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An industrial chemical used in coal-washing influences plankton communities in freshwater microcosms

An Honors Thesis submitted in partial fulfillment of the requirements for Honors in Department of Biology.

By
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Under the mentorship of Dr. Risa Cohen

ABSTRACT

In 2014, 4-methylcyclohexane methanol (MCHM), an industrial chemical used to wash coal, contaminated drinking water in 300,000 homes in West Virginia, USA and raised concerns about toxicity to humans and freshwater ecosystems. The Centers for Disease Control and Prevention determined that a concentration of 1 ppm was safe for human exposure. Despite the concern for human consumption, it is important to determine if MCHM has negative effects on aquatic organisms in contaminated water, particularly plankton communities that comprise the base of freshwater food webs. I exposed freshwater plankton communities in microcosms to 0, 0.5, 1 or 3 ppm MCHM under greenhouse conditions to determine whether environmentally relevant concentrations adversely affect plankton community composition and water quality. Plankton (zooplankton and phytoplankton) and water quality were sampled every 7 days for four weeks. I found that the plankton community changed following exposure to concentrations of 0.5 and 1 ppm MCHM. Although the zooplankton taxa present were the same, the proportion of copepods decreased while rotifers increased. In addition, phytoplankton (as chlorophyll *a*) abundance also decreased at 0.5 and 1 ppm treatment levels. Water column conductivity increased only with initial MCHM addition, and resembled the control treatment after one week. My findings suggest that MCHM contributes to loss of copepod species. Because copepods are a major food source for fish, their decline, followed by an increase in smaller-bodied rotifers, may not only reduce food availability to higher trophic levels, but also decrease species diversity.

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Introduction

Industrial chemicals, when stored improperly, can leak or spill causing immediate danger to employees, surrounding ecosystems, and nearby waterways (Office of Response and Restoration, 2015). Increased use of industrial chemical agents in agriculture, transportation, and energy production results in an increase in point and non-point freshwater pollution (Holt, 2000). For example, over 700 industrial chemical spills occurred in Southern Ontario Canada waterways in 2012 (Cao et al, 2012), and the U.S. National Response Center recorded more than 30,000 chemical and oil spills to freshwater systems in the United States in 2014 (U.S. Coast Guard National Response Center, 2014). Xenobiotic chemicals are threats to freshwater resources because they can alter water quality and disrupt food webs. In the United States, the federal Environmental Protection Agency (EPA, 2015) is responsible for regulating potentially harmful substances and assessing chemical safety regulations for industry. As a part of EPA federal guidelines, at least 60,000 chemicals are exempt from EPA regulation because they were not exclusively manufactured for distribution in commerce and have no commercial purpose apart from the substance or mixture they are associated with (Toxic Control Act, 1976). Under this act, thousands of chemicals go unregulated and untested, including a frothing agent used to wash coal, an organic alcohol known as 4-methylcyclohexane methanol (MCHM) (EPA, 2015).

In 2014, approximately 10,000 gallons of crude MCHM leaked from a holding tank at Freedom Industries into the Elk River in West Virginia, USA (West Virginia Department of Environmental Protection, 2014). The site of contamination occurred 1.5 miles upstream from a public water facility that was the sole drinking water supply for

300,000 West Virginia residents (West Virginia Department of Environmental Protection, 2014). Unfortunately due to its exemption from EPA regulation, the only MCHM toxicity studies conducted were by the Eastman manufacturing company (Eastman, 1998). Eastman's (1998) data suggested that MCHM degrades by approximately 50% within a 28-day period, and that the 48-hr EC_{50} for *Daphnia magna* exposed to crude MCHM was 98 ppm and the 48-hr NOEC was 50 ppm. Initial concentrations 3 days after the 2014 spill ranged from 6-36 ppm and concentrations of 3.4 ppm persisted for approximately twenty days (Whelton et al., 2014), concentrations that were all lower than the concentrations previously found to impair *D. magna* (Eastman, 1998). The Centers for Disease Control and Prevention determined a no adverse effect concentration of 1 ppm in drinking water based on a 28-day rat exposure study (Eastman, 1998; Centers for Disease Control, 2014; Lan, 2015). Studies conducted by Eastman determined the acute effects of MCHM on the zooplankton, *Daphnia magna*, establishing a 48-hr EC_{50} of 98 ppm and a 48-hr NOEC of 50 ppm (Eastman, 1998). However, following the spill a more recent study showed that crude MCHM is more toxic to *Daphnia magna* that previously reported, with a 48-hr EC_{50} of 57 ppm and a 48-hr NOEC of 6.25 ppm (Whelton, 2014). Despite the renewed interest in testing MCHM, all testing to date focused on single species testing of model organisms (rats and *Daphnia* sp.); to my knowledge, no studies including responses of multiple species or other planktonic organisms to MCHM have been conducted.

