Summer 2016

Combined Effects of Tetrakis Hydroxymethyl Phosphonium Chloride and Ammonium on Plankton Community Structure in Blackwater Pond Mesocosms

Marina N. Osier

COMBINED EFFECTS OF TETRAKIS HYDROXYMETHYL PHOSPHONIUM CHLORIDE AND AMMONIUM ON PLANKTON COMMUNITY STRUCTURE IN BLACKWATER POND MESOCOSMS

by

MARINA OSIER

(Under the Direction of Risa A. Cohen)

ABSTRACT

Flame retardant chemicals enter aquatic systems through municipal and industrial wastewater, and despite being detected in surface waters, most have not been thoroughly assessed for environmental risk. Halogenated flame retardants, known to be persistent and highly toxic to aquatic organisms are being phased out, and replaced with new or under-tested organophosphorus flame retardants. Organophosphorus flame retardants, such as tetrakis hydroxymethyl phosphonium chloride (THPC), often have similar applications to and are generally not as persistent as halogenated flame retardants. Single-species toxicity tests of organophosphorus flame retardants reveal a wide range of toxicity values within the range of environmentally relevant concentrations in surface waters. Additionally, other contaminants from industry and agriculture are likely to accompany flame retardant chemicals in surface waters. For example, THPC may be found in combination with ammonia, which is released directly with THPC in textile effluent and is a common component of agricultural fertilizers. Ammonia is a form of inorganic nitrogen that can stimulate primary production, but in excess can shift phytoplankton community composition to dominance by less edible or harmful species. While the effects of excess nutrients on planktonic communities are well studied, recent findings suggest the presence of nutrients can reduce or enhance the effects of other contaminants on

aquatic organisms. Floating, *in situ* mesocosms were dosed with two levels of THPC (0.08 and 0.8 mg L^{-1}) with or without ammonium (0.3 mg L^{-1}) in a fully crossed design to study the effects of THPC and ammonium in combination on plankton communities. I hypothesized that THPC and ammonium affect plankton communities differently alone than in combination. Water samples were collected weekly over four weeks to assess zooplankton community composition and phytoplankton abundance (as chlorophyll *a*) in response to treatment additions. The combination of THPC and ammonium influenced community composition, but each factor alone had no effect. Specifically, low THPC+ammonium decreased the proportion of calanoid copepods with a corresponding increase in rotifers due to lower predation pressure compared to each factor alone one to three weeks post exposure. Total zooplankton abundance changed over time across all treatments, decreasing by more than 50% after one and two weeks of exposure before increasing to abundances approximately 27% higher than initial by week 4. Chlorophyll *a* was not affected by THPC and nutrient addition, but concentrations fluctuated between 5-15 µg L^{-1} in all treatments throughout the duration of the experiment. The interaction of THPC and ammonium suggests that these contaminants in combination can reduce abundance of large zooplankton such as copepods, thereby limiting a food source for planktivorous fish, and releasing smaller crustacean zooplankton and rotifers from predation and competitive pressure.

INDEX WORDS: Contaminant mixtures, Flame Retardant, Chronic Exposure, Ecotoxicology

COMBINED EFFECTS OF TETRAKIS HYDROXYMETHYL PHOSPHONIUM CHLORIDE AND AMMONIUM ON PLANKTON COMMUNITY STRUCTURE IN BLACKWATER POND MESOCOSMS

by

MARINA OSIER

B.S. Forestry Iowa State University, 2013

A Thesis Submitted to the Graduate Faculty of Georgia Southern University in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

STATESBORO, GEORGIA

COMBINED EFFECTS OF TETRAKIS HYDROXYMETHYL PHOSPHONIUM CHLORIDE AND AMMONIUM ON PLANKTON COMMUNITY STRUCTURE IN BLACKWATER POND MESOCOSMS

by

MARINA OSIER

Major Professor: Risa A. Cohen

Committee: Stephen P. Vives
J. Checo Colón-Gaud

Electronic Version Approved:

July 2016

ACKNOWLEDGEMENTS

Many people played a strong role in the development and execution of my thesis project, and I could not have completed this journey without their help along the way. I would like to extend my gratitude to my advisor, Risa Cohen, for her never-ending support for both my project and professional development. She devoted a great deal of her time to helping me develop skills even beyond the scope of my project to help me prepare for the challenges I might meet in the future, for which I am very thankful. I also want to thank my committee members, Stephen P. Vives and Checo Colón-Gaud, for their valuable insight, troubleshooting, and use of equipment for my project. I am also grateful to C. Ray Chandler for allowing me access to his property and for providing his statistical expertise.

Several fellow graduate students helped me through every step of the process. Jason Duff, Steven Riera, Jackman Eschenroeder, and Stefan Petersen, thank you for helping me develop my project and brainstorm ideas. I especially want to thank Matt Carey for allowing me to use his house as a construction zone, helping me build and deconstruct my project, and providing unwavering emotional support.

Lastly, I thank my mom, Toni Osier, for supporting me from afar. Her love and encouragement never waned, and she inspired me to continue to push through every stage of the process.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

COMBINED EFFECTS OF TETRAKIS HYDROXYMETHYL PHOSPHONIUM CHLORIDE AND AMMONIUM ON PLANKTON COMMUNITY STRUCTURE IN BLACKWATER POND MESOCOSMS

INTRODUCTION

Entry of contaminants into freshwaters can alter both community structure and function (Baron et al. 2002; Covich et al. 2004). In particular, contaminants in industrial and wastewater treatment effluent and agricultural runoff degrade water quality and are often toxic to aquatic organisms (Buxton and Kolpin 2002; Fleeger et al. 2003; Stuart et al. 2012; Gavrilescu et al. 2015). These toxic inputs result in the loss of sensitive species and dominance by more tolerant species that may be of lower quality (i.e. less palatable or nutritionally valuable) to consumers (Carpenter 1998; Baron et al. 2002). Such reduction in food quality often leads to less efficient energy transfer from the base of the food web to fish and other organisms of higher trophic levels (Persson et al. 2008), thus it is important to understand the potential effects of chemicals introduced to aquatic systems.

Approximately 80,000 chemicals are registered for use in the United States alone and an estimated 2,000 more introduced each year, but the exposure potential and toxicological risks associated with many of these chemicals are largely understudied (NTP 2016). Recent improvements in analytical techniques has allowed for the measurement of new and previously undetected persistent contaminants in surface waters (Howard and Muir 2010; Maruya et al. 2013). However, the technological ability to measure the concentrations of new chemicals in the environment is still limited in many cases due to lack of accepted methodology and the necessity for focusing on screening for and managing chemicals that are known to cause environmental harm, such as persistent polychlorinated biphenols (PCBs) (Howard and Muir 2010).

Furthermore, the United States Environmental Protection Agency (US EPA) issues National Pollution Discharge Elimination System (NPDES) permits based on the technology available for treating industry-specific pollutants contained in effluent (US EPA 2016a; US EPA 2016c). For example, the textile industry effluent guidelines specify allowable discharges of phenols, chromium, sulfide, and flame retardant chemicals into U.S. surface waters that are not anticipated to reduce water quality (US EPA 2016c). As a result, flame retardant chemicals associated with the textile industry are now detectable in surface waters (Marklund et al. 2005).

Despite their prevalence, flame retardant chemicals are often not thoroughly tested for potential risk to aquatic organisms (Maruya et al. 2013; Schreder and La Guardia 2014). Flame retardant chemicals are among chemicals that are screened for persistence, bioaccumulation potential, and toxicity based on the chemical properties and structure (Strempel et al. 2012); halogenated flame retardant chemicals are known to be highly persistent, have bioaccumulation potential, and are toxic to aquatic organisms (Covaci et al. 2011; Kharlyngdoh et al. 2015). Despite this knowledge, Covaci et al. (2011) suggested thorough environmental risk assessments of flame retardants is limited by the lack of developed analytical methods for quantifying flame retardants in environmental samples that are likely influenced by flame retardant inputs. The textile industry is the second largest industry in the world and water is used for the dyeing and finishing processes including the application of flame retardants and ultimately treated as wastewater in the wet-processing of textiles (Moore and Ausley 2004; Derden and Huybrechts 2013). Thus concentrations of flame retardant chemicals in aquatic systems are linked to textile production, subsequent wastewater discharge, and the persistence of the chemical in the environment (Reemtsma et al. 2008; Covaci et al. 2011; Wolschke et al. 2015; Shi et al. 2016).

There has been a surge in development and use of new flame retardant chemicals to replace the halogenated compounds found to be highly toxic and persist in aquatic systems (van der Veen and de Boer 2012; Cristale et al. 2013). Organophosphorus flame retardants in particular are useful for similar applications and less persistent than halogenated compounds (Covaci et al. 2011; van der Veen and de Boer 2012; Zhang et al. 2016). However, despite recent quantification of organophosphorus flame retardants such as TCPP and TCEP at concentrations ranging from 6.3-26,050 ng L^{-1} in the River Aire, UK and 4.23-87.4 µg L^{-1} at a Japanese waste disposal site (Kawagoshi et al. 2002; Cristale et al. 2013), many organophosphorus flame retardant chemicals have not been evaluated for environmental risk (Marklund et al. 2005). Tetrakis hydroxymethyl phosphonium chloride (THPC), a water-soluble organophosphorus flame retardant formed from the reaction of hydrochloric acid, formaldehyde, and phosphine (NRC 2000; WHO 2000), is used to produce flame and crease resistant clothing (Reeves and Guthrie 1956; Special Materials Company 2010). In the environment, THPC breaks down via photodegradation and hydrolysis into phosphorus oxides, ammonia, and chlorine (WHO 2000). Though the effects of ammonia and chlorine on aquatic organisms have been studied (e.g. Brungs 1974; Fuhui et al. 2010), how THPC influences organisms following discharge into surface waters is largely unknown.

The few existing single-species THPC toxicity tests suggest both acute sublethal and lethal effects on zooplankton and fish, though at different concentrations (WHO 2000, Bartolome and Sanchez-Fortun 2005, Sanchez-Fortun et al. 2005). For example, brine shrimp exposed to THPC for 24 h resulted in LC_{50} values ranging from 0.02 µg L^{-1} for 72-h old individuals to 7.8 µg L^{-1} for 24-h old individuals, indicating variation in sensitivity at different life stages (Bartolome and Sanchez-Fortun et al. 2005). Genotoxic effects, such as double stranded DNA

denaturation in isolated gonad cells from rainbow trout, increased with increasing THPC concentration over the range of 0.2-2.0 µg L^{-1} (Sanchez-Fortun et al. 2005). Much higher toxicity values for *Daphnia magna* (48-h LC_{50} 19,400 µg L^{-1}) and marine and freshwater algae (72-h EC_{50} 160 and 652 µg L^{-1}, respectively) have been reported (WHO 2000). However, all of these tests of THPC have been on single species over acute time frames (≤96 hours). Given that THPC exposure in the environment likely occurs over longer time frames, there is the possibility that chronic exposure results in adverse effects at lower concentrations. For example, the organophosphate insecticide chlorpyrifos negatively affected copepod abundance at a concentration ten times lower than the 96-h LC_{50} values after seven weeks of exposure (van den Brink et al. 1995).

Differential sensitivities across life stages and types of organisms to chemicals can shift zooplankton community dominance toward more tolerant species, ultimately affecting species richness and diversity (Ma et al. 2002; Preston 2002; Ma 2005; Sakamoto et al. 2005; Relyea and Hoverman 2006). Zooplankton taxa respond differently to organophosphorus chemicals (Hanazato and Yasuno 1990; Fulton and Key 2001). For example, large cladocerans were almost entirely lost from zooplankton communities exposed to the organophosphorus insecticide Fention, while rotifers and small cladocerans increased in abundance (Hanazato and Kasai 1995). The authors suggest that this change in zooplankton community composition was due not only to the differential sensitivities across taxa but also indirect effects associated with loss of larger competitors for food resources (Hanazato and Kasai 1995). While the differential effects of THPC on individual species suggests environmental exposure could alter aquatic community composition, testing is necessary to assess the direct (i.e. directly toxic) and indirect effects (e.g. affects the food source of an organism) of THPC (Preston 2002).

