Effects of Oil Spills on Freshwater Zooplankton Communities: Insights from Laboratory Microcosms

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Abstract

Oil spills in aquatic environments have been of increasing concern in recent decades. Particularly, the Enbridge Line 5 pipeline that runs under the Mackinac Straits presents the potential for a disastrous oil spill that would affect over 1000 km of coastline. To assess the potential effects of an oil spill on zooplankton communities in the Great Lakes, two sets of microcosms, one for a Michigan inland lake and another for Lake Huron, were manipulated over an exposure period of three days each. The abundance of alive Cladocera was not observed to decrease as oil content in water increased in both microcosms. Hydrocarbon content within biomass was found to be significantly lower in our control group compared to experimental groups. Chlorophyll-a levels did not differ between control and experimental groups for both microcosms. These results suggest that a longer exposure time was needed to view any significant change in abundance of zooplankton and primary productivity. However, the increased hydrocarbons in biomass suggest that zooplankton were intaking oil into bodies and storing it. We believe a more extensive microcosm study of zooplankton communities is merited to further understand the impacts an oil spill would have on them.

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Oil spills in aquatic environments have been of increasing concern in recent decades. Particularly, the Enbridge Line 5 pipeline that runs under the Mackinac Straits presents the potential for a disastrous oil spill that would affect over 1000 km of coastline. To assess the potential effects of an oil spill on zooplankton communities in the Great Lakes, two sets of microcosms, one for a Michigan inland lake and another for Lake Huron, were manipulated over an exposure period of three days each. The abundance of alive Cladocera was not observed to decrease as oil content in water increased in both microcosms. Hydrocarbon content within biomass was found to be significantly lower in our control group compared to experimental groups. Chlorophyll-a levels did not differ between control and experimental groups for both microcosms. These results suggest that a longer exposure time was needed to view any significant change in abundance of zooplankton and primary productivity. However, the increased hydrocarbons in biomass suggest that zooplankton were intaking oil into bodies and storing it. We believe a more extensive microcosm study of zooplankton communities is merited to further understand the impacts an oil spill would have on them.

Introduction:

Oil spills and their effects on aquatic ecosystems have been an increasing cause of concern in recent decades. Many studies have been conducted on the effect past oil spills have had on aquatic ecosystems, but these have mostly been focused on marine ecosystems (Özhan *et al.*, 2013; Almeda *et al.*, 2013; Won Jung *et al.*, 2010; Bence *et al.*, 1996; Anderson *et al.*,

1974). Only a few studies have been conducted with freshwater ecosystems (Perhar and Arhonditsis, 2014; Klerks, 2004; Bhattacharyya *et al.*, 2003) which are also at great risk of oil spills. One particular system at great risk are the Great Lakes in North America.

The Great Lakes contain 21% of the world's freshwater by volume and are one of the most important commercial areas in the Western hemisphere. An estimated $375 billion in exports from the United States and Canada goes through the Great Lakes – St. Lawrence Seaway every year (Perhar and Arhonditsis, 2014). In the Mackinac Straits, a submerged section of the Enbridge Inc. Line 5 pipeline carries up to 20 million gallons of light crude oil, synthetic crude oil, and natural gas liquids under Lakes Michigan and Huron each day (Alexander and Wallace, 2013). A 2016 study done by the University of Michigan Water Center revealed that up to 1,000 km Lake Michigan-Huron coastline and large area of both lakes would be affected in the case of a Line 5 spill (Fig. 1) (Schwab, 2016). Such oil spills would have drastic effects on all trophic levels of aquatic life found in the Great Lakes (Perhar and Arhonditsis, 2014).

Zooplankton occupy one of the most important trophic levels in aquatic systems. They serve the role of connecting the primary producers, phytoplankton, to higher level consumers such as planktivorous fish (Saiz *et al.*, 2007). Their feeding, growth, and death also have a great influence on nitrogen and phosphorous cycling in aquatic ecosystems (Alcaraz *et al.*, 2010). Disruptions in these processes may significantly affect flows of mass and energy in the system (Alcaraz *et al.*, 2010). Studies have shown that zooplankton react adversely to oil exposures in both marine and freshwater systems (Perhar and Arhonditsis, 2014; Almeda *et al.*, 2013; Won Jung *et al.*, 2010; Klerks *et al.*, 2004; Bhattacharyya *et al.*, 2003; Federle *et al.*, 1972). Klerks (2004) suggested that toxicity was more intense for water-column species, specifically *Cladocera*, than benthic species such as *Chironomidae*. Won Jung (2013) saw *Copepoda* abundance drop dramatically at crude oil concentrations higher than 1,000 ppm. Federle (1972)