Examining the effects of MCHM on microscopic aquatic plants (phytoplankton) and animals (zooplankton) simultaneously is important because there is evidence that introduction of a xenobiotic reduces plankton abundance, potentially altering availability

of resources to higher trophic levels (McQueen, 1986; Krebs, 1994). Removal of specific plankton groups, like copepods, can alter abundance of their smaller zooplankton prey, like rotifers and cladocerans that graze on phytoplankton (Lynch, 1977). Therefore, depletion of a copepod population may increase cladoceran and rotifer abundance and decrease phytoplankton availability (Byron et al., 1984). Another possibility is that removal of cladocerans or an increase in copepod population leads to increased algal abundance and ultimately water column oxygen depletion as the biomass decays (Bushaw-Newton, 1999). For example, Lay et al. (1985) found that petroleum hydrocarbons similar to MCHM in basic structure decreased cladoceran and meiobenthic copepod species diversity in experimental ponds. Different groups of copepods may not all be sensitive to MCHM; Harpacticoid copepod species, *Coullana* sp. and *Paronychocamptus* sp., suffered significant increased mortality rates due to hydrocarbon exposure after 30 days, while other Harpacticoid species, *Cletocamptus* sp. and *Enhydrosoma* sp. increased in abundance, resulting in no apparent overall change in total copepod abundance (Millward et al., 2004). Finally, an organic insecticide, azadirachtin (containing a hydrocarbon structure with constituent alcohol side-groups like MCHM) reduced cladoceran and rotifer populations by 40-70 % and nearly eliminated copepods in pond enclosures in Canada (Kreutzweiser et al., 2002). Therefore, MCHM exposure has the potential to change aquatic communities, emphasizing the need to examine interactions among multiple species that would be missed using single species tests (Relyea, 2005; Côté, 2015).

Furthermore existing acute tests of MCHM exposure do not permit evaluation of adverse effects at sub-lethal concentrations over time periods over which MCHM is

detected following a spill (Rand, 1995). For instance, trace amounts of MCHM were

detected nearly 400 miles away in the Ohio River weeks after the initial spill (Foremane

et al, 2015). Low level chronic exposure (29 days) of aromatic hydrocarbon naphthalene,

similar to MCHM, resulted in significant reduction in total calanoid nauplii produced,

mean size and survival rate compared to 24-h acute testing which had no effect on

reproductive success or mean size (Ott et al., 1979). Low-level exposure may also lead to

chronic effects that alter water quality parameters in addition to altering plankton

communities (Rand, 1995).

MCHM may also affect freshwater plankton communities indirectly via altered

water quality parameters such as pH, dissolved oxygen or conductivity. Following the

Elk River spill, Whelton et al. (2014) found that the pH of tap water from 16 households

was unaffected by MCHM. In contrast with the Eastman Company's (1998) findings that

MCHM was not readily biodegradable after 28 days, Yaun et al. (2016) observed total

aerobic degradation of MCHM after 16 days and only residual percentages of cis-4-

MCHM and trans-4-MCHM in anaerobic conditions using river sediment bacteria,

Bacillus pumilus. Increased water temperatures also favor an increase in MCHM aerobic

biodegradability (Moyer et al, 2004). Structurally similar chemicals, such as

cyclohexandimethanol were shown to increase in conductivity due to the addition of ions

from the chemical (TOXNET, 2014). Therefore MCHM could behave similarly,

increasing conductivity upon addition to the water, and decreasing dissolved oxygen as it

degrades.

The objective of this study was to test the hypothesis that environmentally

relevant concentrations of MCHM affect aquatic plankton abundance and species

composition in freshwater microcosms. I predicted that MCHM concentrations at and below the 1 ppm screening level would alter phytoplankton and zooplankton abundance and community composition, likely through decreases in copepods as observed in hydrocarbon studies, ultimately leading to an increase in smaller-bodied zooplankton grazers thereby decreasing phytoplankton abundance as well. I also predicted MCHM would increase conductivity and decrease DO following MCHM exposure.

Methods

In order to test the hypothesis that MCHM affects aquatic plankton communities at environmentally relevant concentrations, assemblages of freshwater plankton were exposed to concentrations of 0, 0.5, 1 or 3 ppm MCHM. Water containing plankton was pumped from a constructed pond located on the Georgia Southern University campus, Statesboro, GA, USA (32.41° N, 81.78° W) into 200L holding tanks and transported to the Biological Sciences building greenhouse where 10L aliquots were dispensed into 20L cylindrical opaque plastic microcosms. The 10L volume was sufficient to maintain diverse freshwater plankton assemblages over the same time frame as the current study in an experiment with a similar design evaluating cadmium toxicity to plankton (Fernandez-Leborans et al., 1995). MCHM treatments were chosen to reflect 1) the CDC screening level for human consumption in drinking water (1 ppm); 2) an environmentally relevant concentration below the screening level (0.5 ppm); 3) a concentration above the screening level that could occur following a spill; (3 ppm; concentrations in the Elk River were even higher, 6-36 ppm (Whelton et al., 2015) and 4) a no addition control. Treatments were created by pipetting appropriate amounts of purified MCHM (TCI-America CAS-No.: 34885-03-5) into individual microcosms.

Each microcosm was assigned to one of the four treatments with four-fold replication and five sampling times, and randomized by location in the greenhouse. Sampling of water physicochemical parameters and plankton occurred initially and then weekly for one month from October 31 until November 28, 2014. To compensate for evaporation from the open microcosms over the course of the experiment, deionized water was added periodically to maintain the 10L volume. The initial testing immediately after establishing treatments occurred to ensure that plankton abundance and community composition and water column dissolved oxygen and pH started at similar levels, but conductivity was expected to increase due to the addition of MCHM (Mount et al., 1997). The one-month duration of the study was selected to allow for plankton community responses over multiple generations. Typically, copepod species take approximately 2 weeks to develop from the nauplii stage to an adult (Frisch, 2001), and sexual maturity is reached within 18 hrs of hatching in rotifers (Marini, 2002). Cladocerans like *Bosmina* sp. have a total life span of about 25 days with a 2-day gestational period (Korinek, Saha, and Bhattacharya, 1999). Each week, water column pH (Oakton pHTestr), dissolved oxygen (DO) and conductivity (YSI 85 multi-parameter probe) were measured using hand-held instruments in four replicates from each treatment group. The microcosms were then stirred gently with a glass rod to homogenize plankton that might have settled, and a 100 mL water sample was collected to determine phytoplankton abundance. The remaining volume in each microcosm was then filtered through a plankton net (80 µm mesh) to concentrate all of the zooplankton into a 40 mL sample and preserved in 70% ethanol for subsequent identification using microscopy.

