

IMPACTS OF HYDROCARBON SOLUTION ON FRESHWATER PLANKTON AND THE
IMPLICATIONS FOR LAKE SYSTEM COMMUNITIES

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Abstract

Zooplankton and phytoplankton are key members on which aquatic systems are supported. Cultivation of phytoplankton and zooplankton in motor oil contaminated tanks that simulate synthetic crude oil impacts the survival of the zooplankton *Cladocera* through consumption of hydrocarbons and potentially limits photosynthesis in algal communities. This was tested through subjecting water samples from Douglas Lake and Lake Huron with highly concentrated amounts of plankton to different oil concentrations (500ppm and 1000ppm). Counts of living *Cladocera* were taken for 4 days, and each tank was sampled and analyzed for hydrocarbon and chlorophyll content within plankton. Results revealed amounts of hydrocarbon consumed in experimental tanks and suggested lower levels of chlorophyll consumption in the same tanks, implying reduced ability of phytoplankton to photosynthesis and increased zooplankton death as a result of starvation.

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Introduction

The Straits of Mackinac are outlined as the 10km passageway of water that separates Michigan's upper peninsula from the lower and acts as an open connection between Lake Michigan and Lake Huron (Schwab, 2016). With an average depth of 20 m and width of 6 km at its narrowest point, the Straits provide a free exchange of both goods being hauled on shipping freighters and water carried by currents up to 70km into the main bodies of each lake (Anderson and Schwab, 2013). Current direction oscillated between lakes about every 2 to 3 days and flow can reach 1 m/s, with volumetric net flow of up to 80,000 m³/s (Anderson and Schwab, 2013). As a result of this connection, Lake Michigan and Lake Huron function as a singular lake instead of two, together containing almost 8% of the world's surface freshwater supply (Anderson and Schwab, 2013). Because of this, each lake plays an important role in the water quality, ecology, economic gains, and potential contamination of the other. Much of the area surrounding the Straits relies upon ecosystem services provided by the Great Lakes such as increased tourism and fishing in order to thrive.

To the West of the Mackinac Bridge that spans Michigan's peninsulas lies Enbridge Inc. Line 5 oil pipeline, which sits in the benthos and transports up to 20 million gallons of light crude oil, light synthetic crude oil, and natural gas beneath the waters of the Straits each day (Alexander and Wallace, 2013). As the pipeline ages, there is increased awareness and investigation of how a potential oil leak in this critical area would impact the surrounding area and the magnitude of the burden this would place on the ecosystem (Perhar, 2014). It is theorized that spills occurring in freshwater retain oil in sediments for up to as many as 5 years due to reduced energy in comparison to wave action found in coastal marine environments, amplifying the threat to Straits (Bhattacharyya *et al.* 2002).

Zooplankton and phytoplankton play a pivotal role within this ecosystem, functioning as primary and producers and consumers that support a larger food chain (Saiz *et al.* 2007). Understanding how intake of hydrocarbon from oil affects zooplankton impacts both how these chemicals could be bioaccumulated by larger organisms as well as inform how a clean-up procedure is chosen following an oil spill (Klerks *et al.* 2003; Lotufo, 1998).

Materials and Methods

Zooplankton Collection

Zooplankton, phytoplankton, and water were collected from Douglas Lake and Lake Huron at Cheboygan (Fig.1). The experiment was run twice, one time for each site where zooplankton were collected. Starting at 9:00 am, team members stood at the end of piers at each site and cast plankton nets three times before rinsing nets by dipping them into lake water vertically, and emptying water concentrated with zooplankton into buckets. Procedure was repeated until 9 liters of zooplankton concentrate was collected. 126 liters of non-concentrated water was collected as well. Nine 10 gallon tanks were each filled with fourteen liters of normally concentrated water and one liter of highly concentrated. One stone aquarium bubbler was connected to each tank to oxygenate organisms. Used motor oil functioned as substitute for crude oil due to its similar chemical properties (Payne *et al.* 1995). The experiment took place in a temperature controlled room kept at approximately 20 °C. Experiments were kept close to north facing windows to provide ample sunlight to phytoplankton in the tanks.

Experimental Procedure

1500 ml of used motor oil was added to three of the tanks to create a 1000 ppm concentration of oil in the tanks. This was repeated to three more tanks with 750 ml to make tank

concentrations of 500 ppm of oil. The last three tanks functioned as control tanks with no added motor oil in order to account for potential organism death resulting from factors apart from organism death. Disposable pipettes were used to take 15 ml samples from each tank. Samples were placed in clear petri dishes with a centimeter sized grid drawn on the back. Organisms found within each square of the grid were classified, moving from the square at the upper left corner then moving to the square to the right until the entire sample was counted. Initial counts of organisms were taken before any motor oil was added. Motor oil was added and counts were taken once every day for three days. Counts categorized organisms into *Cladocera*, *Copepoda*, *Rotifera*, and other and differentiated between if organisms in each category appeared to living or dead. Phytoplankton was also counted. After the allotted 4-day period, water samples were taken from each tank using a baster and contained in Nalgene bottles before running chemical analysis.

