THE EFFECTS OF MICROPLASTICS ON FRESHWATER PHYTOPLANKTON AND ZOOPLANKTON COMMUNITIES IN A BOREAL LAKE

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Desiree Langenfeld
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Department of Entomology
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ABSTRACT

Langenfeld, Desiree. M.Sc., The University of Manitoba, May 2023. The Effects of Microplastics on Freshwater Phytoplankton and Zooplankton Communities in a Boreal Lake. Major Professor: Michael Paterson.

Our understanding of the potential impacts of microplastics (MPs) on freshwater ecosystems is limited. There is evidence that high MP concentrations can negatively impact phytoplankton and zooplankton under laboratory conditions, but community level effects under natural conditions are unknown. Two large scale in-situ mesocosm (limnocorral) experiments were conducted at the International Institute for Sustainable Development Experimental Lakes Area (IISD-ELA) in northwestern Ontario, Canada to assess the responses of phytoplankton and zooplankton communities to MP additions. In the first experiment, a mixture of common polymers (polyethylene; PE, polystyrene; PS, and polyethylene terephthalate; PET) were added in equal contributions to limnocorrals in a range of environmentally relevant nominal concentrations (0-29,240 MPs/L for all polymers together). In the second experiment, the same mixture of polymers was added to limnocorrals at a total concentration of 29,240 MPs/L each with and without chemical additives and these were compared to controls with no MPs to distinguish if there was a physical or chemical mechanism for MP toxicity. Phytoplankton biomass was not affected in either experiment, but there may be some potential for MPs to affect chlorophyll *a* production and community composition over time. There was weak evidence that zooplankton abundance and biomass were stimulated by MPs in both experiments, and copepod reproduction was slightly reduced. Overall, there was little evidence of significant negative impacts of MPs on the plankton communities.

FOREWORD

The format of this thesis is that of a grouped manuscript style or “sandwich” thesis. This thesis consists of a general introduction and literature review followed by two manuscripts intended for publication; Chapters 2 and 3 summarise experiments that investigate the impacts of microplastics for freshwater plankton communities, contributing novel findings to the existing literature. Chapter 4, the final chapter of this thesis, summarises these findings and provides recommendations for future research.

This thesis is an original work written in its entirety by Desiree Langenfeld. I performed all the statistical analyses, sample collections and zooplankton counts. The pELAstics project was conceived and designed by Drs. Chelsea Rochman, Diane Orihel, Jennifer Provencher, Michael Rennie, Michael Paterson and Matthew Hoffman. Drs. Chelsea Rochman, Michael Paterson, Diane Orihel, and Michael Rennie oversaw the deployment of the limnocorrals. Drs. Scott Higgins and Chelsea Rochman provided phytoplankton and microplastic counts. Dr. Sonya Havens oversaw the chemical analysis of water samples. Drs. Michael Paterson and Scott Higgins provided comments on earlier drafts of the thesis.

CHAPTER 1: INTRODUCTION & LITERATURE REVIEW

1.1 General Introduction

Plastic production has been rapidly increasing over the past 50 years, sparking concern about impacts to aquatic ecosystems (Anderson et al. 2016). As plastic breaks down into smaller pieces called microplastics (MPs), they can interact with a wide variety of organisms, including plankton communities. Zooplankton are an important trophic link in freshwater food webs and have been shown to be negatively impacted by MPs in the laboratory (Lee et al. 2013; Cole et al. 2015; Ogonowski et al. 2016). MPs also have the potential to negatively impact phytoplankton, the foundation of freshwater food webs (Bhattacharya et al. 2010; Sjollema et al. 2016; Mao et al. 2018; Rani-Borges et al. 2021). However, research on the effects of MPs for phytoplankton and zooplankton are almost entirely limited to the laboratory. The main objective of this thesis was to understand the potential impacts of MPs for natural freshwater phytoplankton and zooplankton communities. This thesis investigates the potential impacts of MPs using large in-situ mesocosms (limnocorrals) to assess the responses of phytoplankton and zooplankton communities in response to MP additions.

1.2 Plastic Overview

Plastics are synthetic organic compounds that are made by polymerizing monomers typically derived from fossil fuels, and the process also often involves adding chemical additives to enhance the properties of the material (Lithner et al. 2011; Geyer et al. 2017). Since the commercial development of plastics in the 1930s and 1940s, plastics have become a dominant staple in the consumer marketplace because they are inexpensive to produce, durable and versatile (Jambeck et al. 2015; Geyer et al. 2017). Plastics are an important commodity in the global economy, being used for applications such as food packaging, clothing and textiles, medical supplies, and construction materials (Worm et al. 2017). Some of the most widely produced plastic polymers include polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinylchloride (PVC), polyethylene terephthalate (PET) and polyurethane (PUR) (Geyer et al. 2017). The chemical additives added to plastics commonly include plasticizers, light and heat stabilizers and antioxidants, each contributing to the desirable functional properties of many plastic products (Hahladakis et al. 2018). Plastic packaging, which is predominately composed of PE, PP and PET, constitutes the largest proportion of plastic production and use, followed by construction materials composed of PVC (Geyer et al. 2017). The service lifespan for approximately 40% of plastics is typically less than 1 month, creating a serious plastic waste issue (Hahladakis et al. 2018). It is estimated that only around 30% of all plastics ever produced are currently in use, with a majority of the remainder contained in landfills or in the natural environment (Geyer et al. 2017).

Over the past 50 years, plastic production has been rapidly increasing, resulting in an accumulation of plastic debris in aquatic ecosystems (Anderson et al. 2016). Plastic debris enters the environment from many sources including littering, dumping of plastic waste, abrasion from paints and tires, wastewater effluent, loss during waste collection, agricultural sludge, and from inappropriately managed landfill sites (Duis and Coors 2016). The majority of plastic in the environment comes from land based sources, entering freshwaters before finally being transported to marine environments (Jambeck et al. 2015). It is estimated that 4.8 to 12.7 million metric tons of plastic waste entered the ocean in 2010 and this number is predicted to increase by an order of magnitude by 2025 if no improvements are made to plastic waste management (Jambeck et al. 2015). Almost 10,000 metric tonnes of plastic debris enters the Laurentian Great Lakes alone each year, most of which is accumulated near major population centers (Hoffman and Hittinger 2017). Plastic polymers are slow to degrade, and concentrations in the environment will continue to increase as long as their production and release continues (Duis and Coors 2016). Plastic littered beaches and "garbage patches" in the ocean have increased interest by the public, environmental activist groups, and policy makers (Rochman et al. 2016). Evidence has accumulated of the risks plastic debris poses to aquatic organisms via ingestion, entanglement and smothering (Rochman et al. 2016). Sea turtles, marine mammals and seabirds are of particular concern since they have high rates of contamination (Rochman et al. 2016). Once in the environment, plastics are exposed to weathering processes such as ultraviolet (UV) radiation and mechanical abrasion from wind and waves, which leads to fragmentation and release of chemical additives, creating a different suite of hazards for aquatic organisms (Song et al. 2017).

1.3 Microplastics

Microplastics (MPs), defined as plastics less than 5 mm in size, have been emerging as a contaminant of concern due to the potential risks they pose for aquatic environments and biota (Anderson et al. 2016). MPs may be introduced to aquatic ecosystems as either primary MPs, which are intentionally produced as micro-sized particles, or as secondary MPs, which are formed from larger plastic debris that has been broken down by weathering processes (Anderson et al. 2016). Primary MPs include microbeads used in cosmetics and industrial applications for abrasives (i.e. beadblasting), and plastic pellets (nurdles), which are the precursors for larger plastic products (Botterell et al. 2019). Primary MPs may enter aquatic environments directly from accidental spillage, wastewater effluent, agricultural sludge, and atmospheric deposition (Baldwin et al. 2016). Wastewater treatment plants (WWTP) are not able to effectively remove all MPs and are a major source of MPs in the environment (Thompson 2015). Secondary MPs include fibers released from synthetic clothing and textiles, and fragments of larger plastic debris that breaks down following exposure to UV radiation, heat and abrasion from wind and waves (Thompson 2015). While secondary MPs come in a variety of shapes (e.g. films, foams, flakes), the most common types found in the environment are fragments and fibers (Koelmans et al. 2019).

MPs are ubiquitous contaminants and have been found in marine sediments and surface waters (Thompson 2015) as well as in the sediments and surface waters of freshwater lakes and rivers across the world (Eerkes-Medrano et al. 2015). MPs have been found in remote regions such as surface waters in the Canadian Arctic (Jones-Williams et al. 2021) and pristine mountain catchments in the French Pyrenees through atmospheric deposition (Allen et al. 2019). MP contamination varies spatially in aquatic environments (Eriksen et al. 2013), with concentrations up to 1,770 particles/L reported for surface waters in the Southern North Sea basin (Dubaish and Liebezeit 2013). Over time, MPs typically become coated with a biofilm (a process called biofouling), increasing their density and causing them to sink to the water bottom where they accumulate in sediment (Baldwin et al. 2016; Semcesen and Wells 2021). While a wide size range of MPs exist in the environment, there is a lack of standardization in data collection and methods for detecting particles <333 μm are underdeveloped (Rochman et al. 2016; Besseling et al. 2019). Because of this, MP concentrations in the environment are likely greatly underestimated (Besseling et al. 2019). Due to continuous fragmentation, it is further estimated that nanoplastic (<0.1 μm in size) particle concentrations are 10^{14} times higher than MP particle concentrations (Besseling et al. 2019). Methods for detecting nanoplastics are even less developed than for MPs. MPs are considered complex contaminants due to the many variations in their polymer composition, chemical additives, shape and size and there is currently no scientific consensus on their toxicity in natural environments (Bucci et al. 2020).

1.3.1 Toxicology of Microplastics

MPs can have toxic effects on a wide range of aquatic organisms from primary producers to higher trophic levels including benthic macroinvertebrates, zooplankton, and fish (Cole et al. 2015; Mao et al. 2018; Besseling et al. 2019; Wang et al. 2020). The main pathways of MPs toxicity in aquatic organisms are ingestion, leaching of chemical additives, and increased exposure to other contaminants (Anderson et al. 2016). The most likely interaction between aquatic organisms and MPs is ingestion because their small size makes them available to a wide range of biota (Egbeocha et al. 2018). Exposure to sunlight can result in photo-degradation and oxidation of plastic polymers that results in the leaching of toxic chemical additives such as plasticizers and stabilizers (Lithner et al. 2011; Cole et al. 2011; Horton et al. 2017). Toxicity of leachates from various plastic types is partially reflected in the levels of additives contained in the plastic, with higher additive content having more toxic effects (Gewert et al. 2021). Additionally, plastic exposed to UV light has been shown to produce more toxic chemical leachates than plastic that has not been exposed (Gewert et al. 2021). A study comparing the aquatic toxicity of plastic polymers with and without additives found that raw polymers without additives were non-toxic, while polymers containing common additives were toxic to copepods, microalgae and sea-urchins (Beiras et al. 2021).

The potential toxicity of MPs is also dependent on the monomers they contain, many of which are toxic, including polystyrene (PS), a suspected carcinogen, and polyethylene terephthalate (PET), a suspected endocrine disrupter (Lambert et al. 2017). Polyethylenes such as low density polyethylene (LDPE) are among the least hazardous plastic polymers, while polyurethane (PUR), polyacrylonitrile (PAN) and polyvinyl chloride (PVC) are among the most hazardous (Lithner et al. 2011). MPs that are hydrophobic also have the potential to adsorb organic and inorganic pollutants from the surrounding water, increasing the potential hazard for organisms (Egbeocha et al. 2018). MPs may also undergo trophic transfer and bioaccumulation in the aquatic food web, which could cause cascading effects for ecosystems (Setälä et al. 2014; Au et al. 2017; Chae et al. 2018).

1.3.2 Phytoplankton

Phytoplankton are essential primary producers in freshwater environments and are vital to ecosystem processes such as oxygen production and nutrient cycling, as well as serving as a food source for aquatic herbivores. MPs have been found to have negative impacts on algal growth, chlorophyll *a* production and photosynthesis and they may compromise cell morphology and cause oxidative stress at high concentrations

(Bhattacharya et al. 2010; Sjollema et al. 2016; Mao et al. 2018; Li et al. 2020; Rani-Borges et al. 2021). Toxic effects to phytoplankton may be dependent on the physical or chemical properties of MPs (Rummel et al. 2022). The negative effects of MP exposure may be physically attributed to shading, increased turbidity, and adhesion of MPs to the cell wall (Bhattacharya et al. 2010; Rani-Borges et al. 2021). Phytoplankton may also be negatively impacted due to the physical obstruction of CO_2 and nutrient uptake pathways (Bhattacharya et al. 2010; Prata et al. 2019b).

Chemical leachates from MP additives are also a potential threat to phytoplankton growth and photosynthetic activity (Rani-Borges et al. 2021). For example, Rummel et al. (2022) found that MPs with lower additive content had a less toxic effect on phytoplankton than MPs containing a higher additive load (Rummel et al. 2022). The size of MPs may also impact the severity of effects for phytoplankton. For example, Zhang et al. (2017) found that phytoplankton exposed to MPs 1 μm in size exhibited inhibition of growth, while MPs 1 mm in size had no effect. The negative effects to phytoplankton may only be temporary, with evidence of adaptive responses such as thickening of the cell wall leading to recovery (Yokota et al. 2017; Mao et al. 2018).

In contrast to the negative effects described above, MPs may also serve as a substrate for phytoplankton in a process called biofouling, which could potentially enhance algal growth (Canniff and Hoang 2018). With biofouling, microorganisms attach themselves to MP particles, resulting in a build-up of complex communities of bacteria, protozoa, and algae in the biofilm (Kooi et al. 2017; Vroom et al. 2017; Rogers et al. 2020). Under favorable light conditions, algae account for the majority of biomass in biofilms (Chen et al. 2020). Some studies have shown the potential for MPs to act as a substrate for phytoplankton that can actually increase their growth (Yokota et al. 2017; Canniff and Hoang 2018; Pan et al. 2022) and potentially change algal community structure (Chen et al. 2020; Hitchcock 2022). Biofilm formation begins immediately (Rummel et al. 2017) and is affected by temperature, salinity, nutrients and light irradiance (Nava and Leoni 2021). Biofouling and subsequent aggregate formation with phytoplankton can alter the fate of MPs in a variety of ways, including residence time in the water column, sedimentation, uptake by organisms, and vertical and horizontal transport of pollutants (Long et al. 2015; Rogers et al. 2020). Biofouling can cause otherwise buoyant plastic polymers to sink to bottom sediments where they can come into contact with benthic communities (Horton et al. 2017). Biofouling communities on MPs may also affect nutrient cycling by sorption of or transformation and release of nutrients to the environment (Chen et al. 2020). Biofouling additionally creates an edible coating that makes MPs more attractive as food for grazing herbivores such as zooplankton, potentially increasing their ingestion and making MPs more available to higher trophic levels (Vroom et al. 2017; Egbeocha et al. 2018; Amariei et al. 2022).

The majority of studies to date have focused on species specific responses, but MPs may have the ability to affect phytoplankton community composition (Chen et al. 2020; Hitchcock 2022). Freshwater phytoplankton communities are diverse and different taxonomic groups of phytoplankton may be affected to different extents by MPs. For example, Song et al. (2020) found that *Phaeodactylum tricornutum* growth was reduced by MPs while *Chlorella sp.* was able to adapt. Similarly, a microcosm study looking at the impacts of MPs on a natural phytoplankton community found that chlorophyte abundance was reduced while cyanobacteria abundance increased at high MP concentrations (Hitchcock 2022). An increase in cyanobacteria can result in toxic blooms that contain little nutrition for secondary consumers and cause stress that results in losses of biodiversity (Krztoń et al. 2019). Although there is currently no scientific consensus, current MP concentrations in the environment are not expected to have direct toxicity for phytoplankton (Prata et al. 2019b). However, indirect effects for phytoplankton communities could potentially cause disruptions in aquatic ecosystems in terms of nutrient cycling and predator dynamics (Prata et al. 2019b; Pan et al. 2022).

1.3.3 Zooplankton

Zooplankton are an important link between primary producers and higher trophic level organisms and play an essential role in nutrient cycling and ecosystem structure and functioning (Sodré and Bozelli 2019; Kvale et al. 2021). Zooplankton are a food source for many aquatic invertebrates, adult zooplanktivorous fish, and many fish species during the larval stages (Piscia et al. 2019). Two important and abundant groups of freshwater crustacean zooplankton are cladocerans, which are herbivorous non-selective filter feeders, and copepods, which are selective current feeders and ambush predators (Sodré and Bozelli 2019). Zooplankton are sensitive to anthropogenic stressors and environmental change and are an important indicator for ecological disturbance (Xiong et al. 2020).

Ingestion of MPs by various zooplankton taxa has been demonstrated (Botterell et al. 2019), but the ecotoxicological risks remain uncertain (Kong and Koelmans 2019) and almost all studies to date have been conducted only in the laboratory. Consequences of

MP ingestion include physical blockage and damage of digestive tracts and exposure to chemical additive leachates (Kooi et al. 2017). Ingestion of MPs has been shown to physically impede zooplankton feeding (i.e. food dilution effect), resulting in less algae being consumed and leading to energetic deficiencies, negatively impacting growth and reproduction and causing mortality at high concentrations (Lee et al. 2013; Cole et al. 2015; Ogonowski et al. 2016; Pan et al. 2022). Typically, ingestion and subsequent toxicity increases with higher MP concentrations (Pan et al. 2022). MP shape and morphology also affects physical toxicity, with irregular shapes (fragments and fibers) suggested to be more toxic than spherical MPs (i.e. microbeads; Frydkjær et al. 2017). MPs are typically egested within hours (Cole et al. 2013) but egestion of irregular shaped MPs is typically slower than that of smoother particles such as microbeads (Frydkjær et al. 2017). MP size is also an important physical factor, with smaller MPs typically producing more toxic effects than larger particles because they are more readily ingested and may enter tissues and cells more easily (Rehse et al. 2016). Aside from ingestion, MP particles may also physically adhere to inner and outer tissues and appendages of zooplankton, which may impair filtering and swimming activities and decrease fitness at high concentrations (Cole et al. 2013; Frydkjær et al. 2017).

MP ingestion may also provide a chemical route of exposure and toxicity for zooplankton. For example, exposure to leachates from chemical additives in polystyrene has been found to have toxic effects for zooplankton resulting in reduced reproductive output and mortality (Thaysen et al. 2018). Similarly, Beiras et al. (2021) found that raw polymers (PE, PVC, and PA resins) with no additives had no effects while the same polymers containing conventional chemical additives were toxic to copepods. Still it is uncertain whether the toxic effects of MPs are more physical or chemical in nature, or a combination of the two (Zimmermann et al. 2020).

In contrast to the negative effects described above, some studies have found no negative effects of MP ingestion on zooplankton and there may even be potential for MPs to benefit zooplankton (Vroom et al. 2017; Canniff and Hoang 2018; Beiras et al. 2018). The presence of biofilm on MPs is suspected to provide nutrition to zooplankton (Canniff and Hoang 2018; Amariei et al. 2022). For example, Amariei et al. (2022) found that biofouled MPs had a positive effect on zooplankton growth, while pristine MPs had a negative food dilution effect. This suggests that there may be a trade off for zooplankton, where the positive effects from the added nutrition of biofilm growth may offset the negative effects of MP ingestion (Amariei et al. 2022). Canniff & Hoang (2018) similarly found that survival and reproduction of *Daphnia magna* were unaffected by MP beads, but increased algal growth likely still provided nutrition to zooplankton counteracting any negative effects of the MPs.

Given that MPs may affect the growth, reproduction, and mortality of individual zooplankton in the laboratory there is potential for MPs to impact natural zooplankton communities, but this has rarely been studied. Field studies are primarily limited to the detection of ingested MPs, but the effects on zooplankton in the natural environment are unknown (Botterell et al. 2019). Ingestion of MPs by natural zooplankton has been found for calanoid copepods in the Northeast Pacific Ocean at an encounter rate of 0.029 particles per zooplankton (Desforges et al. 2015) and in the Black Sea at a rate of 0.024 particles per zooplankton (Aytan et al. 2022). Ingestion of MPs has also been detected in the seawaters of Malaysia for cladocerans and cyclopoid and calanoid copepods (Taha et al. 2021). MP ingestion for natural freshwater zooplankton has not yet been studied. MP ingestion by zooplankton in the wild is much lower than in the laboratory likely due to high concentrations and the absence of other food sources in the laboratory. Selectively feeding zooplankton, such as copepods, may actively avoid MPs (Xu et al. 2022).

Only one study to date has looked at the community level effects for zooplankton. Yildiz et al. (2022) used in-situ mesocosms to assess community level effects of a mixture of MPs and found that at environmentally relevant concentrations, zooplankton were unaffected in terms of biomass and species composition. At higher concentrations *Daphnia* biomass was reduced slightly, but all other taxonomic groups were not impacted (Yildiz et al. 2022). While MPs may not directly negatively impact zooplankton at environmentally relevant concentrations, MPs still have the potential to create indirect effects for aquatic ecosystems. For example, ingestion of MPs by zooplankton in the environment may reduce grazing pressure on phytoplankton, resulting in more phytoplankton biomass sinking directly to the benthos, and consequently reducing nutrient recycling via zooplankton excretion (Kvale et al. 2021). MPs could also indirectly alter predator-prey dynamics by altering zooplankton behavior and reproduction (Pan et al. 2022).

1.4 Knowledge Gaps

MP research to date has mainly focused on impacts to marine systems and organisms, while impacts to freshwater environments and biota remain understudied (Blettler et al. 2018; de Sá et al. 2018). This is despite the fact that the majority of plastic waste initially enters freshwaters before being transported to oceans (Besseling et al. 2017). Our understanding of the effects of MPs is also generally limited to laboratory studies with single species, the results of which are not easily translatable to natural communities (Bucci et al. 2020). Laboratory studies have focused on the effects of primary MPs using pristine microbeads that are not representative of the majority of MPs found in the environment (Ogonowski et al. 2016; Au et al. 2017). Secondary MPs such as fragments and fibers are the most commonly identified types of MP particles found in field studies (Duis and Coors 2016; Koelmans et al. 2019). Pristine MP particles used in many experiments may underestimate the uptake and effects on biota under realistic environmental conditions (Horton et al. 2017; Vroom et al. 2017; O’Connor et al. 2020; Amariei et al. 2022).

MP studies also tend to use MP concentrations that are much higher than concentrations currently found in the environment and there is a need to study the effects of environmentally relevant MP concentrations (Horton et al. 2017; Burns and Boxall 2018; Bucci et al. 2020). Comparison of current environmental concentrations and concentrations found to invoke effects in laboratory studies suggests that it is unlikely that MPs are directly causing toxic effects for plankton in the environment (Burns and Boxall 2018). However, indirect effects from MPs could have cascading impacts for aquatic ecosystems (Pan et al. 2022) and population and community level responses are not yet understood (Rochman et al. 2016; Besseling et al. 2019).

1.5 The pELAstic Project

The pELAstic project is a multi-collaborator study led by Dr. Chelsea Rochman (University of Toronto) with the objective of better understanding the physical and chemical fate of MPs and their impact on freshwater ecosystems across all levels of biological organization. A major component of the project included two large scale limnocorral studies that took place during the summers of 2021 and 2022 at the International Institute for Sustainable Development Experimental Lakes Area (IISD-ELA). The IISD-ELA is a remote research station located in northwest Ontario, Canada encompassing 58 freshwater lakes set aside for experimental research. The research conducted at the IISD-ELA has helped to improve our understanding of human impacts on the environment and inform policy and environmental practices for over 50 years. Limnocorrals are in-lake mesocosms that allow researchers to manipulate natural ecosystems and study the effects of anthropogenic contaminants. Both limnocorral studies were conducted in Lake 378, a small oligotrophic boreal lake.

In the first experiment, limnocorrals were dosed with a range of environmentally relevant MP concentrations and the fate and effects of the MPs were observed over a 10-week period. Chapter 2 of this thesis examines the responses of the freshwater phytoplankton and zooplankton communities in this first experiment. In the second experiment, limnocorrals were dosed with MPs containing chemical additives or MPs containing no chemical additives to determine if effects of MPs are physical or chemical in nature. The phytoplankton and zooplankton communities were monitored for 9 weeks, and Chapter 3 discusses the results. This work contributes to our understanding of how

MPs affect natural freshwater communities using environmentally relevant MP types and concentrations.

1.6 Objectives

The primary objective of this thesis is to improve our understanding of how MPs impact phytoplankton and zooplankton communities in freshwater ecosystems. The two limnocorral experiments have the following objectives:

Objective 1: Determine the impacts of a range of environmentally relevant MP concentrations on freshwater plankton communities.

- What are the relationships between MP concentration and phytoplankton biomass, community composition, and chlorophyll *a*?
- What are the relationships between MP concentration and zooplankton abundance, biomass, reproduction, and community composition?

Objective 2: Determine if the impacts of MPs for freshwater plankton communities are due to physical or chemical characteristics of MPs.

- How do MPs with and without additives affect phytoplankton biomass, community composition and chlorophyll *a*?
- How do MPs with and without additives affect zooplankton abundance, biomass, reproduction, and community composition?

CHAPTER 2: EFFECTS OF ENVIRONMENTALLY RELEVENT MICROPLASTIC CONCENTRATIONS ON FRESHWATER PLANKTON COMMUNITIES

2.1 Abstract

Microplastics (MPs) are ubiquitous contaminants within aquatic and terrestrial environments globally, but their potential impacts on freshwater planktonic communities are not well understood. Existing research is primarily limited to laboratory studies that typically look at individual or population level effects using concentrations much higher than what is encountered in natural systems. To assess the potential risk of environmentally relevant MP concentrations in natural freshwater ecosystems, a novel large scale in-lake mesocosm (limnocorral) experiment was conducted at the IISD-Experimental Lakes Area in northwestern Ontario, Canada. A mixture of common polymers (polyethylene; PE, polystyrene; PS, and polyethylene terephthalate; PET), each with distinct colours and buoyancies, were added to 9 limnocorrals in equal contributions in a range of environmentally relevant nominal concentrations (0-29,240 MPs/L) as a single pulse. The phytoplankton and zooplankton communities were monitored for changes in abundance, biomass, and community composition for 68 days. Effects of MPs on phytoplankton biomass and community composition were not detected. However, chlorophyll *a* concentration was negatively impacted after 12 days of exposure but recovered by day 68. Zooplankton ingested PE and PS MPs but the community was not negatively impacted in terms of abundance, biomass or community composition. Calanoid copepod abundance was temporarily stimulated in high MP treatments after 33 days of exposure. There was a small, short term negative impact to *Tropocyclops*

extensus egg production and to copepod nauplii abundance suggesting that MPs could potentially affect copepod reproduction. Overall, there was little evidence of significant negative impacts of MPs on plankton communities.

2.2 Introduction

The accumulation of plastic debris in aquatic environments has sparked concern about potential impacts on biota (Anderson et al. 2016). Microplastics (plastic particles <5 mm in size; MPs) are of particular concern since they are bioavailable to a wide range of organisms (Au et al. 2017) and their potential impacts on aquatic ecosystems are poorly known. MPs can enter the environment either as primary MPs that are manufactured as micro-sized particles, or as secondary MPs that are broken down into smaller pieces from larger plastic debris (Eriksen et al. 2013). Due to continuous fragmentation and resistance to degradation, MPs are persistent contaminants and concentrations are continuously increasing in the environment (Eerkes-Medrano et al. 2015; Horton et al. 2017). Effluent from wastewater treatment plants (WWTP) and stormwater outfalls are some of the most important sources of MPs in freshwater environments (Simmerman and Coleman Wasik 2020).

MPs have been found in surface waters across the world (Eerkes-Medrano et al. 2015). Concentrations found in natural water bodies are variable, with the highest reported concentration in surface waters being 1,770 particles/L in the Southern North Sea basin (Dubaish and Liebezeit 2013). Eriksen et al. (2013) found MP contamination in the Laurentian Great Lakes varied spatially, with concentrations ranging from 0 to

450,000 particles/km^2. Even though MP contamination in freshwater is pervasive, studies are limited (Provencher et al. 2019).

The most abundant polymers found in freshwater environments are polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC,) polyamide (PA) and polyethylene terephthalate (PET), which reflects global plastic demand (Canniff and Hoang 2018; Koelmans et al. 2019). PE is typically positively buoyant and found in surface waters, while PS and PET polymers are more dense and sink to the sediment (Koelmans et al. 2019). Biofilms comprised of bacteria and algae, form on MPs rapidly, which makes MPs more palatable for organisms and increases their sedimentation rates (Rummel et al. 2017). Due to their small size and availability, planktonic communities are likely to directly interact with MPs in the environment (Cole et al. 2013).

At high concentrations, MPs have been shown to negatively impact phytoplankton growth and chlorophyll *a* production in the laboratory (Bhattacharya et al. 2010; Mao et al. 2018; Rani-Borges et al. 2021). Negative effects have been attributed to shading, increased turbidity, and adhesion (Nolte et al. 2017; Rani-Borges et al. 2021). MPs may physically block CO_2 and nutrient uptake pathways causing a reduction in photosynthetic activity (Bhattacharya et al. 2010). Alternatively, MPs have also been shown to have positive effects by acting as a substrate for algal growth via biofouling (Yokota et al. 2017; Canniff and Hoang 2018). There is also potential for MPs to alter phytoplankton community composition (Hitchcock 2022), but this is not fully understood.

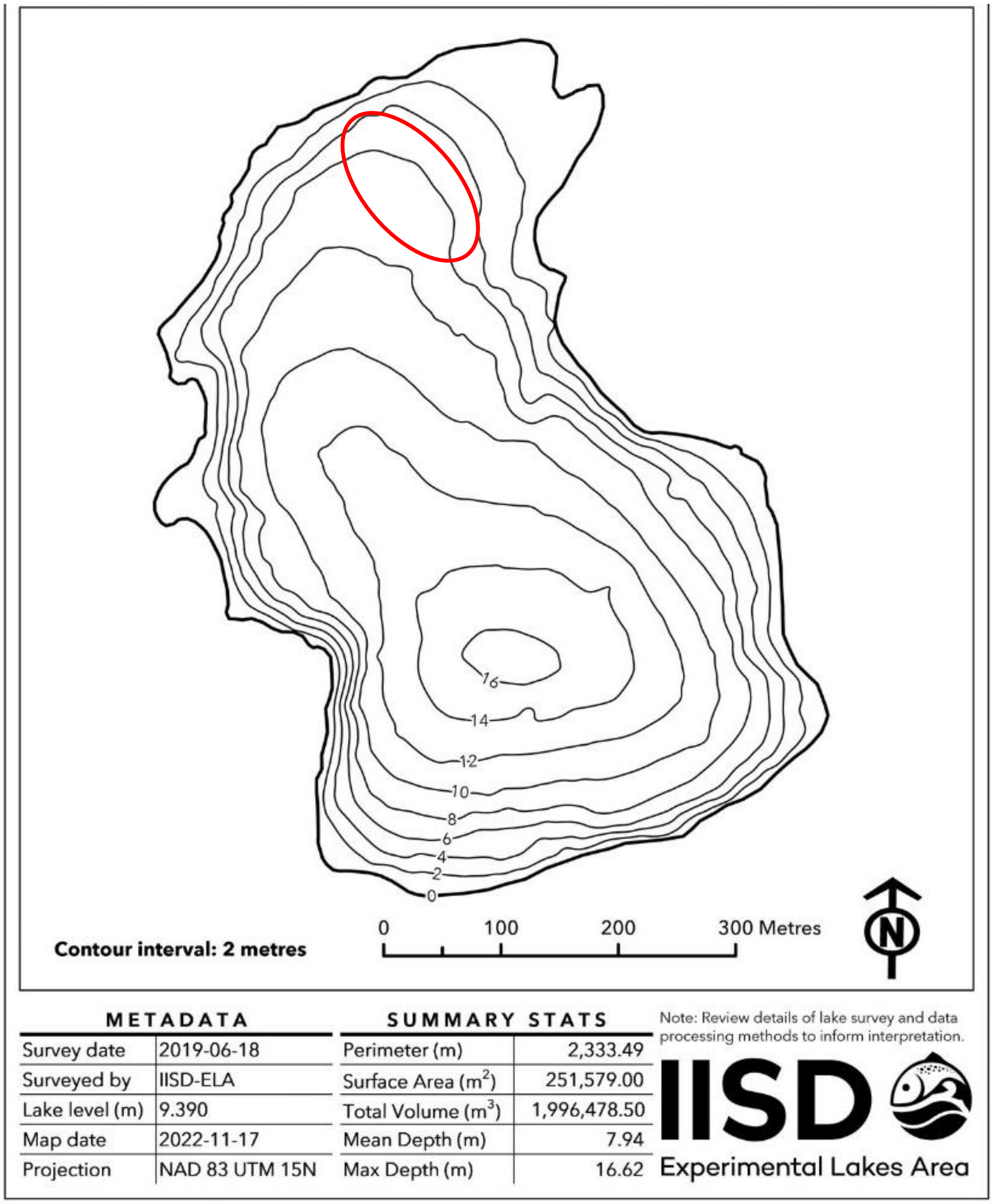
Zooplankton have been negatively impacted by MPs at high concentrations in laboratory studies, with ingestion of MPs leading to reduced growth, decreased reproductive output, and mortality (Lee et al. 2013; Cole et al. 2015; Ogonowski et al. 2016). Alternatively, MPs with biofilm may have positive effects by providing an additional nutrition source (Amariei et al. 2022). Current understanding of how MPs impact zooplankton are largely based on laboratory studies and most have used very high concentrations of one type of MPs (Bucci et al. 2020). Community level effects are not yet understood (Pan et al. 2022). Only one study to date has looked at community level effects and found no significant impacts of environmentally relevant concentrations of MPs (Yildiz et al. 2022).

This chapter examines the effects of a range of environmentally relevant MP concentrations on a freshwater planktonic food web using large in-lake mesocosms (limnocorrals). Aside from two controls with no added MPs, a mixture of MPs was added to limnocorrals using a gradient of environmentally relevant nominal concentrations (6 to 29,240 MPs/L) in a regression design. Phytoplankton and zooplankton were then monitored for 10 weeks to examine potential effects on community dynamics including biomass, species composition, reproduction, and diversity. The following questions are addressed: 1) What are the relationships between MP concentration and phytoplankton biomass, community composition, and chlorophyll *a*? and 2) What are the relationships between MP concentration and zooplankton abundance, biomass, reproduction, and community composition?

2.3 Methods

2.3.1 Study Site

The limnocorral study was conducted in a small, oligotrophic boreal lake (Lake 378; Figure 2.1) at the International Institute for Sustainable Development Experimental Lakes Area (IISD-ELA) located in northwestern Ontario, Canada (49^{o}41’37.88” N, 93^{o}46’32.18” W). The IISD-ELA is a remote field facility consisting of 58 freshwater lakes set aside for scientific research. For over 50 years, research at the IISD-ELA has examined anthropogenic impacts to freshwater ecosystems, including studies on eutrophication (Schindler and Fee 1974), acid rain (Schindler 1988), and oil spills (Cederwall et al. 2020). Lake 378 was chosen based on criteria such as adequate road access, a flat and sandy shoreline, and a well-defined multitrophic food web. Sampling has been conducted on Lake 378 since 2019 to characterize the baseline conditions of the lake’s water chemistry and biota. Lake 378 has a surface area of 251,579 m^2, a total volume of 1,996,478 m^3 and a maximum depth of 16.6 m (Figure 2.1; IISD Experimental Lakes Area, 2022). The phytoplankton community is typically dominated by chrysophytes (S. Higgins, personal communication), and the zooplankton community is dominated by calanoid copepods (*Diaptomus minutus;* M. Paterson, personal communication). The fish community consists of Spottail Shiner (*Notropis hudsonius*), Blacknose Shiner (*Notropis heterolepis*), Northern Pearl Dace (*Margariscus nachtriebi*), Fathead Minnow (*Pimephales promelas*), Slimy Sculpin (*Cottus cognatus*), Yellow Perch (*Perca flavescens*), White Sucker (*Catostomus commersonii*), Burbot (*Lota lota)* and Lake Trout (*Salvelinus namaycush*; L. Hayhurst, personal communication).



METADATA	
Survey date	2019-06-18
Surveyed by	IISD-ELA
Lake level (m)	9.390
Map date	2022-11-17
Projection	NAD 83 UTM 15N

SUMMARY STATS	
Perimeter (m)	2,333.49
Surface Area (m^2)	251,579.00
Total Volume (m^3)	1,996,478.50
Mean Depth (m)	7.94
Max Depth (m)	16.62

Figure 2.1 Bathymetric map of Lake 378, located at the IISD-Experimental Lakes Area in northwestern Ontario, Canada; IISD Experimental Lakes Area, 2022). The red circle represents the approximate location of the limnocorrals in the NE bay of the lake.

2.3.2 Experimental set-up

Nine limnocorrals were deployed in the northeast bay of Lake 378 in May 2021 (Figure 2.1). The limnocorrals were constructed of a food-grade polyethylene curtain containing no chemical additives secured to a 10-sided floating polystyrene collar (Figure 2.2 and Figure 2.3; Curry Industries, Winnipeg, Manitoba, Canada). Each limnocorral was 10 m in diameter and 2 m deep, sealed from the sediment and located in the pelagic (open water) zone of the lake. Rings of PVC pipe were attached around the outside of the polyethylene curtain to maintain its shape. The limnocorrals were filled with lake water using a pump (a total volume of ~150,000 L) and held in place with cement anchors.

Experimental treatments included 2 controls with no added MPs and 7 MP treatments that were randomly assigned to the limnocorrals. A regression approach was used because it allows for the detection of dose-response relationships and nonlinear responses (Cottingham et al. 2005; Kreyling et al. 2018). MPs were added to treatment limnocorrals to generate nominal concentrations ranging from 6 to 29,240 MPs/L in an approximate logarithmic scale, on the assumption that all added MPs were fully mixed into the water column of each limnocorral following addition. The nominal MP treatments bracketed a range from low average concentrations found in the environment to concentrations one order of magnitude above a 2050 projection under a “business as usual” scenario, assuming no major changes are made to mitigate plastic emissions (Dubaish and Liebezeit 2013; Geyer et al. 2017). A mixture of three different types of widely used plastic polymers (polyethylene; PE, polystyrene; PS, and polyethylene terephthalate; PET) were added in equal contributions to the water surface at the start of the experiment (June 2nd, 2021; day 0) as a single pulse. The plastics were produced by

Techmer and ground into MP fragments via a toll grinder under liquid nitrogen by Custom Processing Services with fragment sizes ranging from 37 µm – 1408 µm. The MPs contained common chemical additives (UV stabilizers, antioxidants, pigments), varied in buoyancy (positive, neutral, and negative) and were added as different colours to facilitate tracing (Table 2.1). Wherever possible, equipment made from natural materials was used during deployment and sampling of the limnocorrals to avoid MP contamination (e.g., cork floats, natural fiber ropes).

Two weeks prior to MP additions, limnocorrals were augmented with zooplankton collected from Lake 378 to offset any mortality caused during filling and to establish the community. Fifteen 10 m vertical hauls were collected at the deepest station of L378 using a 0.5 m diameter net with 150 µm mesh and added to each limnocorral. Each limnocorral was stocked with 26 young of the year Yellow Perch, a common planktivorous fish species found in Lake 378. Fish feeding was supplemented with additions of frozen *Mysis*, a freshwater shrimp, every 3 days to reduce predation pressure on the zooplankton community.

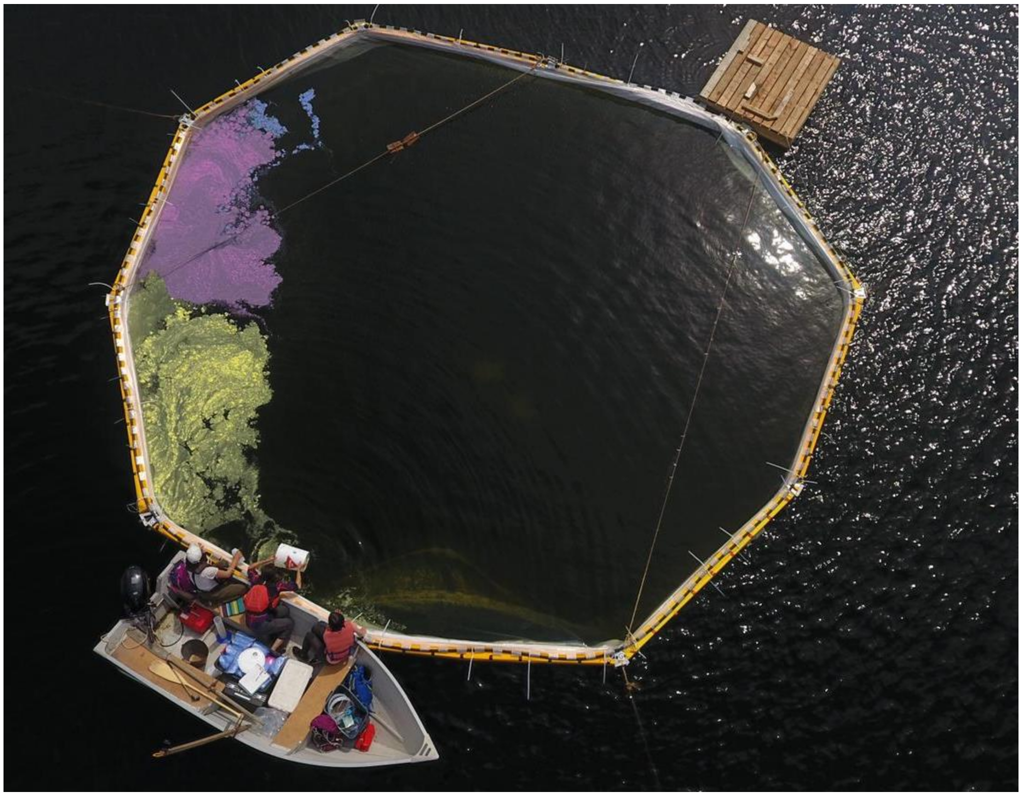


Figure 2.2 Aerial view of a limnocorral during MP additions. Photograph by Scott Higgins.

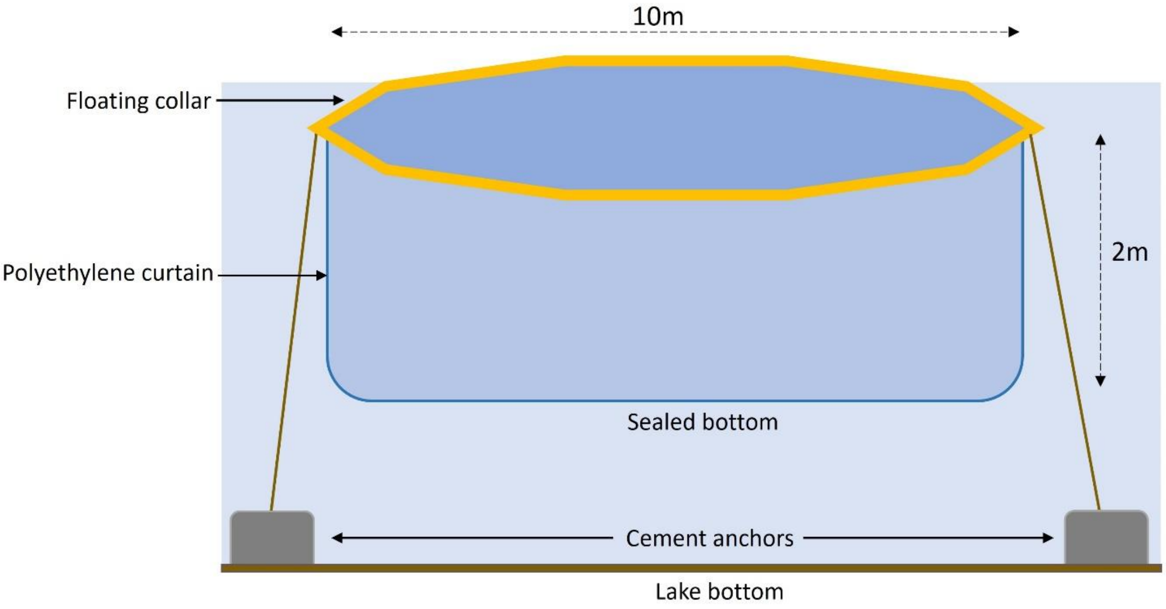


Figure 2.3 Limnocorral construction design.

Table 2.1 Characteristics of the plastics used in the experiment.

	Polyethylene Terephthalate (PET)	**Polystyrene (PS)**	**Linear Low-Density Polyethylene (LLDPE)**
Colour	Blue	Pink	Yellow
Chemical additives	Blue pigment	Red pigment, Titanium dioxide (0.15%), Irgafos 126 Antioxidant (0.1%), N,N Ethylene Bis-stearamide (0.01%)	Yellow pigment, Chimassorb 944 HALS UV (0.05%), Tinuvin 622 HALS UV (0.05%), Irganox B215 Antioxidant (0.025%), Irganoz 168 Antioxidant (0.025%), Benzotriazole-Acetostab 236 (0.05%)
Polymer buoyancy in freshwater	Negative	Neutral	Positive
Size range (µm)	52-1408	48-1408	37-1086

2.3.3 Sample Collection and Analysis

Environmental Parameters. Samples for water chemistry were collected from all limnocorrals one week prior to MP additions (May 24th, 2021) and on 2 occasions after (June 14th and August 9th, 2021). Water samples were collected using a peristaltic pump with plastic tubing from a depth of 1 m, sampled at least 1 m from the walls of the limnocorrals. Total dissolved and particulate nitrogen and phosphorus, dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), soluble reactive silica (SRSi), chlorophyll-*a*, and pH were analyzed by the chemistry laboratory at IISD-ELA using methods from Stainton et al. (Stainton et al. 1977) under the direction of Dr. Sonya Havens-Higgins. Particulate carbon and nitrogen, major ions (chloride, sulfate, calcium, potassium, magnesium, and sodium) and alkalinity were analyzed by the University of Alberta Biochemical Analytical Service Laboratory. Dissolved oxygen (DO) and water temperature were measured weekly using a Yellow Springs International (YSI) EXO2 Multiparameter Sonde at a depth of 1 m. Turbidity was recorded weekly by sampling limnocorral water from a depth of 1 m using a peristaltic pump and analyzed using a MANTECH Turbidity T10 meter in the field.

