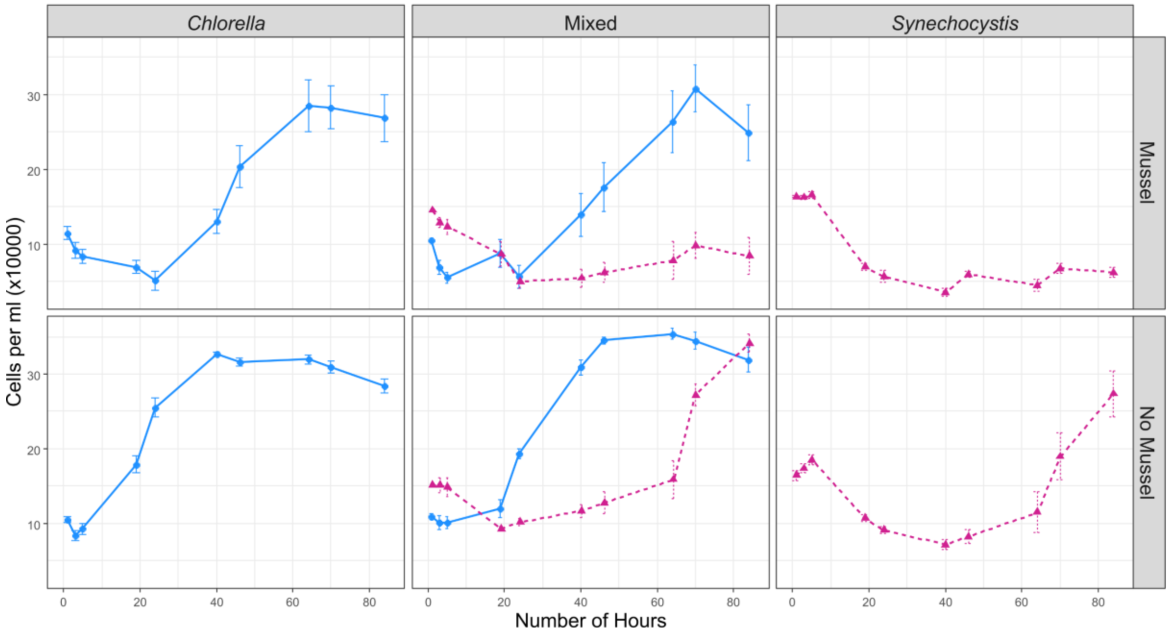


Figure 6.1 Changes in cell numbers over time across all treatment conditions. (a) Changes in the cell numbers over time of *Chlorella* and *Synechocystis* in single algae and mixed species treatments, in both the presence and absence of mussels, in high P conditions. (b) Same condition variations as panel (a), but in Low P conditions. Solid lines and circular points indicate *Chlorella* cell numbers. Dotted lines and triangular points indicate *Synechocystis* cell numbers. The error bars indicate standard error.

6.4 Discussion

The key findings of the study are that both *Chlorella* and *Synechocystis* cell numbers were significantly reduced when zebra mussels were present; the presence of mussels in mixed species treatments caused the number of *Synechocystis* cells to drop more than the number of *Chlorella* cells; *Chlorella* cell number was not affected by the presence of *Synechocystis*; *Synechocystis* reached greater cell numbers when grown in a mixed species culture with *Chlorella* than when grown in monoculture; and the initial levels of P had no effect on the cell numbers of either *Chlorella* or *Synechocystis* in the presence or absence of mussels.

6.4.1 Effect of mussels on cell numbers

The decline of algae and cyanobacteria cell numbers in the presence of zebra mussels is likely due to the filter feeding activity of the mussels. Despite early declines, which are likely due to the algae acclimatising to being removed the stock culture and optimal nutrient conditions, *Chlorella* still reached high densities of 300,000 cells/ml. Zebra mussels have been observed to reduce their filtering activity as food concentration declines, potentially as an energy saving method in response to a reduced food reward to energy expended trade-off (Horgan and Mills, 1997). Reduced feeding could also result from satiation of the mussels (Horgan and Mills, 1997). Released from grazing pressure and given the optimum conditions for photosynthesis, it may be that the *Chlorella* was able to recover and ultimately divide faster than the zebra mussels were grazing. It has been shown that zebra mussels exhibit feeding rates which are proportional to the amount of algae available, except in extremely high algal concentrations such as those present in this experiment and in algae cultivation systems (Kemp and Aldridge, 2018).

Additionally, zebra mussels were shown to affect the cell numbers of *Synechocystis* to a greater extent than *Chlorella,* which suggests that zebra mussels may express a feeding preference for *Synechocystis* over *Chlorella.* Naddafi et al., (2007) illustrated selective feeding by zebra mussels in lake water samples, finding they avoided green algae in preference for cryptophytes, and also that zebra mussels alter their feeding behaviour in relation to algae composition to ingest the phytoplankton with the highest concentrations of long-chain polyunsaturated fatty acids (Naddafi et al., 2007).

This putative preference is interesting as both *Synechocystis* and *Chlorella* are unicellular in morphology and of similar size. At the start of the experiment the average cell size for *Chlorella* was 2.55µm (s.d±0.33) and the average cell size of *Synechocystis sp.* was 2.64µm (s.d±0.38).

Mussels of all sizes, and therefore ages, should be able to graze on these phytoplankton, given their size. Horgan and Mills (1997) showed that phytoplankton morphology did not hamper ingestion rates, with zebra mussels able to ingest unicellular, filaments and globular colonies indiscriminately(Horgan and Mills, 1997). Horgan and Mills (1997) also showed that most particles smaller than the incurrent siphon are subject to grazing (Horgan and Mills, 1997). Given that the siphon diameter of a 10mm mussel, which is significantly smaller than the individuals used in this experiment (35 mm ± 5SE), has a diameter of 2100µm (Wong and Levinton, 2007), both juvenile and adult mussels would have the ability to graze on these phytoplankton.

There is emerging evidence to suggest that *Chlorella* (Fisher et al., 2016) and some cyanobacterial species (Yang and Kong, 2012; Xiao et al., 2018) will form colonies when under grazing pressure from zooplankton, but even under these circumstances the overall size of the observed colonies (18 cells and 180 µm respectively), remain well within the size ranges of particles drawn through the inhalant siphon of a zebra mussel (<2100 µm(Wong and Levinton, 2007)) and either digested or deposited as pseudofaeces. Additionally, these colony forming behaviours have not been documented in the presence of mussels. With regards to any potential predator initiated phenotypic plasticity of both *Chlorella* and *Synechocystis,* any changes in individual cell size or colony formation of either phytoplankton species would likely remain within the grazing capacity of zebra mussels, it is unlikely that any changes in this respect would have altered the outcome of the experiment.

6.4.2 Effect of Chlorella on Synechocystis growth

In the mixed species treatments conducted in both the presence and absence of mussels, *Synechocystis* cell numbers exceeded those observed in monoculture, whereas *Chlorella* achieved similar cell numbers when in a mixed species culture or monoculture. In the absence of mussels *Synechocystis* cell numbers were very similar to *Chlorella* in mixed species treatments by the end of the experiment. Additionally, increases in *Synechocystis* cell number are preceded by increases in *Chlorella*. This may suggest a commensal relationship between *Chlorella* and *Synechocystis* in which *Synechocystis* derives a benefit from *Chlorella* without affecting *Chlorella's* growth. The literature on the symbioses observed in algae-bacteria co-cultures is extensive, as many micro-algae rely on exogenous sources of cobalamin (Vitamin B_{12}), thiamine (Vitamin B_1) and or biotin (Vitamin B_7) to grow (Kazamia et al., 2012b; Xie et al., 2013; Grant et al., 2014). However, there is a dearth of literature studying symbioses between green algae and cyanobacteria. The suggestion from our study that cyanobacteria may derive nutritional benefits from sympatric algae has considerable implications for understanding ecosystem dynamics in freshwater systems and warrants further investigation.

A further compelling potential explanation for the enhanced cyanobacterial growth in co-cultures lacking mussels is Transgressive Overyielding (TO) (Cardinale et al., 2007). TO is thought to occur when the biomass production of a species community exceeds that produced by either species grown in monoculture, however examples of this are elusive (Cardinale et al., 2007). This theory is an extension from niche theory, which argues that species must use resources in a spatiotemporally complementary manner to ensure co-existence. It may be the case that *Chlorella-Synechocystis* co-cultures more efficiently and extensively captured and utilised the available nutrients in the aquaria than *Synechocystis* in monoculture, however because total levels of P and N were not continuously measured, a definitive conclusion on the occurrence of TO cannot be drawn. Additionally, although complementarity may be observed here in allowing *Synechocystis* to contribute to the total biomass in a way that is potentially enhanced by the presence of *Chlorella*, is has been shown TO requires a greater degree of complementarity than simply allows two species to co-exist (Loreau, 2004).

6.4.3 Effect of high P and low P treatments on algae growth

In contrast to the body of research which demonstrates the ability of zebra mussels to mask the effect of P-enrichment on algal biomass (Raikow et al., 2004; Sarnelle et al., 2005; Knoll et al., 2008; Dzialowski and Jessie, 2009), this experiment found no significant difference between algal or cyanobacterial biomass grown under different P concentrations, regardless of the presence of mussels (Table 6.1; Table 6.2). However, at least for *Chlorella*, this finding aligns with research undertaken by Wong et al. (2017) which looked to understand the effect on N and P ratios on biomass and lipid production (Wong et al., 2017). Wong et al., (2017) observed the highest *Chlorella* biomass production in BBM - $N_{control}P_{limted}$ growth media conditions and noted that P did not exhibit a significant effect on biomass production and that P starvation could result in higher amounts of total lipids and positively affect the lipid composition of *Chlorella* cultures (Wong et al., 2017). This may suggest that the expected impact of nutrient loading in freshwater systems on algal biomass and the ability of zebra mussels to mask this effect will vary depending on the species of algae present in the system.

6.4.4 The use of zebra mussels in open raceways

Introduction of zebra mussels to algal raceways could be implemented using meshed cages, as has been suggested for the use of planktivorous fish as biocontrol agents in other studies (Yun et al., 2016). This would be the optimal method for simplifying the recovery of mussel biomass from open algal raceways.

As an invasive species, the risks and benefits of encouraging the proliferation of zebra mussels, in either new or established ranges, need to be carefully assessed on a site-by-site basis in order for this application to be used responsibly and successfully. For example, the risk profile varies vastly when comparing the use of zebra mussels in a closed system within an invaded range, to an open system with links to surrounding waterways, in an uninvaded range. Any decision to encourage zebra mussels should only be taken following discussion and appraisal with the relevant authorities at the regional, national or international level, depending on the perceived risks (McLaughlan and Aldridge, 2013).

It is highly improbable that zebra mussels would be able to proliferate in an algal raceway due to their shallow design. The average depth of algal raceways varies between 0.1-0.3m (James and Boriah, 2010), therefore UV radiation exposure from sunlight would be lethal to the larval stages of zebra mussels (Seaver et al., 2009), for which the lethal dose is 350 mJ/cm^2. In open water settings, translocated adult zebra mussels can persist in very shallow water due to their protective shells, but settlement of veliger larvae and subsequent recruitment is rarely found at depths <0.5m due to larval sensitivity to UV radiation (Aldridge et al., 2014).

Although zebra mussels have lower temperature tolerances than many freshwater mussels, studies by McMahon et al., (1995) suggest that the minimal incipient upper lethal temperature of the zebra mussel is greater than 30°C (McMahon and Ussery, 1995). Serra-Maia et al., (2016) estimate the optimal growth temperature of *Chlorella* to be 23.3°C(Serra-Maia et al., 2016), and Kessler et al., (1985) demonstrated an upper limit of temperature for growth of 14 strains of *Chlorella* in the range of 26 to 30 °C(Kessler, 1985). Therefore, zebra mussels should be able to tolerate most climates suitable for the optimal production of *Chlorella*.

There has been increased attention on the functional role of secondary metabolites produced by phytoplankton as anti-herbivore defences (Hay, 2009). These chemical defence responses include oxidative bursts, the halogenation of molecules, and the synthesis of secondary metabolites such as polyphenols (Potin et al., 2002). We recognise that in order to initiate these defensive responses there will be a metabolic cost which may affect the overall biochemistry, and therefore potentially the protein or lipid content of the phytoplankton. There are some studies which attempt to identify biochemical change in phytoplankton as the result of producing secondary metabolites in response to certain biotic threats, including endophytes (Bouarab et al., 2004), bacteria (Küpper et al., 2002) and zooplankton (Pohnert, 2005) however there are no studies which attempt to look at biochemical changes in response to mussels. Secondary metabolites exhibit both spatial and temporal variability and most are likely to have multiple additional functions, such as anti-fouling (Steinberg and De Nys, 2002) and UV screening (Pavia et al., 1997). This makes interpreting the presence, composition or levels of secondary metabolites specifically produced as a response to predation, and therefore any potential or predictable biochemical impact they may have, a challenge. However, this is an area of research which may warrant further investigation.

6.4.5 Applications in synthetically engineered ecosystems for algae production

The zebra mussels' putative preference for *Synechocystis* over *Chlorella* could have useful application in the field of Synthetic Ecology and in the mass production of algae. Understanding the constraints of this interaction will be key in designing *de novo* communities that could be used to expand the commercial production of algae, such as *Chlorella*, in outdoor production systems where one constraint is competitive suppression by other algae species. The results of this experiment suggest that zebra mussels, at the right density, could dampen the ability of an invasive cyanobacteria to proliferate and bloom further in a *Chlorella* raceway pond system. This is due to the mussels appearing to express a feeding preference for the cyanobacteria over the green algae. Designing systems that can stabilise in a predictable manner is a key concept in Synthetic Ecology. Effective *de novo* ecosystems would enable industrial production of algae, and other crops, to move away from vulnerable monocultures by designing resilient poly-culture communities, utilising organisms from different trophic levels, which can produce similar yields with reduced inputs and less direct management.

In addition to the production of certain species of microalgae in open raceways, zebra mussels may have utility in the production of commercially relevant filamentous macroalgal species. Lawton et al., (2017) identify the cosmopolitan freshwater macroalgae genus *Oedogonium* as an ideal candidate for open pond systems, as it is resilient, competitively dominant and has biomass productivities comparable with microalgae (Lawton et al., 2017). Sakharova et al. (2018) have illustrated that *Oedogonium* is resistant to the grazing of zebra mussels (Sakharova et al., 2018) and so therefore a coculture system in which zebra mussels remove competing microalgae species, reducing competition for light and nutrients, may help to improve *Oedogonium* yields further.

The methodology used in this research provides a useful template for future work on Synthetic Ecology. For example, contrary to the effects observed in this experiment with *Synechocystis*, many studies have linked the invasion of zebra mussels with the promotion and bloom formation of the toxic cyanobacteria *Microcystis aeruginosa* in the temperate lakes of North America (Vanderploeg et al., 2001; Nicholls et al., 2002). However, more recent studies contradict such findings, indicating that nutrients and temperature are more likely causal factors (Raikow et al., 2004; Fernald et al., 2007). Studies which rely on field-based observations make it challenging to identify key drivers of community change because so many interacting factors can be at play. By following the methodology used here, using axenic algal communities and creating these systems *de novo* in isolation, may help to elucidate the true effect of zebra mussels on blooms of this harmful cyanobacteria. Accurate characterisation of species relationships and context dependencies will be key in the application of Synthetic Ecology.