found overall loss in zooplankton abundance and primary production in tundra thaw ponds exposed to oil spills. Overall, the presence of petroleum hydrocarbons, found in all Line 5 oils, reduce egg production, increased mortality, and shift species of zooplankton (Perhar and Arhonditsis, 2014).

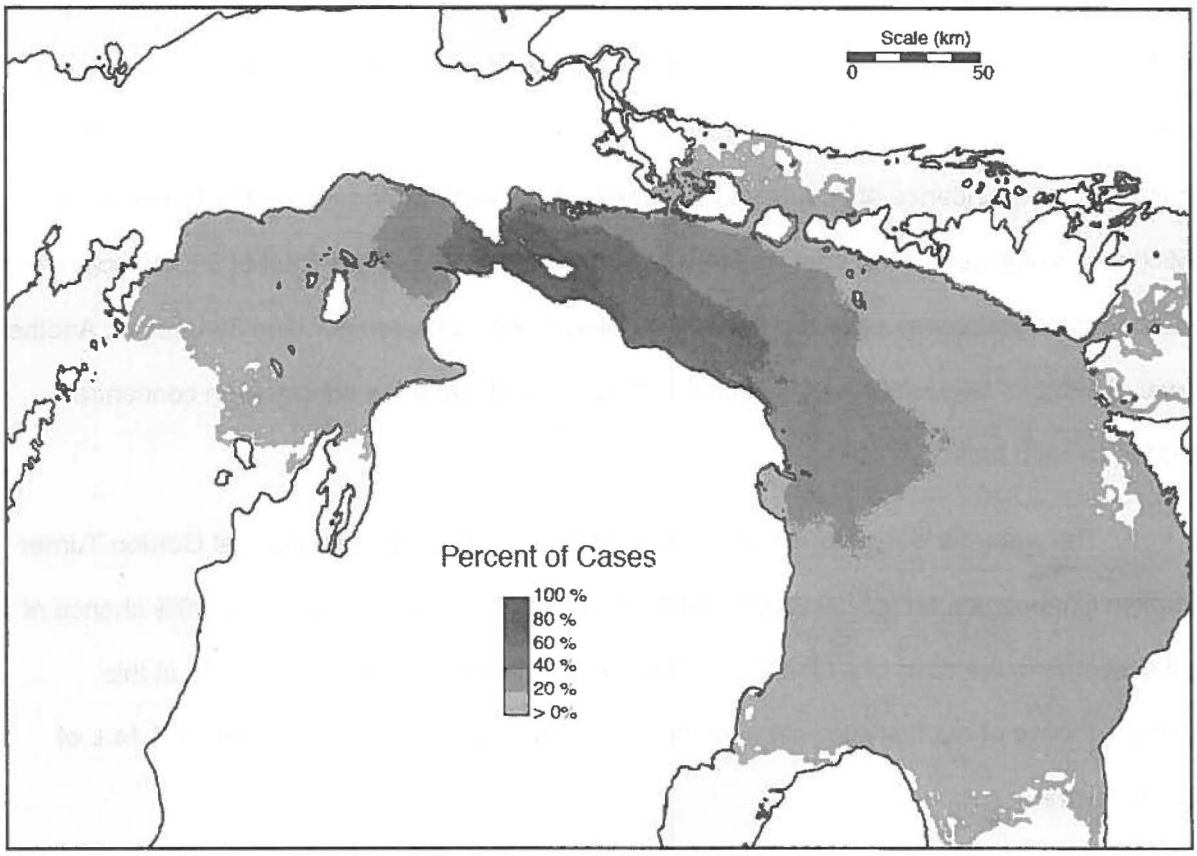

Figure 1. Percent of cases in which oil is present at *any* time after simulated release of oil from pipeline (Schwab 2016).