To establish phytoplankton abundance, 100 ml water samples were vacuum filtered through Whatman GF/F glass fiber filters (nominal pore size 0.7 □m) to concentrate cells. Pigment concentration (chlorophyll *a*) was used as a proxy for abundance and pigments were extracted from the cells collected on the filters in 90% acetone in the dark for 24 hrs at -20°C, followed by analysis using a TD Trilogy fluorometer (Turner Designs, Sunnyvale, CA) according to EPA method 445.0 (Arar and Collins, 1997). Zooplankton samples were stained with Rose Bengal and transferred into 95% ethanol at least 24 hours prior to counting and identification (Goswami, 2004). After homogenization, the 40 ml samples were further subdivided into 10 ml subsamples for zooplankton enumeration and identification using a Bogorov counting chamber under a dissecting microscope at 60x (Goswami; 2004; Riera, 2015). Two 10mL subsamples with a minimum of 48 total individuals were observed for species identification to ensure acceptable accuracy levels according to previously published identification methodology (Postel et al. 2000; Riera, 2015). The number of individuals in each taxonomic group was then doubled to represent the total in the entire sample (Parsons, 1984; Proosdij, 2015). Copepods were identified to order, cladocerans to genus, and rotifers to family using a zooplankton image based-key from the University of New Hampshire Center for Freshwater Biology (Hanely et al., 2013). This level of taxonomic discrimination provides adequate resolution to distinguish the effects of disturbance on zooplankton communities when using the multivariate statistics (Nielsen et al. 1998; Riera, 2015).

To determine if MCHM concentration and duration of exposure affected DO, pH, conductivity, chlorophyll *a* concentration and total zooplankton abundance, data were first tested for normality using the Shapiro-Wilk *W* test and for homogeneity of variances

with Levene's test. Data not meeting assumptions of parametric tests were either log transformed, or nonparametric tests were used. Dissolved oxygen, total zooplankton abundance and log-transformed chlorophyll *a* data were analyzed using analysis of variance (ANOVA), while conductivity and pH were analyzed using nonparametric analogs of ANOVA (either Kruskal-Wallis, or Kruskal-Wallis with the Sheirer-Ray-Hare extension). All ANOVA-type analyses (one or two-way) were conducted using the JMP Pro 10 statistical package (SAS Institute Inc., Cary, NC, USA). In order to analyze the effects of MCHM treatment and time on community composition, zooplankton community data were first square root transformed to compensate for the contributions of dominant species (Clarke and Gorley, 2006; Riera, 2015) followed by permutational multivariate analysis of variance (PERMANOVA) pairwise comparisons using PRIMER v6 with the PERMANOVA+ add-on (PRIMER-E LTD., Plymouth, UK). Data analyses were conducted for the first two weeks of the experiment. Data collected after the second week of the experiment were excluded from analysis due to a climate control malfunction in the greenhouse causing a greater than 10℃ decline in temperature relative to ambient (resulting in temperatures of 5℃-11℃).

Results

A total of 5 taxonomic groups were identified within the zooplankton community (Table 1). Initially, total zooplankton abundance in each microcosm was variable, ranging between 600-1500 individuals (Figure 1), but zooplankton proportion was similar across all treatments (ANOSIM, Global R=0.212) with 60% of the zooplankton community consisting of copepod nauplii (calanoid and cyclopoid). Phytoplankton abundance

(chlorophyll *a* concentration), was approximately $12.2 \pm 0.9\ \mu g\ L^{-1}$ and initially similar across all treatments (one-way ANOVA, $F_{3,15} = 0.80$, $p = 0.51$) (Figure 1).

MCHM altered zooplankton community composition (PERMANOVA, Pseudo-$F_{3,36}$=3.16, p=0.001). The communities exposed to 0.5 and 1 ppm MCHM differed from both the control and the 3 ppm treatment (p<0.05), but no differences occurred between the control and 3 ppm treatments (p>0.05) after one week of exposure (Table 2). The differences appeared to be driven by changes in the rotifer and copepod populations. Rotifers became the most abundant taxonomic group in the 0.5 and 1 ppm MCHM treatments; the proportion of Brachionidae increased from 20% to 60% in the 1 ppm, and from 15% to 50% in the 0.5 ppm treatment. In contrast, cyclopoid copepods (adult and nauplii) were the most abundant taxonomic group in the control and 3 ppm treatment level, comprising 50% of the total zooplankton composition (Figure 2). Proportions of cyclopoid nauplii decreased from 45% to 15% in both the 0.5 and 1 ppm concentrations (Figure 2). The decrease in total nauplii (calanoid and cyclopoid) of 45% in 0.5 ppm and 40% in 1 ppm treatments was not reflected by a similar increase in total adult copepod proportion (which increased by only 12% and 2% in 0.5 and 1 ppm concentrations respectively). Overall the total adult copepod population only accounted for ~20% of zooplankton population composition in 0.5 and 1 ppm treatments compared to the 40% in the control and 3 ppm treatments (Figure 2). Phytoplankton abundance decreased across all treatments after one week, but chlorophyll *a* concentrations were 50%-70% lower in the 0.5 and 1 ppm treatments relative to the control (one-way ANOVA, $F_{3,15}$=6.10, p=0.009).