A further potential application of synthetic ecologies outside of industrial biomass production could be phycoremediation, which focuses on waste water remediation using algae species. Traditionally research into waste water remediation has focussed on using green algae (de-Bashan et al., 2008) and cyanobacteria (Olguín et al., 2003) in monoculture to sequester excess nutrients such as nitrogen, phosphorus and ammonia. However the field of phycoremediation is quickly realising that co-cultures are both more effective at sequestering nutrients and inherently more stable systems (Silva-Benavides and Torzillo, 2012). *Chlorella* has been demonstrated to absorb 45-97% of nitrogen, 28-96% of Phosphorus and reduce the chemical oxygen demand by 61-86% from different types of wastewater including agricultural and sewage (Lau et al., 1996; Valderrama et al., 2002; Aslan and Kapdan, 2006; Feng et al., 2011; Silva-Benavides and Torzillo, 2012). Subsequently there is potential to use mussels to remove these nutrient-accumulating micro algae species, ultimately repackaging and removing excess nutrients as mussel biomass. There has already been significant interest in utilising zebra mussels as tools for managing nuisance algae and cyanobacteria in drinking water reservoirs where the mussel has already established (McLaughlan and Aldridge, 2013) as well as utilising the resultant mussel biomass as a supplement for commercial chicken feed (Claire McLaughlan et al., 2014). The field of phycoremediation could present further applications for zebra mussels. The pursuit of Synthetic Ecology in understanding and modelling the governing environmental intricacies and interspecies relationships of algal communities should allow for the creation of evermore stable and predictable *de novo* communities with utility in industrial applications, from commercially significant algae production, phycoremediation and biofuel production.

Chapter 7

Synthesis

INNS can drive significant effects in recipient ecosystems, and the magnitude and longevity of these impacts can become increasingly pervasive if the INNS express the characteristics of an ecosystem engineer (Emery-Butcher et al., 2020). In this thesis I explored the impact of INNS on vulnerable freshwater ecosystems and took steps to further our understanding of the nuanced relationship between INNS and their environment. The overarching aim of the thesis was to assess whether the changes brought about through the establishment of INNS may be able to drive favourable shifts between alternative equilibria and help to supress cyanobacteria abundance. In this chapter, I briefly summarise the findings of the preceding chapters and consider their general findings with respect to the potential for dreissenids and invasive plants to drive systems towards conditions that would typically be considered as favourable: a clear water state and low cyanobacteria biomass. I then consider potential implications for the management and exploitation of INNS in freshwater ecosystems, consider the ethics of harnessing INNS as ecological engineers, and contemplate directions for future research.

7.1 Chapter summaries

In Chapter 2, through a meta-analysis of the global impacts of INNS on freshwater lake ecosystems, I identified that molluscs were potential drivers of a clear water state in lake ecosystems, encouraging system recovery and delaying ecosystem collapse. Invasive fish and crustaceans, however, were identified as potentially pushing systems towards a turbid state and resisting recovery.

Following on from the finding that invasive molluscs may drive favourable changes between alternative equilibria in shallow lakes, I further assessed the impact of invasive molluscs on freshwater ecosystems, focussing primarily on invasive bivalves. In Chapter 3, through a Progressive-Change BACIPS analysis, I evaluated the potential impact of quagga mussel establishment across a group of UK reservoirs. I identified that impacted sites demonstrated: an increase in overall reactive silica and SRP concentrations; reductions in overall diatom, cyanobacteria and *Aphanizomenon* sp. abundance; and an increase in chlorophyll a concentration. However, no significant changes were seen in overall green algae, *Microcystis* sp., *Anabaena* sp., or *Euglena* sp. abundance, ammonium concentration or turbidity.

In Chapter 4, I investigated the nuances of quagga mussel feeding behaviours. I measured the clearance rate and pseudofaeces production of different populations of quagga mussels resident to either lotic or lentic ecosystems, when exposed to single or mixed phytoplankton communities. The results demonstrated that quagga mussels from both populations expressed a higher clearance rate for the green algae *C. vulgaris* than the cyanobacterium *S. elongatus*. With regard to pseudofaeces production, quagga mussels from a lotic environment produced a greater amount of pseudofaeces at the same phytoplankton concentrations than lentic quagga mussels. For both populations, pseudofaeces production was highest under mixed phytoplankton conditions and more frequently contained the cyanobacteria *S. elongatus* than green algae *C. vulgaris*.

In Chapter 5, I explored the ecological engineering characteristics of invasive non-native plant species, through their allelopathic effect on the average growth rates of cyanobacteria, *Synechocystis* sp., and green algae, *C. vulgaris*. The allelopathic potential of *Hydrocotyle ranunculoides* and *Crassula helmsii*, were compared between the plant in a whole, or crushed state, to attempt to identify if allelochemicals were excreted naturally by the plants. The results uncovered a mixed picture; whole *H. ranunculoides* supressed only the growth rate of *C. vulgaris*, while crushed *H. ranunculoides* had no effect on either phytoplankton species. Conversely, whole *C. helmsii* had no effect on either phytoplankton species, whereas crushed *C. helmsii* supressed the growth rate of both phytoplankton species.

Finally, in Chapter 6, I examined the potential for INNS to play a role in the novel concept of Synthetic Ecologies. I explored harnessing the selective filter feeding behaviour of invasive zebra mussels to drive favourable conditions in the cultivation of the commercially relevant algae species, *C. vulgaris*., in outdoor raceways by supressing the growth of competing cyanobacteria which enter the system. The results indicated that although zebra mussels had a small negative effect on the abundance of the target commercial species *C. vulgaris,* they had a very large negative effect on the abundance of nuisance cyanobacteria, in both mixed and single phytoplankton species conditions. This suggested that zebra mussels' preference for cyanobacteria may be harnessed to maintain a dominance of *C. vulgaris* in outdoor raceways prone to contamination by cyanobacteria.

7.2 INNS in the management and exploitation of freshwater ecosystems and phytoplankton communities

The use of biological management approaches to control HABs and perpetuate the maintenance of a clear water state have attracted much attention and are considered as favourable alternatives to chemical or physical interventions (Sun et al., 2018). Biological management approaches require less direct management and are free of the cost and supply chain issues associated with chemical or physical interventions. Traditional biological management has primarily been restricted to the removal of benthivorous and zooplanktivorous fish and the introduction of piscivorous fish in order to promote filter feeding zooplankton species (Benndorf, 1995; Jeppesen et al., 2007; Peretyatko et al., 2012). However, the grazing capability of zooplankton is limited due to predation by invertebrates and juvenile fish, toxic cyanobacteria and food limitation (Benndorf et al., 2000; Gulati et al., 2008). Therefore, INNS which express stabilising ecological engineering characteristics, in combination with other management tools, may present opportunities to consistently manage phytoplankton communities towards desired end points.

7.2.1 Invasive bivalves

The results outlined in this thesis suggest that, broadly, invasive dreissenid bivalves may drive shifts between alternative stable states through their deleterious impact on phytoplankton abundance, aiding ecosystem recovery to a clear water state and delaying system collapse. However, dreissenids may not impact all phytoplankton taxa equally, as we show that there may be plasticity in dreissenid grazing behaviours and their tolerances and preferences for different phytoplankton species. This plasticity may perhaps be governed, in part, by population level differences influenced by the hydrodynamic environment. Additionally, despite the uniformity across all studies demonstrating that dreissenids reduce phytoplankton abundance, interestingly, in Chapter 3 this was not born out in a comparable reduction in turbidity in invaded systems. As illustrated by the theory of alternative equilibria, turbidity is determined by both phytoplankton abundance and total suspended solids. Therefore, although dreissenids may be able to reduce phytoplankton loads, in highly mixed systems or systems which experience significant benthic disturbances, dreissenids may not be able to overcome the hysteresis, or avoid reaching the tipping point, which results in system recovery or collapse, respectively.

In terms of preventing and controlling HABs by reducing cyanobacterial biomass, the results of this thesis illustrate that, in some cases, dreissenid mussels may play a role in the targeted reduction of cyanobacteria within wider phytoplankton assemblages. However, the effect may be varied and context dependant. The contrast in the effects observed between quagga mussels and zebra mussels and between quagga mussels in mesocosm experiments and in natural systems illustrated in this thesis indicate that the relationship between dreissenid mussels and cyanobacteria is likely to be highly nuanced. The relationship between dreissenids and cyanobacteria abundance in natural systems are likely governed by numerous factors, including: the species, strain and growth rate of the target cyanobacteria; beneficial, deleterious or mutualistic relationships between the cyanobacteria and the wider phytoplankton community; the species, population density, size, and putative phenotypic adaptation of dreissenid mussels; and the abiotic nature of the system, such as nutrient status, hydrodynamic characteristics, and depth. This may present difficulties in making general predictions about the effect of dreissenids on cyanobacteria, which apply across all natural systems. However, this does not invalidate the usefulness of the studies within this thesis, as they provide further insights into how certain systems may respond and how these confounding factors should be, or may be, accounted for.

Many freshwater ecosystems historically contained extensive beds of native mussels which have been lost to multiple stressors, such as sedimentation or drought, leaving these systems devoid of the vital ecosystem services which mussel beds provide (DuBose et al., 2019). In biologically depleted and turbid systems, the establishment of highly resilient dreissenid mussels may represent an opportunity to replace these lost services and drive ecosystem recovery. Moreover, there may be opportunities for these ecological engineers to bring these services to systems where they could not previously exist. For example, dreissenid mussels possess byssus threads which allow them to colonise hard surfaces which are unsuitable for unionid mussels, which require sediment to burrow in. The dreissenid mussels' ability to attach and densely colonise hard surfaces, such as the concrete basins of man-made reservoirs, may enable the provision of ecosystem services which will maintain a favourable clear water state and potentially allow for colonisation by native species and macrophytes through the provision of protection, nutrients, food resources and attachment sites.

There is increased interest in the use of dreissenids as management tools for cyanobacterial blooms, particularly in urban ponds (Waajen et al., 2016) and shallow lakes (Reeders and Bij de Vaate, 1990; Dionisio Pires et al., 2005a), where they have been shown to reduce cyanobacterial abundance. However, the exact impact of quagga mussels and zebra mussels on cyanobacteria is a complex picture. With some studies suggesting they ingest toxic cyanobacteria strains (Dionisio Pires and Van Donk, 2002; Dionisio Pires et al., 2005a, 2005b), selectively reject toxic strains (Vanderploeg et al., 2001), increase the prevalence of HABs (Knoll et al., 2008), and reduce prevalence of HABs (Dionisio Pires et al., 2005a; Waajen et al., 2016). Further studies suggest interactions between dreissenids and cyanobacteria could be specific to the cyanobacterial strain (Vanderploeg et al., 2013), and augmented by temperature (Fernald et al., 2007), light intensity and nutrient ratios (Raikow et al., 2004; Sarnelle et al., 2005; Wojtal-Frankiewicz et al., 2010). It has also been hypothesised that phenotypic and genetic differences between dreissenid populations could be responsible for differential reactions (Vanderploeg et al., 2013; Huisman et al., 2018). This hypothesis is born from the fact that the majority of the differences in response of cyanobacteria to dreissenids is split between continents, with North American studies suggesting dreissenids increase the prevalence of HABs, and European studies suggesting dreissenids supress them.

The results of Chapter 3 and 6 align with other European studies in that it is likely dreissenids could be utilised to suppress cyanobacterial biomass. However, Chapter 4 highlights that the relationship could be complicated by the growth rates of cyanobacterial species, mussel provenance, and their ability of target cyanobacteria to survive and resuspend from pseudofaeces.

As shown in Chapter 6, there is potential for the selective grazing activity of dreissenid mussels to be utilised in Synthetic Ecologies. In closed systems with known abiotic parameters and biotic interactions they could play a role in driving or maintaining predictable phytoplankton communities. Potential applications could exist in managing communities in commercial algae production, flocculating target phytoremediating phytoplankton species in pseudofaeces, or removing nuisance phytoplankton species in water treatment systems.

7.2.2 Invasive allelopathic plants

Despite being a smaller focus of the thesis, I felt that the allelopathic interactions between invasive plants and phytoplankton may serve as a powerful mechanism for maintaining a clear water state, and worthy of investigation. While allelopathic interactions between plants and phytoplankton are widely studied (Hilt and Gross, 2008; Grutters et al., 2017), it is an understudied impact in the context of aquatic plant invasions. Dialysis bag experiments, as employed in this study, are recognised as demonstrating the highest degree of realism, and the ability to grant direct observations of phytoplankton inhibition (Gross et al., 2007). The results of this thesis paint a complex picture concerning allelopathic interactions and suggests that the allelopathic effects of invasive plant species may not be universally suppressive across all phytoplankton taxa. Despite numerous studies suggesting cyanobacteria are more sensitive than green algae and diatoms to allelopathic substances (Gross and Jüttner, 2003; Hilt and Gross, 2008; Jasser, 1995; Planas et al., 1981; and van Donk and van de Bund, 2002), it would appear that this is not universally applicable to all allelopathic substances and cyanobacteria species.

The model of alternative equilibria identifies allelopathic interactions to be one of the key drivers of alternative stable states (Scheffer et al., 1993). It is acknowledged that allelopathic inhibition of cyanobacterial blooms is a novel and promising area of research; however, the concentration of allelochemicals present in natural systems are often not at levels high enough to supress cyanobacterial growth (Lu et al., 2014). Moreover, although many types of allelochemicals have been extracted from plants, artificially producing the quantities required to supress phytoplankton in natural systems is a complicated and time-consuming process (Tan et al., 2019). This is why invasive allelopathic plants may serve as a useful delivery mechanism, as they may be able to reach densities sufficient enough to naturally exudate allelochemicals at environmentally relevant concentrations.

Chapter 5 suggests that the suppressive effect of *H. ranunculoides* on green algae could be leveraged in natural systems to directly suppress green algae, and perhaps indirectly supress cyanobacterial biomass. It is plausible that a floating boom system could be utilised to maintain a population of *H. ranunculoides* within a controlled area to drive systems towards a clear water state. Despite the allelopathic effects of *C. helmsii* only being noted in a crushed state, *C. helmsii* biomass removed from an invaded and environmentally depleted system could be recycled and returned to the system bound in floating bales, in a manner similar to the current use of barley straw (Murray et al., 2010). The implementation of floating bales of barley straw is a common method for suppressing phytoplankton abundance in freshwater ecosystems. Exudates from decomposing barley straw do not kill existing phytoplankton but do inhibit further growth (Gibson et al., 1990).

The success of allelopathic control efforts are highly likely to be species and context specific, eliciting both direct and indirect effects. However, a sophisticated understanding of the differential sensitivity of key phytoplankton species to certain invasive plant species may also allow for some targeted management of phytoplankton populations towards specific desirable end points. Research into the allelopathic management of phytoplankton abundance and cyanobacterial blooms is at an early stage, with most research still conducted at the laboratory scale, and yet to be extended meaningfully to natural systems (Tan et al., 2019). However, the ecological engineering capabilities of some invasive plant species could provide a useful vector for implementing allelopathic controls, given their ability to sustain high abundances in invaded systems.

7.3 The ethics of harnessing Invasive Non-Native Species

Research documenting the impacts of invasive ecosystem engineers is often framed around the negative effects they may have on ecosystem services which serve human society (e.g. Cuhel and Aguilar, 2013; Sainty et al., 1997). This can lead to the overriding perception that INNS always have negative effects on recipient ecosystems, or that the negative impacts of INNS will always outweigh any positive effects which occur. However, as demonstrated by the results of this thesis, as well as noted in the wider literature, not all effects of INNS establishment are decidedly negative (McLaughlan and Aldridge, 2013; Gallardo et al., 2016; Boltovskoy et al., 2020; Emery-Butcher et al., 2020). Therefore, INNS may offer desirable outcomes in systems already highly degraded by anthropogenic impacts, such as eutrophication. There are likely certain situations in which INNS may present an opportunity to impart positive effects on native species, biodiversity or ecosystem service provision.