Photosynthetically Active Radiation (PAR) was measured weekly using a Licor LI190 or LI192 Underwater Quantum sensor and a LI-1400 logger. PAR is the portion of the light spectrum that is utilized by plants for photosynthesis (wavelengths 400-700 nm). Measurements were always taken from the sunny side of the boat to avoid shadows. A surface reading was taken in the air just above the water surface, and then readings were taken at depths of 1 m and 2 m. A second above-surface reading was then taken immediately after completing the profile and if the two above-surface measurements were not within 10% of each other, a new light profile was taken. The light extinction coefficient (Kd) was calculated by regressing the natural log of the percent of PAR vs depth (Fee et al. 1996). Typically, the surface reading is excluded from Kd calculations, however it was included in this analysis to produce three points for the regressions (0, 1 and 2 m depths).

Perch abundances were recorded after 10 weeks since perch predation may have varied among limnocorrals, affecting zooplankton and phytoplankton (de Bernardi et al. 1987). Yellow Perch were retrieved from limnocorrals using a large seine net.

Phytoplankton. Samples for phytoplankton taxonomy and biomass estimation were collected from all limnocorrals prior to MP additions (May 24th, 2021) and on 3 occasions afterwards (June 14th, July 7th, and August 9th, 2021). Phytoplankton samples were collected using the same methods as for water chemistry using a peristaltic pump. Phytoplankton samples from unfiltered limnocorral water were preserved in Lugol's solution and enumerated by David Findlay (Plankton R' Us, Winnipeg, MB, Canada) using methods described in Findlay and Kling (2003) to estimate biomass. The chlorophyll *a*/biomass ratio was calculated by dividing the chlorophyll *a* concentration by the total phytoplankton biomass (first converted to dry weight using a dry weight:wet weight ratio of 0.1). Diversity was calculated using the Inverse Simpson Index (^{2}D), which takes the average proportional abundance of each species (p_i) in a data set with R species, to quantify the effective number of species and is based on the Simpson Index (λ) (Simpson 1949):

$$\frac{1}{\lambda} = \frac{1}{\sum_{i=1}^{R} p_i^2} = {}^2D$$

Zooplankton. Samples for zooplankton taxonomy were collected from all limnocorrals prior to MP additions (June 1st, 2021) and then at weekly intervals for 10 weeks (68 days). Zooplankton samples were collected using a Wisconsin plankton net with a 0.25 m diameter opening and 53 µm mesh, attached to a long pole to access the center of the limnocorrals. Two 1.5 m vertical net hauls were collected from opposite sides of each limnocorral and then combined to provide a representative sample of the zooplankton community (total sample volume of 147 L). The zooplankton samples were preserved in a 5% sugar-formalin solution (Prepas 1978) in the laboratory after narcotization in methanol (Gannon and Gannon 1975) in the field.

Zooplankton were identified and counted to the lowest possible taxonomic level using light microscopy with the support of several keys (Smith and Fernando 1978; Balcer et al. 1984; Witty 2004). Copepod nauplii and stage CI to CIII copepodites were not identified to species. Abundance was calculated from raw counts as number of zooplankton per L. Biomass was estimated by multiplying the density of each taxon and life stage by a fixed mass determined from size-weight regressions developed at IISD-ELA (Paterson et al. 2019). Eggs were enumerated to determine reproductive rates of zooplankton taxa by calculating the ratio of eggs per female for abundant taxa (Taylor 1988). Loose copepod eggs were apportioned to copepod females based on the relative abundance of adult females of copepod taxa in each sample. Only egg ratios for abundant taxa with sufficient egg counts are reported here. Community diversity was calculated using the Inverse Simpson Index, as described above.

Fate of MPs in Water. Samples for MP fate in the water column were collected 24 h, 72 h, weekly for the first 4 weeks and then biweekly thereafter for a control treatment (0 MPs/L), low treatment (6 MPs/L), medium treatment (414 MPs/L) and the highest treatment (29,240 MPs/L). 4 L of limnocorral water was pumped through a peristaltic pump onto a 10 µm filter for three different depths (10 cm, 1m and 2m). In the laboratory, MPs were removed from the filters by rinsing them with deionized water, and then preserved in polypropylene jars with rubbing alcohol. MPs were counted and characterized by polymer type and size fraction (>212, 106-212, and 53-106 µm) at the

Rochman Laboratory at the University of Toronto under the direction of Dr. Chelsea Rochman. Methods for quantifying MPs for the <53 μm size fraction are still under development.

Ingestion of MPs by Zooplankton. Ingestion of MPs by common zooplankton taxa was assessed by picking animals from random subsamples collected for zooplankton taxonomy on day 68 of the experiment. One millilitre subsamples were suspended in tap water and carefully picked with fine metal tweezers under a microscope and placed into clean glass vials. Zooplankton were visually inspected for MPs attached to the carapace and appendages, and only visually "clean" zooplankton were used. Nauplii and stage CI-III copepodites were not included due to their small size. Only abundant species were picked, including *D. minutus*, *Diaphanosoma birgei*, *Tropocyclops extensus*, *Eubosmina sp.* and *Mesocyclops edax*. Where possible, up to 100 individuals of each species were picked from a control (0 MPs/L), low concentration (6 MPs/L), medium concentration (414 MPs/L) and high concentration (29,240 MPs/L) treatments. The number of individuals picked of each species was recorded to determine the number of MP particles ingested per individual zooplankton for each taxon.

The samples were analyzed at the Rochman Laboratory at the University of Toronto under the direction of Dr. Chelsea Rochman. Zooplankton were rinsed into clean polypropene cups and 40 ml of 30% H_2O_2 (hydrogen peroxide) solution was added (Alfonso et al. 2021). Aluminum foil was placed over the polypropylene cups, which were then placed in a temperature-controlled oven at 45°C for 24 hours to digest the zooplankton carapaces. Two blanks were performed following the same methods to check for procedural contamination.

After digestion, MPs were sorted into size fractions using metal sieves (>212, 106-212 and 53-106 μm). The filtrate was collected onto a 1 μm filter to preserve the < 53 μm size fraction for later analysis. For each size fraction, samples were examined under a microscope for MPs, and each particle was picked and analyzed using Raman Spectroscopy to confirm polymer type (Lenz et al. 2015).

2.3.4 Statistical Analysis

All statistical analyses were conducted in R version 4.1.3 (R Core Team 2022). Analyses were conducted using the *vegan* package version 2.6-4 (Oksanen et al. 2022) and plots were produced using the *ggplot2* package version 3.4.0 (Wickham 2016).

Univariate Analyses. Linear regression was used to assess relationships between MP treatment concentrations and variables in the planktonic food web. Phytoplankton biomass, diversity, chlorophyll *a,* and zooplankton abundance, biomass, diversity and reproduction (egg ratios and nauplii abundance) were linearly regressed individually against MP treatment concentration for individual time points throughout the experiment. Diagnostic plots were produced from residuals and visually inspected to ensure the assumptions of linear regression were met for each model. MP treatment concentration was always $\log_{10}$ transformed to meet the assumption of linearity since concentrations were selected to approximately fall on a log scale. Quantile-quantile plots were used to assess normality and scatterplots were used to assess homoscedasticity and potential outliers. The dependent variables were $\log_{10}$ transformed when needed to meet assumptions and improve model fit. An alpha level of 0.05 was used to determine significance.

Other environmental parameters were plotted to visually assess temporal trends. These included turbidity, light attenuation (Kd), water temperature, dissolved oxygen (DO), total phosphorus (TP), total nitrogen (TN), dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), particulate carbon, major ions (calcium, potassium, magnesium, sodium, chloride and sulfate), soluble reactive silica (SRSi), pH, and alkalinity. Total phosphorus (TP) and total nitrogen (TN) were determined from the sum of the dissolved and particulate fractions. Relationships between final Yellow Perch densities and final zooplankton and phytoplankton biomass were inspected for correlations to assess perch predation as a possible covariate.

Multivariate Analyses. Principal response curves (PRC) were used to determine phytoplankton and zooplankton community responses to MPs (Van den Brink and Ter Braak 1999). PRCs are a method of constrained ordination in which Redundancy Analysis (RDA) is used to determine treatment differences in a community over time, contrasted against a control treatment (Van den Brink and Ter Braak 1999). The outputs of a PRC include the canonical coefficients (C_{dt}), which represent the magnitude and direction of the effect on the community relative to the control at a given time, and the species weights (b_k), which represent the affinity of each species with the overall response of the community (Van den Brink and Ter Braak 1999). When C_{dt} and b_k share the same sign, that species is more abundant in the treatment than the control and when C_{dt} and b_k have different signs, that species is less abundant in the treatment than the control (Auber et al. 2017). The magnitude of the species weights indicates the affinity each species has for the overall community trend. Species weights between -0.5 and 0.5 are generally considered to have a weak response, or to have a different response pattern from the community. The matrices used for the PRC analysis included phytoplankton biomass (mg/m^3) for major phytoplankton groups, and zooplankton abundance (number/L) and biomass (μg/L dry weight) for zooplankton taxa. Variables were log$_{10}$ transformed prior to PRC analysis.

The PRC method is non-parametric and has no assumption of any particular distribution, although it assumes linearity of the data. Only the first axis of the PRC is reported here. A permutation test (999 permutations) was used to determine the significance of the first PRC axis, to assess if the PRC can accurately explain a significant portion of the treatment variance over time. The PRC analyses were conducted using the *prc()* function from the *vegan* package in R.

2.4 Results

2.4.1 MP Fate in the Water Column

MP concentrations measured in the water column were much lower than the nominal treatment concentrations even after just 24 hours (Figure 2.4). Actual MP concentrations in the water column varied by time and depth. PS was the most abundant polymer detected 24 hours after MP additions, but after 3 weeks PE was most abundant. Only very low concentrations of PET were found on all days. Low concentrations of MPs (< 4 MPs/L) were detected in the control treatment 24 h after additions and on the last week of the experiment. The majority of MPs in the water column were below 250 µm in length and width.

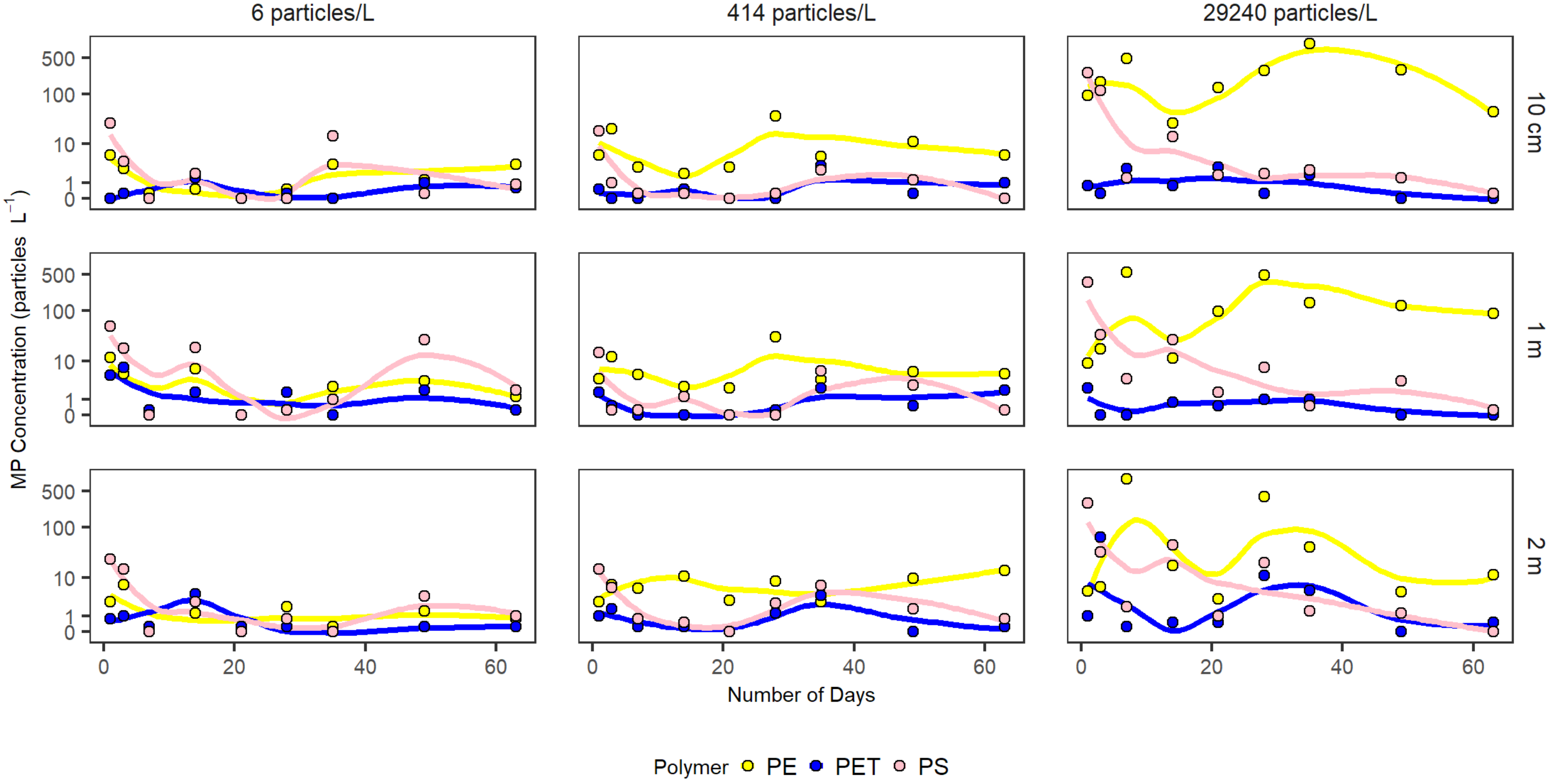


Figure 2.4 Nominal initial MP concentrations versus actual concentrations of MPs at different depths (10 cm, 1 m, 2 m) in the water column throughout the study for the low (6 MPs/L), medium (414 MPs/L) and high (29,240 MPs/L) treatment. Note logarithmic scale. Figure provided by Dr. Chelsea Rochman (University of Toronto).

2.4.2 Environmental Variables

The light extinction coefficient (Kd) decreased slightly over time but was not correlated with MP treatment concentration except on day 26 ($R^2 = 0.71$, $p = 0.004$) and day 61 ($R^2 = 0.72$, $p = 0.004$; Appendix Figure 1), where Kd was lower in the higher MP treatments suggesting higher water clarity with increasing MP concentration. Turbidity was initially similar for all treatments then diverged and became more variable (Appendix Figure 2). Turbidity was generally not significantly correlated with MP treatment concentrations except on day 5 ($R^2 = 0.66$, $p = 0.009$) and day 68 ($R^2 = 0.48$, $p = 0.04$), where turbidity was lower in the higher MP treatments. Kd and turbidity were not correlated with one another. Overall, there was no evidence that light was significantly affected by the MP treatments.

Water temperature increased following the addition of MPs for all treatments and peaked around day 30 during mid-July (Appendix Figure 3). Water chemistry did not vary consistently with MP treatment concentrations. DO at 1m decreased following MP additions, presumably in response to increasing water temperatures (Appendix Figure 4). DO concentrations were never limiting (< 2 mg/L) for zooplankton (Weider and Lampert 1985). TN increased after MP additions but did not vary among treatments (Appendix Figure 5). TP and particulate carbon (Appendix Figure 6 and Appendix Figure 7) did not vary consistently across time or treatment while only small changes in DOC, DIC and alkalinity, were observed (Appendix Figure 8, Appendix Figure 9, and Appendix Figure 10, respectively). pH increased slightly over time and there was a small significant positive relationship with MP treatment concentration on day 12 ($R^2 = 0.62$, $p = 0.012$; Appendix Figure 11). For all treatments, silica (SRSi) concentrations similarly decreased over time (Appendix Figure 12). Concentrations of all major ions (Ca, K, Mg, Na, Cl, and SO_4) increased by day 68 for all treatments but did not differ among treatments (Appendix Figure 13 and Appendix Figure 14).

Twenty-six perch were initially added to each limnocorral but by the end of the experiment final perch densities ranged from 4 to 17 (Table 2.2). Other fish species that were not intentionally added to limnocorrals, mainly Spottail Shiner, were also recorded. Spottail Shiner densities ranged from 0 to 4 across treatments and one small burbot was captured from the 1710 MPs/L treatment. Perch from the 24 MPs/L treatment were not sampled due to logistical issues. The two controls differed the most in their final Yellow Perch densities. Final perch density was not correlated with MP treatment concentration. Zooplankton biomass on day 68 had a weak negative correlation with final perch densities ($R^2 = 0.06$, $p = 0.012$). Phytoplankton biomass had a strong negative correlation with final perch densities ($R^2 = 0.81$, $p = 0.002$). As a result, variations in Yellow Perch densities may have contributed to variations in phytoplankton biomass but less so for zooplankton. Because fish were sampled only at the experiment’s end, the timing of fish mortalities is unknown, and relationships between fish and plankton on other dates cannot be assessed.

Table 2.2 Final Yellow Perch abundances and by-catch for each treatment. Fish in the 24 MPs/L treatment were not sampled (NA).

Treatment (nominal MPs/L)	**Final Yellow Perch abundances**	**By-catch**
Control 1	4	
Control 2	17	
6	9	1 Spottail Shiner
24	NA	NA
100	12	1 Spottail Shiner
414	10	1 Spottail Shiner
1710	10	1 Spottail Shiner, 1 Burbot
7071	9	4 Spottail Shiner
29,240	9	1 Spottail Shiner

2.4.3 Phytoplankton

Chlorophyll *a*. Chlorophyll *a* concentration in the water column on day -9 ranged from 0.66 µg/L to 0.94 µg/L across treatments. Chlorophyll *a* concentration initially decreased slightly for all treatments 12 days after MP additions. On day 68 final chlorophyll *a* concentration ranged from 0.36 to 0.9 µg/L across treatments. There was a significant negative relationship between chlorophyll *a* concentration and MP treatment concentration on day 12 ($R^2 = 0.53$, $p = 0.026$; Figure 2.5). There was no relationship between chlorophyll *a* and MP concentration by day 68 ($P > 0.05$).

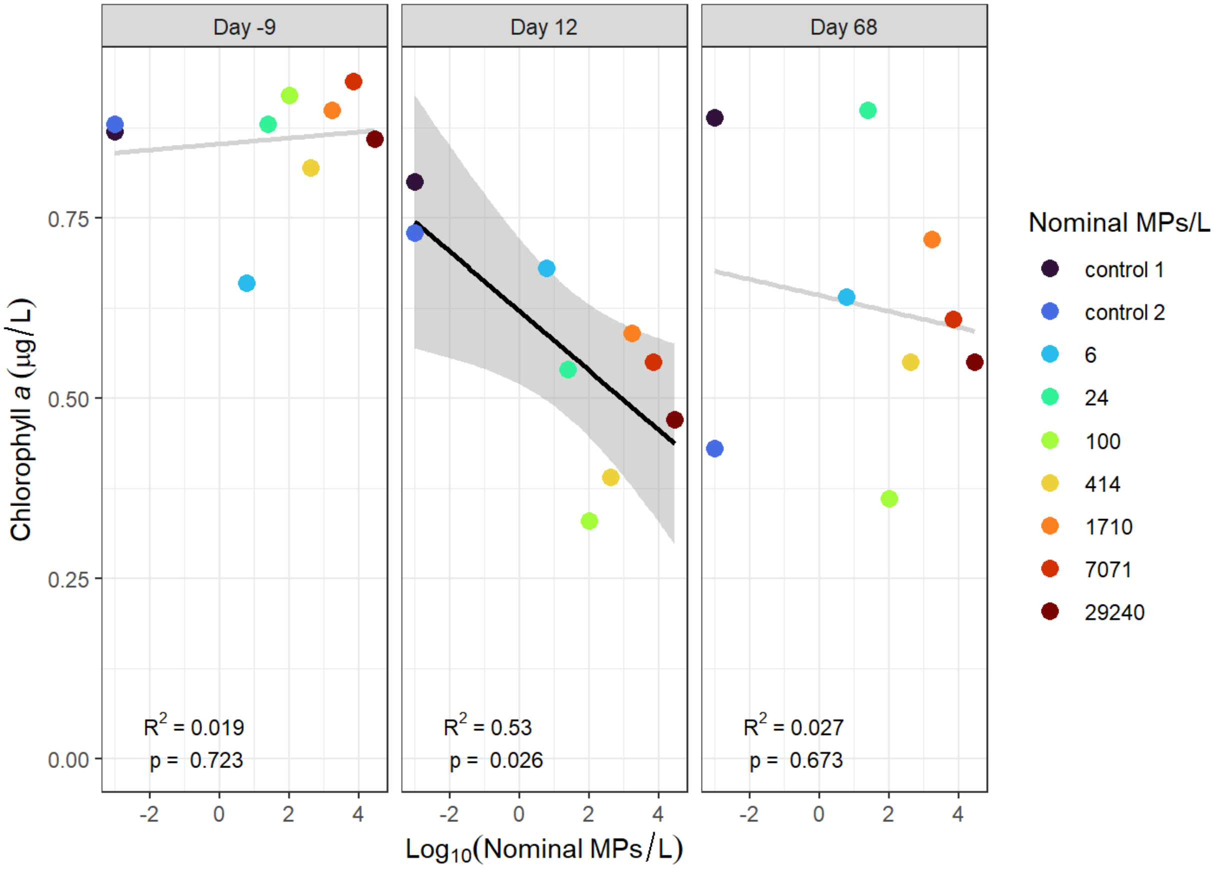


Figure 2.5 Chlorophyll *a* linearly regressed against MP treatment concentration, $\log_{10}$ transformed for three different time points throughout the experiment.

Phytoplankton Biomass and Community Composition. Initial phytoplankton biomass on day -9 ranged from 310 mg/m^3 to 430 mg/m^3 across treatments with the community being dominated by chrysophytes (Figure 2.6). There was a slight decrease in total phytoplankton biomass after MP additions for all treatments, followed by a peak in biomass on day 35. The magnitude of this peak ranged from 265 to 1614 mg/m^3 and was driven by an increase in diatoms. By day 68, phytoplankton biomass decreased to below initial levels for most treatments (29 to 504 mg/m^3). Community composition was initially similar among treatments, but treatments diverged and became considerably different by day 68. Community composition varied the most between the two controls on day 68, with one control treatment dominated by diatoms and the other dominated by chlorophytes.

Linear regression analysis indicated that there were no significant relationships between total phytoplankton biomass and MP treatment concentration throughout the study ($p > 0.05$; Figure 2.7). PRC analysis indicated that biomass-based composition of the phytoplankton community did not differ significantly among MP treatments over time (first PRC axis; $p = 1$; Figure 2.8). Phytoplankton diversity was also not related to MP treatment concentration throughout the experiment ($p > 0.05$; Figure 2.9).

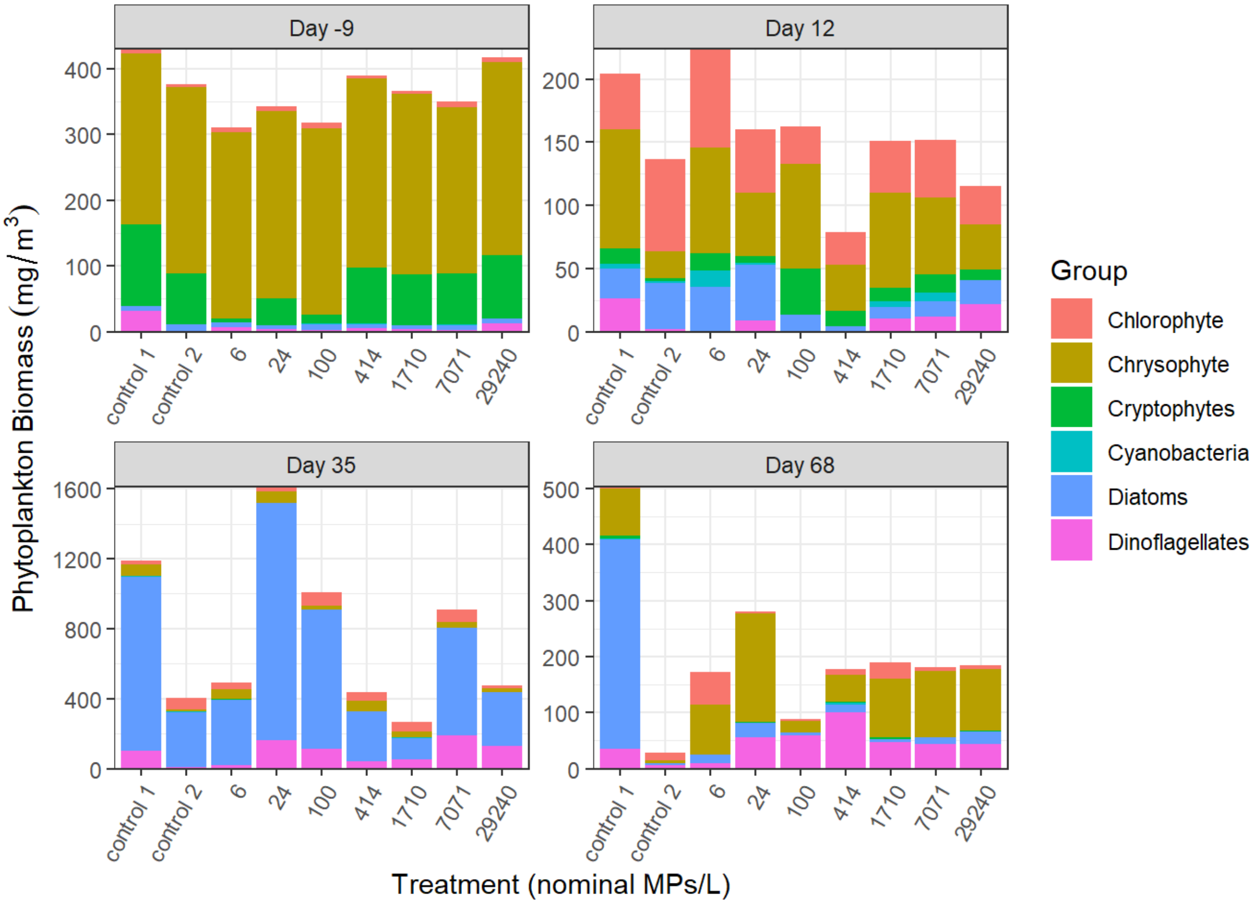


Figure 2.6 Biomass-based phytoplankton community composition (wet weight). Note the different scales for each plot.

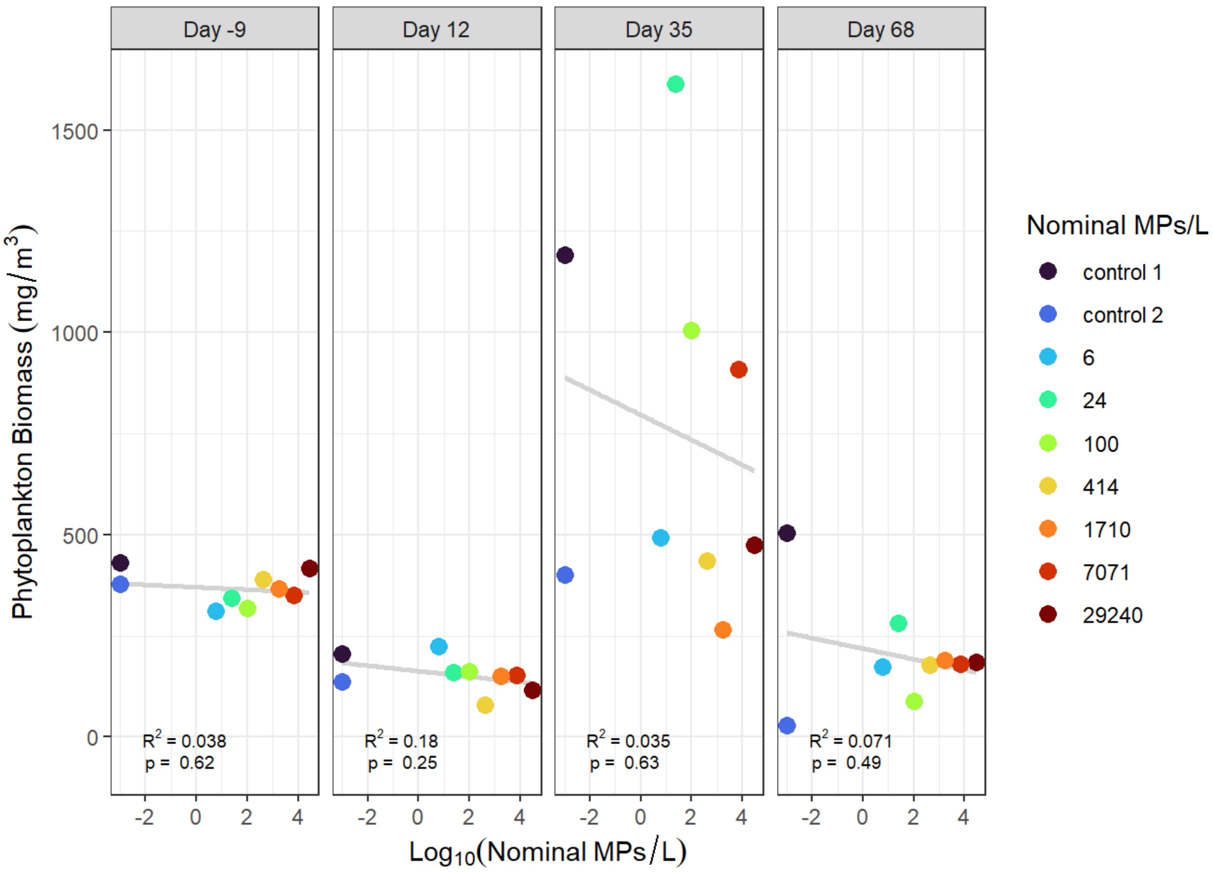


Figure 2.7 Total phytoplankton biomass linearly regressed against $\log_{10}$ MP treatment concentration.

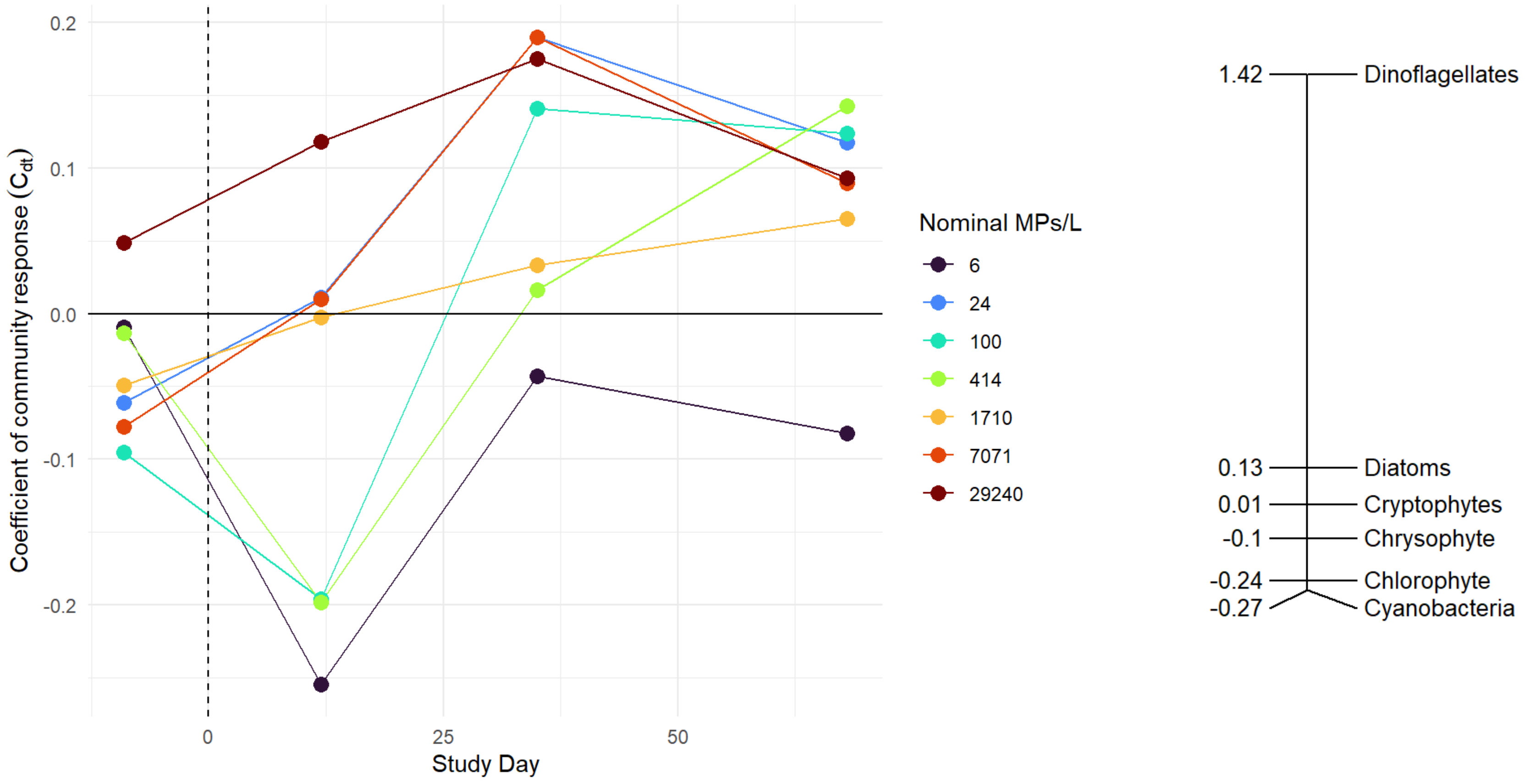


Figure 2.8 Principal response curve (PRC) for biomass-based phytoplankton community composition. Phytoplankton biomass was $\log_{10}$ transformed prior to analysis. The community response (C_{dt}) is displayed on the left side of the y axis and species weights (b_k) are displayed on the right. The solid black horizontal line represents the average of the control treatments (where $C_{dt} = 0$), and the dashed vertical line represents the day of MP addition (day 0). The PRC axis was not significant (p = 1).

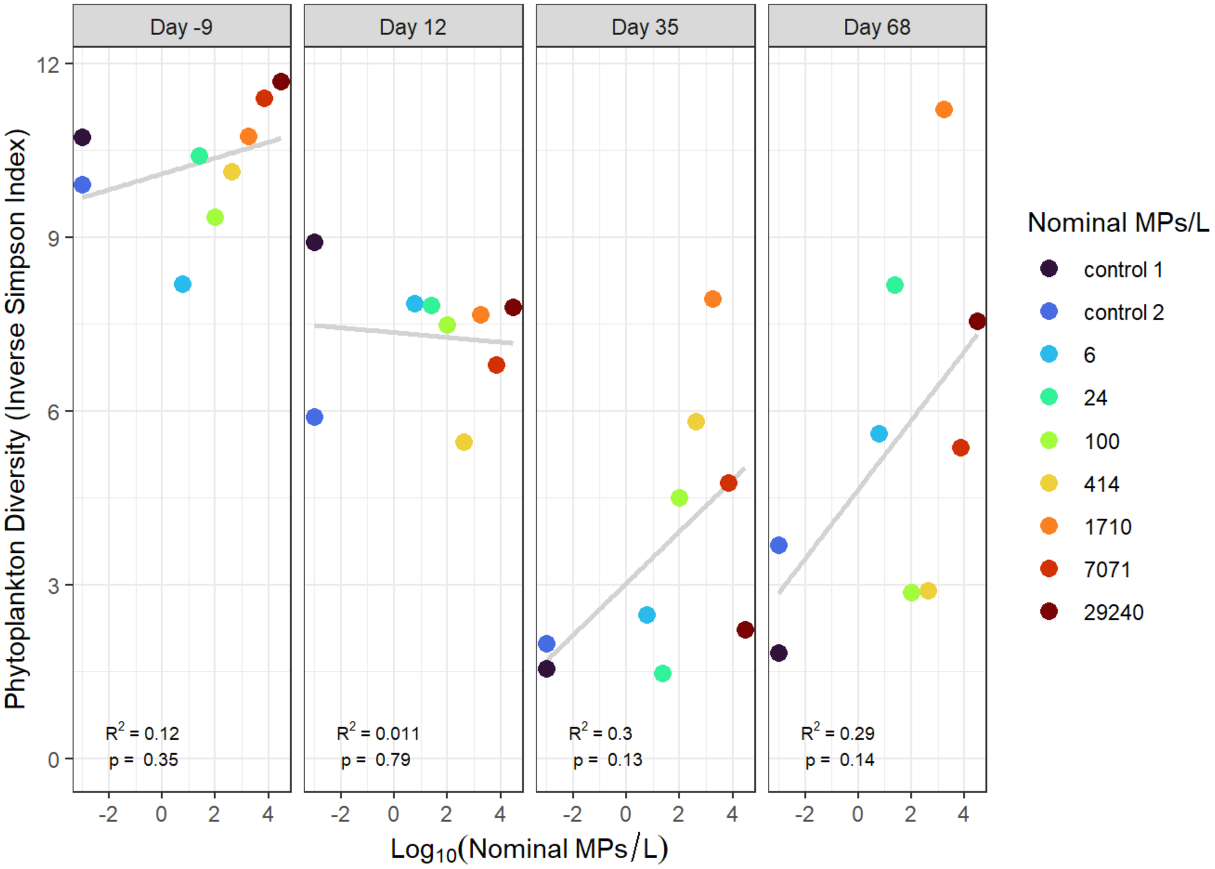


Figure 2.9 Phytoplankton community diversity, measured by the Inverse Simpson Index, versus MP treatment concentration during the experiment.

Chlorophyll *a*/Phytoplankton Biomass Ratio. The chlorophyll *a*/biomass ratio for phytoplankton at the start of the experiment ranged from 0.02 to 0.03 across treatments on day -9. By day 68 the chlorophyll *a*/biomass ratio ranged from 0.02 to 0.15 with the two controls varying the most in their chlorophyll *a* content. The chlorophyll *a*/biomass ratio for phytoplankton was not correlated with MP treatment concentrations on any day ($p > 0.05$; Figure 2.10).

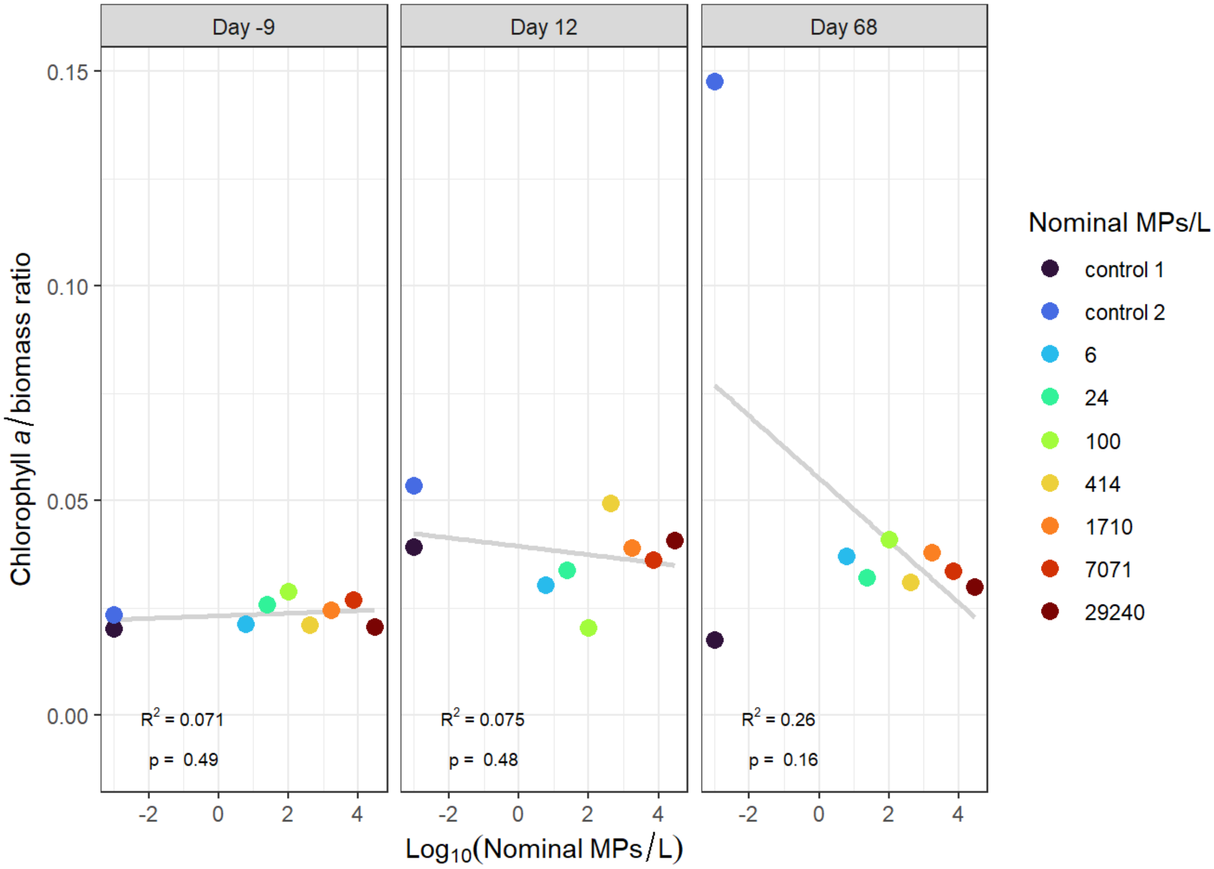


Figure 2.10 Chlorophyll *a*/biomass ratio for phytoplankton versus MP treatment concentration during the experiment.

2.4.4 Zooplankton

MP Ingestion by Zooplankton. All zooplankton taxa examined ingested MPs (Figure 2.11). The number of ingested MP particles ranged from 0.01 to 0.18 particles per individual zooplankton. The highest occurrence of ingested MPs was observed for *Eubosmina sp.* in the 29,240 MPs/L treatment at an occurrence of 0.18 MPs/individual, closely followed by *D. birgei* in the 414 MPs/L treatment with 0.17 MPs/individual. Zooplankton in the MP treatments only ingested PS and PE particles, but one PET particle was detected in *Eubosmina sp*. in the control treatment. *D. birgei* and *D. minutus* in the control treatment also ingested MPs at occurrences of 0.053 and 0.02 particles per individual, respectively. The size of ingested MPs ranged from 18 to 261 µm for the smallest dimension and 29 to 1014 µm for the largest dimension. The average size of the smallest dimension of ingested MPs was 53 µm for *D. birgei*, 56 µm for *T. extensus*, 58 µm for *Eubosmina sp.*, 73 µm for *M. edax* and 136 µm for *D. minutus*. MPs in the 1-53 µm size fraction have not yet been analyzed by the Rochman Laboratory.

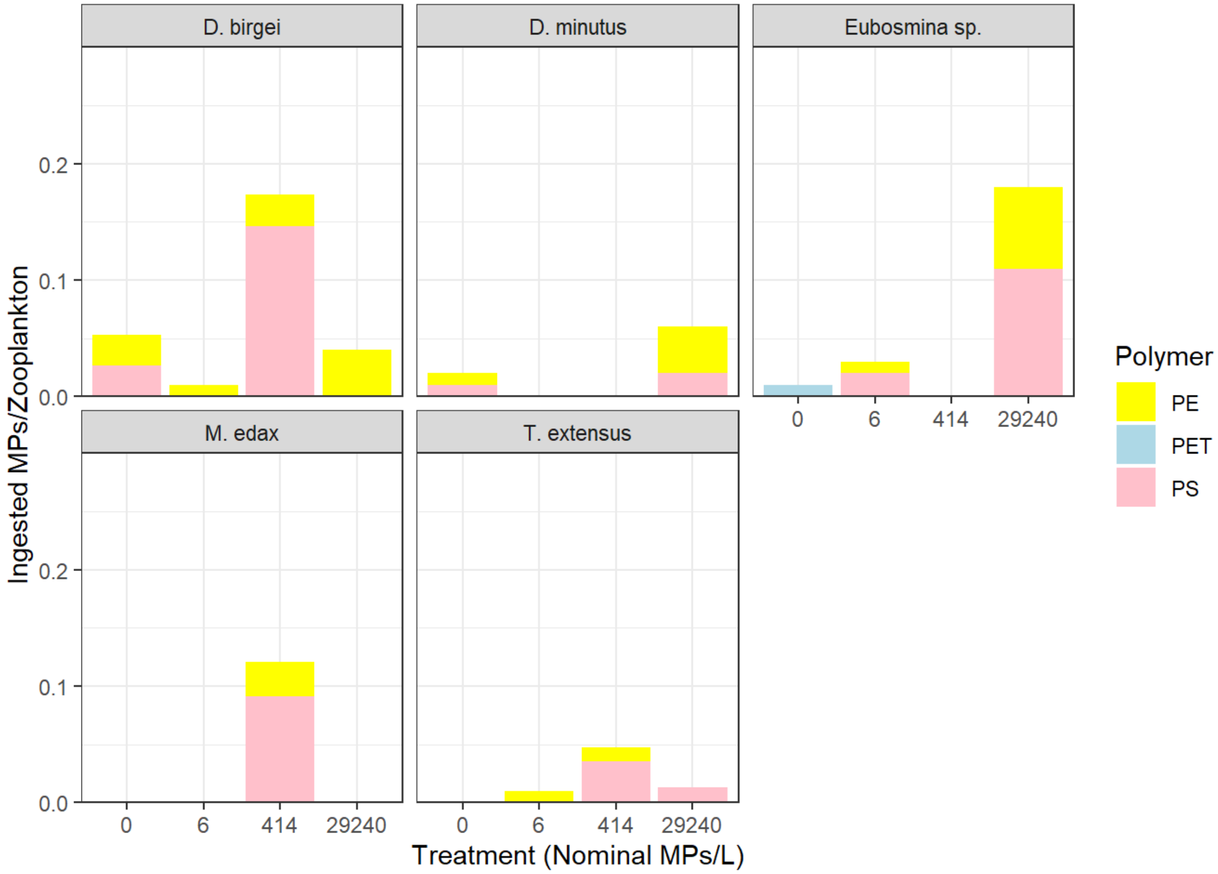


Figure 2.11 MP ingestion for common zooplankton taxa on day 68 of the experiment.