We investigated the effects a potential oil spill and the introduced load of petroleum hydrocarbons would have on both a Michigan inland lake and a Lake Huron zooplankton community. We sought to determine lethality of crude oil on zooplankton, whether zooplankton would absorb the crude oil and if primary productivity decreased after oil exposure. We expected that the exposure of our microcosms to oil hydrocarbons would decrease zooplankton abundance and primary productivity while increasing total hydrocarbon content in zooplankton.

Materials and methods:

Microcosm preparations

A total of two microcosms were ran, each for five days, for the purposes of the experiment. The first microcosm ran from June 22nd to June 27th, 2018 while the second microcosm ran from June 29th to August 3rd, 2018. Water would be sampled at dusk (from 9 – 11 PM ET). For the first microcosm, we sampled water from an inland, warm water lake (Douglas Lake) that is more eutrophic compared to Lakes Michigan and Huron (Lind and Lind, 1993) and has a higher abundance of zooplankton. Plankton tows were performed off the University of Michigan Biological Station boat well (45°33'38.3"N, 84°40'34.6"W). A total of 9 L of zooplankton concentrated water was collected to be distributed evenly between our nine 38-L tanks. Another total of 126 L of lake water was collected to fill tanks and dilute the zooplankton concentrate added to each tank.

The water for our second run was collected from the lighthouse point at Gordon Turner Park in Cheboygan, MI (45°39'28.0"N, 84°27'50.6"W). This area falls within an 80% chance of oil exposure in the case of a Line 5 spill (Fig. 1). Our methods for water sampling at this mirrored those of our first site with each tank receiving 1 L of concentrate water and 14 L of diluted water.

Oil preparation

Motor oil was utilized as a substitute for crude oil in our experiment. To increase diversity of oil treatments two concentrations of oil were used, 500 ppm and 1000 ppm. We added 7.5 g of oil to the 500 ppm tanks and 15 g to the 1000 ppm tanks per the measurements calculated by Boguski (2006).

Microcosm design and set-up

Microcosms comprised of nine tanks that contained 15 L of each of our site's freshwater and were set up in a controlled laboratory environment at UMBS where conditions were kept the same across all tanks with the exception of oil content. The nine tanks were divided into groups of three so as to have three replicates per experimental condition. All tanks had aquarium air pumps to ensure oxygenation of the environment and were set up against a set of three windows to provide solar energy to the system. Each group would have one tank at each window for a total of 3 tanks per window so as to keep light exposure constant across all experimental groups (i.e. each window had one control tank, a 500 ppm tank, and a 1000 ppm tank).

After water was added, zooplankton were left to settle and acclimate for 16 hours and zooplankton counts were taken for each tank. Then, oil was poured in via droppers and stirred gently into the tanks. Conditions remained unchanged for four days and zooplankton counts were taken each day.

Monitoring of zooplankton communities

Zooplankton were counted each day over a four-day period. One zooplankton count was taken for each tank prior to initial oil exposure. One count was done per day per tank for a total of three days after exposure to oil. Counts were taken at the same time each day. To take the water sample, a micropipette was used to obtain 1 mL of water at a time for a total of 3 mL for the Douglas Lake run and 15 mL for the Lake Huron run. Water was stirred before taking samples to homogenize all zooplankton within water. Samples would be placed in a petri dish and under a dissecting microscope where identified using a zooplankton key developed by the University of New Hampshire (2013). Each individual was differentiated between *Cladocera, Copepoda, Rotifera*, or other by physical morphology and was also marked as alive or dead.

Due to the small count of Copepoda and Rotifera, we chose to focus on Cladocera communities for our count results.

Oil absorption tests

On the fourth day after exposure, 1 L water samples were taken from each tank to test for hydrocarbon content within biomass. Each tank's water sample was filtered through a glass fiber filter that collected all zooplankton biomass. The glass fiber filter was then put in a vial filled with acetone where it would dissolve. Total milligrams of hydrocarbons were obtained from a gas chromatographer. A one -way ANOVA and Tukey post hoc were done to determine if there were any significant differences between the mean hydrocarbon content of our control and experimental tanks.

Chlorophyll levels

On the fourth day after exposure, 250 mL water samples were taken from each tank to test for chlorophyll-a within the water, which is a measure of primary productivity. Water samples were passed through a glass fiber filter. Glass fiber filters were once again dissolved in an acetone solution. Chlorophyll-a was then determined by EPA Method 447 (Arar, 1997). After readings were obtained for each tank, a one-way ANOVA and Tukey post hoc was done to determine if there were any significant differences between the mean chlorophyll-a levels of the control and experimental tanks.