Assessment of the impact of INNS should be as balanced as possible and avoid one-sided assessments which attempt to frame all impacts of invasive species as negative. For example, Walsh et al., 2016, estimated that the invasion of the cladoceran *Bythotrephes longimanus* in Lake Mendota (USA) resulted in a significant economic loss due to reductions in water clarity, stating “such extreme cases of economic damages call for increased investment in the prevention and control of invasive species”. However, in 2015, zebra mussels were found to have established in Lake Mendota (Hayranto, 2018), which had been predicted to significantly increase water clarity (Reed-Andersen et al., 2000). Therefore, if the negative effects of one invader, *B. longimanus*, are offset by a second invader, zebra mussels, should the establishment of zebra mussels be labelled as a positive ecosystem service? The filter feeding activity of bivalves is widely accepted as a valuable ecosystem service (Vaughn, 2017), but due to the INNS status of zebra mussels should the effect be framed as a negative consequence? For example, the consumption of phytoplankton by zebra mussels reduces its availability to other filter-feeders (Strayer and Malcom, 2018).

All species, native or invasive, can, and often do, have both beneficial and detrimental effects on biota, the environment, and the economy, all of which should be considered when assessing their overall impact. In the case of dreissenid mussels, their negative impacts on industrial facilities (e.g.

clogging and restricting water flow in pipes and water treatment facilities (Gallardo and Aldridge, 2020)), potential displacement of native filter feeders (Aldridge et al., 2004; Sousa et al., 2011), inconsistent association with HABS (Vanderploeg et al., 2013), and role in invasional meltdowns (Gallardo and Aldridge, 2015), should be weighed against their positive effects on water clarity (Higgins and Vander Zanden, 2010), removal of cyanobacteria (Dionisio Pires et al., 2005a; Waajen et al., 2016), and provision of food resources for native species (Carlsson et al., 2009). Obviously, the magnitude and relevance of these impacts will vary depending on the status of the recipient waterbody. In the extreme, the negative effects of dreissenid mussel establishment would be felt very acutely in a pristine lake ecosystem, highly connected to surrounding waterways, situated within an uninvaded range, which supports highly diverse mussel beds of critically endangered native freshwater mussels. However, dreissenid mussel establishment may be a favourable occurrence in an ecologically depleted lake, with no surface water connections to other waterways, within an invaded range, devoid of any other filter feeding bivalves, where cyanobacterial abundance is high.

The ability to contain INNS within the desired range is a further consideration which must be taken, as the maintenance of a population of INNS, if not appropriately assessed, may increase propagule pressure to the surrounding environment. The deliberate relocation of INNS to uninvaded sites should never be encouraged, due to their ability to displace native species, affect ecosystem functioning and introduce novel parasites (Strayer, 2012; David et al., 2017; Chalkowski et al., 2018). In systems containing established populations of INNS, any recreational activities, such as angling or sailing, should ensure to follow best practice advice when moving equipment between ecosystems. Additionally, inter-basin water transfers between invaded and uninvaded environments should be avoided to prevent further spread of INNS (Gallardo and Aldridge, 2018). The risks of containment, if it is elected to utilise a management strategy harnessing the effects of INNS, varies greatly depending on the type of intervention, for example the risk of spread from maintaining a population of dreissenid mussels is much greater than the risk from utilising crushed invasive plants, which are no longer viable. Although I do not recommend that INNS should be deliberately moved to new sites, I think that there may be positive effects which could be embraced in ecologically depleted systems where INNS are already established, if all the risks are carefully assessed (McLaughlan and Aldridge, 2013).

Our ability to discern whether certain INNS should be embraced or expelled will depend on our capacity to implement research which widely assesses all potential impacts of the INNS in question. A broad and nuanced understanding which accepts the prevalence of the mixed effects of INNS, and acknowledges and recognises positive effects where they exist, will allow for a greater appreciation of biological invasions and recognition of circumstances where their effects may be beneficial. In the case of systems which experience alternative equilibria, the methodology implemented in Chapter 2, whereby a meta-analysis technique of available literature is framed around the species interaction model governing the location of critical thresholds, could play a role in initially highlighting the unbiased potential effects of INNS establishment on ecosystem collapse and recovery.

I do not want to suggest that we should always welcome INNS which may yield favourable outcomes. Where INNS cause significant environmental or economic damage their spread should be actively limited through appropriate interventions. Rather, I suggest that under certain circumstances, within an invaded range and where INNS can be appropriately contained, INNS could be cultivated or allowed to remain within ecologically depleted systems where it can be predicted with a high degree of certainty that their presence will yield a favourable effect.

7.4 Future directions

In order to build on the research presented in this thesis, there are numerous avenues which could be explored to better understand the potential implications of invasive bivalves and allelopathic plants on freshwater ecosystems. A better understanding of the implications of dreissenid invasions on phytoplankton community structure, opposed to simply overall phytoplankton biomass, is essential in being able to predict the characteristics of a system which may lead to suppression or promotion of cyanobacteria dominance. This is traditionally a difficult and time-consuming area of study, as recreating naturally dynamic phytoplankton communities in the lab is extremely challenging. Monitoring and recording detailed changes in phytoplankton community structures, which involve more than two or three phytoplankton species, requires highly specialised identification skills. However, a lot of detailed phytoplankton community data already exists in records maintained by water resource managers. Therefore, the wide-scale implementation of the Progressive Change BACIPS analysis technique to analyse existing long-term reservoir datasets could be a simple and effective way of quickly identifying common trends in naturally occurring phytoplankton community structures associated with INNS establishment.

Additional research in characterising the differences in grazing behaviour noted between dreissenids in North America and Europe could be a fruitful area of investigation. Arranging parallel experiments, ideally in the same location and under the same conditions, to characterise the feeding behaviours of these geographically separated populations of mussels, may help to identify why the literature presents divergent results of their impacts on cyanobacteria abundance. Direct comparisons between dreissenid populations may aid the identification of the putative phenotypic or genotypic differences that may exist between them and allow for a clearer understanding of the traits which may govern favourable outcomes. Following on from the work undertaken by Galbraith et al., (2009) and Silverman (1997), comparison of their gill structures or labial palps, both key structures in governing the feeding behaviour of mussel species, could yield interesting results.

A more nuanced understanding of the fate of phytoplankton egested by dreissenid mussels in pseudofaeces is key to understanding whether selective rejection is a driver of cyanobacterial blooms. Knowledge of which species of phytoplankton remain viable, how long they can remain viable and under which circumstances they can successfully resuspend will allow us to better understand which freshwater ecosystems may benefit from the grazing activity of dreissenid mussels, based on the phytoplankton communities they host.

Distinguishing the potential of allelopathic invasive plants to drive favourable change in freshwater ecosystems would benefit greatly from the design and implementation of a greater number of experiments which attempt to measure or characterise the natural concentrations of allelochemicals exudated by live plants. While quantifying the effect of concentrated plant exudates on phytoplankton growth is useful for identifying plant species with high allelopathic potential (Grutters et al., 2017), results cannot be used to directly predict the effects which may be yielded in natural systems. Identifying the allelopathic effect of live plants on natural phytoplankton communities will provide a greater understanding of how invasive plants with high allelopathic potential may aid ecosystem recovery or reduce cyanobacteria abundance.

Studying the impacts of INNS can provide important insights into how ecosystems function, as the establishment of a single INNS can have widespread effects on the physical and biological structure of recipient ecosystems, which may alter ecosystem functioning. A well-developed understanding of the impacts of INNS will allow for the identification of opportunities to improve management and recognise potential scenarios where favourable impacts of INNS may be harnessed. The expansion of global interconnectivity and increased climate warming are likely to further drive the spread of INNS (Walther et al., 2009; Seebens et al., 2017, 2018), therefore improving our understanding of their impact on freshwater ecosystems will be essential in supporting managers in the effective control and exploitation of INNS in the future.

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Appendix A

Supplementary Information for Chapter 2

Contents

Table A1 Database of references used for meta-analysis. This table contains a list of the authors, the titles, the focal Invasive Non-Native Species (INNS), taxonomic group of the INNS and the data source (G – Gallardo et al. 2016 [1], and T - Tang et al., 2020 [PhD Thesis][2]) of each publication included in this meta-analysis. Superscripts refer to references cited, which follow the table.

Author	Title	INNS	Taxonomic Group	Data Source
Akhurst DJ, Jones GB, Clark M, Reichelt-Brushett A	Effects of fish and macrophytes on phytoplankton and zooplankton community structure in a subtropical freshwater reservoir [3]	*Gambusia holbrooki*, *Cyprinus carpio*	Fish, Fish	T
Albertson LK, Daniels MD	Effects of invasive crayfish on fine sediment accumulation, gravel movement, and macroinvertebrate communities [4]	*Orconectes rusticus*	Crustacean	T
Angeloni NL, Jankowski KJ, Tuchman NC, Kelly JJ	Effects of an invasive cattail species (*Typha glauca*) on sediment nitrogen and microbial community composition in a freshwater wetland [5]	*Typha glauca*	Plant	G
Angradi TR, Hagan SM, Able KW	Vegetation type and the intertidal macroinvertebrate fauna of a brackish marsh: *Phragmites* vs. *Spartina* [6]	*Phragmites australis*	Plant	T
Atalah J, Kelly-Quinn M, Irvine K, Crowe TP	Impacts of invasion by *Dreissena polymorpha* (Pallas, 1771) on the performance of macroinvertebrate assessment tools for eutrophication pressure in lakes [7]	*Dreissena polymorpha*	Mollusc	G
Back CL, Holomuzki JR, Klarer DM, Whyte RS	Herbiciding invasive reed: Indirect effects on habitat conditions and snail-algal assemblages one year post-application [8]	*Phragmites australis*	Plant	G
Badiou PHJ, Goldsborough LG	Ecological impacts of an exotic benthivorous fish, the common carp (*Cyprinus carpio L.*), on water quality, sedimentation, and submerged macrophyte biomass in wetland mesocosms [9]	*Cyprinus carpio*	Fish	T
Bajer PG, Sullivan G, Sorensen PW	Effects of a rapidly increasing population of common carp on vegetative cover and waterfowl in a recently restored Midwestern shallow lake [10]	*Cyprinus carpio*	Fish	G
Baranowska KA, North RL, Winter JG, Dillon PJ	Long-term seasonal effects of dreissenid mussels on phytoplankton in Lake Simcoe, Ontario, Canada [11]	*Dreissena polymorpha*	Mollusc	G
Barbiero RP, Rockwell DC	Changes in the crustacean communities of the central basin of Lake Erie during the first full year of the *Bythotrephes longimanus* invasion[12]	*Bythotrephes longimanus*	Crustacean	G
Bassett IE, Paynter Q, Beggs JR	Invertebrate community composition differs between invasive herb alligator weed and native sedges [13]	*Alternanthera philoxeroides*	Plant	G
Beekey MA, McCabe DJ, Marsden JE	Zebra mussel colonisation of soft sediments facilitates invertebrate communities [14]	*Dreissena polymorpha*	Mollusc	G

Beisner BE, Ives AR, Carpenter SR	The effects of an exotic fish invasion on the prey communities of two lakes[15]	*Osmerus mordax*	Fish	G
Bially A, Macisaac HJ	Fouling mussels (*Dreissena* spp.) colonize soft sediments in Lake Erie and facilitate benthic invertebrates [16]	*Dreissena polymorpha*	Mollusc	G
Bogacka-Kapusta E, Kapusta A	Spatial and diurnal distribution of cladocera in beds of invasive *Vallisneria spiralis* and open water in heated lake [17]	Vallisneria spiralis	Plant	G
Bonello JE, Judd KE	Plant community recovery after herbicide management to remove *Phragmites australis* in Great Lakes coastal wetlands [18]	*Phragmites australis*	Plant	T
Botts PS, Patterson BA, Schloesser DW	Zebra mussel effects on benthic invertebrates: physical or biotic? [19]	*Dreissena polymorpha*	Mollusc	G
Bourdeau PE, Pangle KL, Peacor SD	The invasive predator Bythotrephes induces changes in the vertical distribution of native copepods in Lake Michigan [20]	*Bythotrephes longimanus*	Crustacean	G
Bowen KL, Johannsson OE	Changes in zooplankton biomass in the Bay of Quinte with the arrival of the mussels, *Dreissena polymorpha* and *D. rostiformis bugensis*, and the predatory cladoceran, *Cercopagis pengoi*: 1975 to 2008 [21]	*Dreissena polymorpha*, *D. rostiformis bugensis*, *Cercopagis pengoi*	Mollusc, Mollusc, Crustacean	G
Cardona L	Trophic cascades uncoupled in a coastal marsh ecosystem [22]	*Gambusia holbrooki*	Fish	T
Carlsson NOL, Brönmark C, Hansson LA	Invading herbivory: The golden apple snail alters ecosystem functioning in Asian wetlands [23]	*Pomacea canaliculata*	Mollusc	G
Carvalho F, Pascoal C, Cássio F, Sousa R	Direct and indirect effects of an invasive omnivore crayfish on leaf litter decomposition [24]	*Procambarus clarkii*	Crustacean	T
Cheruvelil KS, Soranno PA, Madsen JD, Roberson MJ	Plant architecture and epiphytic macroinvertebrate communities: The role of an exotic dissected macrophyte[25]	*Myriophyllum spicatum*	Plant	G
Coetzee JA, Jones RW, Hill MP	Water hyacinth, *Eichhornia crassipes* (*Pontederiaceae*), reduces benthic macroinvertebrate diversity in a protected subtropical lake in South Africa [26]	*Eichhornia crassipes*	Plant	G
Cuassolo F, Díaz Villanueva V, Modenutti B	Litter decomposition of the invasive *Potentilla anserina* in an invaded and non-invaded freshwater environment of North Patagonia [27]	*Potentilla anserina*	Plant	T
da Silva LHS, Arcifa MS, Salazar-Torres G, Huszar VL de M	*Tilapia rendalli* increases phytoplankton biomass of a shallow tropical lake [28]	*Tilapia rendalli*	Fish	T
De Winton MD, Taumoepeau AT, Clayton JS	Fish effects on charophyte establishment in a shallow, eutrophic New Zealand lake [29]	Several fish spp.	Fish	G
Driver PD, Closs GP, Koen T	The effects of size and density of carp (*Cyprinus carpio L.*) on water quality in an experimental pond [30]	*Cyprinus carpio*	Fish	G