Zooplankton Abundance. Initial zooplankton abundance on day -1 ranged from 24 to 69 zooplankton/L across treatments (Figure 2.12). There was a slight initial drop in total zooplankton abundance for all treatments following MP additions. Zooplankton abundance fluctuated throughout the experiment but by day 68 returned to similar abundances as before MP additions for all treatments (37 to 78 zooplankton/L). Throughout the experiment, the zooplankton community in all limnocorrals was on average dominated by *Eubosmina sp.*, a small bodied cladoceran, in terms of abundance. On day 33, the community became dominated by *D. birgei*, a larger bodied cladoceran, and *D. minutus*, a calanoid copepod. Although not identified to species, CI – CIII calanoid copepodites were most likely *D. minutus* as other calanoid taxa were rare in the enclosures. *Eubosmina sp*. numbers increased again by day 54.

There was a significant positive relationship between total zooplankton abundance (excluding nauplii) and MP treatment concentration on day 33 ($R^2 = 0.6$, $p = 0.014$; Figure 2.13). All other regressions were not statistically significant. Abundances of cladocerans and cyclopoid copepods had no significant relationships with MPs on any day ($p > 0.05$; Figure 2.14 and Figure 2.15), but a significant positive relationship was observed for the calanoid copepod group on days 19 ($R^2= 0.49$, $p = 0.04$) and 33 ($R^2= 0.7$, $p = 0.005$; Figure 2.16). All other regressions were not statistically significant however in general, calanoid copepod abundance followed a positive trend with MP concentration throughout the experiment.

The PRC for zooplankton abundance was not significant ($p = 0.97$) indicating that abundance-based species composition of the zooplankton community was not related to

MP treatment over time (Figure 2.17). CI to CIII calanoid copepods and *D. minutus* had relatively large positive species weights indicating they had a strong affiliation with the community trend, but that this response was not due to MP treatment.

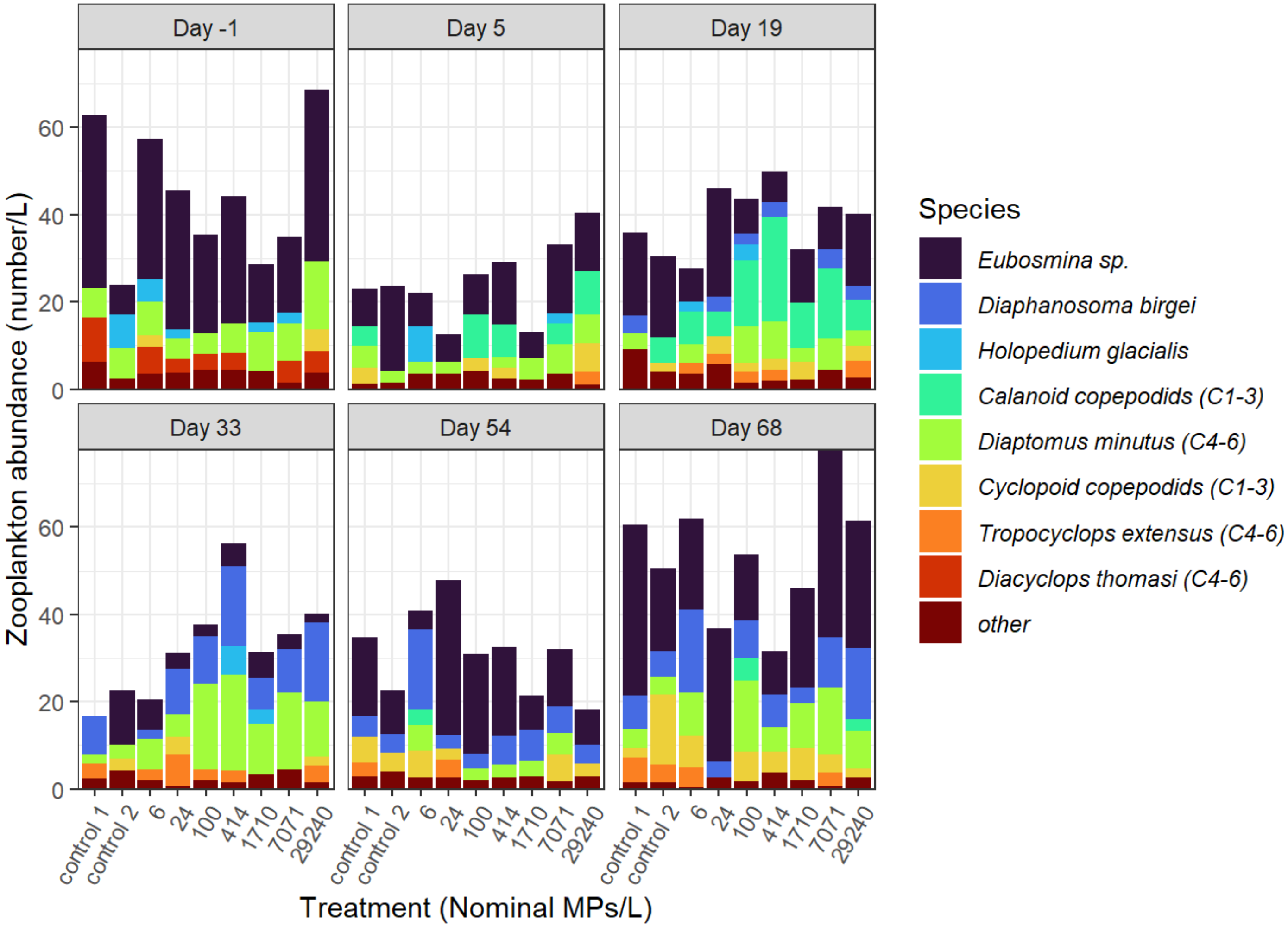


Figure 2.12 Zooplankton abundance (number per L) for each treatment on different days on the experiment.

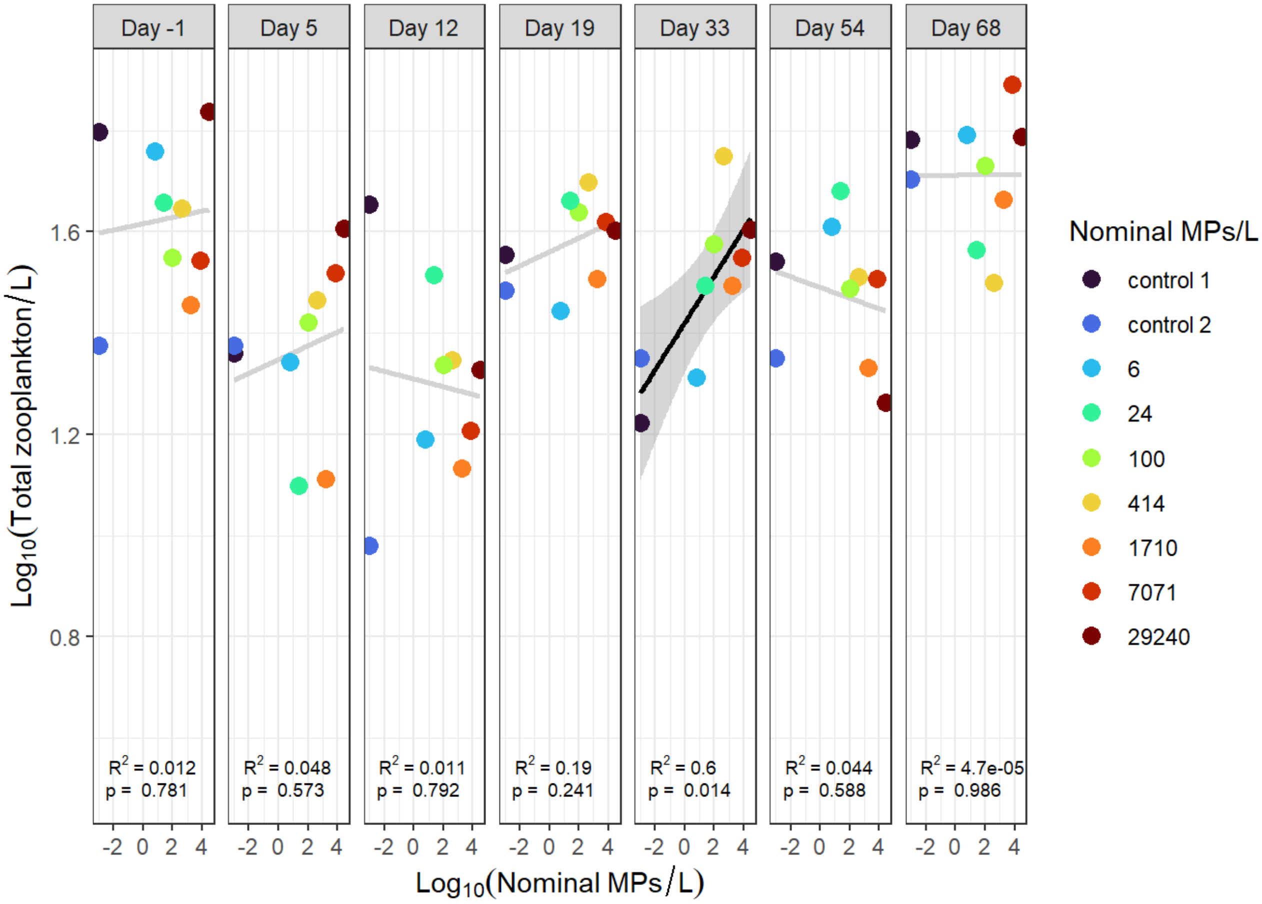


Figure 2.13 Total zooplankton abundance, excluding nauplii, versus MP treatment for 7 time points during the limnocorral experiment.

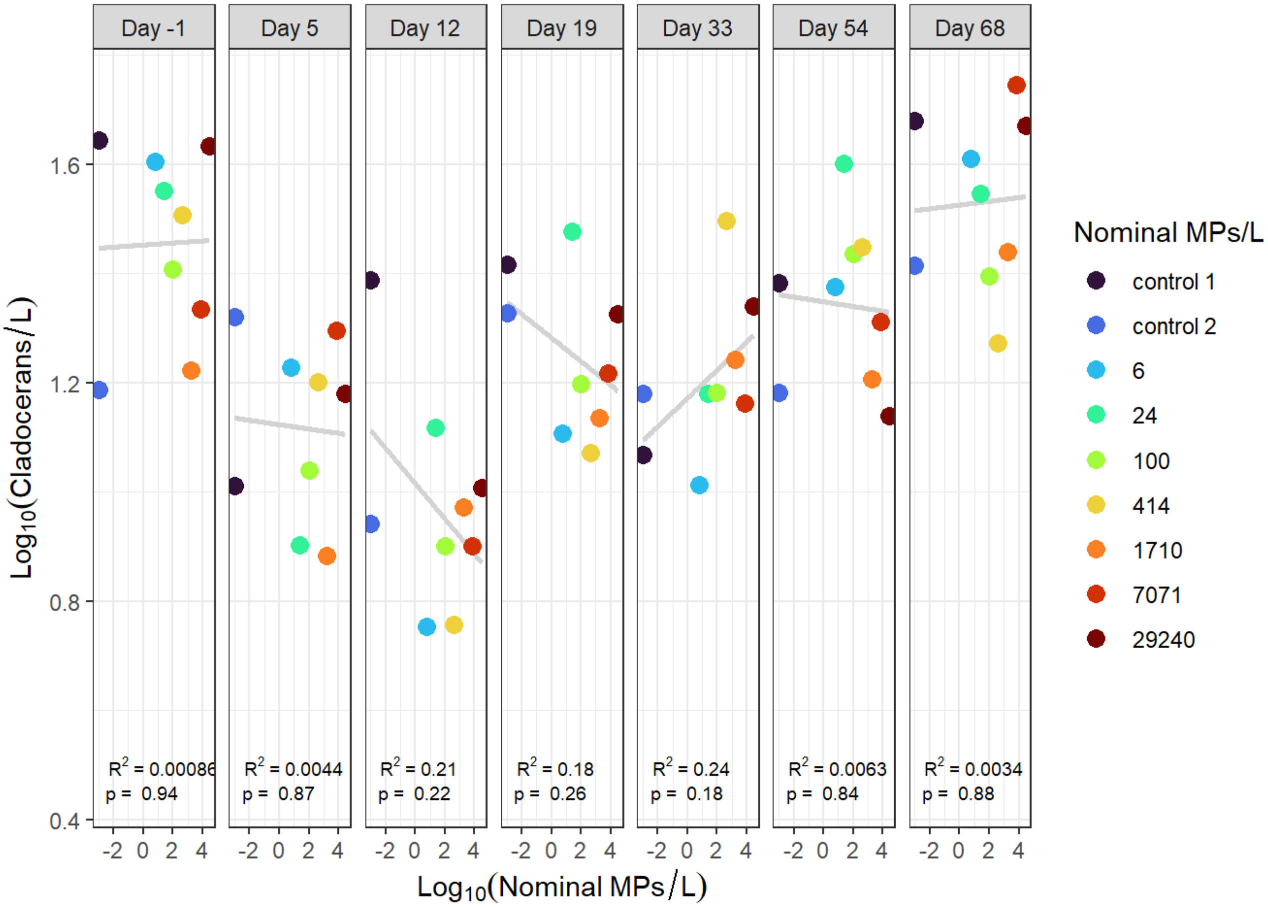


Figure 2.14 Cladoceran abundance versus MP treatment for 7 time points during the limnocorral experiment.

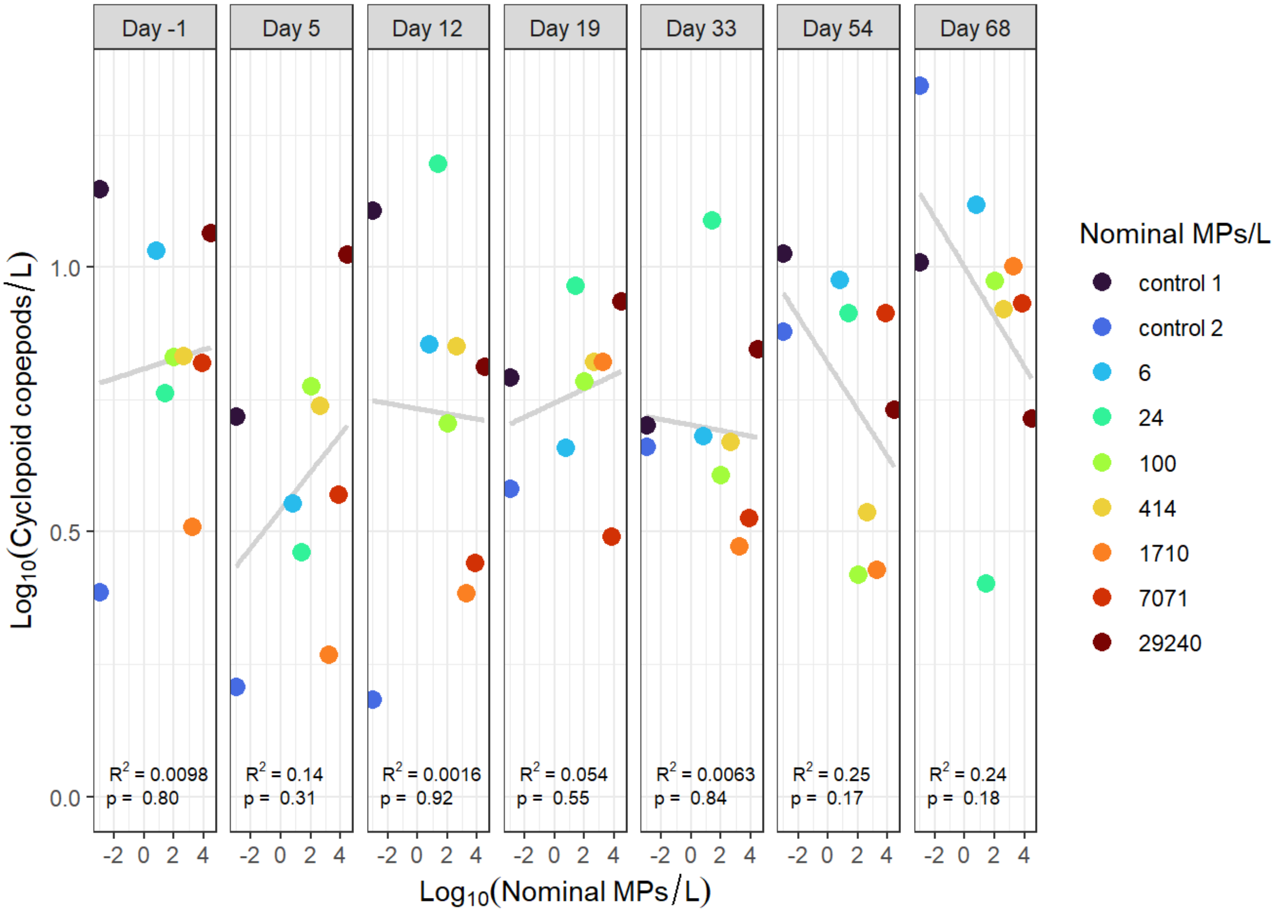


Figure 2.15 Cyclopoid copepod abundance versus MP treatment for 7 time points during the limnocorral experiment.

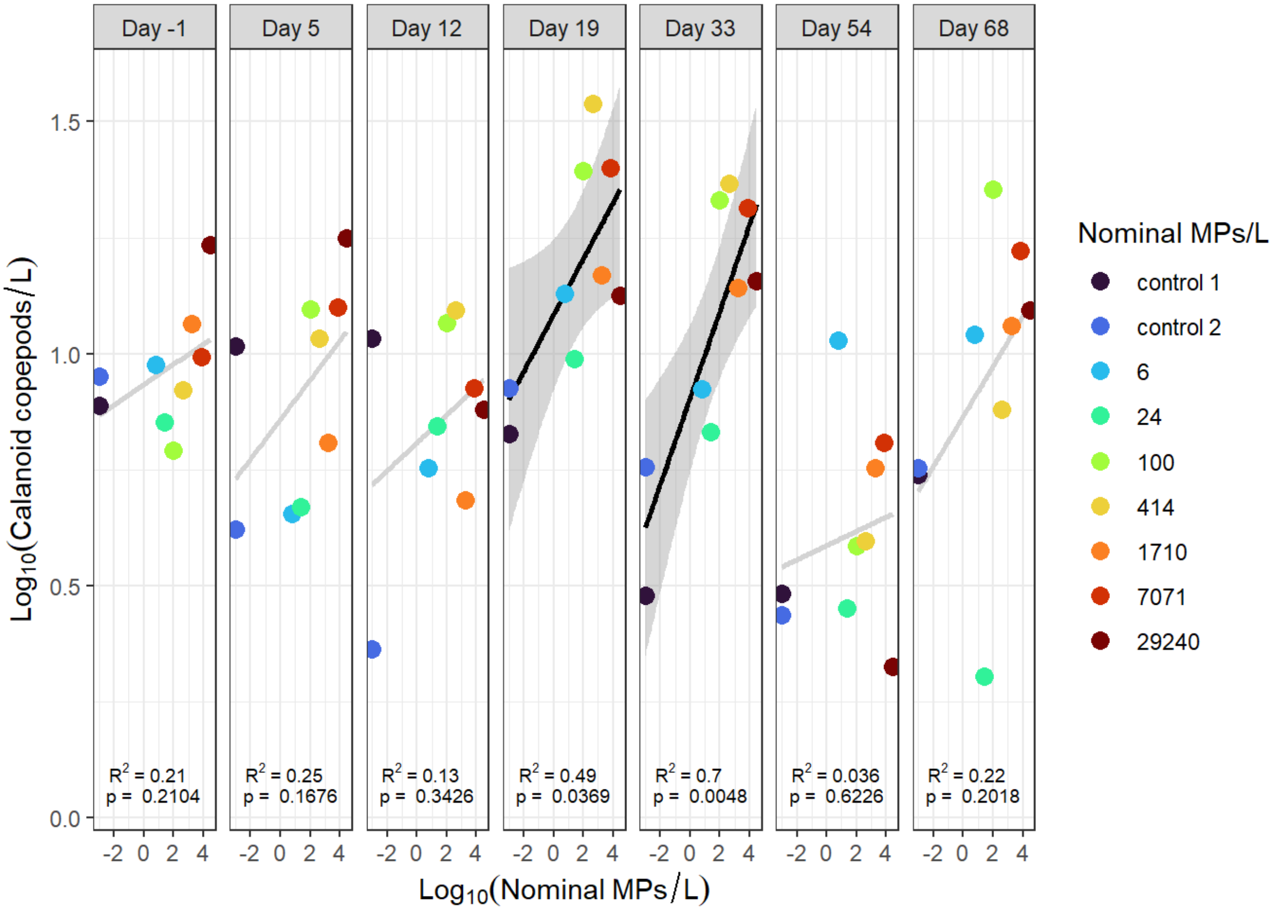


Figure 2.16 Calanoid copepod abundance versus MP treatment for 7 time points during the limnocorral experiment.

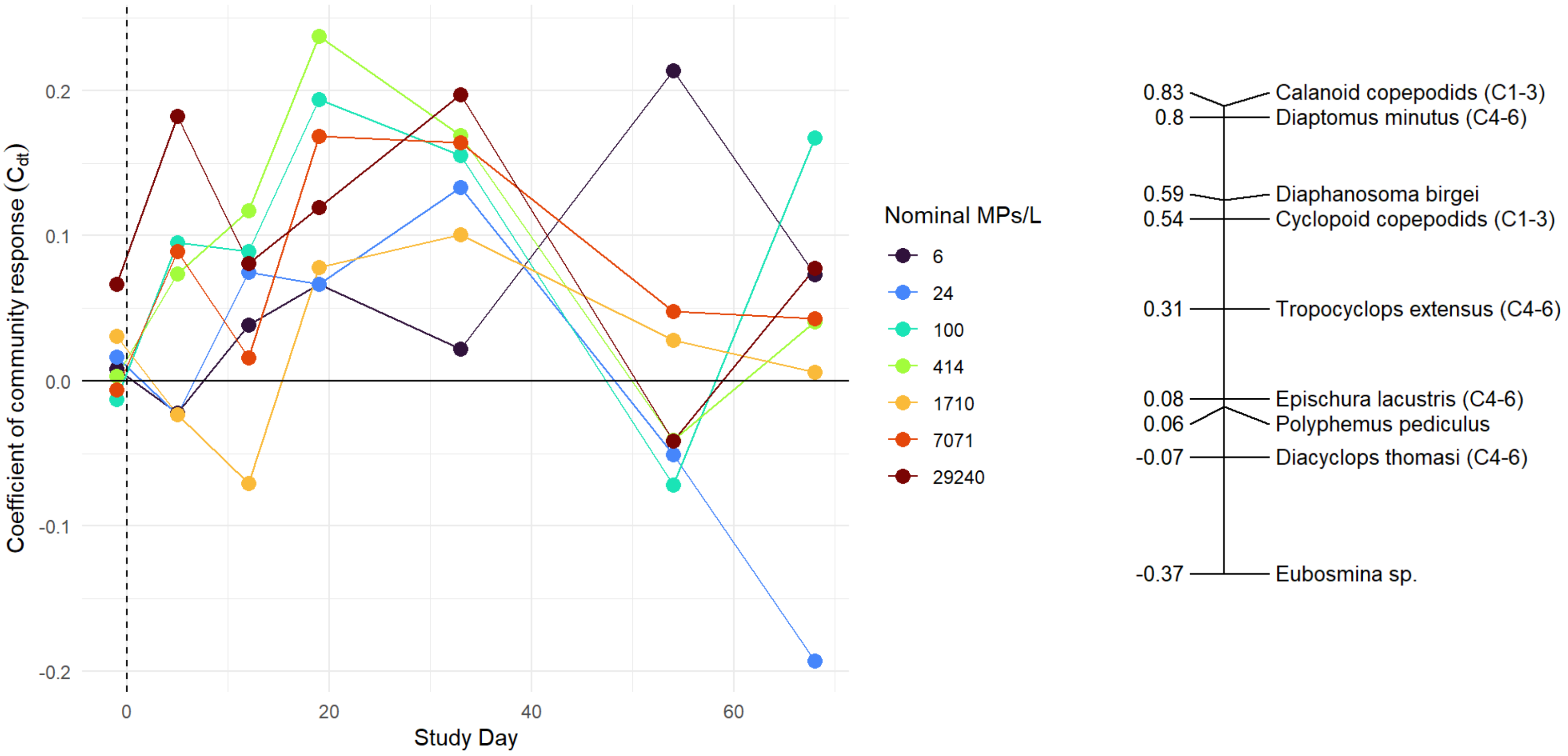


Figure 2.17 Principal response curve (PRC) for abundance-based zooplankton community composition. Abundance was $\log_{10}$ transformed prior to analysis. The community response (C_{dt}) is displayed on the left and species weights (b_k) are displayed on the right. The solid black horizontal line represents the control treatment (where $C_{dt} = 0$), and the dashed vertical line represents the day of MP addition (day 0). The PRC axis was not significant ($p = 0.97$).

Zooplankton Biomass. Initial zooplankton biomass on day -1 ranged from 44 to 96 µg/L (dry weight) across treatments (Figure 2.18). There was an initial decrease in total zooplankton biomass after MP additions for all treatments (including controls) and then temporal fluctuations occurred throughout the study. By day 68 zooplankton biomass was slightly lower across treatments from the start of the experiment (22 to 79 µg/L), and a large shift in biomass-based community composition occurred. In terms of biomass, the zooplankton community was generally dominated by *D. minutus* and *D. birgei* throughout the experiment. Some large bodied species were initially present but declined later in the experiment, including *Diacyclops thomasi*, *Daphnia mendotae* and *Holopedium glacialis*.

There was a significant positive relationship between total zooplankton biomass and MP treatment concentration on day 33 ($R^2 = 0.59$, $p = 0.016$; Figure 2.19). This trend was driven by an increase in calanoid copepod biomass. All other regressions were not statistically significant.

The PRC for zooplankton biomass was not significant ($p = 0.84$) indicating that biomass-based species composition of the zooplankton community was not affected by MP treatment concentration over time (Figure 2.20). The relatively large negative species weight (b_k) for *H. glacialis* indicated that in general, this species had a strong opposite affiliation with the community response in terms of biomass, however this response was not due to effect of MP treatment.

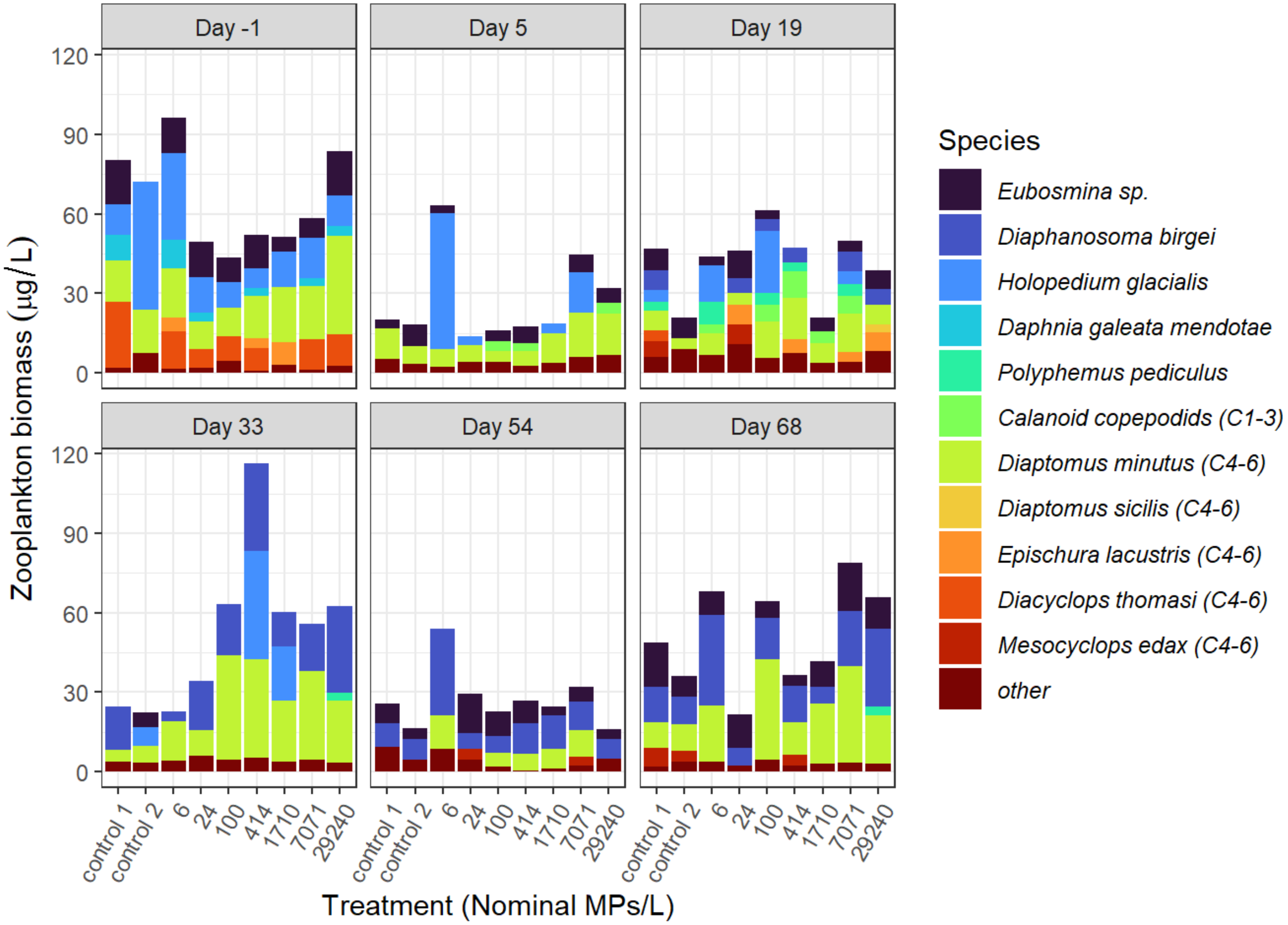


Figure 2.18 Biomass (µg/L dry weight) of zooplankton for each treatment on different days on the experiment.

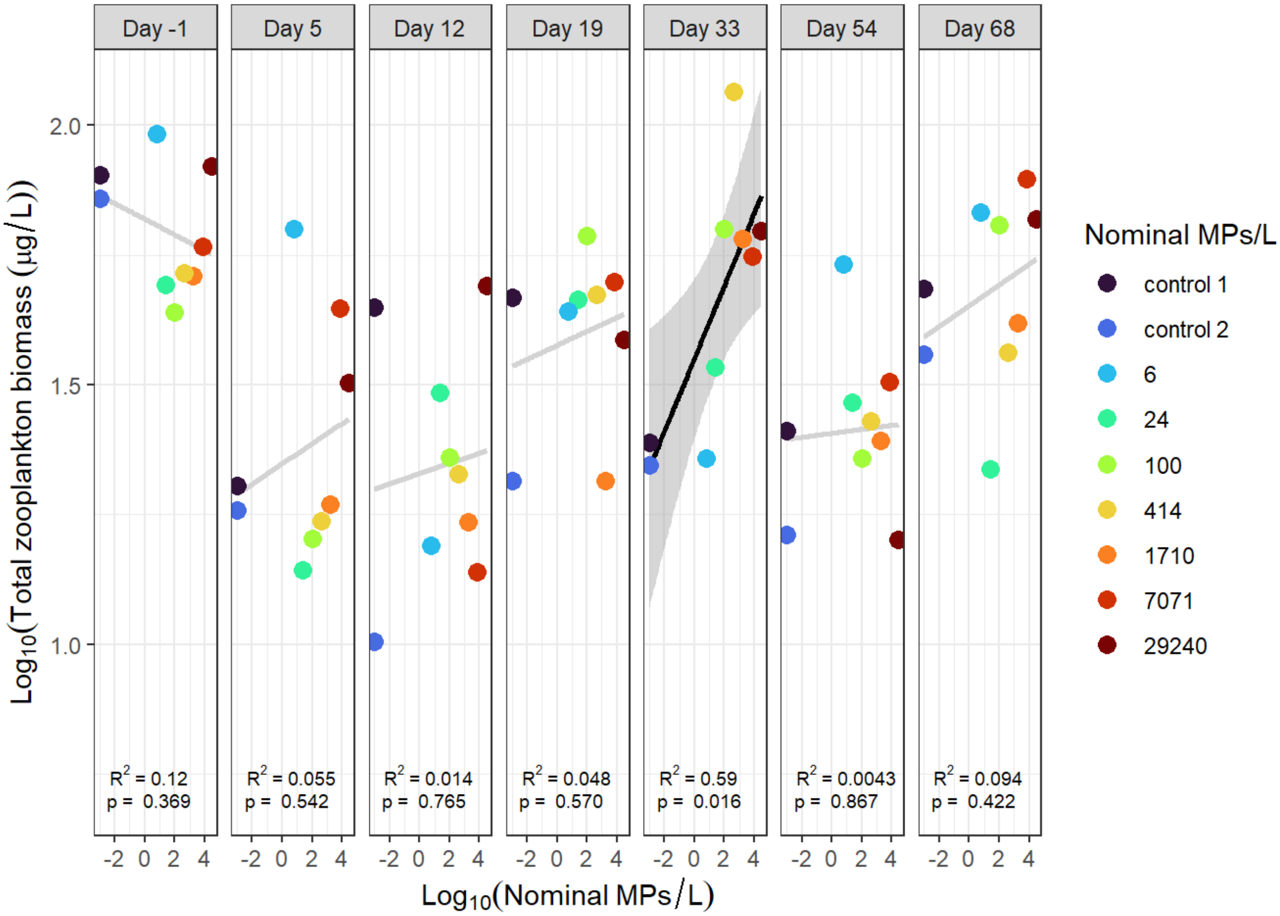


Figure 2.19 Zooplankton biomass versus MP treatment concentration during the limnocorral experiment.

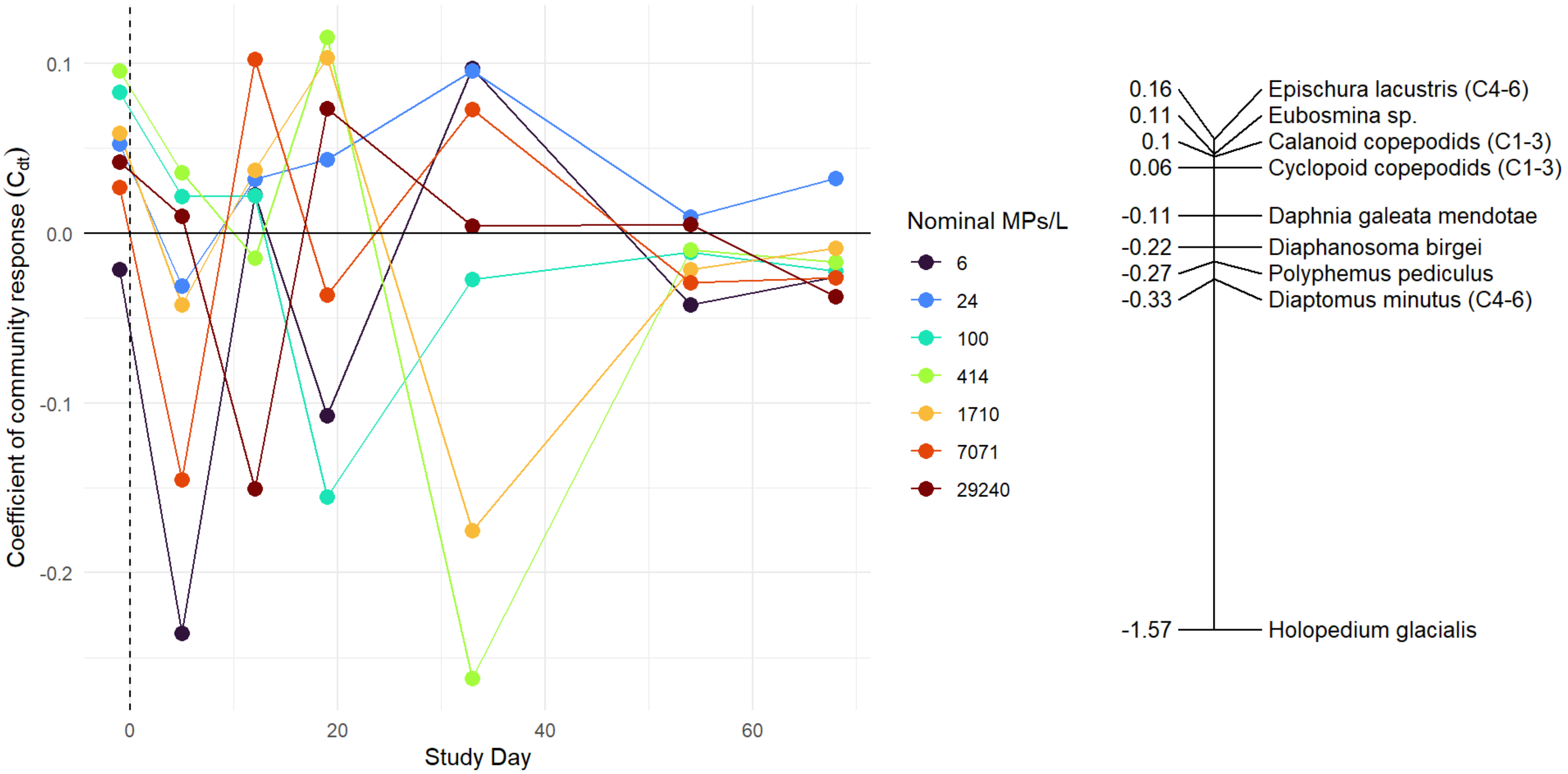


Figure 2.20 Principal response curve (PRC) for biomass-based zooplankton community composition. The community response (C_{dt}) is displayed on the left and species weights (b_k) are displayed on the right. The solid black horizontal line represents the control treatment (where $C_{dt} = 0$), and the dashed vertical line represents the day of MP addition (day 0). Biomass was $\log_{10}$ transformed prior to analysis. The PRC axis was not significant ($p = 0.84$).

Community Diversity. Zooplankton diversity, measured by the Inverse Simpson Index, ranged from 1.78 to 3.40 across treatments at the start of the experiment on day -1 and from 1.39 to 3.44 on day 68. Linear regression analysis indicated that there were no statistically significant relationships between zooplankton diversity and MP treatment concentration throughout the study ($p > 0.05$; Figure 2.21).

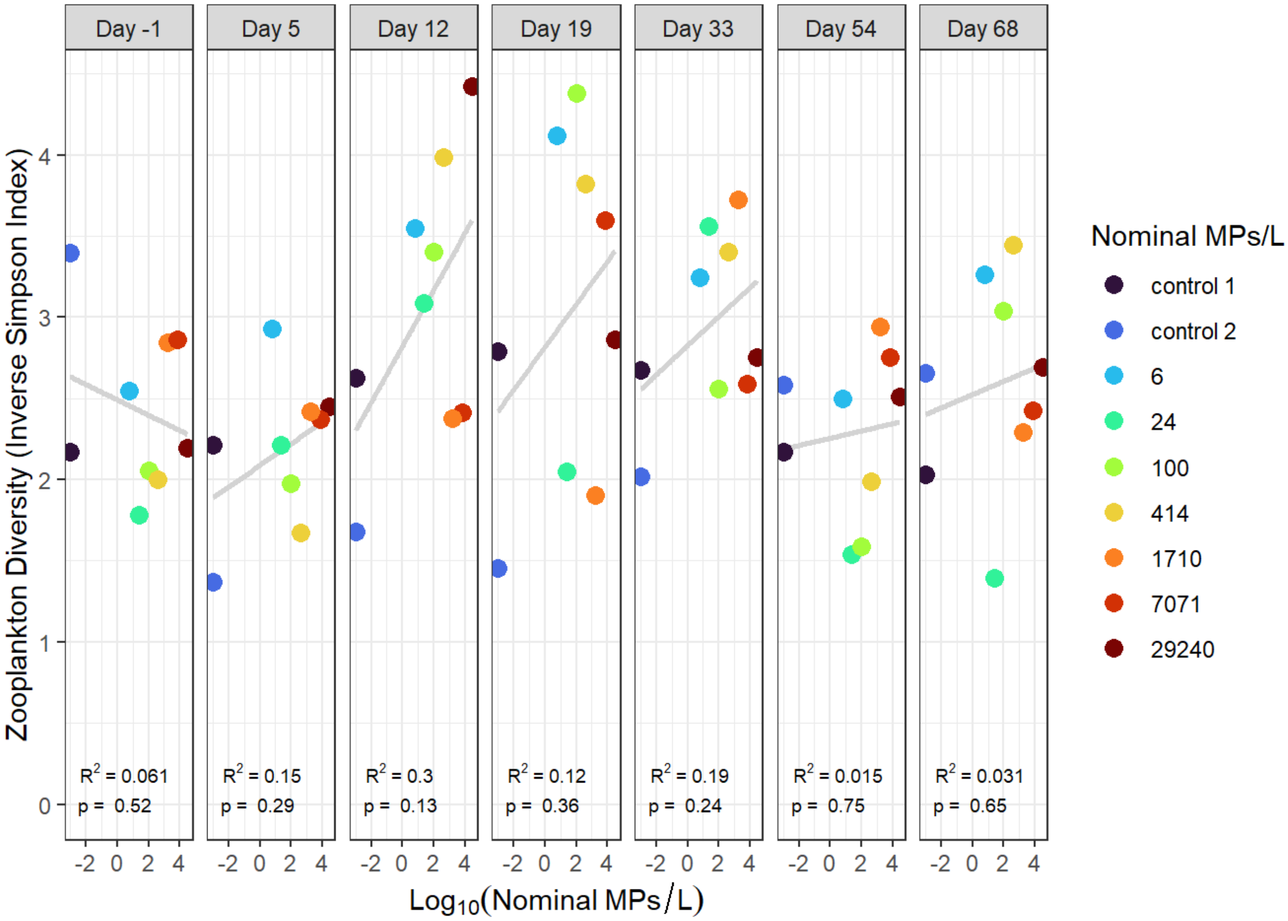


Figure 2.21 Zooplankton community diversity, measured by the Inverse Simpson Index, versus MP treatment concentration during the experiment.

Zooplankton Reproduction. In general, egg counts were low even for abundant taxa, however loose copepod eggs were often abundant. Egg ratios ranged from 0 to 0.37 eggs/female for the cladoceran *Eubosmina sp.* throughout the study. Linear regression indicated no significant relationships between eggs/*Eubosmina sp.* female and nominal MP treatment concentration for any study day ($p > 0.05$; Figure 2.22). The number of eggs per female for the calanoid *D. minutus* ranged from 0 to 6.8 throughout the study, which includes eggs that came from apportioned loose copepod eggs. There were no significant relationships between eggs per *D. minutus* female and MP treatment concentration throughout the study ($p > 0.05$; Figure 2.23). The number of eggs per female, including apportioned loose copepod eggs, for the cyclopoid copepod *T.extensus* ranged from 0 to 10.1 throughout the study. On day -1 the two controls were most variable in the ratio of eggs per *T. extensus* female. Linear regression analysis indicated that on day 19 a statistically significant negative relationship was observed between the ratio of eggs per *T. extensus* female and MP treatment concentration ($R^2 = 0.51$, $p = 0.031$; Figure 2.24).