Results:

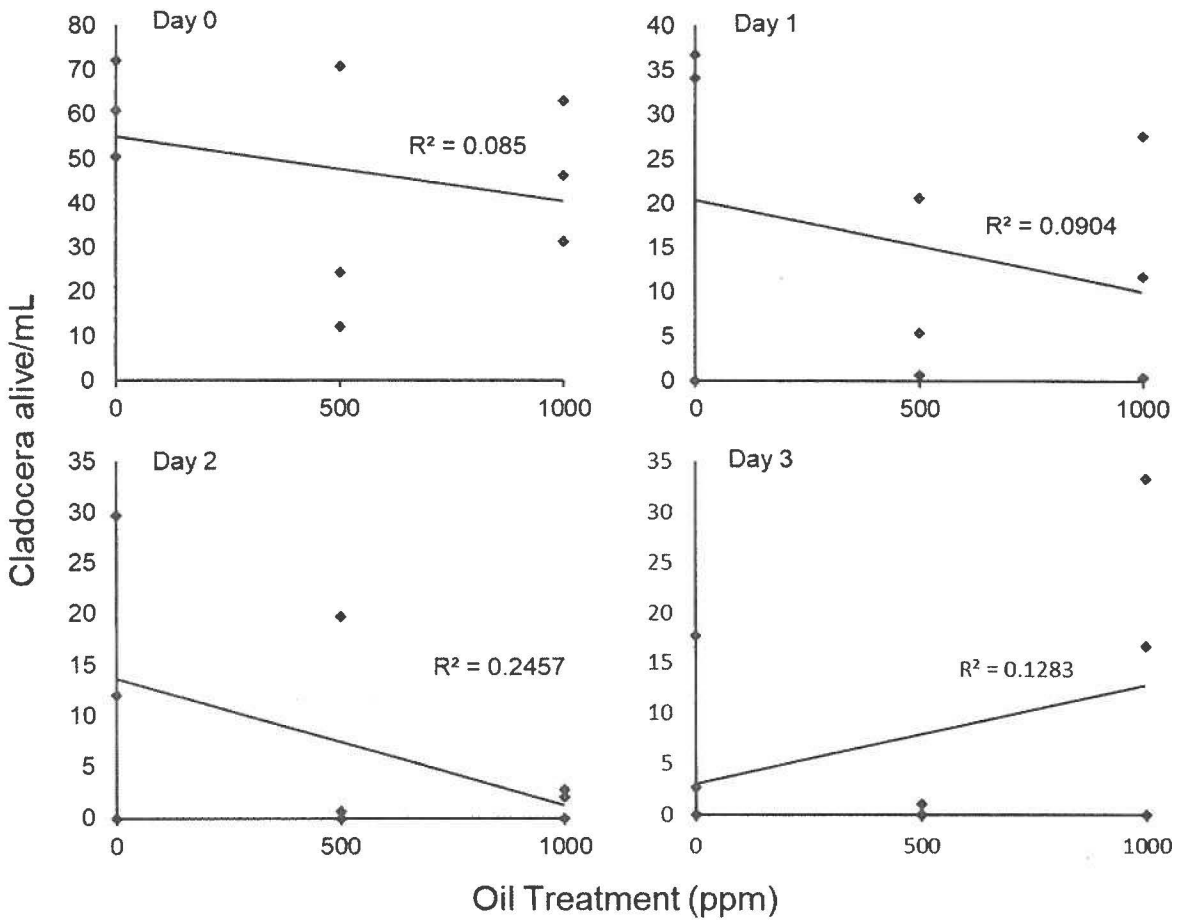

Figure 2. Alive Cladocera counted per mL per treatment per day for Lake Douglas exposure. Three values of counts are seen for each treatment representing each of the three tanks subjected to respective treatment. R^2 values are shown for each trendline. No significant relationship was found between abundance of alive *Cladocera* and treatment group on any day (p = 0.34, 0.43, 0.17, and 0.34 respectively)

Cladocera communities (Lake Douglas exposure)

Alive counts per mL for day 0 (day before oil exposure began) did not exhibit a significant relationship between Cladocera abundance and treatment group (p = 0.34). There was no significant trend as oil concentrations increased on the number alive Cladocera found per treatment for days 1, 2, and 3 after oil exposure (p = 0.43, p = 0.17, and p = 0.34 respectively) (Fig. 2).