Dzialowski AR	Invasive zebra mussels alter zooplankton responses to nutrient enrichment [31]	*Dreissena polymorpha*	Mollusc	G
Ercoli F, Ruokonen TJ, Erkamo E, Jones RI, Hämäläinen H	Comparing the effects of introduced signal crayfish and native noble crayfish on the littoral invertebrate assemblages of boreal lakes [32]	*Pacifastacus leniusculus*	Crustacean	T
Ercoli F, Ruokonen TJ, Koistinen S, Jones RI, Hämäläinen H	The introduced signal crayfish and native noble crayfish have different effects on sublittoral macroinvertebrate assemblages in boreal lakes [33]	*Pacifastacus leniusculus*	Crustacean	T
Figueredo CC, Giani A	Ecological interactions between nile tilapia (*Oreochromis niloticus, L.*) and the phytoplanktonic community of the furnas reservoir (Brazil) [34]	*Oreochromis niloticus*	Fish	G
Fischer JR, Krogman RM, Quist MC	Influences of native and non-native benthivorous fishes on aquatic ecosystem degradation [35]	*Cyprinus carpio*	Fish	G
Foster SE, Sprules WG	Effects of the Bythotrephes invasion on native predatory invertebrates [36]	*Bythotrephes longimanus*	Crustacean	G
Gezie A, Assefa WW, Getnet B, Anteneh W, Dejen E, Mereta ST	Potential impacts of water hyacinth invasion and management on water quality and human health in Lake Tana watershed, Northwest Ethiopia [37]	*Eichhornia crassipes*	Plant	T
Greenfield BK, Siemering GS, Andrews JC., Rajan M, Andrews SP, Spencer DF	Mechanical Shredding of Water Hyacinth (*Eichhornia crassipes*): Effects on Water Quality in the Sacramento-San Joaquin River Delta , California [38]	Eichhornia crassipes	Plant	G
Hager HA, Vinebrooke RD	Positive relationships between invasive purple loosestrife (*Lythrum salicaria*) and plant species diversity and abundance in Minnesota wetlands[39]	*Lythrum salicaria*	Plant	G
Hansen GJA, Hein CL, Roth BM, Vander Zanden MJ, Gaeta JW, Latzka AW, Carpenter SR	Food web consequences of long-term invasive crayfish control [40]	*Orconectes rusticus*	Crustacean	G
Hayden B, Holopainen T, Amundsen PA, Eloranta AP, Knudsen R, Præbel K, Kahilainen KK	Interactions between invading benthivorous fish and native whitefish in subarctic lakes [41]	*Perca fluvialitis,* *Gymnocephalus cernuus*	Fish, Fish	G
Haynes JM, Tisch NA, Mayer CM, Rhyne RS	Benthic macroinvertebrate communities in southwestern Lake Ontario following invasion of Dreissena and Echinogammarus: 1983 to 2000 [42]	*Dreissena polymorpha*	Mollusc	G
Herrera-Martínez Y, Paggi JC, Garcia CB	Cascading effect of exotic fish fry on plankton community in a tropical Andean high mountain lake: A mesocosm experiment [43]	*Oncorhynchus mykiss*	Fish	T

Hinchliffe C, Atwood T, Ollivier Q, Hammill E	Presence of invasive Gambusia alters ecological communities and the functions they perform in lentic ecosystems [44]	*Gambusia* spp	Fish	T
Hogsden KL, Sager EP, Hutchinson TC	The impacts of the non-native macrophyte *Cabomba caroliniana* on littoral biota of Kasshabog Lake, Ontario [45]	*Cabomba caroliniana*	Plant	G
Holmes TG, Tonn WM, Paszkowski CA Scrimgeour GJ	Limited effects of non-native trout on microcrustacean zooplankton in boreal foothills lakes: Comparisons of stocked, unstocked, and fishless lakes[46]	*Oncorhynchus mykiss*	Fish	T
Holomuzki JR, Klarer DM	Invasive reed effects on benthic community structure in Lake Erie coastal marshes [47]	*Typha angustifolia*, *Phragmites australis*	Plant, Plant	G
Horvath TG, Martin KM, Lamberti GA	Effect of zebra mussels, *Dreissena polymorpha*, on macroinvertebrates in a lake-outlet stream [48]	*Dreissena polymorpha*	Mollusc	G
Idrisi N, Mills EL, Rudstam LG, Stewart DJ	Impact of zebra mussels (*Dreissena polymorpha*) on the pelagic lower trophic levels of Oneida Lake, New York [49]	*Dreissena polymorpha*	Mollusc	G
Johnson PTJ, Olden JD, Solomon CT, Vander Zanden MJ,	Interactions among invaders: community and ecosystem effects of multiple invasive species in an experimental aquatic system [50]	*Bellamya chinensis*, *Orconectes rusticus*	Mollusc, Crustacean	G
Kuhns LA, Berg MB	Benthic invertebrate community responses to round goby (*Neogobius melanostomus*) and zebra mussel (*Dreissena polymorpha*) invasion in southern Lake Michigan [51]	*Neogobius melanostomus*, *Dreissena polymorpha*	Fish, Mollusc	G
Kloskowski J	Impact of common carp *Cyprinus carpio* on aquatic communities: direct trophic effects versus habitat deterioration [52]	*Cyprinus carpio*	Fish	G
Knapp RA, Matthews KR, Sarnelle O	Resistance and resilience of alpine lake fauna to fish introductions [53]	Several fish spp.	Fish	G
Knight JC, O'Malley BP, Stockwell JD	Lake Champlain offshore benthic invertebrate community before and after zebra mussel invasion [54]	*Dreissena polymorpha*	Mollusc	T
Kolosovich AS, Chandra S, Saito L, Davis CJ, Atwell L	Short-term survival and potential grazing effects of the New Zealand mudsnail in an uninvaded Western Great Basin watershed [55]	*Potamopyrgus antipodarum*	Mollusc	G
Laguna C, López-Perea JJ, Viñuela J, Florín M, Feliu J, Chicote Á, Cirujano S, Mateo R	Effects of invasive fish and quality of water and sediment on macrophytes biomass, and their consequences for the waterbird community of a Mediterranean floodplain [56]	*Cyprinus carpio*	Fish	T
Lancelotti J, Marinone MC, Roesler I	Rainbow trout effects on zooplankton in the reproductive area of the critically endangered hooded grebe [57]	*Oncorhynchus mykiss*	Fish	T
Larochelle M, Dumont P, Lavoie C, Hatin D	Varying effects of common reed invasion on early life history of Northern Pike [58]	*Phragmites australis*	Plant	T

Lawrence BA, Bourke K, Lishawa SC, Tuchman NC	Typha invasion associated with reduced aquatic macroinvertebrate abundance in northern Lake Huron coastal wetlands [59]	*Typha glauca*	Plant	T
Leyse KE, Lawler SP, Strange T	Effects of an alien fish, *Gambusia affinis*, on an endemic California fairy shrimp, *Linderiella occidentalis*: Implications for conservation of diversity in fishless waters [60]	*Gambusia affinis*	Fish	G
Linares MS, Callisto M, Marques JC	Invasive bivalves increase benthic communities complexity in neotropical reservoirs [61]	*Corbicula fluminea*, *Limnoperna fortunei*	Mollusc, Mollusc	T
Lodge DM, Kershner MW, Aloi JE, Covich AP	Effects of an omnivorous crayfish (*Orconectes rusticus*) on a freshwater littoral food web [62]	*Orconectes rusticus*	Crustacean	G
Lozano SJ, Scharold J V, Nalepa TF	Recent declines in benthic macroinvertebrate densities in Lake Ontario [63]	*Dreissena* spp.	Mollusc	G
Maezono Y, Miyashita T	Impact of exotic fish removal on native communities in farm ponds [64]	*Micropterus salmoides*	Fish	G
Matsuzaki SIS, Usio N, Takamura N, Washitani I	Contrasting impacts of invasive engineers on freshwater ecosystems: An experiment and meta-analysis [65]	*Cyprinus carpio*, *Procambarus clarkii*	Fish, Crustacean	G
Menezes RF, Attayde JL, Lacerot G, KostenS, Coimbra e Souza L, Costa LS, van Nes EH, Jeppesen E	Lower biodiversity of native fish but only marginally altered plankton biomass in tropical lakes hosting introduced piscivorous *Cichla cf. ocellaris* [66]	*Cichla ocellaris*	Fish	G
Mines CH, Ghadouani A, Legendre P, Yan ND, Ivey GN	Examining shifts in zooplankton community variability following biological invasion [67]	*Bythotrephes longimanus*	Crustacean	G
Mörtl M, Rothhaupt KO	Effects of adult *Dreissena polymorpha* on settling juveniles and associated macroinvertebrates [68]	*Dreissena polymorpha*	Mollusc	G
Nalepa TF, Fanslow DL, Lansing MB, Lang GA	Trends in the benthic macroinvertebrate community of Saginaw Bay, Lake Huron, 1987 to 1996: Responses to phosphorus abatement and the zebra mussel, *Dreissena polymorpha* [69]	*Dreissena polymorpha*	Mollusc	G
Nienhuis S, Haxton TJ, Dunkley TC	An empirical analysis of the consequences of zebra mussel invasions on fisheries in Inland, freshwater lakes in Southern Ontario[70]	*Dreissena polymorpha*	Mollusc	T
Nystrom P, Bronmark C, Graneli W	Influence of an exotic and a native crayfish species on a littoral benthic community [71]	Several fish spp., *Pacifastacus leniusculus*, *Oncorhynchus mykiss*	Fish, Crustacean, Fish	G
Ozersky T, Barton DR, Evans DO	Fourteen years of dreissenid presence in the rocky littoral zone of a large lake: Effects on macroinvertebrate abundance and diversity [72]	*Dreissena* spp.	Mollusc	G

Ozersky, T Barton, DR. Hecky, RE. Guildford, SJ.	Dreissenid mussels enhance nutrient efflux, periphyton quantity and production in the shallow littoral zone of a large lake [73]	*Dreissena polymorpha*	Mollusc	G
Pelicice FM, Agostinho AA	Fish fauna destruction after the introduction of a non-native predator (*Cichla kelberi*) in a Neotropical reservoir [74]	*Cichla kelberi*	Fish	G
Peters JA, Lodge DM	Habitat, predation, and coexistence between invasive and native crayfishes: Prioritizing lakes for invasion prevention [75]	*Orconectes rusticus*	Crustacean	G
Pothoven SA, Höök TO, Nalepa TF, Thomas M V, Dyble J	Changes in zooplankton community structure associated with the disappearance of invasive alewife in Saginaw Bay, Lake Huron [76]	*Alosa pseudoharengus*	Fish	G
Preston DL, Henderson JS, Johnson PTJ	Community ecology of invasions: Direct and indirect effects of multiple invasive species on aquatic communities [77]	*Gambusia holbrooki*	Fish	G
Rakauskas V, Šidagytė E, Kesminas V, Kaminskas O	Can the invasive New Zealand mud snail (*Potamopyrgus antipodarum*) threaten fisheries of temperate lakes? A case study from lake Dusia, Lithuania[78]	*Potamopyrgus antipodarum*	Mollusc	T
Rakauskas V, Šidagyte E, Butkus R, Garbaras A	Effect of the invasive New Zealand mud snail (*Potamopyrgus antipodarum*) on the littoral macroinvertebrate community in a temperate mesotrophic lake [79]	*Potamopyrgus antipodarum*	Mollusc	T
Reed T, Wielgus SJ, Barnes AK, Schiefelbein JJ, Fettes AL	Refugia and local controls: Benthic invertebrate dynamics in lower Green Bay, Lake Michigan following zebra mussel invasion [80]	*Dreissena polymorpha*	Mollusc	G
Reissig M, Trochine C, Queimaliños C, Balseiro E, Modenutti B	Impact of fish introduction on planktonic food webs in lakes of the Patagonian Plateau [81]	Several fish spp.	Fish	G
Ricciardi A, Whoriskey FG, Rasmussen JB	The role of the zebra mussel (*Dreissena polymorpha*) in structuring macroinvertebrate communities on hard substrata [82]	*Dreissena polymorpha*	Mollusc	G
Rivera Vasconcelos F, Menezes RF, Attayde JL	Effects of the Nile tilapia *(Oreochromis niloticus L.*) on the plankton community of a tropical reservoir during and after an algal bloom [83]	*Oreochromis niloticus*	Fish	T
Rösch R, Baer J, Brinker A	Impact of the invasive three-spined stickleback (*Gasterosteus aculeatus*) on relative abundance and growth of native pelagic whitefish (*Coregonus wartmanni*) in Upper Lake Constance[84]	*Gasterosteus aculeatus*	Fish	T
Ruokonen TJ, Karjalainen J, Hämäläinen H	Effects of an invasive crayfish on the littoral macroinvertebrates of large boreal lakes are habitat specific [85]	*Pacifastacus leniusculus*	Crustacean	T
Ruokonen TJ, Ercoli F, Hämäläinen H	Are the effects of an invasive crayfish on lake littoral macroinvertebrate communities consistent over time? [86]	*Pacifastacus leniusculus*	Crustacean	T

Scharfy D, Eggenschwiler H, Olde Venterink H, Edwards PJ, Güsewell S	The invasive alien plant species Solidago gigantea alters ecosystem properties across habitats with differing fertility [87]	*Solidago gigantea*	Plant	G
Schilling EG, Loftin CS, Huryn AD	Effects of introduced fish on macroinvertebrate communities in historically fishless headwater and kettle lakes [88]	*Salvelinus fontinalis*, Several fish spp.	Fish, Fish	G
Schrank AJ, Lishawa SC	Invasive cattail reduces fish diversity and abundance in the emergent marsh of a Great Lakes coastal wetland [89]	*Typha* spp.	Plant	T
Shulse CD, Semlitsch RD, Trauth KM	Mosquitofish dominate amphibian and invertebrate community development in experimental wetlands [90]	*Gambusia affinis*	Fish	G
Shurin JB, Cottenie K, Hillebrand H	Spatial autocorrelation and dispersal limitation in freshwater organisms [91]	*Lepomis macrochirus*	Fish	G
Stewart TW, Haynes JM	Benthic macroinvertebrate communities of southwestern Lake Ontario following invasion of Dreissena [92]	*Dreissena polymorpha*	Mollusc	G
Strecker AL, Arnott SE	Invasive predator, Bythotrephes, has varied effects on ecosystem function in freshwater lakes [93]	*Bythotrephes longimanus*	Crustacean	G
Strecker AL, Arnott SE, Yan ND, Girard R	Variation in the response of crustacean zooplankton species richness and composition to the invasive predator *Bythotrephes longimanus* [94]	*Bythotrephes longimanus*	Crustacean	G
Strecker AL, Beisner BE, Arnott SE, Paterson AM, Winter JG, Johannsson OE, Yan ND	Direct and indirect effects of an invasive planktonic predator on pelagic food webs [95]	*Bythotrephes longimanus*	Crustacean	G
Tumolo BB, Flinn MB	Top-down effects of an invasive omnivore: detection in long-term monitoring of large-river reservoir chlorophyll-a [96]	*Hypophthalmichthys molitrix*	Fish	T
Volta P, Jeppesen E, Leoni B, Campi B, Sala P, Garibaldi L, Lauridsen TL, Winfield IJ	Recent invasion by a non-native cyprinid (common bream *Abramis brama*) is followed by major changes in the ecological quality of a shallow lake in southern Europe [97]	*Abramis brama*	Fish	G
Waajen GWAM, Van Bruggen NCB, Pires LMD, Lengkeek W, Lürling M	Biomanipulation with quagga mussels (*Dreissena rostriformis bugensis*) to control harmful algal blooms in eutrophic urban ponds [98]	*Dreissena rostriformis bugensis*	Mollusc	T
Wilson SJ, Ricciardi A	Epiphytic macroinvertebrate communities on Eurasian watermilfoil (*Myriophyllum spicatum*) and native milfoils *Myriophyllum sibericum* and *Myriophyllum alterniflorum* in eastern North America [99]	*Myriophyllum spicatum*	Plant	G

Wittmann ME, Chandra S, Reuter JE, Caires A, Schladow SG, Denton M	Harvesting an invasive bivalve in a large natural lake: species recovery and impacts on native benthic macroinvertebrate community structure in Lake Tahoe, USA [100]	*Corbicula fluminea*	Mollusc	G
Yuckin S, Rooney R	Significant increase in nutrient stocks following *Phragmites australis* invasion of freshwater meadow marsh but not of cattail marsh [101]	*Phragmites australis*	Plant	T
Zambrano L, Perrow MR, Macías-García C, Aguirre-Hidalgo V	Impact of introduced carp (*Cyprinus carpio*) in subtropical shallow ponds in Central Mexico [102]	*Cyprinus carpio*	Fish	G
Zhu, B., Kopco J, Rudstam LG	Effects of invasive European frogbit and its two physical control methods on macroinvertebrates [103]	*Hydrocharis morsus-ranae*	Plant	T

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Table A2 Invasive species grouped by taxonomy. This table outlines the species which are included in each of the taxonomic groupings used in this study.