THPC is not found in the environment alone, and other chemicals likely to enter freshwater systems include pulses of inorganic nutrients (nitrogen and phosphorus) following application of agricultural fertilizers and municipal wastewater discharge (Howarth et al. 2002; Pieterse et al. 2003). In the most extreme cases, up to 80% of the fertilizer applied to agricultural land can be transported to surface waters (Howarth et al. 1996; Carpenter et al. 1998). Nutrients are required to support algal growth to supply food to zooplankton grazers (Smith 1998). However, excess inorganic nitrogen from agricultural fertilizers (e.g. ammonium (NH_4^+)) can be toxic to algal cells when concentrations exceed 100 µM NH_4^+ (Berg et al. 2003; Glibert et al. 2015). Lower concentrations favor opportunistic taxa, such as cyanobacteria, dinoflagellates, and cryptophytes (Paerl 1988; Smith 1998; Berg et al. 2003) that are less palatable to zooplankton grazers (Porter 1973; Porter 1977). Thus, NH_4^+-induced changes in phytoplankton species composition can influence both the abundance of zooplankton grazers and planktivorous fish (Carpenter et al. 1985; Smith 1998).

The effects of NH_4^+ on aquatic organisms can also be influenced by environmental factors. Conditions such as high pH, low temperatures, and low dissolved oxygen can increase the toxicity of ammonia (derived from both THPC degradation and agricultural fertilizer), leading to negative effects on aquatic consumers (US EPA 1999, Camargo and Alonso 2006). Specifically, increased temperature and pH increases the fraction present as un-ionized ammonia (NH_3) relative to the fraction of ionized NH_4^+ (US EPA 1999). The fraction of NH_3 largely determines toxicity to aquatic organisms, while chronic toxicity of NH_4^+ is relatively rare (Constable et al. 2003; Arauzo and Valladolid 2003; Di Lorenzo et al. 2014). The zooplankton *Daphnia simuloides* demonstrated a pattern of differential sensitivity to NH_3 based on age, where 24- and 48-h LC_{50} values were lowest for young individuals (0.5 days old) and increased with

age until individuals reach 5 days old, and declined again by 7 days old (Fuhui et al. 2010). Planarians exhibited 60% mortality after exposure to 0.09 mg L^{-1} NH_3-N for 30 days, and their movement was increasingly inhibited with duration of exposure (Alonso and Camargo 2011). Ammonia exposure can also have sublethal reproductive effects. For example, rotifer fecundity was reduced by 50% following exposure to 2.4 mg L^{-1} ammonia (de Araujo et al. 2000), and copepods exposed to 120 µg L^{-1} ammonia for 9 days had a 50% reduction in egg hatching success compared to non-exposed copepods (Buttino 1994). Because ammonia toxicity can be influenced by environmental conditions, it is also important to study interactions of contaminants under environmental conditions.

Interactions of contaminants and nutrients on aquatic organisms and communities can be complex (Alexander et al. 2013; Lizotte et al. 2013). Contaminants can interact such that their toxicities are 1) additive: the expected toxicity of each added together, 2) synergistic: the combined toxicity of the contaminants is greater than the sum of individual toxicities, 3) antagonistic: the total toxicity of the contaminants is less than the sum of individual toxicities, or 4) potentiation: a contaminant is not toxic at exposure concentration, but becomes toxic in the presence of another contaminant (Newman 2010). Lizotte et al. (2013) found synergistic effects on the phytoplankton community in which phytoplankton abundance decreased with addition of pesticide+nutrient (atrazine, S-metolachlor, and permethrin+nitrogen and phosphorus) treatments compared to pesticide-only treatments in wetland mesocosms, but phytoplankton abundance appeared to recover more quickly after exposure to pesticides in the nutrient+pesticide treatments than in the pesticide-only treatments. It is also possible that nutrients have an antagonistic interaction with a contaminant, or the presence of nutrients ameliorates the negative effects of a contaminant (Qian et al. 2013; Alexander et al. 2013; Rocha et al. 2016). For example,

macroinvertebrates and periphyton exposed to pesticides (chlorpyrifos, dimethoate and imidacloprid) increased in abundance with added nutrients compared to no-nutrient addition treatments (Alexander et al. 2013). Thus it is important to examine the responses of aquatic communities in natural systems to contaminant mixtures to understand potential direct and indirect effects.

The purpose of this study was to investigate the effects of THPC and inorganic nitrogen (as NH_4) alone and in combination on plankton abundance and community composition in floating mesocosms in a blackwater pond in the US southeastern coastal plain. Blackwater systems are characterized by low primary production, relatively low pH (<5), and low dissolved oxygen (<5 mg L^{-1}), particularly in the summer and early fall when temperatures are high (Meyer 1992; Mallin et al. 2004). Blackwater systems are also typically oligotrophic, with average ammonium concentrations of 0.08 mg L^{-1} (in the Ogeechee River, GA, USA; Meyer 1992), but have the potential to receive additional ammonium from agricultural inputs (Meyer 1992; Mallin et al. 2001). Although THPC in textile wastewater is typically discharged into lotic systems, blackwater river systems contain slow-moving backwater areas that resemble lentic systems. I hypothesized that THPC and ammonium affect plankton communities in lentic water differently alone than in combination. I expected that added nutrients either 1) provide nutrients to stimulate growth and allow phytoplankton abundance to withstand or recover from THPC additions or 2) exacerbate adverse effects such as decreased abundance and changes in phytoplankton and zooplankton community composition when compared to ammonium-only and THPC-only treatments. The results from this study provide valuable information on the toxicity of THPC in mixture with a common agricultural nutrient on plankton communities at relevant environmental exposure concentrations.

METHODS

Preliminary Laboratory Experiments: Microalgal exposure to THPC

Two 7-day laboratory tests were conducted to determine sensitivity of the green freshwater microalga *Chlorella* sp. to THPC to aid in determining concentrations to add to field pond mesocosms. The goal was to select environmentally relevant THPC concentrations based on documented flame retardant chemical concentrations in aquatic systems (0.0063 – 87.4 µg L^{-1}) (Kawagoshi et al. 2002; Cristale et al. 2013) and local textile effluent THPC concentrations prior to discharge (790-13,500 µg L^{-1}). Concentrations tested were also anticipated to have adverse effects on a common microalgal species, and therefore possibly other members of the plankton community, either via direct toxicity or indirectly through food limitation and altered competitive interactions supported by published literature on other aquatic organisms (WHO 2000; Bartolome and Sanchez-Fortun 2005). Published 72-h EC_{50} values for the freshwater green alga *Selenastrum capricornutum* were 204 and 652 µg L^{-1} for biomass and growth inhibition, respectively (WHO 2000). Additionally, the published toxicity data on THPC only includes acute tests (≤96 h), but chronic (≥10% of the organism's life span) exposure to compounds often results in lower effect concentrations (Newman 2010; e.g. van den Brink et al. 1995). THP salts (e.g. tetratkis hydroxymethyl phosphonium sulfate) degrade only 20-60% in 30 days (WHO 2000), thus likely to incur chronic THPC exposure. Concentrations of 80 and 800 µg L^{-1} THPC were selected for preliminary experiments to test for chronic effects of THPC on *Chlorella* sp. abundance.

First *Chlorella* sp. was exposed to THPC concentrations of 0.08, 0.32, 1.28, 5.12, 20.48, 81.92 µg L^{-1}, or a no-addition control with six-fold replication. This THPC concentration range encompassed both the low LC_{50} values for brine shrimp and the high end of the concentration range for organophosphorus flame retardants observed in the environment (Kawaogshi et al.

2002; Bartolome and Sanchez-Fortun 2005). In the second experiment, THPC treatments were 80 µg L^{-1} (the highest concentration used in the first experiment), 800 µg L^{-1} to examine the effects of a concentration 10 times higher and similar to low end of concentrations found in effluent prior to discharge, or a no-addition control with four-fold replication to reduce crowding under the light bank. THPC solutions were created by mixing 80% THPC in water (Sigma-Aldrich, CAS: 124-64-1) into natural spring water. Each 300 ml volume glass experimental unit received 200 ml of solution. The experimental units were then inoculated with cultured *Chlorella* sp. cells to achieve initial cell densities of approximately 1.45 X 10^6 cells ml^{-1}, a density low enough to minimize self-shading but high enough to observe responses to treatment additions (Mayer et al. 1998). The microcosms were covered with clear, loose-fitting plastic covers to minimize evaporation while allowing for gas exchange, and randomized by location under a daylight fluorescent light bank with a 14:10 L:D cycle. Average irradiance was 80 µmol m^{-2} s^{-1}, well within the 60-120 µmol m^{-2} s^{-1} typically used in microalgal toxicity tests (Nyholm and Källqvist 1989).

After 7 days of exposure, the microcosms were vigorously mixed to resuspend the nonmotile *Chlorella* sp. cells. The entire volume of each experimental unit was filtered through a Whatman GF/F glass fiber filter (0.7 µm nominal pore size) to collect cells for chlorophyll *a* analysis. The pigments from the cells were then extracted in 90% acetone at -20°C for 24 hours followed by measurement of chlorophyll *a* fluorescence on a Trilogy fluorometer (Turner Designs, CA, U.S.A.) using EPA method 445.0 (Arar and Collins 1997).

The data were tested for normality using the Shapiro-Wilk *W* test and for homogeneity of variances with Levene's test. Data met the assumptions of parametric tests, therefore no transformations were required. Differences in chlorophyll *a* concentration among treatments

were tested using a one-way ANOVA with Tukey-Kramer HSD post-hoc tests (JMP, Pro 10.0.0 SAS Institute Inc., Cary, NC).

THPC treatment influenced chlorophyll *a* concentration (one-way ANOVA, $F_{6,35}$=18.49, $p<0.0001$) but only at the highest concentration of 81.92 µg L^{-1} (Tukey-Kramer HSD, $p<0.05$), with a 16-fold reduction in chlorophyll *a* concentration relative to the control (Figure 1). In the second experiment, only the 800 µg L^{-1} THPC treatment reduced chlorophyll *a* relative to the control (one-way ANOVA, $F_{2,9}$=22.88, p=0.0003). The control treatment had approximately 37 times more chlorophyll *a* than the 800 µg L^{-1} treatment, and while not statistically significant, 2.5 times more than the 80 µg L^{-1} treatment (Figure 2).

The observed chlorophyll *a* reduction in the preliminary laboratory experiments led me to select concentrations of 80 and 800 µg L^{-1} for the pond mesocosm experiment. Furthermore, published toxicity tests demonstrated growth inhibition of algae (and thus the potential to affect zooplankton food resources) within this concentration range (WHO 2000; Bartolome and Sanchez-Fortun 2005). The chosen concentrations were also environmentally relevant; surface water concentrations of other flame retardant chemicals were 0.0063 – 87.4 µg L^{-1} (Kawagoshi et al. 2002; Cristale et al. 2013) and THPC concentrations in the effluent from a nearby textile plant were 790 -13,500 µg L^{-1}.

Field Mesocosm Study Experimental Design

To test how THPC and nutrients alone and in combination influence zooplankton community structure, phytoplankton abundance and water quality, *in situ* pond mesocosms were dosed with THPC and nutrient treatments in a fully crossed design. The treatments were: no-addition control, low THPC (LT), or high THPC (HT), with or without ammonium (A) (n=8). THPC was added at a concentration of 80 µg L^{-1} for the low treatments and 800 µg L^{-1} for the

high treatments. THPC treatment concentrations were within the range of effluent concentrations from a textile plant known to use THPC (0.79-13.5 mg L^{-1}). These concentrations were also determined from toxicity values from published literature for 72-hr EC_{50} values for growth and biomass inhibition for the freshwater green alga *Selenastrum capricornutum* (204 and 652 µg L^{-1}, respectively) (WHO 2000). The freshwater zooplankton *Daphnia magna* was less sensitive to THPC than *Selenastrum carpicornutum*, with 48-h LC_{50} values two orders of magnitude greater (19,400 µg L^{-1}) (WHO 2000). Although the lethal concentration of THPC for *D. magna* did not fall within an environmentally relevant range, sublethal effects may occur at lower concentrations in natural environments, particularly if THPC diminishes their algal food source. Finally, findings from preliminary laboratory tests revealed these concentrations reduced chlorophyll *a* concentration of the freshwater green microalga *Chlorella* sp.