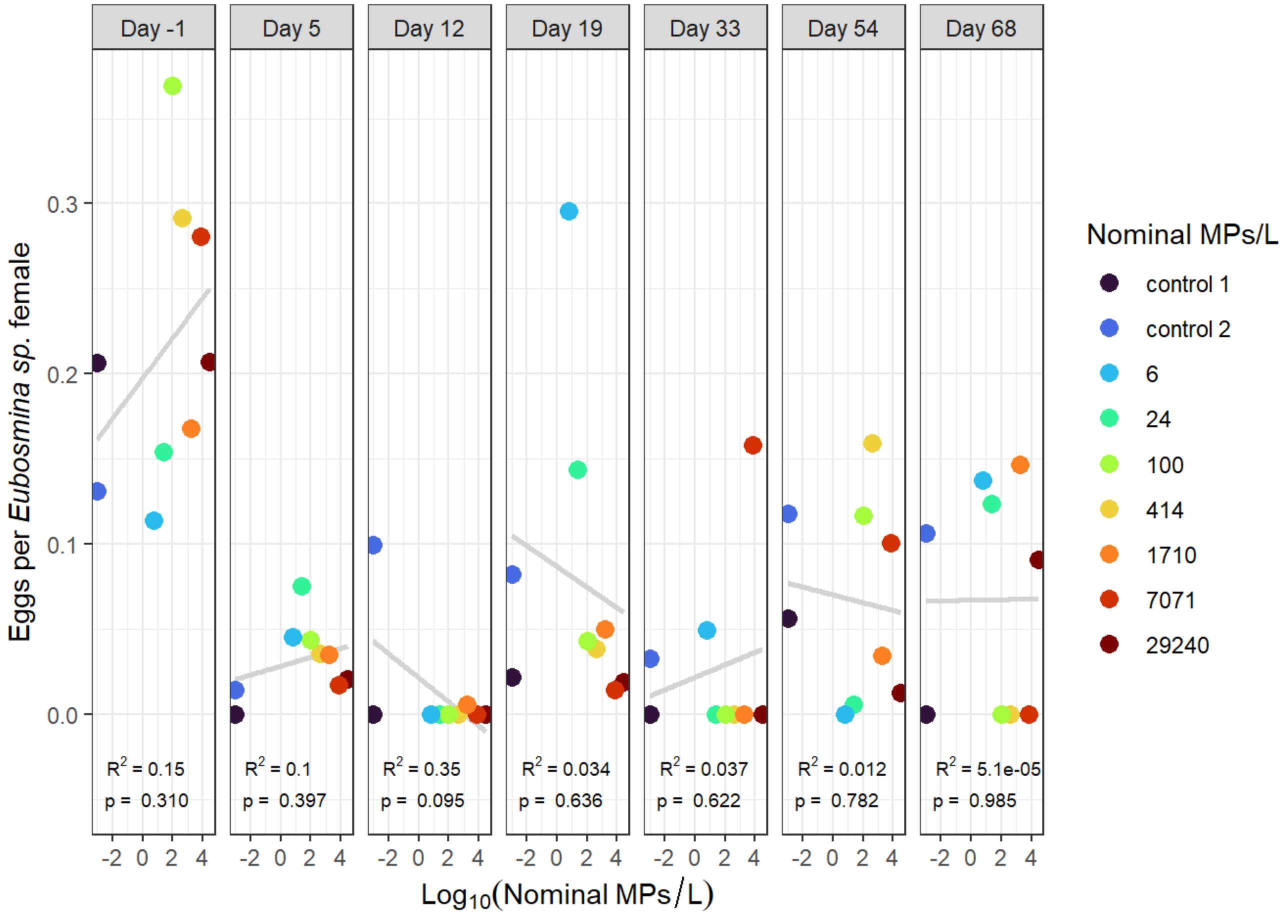


Figure 2.22 Egg ratios (eggs/female) for the cladoceran *Eubosmina sp*. regressed against MP treatment concentration for various days of the study.

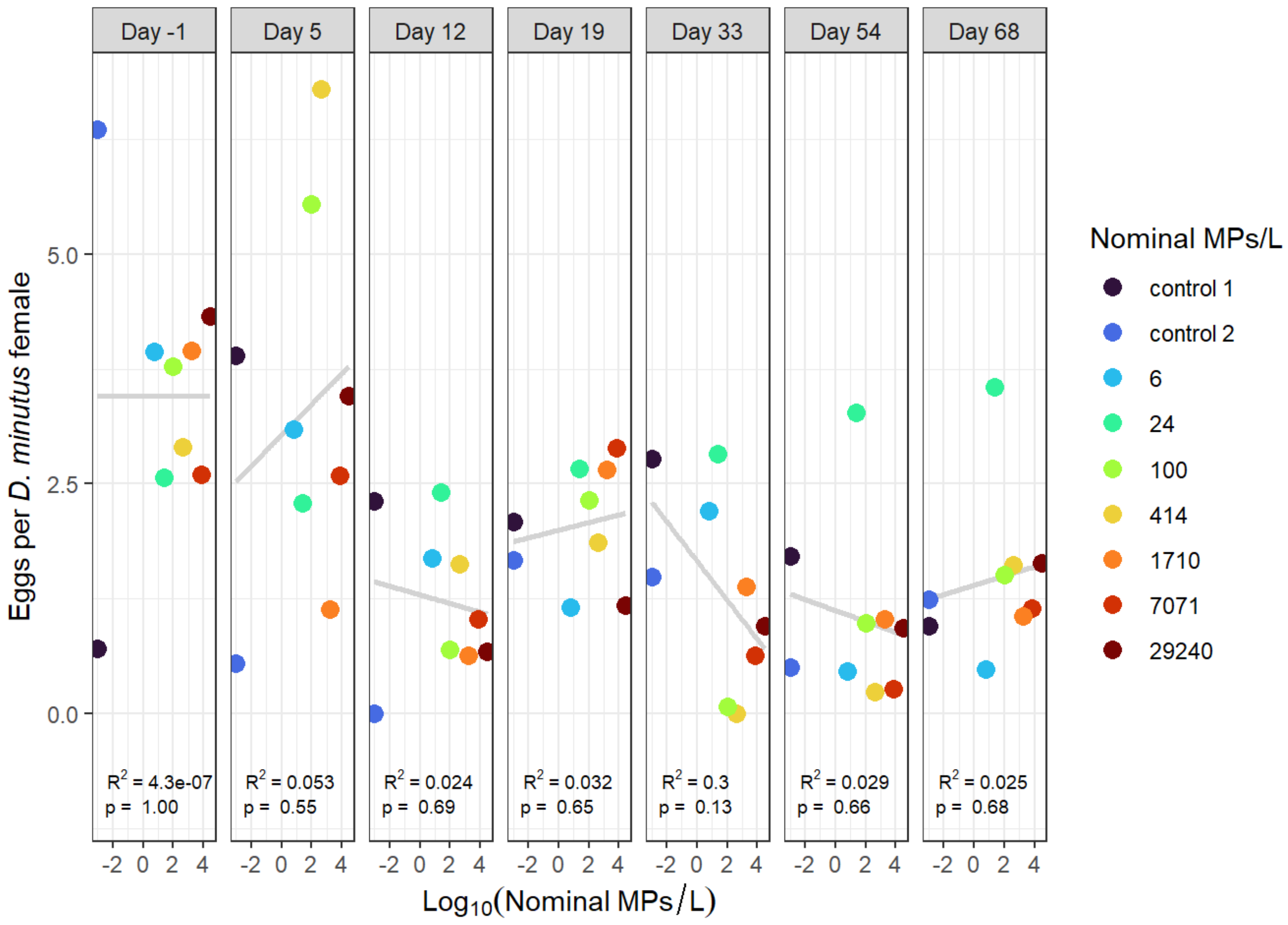


Figure 2.23 Egg ratios (eggs/female) for the calanoid copepod *Diaptomus minutus* regressed against MP treatment concentration for various days of the study.

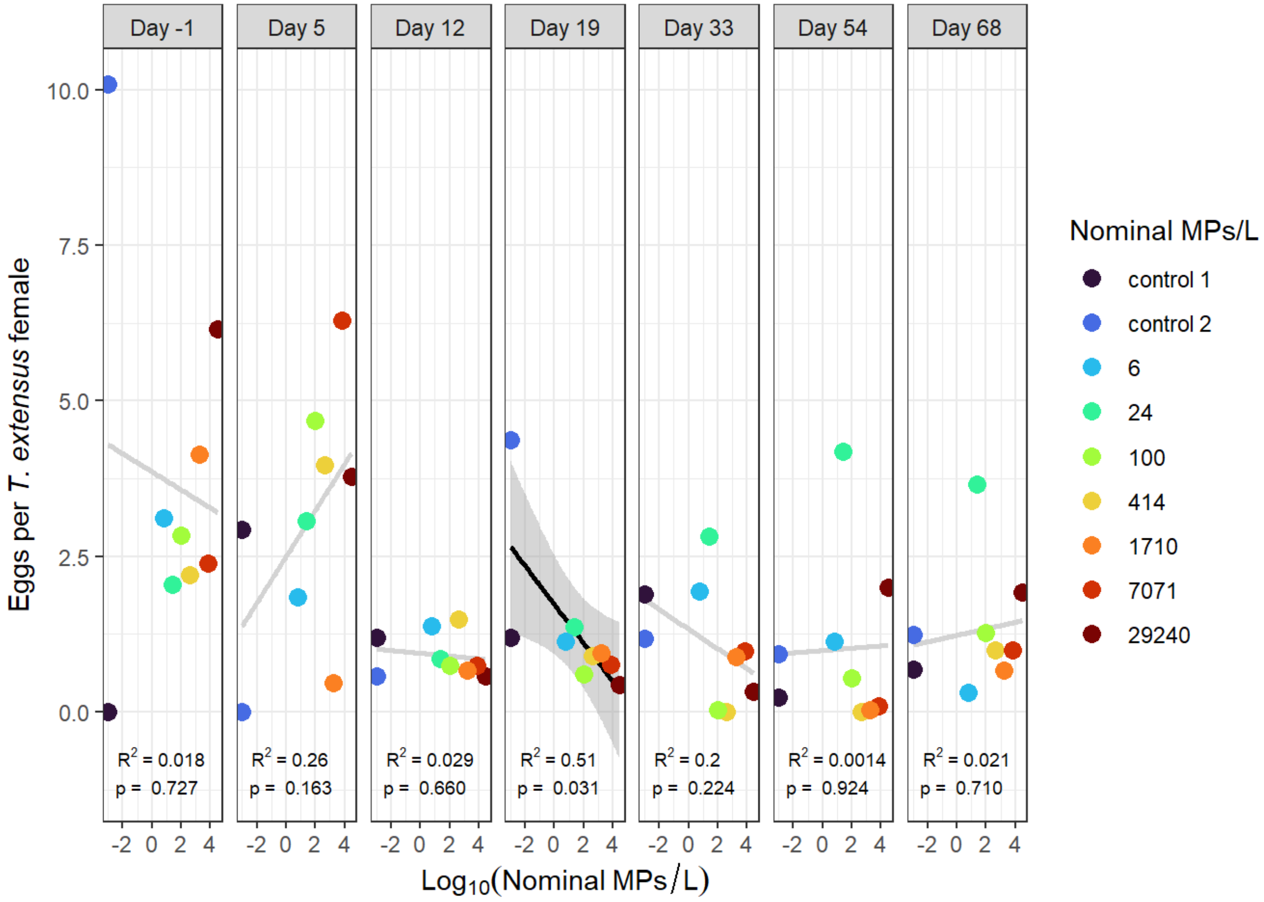


Figure 2.24 Egg ratios (eggs/female) for the cyclopoid copepod *Tropocyclops extensus* regressed against MP treatment concentration for various days of the study.

Initial abundances of copepod nauplii ranged from 47 to 93 nauplii/L across treatments. Abundance of copepod nauplii initially increased after MP additions for all treatments, followed by a sharp decline and then nauplii abundances remained low for the remainder of the experiment (Figure 2.25). Final nauplii abundances ranged from 9 to 49 nauplii/L across treatments. Linear regression analysis indicated that on day 12 a statistically significant positive relationship was observed between nauplii abundance and MP treatment concentration ($R^2 = 0.51$, $p = 0.032$) and on day 54 a statistically significant negative relationship was observed ($R^2 = 0.57$, $p = 0.019$; Figure 2.26).

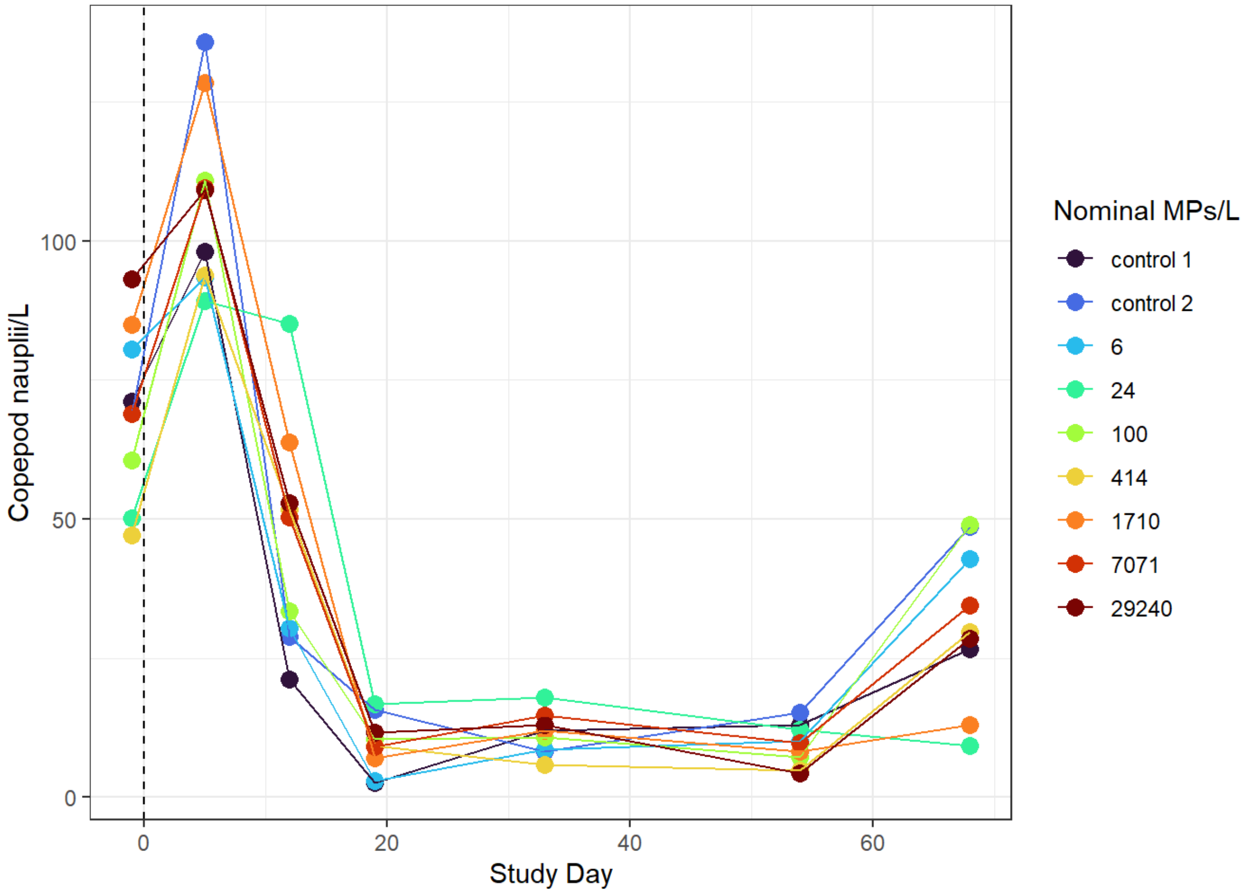


Figure 2.25 Copepod nauplii abundance throughout the study for each treatment.

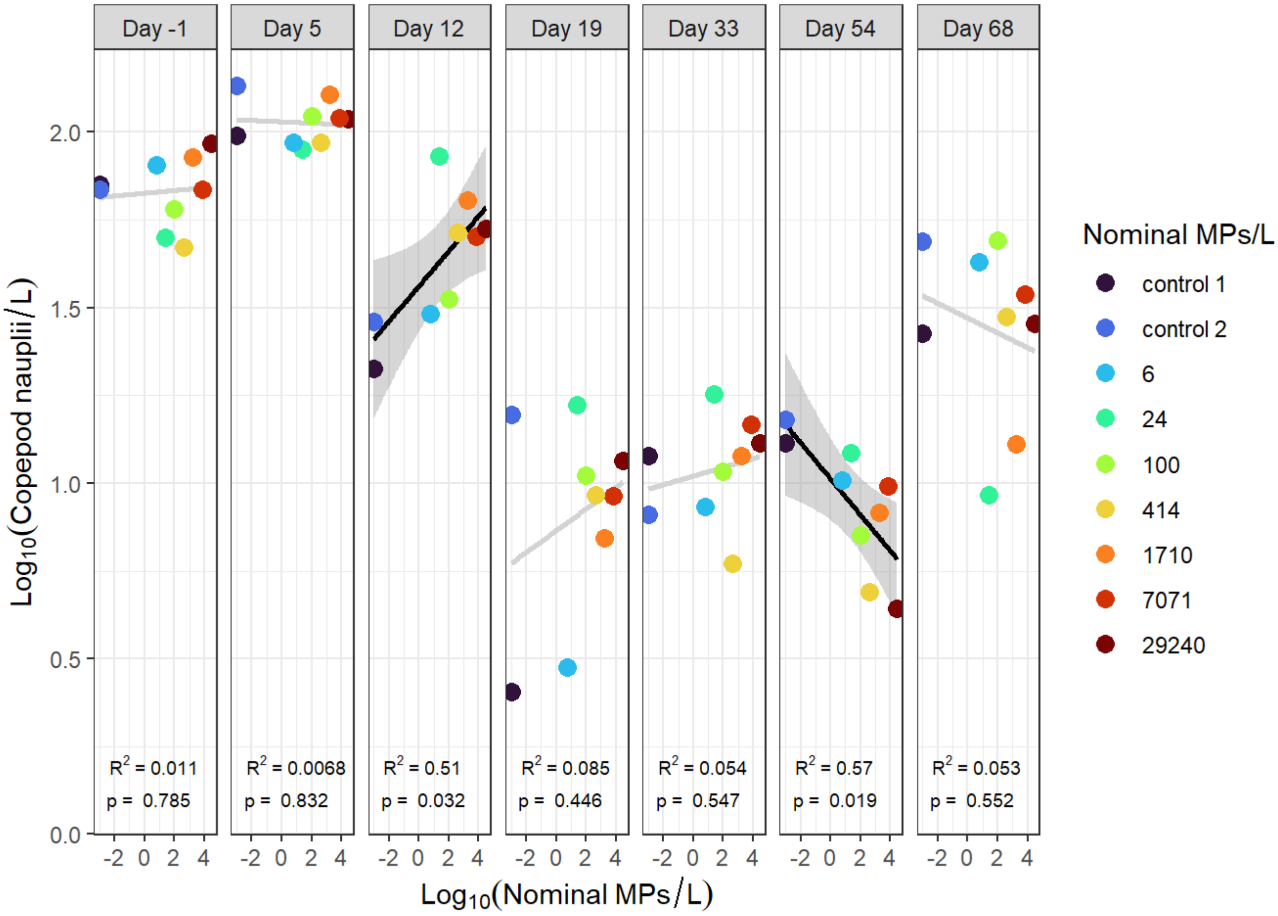


Figure 2.26 Copepod nauplii abundance versus MP treatment concentration during the experiment.

2.5 Discussion

The objective of this chapter was to evaluate the effects of environmentally relevant MP concentrations on a natural freshwater plankton community. Overall, there was little evidence that MP concentration altered phytoplankton biomass or community composition. Results for other parameters were variable but suggestive of possible negative impacts. For example, there may be potential for MPs to temporarily negatively affect chlorophyll *a* concentration. Total zooplankton abundance, biomass, and species composition were not negatively impacted by MP treatment concentration. However MPs may have had taxa specific responses including positive effects on calanoid copepod abundance and negative effects on *Tropocyclops extensus* egg production. However, these statistically significanttaxa specific responses must be considered in the light of the many comparisons undertaken, which increase the probability that some statistically significant relationships may have occurred by chance alone. Overall, despite the large gradient of MP concentrations assessed, there was little evidence that environmentally relevant MP concentrations had significant impacts to zooplankton abundance, biomass, or community structure.

2.5.1 MP Fate in the Water Column

Concentrations of MPs in the water column were considerably lower than nominal concentrations based on the assumption of a homogenous distribution within the water column of each limnocorral. For example, in the treatment with the highest nominal concentration (29,240 MPs/L), actual concentrations in the water column were almost always less than 500 MPs/L, more than an order of magnitude lower than the nominal concentration. Presumably this was due to the settlement of particles to the bottom, adsorption to limnocorral walls, and from buoyant MPs floating on the surface and being removed from limnocorrals by wind and waves. Yellow PE MPs were observed on the surface in the high MP treatments for the entire 10-week experiment. PE and PS particles were detected throughout the water column, but PET had very low relative detection throughout the study. The high density of the PET polymer probably caused these particles to sink rapidly after additions. MPs in the limnocorrals were not homogenously distributed due to differing buoyancies of the polymers, and wind and wave action. Therefore, the actual exposure for pelagic organisms in this study was much lower than nominal concentrations. Although observed concentrations were far below nominal concentrations, maximum concentrations in the limnocorrals were still equivalent to the highest concentrations observed in natural systems (Eerkes-Medrano et al. 2015). A similar study using in-situ 1 m x 1 m mesocosms also found that MP concentrations declined rapidly in the water column and were up to 100 times lower than initial expectations after 50 days (Yildiz et al. 2022).

Low concentrations of MPs (< 4 MPs/L) were detected in the control treatment even though no MPs were purposely added. Although limnocorrals were always sampled in order from low to high MP concentration to minimize contamination, and sampling equipment was rinsed thoroughly after each day, it is possible that MPs floating on the water surface were blown by the wind into control limnocorrals or were introduced on sampling equipment.

MP concentrations were determined in only 3 of the study limnocorrals. As a result, all statistical analyses were conducted on the assumption that nominal treatment densities were correlated with MP exposure over the duration of the experiment.

2.5.2 Environmental Variables

Environmental parameters measured in the limnocorrals were not correlated with MP treatment concentrations. The decrease in silica (SRSi) on Day 68 in all enclosures was most likely a response to the increased growth of silicious diatoms. Increases in ion concentrations over time probably reflect the effect of evaporation. MPs may increase turbidity and attenuate light at high concentrations (Bhattacharya et al. 2010; Rani-Borges et al. 2021), but no significant increases in turbidity or light attenuation were observed, likely because the MP concentrations were too low to cause shading, and in fact water clarity was often highest in the highest MP treatment.

Final Yellow Perch densities were not correlated with MP treatment concentrations, and in fact the greatest variation occurred between the two controls. Perch density was significantly negatively correlated with phytoplankton biomass, but not with zooplankton biomass, likely due to the fish being supplemented with frozen *Mysis*. It is possible that decomposition of dead fish or unused *Mysis* contributed to nutrients that enhanced phytoplankton growth, which could explain why there was higher phytoplankton biomass in limnocorrals with fewer fish.

2.5.3 Phytoplankton Response

Total phytoplankton biomass was not significantly related to MP treatment on any day. Chlorophyll *a* was negatively impacted by MP treatment on day 12 only. Given the relatively weak relationship and multiple statistical comparisons used in this study, this result may be a chance occurrence. Additionally, chlorophyll *a*/biomass ratios were not significantly related to nominal MP concentrations.

Some studies have shown that MPs may negatively impact phytoplankton chlorophyll *a* (Besseling et al. 2014; Wu et al. 2019; Tunali et al. 2020), suggesting that photosynthesis may be hindered (Rani-Borges et al. 2021). Any negative effect of MPs on chlorophyll *a* in this study was not likely due to light attenuation or turbidity since MPs did not negatively affect water clarity. Adsorption of MPs to algal cells may obstruct the pathways for CO_2 and nutrient uptake (Bhattacharya et al. 2010) and cause oxidative stress in phytoplankton (Mao et al. 2018). By day 68 of this study, chlorophyll *a* concentration was no longer related to MP concentration, suggesting chlorophyll *a* concentration may be only temporarily affected by MPs, if at all. Mao et al. (2018) similarly found that photosynthetic activity was temporarily negatively affected by MPs and algal cells could recover over time by thickening the cell wall.

The PRC analysis showed no significant treatment effects of MPs on phytoplankton community composition over time. Phytoplankton diversity was also not related to MP treatment concentration on any day. While MPs have negatively impacted phytoplankton in some laboratory studies (Rani-Borges et al. 2021), others have alternatively found no impact or even positive effects (Long et al. 2017; Yokota et al. 2017; Canniff and Hoang 2018). Overall, these results suggest that environmentally relevant MP concentrations are likely not a threat to freshwater phytoplankton communities.

There was considerable variation in total phytoplankton biomass and species composition among treatments and especially between the two controls, which made it difficult to decipher any modest responses to MPs. This variation may have been due to the differing perch densities since final phytoplankton biomass was strongly negatively correlated with final perch density. The exact mechanism is unclear but may have been caused by nutrients introduced from decaying fish or unused *Mysis* food pellets. Chrysophytes initially dominated in all limnocorrals and diatoms became the dominant group by day 35. IISD-ELA lakes are typically dominated by chrysophytes (Findlay et al. 2001) as was observed at the experiment's start. The increase in diatoms may reflect their ability to colonize and grow on plastic surfaces (Reisser et al. 2014) such as the limnocorral walls. Many of the dominant diatom taxa in this study are typically found in the littoral zone (Dave Findlay, pers. comm.).

2.5.4 Zooplankton Community Response

MP Ingestion. On day 68 of the experiment, abundant zooplankton taxa including cladocerans, and calanoid and cyclopoid copepods had MPs in their guts. The number of particles per individual zooplankton ranged from 0 to 0.18 MPs/zooplankton (average of 0.038) across treatments, which is within the range of what has been found in nature by other researchers (0.029 MPs/copepod, Desforges et al. 2015; 0.024 MPs/copepod, Aytan et al. 2022). The 29,240 and 414 MPs/L treatments had the highest average numbers of MPs in zooplankton (0.059 and 0.068 MPs/zooplankton, respectively) while the 6 MPs/L treatment had the lowest (average 0.01 MPs/L). MPs were also encountered in some zooplankton in the control treatment, although at very low average numbers (0.016

MPs/zooplankton). Zooplankton in the MP treated limnocorrals only ingested PE and PS MPs. PET was negatively buoyant and typically detected at very low concentrations in the water column, so this polymer was largely unavailable to grazing zooplankton.

Cladocerans had more MPs in their guts on average (0.06 MPs/L) than calanoid and cyclopoid copepods (0.02 MPs/L) possibly because copepods are more discriminant feeders than cladocerans (Demott 1986; Sodré and Bozelli 2019). Some marine calanoids have even been shown to select against ingesting MPs (Xu et al. 2022). In general, zooplankton have been shown to egest MPs within minutes (Ogonowski et al. 2016) to hours (Vroom et al. 2017) after ingestion so most MPs were unlikely retained for extended periods of time. It is likely that the low numbers of ingested MPs found in zooplankton was due to low exposure, avoidance, and rapid egestion of MPs.

The size of MP particles encountered in zooplankton ranged between 29 to 1010 µm (mean of 145 µm) in length and 18 to 261 µm (mean of 68 µm) in width across taxa. Large MP particles have been observed to be ingested by Daphniids (1430 µm long fibers; Jemec et al. 2016) but it is likely that most ingested particles would fall below 100 µm (Kokalj et al. 2018). Methods for analyzing the smallest size MP fraction (1 to 53 µm) are still under development, so the full size range of ingested particles may be greater than reported here.

Zooplankton Abundance and Biomass. No negative effects of MP treatment concentration on total zooplankton abundance or biomass were detected. Similarly, a smaller scale mesocosm study by Yildiz et al. (2022) also found no impacts of MPs on total zooplankton abundance or biomass at environmentally relevant concentrations. On day 35 of the study, positive relationships were observed between total zooplankton abundance and biomass and MP treatment concentration. Given the multiple comparisons involved in these analyses, it is possible that this result simply occurred by chance. Alternatively, this positive relationship may have arisen as a result of biofilm growth on the added MPs. It has been shown that MPs containing a biofilm coating can have a positive effect on zooplankton, while pristine MPs with no such biofilm have negative effects (Amariei et al. 2022). Initial biofilm formation begins within hours of MP addition (Rummel et al. 2017) but can take weeks to accumulate a substantial coating (Amariei et al. 2022). This may explain why there was not a positive effect on zooplankton until day 35. Zooplankton also selectively ingest aged MPs with biofilm development (Demott 1986; Vroom et al. 2017). After day 35 and beyond, there was no longer a positive relationship between MP treatment concentration and zooplankton abundance or biomass, suggesting that this may be only a temporary response.

Zooplankton Community Composition and Diversity. The PRCs for zooplankton species composition based on abundance and biomass were not statistically significant, indicating that species composition did not significantly differ among treatments over time. Zooplankton diversity was also not affected by MP treatment concentration. Negative impacts to various zooplankton taxa have been found in the laboratory (Botterell et al. 2019), which could impact diversity in natural environments if some species are more sensitive to MPs than others, however that was not the case in this study. Yildiz et al. (2022) also found no effect of MPs on zooplankton species composition in their mesocosm study.

Zooplankton Reproduction. MP treatment concentration had a small but temporary negative impact on the ratio of eggs per female for the cyclopoid copepod *T. extensus*. MP treatment concentration did not have an effect on the ratio of eggs per female for the cladoceran *Eubosmina sp.* or the calanoid copepod *D. minutus*. While there doesn't appear to be a significant negative effect on the number of eggs produced per female for these species, the hatching success of the eggs is unknown. Cole et al. (2015) found that MPs did not affect egg production but negatively impacted the hatching success of calanoid copepods. Similarly, Lee et al. (2013) suggested that MPs may inhibit the fertilization of copepod eggs. Copepod nauplii abundance could be used as a proxy for hatching success in this study to predict the reproductive output for copepods exposed to MPs. After 12 days of MP exposure, nauplii abundance had a positive relationship with MP treatment concentration, but after 54 days of exposure nauplii had a negative relationship with MP concentration. These impacts for nauplii abundance were small and did not appear to affect the abundance of older stages later in the experiment, but future studies should further investigate the potential for MPs to impact copepod reproduction.

2.6 Conclusion

These results suggest that common MPs found in the environment (PE, PS and PET fragments) are unlikely to negatively impact natural freshwater plankton communities at environmentally relevant concentrations. Phytoplankton biomass and species composition were not affected by MPs, and although a negative impact to chlorophyll *a* concentration was initially observed 12 days after of exposure, MPs did not impact chlorophyll *a* concentration after 68 days. There was no effect of MPs on the chlorophyll *a*/biomass ratio, suggesting that chlorophyll *a* production is not significantly affected by MPs. Zooplankton ingested MPs in low numbers and were not negatively impacted in terms of abundance, biomass, or diversity. In some instances, MPs may increase calanoid copepod abundance, possibly from ingestion of biofilm-coated MPs. The ratio of eggs per female for the cyclopoid copepod *T. extensus* was minimally reduced after 19 days and copepod nauplii abundance was minimally reduced after 54 days. Both recovered suggesting that copepod reproduction may be only marginally temporarily impacted by MPs, if at all. In summation, these results challenge those of laboratory experiments but are consistent with the lone study using more realistic conditions (Yildiz et al. 2022). More field-based experiments using complete food webs are needed to better understand the potential impacts of MPs on natural aquatic communities.

CHAPTER 3: EFFECTS OF MICROPLASTICS WITH AND WITHOUT CHEMICAL ADDITIVES ON FRESHWATER PLANKTON COMMUNITIES

3.1 Abstract

There is growing concern about the potential impacts of microplastics (MPs) on aquatic organisms. Zooplankton ingest MPs and may be negatively affected by reduced feeding and exposure to chemical additives. Phytoplankton have been shown to respond to MPs both negatively and positively. However, the mechanisms for effects are not fully understood and existing research is limited to the laboratory. The objective was to assess whether any effects of MPs are due to chemical additives or the physical properties of the particles themselves for natural freshwater phytoplankton and zooplankton communities. A large scale limnocorral experiment was conducted in Lake 378 at the IISD-Experimental Lakes Area in northwestern Ontario, Canada. An equal mixture of common polymers (polyethylene; PE, polyethylene terephthalate; PET, and polystyrene; PS), with and without chemical additives were added to limnocorrals as a single total pulse of 29,240 total MPs/L and were contrasted with a control treatment with no added MPs. The phytoplankton and zooplankton communities were monitored for 62 days following MP additions. Phytoplankton biomass and chlorophyll *a* concentration were not affected by MP treatment, however phytoplankton community composition was affected by MPs on day 62. Zooplankton abundance, biomass and species composition were not significantly affected by MPs either with or without chemical additives. There was a small negative impact on *Tropocyclops extensus* egg production over time. *Chaoborus* larval abundance was temporarily higher in enclosures containing MPs without additives. Overall, there

was little evidence of significant negative impacts of MPs, with or without chemical additives, on natural phytoplankton or zooplankton communities.

3.2 Introduction

Plastics are largely composed of organic polymers derived from fossil fuels, and their potential impacts on aquatic ecosystems are a growing concern. Global plastic demand has been rapidly increasing over the past 50 years, and as a result of poor waste management practices and slow degradation, plastic has been accumulating in aquatic environments (Anderson et al. 2016; Duis and Coors 2016). Some of the most widely produced polymers include polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET) and polystyrene (PS), which are predominantly used for packaging, and polyvinylchloride (PVC), which is used in construction (Geyer et al. 2017). Chemical additives such as plasticizers, stabilizers, antioxidants, flame retardants, and colorants are added to plastics to contribute to their desirable functional properties (Geyer et al. 2017; Hahladakis et al. 2018). For example, antioxidants and ultraviolet (UV) stabilizers are used to slow the degradation of plastics, and plasticizers are used to increase plasticity and reduce brittleness (Hahladakis et al. 2018). The amount of additives differ for various polymer types, with PVC typically containing high amounts of plasticizers (10-70%) while PE typically contains only low percentages of antioxidant and stabilizer additives (0.05-3%; Hahladakis et al. 2018). Many polymers and plastic additives are considered toxic and may be carcinogenic or endocrine disrupting compounds (Lithner et al. 2011; Cole et al. 2011; Horton et al. 2017). In the environment, plastic debris is exposed to weathering processes such as UV radiation and abrasion from wind and waves, which results in fragmentation and release of chemical additives (Song et al. 2017).

Microplastics (plastic particles < 5 mm; MPs) are of particular concern since they are available to a wide range of organisms (Egbeocha et al. 2018). MPs are ubiquitous in freshwater environments across the world (Eerkes-Medrano et al. 2015) and are found even in remote regions such as the Canadian Arctic (Jones-Williams et al. 2021). MPs may be released directly into the environment as micro-sized particles (primary MPs) or broken down by weathering processes into smaller fragments from larger plastic debris (secondary MPs). Due to constant fragmentation of plastic into smaller and smaller particles, MP debris in the environment will continue to increase (Song et al. 2017; Horton et al. 2017). MPs vary in size, shape and chemical composition making them complex contaminants and there is no clear consensus on their toxicity in natural environments (Bucci et al. 2020). There is growing evidence to suggest MPs can have toxic effects for a wide range of aquatic organisms, including primary producers, macroinvertebrates, zooplankton and fish (Cole et al. 2015; Mao et al. 2018; Besseling et al. 2019; Wang et al. 2020). It is uncertain if the impacts of MPs are driven by the particles themselves or from exposure to chemical additives (Zimmermann et al. 2020).

3.2.1 Physical Effects

Phytoplankton, an essential group of primary producers in aquatic ecosystems, are known to adsorb to MPs and form aggregates (Long et al. 2017; Yokota et al. 2017; Mao et al. 2018). Adsorption of MPs by phytoplankton may obstruct CO_2 and nutrient uptake pathways and shade cells, leading to reduced growth and chlorophyll *a* production, and oxidative stress at high concentrations (Bhattacharya et al. 2010; Sjollema et al. 2016; Mao et al. 2018; Prata et al. 2019b; Rani-Borges et al. 2021). MPs can also provide a substrate for phytoplankton growth in a process called biofouling, where microorganisms attach themselves to MP particles and form complex communities (Kooi et al. 2017; Canniff and Hoang 2018). Biofilm growth additionally promotes the ingestion of MPs by grazing organisms such as zooplankton (Vroom et al. 2017). MPs are readily ingested by zooplankton and can be transferred through food webs (Setälä et al. 2014). Reduced feeding from MP ingestion (food dilution) is thought to be the predominant physical mechanism for MP effects for zooplankton (Cole et al. 2015; Amariei et al. 2022). MPs can physically block the guts of zooplankton and provide a false sense of satiation, reducing food intake which contributes to adverse effects on growth and reproduction at high concentrations (Lee et al. 2013; Cole et al. 2015; Ogonowski et al. 2016; Pan et al. 2022). However, weathered MPs with a biofilm coating have been shown to offset the negative impacts of food dilution and may even benefit zooplankton by providing an added nutrition source (Vroom et al. 2017; Canniff and Hoang 2018; Amariei et al. 2022).

3.2.2 Effects of Chemical Additives

MPs have the potential to impact phytoplankton growth and photosynthetic activity via exposure to chemical additives that may leach into water (Rani-Borges et al. 2021). Research on the impacts of plastic leachates on phytoplankton is limited (Delaeter et al. 2022), however it has been suggested that MPs containing high amounts of chemical additives are likely more toxic than MPs with a lower amounts (Capolupo et al.

2020; Rummel et al. 2022). Additionally, toxicity of chemical additive leachates likely varies for different phytoplankton species and polymer types (Delaeter et al. 2022). For example, leachate from polystyrene enhanced photosynthetic activity for some chlorophyte species (Chae et al. 2020) but inhibited others (Schiavo et al. 2021). Likewise, diatom species have shown varying responses to plastic leachates from different polymers, with PET being non toxic, while other polymers such as PVC, PP and PS were found to be toxic; PET contains very little additives compared to other polymers (Capolupo et al. 2020; Piccardo et al. 2020).

Similar to the effects of chemical additives of MPs on phytoplankton, the effects to zooplankton are also complex and may depend on the polymer and type and amount of additives present. For example, Zimmermann et al. (2020) found that for PVC, the toxic effects on *Daphnia magna* reproduction were due to extracted chemicals but not the particles themselves, whereas for bio-polymers polyurethane (PUR) and polylactic acid (PLA) toxicity was induced by the particles and not the chemicals. Similarly, PE, PVC and PA polymers were only found to be toxic to copepods when containing chemical additives (Beiras et al. 2021). In terms of polymer type, PVC and PP may have more toxic leachates for zooplankton than PS and PET, which is likely a reflection of the levels of additives (Gewert et al. 2021). MP particles and chemical additives likely produce different modes of toxicity for zooplankton; physically, MP particles may cause food deficiency leading to reduced growth and reproductive output, while some chemical additives may act as an endocrine disrupter and affect reproduction (Song et al. 2022). There is also potential for MPs to indirectly affect trophic food web structure which could have cascading impacts for aquatic ecosystems (Setälä et al. 2014; Au et al. 2017; Chae et al. 2018). In general, the mechanisms of MP toxicity are not fully understood, and studies are largely limited to the laboratory.

This chapter investigates the physical and chemical impacts of MPs on natural freshwater zooplankton and phytoplankton communities using in-situ limnocorrals. A mixture of three common polymers (PE, PET, and PS) was added as secondary MP fragments with and without chemical additives to assess their physical and chemical impacts. In addition to the MP treated limnocorrals, a control treatment had no added MPs. Phytoplankton biomass, community composition and chlorophyll *a* concentration, and zooplankton abundance, biomass, reproduction, and community composition were monitored for 9 weeks after MP additions to examine the responses of the plankton community to MPs with and without chemical additives.

3.3 Methods

3.3.1 Study Design

Nine limnocorrals (10 m diameter and 2 m deep) were deployed in Lake 378 in May 2022 at the International Institute for Sustainable Development Experimental Lakes Area (IISD-ELA) located in northwestern Ontario, Canada (49^{o}41’37.88” N, 93^{o}46’32.18” W). Limnocorrals were constructed of a food-grade non-permeable polyethylene curtain containing no chemical additives that were secured to a 10-sided floating polystyrene collar (Curry Industries, Winnipeg, Manitoba, Canada). The limnocorrals had sealed bottoms and were filled with lake water using a pump (a total volume of ~150,000 L) and held in place with cement anchors in the pelagic zone of the lake (Figure 2.3).

Three treatments were randomly assigned to limnocorrals and replicated in triplicate. Treatments included a control with no added MPs, MPs containing additives, and MPs without additives (Figure 3.1). Both MP treatments used the same mixture of PE, PET and PS fragments as in the first experiment (Chapter 2). MPs in the additive treatment contained common chemical additives including UV stabilizers, antioxidants, and pigments (Table 2.1), whereas MPs in the non-additive treatment contained no chemical additives. Both MP treatments were added at the highest dose used in the first experiment (29,240 particles/L). Added MPs ranged in size from 10 µm – 1900 µm and each polymer type varied in buoyancy (PE was positively buoyant, PET was negatively buoyant, and PS was neutrally buoyant). MPs were added as a single pulse to the water surface at the start of the experiment (June 9th; day 0) by first wetting the MPs to prevent them from becoming airborne, then evenly dispersing them around the limnocorrals.

The limnocorrals contained natural communities of bacteria, phytoplankton, periphyton and zooplankton and were also stocked with 26 young of year Yellow Perch, a common planktivorous fish species found in Lake 378. Two weeks before MP additions, the zooplankton community was augmented with 15 vertical net tows each from Lake 378 to ensure sufficient abundances of zooplankton were present. Fish feeding was not supplemented for this limnocorral experiment to assess potential food dilution effects with Yellow Perch.

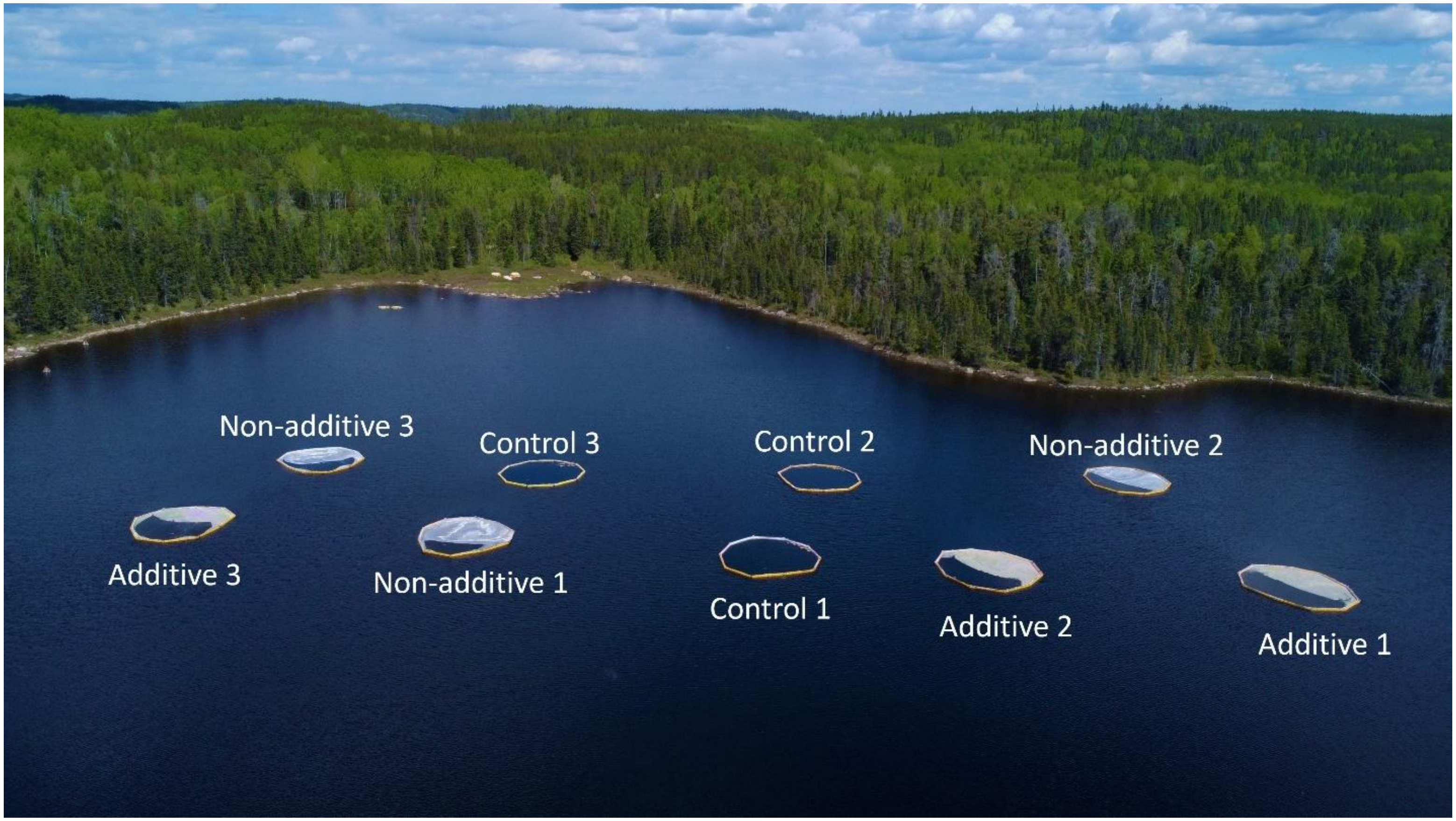


Figure 3.1 Drone photograph of the mesocosms in Lake 378. Treatments included a control treatment (no MPs), an additive treatment (MPs containing chemical additives) and a non-additive treatment (MPs containing no additives). Treatments were randomly assigned to limnocorrals. Photograph by Garth Covernton.

3.3.2 Sample Collection and Analysis

Samples were collected from all limnocorrals one day prior to MP additions (June 8th, 2022; day -1), and then at 2 weeks (June 22nd; day 13), 5 weeks (July 12th; day 33) and 9 weeks (August 10th; day 62) after additions. Water chemistry parameters including total dissolved nitrogen (TDN), total phosphorus (TP), and chlorophyll *a* were collected and analyzed using the same methods as previously described in Chapter 2. A Yellow Springs International (YSI) EXO2 Multiparameter Sonde was used to collect temperature and dissolved oxygen measurements weekly at a depth of 1 m.

Turbidity was recorded weekly by collecting limnocorral water from a depth of 1 m using a peristaltic pump and analyzed using a MANTECH Turbidity T10 meter in the field. Photosynthetically Active Radiation (PAR) was measured weekly using methods described previously in Chapter 2. A PAR reading was taken in the air just above the water surface, and then at depths of 0.5 m, 1 m, 1.5 m and 2 m. The light extinction coefficient (Kd) was calculated by regressing the natural log of the percent of PAR for each depth (Fee et al. 1996).