Cladocera communities (Lake Huron exposure)

Alive counts per mL for day 0 (day before oil exposure began) did not exhibit a significant relationship between Cladocera abundance and treatment group ($p = 0.76$). There was no significant trend as oil concentrations increased on the number alive Cladocera found per treatment for days 1, 2, and 3 after oil exposure ($p = 0.37$, $p = 0.48$, and $p = 0.45$ respectively) (Fig. 3).

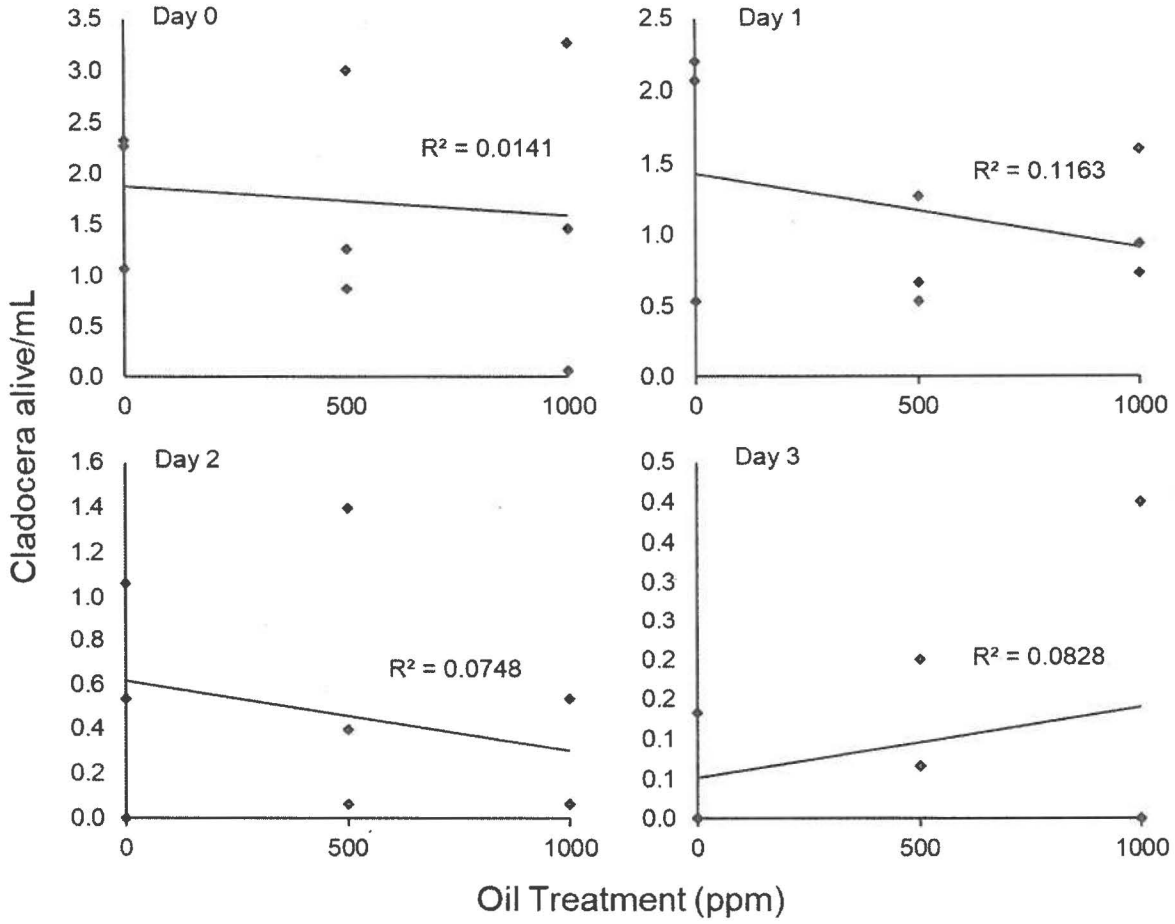

Figure 3. Alive Cladocera counted per mL per treatment per day for Lake Huron exposure. Three values of counts are seen for each treatment representing each of the three tanks subjected to the respective treatment. R^2 values are shown for each trendline. No significant relationship was found between abundance of alive *Cladocera* and treatment group on any day ($p = 0.76$, 0.37, 0.48, and 0.45 respectively).