Fish Species	
Cyprinus carpio	*Salvelinus fontinalis*
Gambusia affinis	*Oncorhynchus mykiss*
Gymnocephalus cernuus	*Micropterus salmoides*
Gambusia holbrooki	*Micropterus sp.*
Lepomis macrochirus	*Cichla ocellaris*
Neogobius melanostomus	*Cichla kelberi*
Procambarus clarkii	*Oreochromis niloticus*
Abramis brama	*Hypophthalmichthys molitrix*
Alosa pseudoharengus	*Gambusia spp*
Perca fluvialitis	*Tilapia rendalli*
Osmerus mordax	*Gasterosteus aculeatus*
Plant Species	
Alternanthera philoxeroides	*Lythrum salicaria*
Hydrocotyle ranunculoides	*Cabomba caroliniana*
Ludwigia grandiflora	*Solidago gigantea*
Myriophyllum aquaticus	*Eichhornia crassipes*
Gambusia affinis	*Phalaris arundinacea*
Typha angustifolia	*Typha sp.*
Phragmites australis	*Urochloa subquadripara*
Myriophyllum spicatum	*Potentilla anserina*
Vallisneria spiralis	*Hydrocharis morsus-ranae*
Typha glauca	
Molluscs	
Dreissena polymorpha	*Potamopyrgus antipodarum*
Bellamya chinensis	*Dreissena rostriformis bugensis*
Corbicula fluminea	*Limnoperna fortunei*
Pomacea canaliculata	
Crustaceans	
Procambarus clarkii	*Pacifastacus leniusculus*
Orconectes rusticus	*Cercopagis pengoi*
Bythotrephes longimanus	*Dikerogammarus villosus*

Table A3 Summary of results for the sensitivity analyses and publication bias. Change indicates if after removing potential outliers the outcome of the analysis was significantly different. The symbol "-" denotes effects excluded from the analysis due to their being <5 cases available.

Effect size metric	**Random-effects model (before)**			**Potential publication bias test**		**Outliers**	**Random-effects model (after)**			
	n	**SMD**	**±95% CI**	**z-value**	**p-value**	**n**	**n**	**SMD**	**±95% CI**	**Change**
Impacts of invasive fish										
Plants	16	-1.1516	0.7564	-0.2853	0.7754	2	14	-1.3033	0.7454	No
Fish	9	-0.5046	1.2588	-0.4	0.6892	1	8	-0.6481	1.0184	No
Phytoplankton	37	0.6894	0.5437	-3.0404	0.0024	2	35	0.6038	0.5428	No
Zooplankton	64	-0.0838	0.4703	2.0037	0.0451	9	55	0.008	0.3806	No
Benthic Invertebrates	21	-1.2005	0.6276	0.9909	0.3218	2	19	-1.1824	0.6159	No
Nutrients	44	0.7405	0.5108	-1.9322	0.0533	4	40	0.6342	0.4099	No
Impacts of invasive plants										
Plants	8	0.9703	1.8713	-0.7353	0.4622	2	6	0.2886	1.158	No
Fish	4	-	-	-	-	-	-	-	-	-
Phytoplankton	2	-	-	-	-	-	-	-	-	-
Zooplankton	1	-	-	-	-	-	-	-	-	-
Benthic Invertebrates	27	-0.5229	0.7107	0.9393	0.3476	0	27	-0.5229	0.7107	No
Nutrients	17	0.5608	0.7789	0.9211	0.357	4	13	0.4236	0.5809	No
Impacts of invasive molluscs										
Plants	2	-	-	-	-	-	-	-	-	-
Fish	4	-	-	-	-	-	-	-	-	-
Phytoplankton	19	-0.7542	0.6571	0.6598	0.5094	0	19	-0.7542	0.6571	No
Zooplankton	16	-1.8529	2.3082	3.4177	0.0006	6	10	-1.5186	1.8405	No
Benthic Invertebrates	35	1.0943	0.8833	1.1965	0.2315	7	28	1.3144	0.6949	No
Nutrients	12	0.0544	1.2872	2.768	0.0056	0	12	0.0544	1.2872	No
Impacts of invasive crustaceans										
Plants	6	-1.7791	1.5036	2.3006	0.0214	0	6	-1.7791	1.5036	No
Fish	0	-	-	-	-	-	-	-	-	-
Phytoplankton	11	0.1744	0.682	-0.6978	0.4853	0	11	0.1744	0.682	No
Zooplankton	10	-0.9612	0.9331	2.1538	0.0313	2	8	-0.7958	0.7917	No
Benthic Invertebrates	48	-0.2769	0.5236	-3.4834	0.0005	2	46	-0.3048	0.4767	No
Nutrients	5	-0.6191	1.1608	1.4746	0.1403	0	5	-0.6191	1.1608	No

Supplementary Methods

Collation of publications and inclusion criteria

Tang et al., (2020 [*PhD Thesis*]) collated additional published literature in order to supplement the publicly available database published by Gallardo et al., 2016(Gallardo et al., 2016). Tang et al., (2020[*PhD Thesis*]) implemented a literature search, following the methodology outlined by Gallardo et al., (2016), on Scopus (http://www.scopus.com/) for the terms: (invas* OR alien OR non-native OR exotic) AND (lake OR river OR estuary OR wetland OR reservoir) AND (impact OR effect). An additional 3092 potential records published between 24 February 2014 and 30 December 2019 were considered for inclusion. Tang et al., (2020 [*PhD Thesis*]) used the following criteria, in alignment with Gallardo et al., (2016), for including additional records in the dataset: First, the study focused on taxa, including all animals, plants (including phytoplankton), and micro-organisms such as bacteria that spend any stage of their life cycle in freshwater water; Second, only field studies were included; Third, only studies that quantitatively compared control and experimental treatments or conditions were included; Fourth, only studies that reported measurements of mean, variability around the mean, and the numbers of replicates were included. After this criteria screening, Tang et al., (2020[*PhD Thesis*]) identified an additional 51 studies that assessed the impact of aquatic invasive species in freshwaters. Therefore, the final dataset with all published data collated up until 30 December 2019 contained 920 observations, across 167 studies for 68 invasive non-native species.

Collation of dataset

For each of the additional articles, Tang et al., (2020 [*PhD Thesis*]), used the following criteria to extract data into the database for meta-analysis, in line with those implemented by Gallardo et al., (2016):

1. When multiple experimental treatments (e.g. several NNIS and densities of NNIS), control treatments (e.g. native species vs. no species), habitat types, and sampling locations were examined separately within the same article, they were entered into the database as separate observations (i.e. each observation was a row in the combined database), as each of these represents different observations of ecological impact.

2. When response variables were measured at multiple time points, only the data from the first and last available sampling events were used.

3. For studies reporting native community measures, such as abundance and diversity, and data on individual native species, data on community measures were extracted along with data on the two most abundant or focal native species.

4. Impacts of NNIS were classified using the following metrics: abundance (comprising density, biomass, and coverage), richness (such as species richness and the Shannon and the Simpson indices), biogeochemical elements (including total nitrogen, nitrate, ammonia, and organic nitrogen, total phosphorus, and phosphate), organic matter, and pollution (including water turbidity and heavy metal concentration).
5. When multiple recipient community measures were reported (e.g., species richness and the Shannon and the Simpson indices), species richness was extracted.
6. Mean and variation around the mean (e.g. standard deviations, standard errors, or confidence intervals) of the response variables were extracted from tables, text from the published paper, or by using the program WebPlotDigitizer (https://automeris.io/WebPlotDigitizer/) when data were only shown in plots.
7. The type of invaders was also classified for each NNIS by its trophic position into five major groups: primary producer, herbivore, omnivore, detritivore, and predator.
8. Given that many affected organisms were not reported at the species level, the type of organisms in recipient ecosystems were classified into seven functional (rather than trophic) groups following Gallardo et al. (2016): bird, fish, benthic invertebrate, zooplankton, phytoplankton, macroflora (i.e. vascular plants and seaweed), and microbe (i.e. bacteria and fungi).

Appendix B

Supplementary Information for Chapter 3

Contents

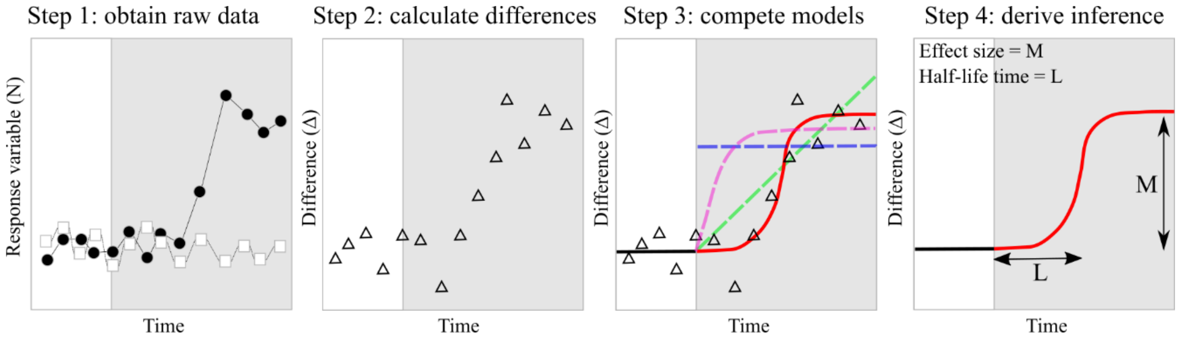
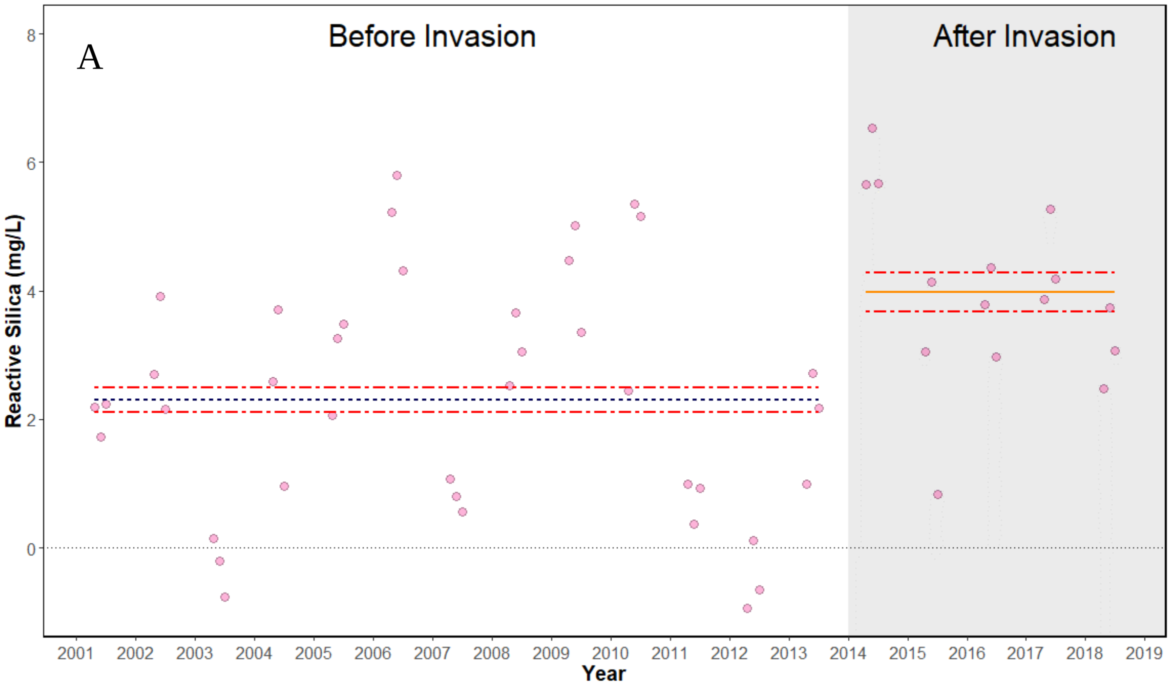
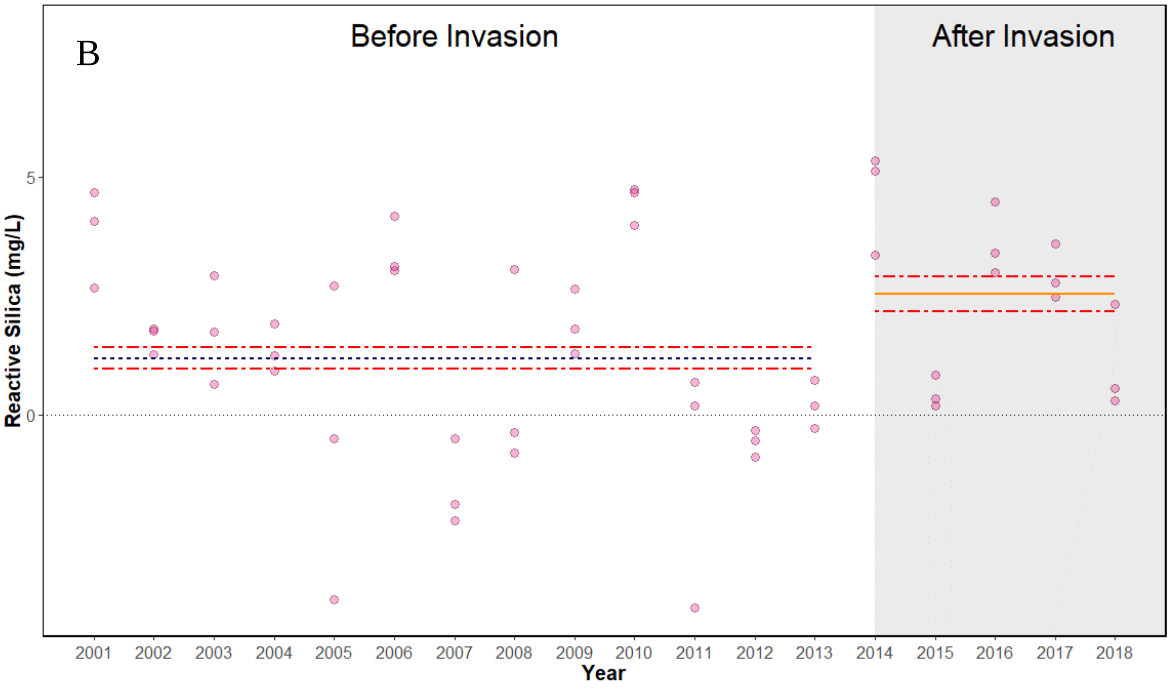


Figure B1 The steps required to undertake Progressive-Change BACIPS analysis. Step 1: a response variable (N), such as cyanobacterial biomass (cells/ml), is measured simultaneously at both impacted (black circles) and control (white squares) sites at multiple time points both before (white background) and after (grey background) the impact; Step 2: calculate the differences in the response variable at each timepoint (white triangles); Step 3: competing models are fitted to the differences; Step 4: the best model (lowest AICc score) is used to draw inferences about the shape and effect size (M) of the impact. Figure is adapted from Thiault et al., (2017).