Chemical Analysis

Zooplankton was separated from water samples through fiberglass filters and analyzed for amounts of hydrocarbon and chlorophyll within the zooplankton to determine food and oil uptake. Hydrocarbon content and chlorophyll were detected from separate sample filters using gas chromatography.

Statistical Analysis

Each of the two trials was analyzed separately for statistics and each treatment of differing oil concentration was averaged for counts of living organisms. A linear regression was used to compare the average living counts of *Cladocera* to the concentration of oil they were exposed to. *Cladocera* were used because they were frequently samples during both test runs whereas other categories of zooplankton were not as abundant or even present in all samples

alive or dead. The assumptions of an ANOVA were tested using a Q-Q plot and a test of homogeneity of variances. An ANOVA was used to compare the mean of the natural log of hydrocarbons in the control and two treatment samples, and a Tukey post hoc test was used to identify significant differences. This process was repeated for testing the mean of natural log chlorophyll content. A p-value of 0.05 was used in all tests.

Results

Trial 1 Douglas Lake

We observed a negative relationship between living *Cladocera* per mL and the concentration of oil, excluding counts from day 3, and this trend was not significant (Fig.2). The mean of hydrocarbon content from the two experimental groups was significantly higher than the control (Tukey 500ppm $p=.044$, 1000ppm $p=.028$), through no discernable difference between content in the 500ppm and 1000ppm samples (Tukey $p=.926$) (Fig.3). The mean of chlorophyll content from the two experimental trials was lower than that of the control group, but ultimately were not statistically different from the control or from each other in the ANOVA test ($p=.268$) (Fig.4).

Trial 2 Lake Huron

Similarly, trial 1 results, there was a negative relationship between living *Cladocera* per mL and the concentration of oil in tank for all days except day 3, where we observed a positive relationship though none of these relationships were statistically significant (Fig.5). Like trial 1, the mean of hydrocarbon content from the two experimental groups was significantly higher than the control group (Tukey 5000ppm $p=.010$, 1000ppm $p=.042$), though there was no statistic difference between the two experimental tanks (Tukey $p=.471$) (Fig.3). The mean chlorophyll

content was lower than that of the control mean, and the differences bordered on statistical significance using an ANOVA test ($p=.062$) (Fig.4).

Discussion

Since Lake Douglas highly eutrophic compared to Lake Huron we expected the living counts of *Cladocera* to be less in trial 1 than trial 2 due to early onset suffocation (Lind and Lind, 1993). While overall number of *Cladocera* counted was much higher in the Douglas Lake trial than the Lake Huron trial, the results followed the same trend. Though results from both tanks were not significant for the relationship between living *Cladocera* per mL and oil content the observable trend followed prediction of increased oil correlating to a decrease in living organisms found. The slightly higher counts from the control group on day 0 when no oil had been added may have been due to inconsistencies when counting or inconsistency concentration of organism density in the water when water was first transfer to each tank. Since water was homogenized and alternately poured in each of the 9 tanks in random order, it is likely that bias during sample counting impacted the results. In order to combat this bias, counters could have been given samples instead of collecting them in order to make counts blind and reduce inflation of control counts. A possible explanation of how there was an observed positive relationship between alive *Cladocera* per mL and oil content could be that increased movement of *Cladocera* toward light in an attempt to escape oil made them both easier to identify as alive and counted multiple times due to their high mobility across the viewing area, inflating counts.

Hydrocarbon results were expected and indicate that *Cladocera* eventually consumed oil likely attached to algae instead of dying from affixation before any foreign chemicals entered these organisms. Since there was no significant difference between the 500ppm treatment and the

1000ppm in either trial, it may have been valuable to run the experiment with a lower amount of oil in order to define a concentration level at which *Cladocera* were able to avoid consuming hydrocarbons.

Though not statistically significant, the reduced amount of chlorophyll consumed in the samples from both trials could have been the consequence of *Cladocera* trying to consume as little as possible from their detection of a chemical substance or because *Cladocera* were killed before they had the opportunity to eat as much as individuals in the control tanks. In a study of the effect of crude oil contamination on chlorophyll production of plants it was found that chlorophyll content of leaves grown in oil was lower than that of control plants (Baruah *et al.* 2014). If algae in the two treatments was unable to photosynthesize, *Cladocera* could have been limited by unavailability of a food supply as oil limited algae growth.

Though further testing would be required to understand how the toxicity of oil altered the relationship between the primary producers and primary consumers in this system, the results suggest a multi-tiered reduction in survival and function in at least two trophic levels. The question of how a release of oil into a freshwater system might affect other players in the system even higher up the trophic scale could be further investigated and highly valuable to understanding potential ecosystem threats.