Phytoplankton biomass and community composition, and zooplankton abundance, biomass, reproduction and community composition were analyzed using methods previously described in Chapter 2. Yellow Perch were captured using a seine net at the end of the experiment.

3.3.3 Statistical Analysis

All statistical analyses were conducted in R version 4.1.3 (R Core Team 2022). Analyses were conducted using the *vegan* package version 2.6-4 (Oksanen et al. 2022) and *rstatix* package version 0.7.1 (Kassambara 2022). Plots were produced using the *ggplot2* package version 3.4.0 (Wickham 2016).

Univariate Analyses. One-way repeated-measures analysis of variance (RM ANOVA) was used to look at treatment-dependent differences of variables in the planktonic food web over time using the *anova_test()* function from the *rstatix* package in R. Prior to analysis, data from before MP additions (day -1) were filtered out. Assumptions of normality and sphericity were assessed using the Shapiro-Wilk normality test and the Mauchly's test of sphericity. The Greenhouse-Geisser epsilon correction was used to correct for violations of sphericity (automatically applied in the model). Variables were $\log_{10}$ transformed where necessary to meet model assumptions. If the RM ANOVA was significant, *post-hoc* testing was performed using one-way ANOVA at each time point to determine the main treatment effects, and then t-tests were used to examine pairwise comparisons. The false discovery rate (FDR) was applied to the p-values to correct for multiple comparisons (Pike 2011). In instances where only one time point was analyzed, a one-way ANOVA with Tukey's honestly significant difference (Tukey HSD) *post-hoc* test was used. An alpha level of 0.05 was used to determine significance.

Multivariate Analyses. Principal response curves (PRC) were used to determine if community composition of the phytoplankton and zooplankton communities differed among treatments over time (Van den Brink and Ter Braak 1999). The matrices used for PRC analysis included biomass-based phytoplankton community composition and abundance and biomass-based zooplankton community composition. Variables were $\log_{10}$ transformed prior to analysis. Nauplii were excluded from the PRC analysis. The significance of the first PRC axis was assessed using a permutation test (999 permutations). If a significant result was detected, redundancy analysis (RDA) was performed on each day to determine where significant effects occurred. A more detailed explanation of PRC analysis can be found in Chapter 2.

3.4 Results

3.4.1 Environmental Parameters

Mean water temperature increased from 16.1-16.5 ^{o}C on day -1 and peaked on day 41 at 24.4 ^{o}C during mid-July but did not differ among treatments (Appendix Figure 15). Dissolved oxygen (DO) at 1 m was similar among limnocorrals and decreased from a mean of 9.98-10.0 µg/L in June (day -1) to 8.01 to 8.05 µg/L in July (day 41; Appendix Figure 16). DO concentrations were never limiting for zooplankton (Weider and Lampert 1985).

Mean turbidity at the start of the experiment ranged from 0.55 to 0.65 NTU across treatments (Appendix Figure 17). After MP additions, MP treated limnocorrals had much more variation in turbidity among replicates while control treatments remained relatively invariant throughout. By day 62, mean turbidity ranged from 0.43 to 0.46 across treatments. RM ANOVA detected a significant treatment effect over time ($p = 0.007$; Table 3.2). *Post-hoc* tests indicated that turbidity was significantly higher in the non-additive and additive MP treatments compared to the control on day 13 ($p = 0.05$), and higher only in the additive MP treatment on day 33 ($p = 0.049$) and day 55 ($p = 0.01$).

The mean light extinction coefficient (Kd) at the start of the experiment ranged from 0.90 to 1.24 across treatments and decreased slightly by the end of the experiment, ranging from 0.57 to 0.67 on day 62 (Appendix Figure 18). RM ANOVA indicated that there was no significant effect of treatment on Kd over time (p = 0.56; Table 3.2).

Total phosphorus (TP) declined slightly from the start of the study but there from means of 7.47-11.5 µg/L to 5.2-5.9 µg/L across treatments (Appendix Figure 19). RM ANOVA indicated that there was no significant difference in TP concentrations among treatments over time (p = 0.90). Total dissolved nitrogen (TDN) ranged from means of 260 to 264 µg/L at the start of the study and did not change much over time, with final mean TDN ranging from 257 to 263 µg /L across treatments on day 62 (Appendix Figure 20). There was no significant difference of treatment over time (RM ANOVA; p = 0.93).

Final Yellow Perch abundances were similar for most limnocorrals except for one of the non-additive MP treated limnocorrals where only 10 perch were retrieved (Table 2.2). This limnocorral also had 15 Spottail Shiner. After the experiment, a small tear was found in the wall of this limnocorral, likely allowing fish to come in and out throughout the experiment. Final Yellow Perch abundance was not correlated with final phytoplankton biomass or final zooplankton abundance or biomass and did not significantly differ among treatments (one-way ANOVA, F = 3.7, p = 0.09).

Table 3.1 Final Yellow Perch abundances and by catch for each treatment.

Treatment	**Final Yellow Perch abundances**	**By catch**
Control 1	20	1 Spottail Shiner
Control 2	26	1 Spottail Shiner
Control 3	26	
Non-additive 1	18	
Non-additive 2	10	15 Spottail Shiner
Non-additive 3	22	
Additive 1	24	
Additive 2	25	
Additive 3	26	1 Spottail Shiner

3.4.2 Phytoplankton

Chlorophyll *a*. Chlorophyll *a* concentration remained relatively invariant throughout the experiment, with mean concentrations on day -1 ranging from 1.94 to 2.22 µg/L and final concentrations on day 62 ranging from 1.70 to 1.95 µg/L across treatments (Figure 3.2). RM ANOVA indicated that there was no significant effect of treatment over time ($p = 0.07$; Table 3.2).

Chlorophyll *a*/Biomass Ratio. The mean chlorophyll *a*/ phytoplankton biomass ratio ranged from 0.03 to 0.04 across treatments on day -1 and from 0.04 to 0.06 on day 62 (Figure 3.3). RM ANOVA indicated a significant interaction between treatment and time ($p = 0.048$; Table 3.2), however there were no significant pairwise differences between treatments on any day ($p > 0.05$).

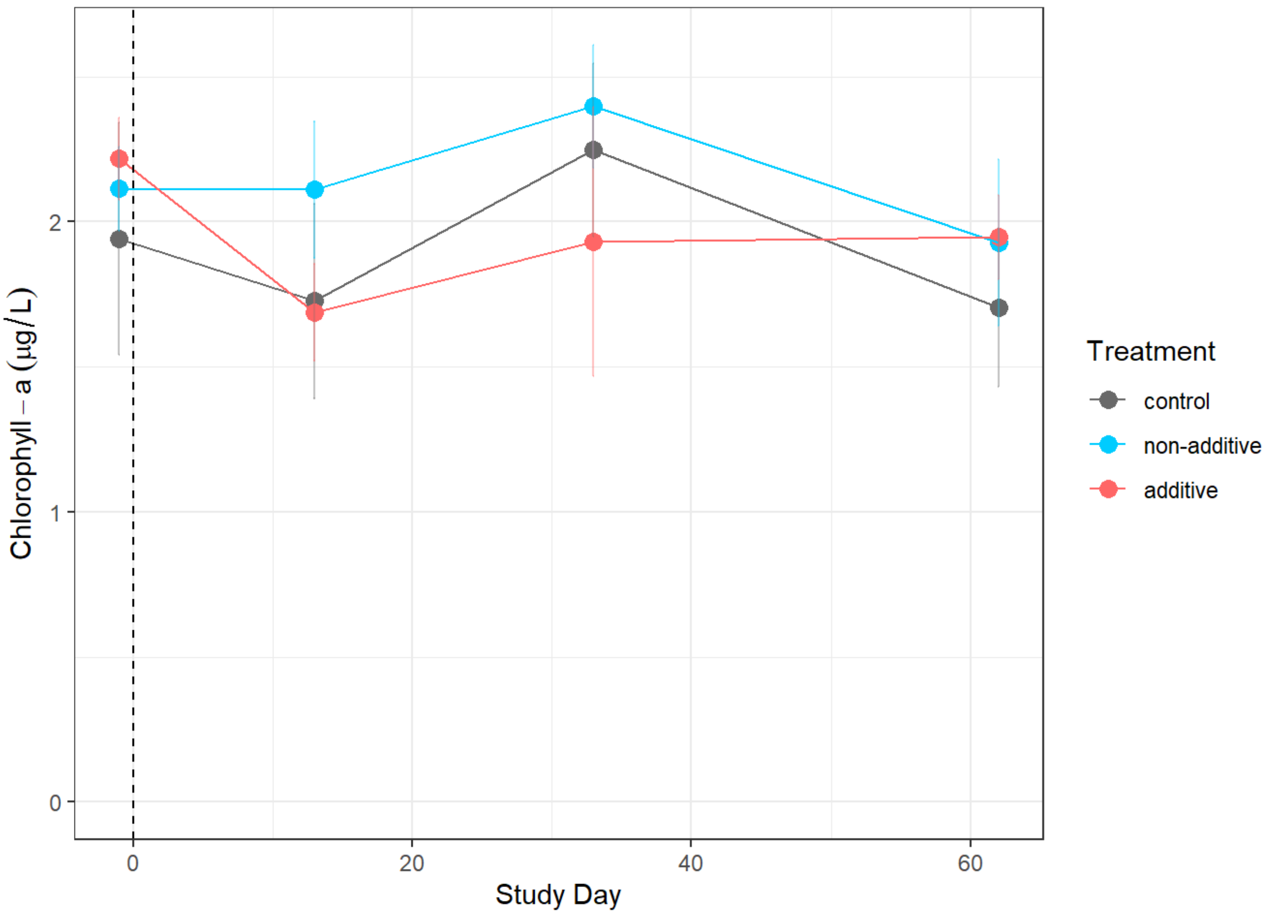


Figure 3.2 Chlorophyll *a* concentration throughout the study (mean ± 1 SD, n =3). There was no significant impact of treatment over time (RM ANOVA; p = 0.07).

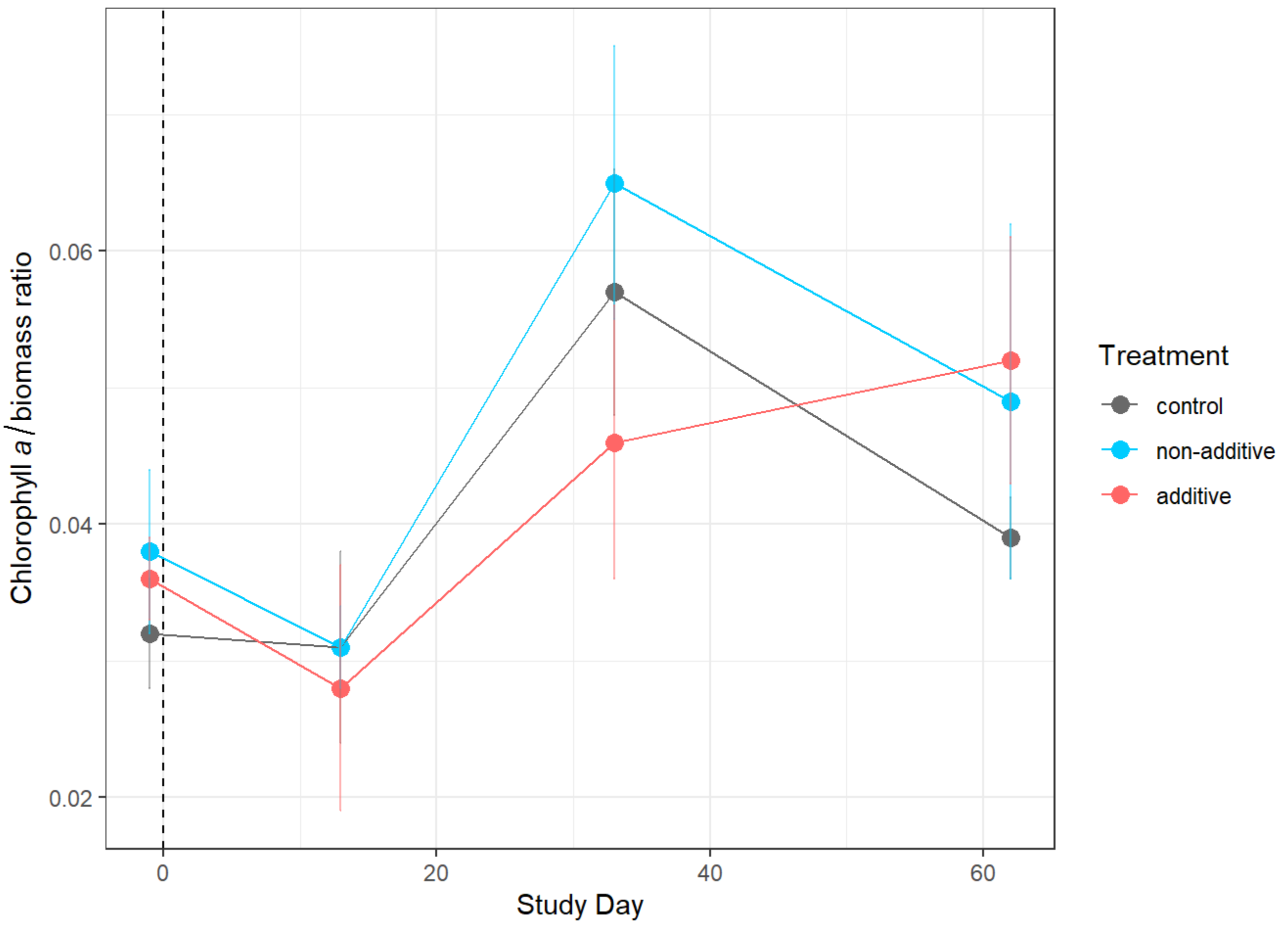


Figure 3.3 Chlorophyll *a*/phytoplankton biomass ratio throughout the study (mean ± 1 SD, n = 3). There was a significant interaction of treatment and time (RM ANOVA; p = 0.048), but no significant differences between treatments on any given day (pairwise t-tests; p > 0.05).

Phytoplankton Biomass. Mean total phytoplankton biomass ranged from 568 to 625 mg/m^3 on day -1 and was slightly reduced by day 62, ranging from 380 to 438 mg/m^3 across treatments (Figure 3.4). RM ANOVA indicated there was no significant effect of treatment over time (p = 0.90; Table 3.2).

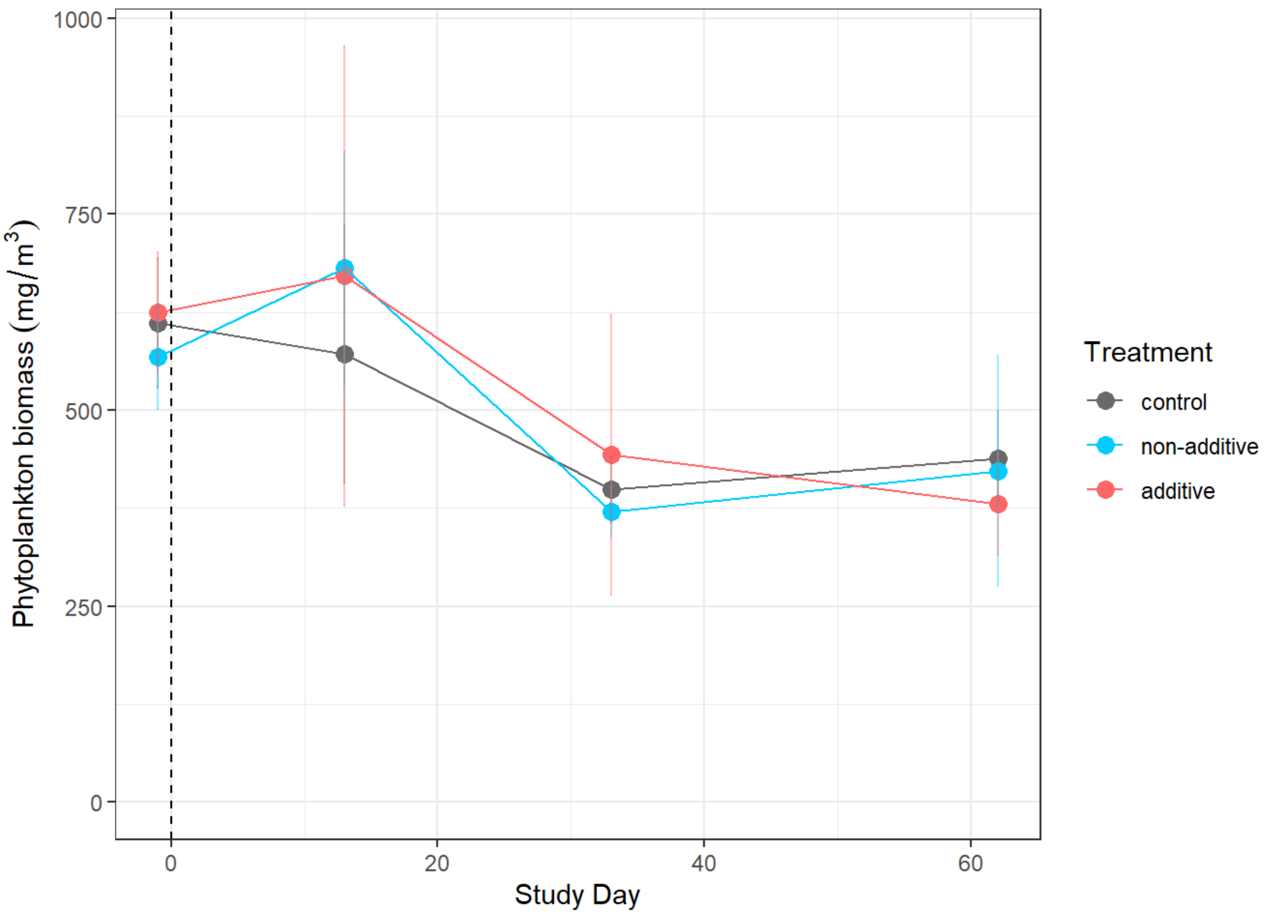


Figure 3.4 Total phytoplankton biomass throughout the study (mean ± 1 SD, n =3). There was no significant impact of treatment over time (RM ANOVA; p = 0.90).

Phytoplankton Community Composition. The phytoplankton community was initially dominated by chrysophytes and then shifted to higher proportions of diatoms on day 33 (Figure 3.5). By day 62, dinoflagellates became dominant. The PRC indicated a significant effect on community composition over time (first PRC axis, $F = 8.98$, $p = 0.001$; Figure 3.6). Conditional variance (time) accounted for 82% of the total variation, constrained variance (treatment and time interaction) accounted for only 6.7 % of the total variation and unconstrained variance (variation between replicates) accounted for 11.3 % of the total variation. RDA was performed on each date as a *post-hoc* test and was only significant on day 62 ($p = 0.016$, permutations = 999). Cyanobacteria and diatoms were the most impacted algal groups as shown by their relatively large species weights ($b_k = 0.88$ and 0.51 respectively) and were more abundant in the MP treatments compared to the control. All other groups had low species weights ($b_k < 0.5$).

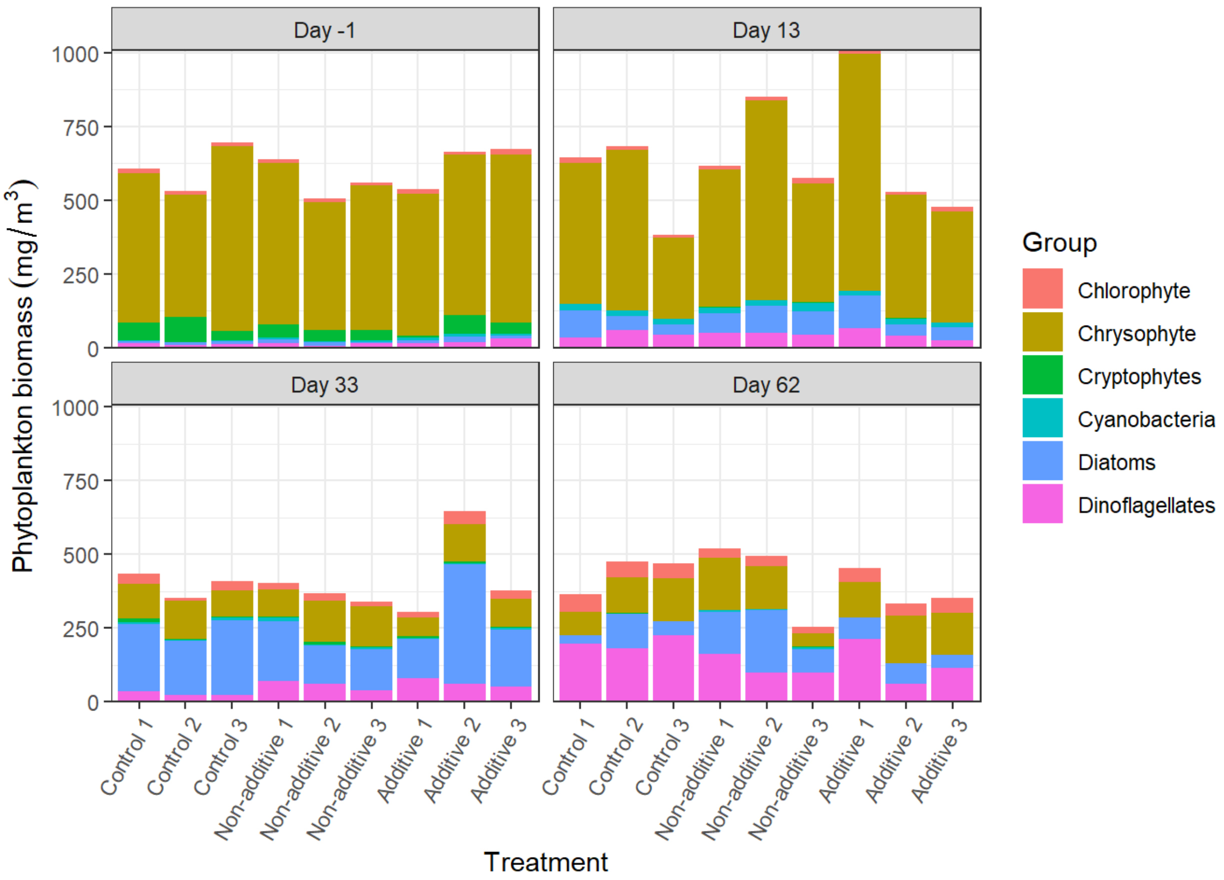


Figure 3.5 Biomass-based phytoplankton community composition for different days of the study.

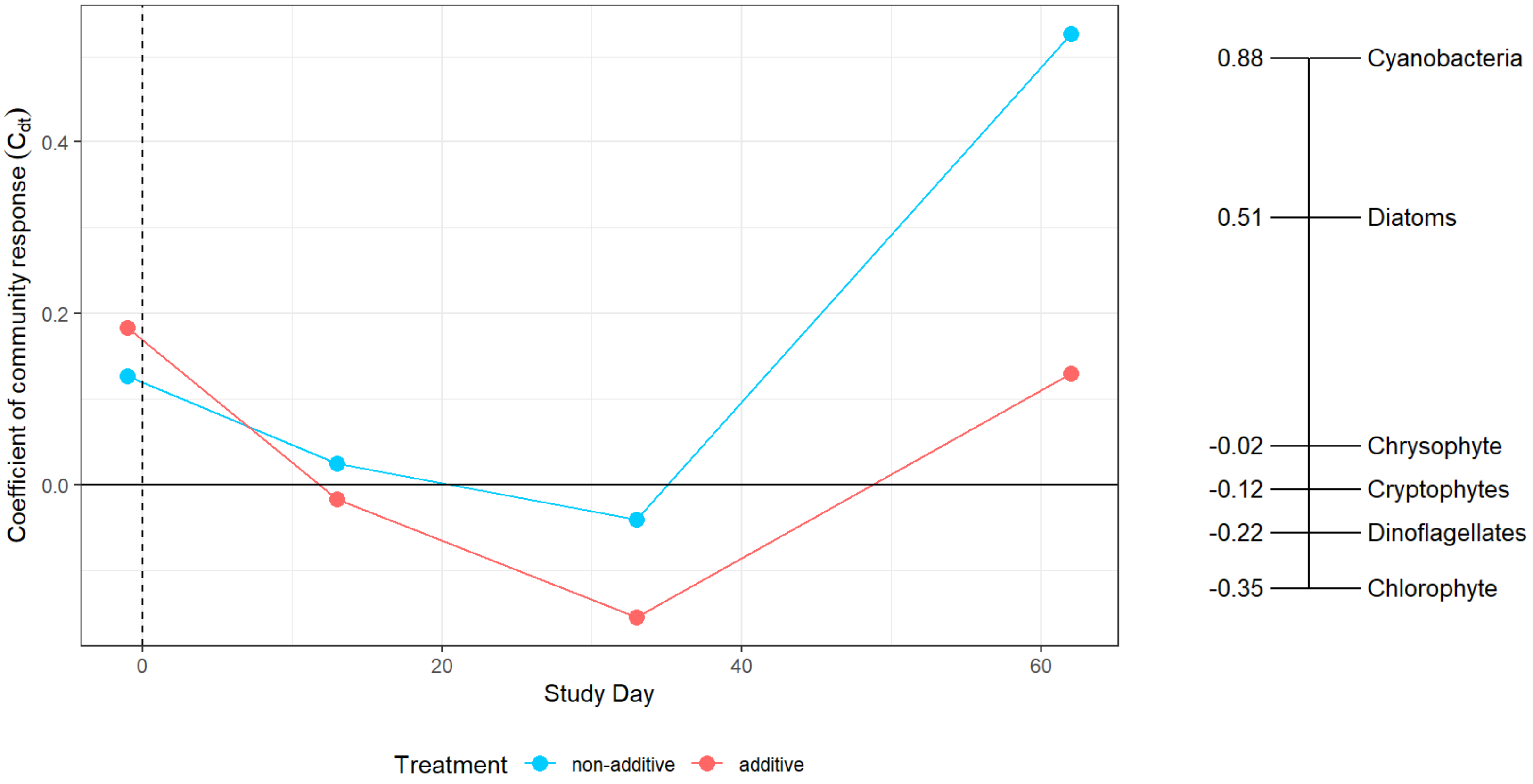


Figure 3.6 Principal response curve (PRC) for phytoplankton biomass-based community composition. The community response (C_{dt}) is displayed on the left and species weights (b_k) are displayed on the right. The solid black horizontal line represents the control treatment (where $C_{dt} = 0$), and the dashed vertical line represents the day of microplastic addition (day 0). Biomass was $\log_{10}$ transformed prior to analysis. The PRC axis was significant ($F = 8.98$, $p = 0.001$) and RDA indicated only a significant effect of treatment on day 62 ($p = 0.016$).

<u>Phytoplankton Diversity</u>. Mean phytoplankton diversity (Inverse Simpson Index) ranged from 9.5 to 10 across treatments on day -1 and decreased to 3.7 to 6.7 on day 33 (Figure 3.7). RM ANOVA indicated no significant effect of treatment on diversity over time (p = 0.079; Table 3.2).

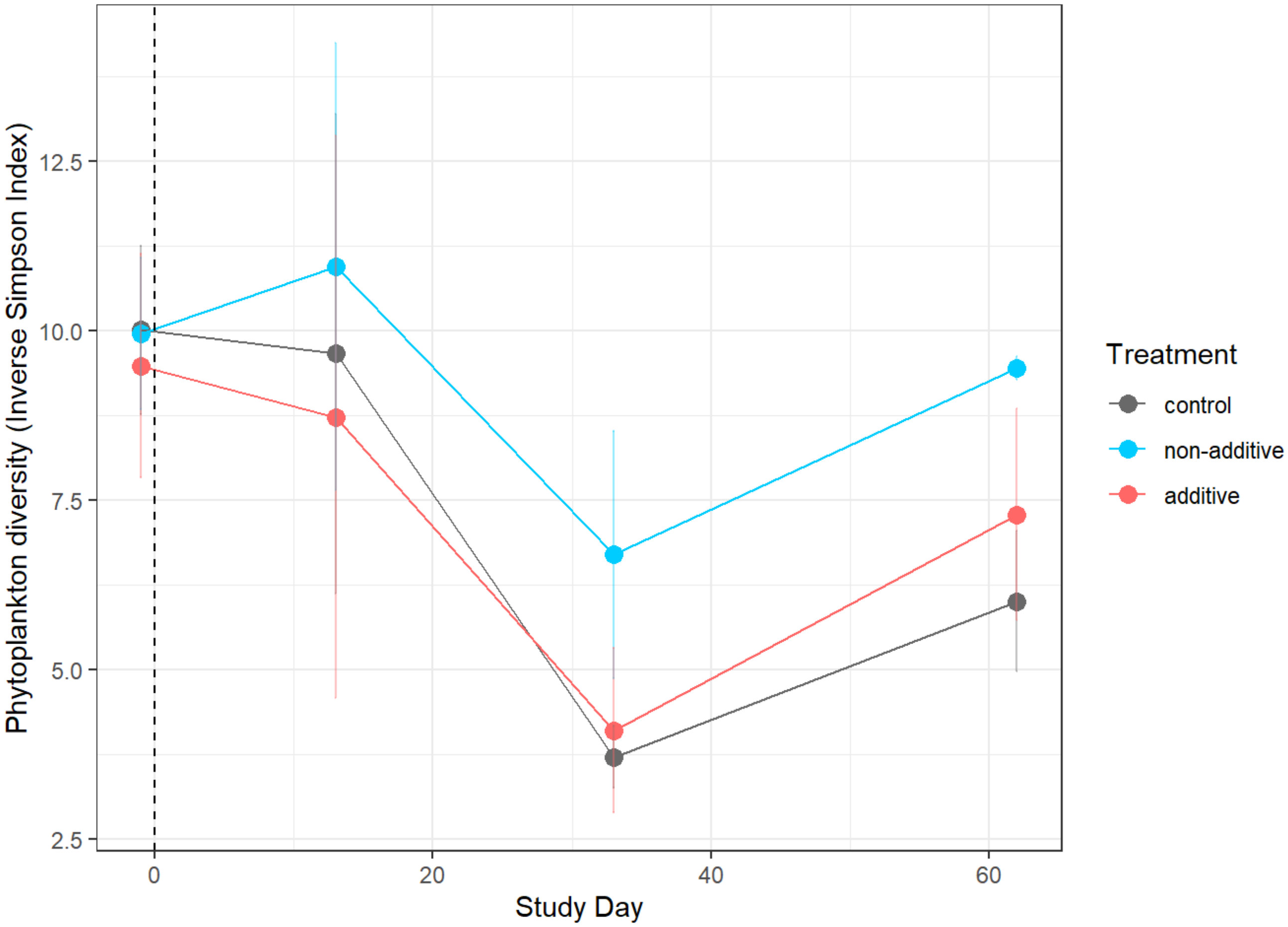


Figure 3.7 Phytoplankton diversity, represented by the Inverse Simpson Index, throughout the study (mean ± 1 SD, n = 3). There was no significant effect of treatment over time (RM ANOVA; p = 0.079).

3.4.3 Zooplankton

Zooplankton Abundance. Mean zooplankton abundance was very low at the start of the experiment ranging from 2.26 to 3.58 total zooplankton/L across treatments (Figure 3.8). After MP additions, zooplankton abundance increased for all treatments and peaked on day 33 with means ranging from 66.4 to 83.8 zooplankton/L across treatments. RM ANOVA indicated a marginally significant treatment effect over time ($p = 0.048$; Table 3.2), however *post-hoc* testing indicated that there were no significant differences between treatments at any time point ($p > 0.05$). When separated by taxonomic group, there were no significant differences for Calanoid ($p = 0.19$) or Cyclopoid copepod ($p = 0.087$) abundances over time (Figure 3.9). RM-ANOVA indicated there was a significant effect of treatment for Cladoceran abundance over time ($p = 0.01$; Table 3.2), however *post-hoc* testing indicated no significant differences between treatments on any day of the study ($p > 0.05$). The different outcomes presumably reflect the greater power of the RM-ANOVA approach.

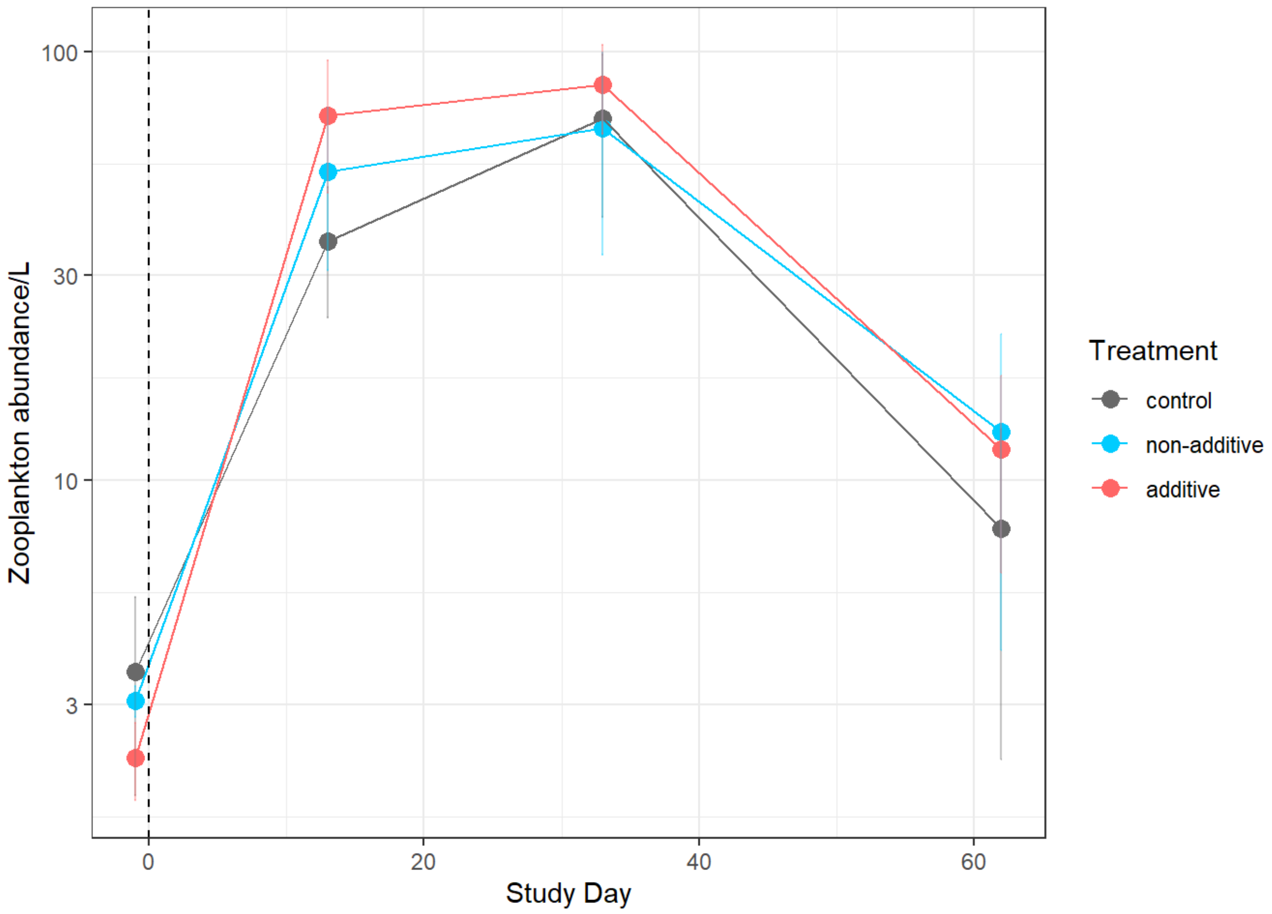


Figure 3.8 Zooplankton abundance throughout the study (mean ± 1 SD, n = 3). There was a significant treatment effect over time (RM ANOVA; $p = 0.048$) but there were no significant differences among treatments on any study day ($p > 0.05$). Note y axis is on a $\log_{10}$ scale.

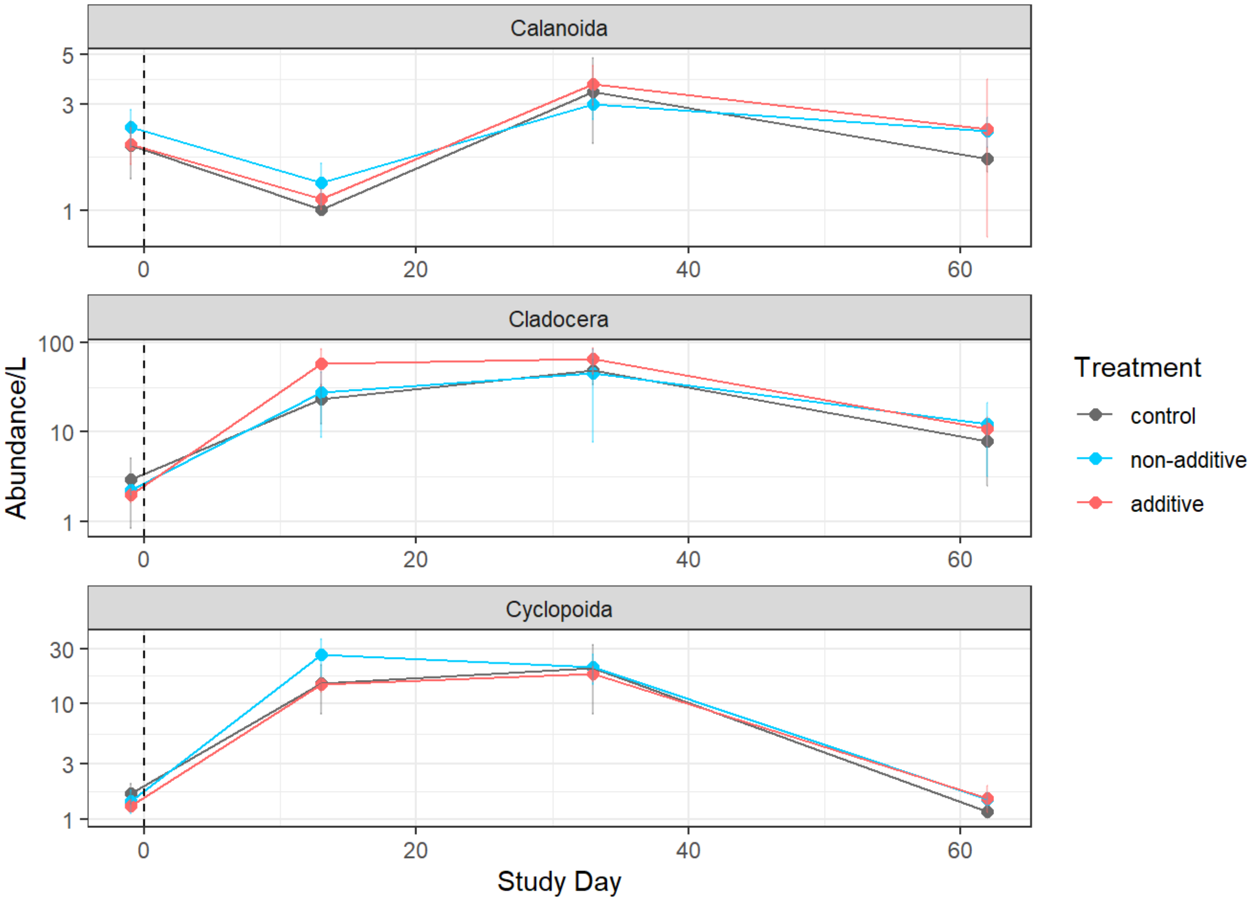


Figure 3.9 Abundances of zooplankton groups throughout the study (mean ± 1 SD, n = 3). There were no significant differences among treatments at any time point for Calanoid or Cyclopoid copepods (RM ANOVA; $p > 0.05$). There was a significant treatment effect over time for Cladocerans (RM ANOVA; $p = 0.01$), however there was no differences between treatments on any study day ($p > 0.05$). Note y axis is on a $\log_{10}$ scale.

Zooplankton Biomass. Mean zooplankton biomass at the start of the experiment ranged from 2.26 to 2.86 µg/L (dry weight) across treatments and peaked on day 33, with mean biomass ranging from 28.6 to 35.6 µg/L across treatments (Figure 3.10). Mean zooplankton biomass on day 62 ranged from 5.02 to 8.04 µg/L across treatments. RM ANOVA indicated a significant treatment effect over time ($p = 0.016$; Table 3.2). *Post-hoc* testing indicated that biomass did not significantly differ among treatments on any day of the study ($p > 0.05$).

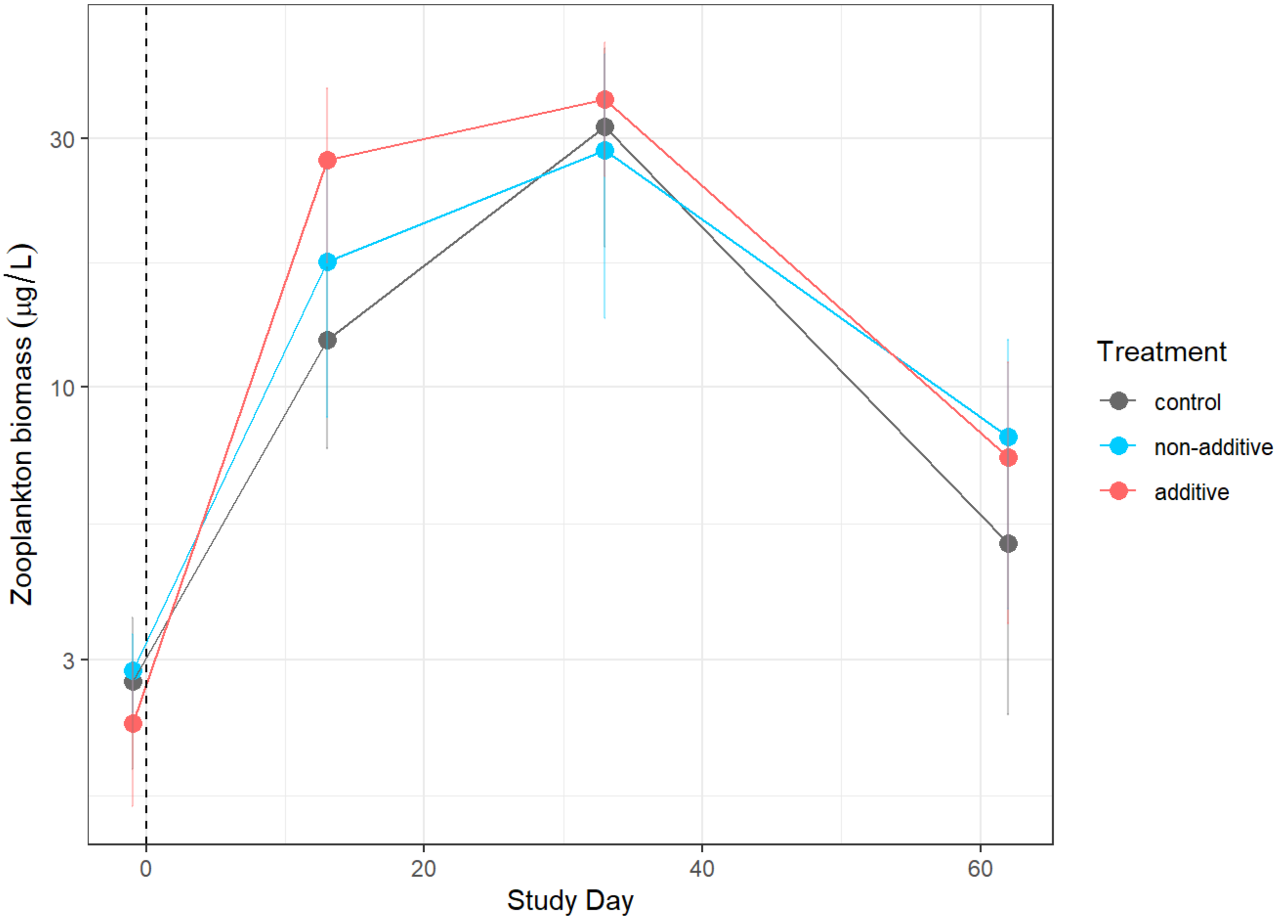


Figure 3.10 Zooplankton biomass throughout the study (mean ± 1 SD, n = 3). There was a significant effect of treatment over time (RM ANOVA; $p = 0.016$) but there were no significant differences between treatments on any study day ($p > 0.05$). Note the y-axis is on a $\log_{10}$ scale.

Zooplankton Community Composition. *Eubosmina sp.*, a small-bodied cladoceran species, was the most abundant taxa throughout the experiment for all treatments, followed by *Tropocyclops extensus*, a small-bodied cyclopoid copepod (Figure 3.11). *Diaptomus minutus*, a calanoid copepod, was only relatively abundant on day -1. *Eubosmina sp*. also generally dominated in terms of biomass, although *D. minutus* made up a large proportion of biomass on day -1 and day 62 (Figure 3.12).

The PRC for abundance-based zooplankton community composition was not significant (p = 0.19) indicating that there was no effect of treatment on species composition over time (Figure 3.13). *Eubosmina sp*. had a relatively large species weight (b_k) indicating that this species had a strong affiliation for the community response, but that this was not related to the treatments.

The PRC for biomass-based zooplankton community composition was not significant (p = 0.14) indicating that there was no effect of treatment on biomass-based species composition over time (Figure 3.14). *Eubosmina sp.* had an opposite affiliation with the community trend in terms of biomass while *D. birgei* had a strong positive affiliation with the community response, however this was not related to the MP treatments.