After 2 weeks, the communities in the 0.5 and 1 ppm treatments were still different from the control (p<0.05), but the 0.5 and 3 ppm treatments were similar, while 0.5 ppm and 1 ppm differed from one another (p< 0.05) (Figure 2, Table 2). Brachionidae decreased by 20% in 0.5 ppm and 40% 1 ppm treatments (Figure 1). However, the Brachionidae became the most abundant species in the in the control and 3 ppm communities, increasing by an average of 45%, similar to the Brachionidae proportion observed in the 0.5ppm and 1 ppm after one week. Total copepod adult and nauplii (calanoid and cyclopoid) proportions increased the most (~80%) in the 1 ppm treatment (Figure 2), likely contributing to the significant difference compared to all of the other treatments (Table 2). In contrast, the 0.5 ppm community more closely resembled the 3 ppm treatment in terms of rotifer and copepod abundance, but was still different from the control communities (Figure 1, Table 2). Less than 10 *Daphnia* sp. and 10 *Bosmina* sp. individuals occurred in each treatment level at both time points post-MCHM exposure (Figure 2), contributing less than 1% to the total community composition (Figure 2). Phytoplankton abundance in all treatment levels decreased by 75% from initial measurements to a mean of 2.9 ± 0.65 µg L^{-1} with no difference in chlorophyll *a* concentrations across treatment levels after two weeks (one-way ANOVA, $F_{3,15}=1.38$, p=0.30) (Figure 1).

Water column conductivity increased relative to the control by approximately 5 µS cm^{-1} immediately upon addition of MCHM, regardless of concentration (Kruskal-Wallis, $\chi^2_3=9.61$, p=0.02), but by the end of one week was similar across treatments (one-way ANOVA, $F_{3,14}=0.12$, p=0.95) (Table 3). Dissolved oxygen and pH (Table 4)

remained within ranges of 6-8 mg L^{-1} and 6.5-8.2 respectively over the course of the two week exposure to treatments (Table 3).

Discussion

I hypothesized that MCHM concentration of 1 ppm and below influence zooplankton abundance and community composition. Zooplankton community composition can be altered by either species replacement, resulting in a completely different set of species present, or a change in species abundance and proportions (Relyea and Hoverman, 2006). I found the same taxonomic groups present before and after MCHM exposure, however, the relative abundance of each group changed throughout the two weeks. The 0.5 and 1ppm treatment levels community composition differed from the control at both testing points. Both community compositions differed from the control with a decrease in copepods and chlorophyll *a*, and decrease in rotifers, but began to differ from one another due to an increase in copepods in the 1 ppm treatment level. There was an overall slight increase in adult copepod population but that rate in development from nauplii to adult did not show accurate compensation for the increased nauplii losses. Furthermore, while it was unexpected that the 3 ppm concentration did not alter the zooplankton community, other toxicity studies have shown that lower concentrations of a toxicant can have greater adverse effects compared to effects at higher concentrations (Stiff, 1971).

One possible reason for the decline in copepods could be that copepods have a higher sensitivity to MCHM than other zooplankton groups. Although copepods were generally less sensitive or of equal sensitivity to aromatic hydrocarbons and alcohols compared to cladocerans (Sanchez-Bayo, 2006), copepods displayed a higher mortality

rate compared to cladocerans and rotifers when exposed to azadirachtin, a hydrocarbon with constituent alcohol side-groups like MCHM (Kreutzweiser et al., 2002). Also, increased copepod mortality rates (Millward et al., 2004), decreased meiobenthic copepod species diversity, and increased phytoplankton abundance (Fleeger et al., 2003; Lay et al 1985) were reported following hydrocarbon exposure in experimental ponds and microcosms. In addition, chronic exposure of organic insecticides directly delayed development of nauplii into adults by 4 days compared to the control (Bejarano, 2005). In light of previous findings, higher sensitivity to MCHM is a likely explanation for the decline in copepods. However, the observed changes in other taxonomic groups may not be solely attributed to low sensitivity to MCHM, but enhanced by indirect effects associated with the loss of copeopds.

A decline in copepod abundance could indirectly affect biomass at other trophic levels via top-down or bottom-up effects (Baum and Worm, 2009; Eby et al., 2006). For example, when high reproductive rates of planktivorous fish increased predation on copepods, rotifer abundance increased due to release from copepod predation, consequently shifting community composition (Lynch, 1997) Subsequently, phytoplankton were found to increase in freshwater systems as a result of zooplankton declines following exposure to hydrocarbon pollutants (Byron et al., 1984; Lay et al., 1985). Furthermore, a zooplankton community consisting of small-bodied species (0.2-0.6 mm), triggers bottom-up effects, decreasing the quantity of food available to visual predators like planktivorous fish in ponds and rivers that rely on food resources at least 1 mm in size (Brooks and Dodson 1965; Krebs, 1994).

Finally, potential food limitation cannot be ruled out as a cause of copepod population decline. Coincident with increased rotifer (Brachionidae) abundance, phytoplankton decreases were greater in the 0.5 and 1 ppm treatments compared to the control. This decrease in phytoplankton could have resulted from increased grazing pressure from the rotifer population (Lynch 1977), but there could also have been additional phytoplankton losses from direct toxicity of MCHM. Phytoplankton species display a range of sensitivities to aromatic hydrocarbons at low levels that may result in growth inhibition (Dustan et al., 1975). Chlorophyll a concentrations in 0.5 and 1 ppm averaged 3 µg L^{-1}, which could be seen as limiting considering Kimmerer (2005) determined a chlorophyll a concentration above 12±6 µg L^{-1} as an adequate food supply. Limited phytoplankton abundance has the potential to increased competition between copepods and rotifers (Gilbert, 1988).