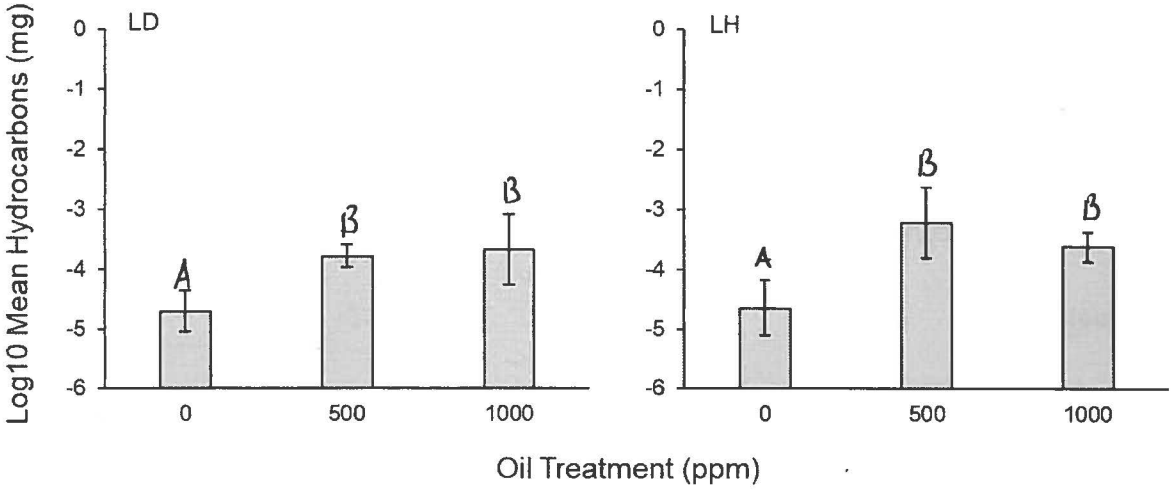

Figure 4. Hydrocarbon content (mg) measured for each treatment for both Lake Douglas (LD) and Lake Huron (LH) exposure. Values are means with error bars representing 2 standard errors from the mean. Different letters indicate a significant difference between them.

Hydrocarbon content

Mean hydrocarbon content (mg) was found to be significantly different between our samples for both Lake Douglas and Lake Huron ($p = 0.023$ and $p = 0.011$ respectively). A Tukey post hoc revealed that the control group (A) had a significantly lower hydrocarbon content compared to our 500 ppm (B) and 1000 ppm (B) groups ($p = 0.010$ and $p = 0.042$ respectively) (Fig. 4).