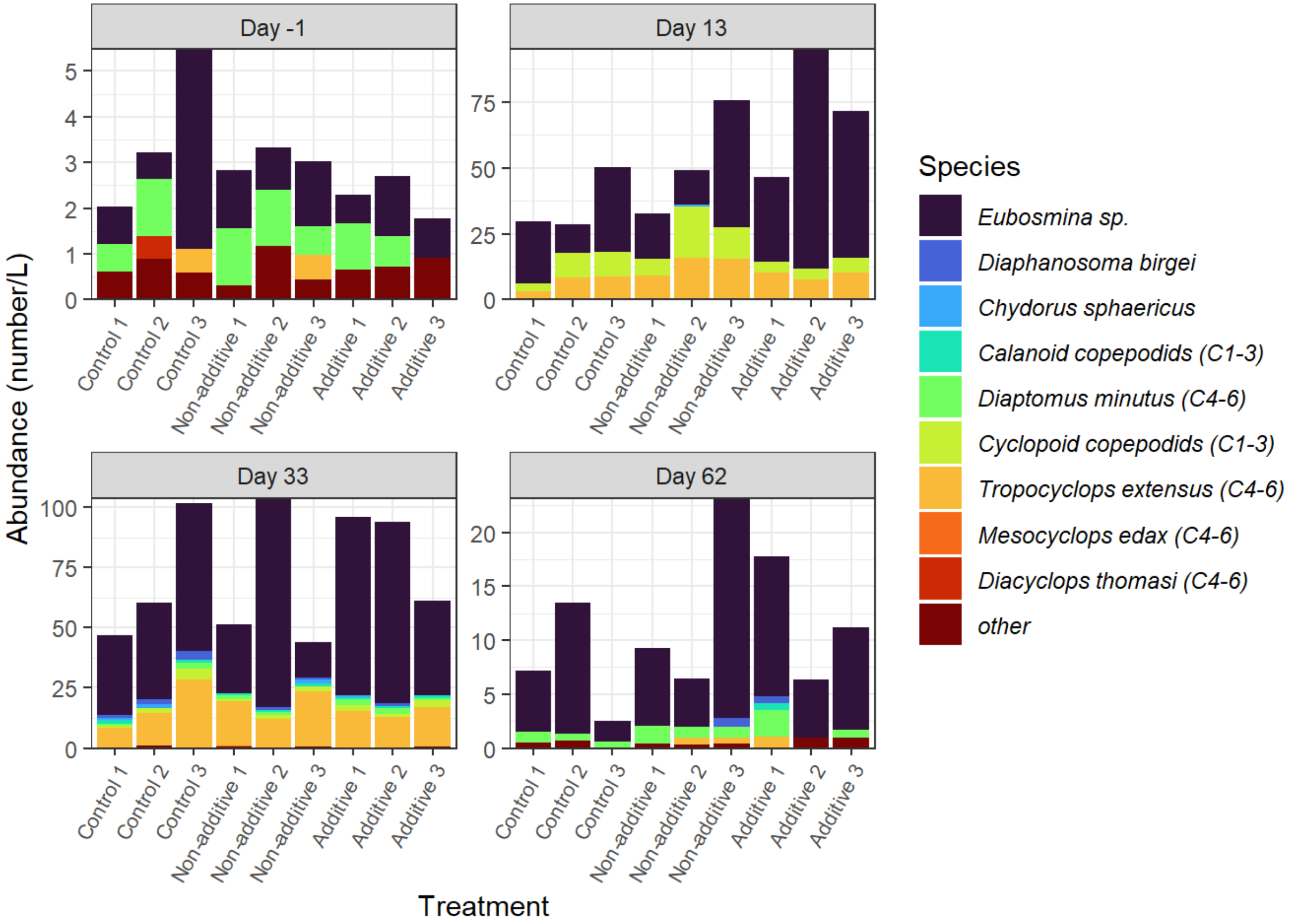


Figure 3.11 Abudance-based zooplankton species composition. Taxon with less than 0.5 animals/L are included in the “other” category. Note the different scales.

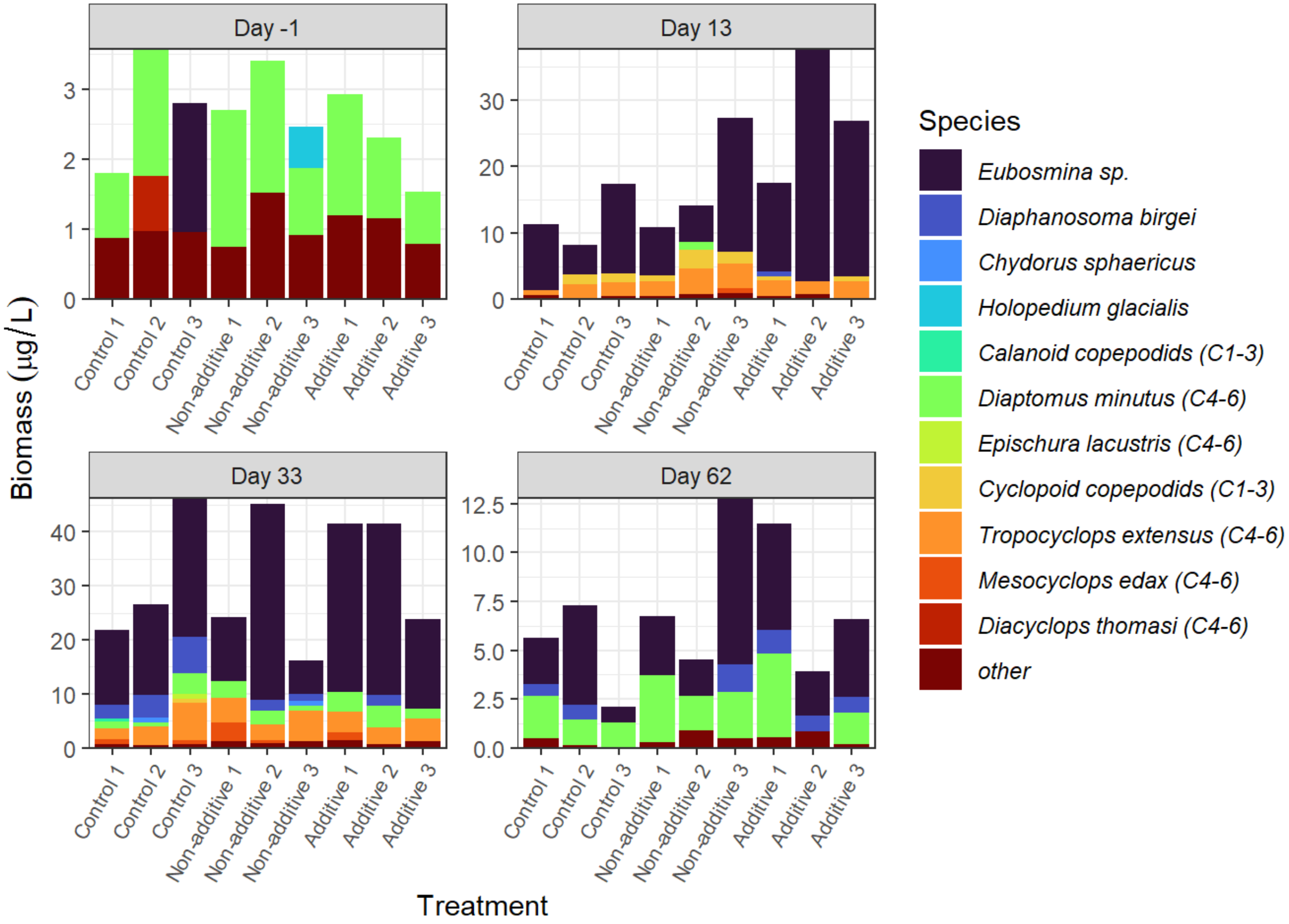


Figure 3.12 Biomass-based species composition. Taxon with less than 0.5 µg /L were included in the “other” category. Note the different scales.

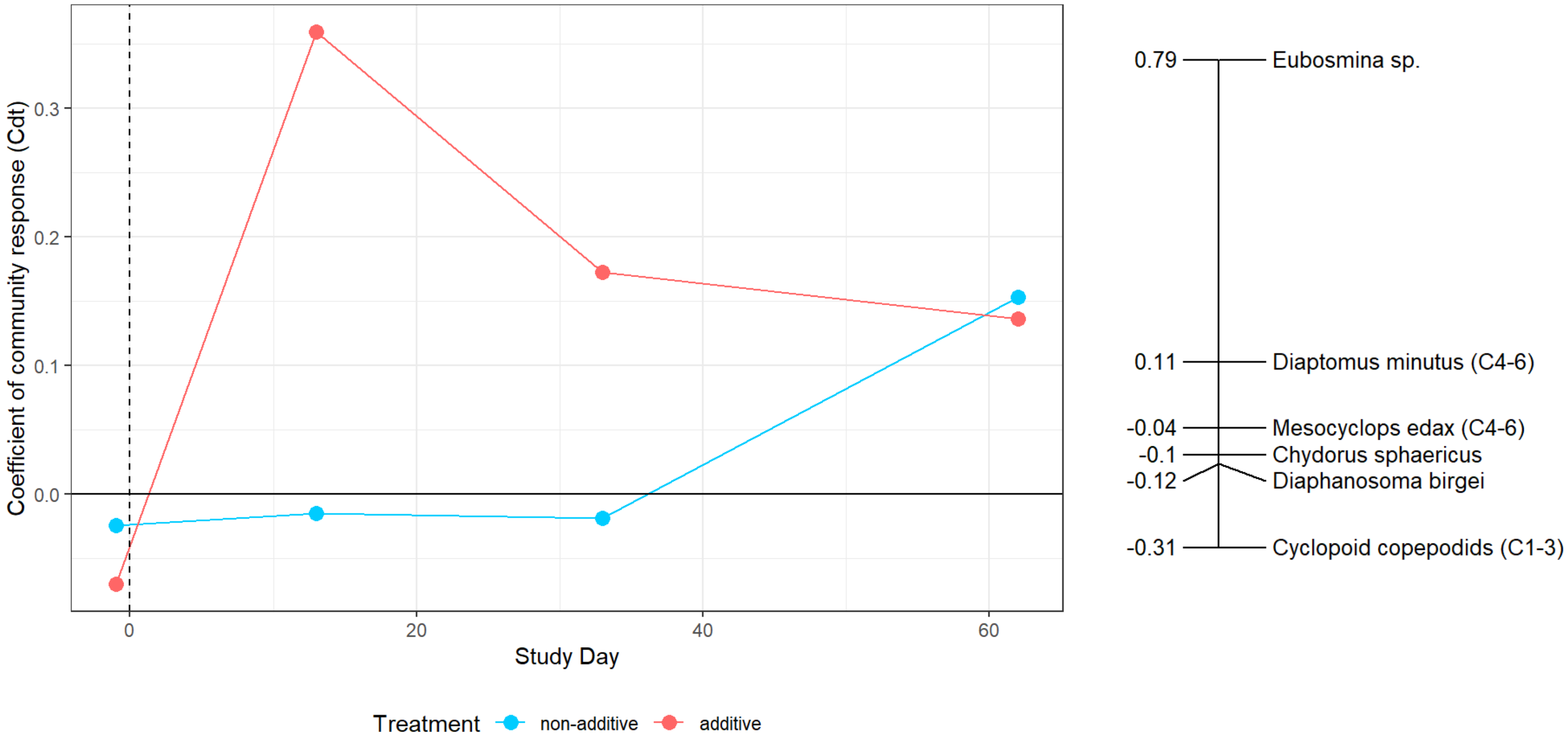


Figure 3.13 PRC for abundance-based zooplankton community composition. The community response (C_{dt}) is displayed on the left and species weights (b_k) are displayed on the right. The solid black horizontal line represents the control treatment (where $C_{dt} = 0$), and the dashed vertical line represents the day of microplastic addition (day 0). Abundance was $\log_{10}$ transformed prior to analysis. The PRC axis was not significant ($p = 0.16$).

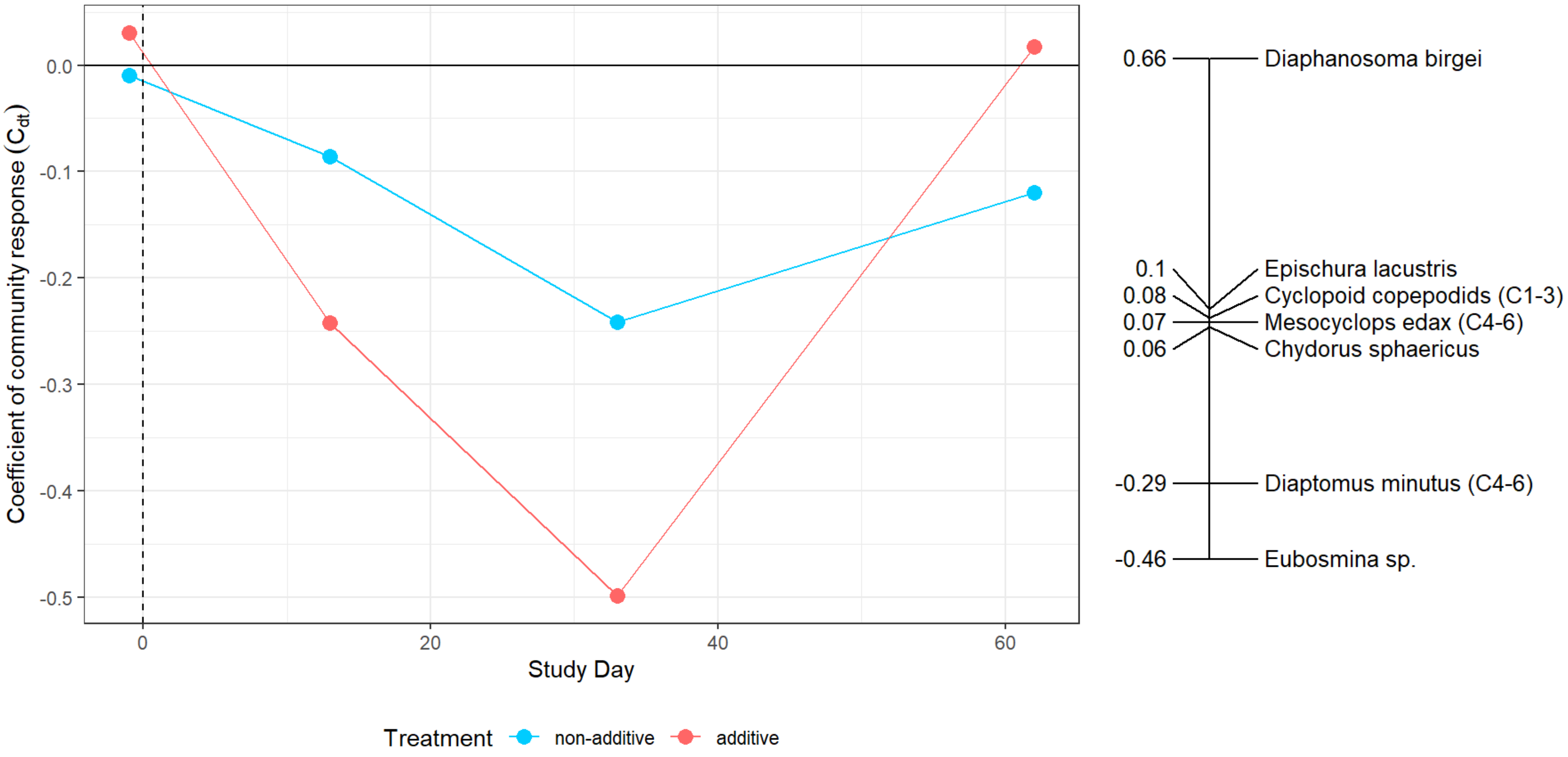


Figure 3.14 PRC for biomass-based species composition. The community response (C_{dt}) is displayed on the left and species weights (b_k) are displayed on the right. The solid black horizontal line represents the control treatment (where $C_{dt} = 0$), and the dashed vertical line represents the day of microplastic addition (day 0). Biomass was $\log_{10}$ transformed prior to analysis. The PRC axis was not significant ($p = 0.14$).

Zooplankton Diversity. Mean zooplankton diversity, represented by the Inverse Simpson Index, ranged from 2.43 to 2.62 on day -1 and decreased after MP additions for all treatments, remaining relatively low for the remainder of the experiment (Figure 3.15). Mean diversity on day 62 ranged from 1.44 to 1.58 across treatments. RM ANOVA indicated that there was no significant effect of treatment over time (p = 0.18; Table 3.2).

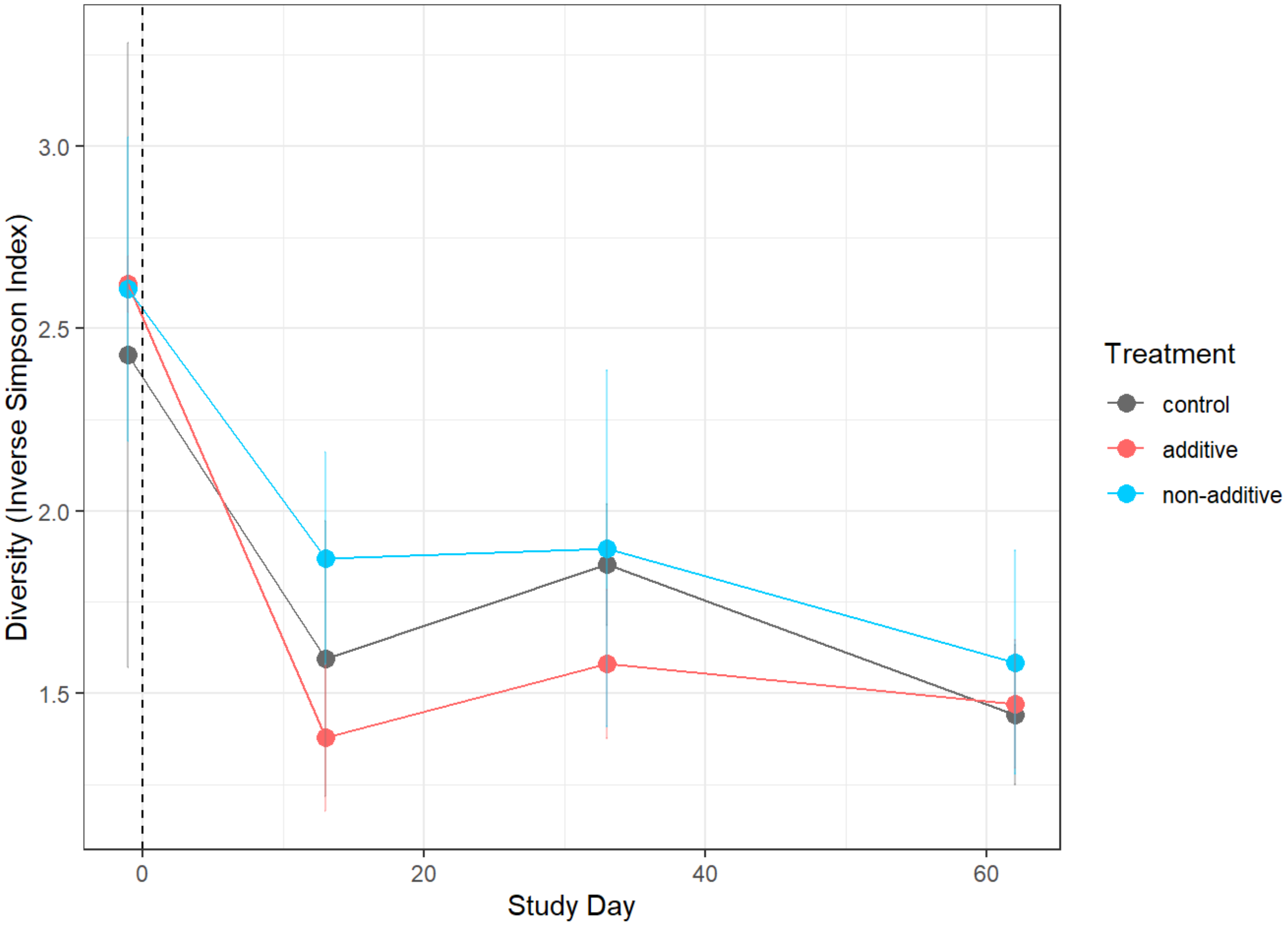


Figure 3.15 Zooplankton community diversity, represented by the Inverse Simpson Index, throughout the study (mean ± 1 SD, n = 3). There was no significant effect of treatment over time (RM ANOVA; p = 0.18).

Zooplankton Reproduction. Egg ratios (eggs per female) for common zooplankton taxa were only statistically assessed where there were sufficient egg counts. On day -1, mean eggs per female for the cladoceran *Eubosmina sp*. ranged from 0.35 to 0.63 and on day 62 ranged from 0.14 to 0.28 across treatments (Figure 3.16). RM ANOVA indicated that eggs per female for *Eubosmina sp*. did not differ among treatments over time (p = 0.84; Table 3.2).

Mean eggs per female for the cyclopoid copepod *T. extensus*, including apportioned loose copepod eggs, ranged from 0.82 to 8.23 throughout the study (Figure 3.17). After MP additions on day 13, egg ratios for *T. extensus* females in the control treatment increased while egg ratios in both MP treatments remained low. Egg ratios decreased for all treatments by day 62. RM ANOVA indicated a significant treatment effect over time (F = 7.85, p = 0.021; Table 3.2). *Post-hoc* testing using pairwise t-tests found no significant differences between treatments on any day when adjusted for multiple comparisons (p > 0.05).

Mean eggs per female for the calanoid copepod *D. minutus*, including apportioned loose copepod eggs, ranged from 1.8 to 7.7 throughout the study, peaking on day 33 (Figure 3.18). Day 13 was excluded from analysis because *D. minutus* adult female abundance was low and even absent in some limnocorrals on this day. RM ANOVA indicated that there was no treatment effect over time (p = 0.207; Table 3.2).

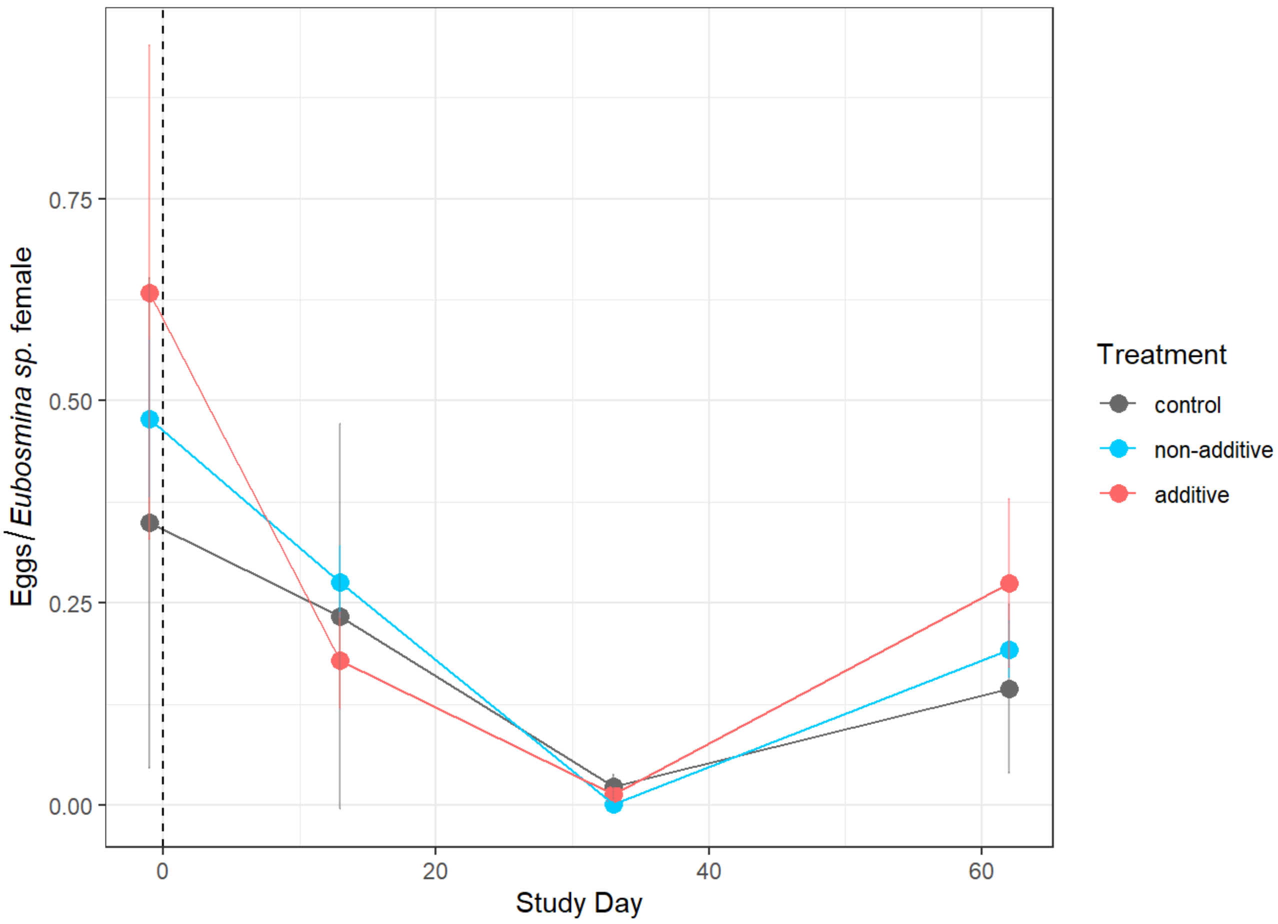


Figure 3.16 Eggs per female for *Eubosmina sp.* throughout the study (mean ± 1 SD, n = 3). There was no effect of treatment over time (RM ANOVA; p = 0.84).

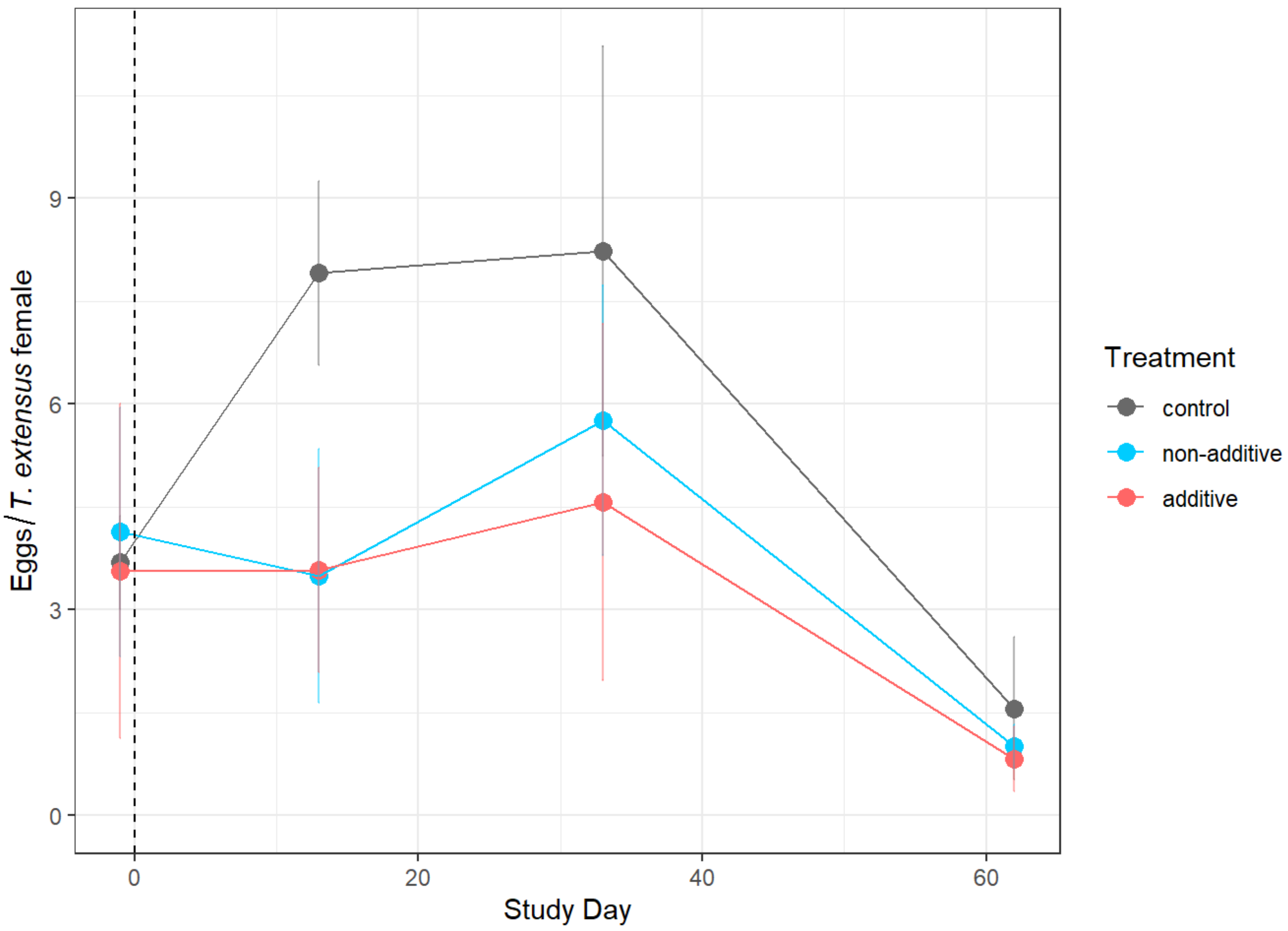


Figure 3.17 Eggs per female for *Tropocyclops extensus* throughout the study (mean ± 1 SD, n = 3). There was a significant effect of treatment over time (RM ANOVA; p = 0.021), however *post-hoc* testing found no significant differences between treatments on any day (p > 0.05).

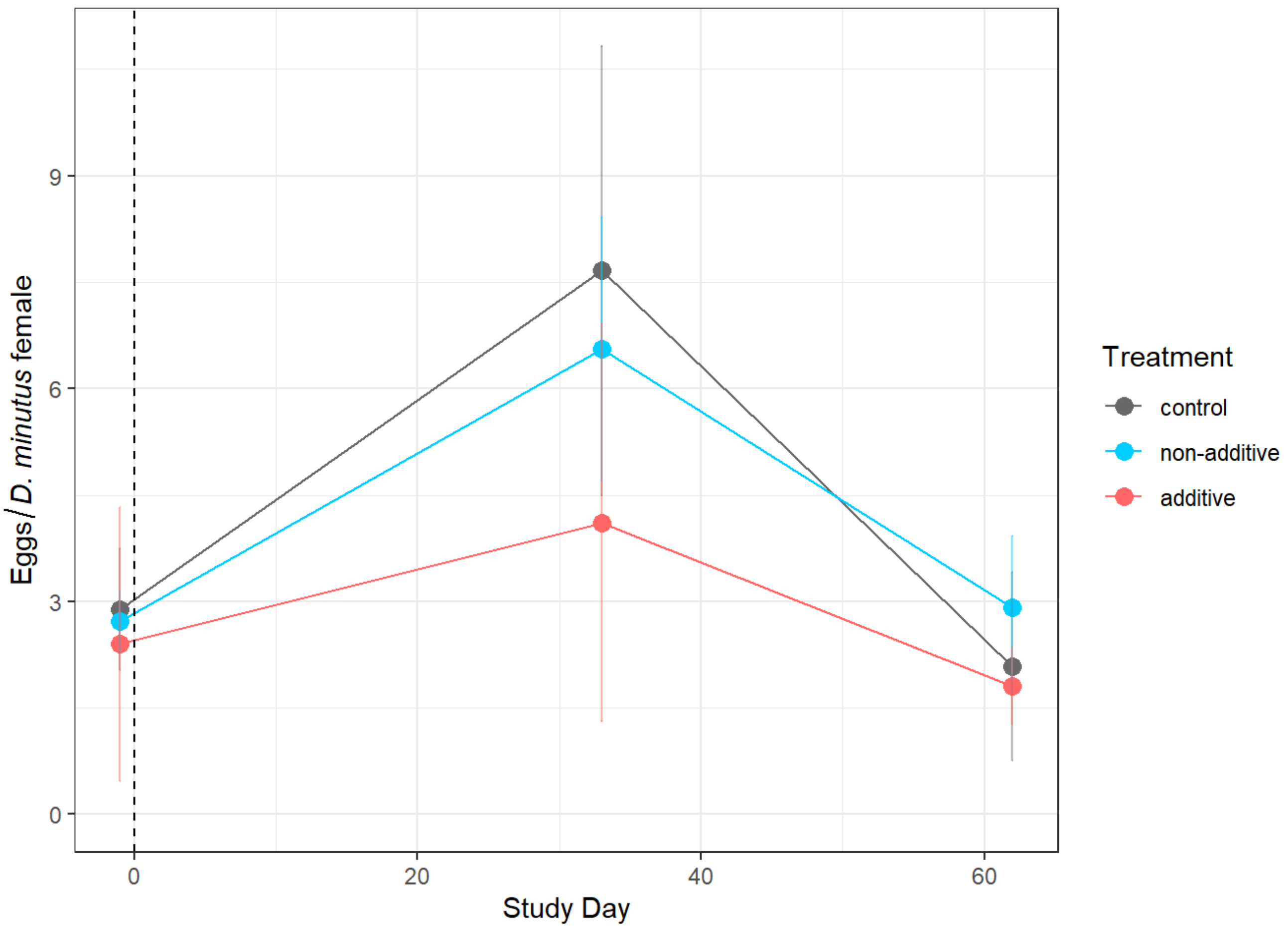


Figure 3.18 Eggs per female for *Diaptomus minutus* throughout the study (mean ± 1 SD, n = 3). There was no effect of treatment over time (RM ANOVA; $p = 0.207$).

Copepod nauplii abundance was very low prior to microplastic additions with means ranging from 0.71 to 1.12 nauplii/L across treatments and peaked on day 33 with means of 69.5 to 121.0 nauplii/L across treatments (Figure 3.19). By day 62 mean nauplii abundance was reduced to 0.13 to 0.47 nauplii/L across treatments. RM ANOVA indicated that there was no significant effect of treatment over time (p = 0.13; Table 3.2).

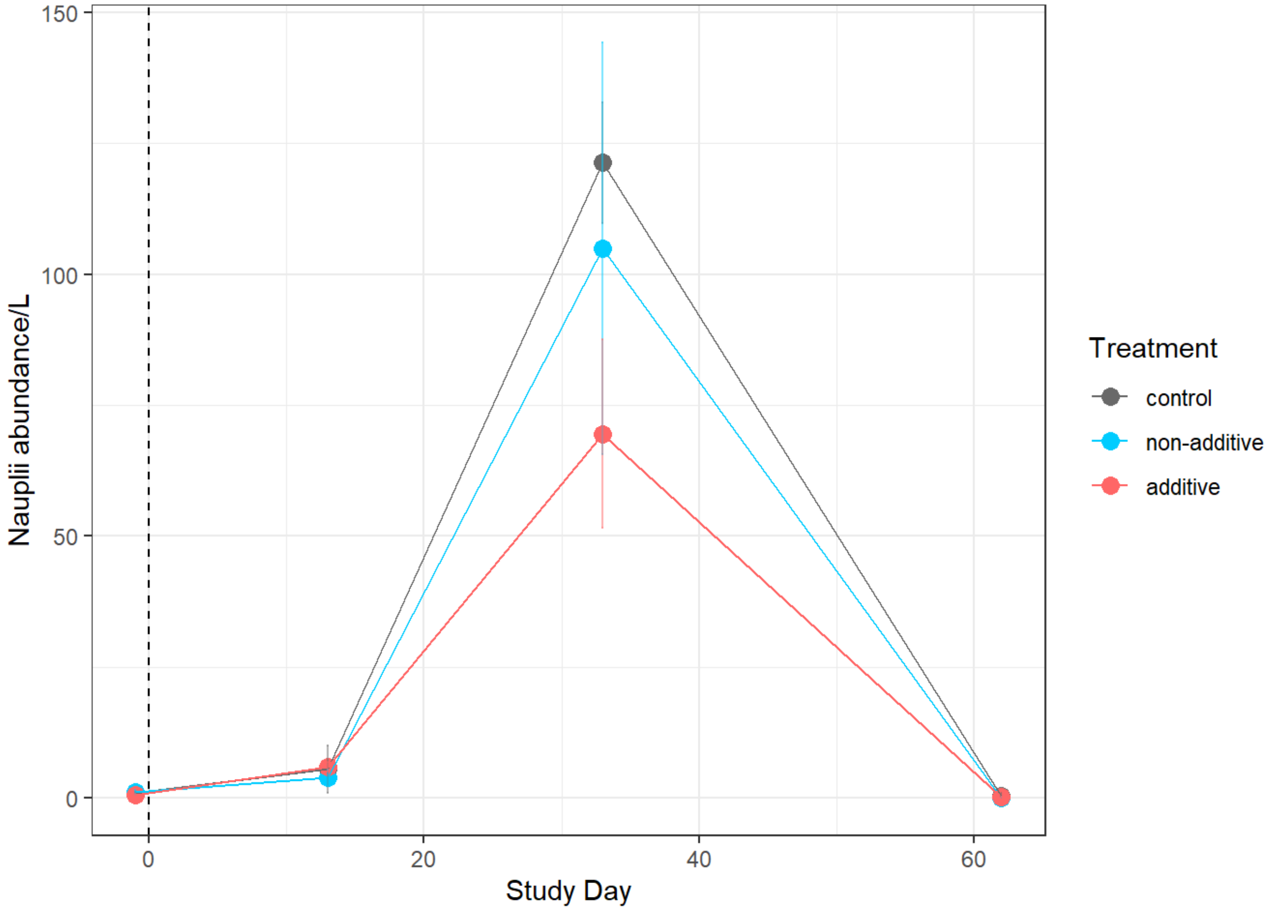


Figure 3.19 Copepod nauplii abundance throughout the study (mean ± 1 SD, n = 3). There was no significant difference among treatments over time (RM ANOVA; p = 0.13).

3.4.4 **Insect Larvae**

Larvae of the Phantom Midge, *Chaoborus* spp., were typically not found or only found in very low abundances in zooplankton samples. On day 33, however, large numbers of small *Chaoborus* larvae were encountered in some limnocorrals (up to 2.58 *Chaoborus*/L). These were instar 1-2 larvae that were probably hatched from recently deposited eggs. *Chaoborus* abundances then remained low for the remainder of the experiment. On day 33, a one-way ANOVA indicated a significant effect of treatment ($F = 13.6$, $p = 0.006$), with greater larval abundances in the non-additive MP treatment compared to the control (Tukey HSD *post-hoc* test; $p = 0.006$) and the additive MP treatment ($p = 0.025$; Figure 3.20). There was no significant difference between the additive treatment and the control ($p > 0.05$).

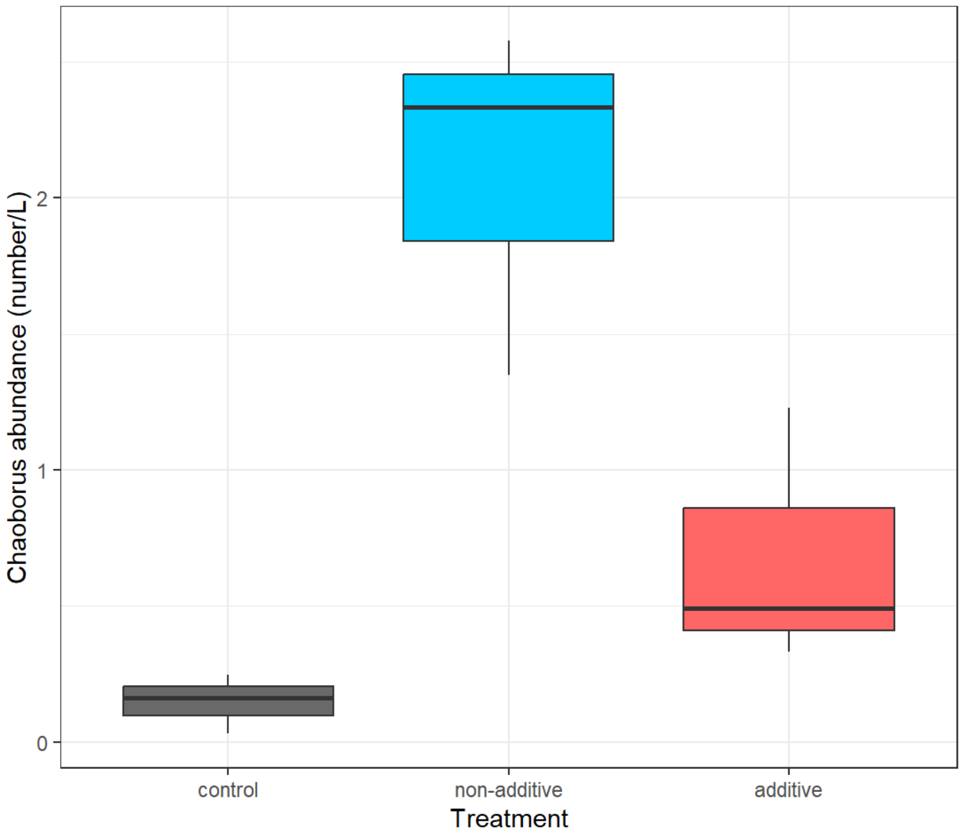


Figure 3.20 *Chaoborus* larvae abundance (mean ± 1 SD, n = 3) was significantly higher in the non-additive MP treatment compared to the control ($p = 0.006$) and the additive MP treatment ($p = 0.025$) on day 33.

Table 3.2 Results of one-way repeated-measures analysis of variance (RM ANOVA) for variables in the planktonic food web.

	Treatment		**Time x Treatment**		***Post-hoc***
Variable	F	P	F	P	Pairwise t-tests
Turbidity	**12.86**	**0.007**	1.20	0.309	Control < non-additive (day 13): p = 0.050; control < additive (day 13): p = 0.050; control < additive (day 33): p = 0.049; control < additive (day 55): p = 0.010.
Light extinction (Kd)	0.65	0.555	0.72	0.761	
Chlorophyll *a* concentration	4.38	0.067	0.87	0.510	
Chlorophyll *a*/phytoplankton biomass ratio	0.97	0.432	**3.31**	**0.048**	P > 0.05 for all days.
Total phytoplankton biomass	0.11	0.900	0.33	0.852	
Phytoplankton diversity	4.001	0.079	0.22	0.924	
Total zooplankton abundance	**5.28**	**0.048**	0.31	0.867	P > 0.05 for all days.
Calanoid abundance	2.22	0.190	1.62	0.233	
Cyclopoid abundance	3.77	0.087	1.59	0.272	
Cladoceran abundance	**11.01**	**0.010**	0.41	0.796	P > 0.05 for all days.
Total zooplankton biomass	**8.89**	**0.016**	0.49	0.747	P > 0.05 for all days.
Zooplankton diversity	2.31	0.181	0.33	0.910	
Eubosmina egg ratio	0.18	0.839	0.97	0.458	
T. extensus egg ratio	**7.85**	**0.021**	1.18	0.367	P > 0.05 for all days.
D. minutus egg ratio	2.19	0.207	0.66	0.558	
Nauplii abundance	2.91	0.131	3.19	0.112	

Bold values highlight overall significance at the $\alpha = 0.05$ level. P values were adjusted with FDR for pairwise comparisons.

3.5 Discussion

The objective of this chapter was to assess the potential effects of MPs with and without chemical additives on freshwater phytoplankton and zooplankton communities to evaluate the mechanism of toxicity for MPs. MPs either with or without chemical additives did not impact total phytoplankton biomass, but there may be potential for MPs to affect community composition and the chlorophyll *a*/biomass ratio over time. Zooplankton abundance, biomass, and species composition were not negatively impacted by MPs, however there may be potential for MPs to positively impact cladoceran abundance and decrease *T. extensus* egg production. Overall, there is little evidence that MPs either with or without chemical additives have strong negative impacts for freshwater plankton communities. Although analyses are not yet complete, it is likely that actual exposure of biota to MPs were far lower than the nominal MP concentrations, as found in the 2021 experiment (Chapter 2).

3.5.1 Environmental Parameters

MP treatment had a significant impact on turbidity over time, with higher turbidity in the MP treatments compared to the control on some days. Visually, it was evident that MPs did not mix homogenously due to differing buoyancies and adsorption to limnocorral walls, which may account for the large variability in both MP treatments while the control treatment stayed relatively invariant. Although MPs influenced turbidity, MPs did not affect light attenuation in the water column. Turbidity is typically a good predictor of light attenuation (Henderson and Bukaveckas 2022), however turbidity and light attenuation were not correlated in this study. All other environmental parameters including nutrient concentrations did not differ significantly among treatments.

Final Yellow Perch densities were generally consistent among treatments except for one of the non-additive limnocorrals that had lower perch densities and many Spottail Shiner due to a tear found in the curtain wall at the end of the experiment. Final Yellow Perch densities were not affected by MP treatment and were not correlated with final phytoplankton biomass or zooplankton abundance or biomass, suggesting fish were not a significant covariate for phytoplankton or zooplankton in this experiment.

3.5.2 Phytoplankton Community Response

Chlorophyll *a* concentration in the water column was not impacted by MPs with or without chemical additives, however there was a significant interaction between treatment and time for the chlorophyll *a*/biomass ratio. This interaction was of borderline significance and there was no effect of treatment on any study day, suggesting only a weak time-dependent response. The chlorophyll *a*/biomass ratio increased from day 33 to 62 for the additive treatment while the control and non-additive treatment decreased, suggesting a positive interaction with the additive treatment. It has been found in other studies that MPs can reduce chlorophyll *a* production at high concentrations (Besseling et al. 2014; Li et al. 2020) and these results suggest there are likely no negative impacts to chlorophyll *a* at these lower MP concentrations.