Ammonium was added as NH_4Cl at a concentration of 0.3 mg L^{-1} according to average concentrations measured in blackwater systems in the coastal plain of the North Carolina (0.003-0.110 mg L^{-1}) and Georgia, USA (0.07-0.27 mg L^{-1}) (Meyer 1992; Mallin et al. 2001; unpublished data from the Ogeechee River, GA, USA). The treatments were added only at the beginning of the experiment to simulate pulsed discharge of nutrients and chemicals that occurs with rain events and fluctuations in daily textile production rates and subsequent release from wastewater treatment (Moore and Ausley 2004; Constable et al. 2003). Ammonium concentrations were measured in each of the mesocosms immediately after addition to assess the accuracy of the nominal concentration (0.3 mg L^{-1}), and at the end of the experiment. Ammonium was measured using the protocol outlined in Holmes et al. (1999). Briefly, 80 ml samples were collected from each mesocosm, and 20 ml of "working reagent" (orthophthaldialdehyde, sodium tetraborate, and sodium sulfite) was added to each sample in the

field. The samples were then stored in the dark at ambient temperatures, transported to the laboratory, incubated for at least 2 hours followed by measurement of absorbance on a Trilogy fluorometer (Turner Designs, CA, U.S.A.). The average ammonium concentration immediately after treatment administration in ammonium addition treatments (A, LT+A, HT+A) was 0.31 (±0.002) mg L^{-1}; in all other treatments (C, LT, HT) the average ammonium concentration was 0.003 (±0.0001) mg L^{-1}.

The study was conducted in for one month in August 2015 to examine how THPC and ammonium affect plankton communities during a period of high summer temperatures and potentially low dissolved oxygen concentrations in a blackwater system. The one-month duration of the experiment was selected to ensure new generations of zooplankton could be observed; reproductive timing of zooplankton is approximately 16 days for many species of copepods and cladocerans (Allan 1976, Gillooly 2000). The mesocosms were established in a 9000 m^2 blackwater pond with an average depth of 1.2 meters located in in the floodplain of the Ogeechee River in Bulloch County, Georgia, USA (32.7011, -81.0411). Each mesocosm was constructed according to the design of Riera and Cohen (in press) (Figure 3). Briefly, a 1-meter length of 0.73 m diameter transparent polyethylene tubing (Uline, Wisconsin, USA) was heat-sealed at the bottom to create a bag, and secured to a ring of irrigation tubing at the top to hold the bag open and to create a cylinder capable of holding 400 liters. The cylinders were secured to four styrofoam floats and a ring of corrugated drainage tubing to ensure the opening of the cylinder remained above the surface of the water to contain treatments and prevent overtopping by elevated water level following rain events (Willis et al. 2004; Medina et al. 2004). The mesocosms were covered with clear lids made from 91% transparent plastic greenhouse film (AT Films, Alberta, Canada) attached to another loop of irrigation tubing angled over the tops of

the mesocosms to allow gas exchange, while keeping rain and other debris from entering and diluting the treatments (Willis et al. 2004). Additionally, a mesocosm control (MC) treatment (n=8) was established to test for mesocosm container effects. The MC units were constructed the same way as the other mesocosms, but 20, 3-cm diameter holes were cut into the sides of the bag to allow for exchange of water and organisms.

Each mesocosm was filled with 340 liters of water pumped from approximately 0.5 meters below the surface of the water with a 12 v pump (Delavan model 5850-201C, Minnesota, USA) with 7 mm mesh over the intake hose to exclude large plant material. Mesocosm volume was limited by pond depth, but the 340 L volume ensured that weekly sampling removed plankton from less than 1% of the total water volume. The volume in this study was comparable to a study on plankton trophic interactions in which 2.1% (6.4 L) of the 300 L volume mesocosm volume was removed with an integrated water sampler each week over a five-week period (Lennon et al. 2003). The mesocosms were arranged in rows of 14 and secured to transect ropes staked to either side of the pond. Mesocosms were spaced 1.5 meters from neighboring mesocosms and 20 meters from the edge of the pond to avoid shading effects from neighboring containers and trees (Willis et al. 2004) (Figure 3). After placement and filling, the plankton communities in the mesocosms were allowed to acclimate for 7 days. The mesocosms were then randomly assigned to treatments.

Field Sampling and Laboratory Processing

Water physicochemical measurements (dissolved oxygen, pH, conductivity, and temperature) were obtained between 0800 and 1000 h using a hand-held meter (YSI ProPlus, Yellow Springs, Ohio, USA) initially and weekly after treatment additions until the end of the

experiment. Dissolved oxygen and pH were measured to monitor changes associated with phytoplankton primary production, microbial activity, and chemical reactions caused by treatment addition (Smith and Piedrahita 1988; Dodson 2005), and conductivity served as a measure of ion concentrations associated with the presence of NH_4^+ and byproducts of THPC degradation. Phytoplankton samples were collected weekly from each mesocosm with an integrated water sampler to sample the entire water column and account for variation in phytoplankton with depth (Lewis and Saunders 1979). A 1.5-meter long PVC tube (diameter 5 cm) was inserted vertically into the center of the mesocosm, stoppered at the top of the tube to contain the water inside by suction, and brought to the surface. The 2 L volume of water was released into a bucket, homogenized by mixing, and a one-liter subsample withdrawn to take to the laboratory in a dark cooler on ice for chlorophyll *a* analysis. A subsample of 100 ml was filtered through Whatman GF/F glass fiber filters with 0.7µm nominal pore size to collect algal cells. The pigments from the cells were then extracted using 90% acetone at -20˚C for 24 hours followed by measurement of chlorophyll *a* fluorescence on a Trilogy fluorometer (Turner Designs, CA, U.S.A.) using EPA Method 445.0 (Arar and Collins 1997).

Zooplankton samples were collected from one vertical tow (plankton net mesh size of 80 µm and diameter of 12 cm) from bottom to top of each mesocosm (~11 L of water) because zooplankton abundance and composition varies spatially and temporally in the water column (Allan 1976, Goswami 2004). The zooplankton samples were preserved in 4% buffered formalin immediately after collection and transported to the lab for identification and enumeration (Goswami 2004). At least 24 hours prior to identification, the samples were stained with Rose Bengal solution, divided into quarter subsamples, and identified in a Borgorov chamber at 60X magnification using a Leica M80 dissecting scope (Leica Microsystems, Wetzlar, Germany)

(Thiel and Sauer 1999; Postel et al. 2000). The magnification and subsampling techniques used ensured that the zooplankton counts fell within the range of 80-120 individuals required to adequately distinguish differences in zooplankton abundance between samples according to published methodology (Postel et al. 2000). Copepods were identified to order, and cladocerans and rotifers were identified to family using a key to North American zooplankton (Haney et al. 2013). Nielsen et al. (1998) found this level of identification was sufficient to detect differences in zooplankton community structure using multivariate analyses.

Statistical Analyses

Data were tested for normality using the Shapiro Wilk *W* test and for homogeneity of variances with Levene's test. Only conductivity and DO required log transformations to meet the assumptions of parametric tests. Water column temperature, pH, and chlorophyll *a* and log-transformed conductivity and DO were analyzed using two-way ANOVA with a repeated measures response design. The unadjusted univariate F tests were used for chlorophyll *a* because the Chi-square value for the sphericity test was not significant (>0.05). The adjusted univariate Geisser-Greenhouse epsilon F tests, in which the degrees of freedom were adjusted by an epsilon factor, were used for temperature, pH, conductivity, and DO because the Chi-square value was significant (<0.05).

The water column physiochemical parameters and chlorophyll *a* were also compared between the MCs and no-addition control using one-way ANOVA in order to assess whether the mesocosm structure affected these variables. No differences in temperature were observed due to the mesocosm structure, but slight differences in pH (~0.1 lower in MC) and conductivity (~1.2 $\mu S\ cm^{-1}$ higher in MC) occurred, likely due to the ion exchange with the surrounding pond water (Table 1). pH was never below values shown to have sublethal reproductive effects on *Daphnia*

pulex (<5.0) in either the MC or no-addition control. Dissolved oxygen concentrations were an average of 1.72 mg L^{-1} lower in the MC units that allowed exchange with the pond than in the no-addition control treatment, possibly because decomposing macrophytes were able to enter through holes from the adjacent pond. Chlorophyll *a* concentrations were ~20 µg L^{-1} higher in MC units over the course of the experiment, likely because approaching the mesocosms for sampling caused significant disturbance of the algal growth on the surrounding macrophytes and disruption of macrophyte fragments, that subsequently flowed through the holes in the mesocosms. The differences in water quality parameters between MC and no-addition control remained consistent and were likely not biologically significant, and differences in chlorophyll *a* concentrations were explainable; therefore the MC treatment was excluded from further statistical analysis. All analyses on water physicochemical parameters were conducted using JMP, Pro 10.0.0 (SAS Institute Inc., Cary, NC).

Zooplankton community assemblage data were square-root transformed to avoid over-representation by the most dominant species (Clarke and Warwick 2001) and a "dummy species" pretreatment was added to all samples to avoid undefined Bray-Curtis coefficients resulting from samples that had zero individuals in some taxonomic groups (Clarke et al. 2006). Following pretreatment, the data were used to make a Bray-Curtis resemblance matrix for Permutational MANOVA (PERMANOVA) (PRIMER-E Ltd, Plymouth, UK) analysis (Clarke and Gorley 2006). PERMANOVA is a non-parametric multivariate test that analyzes time and treatment levels as factors in a repeated measures design. Initial zooplankton data collected were analyzed with ANOSIM, and there were no differences across ammonium (ANOSIM, Global R=-0.034, Significance level=0.75) and THPC (Global R=0.026, Significance level=0.25) treatments at the start of the experiment. The zooplankton community composition in the mesocosm control

treatments was also similar to the no-addition control at the start of the experiment (PERMANOVA; pseudo-$F_{1,15}$=1.1634; p=0.3304), thus community data from the mesocosms with flow were excluded from subsequent statistical analyses.

RESULTS

Plankton Community

Zooplankton community composition changed over the course of the experiment (PERMANOVA; pseudo-$F_{3,126}$=38.601; p=0.0001). Ammonium or THPC alone did not alter plankton communities, but an interaction between ammonium and THPC occurred (Table 2). The combination of THPC and ammonium appeared to influence the proportion of copepods, rotifers and sidids between one and three weeks post-exposure to treatments (Figure 4). For example, the LT+A communities contained 80% more brachionidae (rotifers) than LT-only, and 60% more than A-only communities after one week, with a concomitant decrease in calanoid copepods (Figure 5). Rotifer abundance in the LT+A treatment continued to average ~85% higher than LT and ~81% higher than A-only at three weeks post exposure. In both the LT+A and HT treatments, ~10% of the community consisted of rotifer two and three weeks post-exposure, compared to 5% or less in the communities in all of other treatments. However, HT averaged approximately twice as many sidids and half as many calanoid copepod nauplii as LT+A in week 3. Comparison of HT-only and HT+A revealed 48% fewer rotifer and 19% more calanoid copepods in HT+A.

Total abundance (average number of individuals L^{-1}) across all treatments decreased over the first week of the experiment by an average of 58%, followed by an average increase by ~27% over initial abundance at the end of the experiment (Figure 6; Table 2). The most abundant

taxonomic group was calanoid copepods, comprising approximately 50% of the community across all treatments with an average abundance of 4.37 ± 0.12 individuals L^{-1} (Figure 5, 6). Sidids and calanoid copepod nauplii increased by approximately 80% and 78%, respectively, between two and four weeks post-exposure to treatments. Rotifer, bosminid, and daphnid proportions generally decreased by approximately 3.5% from an average of ~0.38 individuals L^{-1} at initial sampling to approximately ~0.14 individuals L^{-1} across treatments except HT by the end of the experiment (Figure 5).

Phytoplankton chlorophyll a

An interaction between time and THPC treatment on chlorophyll *a* concentration occurred (Table 3). Concentrations never dropped below ~5 µg L^{-1} in any treatment, but patterns were difficult to discern due to high variability in chlorophyll *a* concentration throughout the experiment (5-15 µg L^{-1}) (Figure 7). The chlorophyll *a* concentration in the control and HT decreased from approximately 10 to 5 µg L^{-1} from initial to week 1, but the chlorophyll in HT was more variable and increased again to ~10 µg L^{-1} while concentrations stayed low (~5 µg L^{-1}) in the control (Figure 7).

Water Quality

Water column conductivity was affected by both ammonium and THPC treatment additions (Table 3). THPC and ammonium appeared to have additive effects given that the conductivity difference between HT+A and the control was ~2 µS cm^{-1} and ~1 µS cm^{-1} between A-only and HT-only and the control (Figure 8). Furthermore, the conductivity in LT and the control each averaged 22.3 (±0.07) µS cm^{-1}, and LT+A and A were comparable (23.1 ± 0.1 and

23.0 ± 0.1 µS cm^{-1}, respectively). Conductivity in all treatments that received ammonium appeared consistently elevated over the control for the duration of the experiment (Figure 8).