In addition to MCHM effects on the plankton community, I predicted MCHM would increase conductivity and decrease DO over the course of the experiment (TOXNET, 2014). DO did not decrease in the experiment, likely due to the concurrent decrease in temperature (Benson, 1980). While initial additions of MCHM elevated conductivity indicating MCHM presence, conductivity was similar across all treatments regardless of concentration, most likely due to the small difference between concentrations. Thus the absence of conductivity differences relative to the control after one week suggested the chemical was no longer present at detectable levels, potentially a result of rapid volatilization and release from solution as carbon dioxide (Eastman, 1998). Despite the rapid rate of volatilization, it is possible for even brief exposure to MCHM to have lasting effects. Adults exposed to MCHM might have altered reproductive output, or

offspring vitality (Ott et al. 1978). Furthermore, different life stages have differential

sensitivity to chemical exposure; juveniles may experience sublethal effects including

decreased growth rates, reproductive output, and ultimately survival (Rand, 1995), which

is similar to what we observed for copepod nauplii in this study.

The findings from this study suggest that exposure to MCHM at and below the

"safe" screening level of 1 ppm set by the CDC affects zooplankton abundance and

community composition. The current study demonstrated the potential to reduce copepod

abundance, thereby increasing rotifers, and decreasing phytoplankton abundance. If

copepods are susceptible to MCHM, reduction in abundance and altered species

proportions could reduce food availability to higher trophic levels as well as species

diversity.

Literature Cited

Agrawal, A. A. (1998). Algal defense, grazers, and their interactions in aquatic trophic cascades. *Acta Oecologica*, *19*(4), 331-337**.**

Arar, E. J., & Collins, G. B. (1997). *Method 445.0: In vitro determination of chlorophyll a and pheophytin a in marine and freshwater algae by fluorescence*. United States Environmental Protection Agency, Office of Research and Development, National Exposure Research Laboratory.

Barbour, M. T., Gerritsen, J., Snyder, B. D., & Stribling, J. B. (1999). Rapid bioassessment protocols for use in streams and wadeable rivers. *USEPA, Washington*.

Baum, J. K., & Worm, B. (2009). Cascading top-down effects of changing oceanic predator abundances. *Journal of Animal Ecology*, *78*(4), 699-714.

Bejarano, A. C., Chandler, G. T., & Decho, A. W. (2005). Influence of natural dissolved organic matter (DOM) on acute and chronic toxicity of the pesticides chlorothalonil, chlorpyrifos and fipronil on the meiobenthic estuarine copepod Amphiascus tenuiremis. *Journal of Experimental Marine Biology and Ecology*, *321*(1), 43-57.

Benson, B. B., & Krause, D. (1980). The concentration and isotopic fractionation of gases dissolved in freshwater in equilibrium with the atmosphere. 1. Oxygen. *Limnology and Oceanography*, *25*(4), 662-671.

Biswas, A., Saha, R. K., Sengupta, A., & Saha, H. (2014). Life Cycle of a New Bosminid Cladocera: Bosmina (Bosmina) tripurae (Korinek, Saha, and Bhattacharya, 1999). *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, *84*(4), 953-960.

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

Bushaw-Newton, K. L., & Moran, M. A. (1999). Photochemical formation of biologically available nitrogen from dissolved humic substances in coastal marine systems. *Aquatic Microbial Ecology*, *18*(3), 285-292.

Byron, E. R., Folt, C. L., & Goldman, C. R. (1984). Copepod and cladoceran success in an oligotrophic lake. *Journal of plankton research*, *6*(1), 45-65.

Cao, W., Li, J., & Joksimovic, D. (2012). Characteristics of urban chemical spills in Southern Ontario. *Water Quality Research Journal of Canada*, *47*(2), 166-177.

Centers for Disease Control and Prevention. (2014). Emergency Preparedness and Response. Retrieved September 2014.

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

Côté, R. P., & Wells, P. G. (2012). *Controlling chemical hazards: Fundamentals of the management of toxic chemicals*. Springer Science & Business Media.

Dunstan, W. M., Atkinson, L. P., & Natoli, J. (1975). Stimulation and inhibition of phytoplankton growth by low molecular weight hydrocarbons. *Marine Biology*, *31*(4), 305-310.

Eastman Company. (1998). Crude MCHM Studies, http://www.eastman.com/Pages/Eastman-Crude-MCHM-Studies.aspx (accessed Oct. 3, 2015

Eby, L. A., Roach, W. J., Crowder, L. B., & Stanford, J. A. (2006). Effects of stocking-up freshwater food webs. *Trends in Ecology & Evolution*, *21*(10), 576-584.

Environmental Protection Agency. (2016). Regulations and Guidelines. Retrieved March 2016.

Fernandez-Leborans, G., & Novillo, A. (1995). Hazard evaluation of lead using marine protists. *Encyclopedia of environmental control technology*, *9*, 761-830.

Fleeger, J. W., Carman, K. R., & Nisbet, R. M. (2003). Indirect effects of contaminants in aquatic ecosystems. *Science of the Total Environment*, *317*(1), 207-233.

Foreman, W. T., Rose, D. L., Chambers, D. B., Crain, A. S., Murtagh, L. K., Thakellapalli, H., & Wang, K. K. (2015). Determination of (4-methylcyclohexyl) methanol isomers by heated purge-and-trap GC/MS in water samples from the 2014 Elk River, West Virginia, chemical spill. *Chemosphere*, *131*, 217-224.

Frisch, D. (2001). Life cycles of the two freshwater copepods Cyclops strenuus Fischer and Cyclops insignis Claus (Cyclopoida, Copepoda) in an amphibious floodplain habitat. *Hydrobiologia*, *453*(1), 285-293.