Total phytoplankton biomass was not impacted by either MP treatment. While phytoplankton growth and biomass may be negatively impacted by MPs at extremely high concentrations used in some laboratory studies (Bhattacharya et al. 2010; Mao et al.

2018; Prata et al. 2019a), the results in this chapter suggest this is not the case for the MP concentrations used in this study. The concentrations, although higher than what is found in most natural freshwaters (Eerkes-Medrano et al. 2015), were likely not high enough to harm phytoplankton. Based on results presented in Chapter 2, it is likely that water-column MP concentrations were far less than nominal concentrations used in the experimental design. Although this mixture of PE, PS and PET did not impact phytoplankton biomass, it is possible that other polymer types could potentially impact biomass and this should be investigated in future studies.

It has been suggested that MPs could potentially alter phytoplankton community structure (Chen et al. 2020; Hitchcock 2022). The PRC for biomass-based phytoplankton community composition indicated a significant effect on community composition over time. The community composition only significantly differed on day 62 and was largely driven by a slight increase in cyanobacteria and diatoms in the non-additive treatment. Hitchcock (2022) also found that high concentrations of MPs increased cyanobacteria growth. However, MP treatment accounted for very little of the variation among treatments indicating only a weak overall response. Other variables such as time and enclosure effects contributed to more variation among treatments in the experiment. The results suggest that there is some potential for MPs to alter phytoplankton community composition over time, but this needs to be further investigated to better understand this response.

3.5.3 Zooplankton Community Response

Significant positive treatment effects were detected over time for zooplankton abundance and biomass by RM ANOVA, however these effects were small and of borderline statistical significance, suggesting only a weak relationship. The polymers used in this study (PE, PS and PET) have relatively low chemical additive loads (Hahladakis et al. 2018) and additives were likely not in high enough concentrations to induce a toxic chemical impact. Other polymers with higher additive content such as PVC may potentially produce toxic effects (Gewert et al. 2021). Likewise, concentrations of MPs in the water column were likely too low to induce significant physical blockages and reduction of food intake for zooplankton. Physical impacts are typically induced only at very high concentrations (Cole et al. 2015; Ogonowski et al. 2016; Pan et al. 2022). Additionally, it is possible that biofilm growth on MPs may have offset any possible negative food dilution effects (Amariei et al. 2022). Even though the added concentrations of MPs were higher than what is typically found in nature (Eerkes-Medrano et al. 2015), the actual concentrations in the water column were likely far lower due to settlement of particles and adhesion to the limnocorral walls (Yildiz et al. 2022; Elagami et al. 2023).

Zooplankton community composition and diversity were also not impacted by either MP treatment. While very little research has been done on the potential impacts to zooplankton community composition, these results reflect those of a recent smaller scale mesocosm study that also found no impact to zooplankton community composition (Yildiz et al. 2022).

MP treatment did not impact the number of eggs per female for the cladoceran *Eubosmina sp.* or the calanoid copepod *D. minutus* but had a negative impact on the eggs per female for the cyclopoid copepod *T. extensus* over time. This effect of MP treatment was weak and did not hold up to *post-hoc* tests, however eggs per female were lower in both MP treatments on day 13. Copepod nauplii abundance was also lowest in the additive MP treatment on day 33, which is likely a reflection of the reduced number of copepod eggs produced in the weeks prior, however this difference in nauplii was not statistically significant. While these results are of borderline statistical significance, potential impacts to cyclopoid copepod reproduction should be examined further in future studies.

3.5.4 Insect Larvae

Chaoborus larvae were significantly more abundant in the MP treatment without additives on day 33. The *Chaoborus* larvae were early stage and had probably hatched from recently oviposited eggs. Early instar *Chaoborus* do not typically vertically migrate (Barth et al. 2014), which may explain their abundance in the zooplankton samples. Later instar *Chaoborus* are not usually encountered in zooplankton samples collected during the day because they migrate to just above the sediment-water interface at this time. One possible explanation for the increase in early stage *Chaoborus* larvae may be that MPs increased oviposition sites for *Chaoborus*. There is no research into this subject, however this phenomenon has been observed for other aquatic insects. For example, MPs have been shown to increase oviposition for the marine water strider *Halobates micans* by providing a substrate for them to lay their eggs (Majer et al. 2012). Future studies should examine the potential of MPs to increase oviposition of aquatic insects such as *Chaoborus*.

3.6 Conclusion

Laboratory studies suggest MPs can both physically and chemically affect phytoplankton and zooplankton from exposure to chemical additives and from the particles themselves, however the impacts for natural planktonic communities are unknown. The results suggest that MP fragments of common polymer types (PE, PS and PET) either with or without chemical additives are unlikely to have ecologically significant effects to phytoplankton or zooplankton communities at the concentration used in this study. A weak treatment effect over time was observed for the chlorophyll *a*/biomass ratio for phytoplankton, but MPs did not impact total biomass. Phytoplankton community composition was affected by MPs after 62 days, but the response was weak and phytoplankton diversity was not affected. A weak positive treatment effect was detected for total zooplankton abundance and biomass over time, but there was no impact to community composition or diversity. There may be potential for MPs to negatively impact cyclopoid copepod reproduction in terms of the number of eggs produced per female over time, however the results from this study were of borderline significance and this requires further investigation. Overall MPs with and without chemical additives did not appear to pose a significant risk to phytoplankton and zooplankton communities over the time frame examined.

CHAPTER 4: SUMMARY AND FUTURE RECCOMENDATIONS

4.1 Summary

In this thesis, the responses of freshwater phytoplankton and zooplankton communities to microplastics (MPs) were examined in two large scale limnocorral experiments that were conducted at the IISD-Experimental Lakes Area in 2021 and 2022. In Experiment 1 (Chapter 2) the impacts of a range of environmentally relevant MP concentrations using a mixture of three common polymer types (PE, PS and PET) was assessed. In Experiment 2 (Chapter 3), the chemical and physical impacts of MPs were assessed for freshwater phytoplankton and zooplankton communities using the same mixture of polymers (PE, PS and PET) with and without chemical additives compared to a control with no MPs. Results from both experiments are summarised in Table 4.1.

Table 4.1 Comparison of statistically significant results between Experiment 1 (Chapter 2) and Experiment 2 (Chapter 3) for MP treatment effects.

Variable	**Experiment 1**	**Experiment 2**
Turbidity	Turbidity slightly lower in MP treatments on days 5 and 68.	Higher turbidity in MP treatments over time.
Light attenuation (Kd)	Kd lower in MP treatments on days 26 and 61.	No effect.
Chlorophyll *a*	Negative effect on day 12.	No effect.
Chlorophyll *a*/phytoplankton biomass ratio	No effect.	Small time dependant treatment interaction.
Total phytoplankton biomass	No effect.	No effect.
Phytoplankton community composition	No effect.	Small effect on composition on day 68.
Phytoplankton diversity	No effect.	No effect.
Total zooplankton abundance	Positive effect on day 33.	Small positive effect over time.
Calanoid copepod abundance	Positive effect on days 19 and 33.	No effect.
Cyclopoid copepod abundance	No effect.	No effect.
Cladoceran abundance	No effect.	Small positive effect over time.
Total zooplankton biomass	Positive effect on day 33.	Small positive effect over time.
Zooplankton community composition	No effect.	No effect.
Zooplankton diversity	No effect.	No effect.
Eubosmina sp. egg ratio	No effect.	No effect.
Tropocyclops extensus egg ratio	Negative effect on day 19.	Small negative effect over time.
Diaptomus minutus egg ratio	No effect.	No effect.
Nauplii abundance	Positive effect on day 12, negative effect on day 54.	No effect.

4.2 Comparison of Results

4.2.1 Environmental Parameters

Water chemistry parameters including temperature, dissolved oxygen and nutrients did not differ among treatments in either experiment, suggesting that MPs did not alter nutrient cycling. Turbidity was slightly lower in the MP treatments in Experiment 1 on various days but was higher in the MP treatments in Experiment 2. Other studies have suggested that MPs may impact turbidity and light at high concentrations (Bhattacharya et al. 2010; Rani-Borges et al. 2021), but there were no consistent impacts to turbidity or light in these experiments. It is likely that the MP concentrations used in these experiments were too low to significantly impact light in the water column. Other factors such as particulate nutrients and plankton likely had a stronger influence on turbidity and light than the MP particles.

Final perch densities in Experiment 1 were highly variable among limnocorrals, especially between the two controls, and perch were an important covariate for phytoplankton biomass. In Experiment 2, final perch densities were more similar among limnocorrals and did not covary significantly with total phytoplankton or zooplankton biomass. Planktivorous fish are known to alter plankton communities (Amaral et al. 2021) and variations in perch densities likely contributed to a considerable amount of variation among limnocorrals. In both experiments variable amounts of Spottail Shiner had also entered several limnocorrals likely contributing to variation.

4.2.2 Phytoplankton

In Experiment 1, there was a temporary reduction in chlorophyll *a* concentration in MP treatments, however there was no change in chlorophyll *a*/biomass ratios. In Experiment 2, chlorophyll *a* concentration was unaffected by MP treatment but there was a small time-dependent treatment interaction for the chlorophyll *a*/biomass ratio that appeared to increase the ratio in the additive treatment. These conflicting results indicate that there was no consistent impact of MPs on chlorophyll *a* production at the concentrations used in these experiments, which are lower than what has been observed to induce negative effects in the laboratory (Besseling et al. 2014; Li et al. 2020). This suggests that environmentally relevant concentrations of PE, PS and PET fragments with or without chemical additives likely do not have a strong impact on chlorophyll *a* production.

Phytoplankton biomass was not affected by MPs in either experiment. While other researchers have found both negative (Bhattacharya et al. 2010; Sjollema et al. 2016; Mao et al. 2018; Rani-Borges et al. 2021; Rummel et al. 2022) and positive (Yokota et al. 2017; Canniff and Hoang 2018; Pan et al. 2022) effects of MPs on phytoplankton, my results suggest that MPs may be benign at realistic concentrations, inducing neither a negative nor positive response for total phytoplankton biomass. Some of the suggested reasons for a decrease in phytoplankton growth in the literature are the potential for MPs to increase turbidity, reduce light from shading, adhere to the cell wall, and expose algae to chemical leachates (Bhattacharya et al. 2010; Prata et al. 2019b; Rani-Borges et al. 2021; Rummel et al. 2022). While the second experiment indicated some increase in turbidity over time, light was not reduced in either study, which may be one reason why phytoplankton biomass was unaffected. MP additive concentrations were also likely too low to cause chemical stress to phytoplankton, since MPs with a lower additive load are less toxic to phytoplankton (Rummel et al. 2022).

MPs did not affect phytoplankton diversity in either experiment. Phytoplankton community composition was not affected by MPs in Experiment 1 but was marginally affected in Experiment 2 due to a slight increase in cyanobacteria and diatoms in the non-additive treatment on day 62. Hitchcock (2022) and Yokota et al. (2017) also found cyanobacteria increased in response to high MP concentrations. While only a marginal shift in community composition was observed in the second experiment, this indicates that there may be potential for MPs to alter phytoplankton community composition in aquatic ecosystems over time. Slight changes in community composition could have the potential to disrupt nutrient cycling and trophic interactions in aquatic ecosystems (Prata et al. 2019b; Pan et al. 2022) and this should be explored further in future studies.

4.2.3 **Zooplankton**

Both experiments showed some potential for MPs to positively affect zooplankton abundance and biomass. There was a positive effect on total zooplankton abundance and biomass in both experiments, but the magnitude of the effects in both cases was small. In Experiment 1, this positive effect was driven by an increase in calanoid copepods (mainly *Diaptomus minutus*) while in the second experiment this trend was driven by cladocerans (mainly *Eubosmina sp.*). Calanoid abundance in Experiment 2 was very low compared to Experiment 1 making it difficult to directly compare results between experiments for this species. While many laboratory studies have shown that MPs may negatively impact zooplankton (Botterell et al. 2019), others have also suggested that MPs may positively affect zooplankton if there is sufficient biofilm growth (Vroom et al. 2017; Canniff and Hoang 2018; Amariei et al. 2022). It is possible that biofilm growth on MPs marginally stimulated zooplankton and this should be explored in future studies for various zooplankton groups.

MPs did not affect zooplankton community composition in either study based on the principal response curves (PRCs) nor did MPs affect zooplankton diversity. These results reflect those of a similar smaller scale in-situ mesocosm experiment that found no impact to zooplankton community composition in response to MPs (Yildiz et al. 2022). The copepod communities in each experiment were quite different from each other. In Experiment 1, the dominant copepod species was *D. minutus*, a calanoid copepod, and in the second, it was *T. extensus*, a small cyclopoid copepod. Calanoid abundance was positively affected by MPs in Experiment 1, but no clear trend was evident in Experiment 2 for this group, due to low abundance. This difference in composition in the 2 experiments could be related to fish predation, since higher densities of perch were present at the end of Experiment 2 compared to Experiment 1 and no supplemental food was provided. Zooplanktivorous fish are known to select for larger bodied zooplankton, contributing to altered species composition and size distribution of the community (de Bernardi et al. 1987; Amaral et al. 2021). Zooplankton diversity was also lower in the second experiment, and this may also be attributed to perch predation.

Both experiments showed some potential for MPs to negatively impact copepod reproduction. Laboratory studies have also shown that MPs can negatively impact copepod reproduction at high concentrations (Lee et al. 2013; Cole et al. 2015) and my results suggest this effect may also occur at the lower concentrations used in this study. In both experiments the ratio of eggs per female for the cyclopoid copepod *T. extensus* was reduced by MPs, indicating that the reproductive output of this species may be vulnerable to MPs at environmentally relevant concentrations. The mechanism for this negative effect on *T. extensus* egg production is unclear since both MP treatments with and without chemical additives appeared to reduce egg production similarly, but pairwise comparisons were not significant. Copepod nauplii abundance was at first stimulated and then reduced by MPs in Experiment 1, and was not significantly affected at all in the second experiment, making it unclear what the potential impacts for nauplii may be. Future studies should examine how MPs affect copepod reproduction over longer periods of time to assess if there are multi-generational effects for egg production and nauplii abundance.

4.3 **Extrapolation to Natural Systems**

The limnocorrals used in this study were more realistic than laboratory studies, however, still differ from natural ecosystems. The limnocorrals allowed for indirect food web interactions, which are a key part of natural ecosystem responses that are not typically studied in the laboratory. However, the limnocorrals were closed-bottomed and lacked a benthic habitat. In natural systems, settled MPs would come into direct contact with the benthos community, and this component was missing from the mesocosms. Planktonic communities in mesocosm studies diverge over time and this divergence is suggested to be mainly driven by species interactions rather than by external factors

(Benincà et al. 2008). Since the limnocorrals were in-situ this gave us limited control over environmental variation. Some of this variation was due to differing Yellow Perch densities, especially in the first experiment. It is also important to note that the limnocorrals themselves were constructed of plastic and the close proximity of the mesocosm walls likely altered the plankton communities, favoring more littoral species. While the study duration was long compared to most laboratory studies, it was likely not long enough to allow for long term indirect effects such as changes to fish populations and seasonal effects.

MPs did not mix homogenously and settled quickly from the water column since they were only added as a single pulse. In addition, water column concentrations were lower than expectations based on complete mixing, providing a reduced exposure landscape than was intended. Because MP concentrations were assessed in only a subset of limnocorrals, conclusions presented here are based on nominal treatment concentrations rather than actual concentrations of MPs. In the environment, MP concentrations are continuously increasing due to constant inputs and fragmentation of plastic (Duis and Coors 2016). More frequent additions of MPs could be used to look at long term constant MP exposure in future studies. This study only looked at 3 types of polymers (PE, PS, and PET) that were a restricted size range (10-1900 μm) of fragments. Other types of polymers (e.g., PVC), morphologies (e.g., fibers) and sizes (e.g., < 1 μm) may prove to be harmful for plankton communities and deserves further attention.

Lake 378 is a small oligotrophic lake located in the boreal shield. Other lakes or water bodies with different environmental conditions such as eutrophic lakes or turbid rivers may respond differently to MPs. While the results from the mesocosm studies may not be directly extrapolated to natural systems they can be used to bridge the gap between laboratory studies and real-world conditions. My results are consistent with the results of the only other mesocosm study to examine the effects on MPs on a plankton community (Yildiz et al. 2022). This study was undertaken in a lake in Turkey, which is very different from Lake 378.

4.4 Contributions to Knowledge and Future Recommendations

My research contributes to a growing collection of knowledge on the potential impacts of MPs for aquatic organisms. In general, results in the literature are conflicting, and this has only proven that the effects of MPs are complex and not easily understood. The field of MP research is complicated by the complexity of MPs as multi-faceted contaminants. The many combinations of polymer types, sizes, morphologies and chemical compositions create a difficult path to navigate for researchers. The mixture of MPs used in this study include the most widely detected polymers found in the environment, however it is essential to study the effects of other combinations of polymers and additives. As the first large-scale mesocosm experiment looking at the fate and effects of MPs for a pelagic food web, this research has helped us to better understand the potential real-world implications of MPs in freshwater environments. While mesocosm studies cannot fully replicate the conditions in natural systems, they still provide an idea of the potential community interactions and complex food web interactions that would not be possible under laboratory conditions. While my research provides a step forward, there is a long way to go to fully understanding the complex ways that MPs impact freshwater communities. More mesocosm experiments and whole ecosystem experiments are the next step in looking at the long-term multidimensional impacts of MPs. A whole lake experiment will be conducted starting in summer 2023 to assess the long-term fate and effects of MPs in an experimental lake (Lake 378) at IISD-ELA. This type of whole ecosystem research is essential for furthering our knowledge of indirect biological responses and food web interactions (Carpenter et al. 1995).

Most laboratory studies use concentrations that are much higher than what is environmentally relevant, however my study used concentrations that may be found in nature. It is important for researchers to use what is environmentally relevant instead of only using concentrations that are high enough induce a significant result. A serious problem in MP research is the way that studies with non-significant results are often overlooked or not published. However non-significant results are equally as important as significant ones and should hold equal weight.

4.5 Conclusion

The potential impacts of MPs for freshwater ecosystems are poorly understood as described in Chapter 1. Most studies to date have been based in the laboratory, typically using unrealistic concentrations, and assessing individual and population level effects for marine species. This study is the first large-scale in-lake mesocosm study to evaluate the effects of MPs in natural freshwater planktonic food webs. The results presented here suggest that this mixture of polymer types (PE, PS, and PET) at environmentally relevant concentrations are unlikely to pose a significant direct risk to natural freshwater plankton communities. However, subtle changes to phytoplankton community composition and zooplankton abundance, biomass and reproduction indicate the potential for effects over time. More field-based research is needed to better understand these potential impacts to planktonic food webs over longer periods and for different polymer types. This work may help to inform policy surrounding MPs in freshwater ecosystems and provide a direction for future research.

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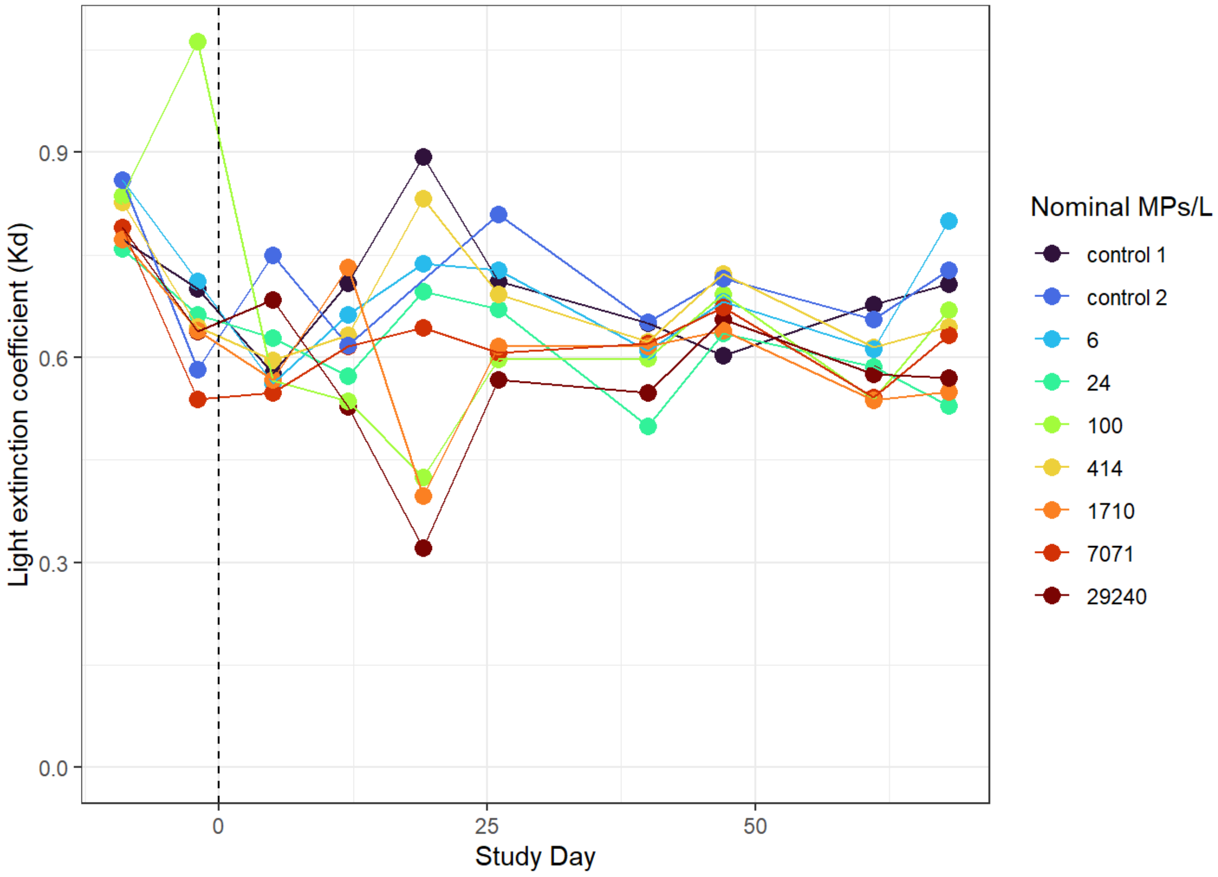
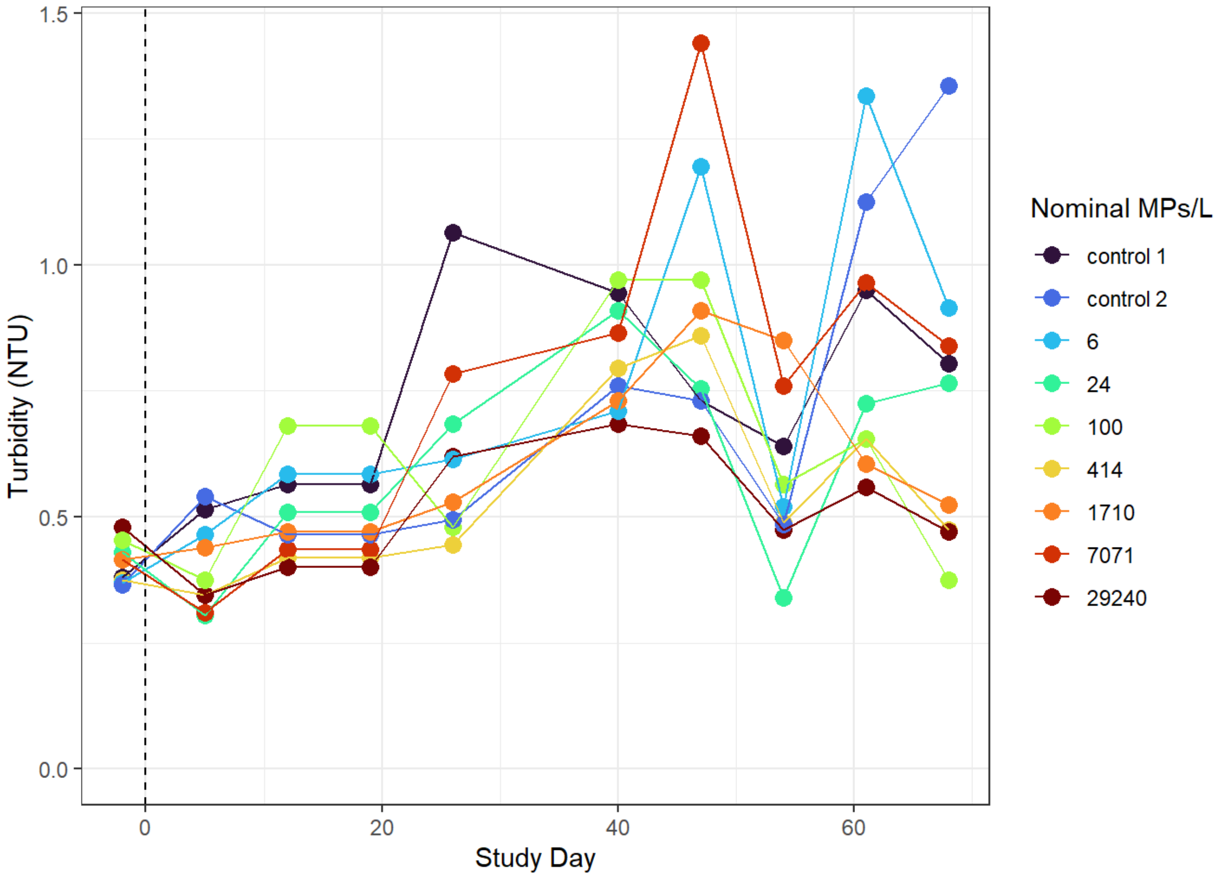
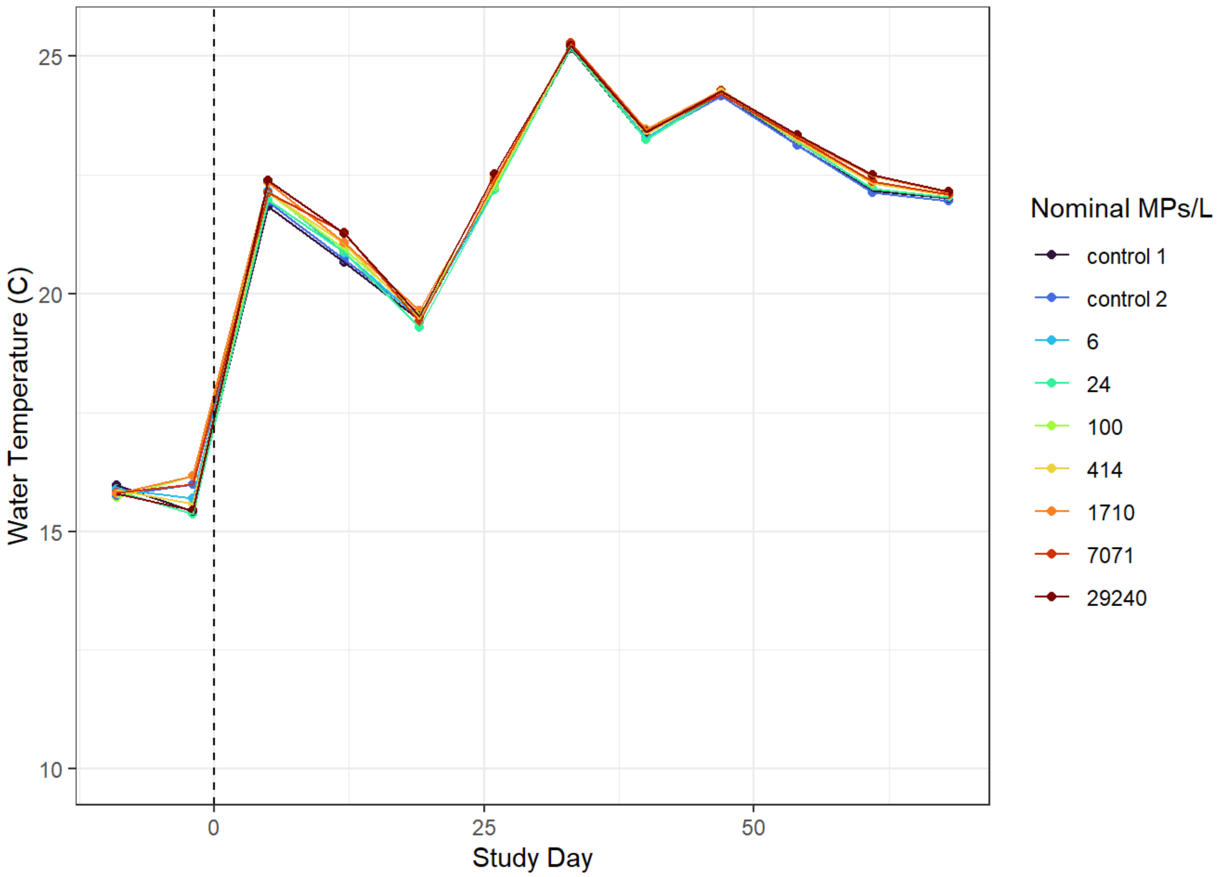
APPENDIX



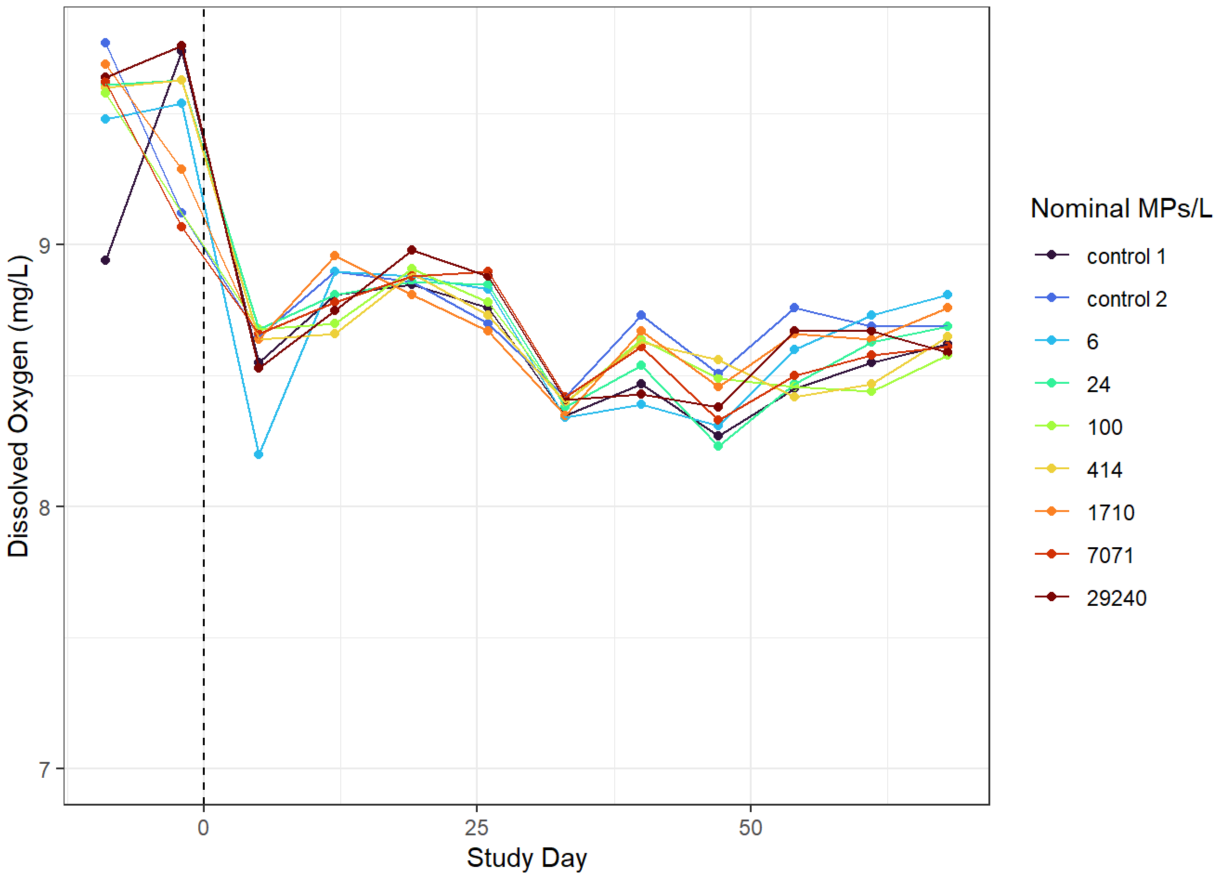
Appendix Figure 1 Light extinction coefficient (Kd) throughout the Chapter 2 study. The dashed line represents the day of MP additions on day 0. In general, there was no statistically significant relationship between K_d and nominal MP concentrations except for negative relationships that were observed on day 26 (R^2= 0.71, p = 0.004) and day 61 (R^2= 0.72, p = 0.004).



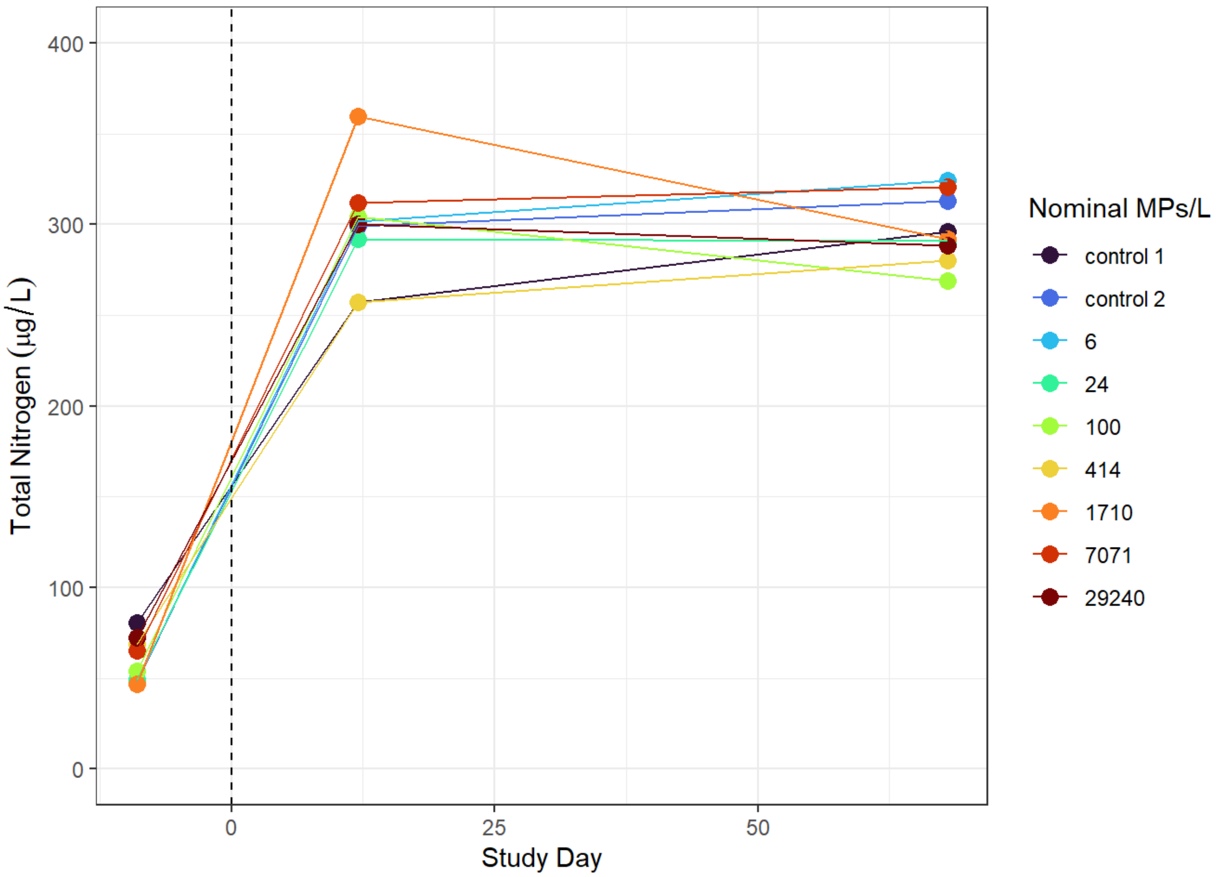
Appendix Figure 2 Turbidity throughout the Chapter 2 study. The dashed line represents the day of MP additions on day 0. In general turbidity did not significantly differ among treatments except for negative relationships that were observed on day 5 (R^2 = 0.66, p = 0.009) and day 68 (R^2 = 0.48, p = 0.04).



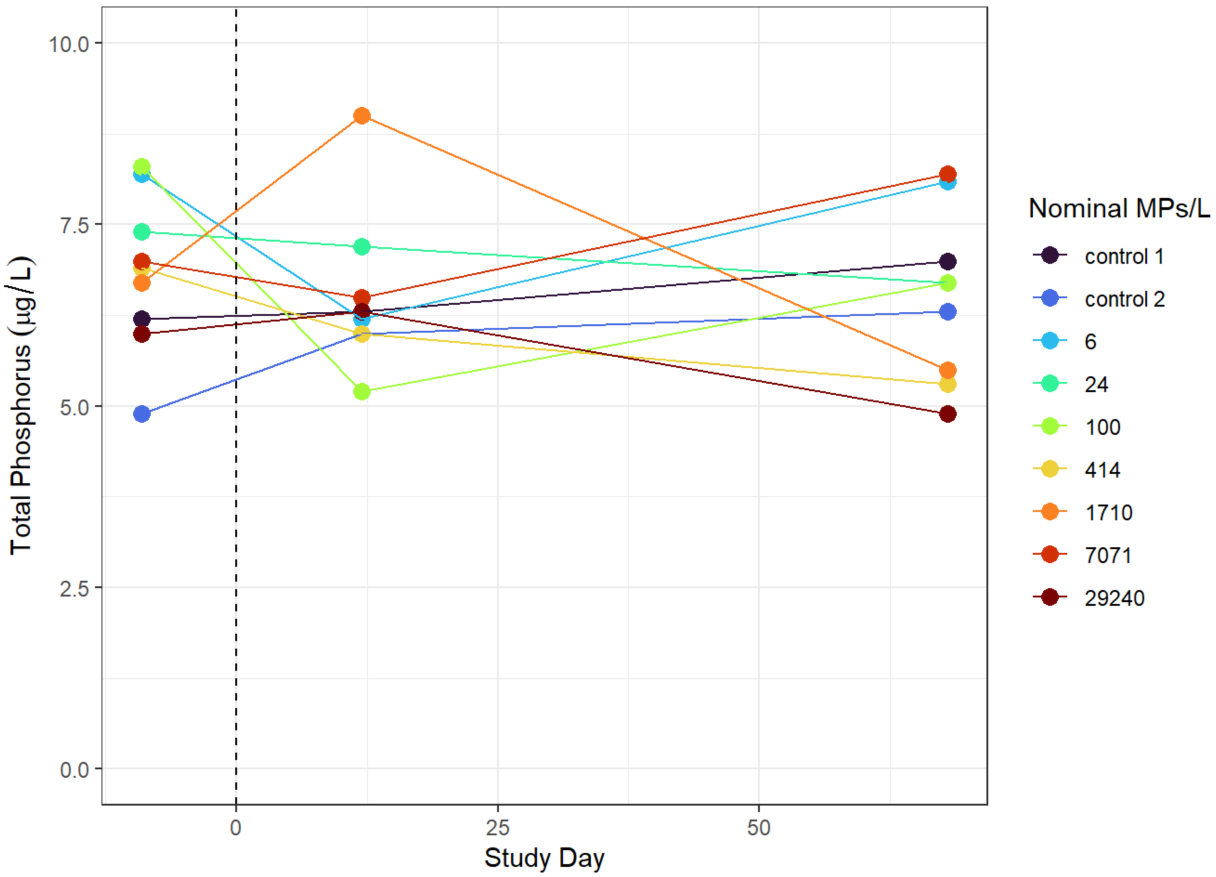
Appendix Figure 3 Water temperature (ºC) at 1 m measured weekly throughout the Chapter 2 study. The dashed line represents the day of MP additions (day 0).



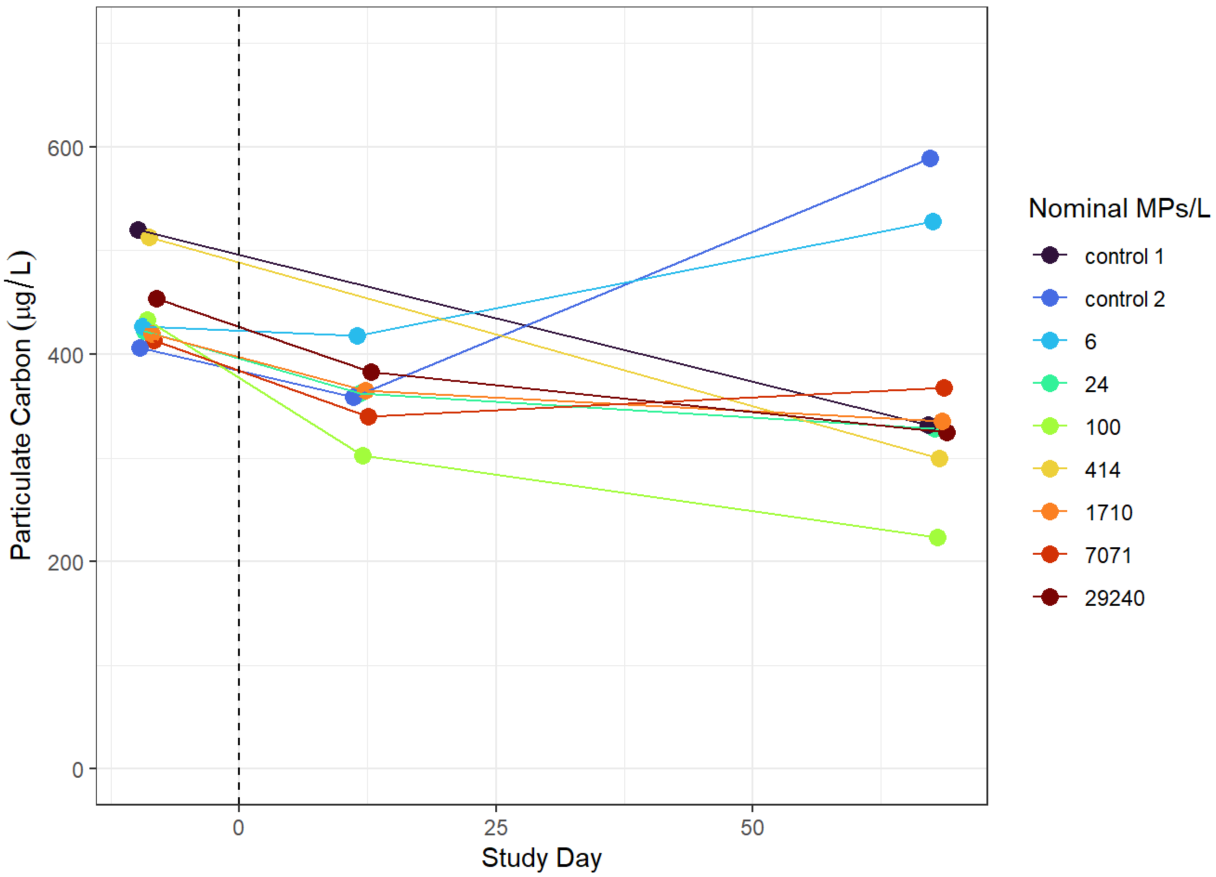
Appendix Figure 4 Dissolved oxygen measured weekly throughout the Chapter 2 study at 1 m depth. The dashed line represents the day of MP additions (day 0).



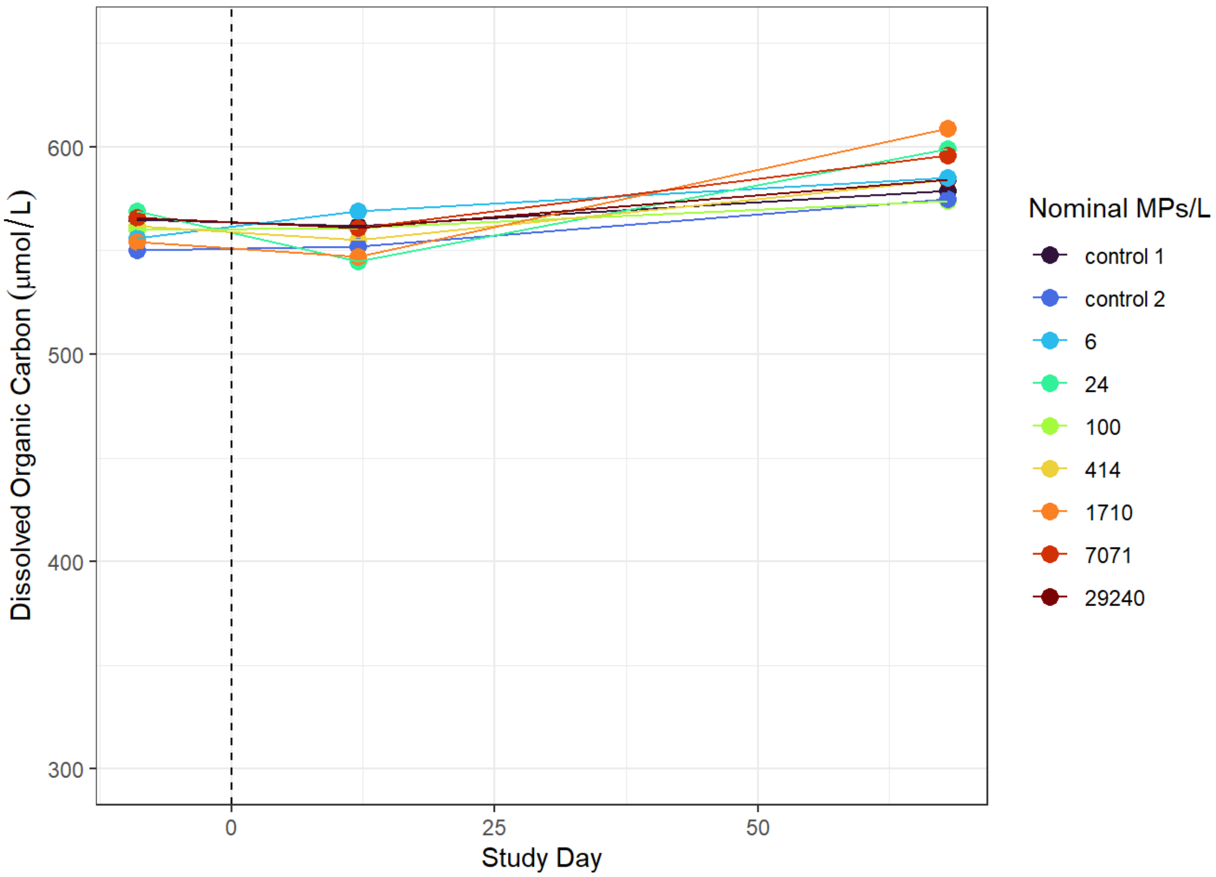
Appendix Figure 5 Total nitrogen concentrations throughout the Chapter 2 study. The dashed line represents the day of MP additions (day 0). Statistically significant relationships between total nitrogen and nominal MP concentrations were not observed on any day.