The pH increased over the course of the experiment across all treatments (repeated measures ANOVA, Table 3) from an average of 5.2 ± 0.01 to 5.4 ± 0.02 (Table 4). Ammonium and THPC additions also influenced water column pH (Table 3). The treatments with ammonium addition (A, HT+A, and LT+A) generally had pH values 0.1-0.2 units lower than the control at the end of the experiment, with the pH in HT+A < LT+A < A < C (Table 4).

Dissolved oxygen increased by an average of 1.3 ± 0.05 mg L^{-1}, reaching 5.09 ± 0.03 mg L^{-1} by the end of the experiment across all treatments (Table 3; Figure 9) with a concurrent decrease in temperature of approximately 3˚C (Figure 9). THPC and ammonium treatment additions did not influence dissolved oxygen or temperature (Table 3).

DISCUSSION

My hypothesis that THPC and ammonium affect plankton communities differently alone than in combination was supported. Toxicity testing on communities provides a more realistic assessment of the effects of contaminants than single-species tests because they provide insight into not only abundance changes, but also indirect effects of species interactions resulting in changes in community composition (Cairns et al. 1996; Preston 2002). The combination of THPC and ammonium affected zooplankton community composition, primarily by decreasing the proportion of copepods and increasing the proportion of rotifers particularly in LT+A and HT compared to LT- and A-only treatments. The observed decreases in copepod abundance may have been a result of direct toxicity to treatment addition, as copepods are often more sensitive to organophosphorus and organochlorine chemicals than cladocerans and rotifers (Medina et al. 2004; Willis et al. 2004; Sanchez-Bayo 2006). The increase in rotifer abundance may also in part

be explained by the decreased copepod abundance in LT+A and HT. Copepods are selective raptorial feeders that prey upon smaller zooplankton (Williamson and Butler 1986; Šorf and Brandl 2012), and increases in rotifers and small crustacean zooplankton have been associated with copepod reduction due to fish predation and pollutants in other studies (Vanni 1987; Hanazato and Yasuno 1990; Paul and Schindler 1994; Thorp and Casper 2003; Devetter and Seda 2006).

I predicted that THPC addition alone would affect phytoplankton communities at the environmentally relevant concentrations used in this study. I found that THPC concentrations of 80 and 800 µg L^{-1} THPC decreased chlorophyll *a* concentrations in the microalga *Chlorella* sp. after 7 days. However, I did not observe a significant decrease in phytoplankton chlorophyll *a* in the mesocosm study. It is possible that green algae such as *Chlorella* sp. were not abundant in the mesocosm experiment while diatoms and cyanobacteria are more likely to dominate the assemblage in southeastern US blackwater systems (Mallin 1984; Mallin et al. 2001; Quinlan and Phlips 2007). Furthermore, because phytoplankton taxa exhibit differential sensitivities to organophosphorus chemicals (Sabater and Carrasco 2001), the THPC concentrations chosen for this experiment may not have been high enough to reduce overall chlorophyll *a* concentration. Finally, in some cases chlorophyll *a* content within algal cells can increase under stressful conditions such as light limitation (Phlips et al. 2000) and toxic stress from metal exposure (Franqueira et al. 2000; Hadjoudja et al. 2009); thus THPC effects on the phytoplankton community were not detectable. Therefore, it may be important to examine changes in phytoplankton physiology and species composition to assess algal responses to THPC addition.

The ammonium concentration used in this study was expected to increase algal abundance and food availability to zooplankton at the concentrations greater than the 0.196 mg

L^{-1} N that stimulated algal production in blackwater streams in North Carolina. Although the absence of an increase in chlorophyll *a* was unexpected, it could have been due to the form of inorganic nitrogen used. Despite evidence that ammonium is the preferred form of inorganic nitrogen for primary producers, more recent studies suggest some phytoplankton, such as diatoms, preferentially utilize nitrate (e.g. Glibert et al. 2015). It is also possible that 0.3 mg L^{-1} ammonium may not have been high enough to stimulate phytoplankton production, for example Maberly et al. (2002) used 2.28 mg L^{-1} NH_4^+ to consistently stimulate phytoplankton production in laboratory bioassays. Alternatively, the ammonium could have stimulated the heterotrophic or periphyton communities instead of phytoplankton production (Mallin et al. 2001; Vadeboncoeur and Steinman 2002). For example, in a study of the effects of nutrient additions on autotrophic and heterotrophic production in blackwater systems, use of added nutrients by the heterotrophic community was the proposed reason for overall increased ATP production without a corresponding increase in chlorophyll *a* (Mallin et al. 2001). It is also possible that the ammonium treatment added was utilized by periphyton since periphyton often outcompete phytoplankton for nutrients in oligotrophic systems (Hansson 1990; Vadeboncoeur and Steinman 2002). I did not analyze periphyton abundance, but observed what appeared to be greater (i.e. denser) accumulation of periphyton on the mesocosms with ammonium addition at the conclusion of the experiment.

Though the phytoplankton community was not stimulated by ammonium addition, zooplankton were likely not food limited due to THPC addition given that the average chlorophyll *a* never decreased below the 5 µg L^{-1} concentration observed to be limiting for zooplankton growth and reproduction (Escribano et al. 1997). However, food limitation cannot be completely ruled out because total chlorophyll *a* typically does not indicate species loss or

substitution in the phytoplankton community (Hurlbert et al. 1972; Kerfoot et al. 1988; Phlips et al. 2000). For example, Hurlbert et al. (1972) observed differential sensitivities between phytoplankton species to an organophosphorus insecticide, including instances of decline in some species resulting in increased abundance of others, particularly *Schroederia* sp. and *Euglena* sp., not reflected in total phytoplankton cell counts. In the present study, THPC and ammonia (including ammonium addition and degradation from THPC) may have removed larger and more edible phytoplankton species either through toxicity or promoting growth of smaller species. Toxicity tests with THPC demonstrated a reduction of biomass of the freshwater green alga *Selenastrum capricornutum* at 0.204 mg L^{-1} (WHO 2000) and increased ammonia availability tends to favor cyanobacteria and smaller phytoplankton species (Gilbert et al. 2015); thus, it is reasonable to expect that treatment additions may have caused changes in phytoplankton community composition rather than total abundance. Additionally, periphyton can be an important supplementary food source for zooplankton, particularly cladocerans (Masclaux et al. 2012). Cladocerans are primarily filter feeders that feed on suspended phytoplankton, but increased biomass, reproduction, and competitive advantages were observed when the phytoplankton food source was supplemented with periphyton (Siehoff et al. 2009; Masclaux et al. 2012). Future studies should consider the effect of THPC on periphyton and phytoplankton and community composition.

The observed changes in abundance of all taxa over the course of the experiment regardless of treatment were consistent with zooplankton life history characteristics and successional changes as a result of fish exclusion from the mesocosms (Brooks and Dodson 1965; Vanni 1987). Average generation time among cladocerans is approximately two weeks, and 18 days for copepods at 25˚C (Allan 1976), and I observed increases in abundance of sidids

within a three-week time frame. Furthermore, the relatively large increases in total zooplankton abundance in weeks 3 and 4 corresponded with the timing of summer peaks in zooplankton abundance recorded during a three-year study in a blackwater impoundment in South Carolina (Mallin 1984). Calanoid copepods often dominate the plankton in oligotrophic lakes due to more efficient food capture and complex escape behaviors from predation compared to cladocerans (Allan 1976; Byron et al. 1984; Dodson 2005), thus their abundance (making up at least 50% of the samples) in the initial zooplankton community composition was not surprising. However, fish exclusion from the mesocosms can partially explain why large cladocerans abundance increased nearly five-fold as the experiment progressed. Planktivorous fish preferentially feed on large zooplankton, thus zooplankton communities influenced by fish predation generally tend to be dominated by smaller zooplankton taxa or select for a smaller mature body size (Brooks and Dodson 1965; Vanni 1987).

The similarity in community composition across treatments at the end of the experiment may have been due to recovery following degradation of the single dose of THPC and utilization of ammonium. The World Health Organization (2000) reported degradation rates of 20-60% over 30 days for tetrakis hydroxymethyl phosphonium salts (e.g. THP sulfate), thus although I was unable to measure THPC concentrations it is possible that significant degradation of THPC occurred after four weeks. Ammonium concentrations were below detection in all treatments at the end of the experiment, therefore plankton communities may have also recovered from any changes induced by THPC + ammonium additions. Although copepod abundances decreased following some treatment additions, they but were not completely eliminated allowing for recovery following degradation. Cladoceran abundance recovered following degradation of the insecticide carbaryl (similar mode of toxicity to organophosphorus chemicals) within 40 and 60

days in the low and high dose treatments, respectively, while no recovery occurred within the 70 day duration after dosing with the slower-degrading organophophate insecticide Fenthion (Hanazato and Yasuno 1990; Hanazato and Kasai 1995).

Treatment additions were expected to affect water chemistry parameters (pH, DO, temperature, and conductivity), but not to harm aquatic organisms. Treatment additions did not affect dissolved oxygen over the course of the experiment, and the increase in dissolved oxygen concentration by the final week across all treatments was likely attributed to the 3˚C decrease in water temperature. Ammonium addition decreased pH likely due to the addition of H^+ ions with NH_4^+ (US EPA 1999), but the difference from the control was small (~0.1) and pH values were not low enough to harm large zooplankton such as daphnids (Walton et al. 1982). Conductivity increased similarly with ammonium and THPC addition, suggesting that the ion concentrations associated with each were similar. The electrical conductivity of water is often higher when influenced by wastewater effluent and agricultural runoff, and ammonium concentrations and conductivity are positively correlated (Stevens et al. 2005; Morrison et al. 2001; Moral et al. 2005). Thus, the increase in conductivity in the current study was expected. However, none of the parameters measured in this experiment suggest that water quality changes indirectly affected zooplankton communities.

In conclusion, I found that the combination of THPC and ammonium altered zooplankton communities in a way that was not predicted from the effects of each factor alone. The reduction in number of calanoid copepods and coincident increase in rotifers in the presence of LT+A one to three weeks post-exposure has important implications for energy transfer to other trophic levels because large zooplankton can be an important food source for planktivorous fish (Brooks and Dodson 1965; Vanni 1987). My results also suggest that repeated doses of THPC and

ammonium associated with continuous discharge from textile plants might depress copepod abundances indefinitely, resulting in altered aquatic species composition. This study clearly underscores the importance of using field-based community testing to provide information about potential risk of contaminant mixtures to aquatic communities.

REFERENCES

Allan JD (1976) Life history patterns in zooplankton *Amer Nat* 110: 971 165-180

Alexander AC, Luis AT, Culp JM, Baird DJ, Cessna AJ (2013) Can nutrients mask community responses to insecticide mixtures? *Ecotoxicology* 22: 1085-1100

Alonso Á, Camargo JA (2011) The freshwater planarian *Polycelis feline* as a sensitive species to assess the long-term toxicity of ammonia *Chemosphere* 84: 533-537

Arar EJ, Collins GB (1997) *In vitro* determination of chlorophyll *a* and pheophytin *a* in marine and freshwater algae by fluorescence. U.S. EPA. Cincinnati, Ohio

de Araujo AB, Snell TW, Hagiwara A (2000) Effect of unionized ammonia, viscosity and protozoan contamination on the enzyme activity of the rotifer *Brachionus plicatilis* *Aquacult Res* 31: 359-365

Arauzo M, Valladolid M (2003) Short-term harmful effects of unionized ammonia on natural populations of *Moina micrura* and *Brachionus rubens* in a deep waste treatment pond *Water Res* 37: 2547-2554

Baron JS, Poff NL, Angermeier PL, Dahm CN, Gleick PH, Hairston Jr. NG, Jackson RB, Johnston CA, Richter BD, Steinman AD (2002) Meeting ecological and societal needs for freshwater *Ecol Appl* 12:5 1247-1260

Bartolome MC, Sanchez-Fortun S (2005) Effects of selected biocides used in the disinfection of cooling towers on toxicity and bioaccumulation in *Artemia* larvae *Environ Toxicol Chem* 24:12 3137-3142

Berg GM, Balode M, Purina I, Bekere S, Bechemin C, Maestrini SY (2003) Plankton community composition in relation to availability and uptake of oxidized and reduced nitrogen *Aquat Microb Ecol* 30: 263-274

Brooks JL, Dodson SI (1965) Predation, body size, and composition of plankton *Science* 150:3692 28-35

Brungs WA (1974) Effects of residual chlorine on aquatic life *Water Pollut Control* 45:10 2180-2193