Gilbert, J. J. (1988). Suppression of rotifer populations by Daphnia: a review of the evidence, the mechanisms, and the effects on zooplankton community structure. *Limnology and Oceanography*, *33*(6), 1286-1303.

Goswami, S. C. (2004). Zooplankton Methodology, Collection & identification-A field manual.

Haney, J.F. *et al*. "An-Image-based Key to the Zooplankton of North America" version 5.0 released 2013. University of New Hampshire Center for Freshwater Biology <cfb.unh.edu> 18 Nov 2014

Holt, M. S. (2000). Sources of chemical contaminants and routes into the freshwater environment. *Food and chemical toxicology*, *38*, S21-S27.

Huisman, J. (1999). Population dynamics of light-limited phytoplankton: microcosm experiments. *Ecology*, *80*(1), 202-210.

Jang, S. J., Baglama, J., & Rick, J. (2006). Nutrient-phytoplankton-zooplankton models with a toxin. *Mathematical and computer modeling*, *43*(1), 105-118.

Kannel, P. R., Lee, S., Lee, Y. S., Kanel, S. R., & Khan, S. P. (2007). Application of water quality indices and dissolved oxygen as indicators for river water classification and urban impact assessment. *Environmental Monitoring and Assessment*, *132*(1-3), 93-110.

Kimmerer, W.J., Nicolini, M.H., Ferm, N. and Peñalva, C., 2005. Chronic food limitation of egg production in populations of copepods of the genus Acartia in the San Francisco Estuary. Estuaries, 28:541–550.

Kořínek, V., Saha, R. K., & Bhattacharya, T. (1999). A new member of the subgenus Sinobosmina Lieder, 1957: Bosmina tripurae sp. nov.(Crustacea, Cladocera) from India. *Hydrobiologia*, *392*(2), 241-247.

Krebs, C. J. (1994). Ecology. Addison-Wesley Educational Publishers, Inc., Menlo Park, CA.

Kreutzweiser, D. P., Back, R. C., Sutton, T. M., Thompson, D. G., & Scarr, T. A. (2002). Community-level disruptions among zooplankton of pond mesocosms treated with a neem (azadirachtin) insecticide. *Aquatic toxicology*, *56*(4), 257-273.

Lan, J., Hu, M., Gao, C., Alshawabkeh, A., & Gu, A. Z. (2015). Toxicity assessment of 4-Methyl-1-cyclohexanemethanol and its metabolites in response to a recent chemical spill in West Virginia, USA. *Environmental science & technology*, *49*(10), 6284-6293.

Lay, J. P., Schauerte, W., Peichl, L., Klein, W., & Korte, F. (1985). Influence of benzene on the phytoplankton and on Daphnia pulex in compartments of an experimental pond. *Ecotoxicology and environmental safety*, *10*(2), 218-227.

Li, J. (2005). Urban spill management planning in the Greater Toronto area. *Environmental Informatics Archives*, *3*, 67-75.

Lynch, M. 1977. Zooplankton competition and plankton community structure. Limnol. Oceanogr. 22: 775-777.

Marini, F. Rotifer Culture Requirements.

McQueen, D. J., Post, J. R., & Mills, E. L. (1986). Trophic relationships in freshwater pelagic ecosystems. *Canadian Journal of Fisheries and Aquatic Sciences*, *43*(8), 1571-1581.

Michaud, J. P. (1991). *A citizen's guide to understanding and monitoring lakes and streams*. Puget Sound Water Quality Authority.

Millward, R. N., Carman, K. R., Fleeger, J. W., Gambrell, R. P., & Portier, R. (2004). Mixtures of metals and hydrocarbons elicit complex responses by a benthic invertebrate community. *Journal of Experimental Marine Biology and Ecology*, *310*(1), 115-130.

Mount, D. R., Gulley, D. D., Hockett, J. R., Garrison, T. D., & Evans, J. M. (1997). Statistical models to predict the toxicity of major ions to Ceriodaphnia dubia, Daphnia magna and Pimephales promelas (fathead minnows). *Environmental Toxicology and Chemistry*, *16*(10), 2009-2019.

Moyer, E., & Kostecki, P. T. (Eds.). (2004). *MTBE remediation handbook*. Springer Science & Business Media.

National Center for Biotechnology Information. PubChem Compound Database; CID=118193, https://pubchem.ncbi.nlm.nih.gov/compound/118193 (accessed Apr. 10, 2016)

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

Nematode (Caenorhabditis Elegans) Toxicity. *National Toxicology Program*. 10 Mar. 2015. Web. 10 Apr. 2015

Office of Environment and Hertiage. (2014). Water Quality. Retrieved May 2014.

Office of Response and Restoration. (2015). Freshwater Restoration. Retrieved March 2016.

Ott, F. S., Harris, R. P., & O'hara, S. C. M. (1978). Acute and sublethal toxicity of naphthalene and three methylated derivatives to the estuarine copepod, Eurytemora affinis. *Marine Environmental Research*, *1*(1), 49-58.

Parsons, F., Wood, P. R., & DeMarco, J. (1984). Transformations of tetrachloroethene and trichloroethene in microcosms and groundwater.*Journal (American Water Works Association)*, 56-59.

Postel L, Fock H, Hagen W (2000) 4 - Biomass and abundance. In: Huntley RHWLRS (ed) ICES Zooplankton Methodology Manual. Academic Press, London, pp 83- 192.

Proosdij, A. S., Sosef, M. S., Wieringa, J. J., & Raes, N. (2015). Minimum required number of specimen records to develop accurate species distribution models. *Ecography*.