Reference

Thiault, L., Kernaléguen, L., Osenberg, C.W., Claudet, J., 2017. Progressive‐Change BACIPS: a flexible approach for environmental impact assessment. *Methods Ecol. Evol.* 8, 288–296. https://doi.org/10.1111/2041-210X.12655


A
Before Invasion
After Invasion
Reactive Silica (mg/L)
8
6
4
2
0
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018
2019
Year


B
Before Invasion
After Invasion
Reactive Silica (mg/L)
5
0
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018
Year

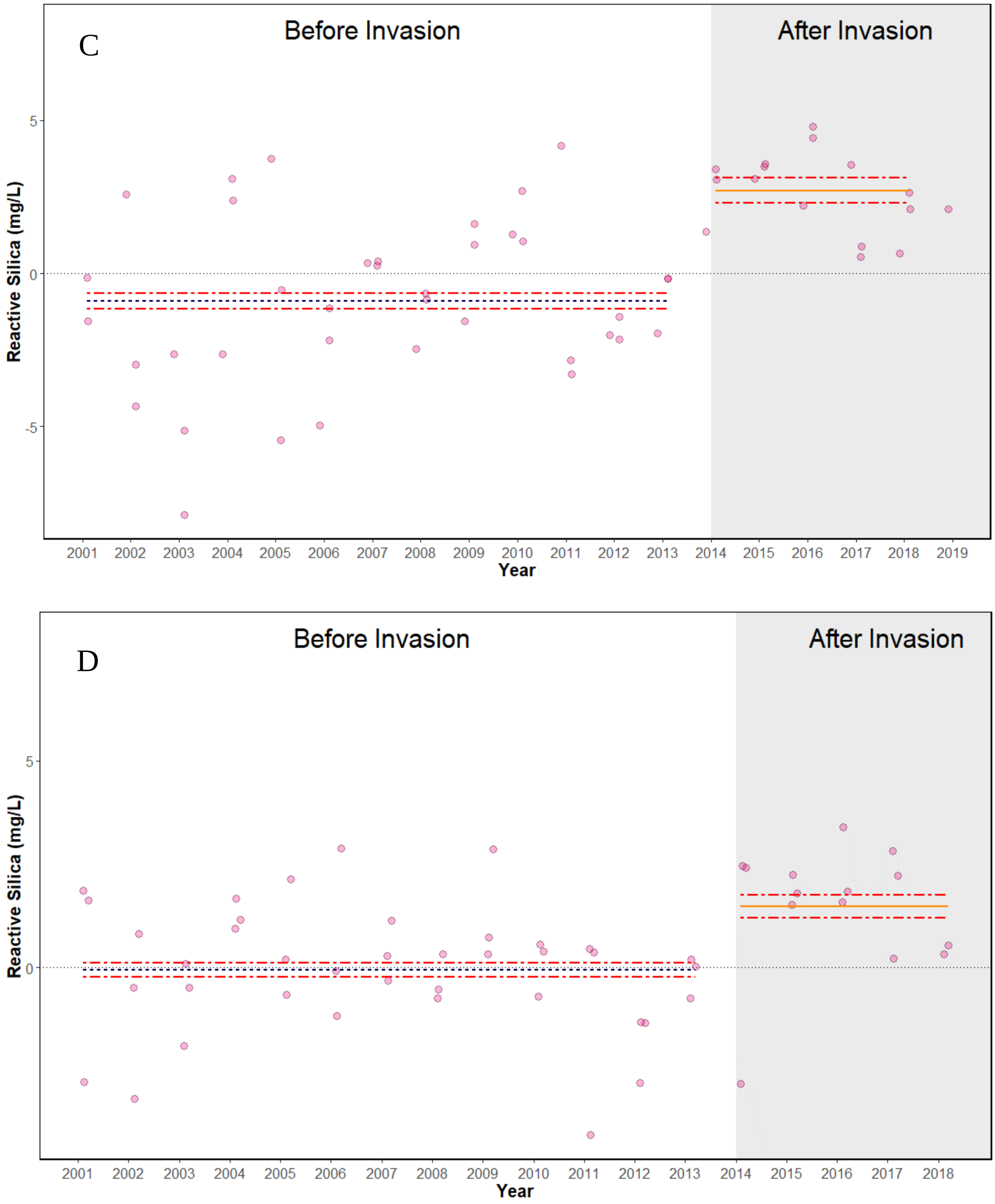


Figure B2 The changes in reactive silica (mg/L) before (white background) and after (grey background) the arrival of quagga mussels. (A) Spring; (B) Summer; (C) Autumn; (D) Winter. The points demonstrate the differences between the impact and control sites (impact-control), values above or below the horizontal dotted line at zero indicate where the value of the variable is higher or lower at impacted sites relative to control sites, respectively. The blue dashed line and the orange solid line indicate the GLM predicted values for the response variable before and after the arrival of quagga mussels, respectively. The red two-dashed line illustrates the 95% confidence interval of the GLM predicted values.

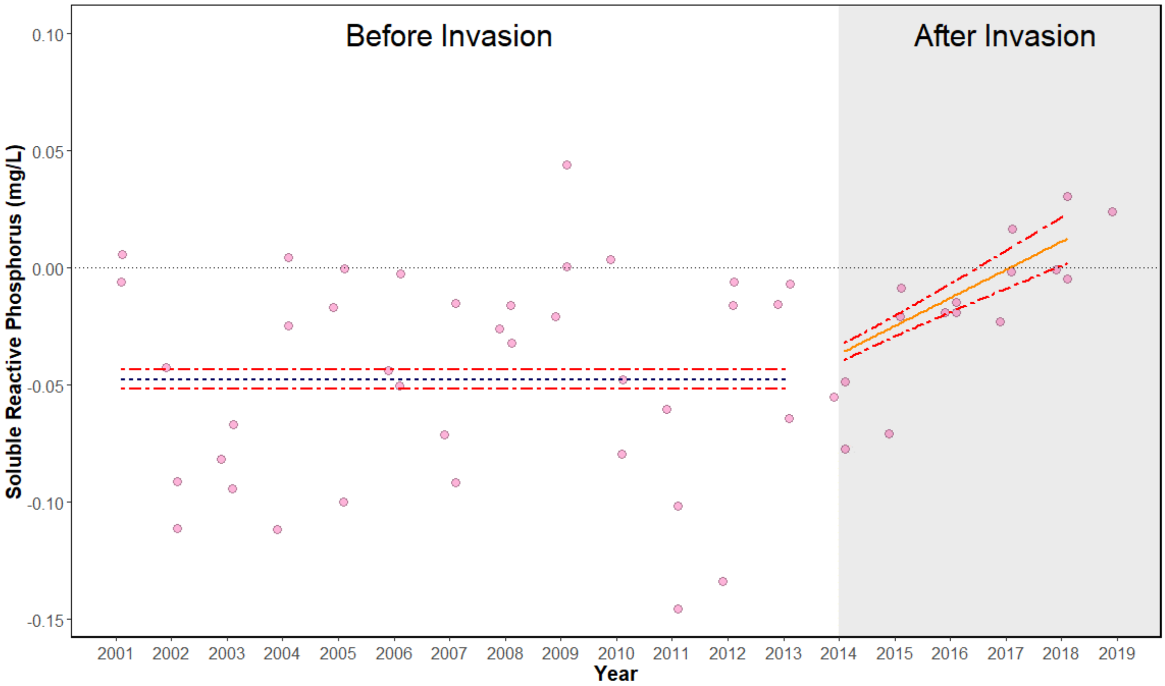
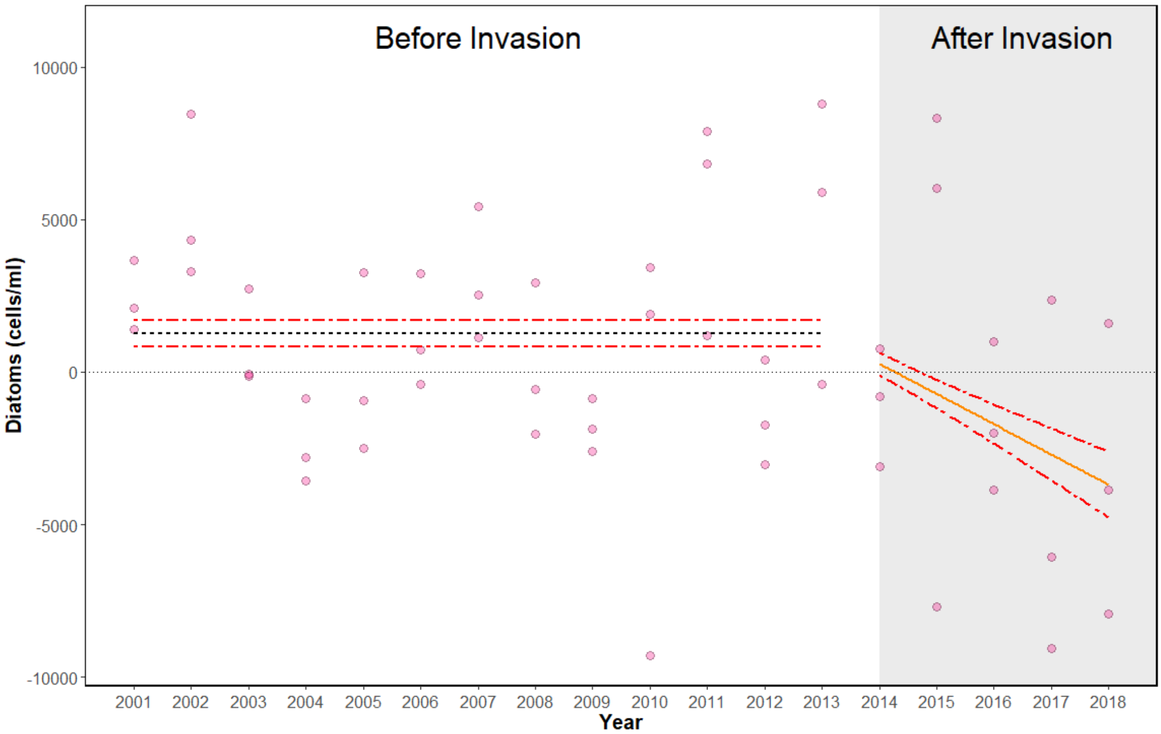
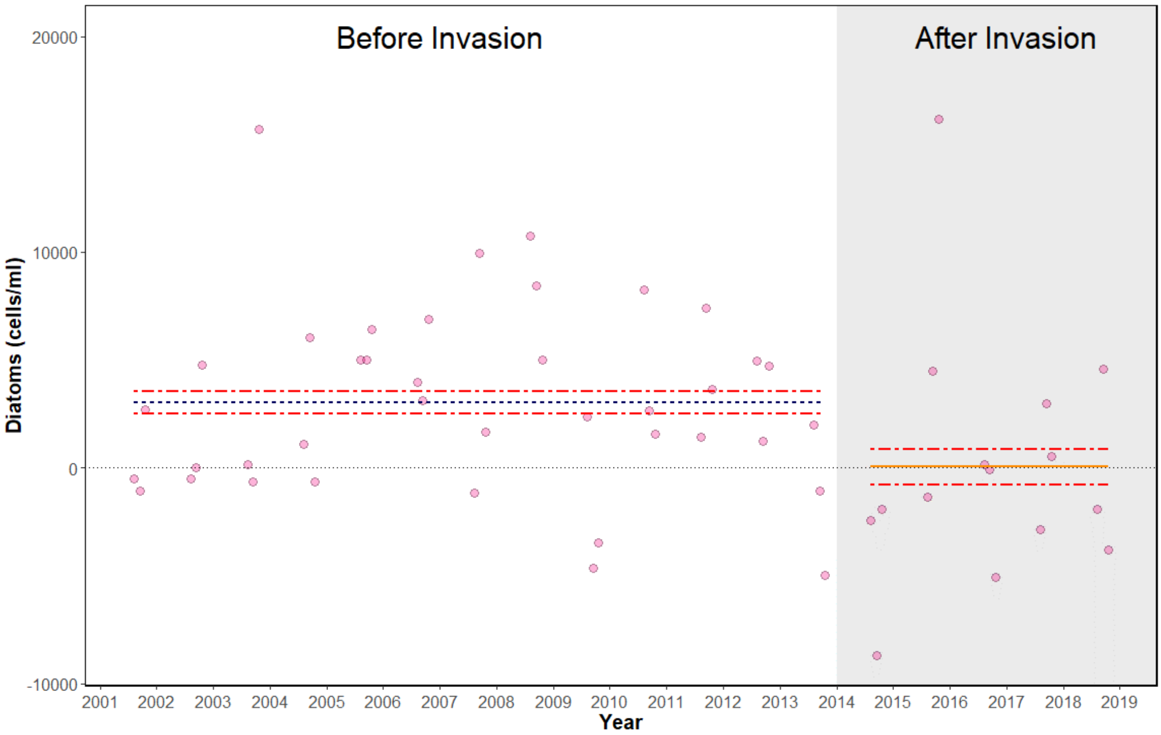


Figure B3 The changes in soluble reactive phosphorus (mg/L) before (white background) and after (grey background) the arrival of quagga mussels during autumn. The points demonstrate the differences between the impact and control sites (impact-control), values above or below the horizontal dotted line at zero indicate where the value of the variable is higher or lower at impacted sites relative to control sites, respectively. The blue dashed line and the orange solid line indicate the GLM predicted values for the response variable before and after the arrival of quagga mussels, respectively. The red two-dashed line illustrates the 95% confidence interval of the GLM predicted values.


Before Invasion
After Invasion
Diatoms (cells/ml)
10000
5000
0
-5000
-10000
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018
Year


Before Invasion
After Invasion
Diatoms (cells/ml)
20000
10000
0
-10000
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018
2019
Year

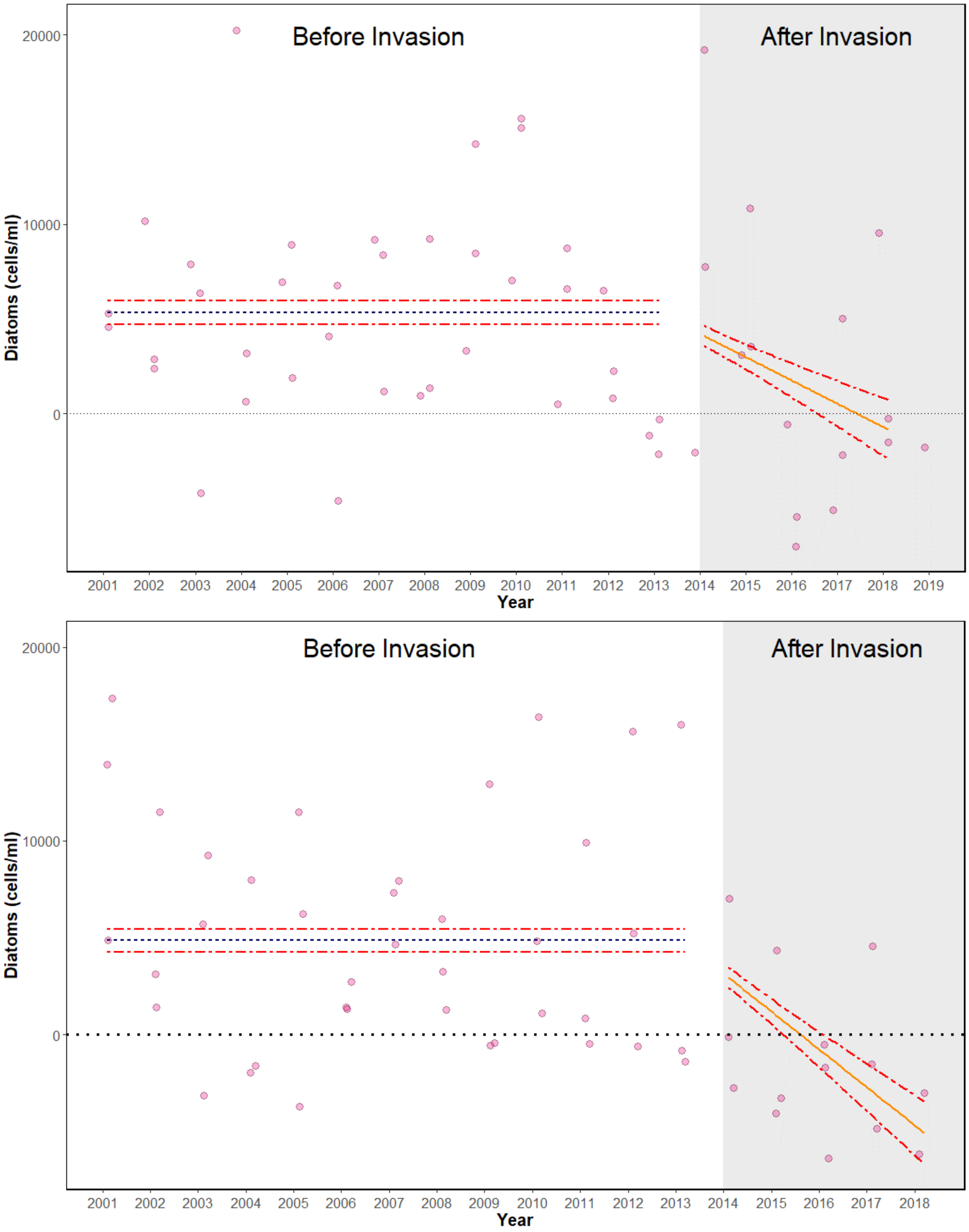


Figure B4 The changes in diatom abundance (cells/ml) before (white background) and after (grey background) the arrival of quagga mussels. (A) Spring; (B) Summer; (C) Autumn; (D) Winter. The points demonstrate the differences between the impact and control sites (impact-control), values above or below the horizontal dotted line at zero indicate where the value of the variable is higher or lower at impacted sites relative to control sites, respectively. The blue dashed line and the orange solid line indicate the GLM predicted values for the response variable before and after the arrival of quagga mussels, respectively. The red two-dashed line illustrates the 95% confidence interval of the GLM predicted values.