Buttino I (1994) The effect of low concentrations of phenol and ammonia on egg production rates, fecal pellet production and egg viability of the calanoid copepod *Acartia clause Mar Biol* 119: 629-634

Buxton HT, Kolpin DW (2002) Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams *USGS Fact Sheet*

Byron ER, Folt CL, Golman CR (1984) Copepod and cladoceran success in an oligotrophic lake *J Plankton Research* 6:1 45-65

Cairns J Jr, Bidwell JR, Arnegard ME (1996) Toxicity testing with communities: microcosms, mesocosms, and whole-system manipulations *Rev Environ Contam T* 147: 45-69

Camargo JA, Alonso A (2006) Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: A global assessment *Environ Int* 32: 831-849

Carpenter SR, Kitchell JF, Hodgson JR (1985) Cascading trophic interaction and lake productivity *BioScience* 35:10 634-639

Carpenter SR, Caraco NF, Correll DL, Howarth RW, Sharpley AN, Smith VH (1998) Nonpoint pollution of surface waters with phosphorus and nitrogen *Ecol Appl* 8:3 559-568

Clarke K, Gorley R (2006) User manual/tutorial PRIMER-E Ltd, Plymouth: 93

Clarke KR, Somerfield PJ, Chapman MG (2006) On resemblance measures for ecological studies, including taxonomic dissimilarities and a zero-adjusted Bray-Curtis coefficient for denuded assemblages *J Exp Mar Biol Ecol* 330: 55-80

Clarke KR, Warwick RM (2001) Change in marine communities: an approach to statistical analysis and interpretation. PRIMER-E Ltd 2nd ed. Plymouth Marine Laboratory, UK

Constable M, Charlton M, Jensen F, McDonald K, Craig G, Taylor KW (2003) An ecological risk assessment of ammonia in the aquatic environment *Hum Ecol Risk Assess* 9:2 527-548

Covaci A, Harrad S, Abdallah MA-E, Ali N, Law RJ, Herzke D, de Wit CA (2011) Novel brominated flame retardants: a review of their analysis, environmental fate and behavior *Environ Int* 37: 532-556

Covich AP, Austen MC, Bärlocher F, Chauvet E, Cardinale BJ, Biles CL, Inchausti P, Dangles O, Solan M, Gessner MO, Statzner B, Moss B (2004) The role of biodiversity in the functioning of freshwater and marine benthic ecosystems. *BioScience.* 54:8 767-775

Cristale J, Katsoyiannis A, Sweetman A, Jones KC, Lacorte S (2013) Occurrence and risk assessment of organophosphorus and brominated flame retardants in the River Aire (UK) *Environ Pollut* 179: 194-200

Derden A, Huybrechts D (2013) Brominated flame retardants in textile wastewater: reducing Deca-BDE using best available techniques *J Clean Prod* 53: 167-175

Devetter M, Seda J (2006) Regulation of rotifer community by predation of *Cyclops vicinus* (Copepoda) in the Římov Reservoir in spring *Internat Rev Hydrobiol* 91:1 101-112

Di Lorenzo T, Di Marzio WD, Sáenz ME, Baratti M, Dedonno AA, Iannucci A, Cannicci S, Messana G, Galassi DMP (2014) Sensitivity of hypogean and epigean freshwater copepods to agricultural pollutants *Environ Sci Pollut Res* 21:4643-4655

Dodson S (2005) Introduction to limnology 1st ed McGraw-Hill, New York, NY

Escribano R, Irribarren C, Rodriguez L (1997) Influence of food quantity and temperature on development and growth of the marine copepod *Calanus chilensis* from northern Chile *Marine Biology* 128: 281-288

Fleeger JW, Carman KR, Nisbet, RM (2003) Indirect effects of contaminants in aquatic ecosystems *Sci Total Environ* 317: 207-233

Franqueira D, Orosa M, Torres E, Herrero C, Cid A (2000) Potential use of flow cytometry in toxicity studies with microalgae *Sci Total Environ* 247: 119-126

Fuhui X, Wei Y, Yafen C, Zhou Y (2010) Acute toxicity of nitrite and ammonia to *Daphnia similoides* of different developmental stages: using the modified Gaussian Model to describe *Bull Environ Contam Toxicol* 84: 708-711

Fulton MH, Key PB (2001) Acetylcholinesterase inhibition in estuarine fish and invertebrates as an indicator of organophosphorus insecticide exposure and effects *Environ Toxicol Chem* 20:1 37-45

Gavrilescu M. Demnerova K, Aamand J, Agathos S, Fava F (2015) Emerging pollutants in the environment: present and future challenges in biomonitoring, ecological risks and bioremediation. *New Biotechnology.* 32:1 147-156

Gillooly JF (2000) Effect of body size and temperature on generation time in zooplankton. *J Plankton Research.* 22:2 241-251

Glibert PM, Wilkerson FP, Dugdale RC, Raven JA, Dupont CL, Leavitt PR, Parker AE, Burkholder JM, Kana TM (2015) Pluses and minuses of ammonium and nitrate uptake and assimilation by phytoplankton and implications for productivity and community composition, with emphasis on nitrogen-enriched conditions *Limnol Oceanogr* 61: 165-197

Goswami SC (2004) Zooplankton methodology, collection, and identification – a field manual.

Hadjoudja S, Vignoles C, Deluchat V, Lenain J-F, Le Jeune A-H, Baudu M (2009) Short term copper toxicity on *Microcystis aeruginosa* and *Chlorella vulgaris* using flow cytometry *Aquat Toxicol* 94: 255-264

Hanazato T, Kasai F (1995) Effects of the organophosphorus insecticide Fenthion on phyto- and zooplankton communities in experimental ponds *Environ Poll* 88: 293-298

Hanazato T, Yasuno M (1990) Influence of persistence period of an insecticide on recovery patterns of a zooplankton community in experimental ponds *Environ Poll* 67: 109-122

Haney JF et al. (2013) An-image-based key to the zooplankton of North America. Version 5.0. University of New Hampshire Center for Freshwater Biology. <cfb.unh.edu>

Hansson L-A (1990) Quantifying the impact of periphytic algae on nutrient availability for phytoplankton *Freshwater Biol* 24: 265-273

Holmes RM, Aminot A, Kérouel R, Hooker BA, Peterson BJ (1999) A simple and precise method for measuring ammonium in marine freshwater ecosystems *Can J Fish Aquat Sci* 56:10 1801-1808

Howard PH, Muir DCG (2010) Identifying new persistent and bioaccumulative organics among chemicals in commerce *Environ Sci Technol* 44: 2277-2285

Howarth RW, Billen G, Swaney D, Townsend A, Jaworski N, Lajtha K, Downing JA, Elmgren, R, Caraco N, Jordan T, Berendse F, Freney J, Kudeyarov V, Murdoch P, Zhao-Liang Z 1996. Regional nitrogen budgets and riverine N & P fluxes for the drainages to the North Atlantic Ocean: natural and human influences. *Biogeochemistry.* 35:1 75-139

Howarth RW, Sharpley A, Walker D (2002) Sources of nutrient pollution to coastal waters in the United States: implications for achieving coastal water quality goals *Estuaries* 25:4b 656-676

Hurlbert SH, Mulla MS, Willson HR (1972) Effects of an organophosphorus insecticide on the phytoplankton, zooplankton, and insect populations of fresh-water ponds *Ecol Monogr* 42:3 269-299

Kawagoshi Y, Nakamura S, Fukunaga I (2002) Degradation of organophosphoric esters in leachate from sea-based solid waste disposal site *Chemosphere* 48(2): 219-225

Kerfoot WC, Levitan C, DeMott WR (1988) Daphnia-phytoplankton interactions: density-dependent shifts in resource quality *Ecology* 69:6 1806-1825

Kharlyngdoh JB, Pradhan A, Asnake S, Walstad A, Ivarsson P, Olsson P-E (2015) Identification of a group of brominated flame retardants as novel androgen receptor antagonists and potential neuronal and endocrine disrupters *Environ Int* 74: 60-70

Lizotte Jr. RE, Testa III S, Locke MA, Steinriede Jr. RW (2013) Responses of phytoplankton and *Hyalella Azteca* to agrichemical mixtures in a constructed wetland mesocosm *Arch Environ Con Tox* 65: 474-485

Lennon JT, Smith VH, Dzialowski AR (2003) Invasibility of plankton food webs along a trophic state gradient *Oikos* 103:191-203

Lewis WM, Saunders JF (1979) Two new integrating samplers for zooplankton, phytoplankton,

and water chemistry *Archiv Fur Hydrobiologie* 85:244-249

Ma J (2005) Differential sensitivity of three cyanobacterial and five green algal species to organotins and pyrethroids pesticides *Sci Total Environ* 341:109-117

Ma J, Zheng R, Xu L, Wang S (2002) Differential sensitivity of two green algae, *Scenedesmus obliqnus* and *Chlorella pyrenoidosa,* to 12 pesticides *Ecotox Environ Safe* 52:57-61

Maberly SC, King L, Dent MM, Jones RI, Gibson CE (2002) Nutrient limitation of phytoplankton and periphyton growth in upland lakes *Freshwater Biol* 47: 2136-2152

Mallin MA (1984) The plankton community of an acid blackwater South Carolina power plant impoundment *Hydrobiologia* 112: 167-177

Mallin MA, Cahoon LB, Parsons DC, Ensign SH (2001) Effect of nitrogen and phosphorus loading in Coastal Plain blackwater rivers. *J Freshwater Ecol* 16:3 455-464

Mallin MA, McIver MR, Ensign SH, Cahoon LB (2004) Photosynthetic and heterotrophic impacts of nutrient loading to blackwater streams *Ecolog Appl* 14:3 823-838

Marklund A, Andersson B, Haglund P (2005) Organophosphorus flame retardants and plasticizers in Swedish sewage treatment plants *Environ Sci Technol* 39: 7423-7429

Maruya KA, Schlenk D, Anderson PD, Denslow ND, Drewes JE, Olivieri AW, Scott GI, Snyder SA (2013) An adaptive, comprehensive monitoring strategy for chemicals of emerging concern (CECs) in California's aquatic ecosystems *Integrated Environmental Assessment and Management* 10:1 69-77

Masclaux H, Bec A, Bourdier G (2012) Trophic partitioning among three littoral microcrusaceans: relative importance of periphyton as a food source *J Limnol* 71:2 261-266

Mayer P, Frickmann, J, Christensen ER, Nyholm N (1998) Influence of growth conditions on the results obtained in algal toxicity tests. *Environ Toxicol Chem* 17:6 1091-1098

Medina M, Barata C, Telfer T, Baird DJ (2004) Effects of cypermethrin on marine plankton communities: a simulated field study using mesocosms. *Ecotox Environ Safe* 58: 236-245

Meyer JL (1992) Seasonal patterns of water quality in blackwater rivers of the coastal plain, southeastern United States. Pages 250-275 in C.D. Becker and D. A. Neitzel, editors. *Water Quality in North American River Systems.* Batelle, Columbus, Ohio, USA.

Moore SB, Ausley LW (2004) Systems thinking and green chemistry in the textile industry: concepts, technologies, and benefits *Journal of Cleaner Production* 12: 585-601

Moral R, Perez-Murcia MD, Perez-Espinosa A, Moreno-Caselles J, Paredes C (2005) Estimation of nutrient values of pig slurries in Southeast Spain using easily determined properties *Waste Manage* 25: 719-725

Morrison G, Fatoki OS, Persson L, Ekberg A (2001) Assessment of the impact of point source pollution from the Keiskammakoek Sewage Treatment Plant on the Keiskamma River – pH, electrical conductivity, oxygen-demanding substance (COD) and nutrients *Water SA* 27:4 475-480

[NRC] National Research Council (2000) Tetrakis (hydroxymethyl)phosphonium salts. In Toxicological Risks of Selected Flame-Retardant Chemicals. National Academic Press, Washington, DC, pp 417–439.

[NTP] National Toxicology Program (2016) U.S. Department of Health and Human Services <http://ntp.niehs.nih.gov/about/index.html>

Newman, MC (2010) Fundamentals of Ecotoxicology. 3rd ed. Boca Raton (FL): CRC Press, Taylor and Francis Group.