Rand, G.M. (1995). Fundamentals of aquatic toxicology: *Effects, Environmental Fate, and Risk Assessment*

Relyea, R. A. (2005). The impact of insecticides and herbicides on the biodiversity and productivity of aquatic communities. *Ecological applications*, *15*(2), 618-627.

Relyea, R., & Hoverman, J. (2006). Assessing the ecology in ecotoxicology: a review and synthesis in freshwater systems. *Ecology Letters*, *9*(10), 1157-1171.

Riera, Steven F. jr., "Alkyl Polyglucoside Compound Influences Freshwater Plankton Community Structure in Floating Mesocosms" (2015). Electronic Theses & Dissertations. Paper 1281. http://digitalcommons.georgiasouthern.edu/etd/1281

Rudolf, A., Ahumada, R., & Pe'rez, C. (2002). Dissolved oxygen content as an index of water quality in San Vicente Bay, Chile (36 degrees, 450S). *Environmental Monitoring and Assessment*, 78, 89–100.

Sanchez-Bayo, F. (2006). Comparative acute toxicity of organic pollutants and reference values for crustaceans. I. Branchiopoda, Copepoda and Ostracoda. *Environmental pollution*, *139*(3), 385-420.

Stiff, M. J. (1971). The chemical states of copper in polluted fresh water and a scheme of analysis to differentiate them. *Water Research*, *5*(8), 585-599.

TOXNET. (2014). 4-Methylcyclohexanemethanol database. Retrieved June 2014.

U.S. Coast Guard National Response Center. 2014 Reports.

U.S. Energy Information Administration - EIA - Independent Statistics and Analysis. *U.S. Energy Information Administration (EIA)*. N.p., June 2014. Web. 11 Apr. 2015.

Whelton, A. J., McMillan, L., Connell, M., Kelley, K. M., Gill, J. P., White, K. D., & Novy, C. (2014). Residential Tap Water Contamination Following the Freedom Industries Chemical Spill: Perceptions, Water Quality, and Health Impacts. *Environmental Science & Technology*.

WV Department of Environmental Protection. (2014). Elk River Chemical Spill. Retrieved January 2015.

Yuan, L., Zhi, W., Liu, Y., Smiley, E., Gallagher, D., Chen, X., ... & Zhang, H. (2016). Aerobic and anaerobic microbial degradation of crude (4-methylcyclohexyl) methanol in river sediments. *Science of The Total Environment*, *547*, 78-86.

Table 1. Classification of zooplankton taxonomic groups found in the constructed pond located on the Georgia Southern University campus, Statesboro, GA, USA.

Common Name	Scientific Classification	Size Range (mm)	Trophic level
Copepod	Cyclopoid	1.2-3	Predator
Copepod	Calanoid	1-1.5	Predator
Cladoceran	*Daphnia* sp.	1 to 5	Herbivore
Cladoceran	*Bosmina* sp.	0.4 -0.6	Herbivore
Rotifer	Brachionidae.	0.1-0.5	Herbivore

Table 2. PERMONVA pairwise comparisons of plankton communities across the 4 different MCHM treatments after one and two weeks of exposure (n=4).

Weeks	Treatments	Permutation P-Values	t-val
1	0, 0.5	0.0297	2.578
	0, 1	0.0541	2.3352
	0, 3	0.8243	0.44379
	0.5, 1	0.801	0.75865
	0.5, 3	0.029	3.4053
	1, 3	0.0289	2.6146
2	0, 0.5	0.0311	2.1158
	0, 1	0.0308	3.1247
	0, 3	0.2004	1.3343
	0.5, 1	0.0285	1.9944
	0.5, 3	0.3394	1.0823
	1, 3	0.0287	2.0131

**Table 3.** Mean water column conductivity initially, and one week post MCHM addition ± one standard deviation (n=4).

Treatment Level (ppm)	Initial	Week 1
0	74.7 ±1.48	69.2 ±1.89
0.5	82.8 ±0.28	69.2±4.79
1	82.9 ±0.14	70.8 ±5.78
3	82.9 ±0.58	67.9±1.36

Table 4. Observed ranges of temperature, pH and dissolved oxygen initially, and one and two weeks post MCHM exposure (n=4).