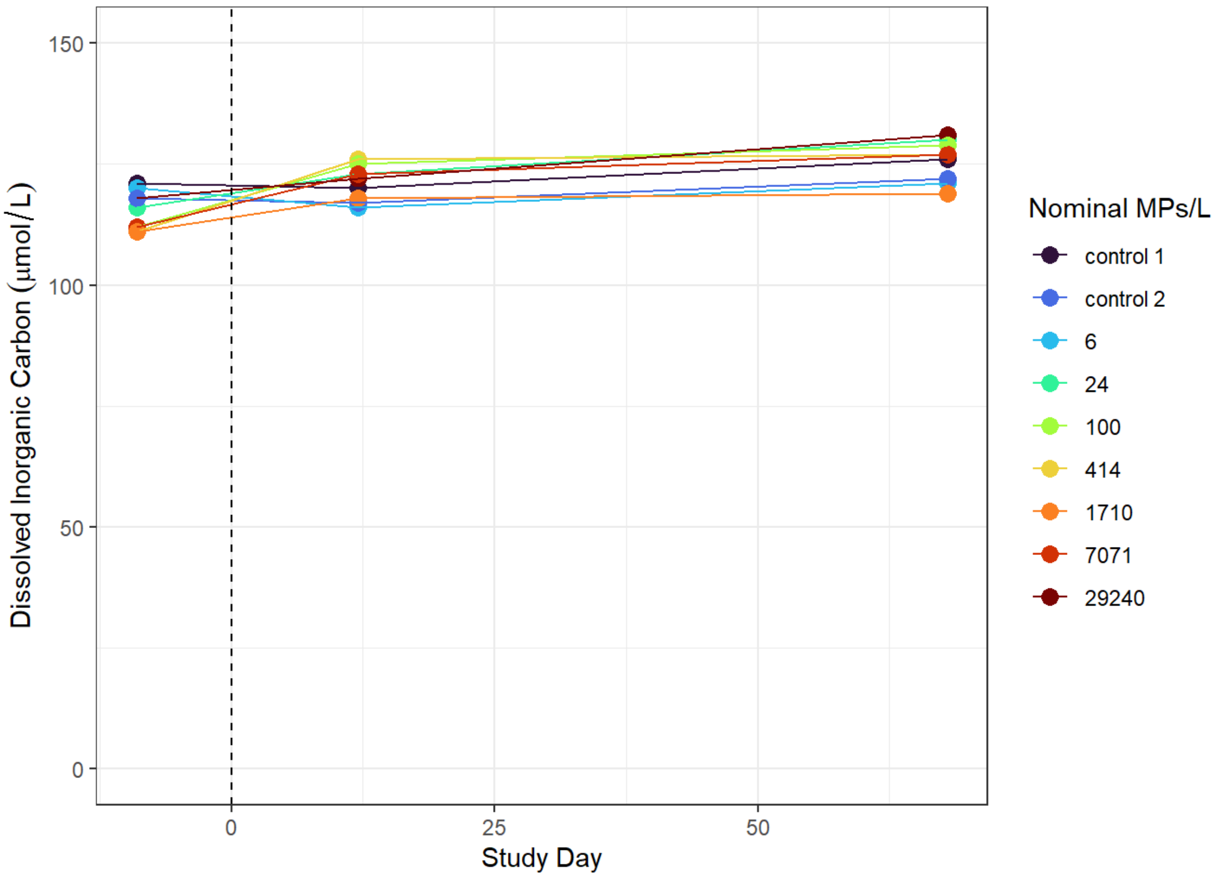
Appendix Figure 6 Total phosphorus concentrations throughout the Chapter 2 study. The dashed line represents the day of MP additions (day 0). Statistically significant relationships between total phosphorus and nominal MP concentrations were not observed on any day.



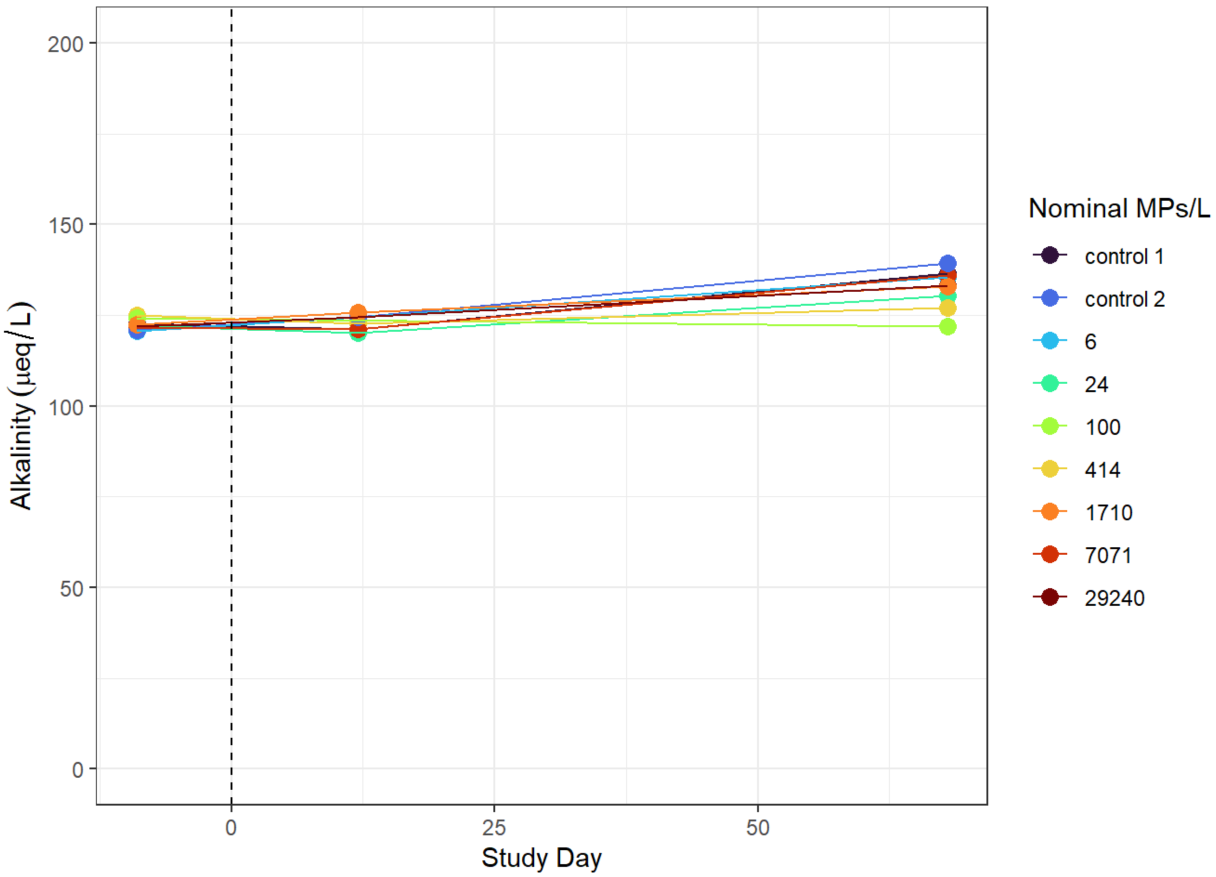
Appendix Figure 7 Particulate carbon concentrations for each treatment throughout the Chapter 2 study. The dashed line represents the day of MP additions (day 0). There were missing values for control 1 and the 414 MPs/L treatment on day 12. Statistically significant relationships between particulate carbon and nominal MP concentrations were not observed on any day.



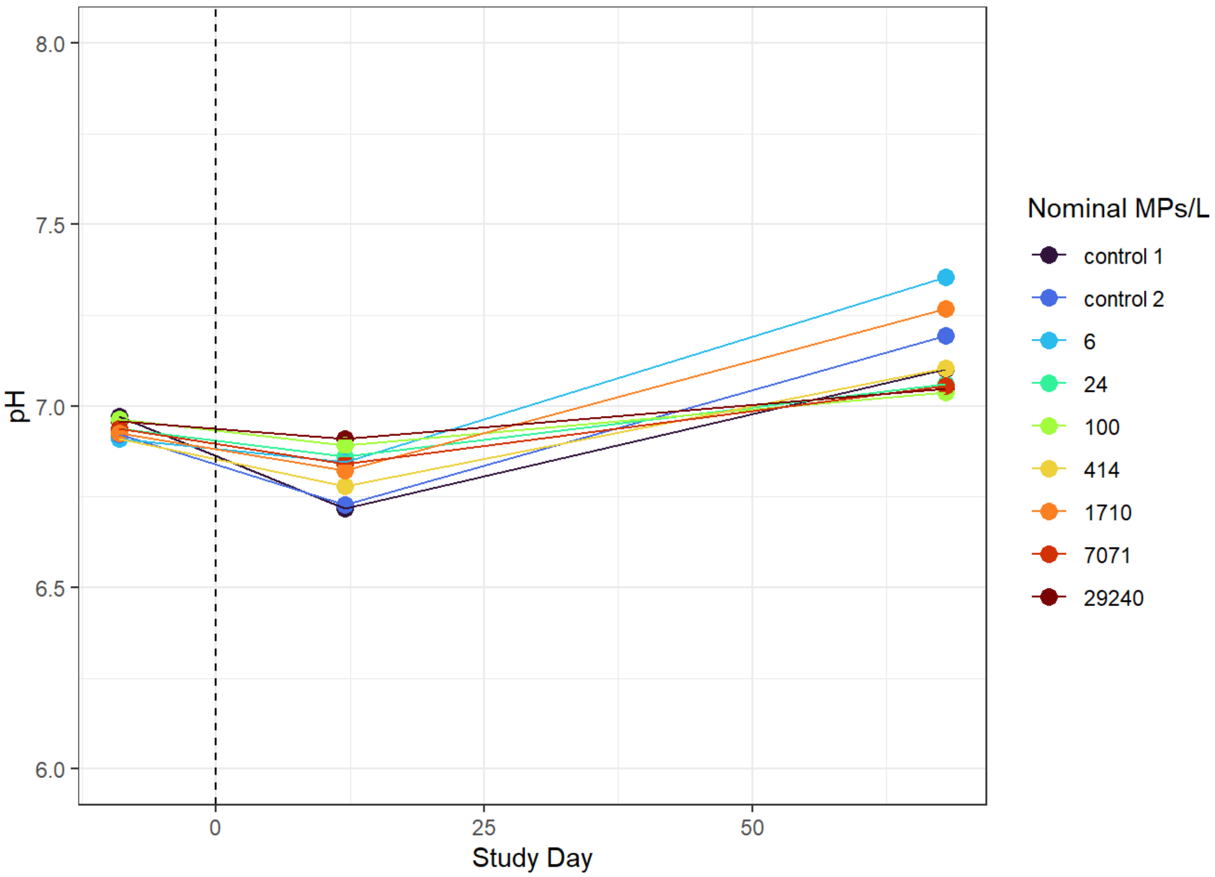
Appendix Figure 8 Dissolved organic carbon (DOC) concentrations for each treatment throughout the Chapter 2 study. The dashed line represents the day of MP additions (day 0). Statistically significant relationships between DOC and nominal MP concentrations were not observed on any day.



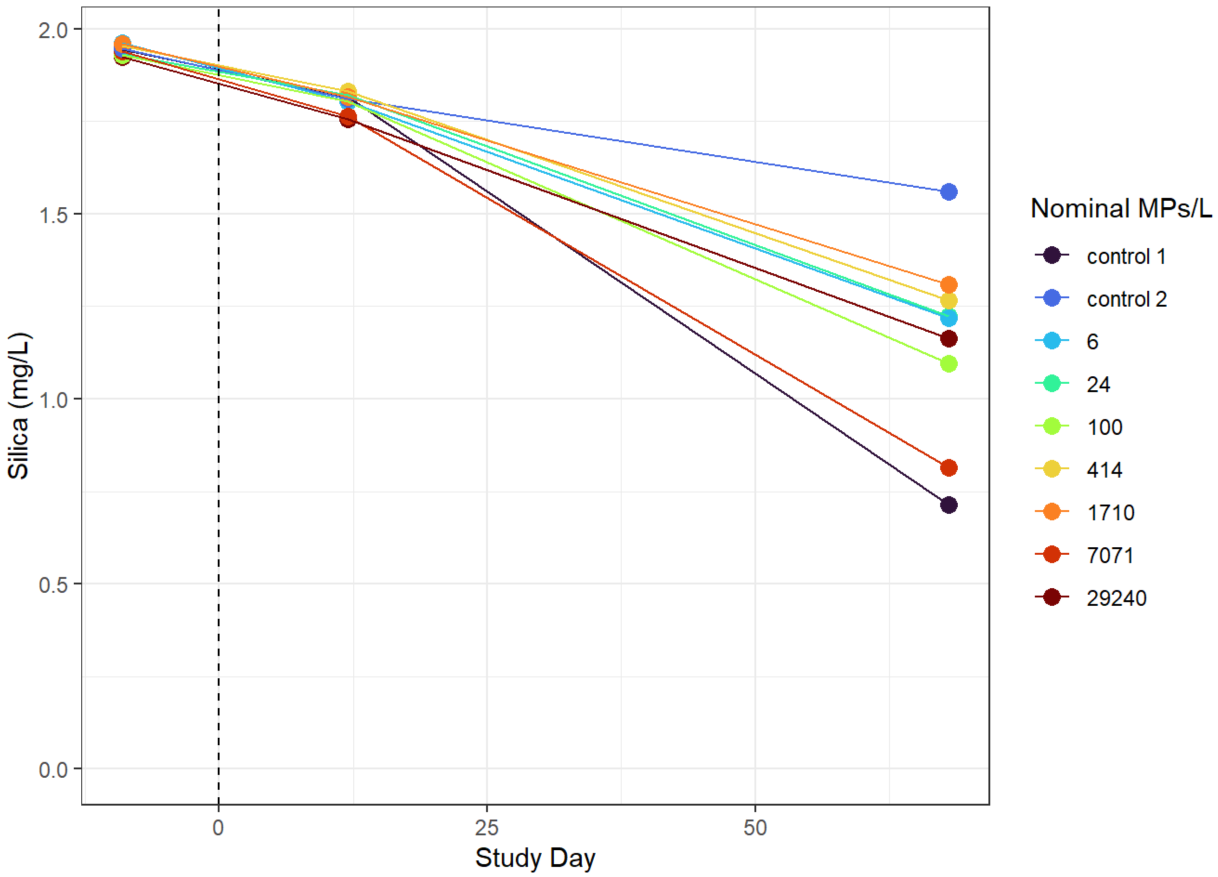
Appendix Figure 9 Dissolved inorganic carbon (DIC) concentrations for each treatment throughout the Chapter 2 study. The dashed line represents the day of MP additions (day 0). Statistically significant relationships between DIC and nominal MP concentrations were not observed on any day.



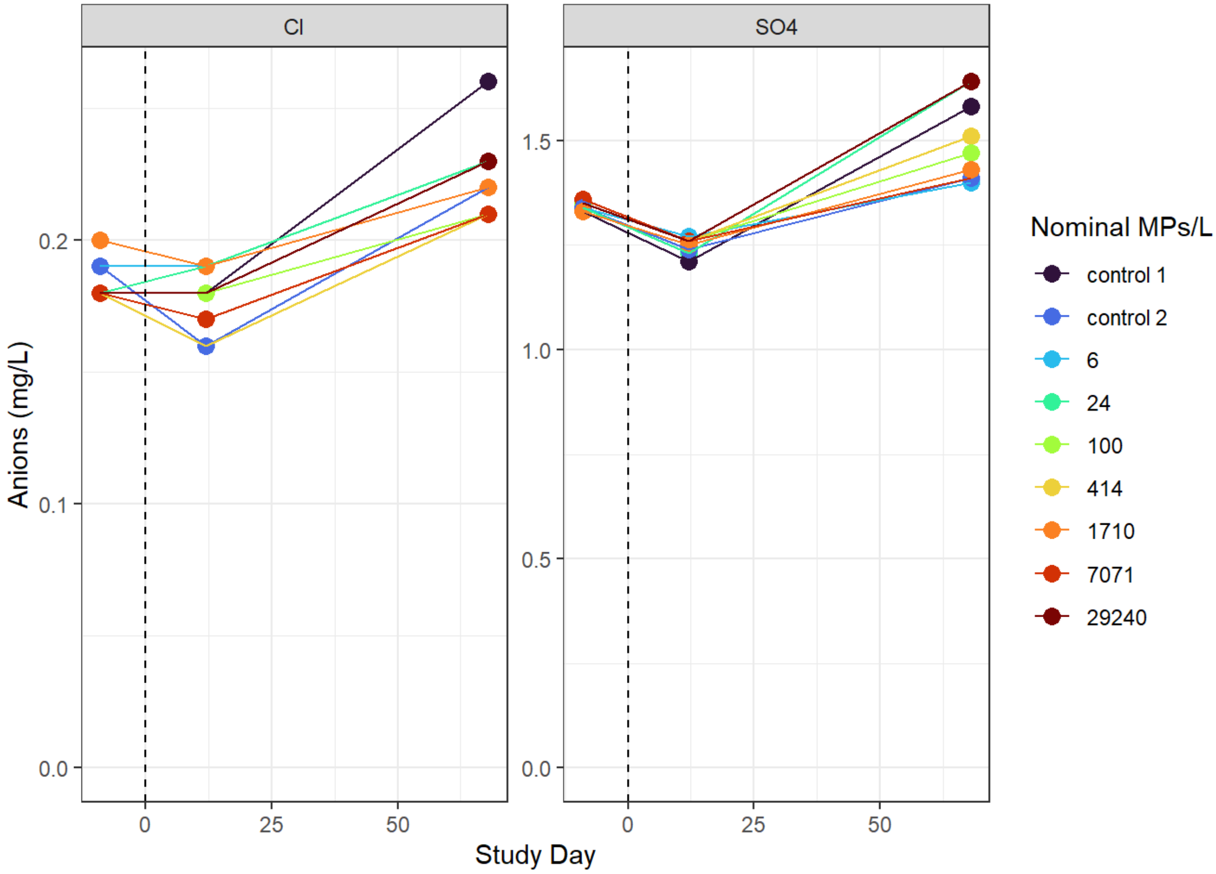
Appendix Figure 10 Alkalinity measurements for each treatment throughout the Chapter 2 study. The dashed line represents the day of MP additions (day 0). Statistically significant relationships between alkalinity and nominal MP concentrations were not observed on any day.



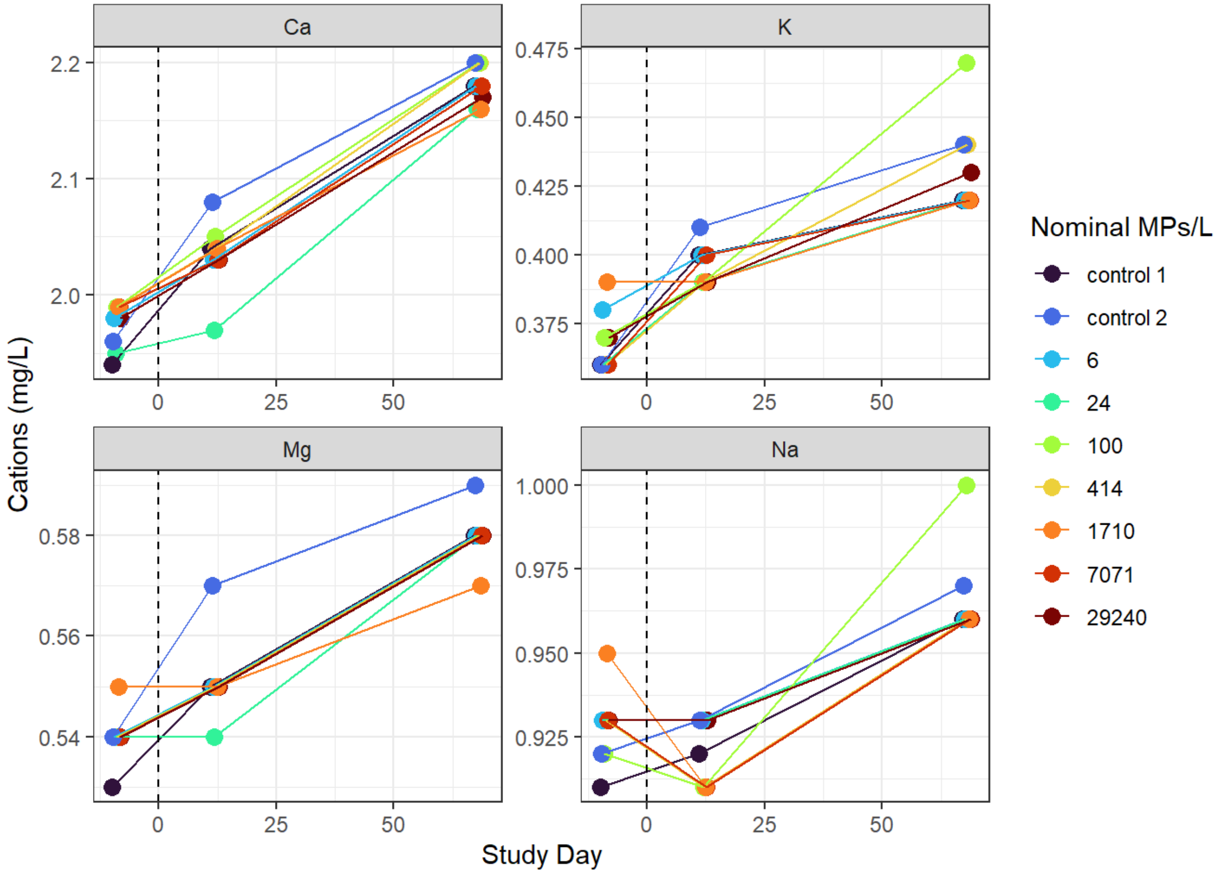
Appendix Figure 11 pH measurements for each treatment throughout the Chapter 2 study. The dashed line represents the day of MP additions (day 0). There was a statistically significant positive relationship on day 12 ($R^2 = 0.62$, $p = 0.012$).



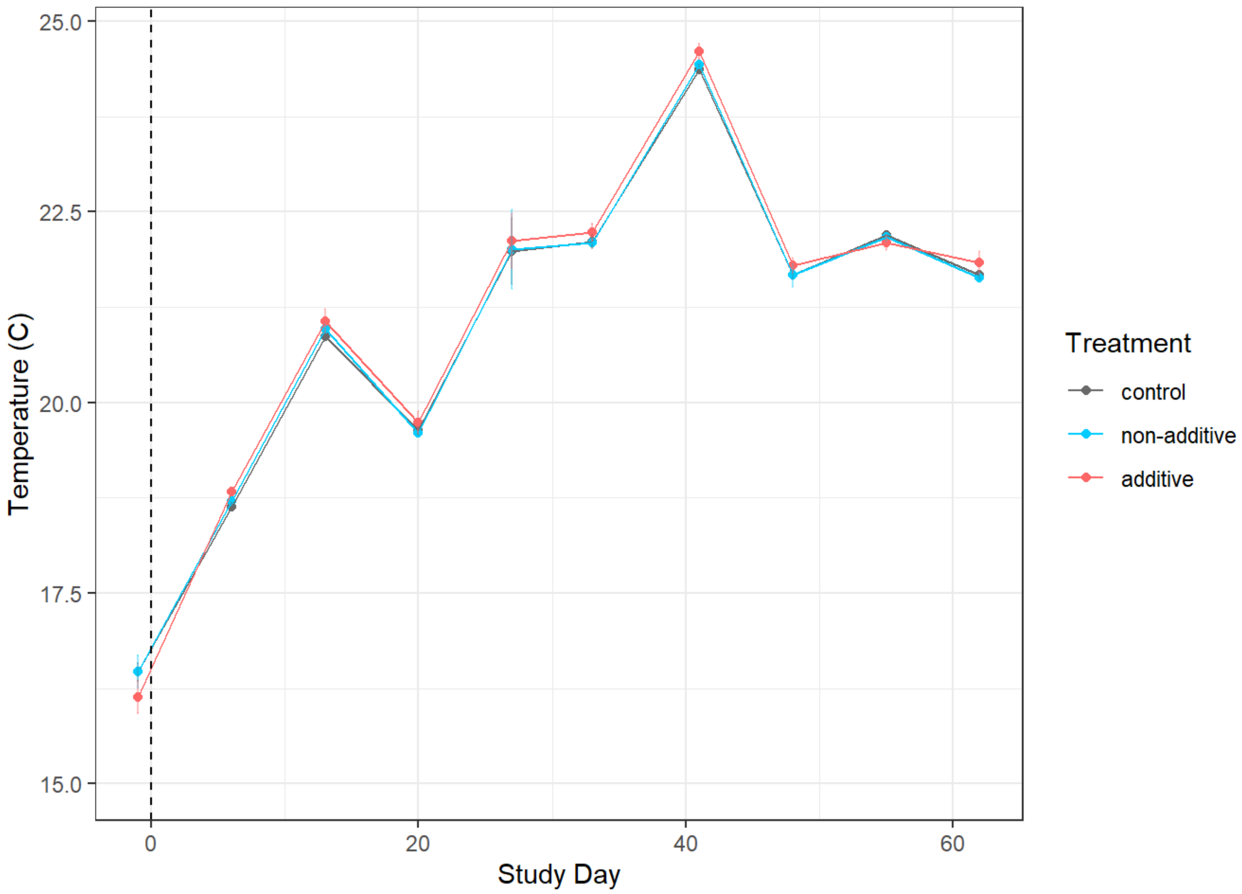
Appendix Figure 12 Soluble reactive silica (SRSi) concentrations for each treatment throughout the Chapter 2 study. The dashed line represents the day of MP additions (day 0). Statistically significant relationships between soluble reactive silica and nominal MP concentrations were not observed on any day.



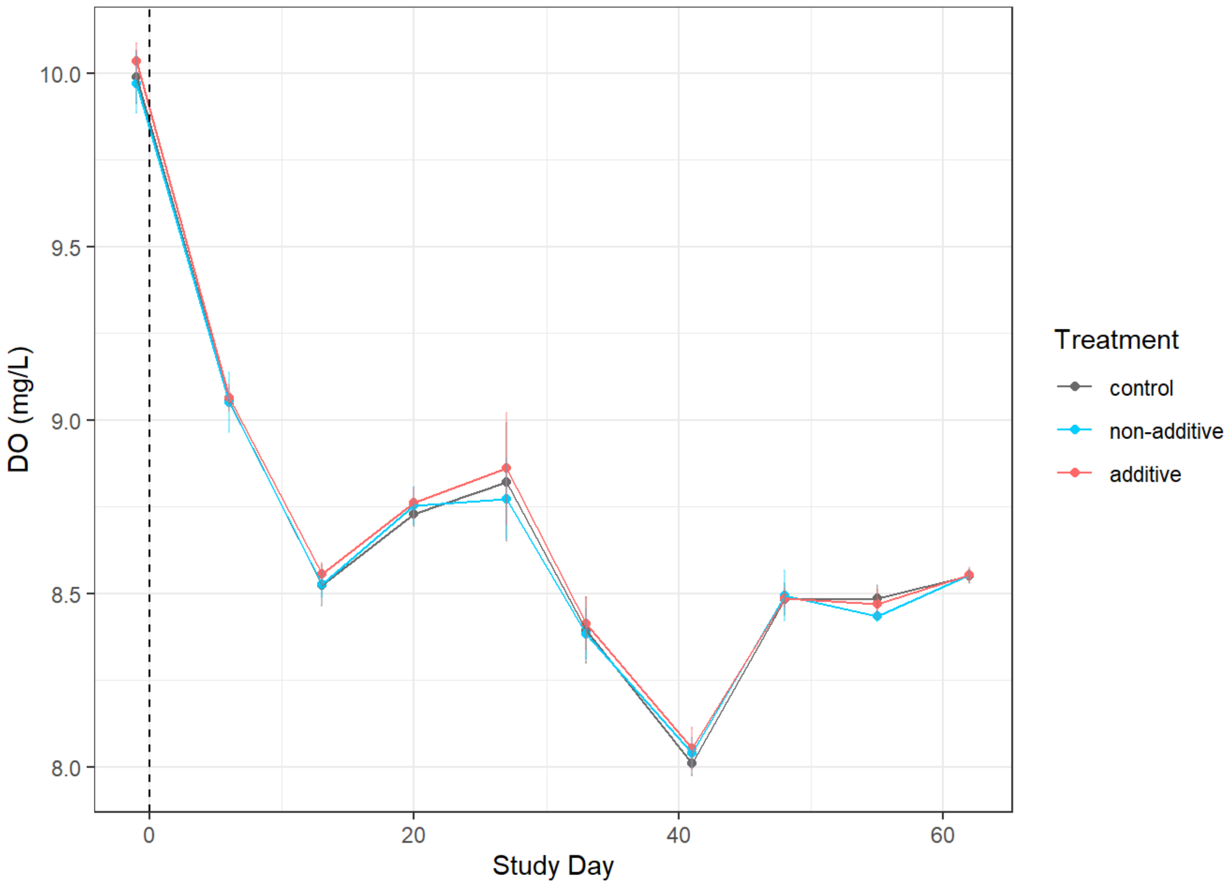
Appendix Figure 13 Anion concentrations for chloride (Cl) and sulfate (SO4) for each treatment throughout the Chapter 2 study. The dashed line represents the day of MP additions (day 0). Statistically significant relationships with nominal MP concentrations were not observed on any day.



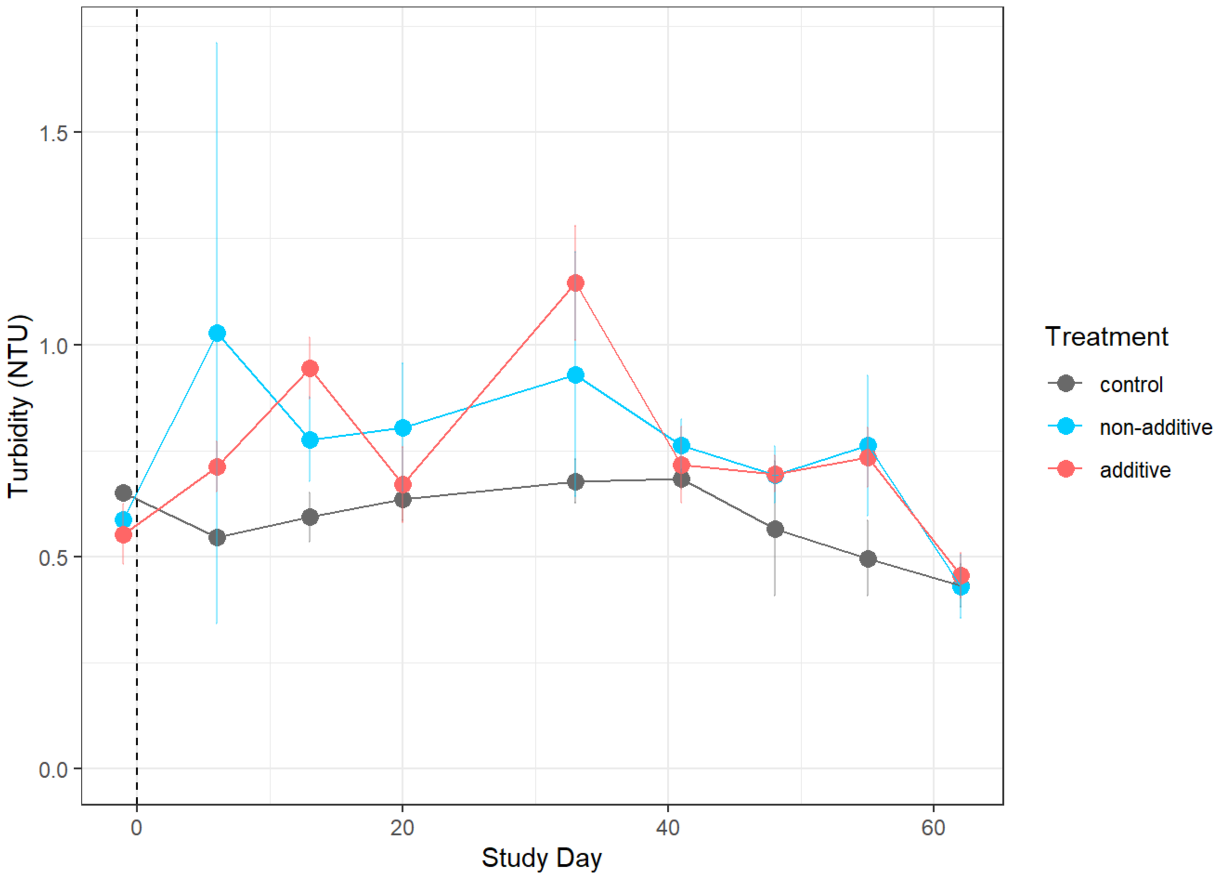
Appendix Figure 14 Cation concentrations for calcium (Ca), potassium (K), magnesium (Mg) and sodium (Na) for each treatment throughout the Chapter 2 study. The dashed line represents the day of MP additions (day 0). Statistically significant relationships with nominal MP concentrations were not observed on any day.



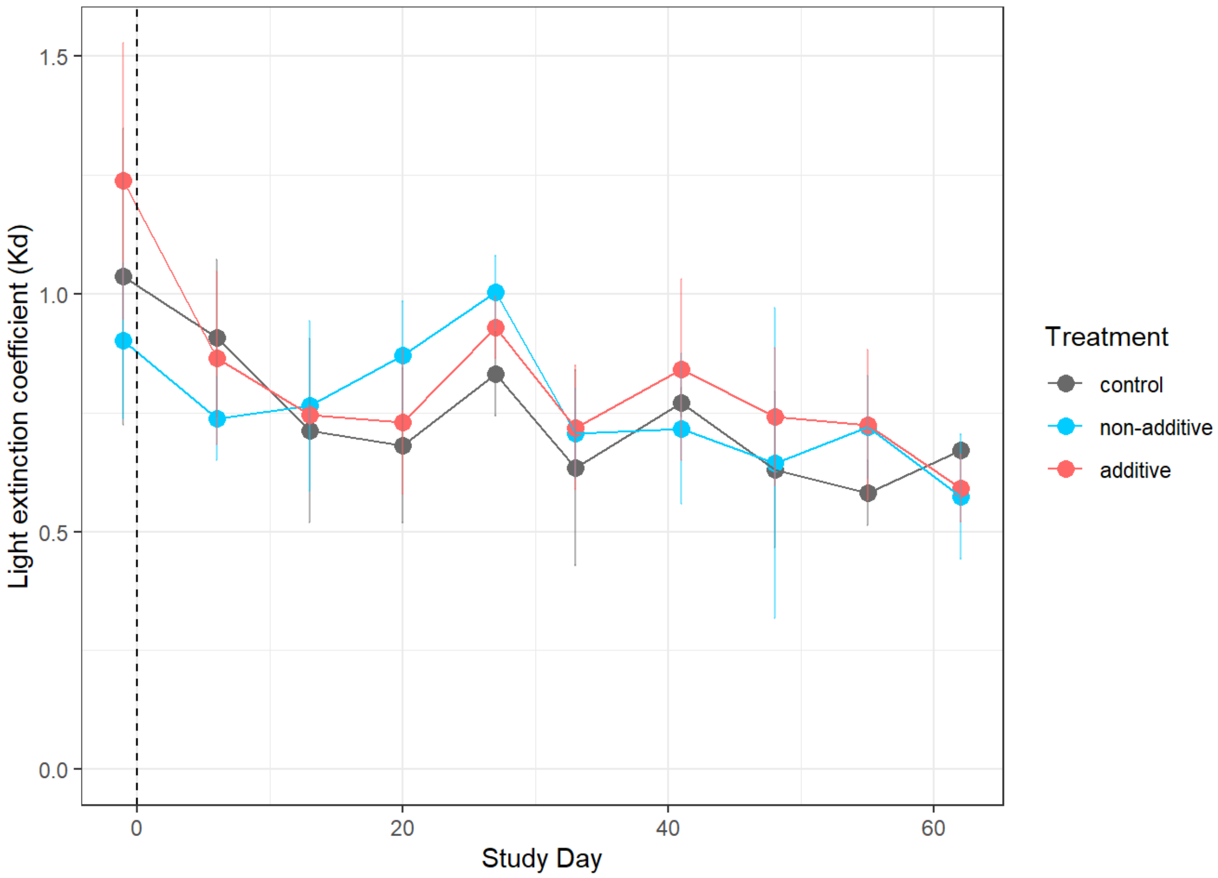
Appendix Figure 15 Water temperature (Celsius) at 1 m throughout the Chapter 3 study (mean ± 1 SD, n = 3).



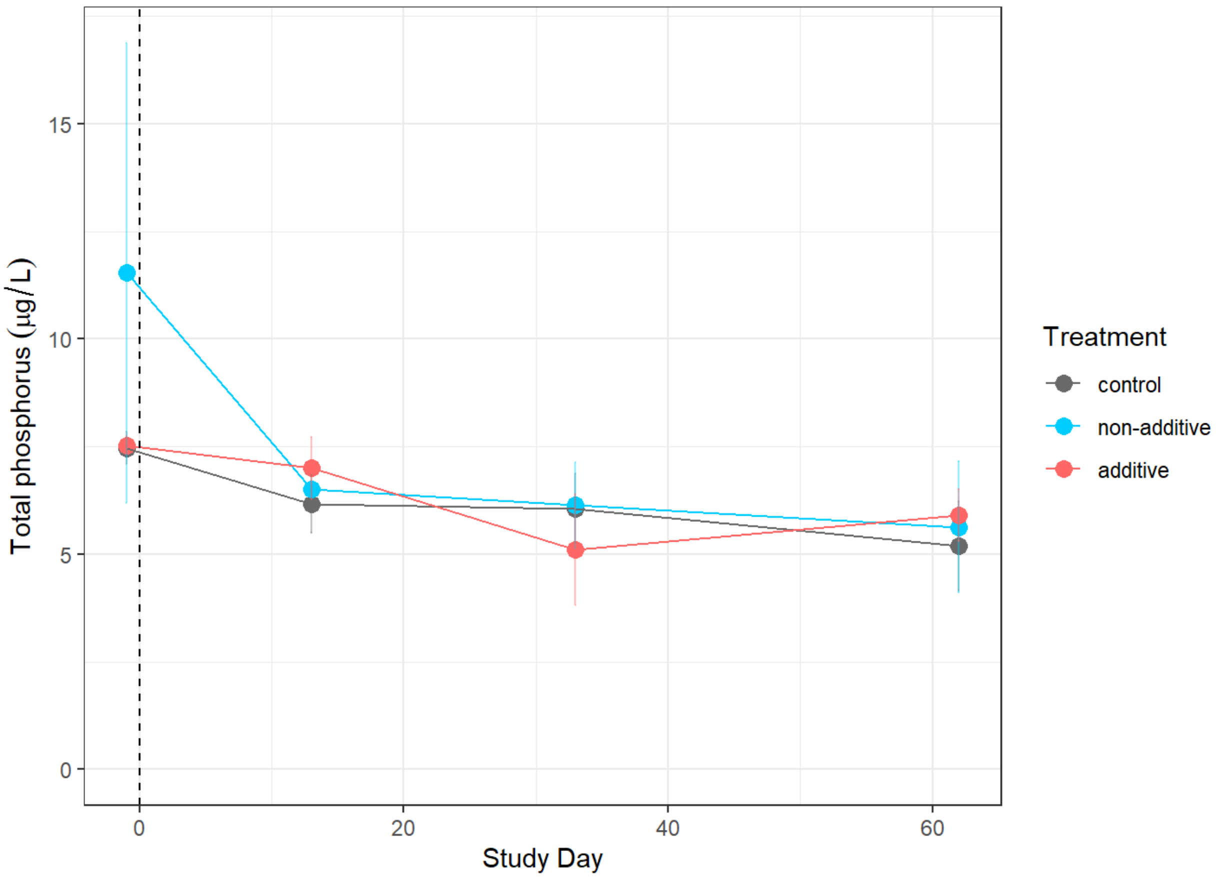
Appendix Figure 16 Dissolved oxygen (DO) at 1 m throughout the Chapter 3 study (mean ± 1 SD, n = 3).



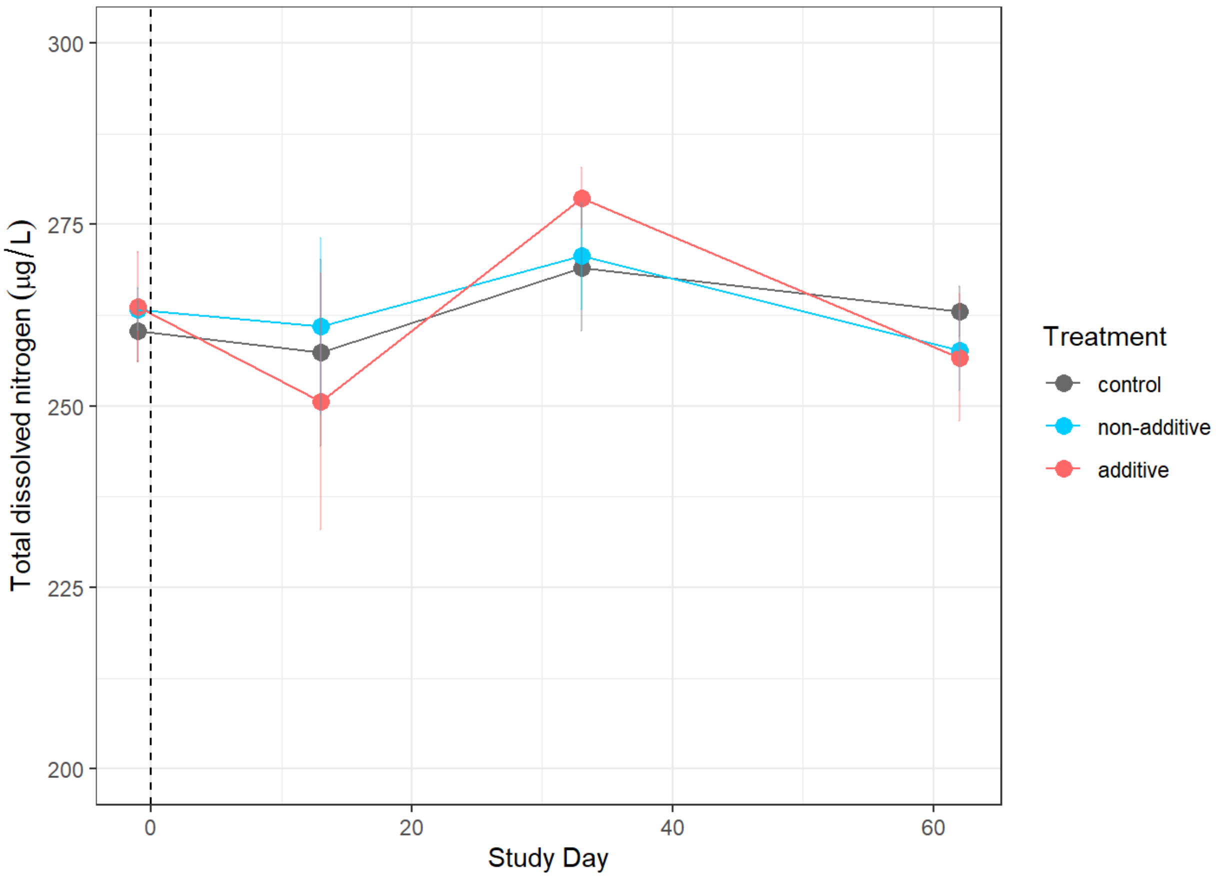
Appendix Figure 17 Turbidity throughout the Chapter 3 study (mean ± 1 SD, n =3). Turbidity was significantly higher than the control in the non-additive and additive treatments on day 13 (p = 0.05), and in the additive treatment on day 33 (p = 0.049) and day 55 (p = 0.01).



Appendix Figure 18 The light extinction coefficient (Kd) throughout the Chapter 3 study (mean ± 1 SD, n =3). There was no significant difference among treatments over time (RM ANOVA; $p = 0.56$).



Appendix Figure 19 Total phosphorus (TP) throughout the Chapter 3 study (mean ± 1 SD, n =3). TP did not differ between treatments over time (RM ANOVA; p = 0.9).



Appendix Figure 20 Total dissolved nitrogen (TDN) throughout the Chapter 3 study (mean ± 1 SD, n =3). TDN did not differ between treatments over time (RM ANOVA; p = 0.93).