Nielsen DL, Shiel RJ, Smith FJ (1998) Ecology versus taxonomy: is there a middle ground? *Hydrobiologia.* 387/388: 451-457

Nyholm N, Källqvist T (1989) Methods for growth inhibition toxicity tests with freshwater algae. *Environ Toxicol Chem* 8: 689-703

Paerl HW (1988) Nuisance phytoplankton blooms in coastal, estuarine, and inland waters *Limnol Oceanogr* 33:4 823-847

Paul AJ, Schindler DW (1994) Regulation of rotifers by predatory calanoid copepods (subgenus *Hesperodiaptomus*) in lakes of the Canadian Rocky Mountains. *Can J Fish Aquat Sci* 51: 2520-2528

Persson J, Vrede T, Holmgren S (2008) Responses in zooplankton populations to food quality and quantity changes after whole lake nutrient enrichment of an oligotrophic sub-alpine reservoir *Aquat Sci* 70: 142-155

Phlips EJ, Cichra M, Aldridge FJ, Jembeck J, Hendrickson J, Brody R (2000) Light availability and variations in phytoplankton standing crops in a nutrient-rich blackwater river *Limnol Oceanogr* 45:4 916-929

Pieterse NM, Bleuten W, Jørgensen SE (2003) Contribution of point sources and diffuse sources to nitrogen and phosphorus loads in lowland river tributaries *J Hydrol* 271: 213-225

Porter KG (1973) The plant-animal interface in freshwater ecosystems: microscopic grazers feed differentially on planktonic algae and can influence their community structure and succession in ways that are analogous to the effects of herbivores on terrestrial plant communities *Amer Sci* 65:2 159-170

Porter KG (1977) Selective grazing and differential digestion of algae by zooplankton *Nature* 244: 179-180

Postel L, Fock H, Hagen W (2000) Biomass and abundance. In Huntley RHWLRS (ed) ICES Zooplankton Methodology Manual. Academic Press, London, pp 83-192

Preston BL (2002) Indirect effects in aquatic ecotoxicology: implications for ecological risk assessment *Environmental Management* 29:3 311-323

Qian H, Sun Z, Sun L, Jiang Y, Wei Y, Xie J, Zhengwei F (2013) Phosphorus availability changes chromium toxicity in the freshwater alga *Chlorella vulgaris Chemosphere* 93: 885-891

Quinlan EL, Phlips EJ (2007) Phytoplankton assemblages across the marine to low-salinity transition zone in a blackwater dominated estuary *J Plankton Res* 29:5 401-416

Relyea R, Hoverman J (2006) Assessing the ecology in ecotoxicology: a review and synthesis in freshwater systems *Ecology Letters* 9:1157-1171

Reemtsma T, Quintana JB, Rodil R, García-López M, Rodríguez I (2008) Organophosphorus flame retardants and plasticizers in water and air I. occurrence and fate *Trends Anal Chem* 27:9 727-737

Reeves WA, Guthrie JD (1956) Intermediate for flame-resistant polymers: reactions of tetrakis(hydroxymethyl) phosphonium chloride *Product and Process Development* 48:1 64-67

Riera S (2015) Alkyl polygucoside compound influences freshwater plankton community structure in floating mesocosms. M.S. Thesis. Georgia Southern University

Riera S, Cohen RA (in press) Alkyl polyglucoside compound influences freshwater plankton community structure in floating field mesocosms *Ecotoxicology*

Rocha GS, Lombardi AT, da Graça G Melão M (2016) Influence of phosphorus on copper toxicity to *Selenastrum gracile* (Reinsch) Korshikov *Ecotox Environ Safe* 128: 30-35

Sabater C, Carrasco JM (2001) Effects of the organophosphorus insecticide fenitrothion on growth in five freshwater species of phytoplankton *Environ Toxicol* 16: 314-320

Sakamoto M, Chang KH, Hanazato T (2005) Differential sensitivity of a predacious cladoceran (*Leptodora*) and its prey (the cladoceran *Bosmina*) to the insecticide carbaryl: results of acute toxicity tests. *Bull Environ Contam Toxicol* 75:28-33

Sanchez-Bayo F (2006) Comparative acute toxicity of organic pollutants and reference values for crustaceans. I. Branchiopoda, Copepoda, and Ostracoda *Environ Pollut* 139: 385-420

Sanchez-Fortun S, Llorente MT, Castano A (2005) Genotoxic effect of selected biocides on RTG-2 fish cells by means of a modified Fast Micromethod Assay *Aquat Toxicol* 73: 55-64

Schreder ED, La Guardia MJ (2014) Flame retardant transfers from U.S. households (dust and laundry wastewater) to the aquatic environment *Environ Sci Technol* 48: 11575-11583

Shi Y, Gao L, Li W, Wang Y, Liu L, Cai Y (2016) Occurrence, distribution and seasonal variation of organophosphate flame retardants and plasticizers in urban surface water in Beijing, China *Environ Pollut* 209: 1-10

Siehoff S, Hammers-Wirtz M, Strauss T, Ratte HT (2009) Periphyton as alternative food source for the filter-feeding cladoceran *Daphnia magna Freshwater Biol* 54: 15-23

Smith VH (1998) Cultural eutrophication of inland, estuarine, and coastal waters in Successes, limitations, and frontiers in ecosystem science. Springer, New York (NY).

Smith DW, Piedrahita RH (1988) The relation between phytoplankton and dissolved oxygen in fish ponds *Aquaculture* 68: 249-265.

Special Materials Company (2010) MSDS. http://www.flame-retardant.net/thpc_tetrakis_hydroxymethyl_phosphonium_chloride_msds.html

Šorf M, Brandl Z (2012) The rotifer contribution to the diet of *Eudiaptomomus gracilis* (G. O. Sars, 1863) (Copepoda, Calanoida). *Crustaceana.* 85:12-13 1421-1429

Stevens RJ, O'bric CJ, Carton OT (1995) Estimating nutrient content of animal slurries using electrical conductivity *J Agr Sci* 125:2 233-238

Strempel S, Scheringer M, Ng CA, Hungerbühler K (2012) Screening for PBT chemicals among the "existing" and "new" chemicals of the EU *Environ Sci Technol* 46: 5680-5687

Stuart M, Lapworth D, Crane E, Hart A (2012) Review of risk from potential emerging contaminants in UK groundwater *Sci Total Environ* 416: 1-21

Thiel P, Sauer J (1995) Long term resource monitoring program procedures: Macroinvertebrate monitoring. National Biological Service Environmental Management Technical Center, Onalaska, Wisconsin

Thorp JH, Casper AF (2003) Importance of biotic interactions in large rivers: an experiment with planktivorous fish, dreissenid mussels and zooplankton in the St Lawrence River *River Research and Applications* 19: 265-279

US EPA (1999) Update of ambient water quality criteria for ammonia – Technical Version – 1999 EPA-823-F-99-024, US EPA, Washington DC, USA.

US EPA (2016)a Effluent Guidelines. Centralized Waste Treatment Effluent Guidelines. <https://www.epa.gov/eg/centralized-waste-treatment-effluent-guidelines>

US EPA (2016)b Effluent Guidelines: Industrial Effluent Guidelines. <https://www.epa.gov/eg/industrial-effluent-guidelines>

US EPA (2016)c National Pollution Discharge Elimination System. NPDES Permit Limits. <https://www.epa.gov/npdes/npdes-permit-limits>

Vadeboncoeur Y, Steinman AD (2002) Periphyton function in lake ecosystems *The Scientific World* 2: 1449-1468

van den Brink PJ, van Donk E, Gylstra R, Crum SJH, Brock TCM (1995) Effects of chronic low concentrations of the pesticides chlorpyrifos and atrazine in indoor freshwater microcosms *Chemosphere* 31:5 3181-3200

van der Veen I, de Boer J (2012) Phosphorus flame retardants: properties, production, environmental occurrence, toxicity and analysis *Chemosphere* 88: 1119-1153

Vanni MJ (1987) Effects of food availability and fish predation on a zooplankton community *Ecol Monogr* 57:1 61-88

Walton W, Compton S, Allan J, Daniels R (1982) The effect of acid stress on survivorship and reproduction of *Daphnia pulex* (Crustacea: Cladocera) *Can J Zoo* 60: 573-579

Williamson CE, Butler NM (1986) Predation on rotifers by the suspension-feeding calanoid copepod *Diaptomus pallidus Limnol. Oceanogr* 31:2 393-402

Willis KJ, van den Brink PJ, Green JD (2004) Seasonal variation in plankton community responses of mesocosms dosed with pentachlorophenol. *Ecotoxicology.* 13: 707-720

[WHO] World Health Organization (2000) Flame retardants: Tris (2-butoxyethyl) phosphate, tris (2-ethylhexyl) phosphate and tetrakis (hydroxymethyl) phosphonium salts. *Environ Health Criter* 218: 121.

Wolschke H, Sühring R, Zhiyong X, Ebinghaus R (2015) Organophosphorus flame retardants and plasticizers in the aquatic environment: A case study of the Elbe River, Germany *Envion Poll* 206: 488-493

Zhang X, Sühring R, Serodio D, Bonnell M, Sundin N, Diamond ML (2016) Novel flame retardants: Estimating the physical-chemical properties and environmental fate of 94 halogenated and organophosphate PBDE replacements *Chemosphere* 144: 2401-2408

Table 1: Average pH, conductivity, and dissolved oxygen concentrations each week during exposure to either the no-addition control (C) or mesocosm control (MC) treatments. Numbers in parentheses represent one ± standard error of the mean (SEM).

Week	**pH**		**Conductivity ($\mu S\ cm^{-1}$)**		**Dissolved Oxygen ($mg\ L^{-1}$)**	
	C	**MC**	**C**	**MC**	**C**	**MC**
0	5.2 (0.02)	5.1 (0.02)	22.6 (0.06)	23.8 (0.14)	3.8 (0.07)	3.0 (0.28)
1	5.2 (0.03)	5.1 (0.02)	22.6 (0.08)	23.5 (0.08)	4.6 (0.12)	3.1 (0.23)
2	5.2 (0.03)	5.1 (0.03)	22.6 (0.09)	22.8 (0.03)	4.7 (0.08)	3.0 (0.22)
3	5.5 (0.03)	5.4 (0.01)	22.6 (0.17)	25.6 (0.09)	4.6 (0.15)	2.1 (0.18)
4	5.5 (0.03)	5.5 (0.01)	22.6 (0.21)	23.2 (0.05)	5.0 (0.10)	2.8 (0.11)

Table 2: Two-way PERMANOVA output for zooplankton community assemblages across THPC and ammonium (NH_4^+) treatments over time (n=8). Asterisks indicate significant p-values.

Factor	DF	Pseudo-F	P(perm)
THPC	2, 42	0.9543	0.4796
NH_4^+	1, 42	0.7723	0.5543
THPC*NH_4^+	2, 42	2.0327	0.0358*
Time	3, 126	38.601	0.0001*
Time*THPC	6, 126	0.8362	0.7086
Time*NH_4^+	3, 126	1.2520	0.2339
Time*THPC*NH_4^+	6, 126	1.0928	0.3534

Table 3: Repeated measures ANOVA output showing the effects of THPC and ammonium over time on temperature, dissolved oxygen, conductivity, pH, and chlorophyll *a*. T represents THPC and A represents ammonium. Significant p-values are marked with asterisks.

	Factor	DF	F-ratio	p-value
pH	THPC	2, 42	8.6726	0.0007*
	NH_4^+	1, 42	40.0174	<0.0001*
	THPC*NH_4^+	2, 42	2.0634	0.1397
	Time	3, 134	283.4160	<0.0001*
	Time*THPC	6, 134	1.3539	0.2349
	Time* NH_4^+	3, 134	9.4784	<0.0001*
	Time*THPC* NH_4^+	6, 134	0.5818	0.7545
Conductivity	THPC	2, 42	21.1482	<0.0001*
	NH_4^+	1, 42	80.6391	<0.0001*
	THPC*NH_4^+	2, 42	2.6702	0.0810
	Time	2, 98	46.4023	<0.0001*
	Time*THPC	5, 98	7.4560	<0.0001*
	Time* NH_4^+	2, 98	28.8897	<0.0001*
	Time*THPC* NH_4^+	5, 98	1.4798	0.1453
Dissolved Oxygen	THPC	2, 42	1.3579	0.2682
	NH_4^+	1, 42	0.0585	0.8101
	THPC*NH_4^+	2, 42	2.0332	0.1436
	Time	3, 128	100.2401	<0.0001*
	Time*THPC	6, 128	0.7193	0.6370
	Time* NH_4^+	3, 128	1.1408	0.3356
	Time*THPC* NH_4^+	6, 128	0.6290	0.7094
Temperature	THPC	2, 42	0.0901	0.9140
	NH_4^+	1, 42	0.0046	0.9460
	THPC*NH_4^+	2, 42	2.4854	0.0955
	Time	3, 108	792.6607	<0.0001*
	Time*THPC	5, 108	0.8888	0.4938
	Time* NH_4^+	3, 108	0.6123	0.5842
	Time*THPC* NH_4^+	5, 108	1.5701	0.1727
Chlorophyll *a*	THPC	2, 42	1.1513	0.3260
	NH_4^+	1, 42	0.8158	0.3716
	THPC*NH_4^+	2, 42	0.2163	0.8064
	Time	4, 168	4.8092	0.0011*
	Time*THPC	8, 168	2.3031	0.0229*
	Time* NH_4^+	4, 168	1.4076	0.2336
	Time*THPC* NH_4^+	8, 168	1.4004	0.1996

Table 4: Average pH in each treatment over four weeks (n=8). C represents no-addition control, A represents ammonium, LT represents low THPC, and HT represents high THPC. Numbers in parentheses represent ± one SEM.