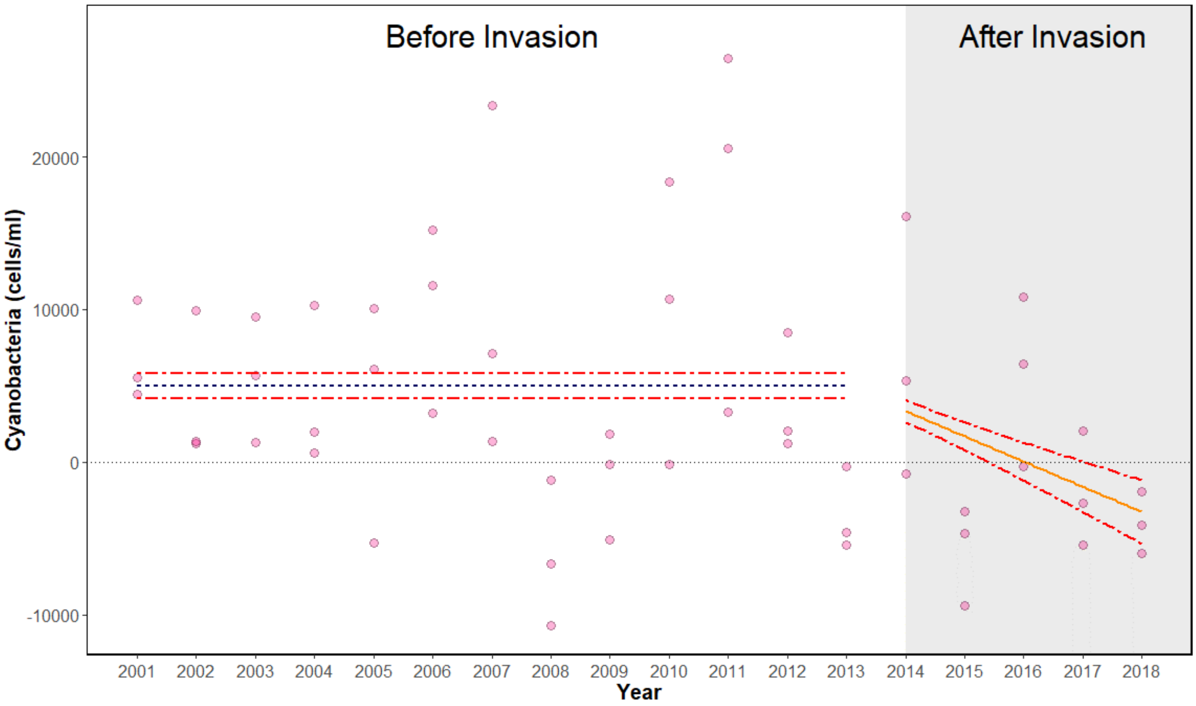


Figure B5 The changes in cyanobacteria abundance (cells/ml) before (white background) and after (grey background) the arrival of quagga mussels during autumn. The points demonstrate the differences between the impact and control sites (impact-control), values above or below the horizontal dotted line at zero indicate where the value of the variable is higher or lower at impacted sites relative to control sites, respectively. The blue dashed line and the orange solid line indicate the GLM predicted values for the response variable before and after the arrival of quagga mussels, respectively. The red two-dashed line illustrates the 95% confidence interval of the GLM predicted values.

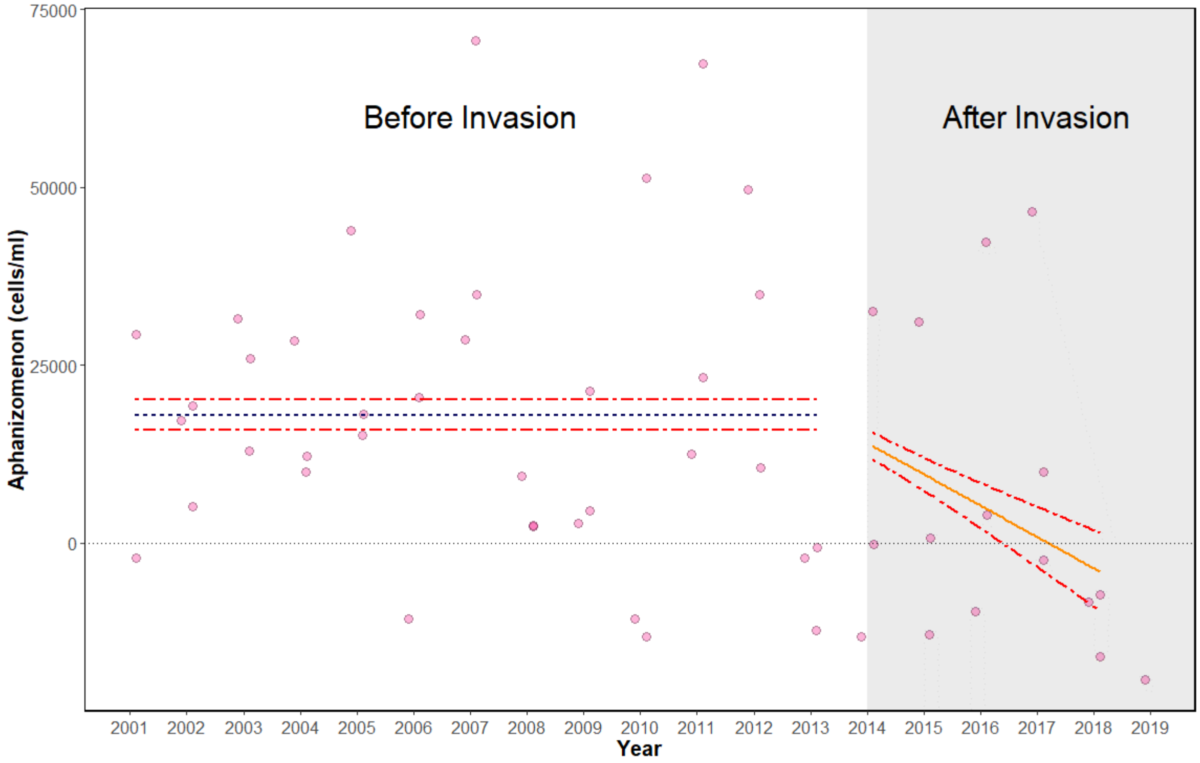


Figure B6 The changes in Aphanizomenon sp. abundance (cells/ml) before (white background) and after (grey background) the arrival of quagga mussels during autumn. The points demonstrate the differences between the impact and control sites (impact-control), values above or below the horizontal dotted line at zero indicate where the value of the variable is higher or lower at impacted sites relative to control sites, respectively. The blue dashed line and the orange solid line indicate the GLM predicted values for the response variable before and after the arrival of quagga mussels, respectively. The red two-dashed line illustrates the 95% confidence interval of the GLM predicted values.

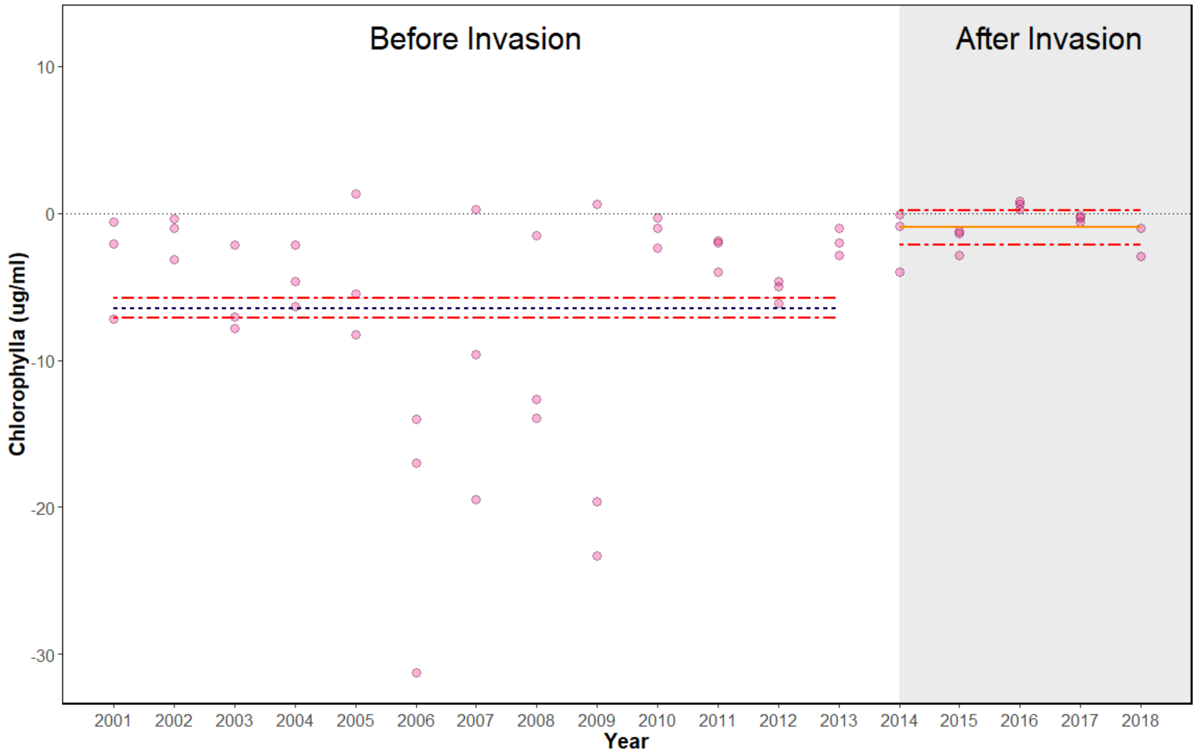


Figure B7 The changes in Chlorophyll a abundance (ug/ml) before (white background) and after (grey background) the arrival of quagga mussels during winter. The points demonstrate the differences between the impact and control sites (impact-control), values above or below the horizontal dotted line at zero indicate where the value of the variable is higher or lower at impacted sites relative to control sites, respectively. The blue dashed line and the orange solid line indicate the GLM predicted values for the response variable before and after the arrival of quagga mussels, respectively. The red two-dashed line illustrates the 95% confidence interval of the GLM predicted values

Appendix C

Supplementary Information for Chapter 5

Contents

Table C1 Previous Work on allelopathic effects of aquatic plants on algae and cyanobacteria. This table outlines all the current work which has been undertaken concerning the allelopathic effect of aquatic plants on algae and cyanobacteria. It outlines the species of plant used, the method applied to the plant material, the allelochemicals found to be contained within the relevant species, and the algae and/or cyanobacteria the plant was shown to affect. This table is adapted from Mohamed, 2017, with additional experimental results added. The list of references cited follow the table.

Species	*Plant material tested*	*Allelochemicals Contained*	*Algae and/or Cyanobacteria Inhibited*	*Reference*
Acorus calamus	Extract	Phenylpropanes	*Anabaena flos-aquae, Aphanizomenon flos-aquae, Microcystis aeruginosa*	(Greca et al., 1989; Zhang, 2015; Zhang et al., 2016)
Acorus gramineus	Extract	Phenylpropanes	*Microcystis aeruginosa*	(Greca et al., 1989; Nakai, S., Zou, G., Okuda, T., Tsai, T.-Y., Song, X., Nishijima, W., Okada, 2010)
Acorus tatarinowii	Exudate	Phenylpropanes	*Anabaena flos-aquae, Synecococcus leopoliensis, Microcystis aeruginosa*	(Greca et al., 1989; He and Wang, 2001)
Arundo donax	Extract	Gramine (N,N-dimethyl-3-amino-methylindole)	*Microcystis aeruginosa*	(Hong et al., 2008, 2009)
Eleocharis acicularis	Co-cultivation	Unknown	*Anabaena flos-aquae, Microcystis aeruginosa, Phormidium tenue*	(Nakai et al., 1999)
Phragmites australis	Extract	Unknown	*Microcystis aeruginosa, Scenedesmus acutus*	(Nakai, S., Zou, G., Okuda, T., Tsai, T.-Y., Song, X., Nishijima, W., Okada, 2010; Chicalote-Castillo et al., 2017)
Phragmites communis	Extract Fraction	Ethyl 2-methylacetoacetate, phenolic acids (p-coumaric acid, ferulic acid), caffeic acid (gallic acid), fatty acid (stearic acid)	*Microcystis aeruginosa, Phormidium* sp.	(Zhou et al., 2004; Li and Hu, 2005)
Scirpus tabernaemontani	Extract	Unknown	*Microcystis aeruginosa*	(Nakai, S., Zou, G., Okuda, T., Tsai, T.-Y., Song, X., Nishijima, W., Okada, 2010)

Thalia dealbata	Extract	Unknown	*Anabaena flos-aquae, Microcystis aeruginosa*	(Zhang et al., 2011b)
Typha latifolia	Extract	Steroids, fatty acids.	*Anabaena flos-aquae, Microcystis aeruginosa*	(Aliotta et al., 1990)
Typha angustifolia	Extract	Phenic acids (o-hydroxycinnamic acid, syringic acid and isoferulic acid	*Synechococcus leopoliensis*	(Zhang et al., 2011a)
Brasenia scherberi	Extract	Unknown	*Anabaena flos-aquae*	(Elakovich and Wooten, 1987)
Cambomba caroliniana	Co-cultivation	Unknown	*Anabaena flos-aquae, Microcystis aeruginosa, Phormidium tenue*	(Nakai et al., 1999)
Eichhornia crassipes	Extract	N-phenyl-1-naphthylamine, linoleic acid, benzoindenone	*Anabaena azollae, Microcystis aeruginosa*	(Wu et al., 2012)
Lemma minor	Co-cultivation	Unknown	*Microcystis aeruginosa*	(Jang et al., 2007)
Nelumbo nucifera	Extract	Propanamide	*Microcystis aeruginosa*	(Hong et al., 2008; He, L.S., Meng, F.L., Diao, X.J., Li, Y.W., Meng, R., Xi, B.D., Shu, 2013)
Pista stratiotes	Extract	Polyphenols, linoleic acid, linolenic acid, fatty acids, steroidal ketones	*Microcystis aeruginosa*	(Aliotta et al., 1991; Wu et al., 2013)
Stratiotes aloides	Extract	Moderately lipophilic non-phenolic compounds	*Anabaena variabilis, Microcystis aeruginosa, Synechococcus elongatus*	(Mulderij et al., 2007; Mohamed and Al Shehri, 2010)
Ceratophyllum demersum	Extract	Element sulfides, labile sulfur compounds	*Microcystis aeruginosa, Anabaena* sp., *Synechococcus elongatus, A. variabilis.*	(Gross et al., 2003; Hong et al., 2008)
Egeria densa	Co-cultivation	Unknown	*Cyanobacteria*	(Nakai et al., 1999)
Elodea canadensis	Exudate/Extract	Phenolic compounds	*Epiphytic cyanobacteria*	(Erhard and Gross, 2006)
Elodea nuttalii	Exudate/Extract	Phenolic compounds	*Epiphytic cyanobacteria*	(Erhard and Gross, 2006)