	Treatment	Temperature °C	pH	Dissolved Oxygen
Week 0	0	19-20	8.1-8.2	6.7-7.0
	0.5	19-20	7.9-8.1	6.9-7.7
	1	19-20	7.8-7.9	6.5-7.0
	3	19-20	7.9-8	7.2-7.5
Week 1	0	15-16	7.5-7.7	7.3-7.5
	0.5	15-16	7.7-7.8	7.2-7.8
	1	15-16	7.9-7.9	7.2-7.6
	3	15-16	7.9	7.1-7.8
Week 2	0	10-11	7.7-7.9	7.9-8.0
	0.5	10-11	7.8-7.9	7.1-7.9
	1	10-11	8	7.6-8.1
	3	10-11	8	7.6-8.0
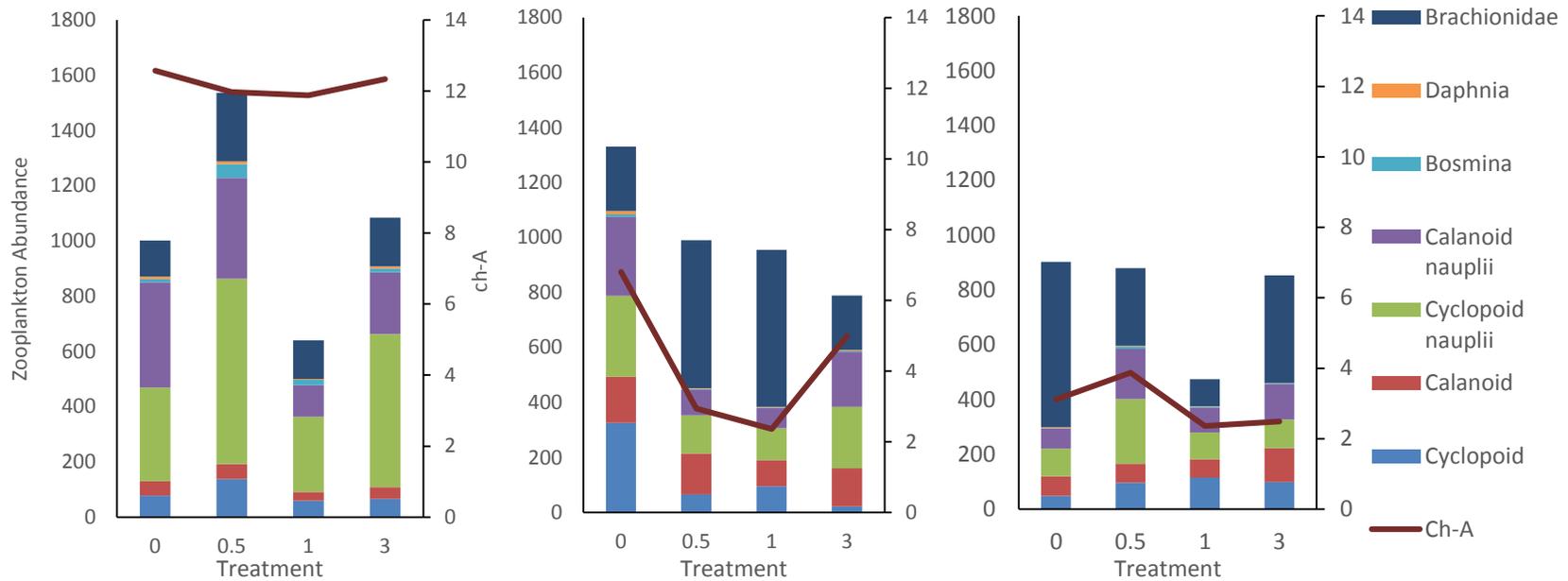
Figure 1. Mean abundance of individuals belonging to each zooplankton group and chlorophyll *a* concentration initially (A) and after 1 (B) and 2 (C) weeks of exposure to MCHM treatments (n=4).
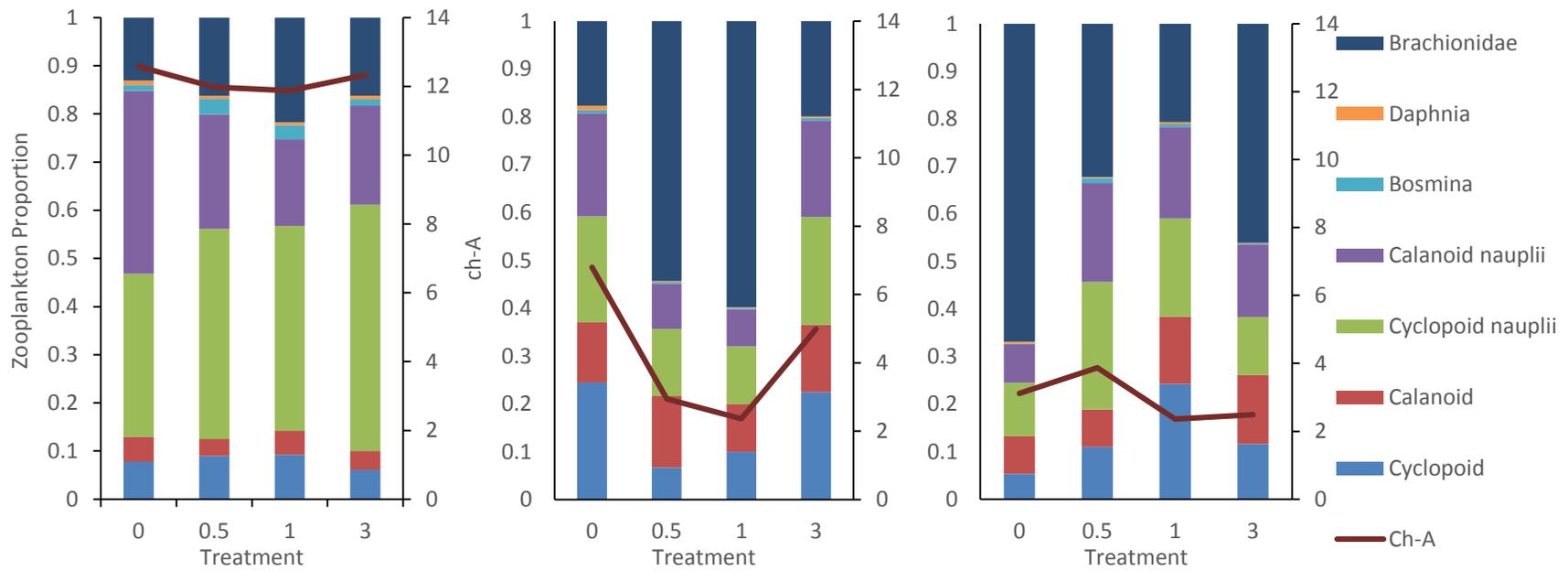
Figure 2. Average proportion of zooplankton groups within the community and chlorophyll *a* concentration initially (A) and after 1 (B) and 2 (C) weeks of exposure to MCHM treatments (n=4).