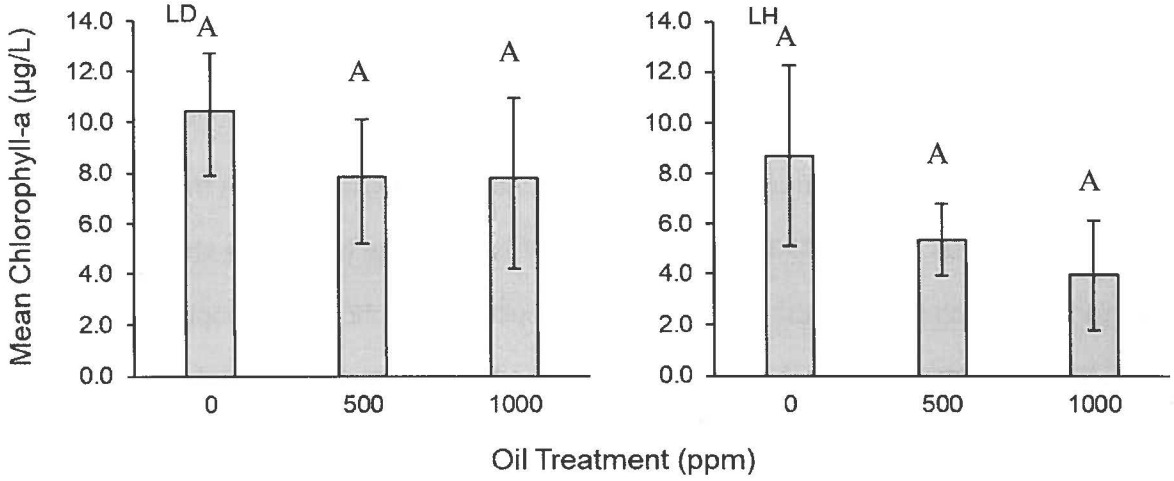

Figure 5. Chlorophyll-a measured for each treatment for both Lake Douglas (LD) and Lake Huron (LH) exposure. Values are means with error bars representing 2 standard errors from the mean. Different letters indicate a significant difference between them. No significant difference found for both Lake Douglas and Lake Huron ($p = 0.41$ and $p = 0.093$ respectively)

Chlorophyll Levels

Mean chlorophyll-a levels (µg/L) were not significantly different between treatments for both Lake Douglas and Lake Huron ($p = 0.41$ and $p = 0.093$). Mean chlorophyll-a levels seemed to be higher in the control compared to experimental groups (Fig. 5).

Discussion:

We believe the lack of a significant decrease in abundance of alive *Cladocera* this to be due to the short duration of both exposures as abundance of alive Cladocera was only taken up to three days after oil exposure and studies have shown that Cladocera would begin to die three to four days after oil exposure (Bhattacharyya *et al.*, 2003). Our exposure time might have been too short to start seeing significant differences between the control group and experimental groups regarding abundance of alive zooplankton.

Hydrocarbon content within zooplankton biomass was significantly higher in experimental groups for both Lake Douglas and Lake Huron which suggests that the zooplankton were intaking oil hydrocarbons into their bodies. Our results for this fall within the same as other studies that showed hydrocarbon absorption by zooplankton (Perhar and Arhonditsis, 2014; Klerks *et al.*, 2004). Initial exposure of zooplankton to oil hydrocarbons leads to rapid dilution by zooplankton until an equilibrium state is established where they will cease to intake hydrocarbons (Rotufo, 1998). Many species of zooplankton have even shown intaking hydrocarbons which then contaminate eggs and as such reduce fitness of zooplankton (Rotufo, 1998). Finally, hydrocarbon intake by zooplankton results in hydrocarbon transfer to higher trophic levels such as planktivorous fish but such transfers have been found to be minimal after digestion with minimal amounts reaching the circulatory system (Perhar and Arhonditsis, 2014).

Chlorophyll levels were not significantly different between control and experimental groups for both the Lake Douglas run and Lake Huron run. This may have been due to our low sample size, the number of tanks per group, played a big factor in this result as it conflicts with previous studies. The literature has suggested that crude oil reduced the levels of chlorophyll-a found in a sample (Baruah *et al.*, 2014; Özhan *et al.*, 2014). In addition to reduced concentrations of chlorophyll-a in samples, studies have also shown that phytoplankton communities undergo change in structure following an exposure to crude oil (Perhar and Arhonditsis, 2014). However, these studies were done in longer running experiments which suggest that our run time of three days was not enough to affect chlorophyll-a levels in our samples.

Overall, our limited sample size and short microcosm exposures seem to have played a factor in the results we obtained. We suggest running microcosms for longer durations and with an increased number of tanks per treatment to reduce data skewing from one tank as was apparent in a set of our Lake Douglas tanks. These suggestions should be taken into consideration for future studies done on zooplankton communities within the Great Lakes after an oil spill.

An oil spill from Line 5 at the Mackinac Straits would result in a massive environmental issue that would not only affect zooplankton and phytoplankton communities but every trophic level of the freshwater ecosystem. Everything from microscopic phytoplankton to large aquatic fauna such as birds and river otters. It has also been found that commonly used chemical dispersant cause even more damaging effects to the flora and fauna in these ecosystems (Perhar and Arhonditsis, 2014). Not only would an oil spill from Line 5 be disastrous to life in Lakes Michigan and Huron, they would also cause a huge economic impact as these lakes are of huge economic importance to both the United States and Canada. As a result, a clear understanding on potential oil spills in freshwater environments is critical to create management

plans in the case of disasters. Continued innovation in response tactics would also be needed so as to not cause more extensive damage to these systems via the use of chemical dispersants.

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