Hydrilla verticillata	Exudate/Extract	Phenolic compound (vanillic acid, protocatechic acid, ferulic acid, caffeic acid)	*Microcystis aeruginosa*	(Wang, L.X., Zhang, L., Zhang, Y.X., Jin, C.Y., Lu, C.M., Wu, 2006; Y. Gao, B.Liu, D.Xu, Q. Zhou, C. Hu, F. Ge, L. Zhang, 2011; Zhang et al., 2012)
Limnophila sessiliflora	Co-cultivation	Unknown	*Cyanobacteria*	(Nakai et al., 1999)
Myriophyllum brasiliense	Extract	Polyphenol-like alleochemicals	*Microcystis aeruginosa*	(Saito et al., 1989)
Myriophyllum spicatum	Co-cultivation	Tellimagrandin II, pyrogallic acid, gallic acid, ellagic acid, (+)-catechin	*Microcystis aeruginosa*	(Nakai et al., 2005; Zhu et al., 2010)
Myriophyllum verticillatum	Extract	a-asarone, phenylpropane, glycoside-like allelochemicals	*Microcystis aeruginosa, Limnothrix redeke*	(Aliotta et al., 1992; Hilt, 2006)
Najas marina	Extract	Hydrophilic and moderately lipophilic allelochemicals	*Anabaena variabilis, Synechococcus elongatus*	(Gross et al., 2003)
Potamogeton malaianus	Co-cultivation/Exudate	Diterpenes, linolenic acid	*Microcystis aeruginosa*	(Hu and Hong, 2008; Zhang, S.H., Cheng, S.P., Wang, H.Q., He, F., Wu, 2009)
Potamogeton maackianus	Co-cultivation/Exudate	Diterpenes, linolenic acid	*Microcystis aeruginosa*	(Hu and Hong, 2008; Zhang, S.H., Cheng, S.P., Wang, H.Q., He, F., Wu, 2009)
Potamogeton pectinastusm	Co-cultivation/Exudate	Diterpenes, linolenic acid	*Microcystis aeruginosa*	(Hu and Hong, 2008; Zhang, S.H., Cheng, S.P., Wang, H.Q., He, F., Wu, 2009)
Potamogeton pusillus	Exudate	Unknown	*Microcystis aeruginosa*	(Takeda et al., 2011)
Potamogeton lucens	Extract	Unknown	*Anabaena variabilis*	(Jasser, 1995)
Potamogeton crispus	Co-cultivation/Exudate/Extract	Unknown	*Anabaena variabilis* *Microcystis aeruginosa*	(Pakdel et al., 2013)
Potamogeton oxyphyllys	Co-cultivation/Exudate/Extract	Unknown	*Anabaena variabilis* *Microcystis aeruginosa*	(Nakai et al., 1999)

Vallisneria denseserrulata	Co-cultivation	2-ethyl-3-methylmaldeimide, carotene derivatives	*Microcystis aeruginosa*	(Xian et al., 2006; Gao, Y., Liu, B., Xu, D., Zhou, Q., Hu, C., Ge, F., Zhang, L., Wu, 2011)
Chara aspra	Extract	4-methylthio-1,2-dithiolane and 5-hydroxy-1,2,3-trithiane.	*Anabaena cylindrica, A. torulosa, Anabaenopsis elenkinii, M. aeruginosa, Synechococcus* sp.	(Berger, J., Schagerl, 2004; Złoch et al., 2018)
Chara globularis	Extract	4-methylthio-1,2-dithiolane and 5-hydroxy-1,2,3-trithiane.	*Anabaena cylindrica, A. torulosa, Anabaenopsis elenkinii, M. aeruginosa*	(Berger, J., Schagerl, 2004; Złoch et al., 2018)
Nitellopsis obtuse	Extract	4-methylthio-1,2-dithiolane and 5-hydroxy-1,2,3-trithiane.	*Anabaena cylindrica, A. torulosa, Anabaenopsis elenkinii, M. aeruginosa*	(Berger, J., Schagerl, 2004; Złoch et al., 2018)
Nitella gracilis	Extract	4-methylthio-1,2-dithiolane and 5-hydroxy-1,2,3-trithiane.	*Anabaena cylindrica, A. torulosa, Anabaenopsis elenkinii, M. aeruginosa*	(Berger, J., Schagerl, 2004; Złoch et al., 2018)
Chara australis	Extract/Exudate	Unknown	*Anabaena variabilis*	(Pakdel et al., 2013)
Chara hispida	Exudate	Unknown	*Pseudanabaena* sp.	(Rojo et al., 2013)
Chara vulgaris	Exudate	Unknown	*Pseudanabaena* sp.	(Rojo et al., 2013)
Chara baltica	Exudate	Unknown	*Pseudanabaena* sp., *Synechococcus* sp.	(Rojo et al., 2013; Złoch et al., 2018)
Nitella hyalina	Exudate	Unknown	*Pseudanabaena* sp.	(Rojo et al., 2013)
Alternanthera philoxeroides	Pure Chemicals	Coumarin, L-hydroxybenzoic acid, protocatechuic acid, stearic acid, and L-aminobenz enesulfonic acid	*Chlorella pyrenoidosa*	(Zuo et al., 2016)
Chara canescens	Extract	Unknown	*Synechococcus* sp.	(Złoch et al., 2018)
Schoenoplectus californicus	Extract	Unknown	*Microcystis aeruginosa, Scenedesmus acutus*	(Chicalote-Castillo et al., 2017)

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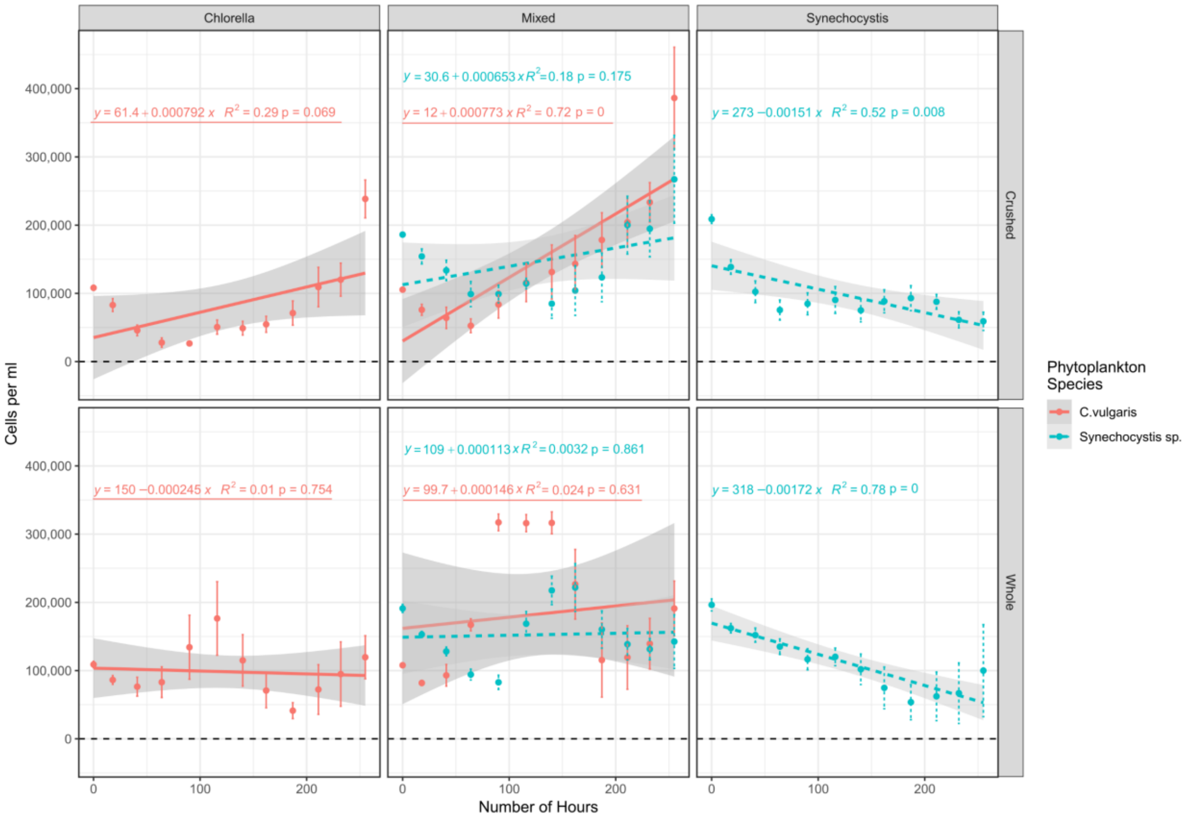
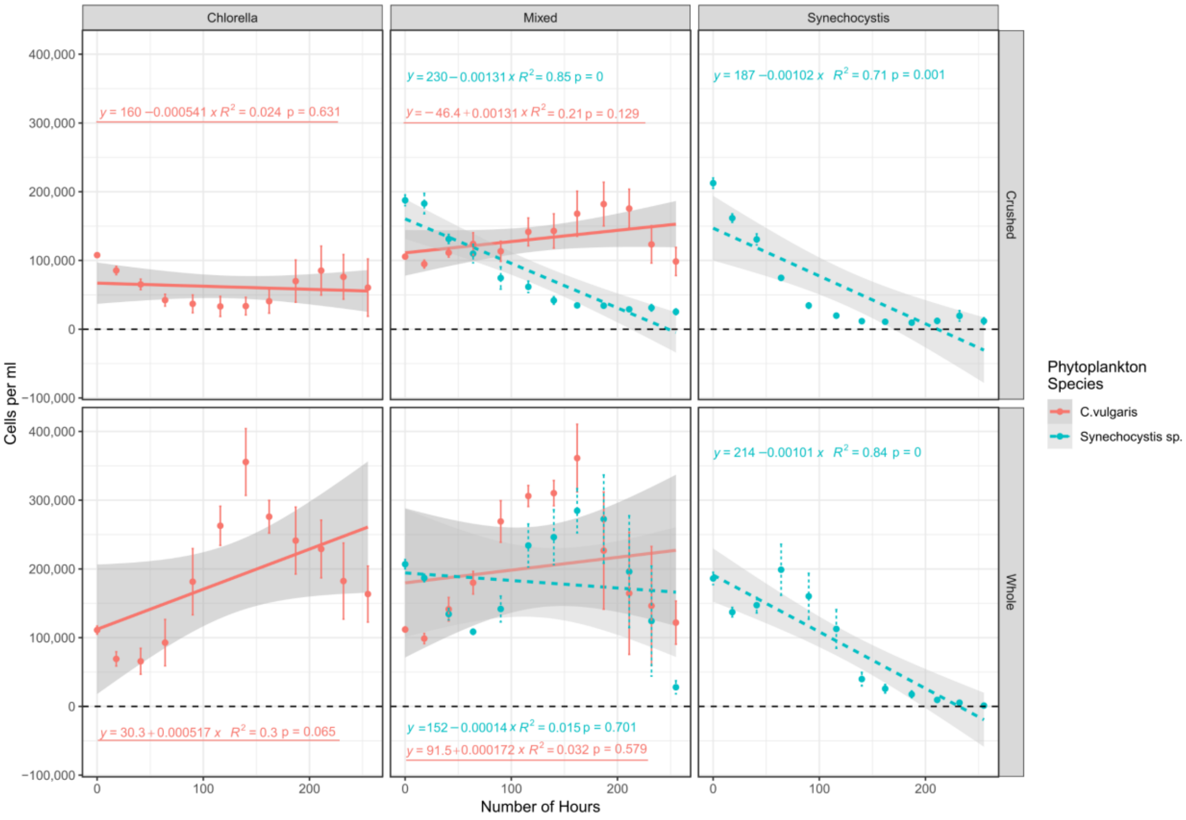
(a) Hydrocotyle ranunculoides



(b) Crassula helmsii



(c) Fake Plant

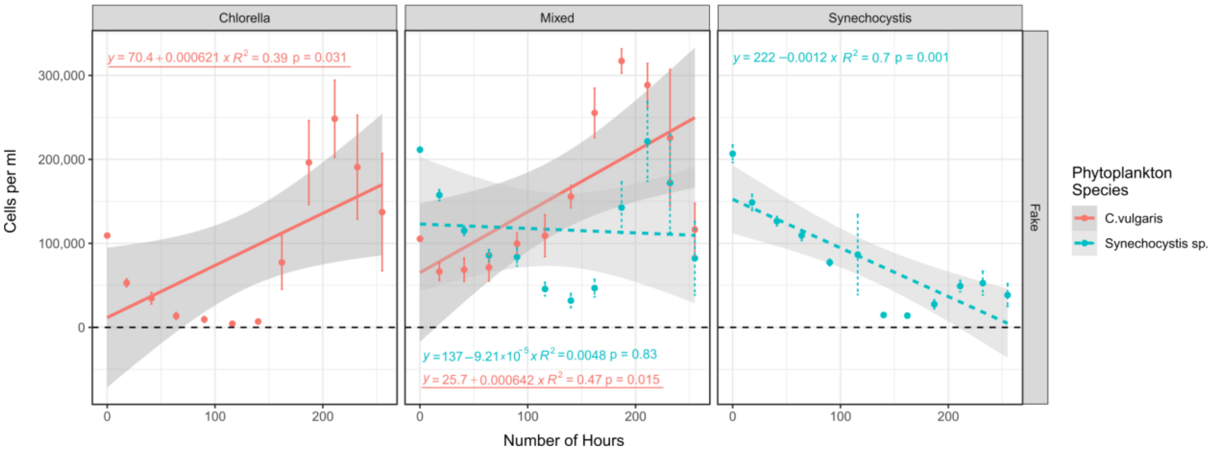


Figure C1 The change in the number of phytoplankton cells over time under varying conditions. All figures are linear regression models indicating the average change in the number of cells between the different treatments. The shaded grey area represents the 95% confidence interval. The dotted line across the bottom of each graph represents 0 cells per ml. The solid red line represents *Chlorella* cell numbers, and the dashed blue line represents *Synechocystis* cell numbers. The red and blue circles represent the average *Chlorella* and *Synechocystis* cell numbers at each time point respectively, the error bars indicate the standard error. The underlined red numbers indicate the intercept, coefficient, R^2 value and P value of the regression models relating to *Chlorella*. The blue, non-underlined numbers, represent the same values for the regression models relating to *Synechocystis*. Figures (a), (b) and (c), represent the results for each plant type, *H. ranunculoides*, *C. helmsii* and *Fake plant* respectively. The vertical facets (*Chlorella*, *Mixed* and *Synechocystis*) refer to monoculture treatments for each species, and the co-culture treatments. The horizontal facet (*Crushed*, *Whole* and *Fake*) refers to the state of the plant.