Week	Treatment	pH
0	C	5.2 (0.02)
	A	5.2 (0.02)
	LT	5.2 (0.02)
	LT+A	5.2 (0.02)
	HT	5.2 (0.02)
	HT+A	5.1 (0.02)
1	C	5.2 (0.03)
	A	5.2 (0.03)
	LT	5.2 (0.02)
	LT+A	5.1 (0.02)
	HT	5.2 (0.02)
	HT+A	5.0 (0.03)
2	C	5.2 (0.03)
	A	5.1 (0.03)
	LT	5.1 (0.02)
	LT+A	5.0 (0.02)
	HT	5.1 (0.02)
	HT+A	5.0 (0.03)
3	C	5.5 (0.03)
	A	5.4 (0.03)
	LT	5.5 (0.02)
	LT+A	5.3 (0.03)
	HT	5.5 (0.01)
	HT+A	5.2 (0.03)
4	C	5.5 (0.03)
	A	5.4 (0.04)
	LT	5.5 (0.02)
	LT+A	5.3 (0.04)
	HT	5.5 (0.03)
	HT+A	5.3 (0.05)

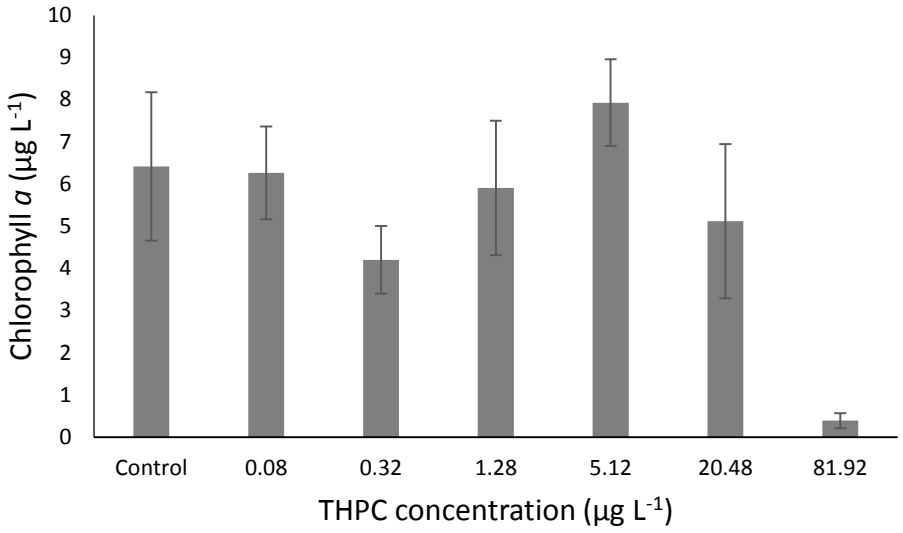

Figure 1: Average chlorophyll *a* concentration after 7 days of *Chlorella* sp. exposure to 0, 0.08, 0.32, 1.28, 5.12, 20.48, or 81.92 µg L^{-1} THPC. Error bars are ± one standard error of the mean (SEM) and n=6.

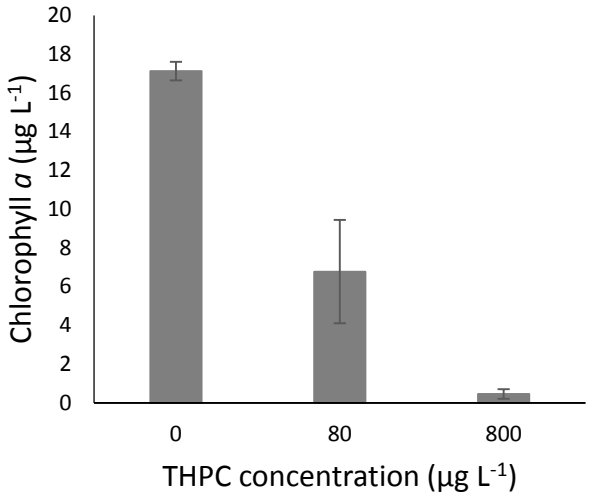

Figure 2: Average chlorophyll *a* concentration of *Chlorella* sp. after 7 days of exposure to 0, 80, or 800 µg L^{-1} THPC. Error bars are ± one SEM and n=4.

Figure 3: Experimental set-up in the pond (A), mesocosm unit (B), and mesocosm lid (C). Photo credits: S. Riera and S. Petersen.

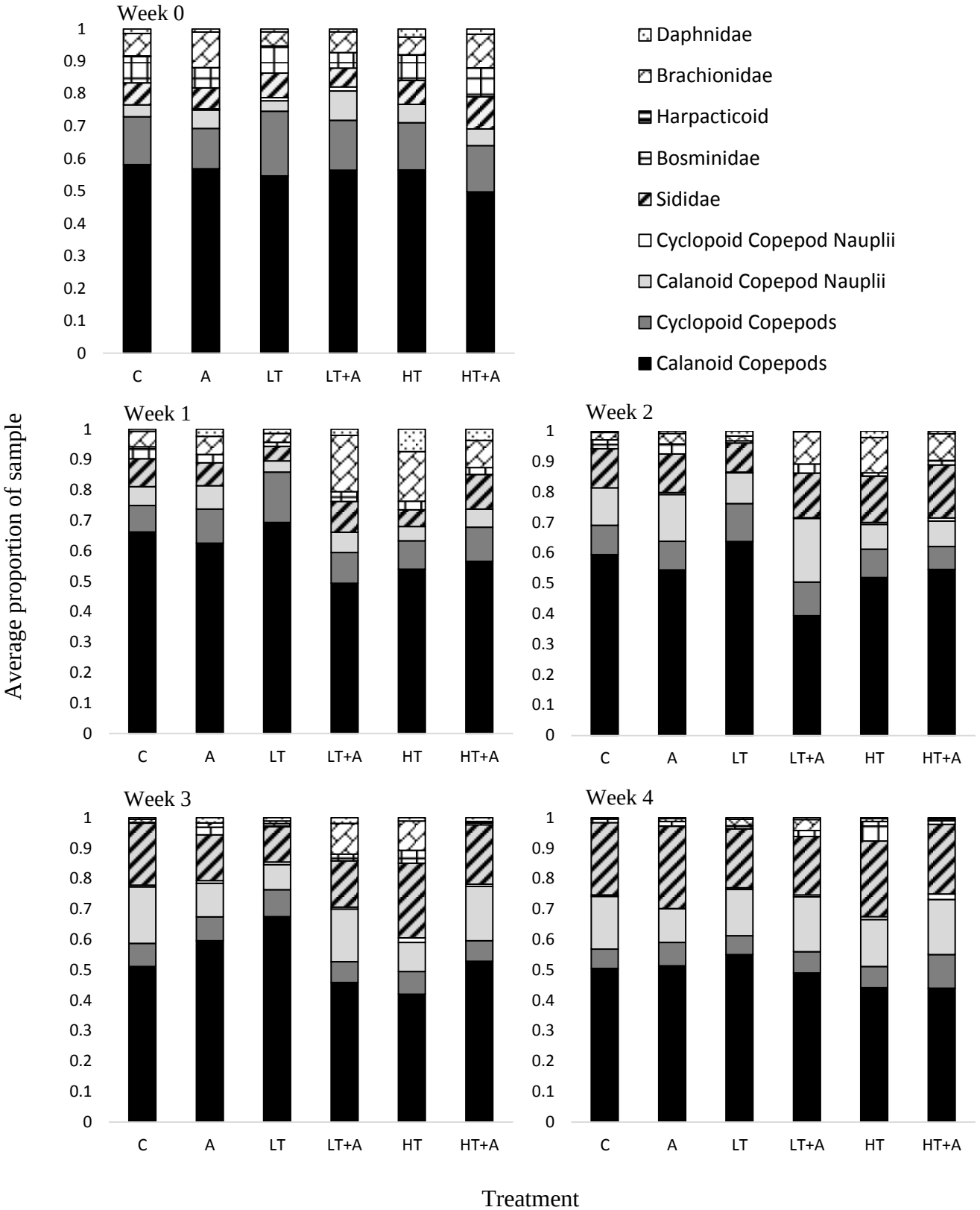

Figure 4: Average proportion of zooplankton taxa observed each week in control (C), ammonium (A), low THPC (LT), high THPC (HT), or the combination of ammonium and THPC (LT+A, HT+A) treatments, n=8.

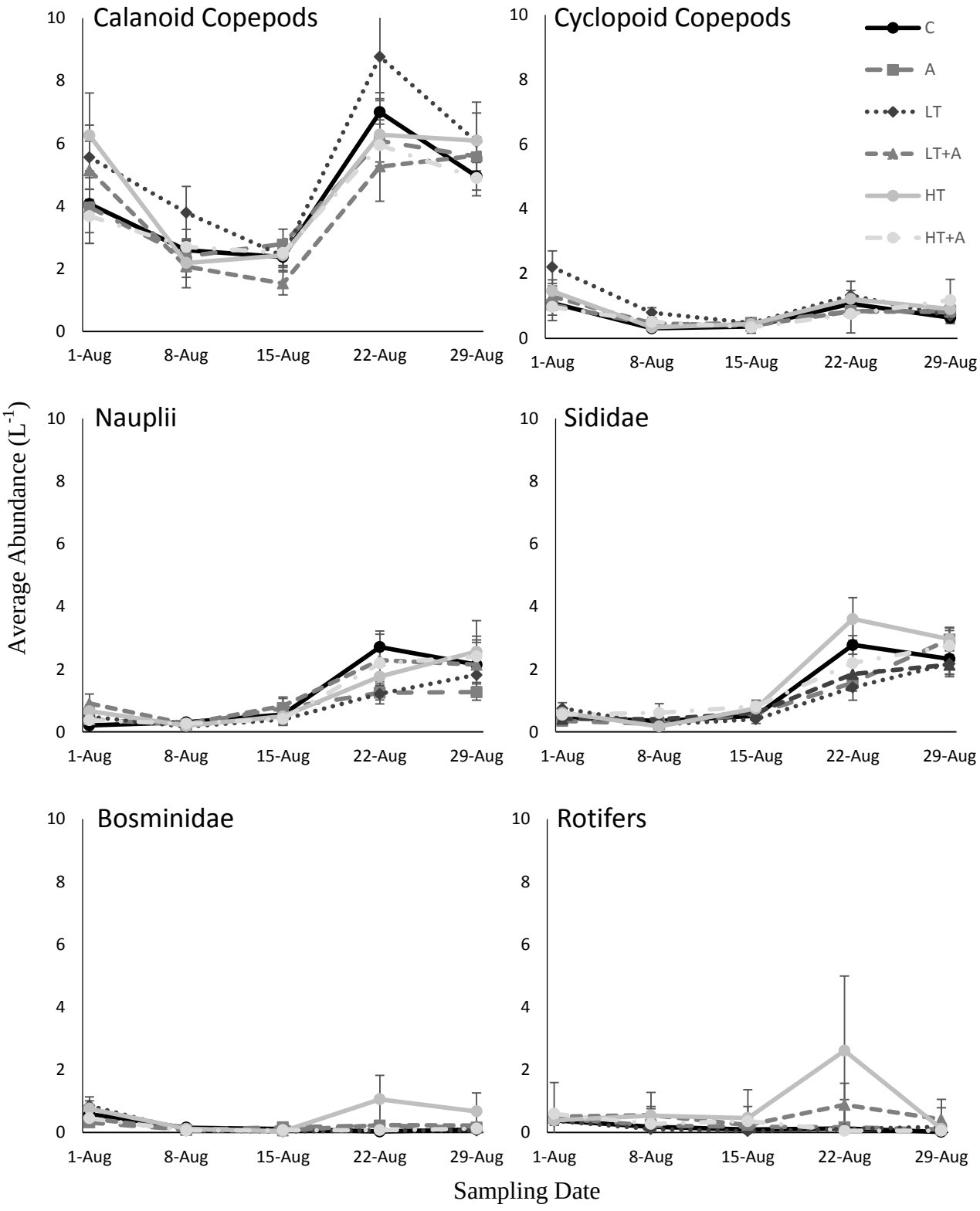

Figure 5: Abundance (average individuals L^{-1}) of zooplankton taxa over four weeks of exposure to control (C), ammonium (A), low THPC (LT), high THPC (HT), or the combination of ammonium and THPC (LT+A, HT+A) treatments. Error bars are ± one SEM and n=8.

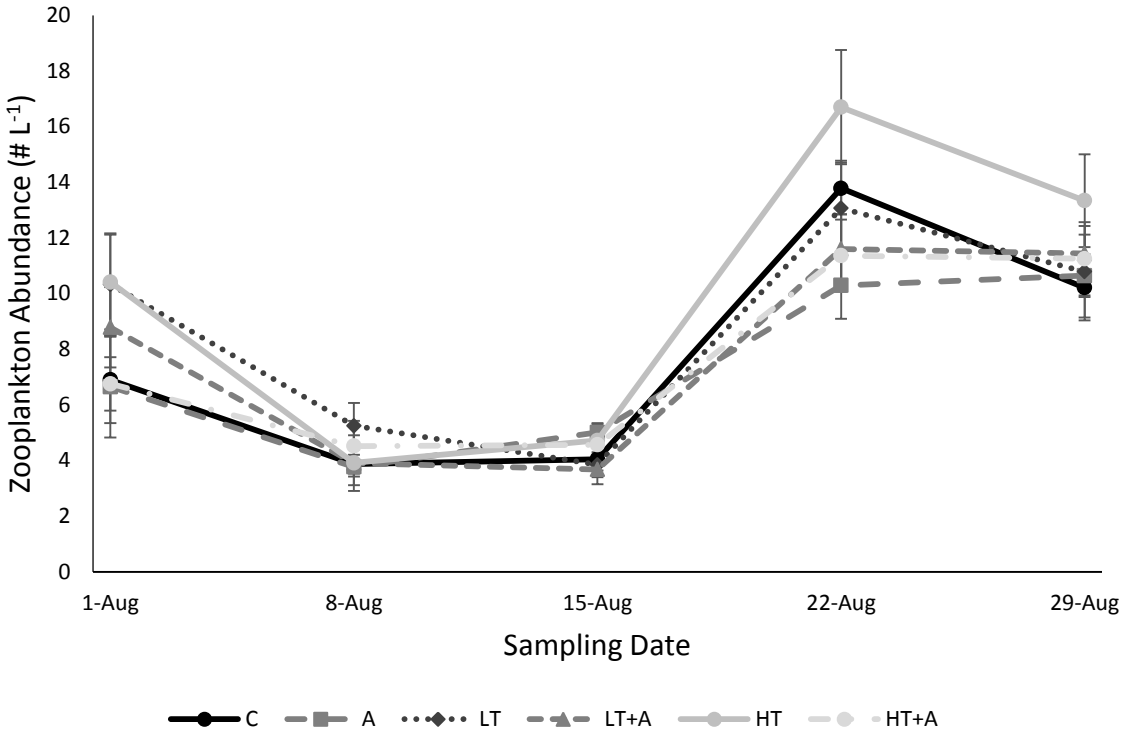

Figure 6: Total average zooplankton abundance (individuals L^{-1}) over four weeks of exposure to treatments. Treatments were control (C), ammonium (A), low THPC (LT), high THPC (HT), or the combination of ammonium and THPC (LT+A, HT+A). Error bars are ± one SEM and n=8.

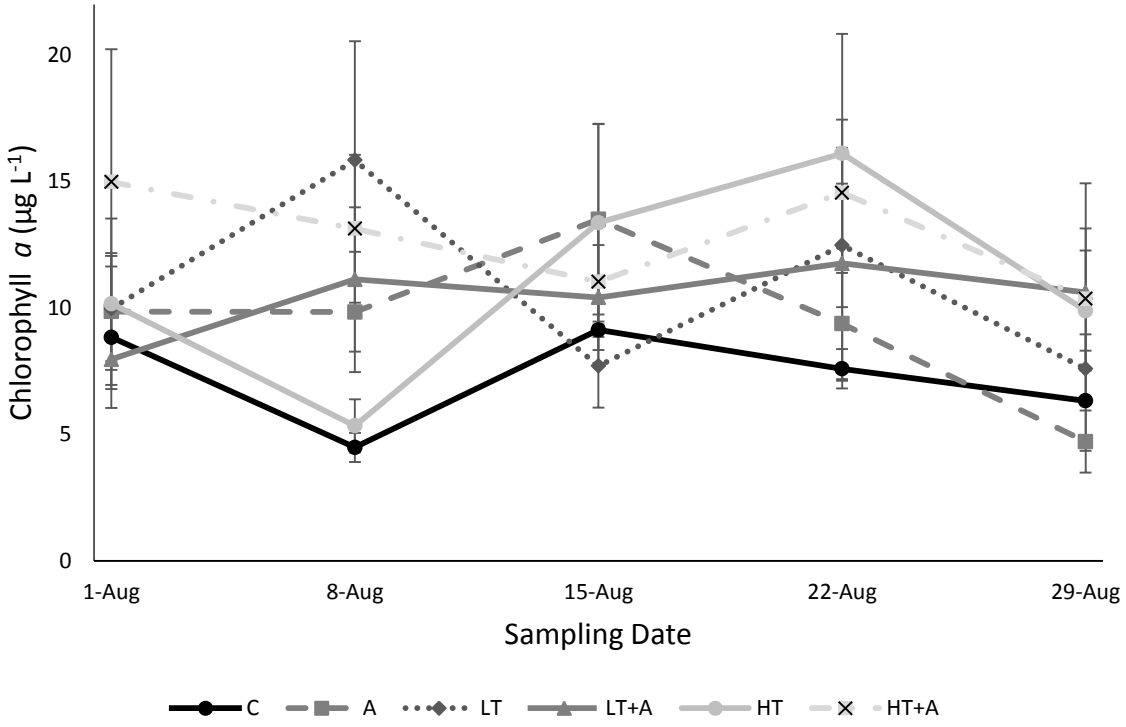

Figure 7: Average chlorophyll *a* concentrations over four weeks of exposure to control (C), ammonium (A), low THPC (LT), high THPC (HT), or the combination of ammonium and THPC (LT+A, HT+A) treatments. Error bars are ± one SEM and n=8.

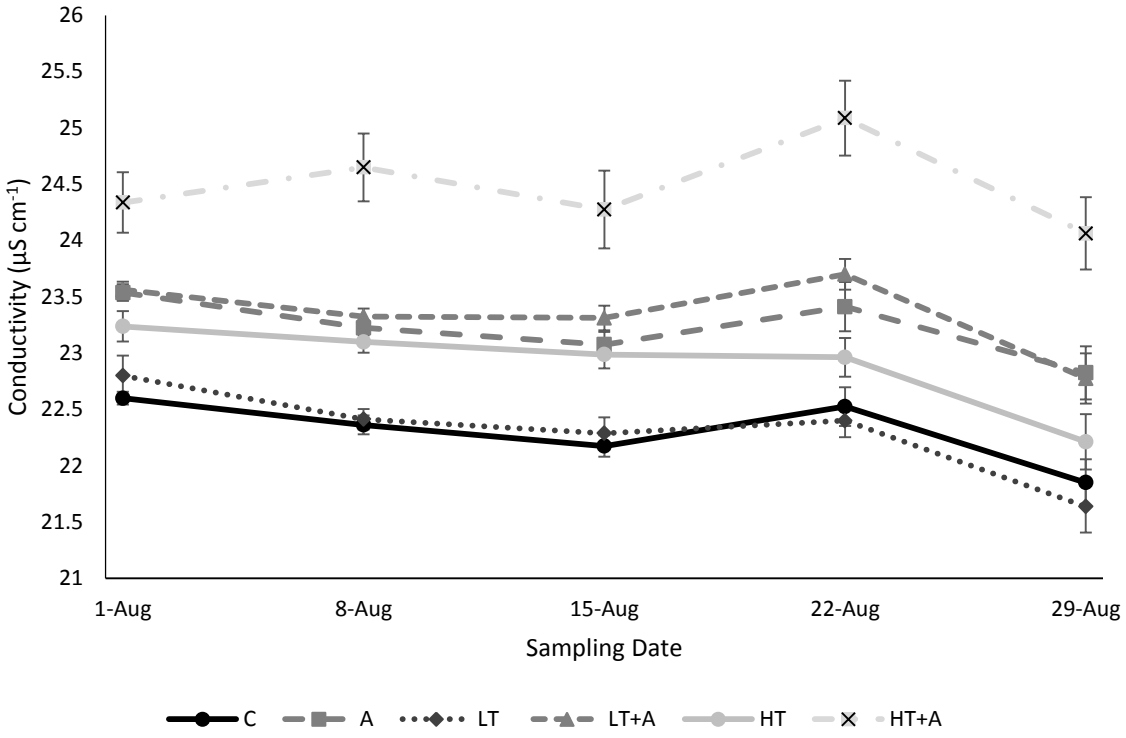

Figure 8: Average conductivity over four weeks of exposure to control (C), ammonium (A), low THPC (LT), high THPC (HT), or the combination of ammonium and THPC (LT+A, HT+A) treatments. Error bars are ± one SEM and n=8.

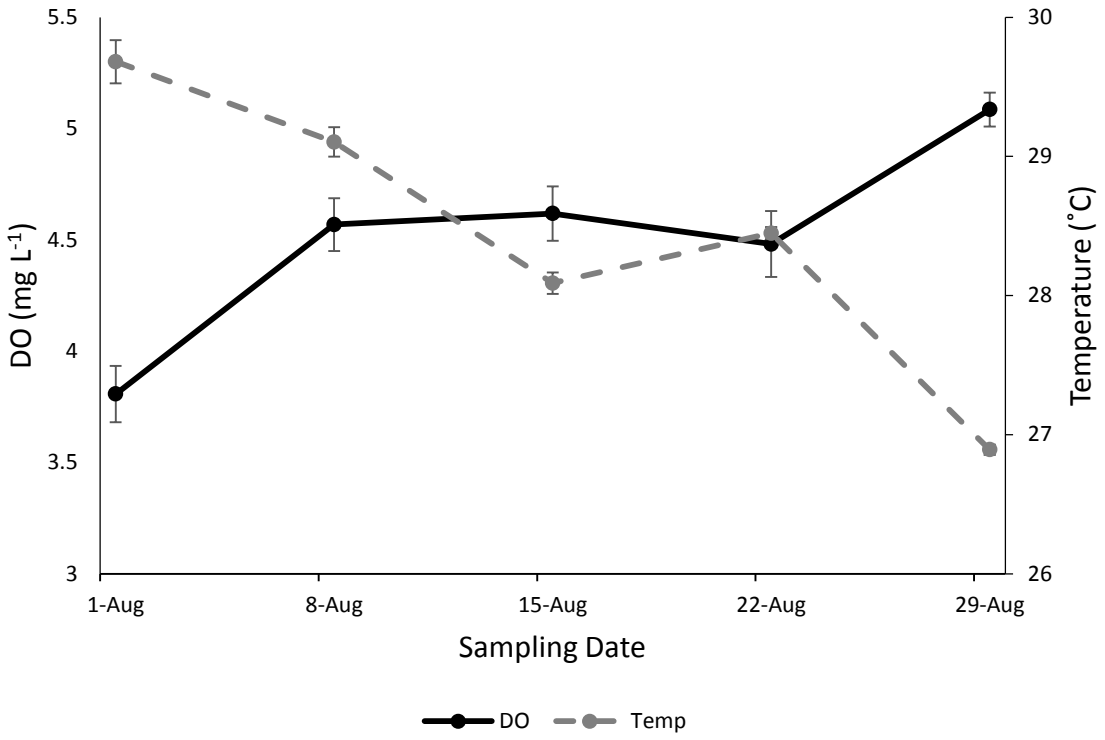

Figure 9: Average temperature and dissolved oxygen (DO) concentrations across all treatments over the four-week experiment. Error bars are ± one SEM.