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Tables and Figures

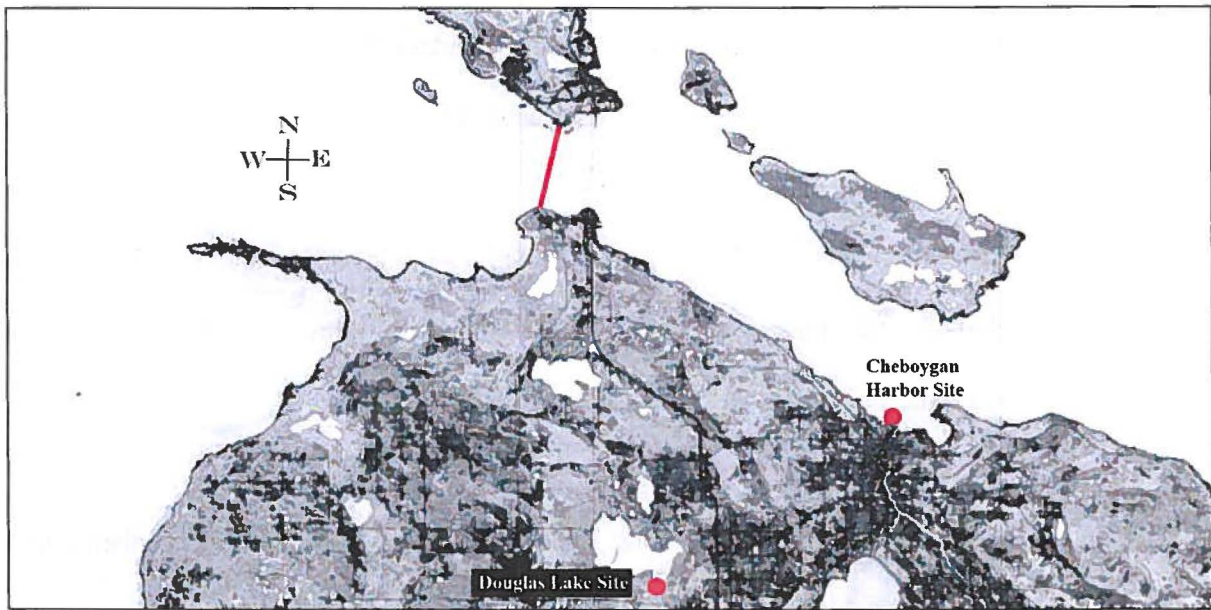


Figure 1. Map of zooplankton sampling sites (red circles) in the northern lower peninsula of Michigan. The location of Pipeline 5 is represented by the red line.

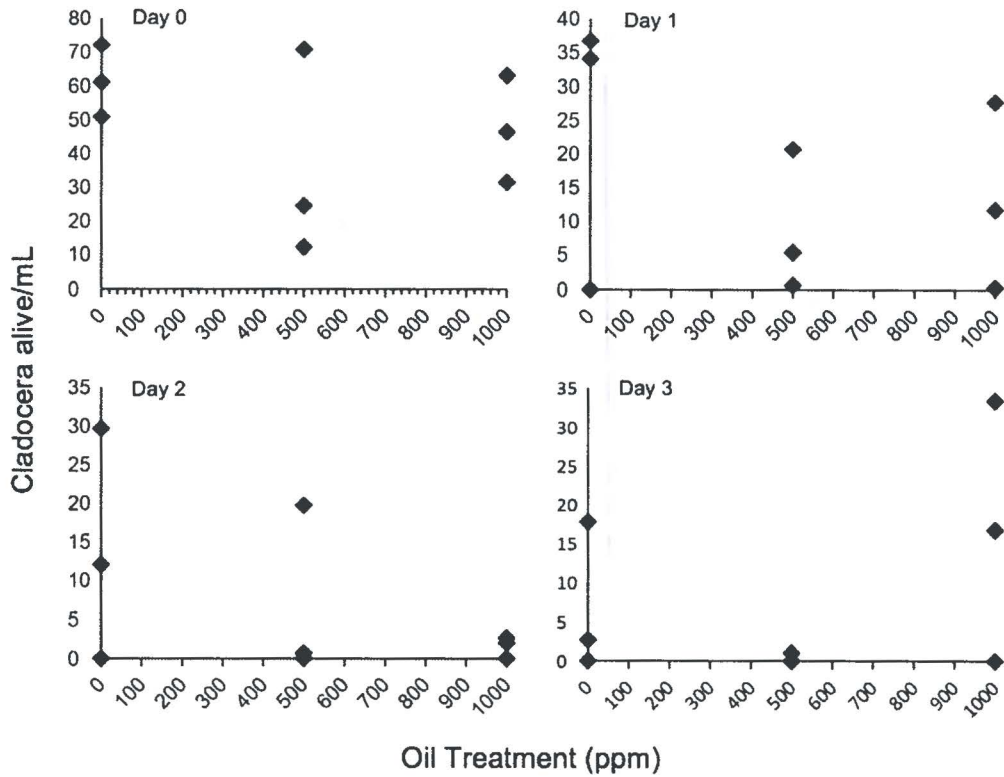


Figure 2. Regression from initial run of the experiment (Douglas Lake) of living *Cladocera* in each oil exposure, 0ppm, 500ppm, and 1000ppm. Black squares represent the amount of living *Cladocera* (y-axis) to the oil exposure amounts (x-axis) for each tank per day.

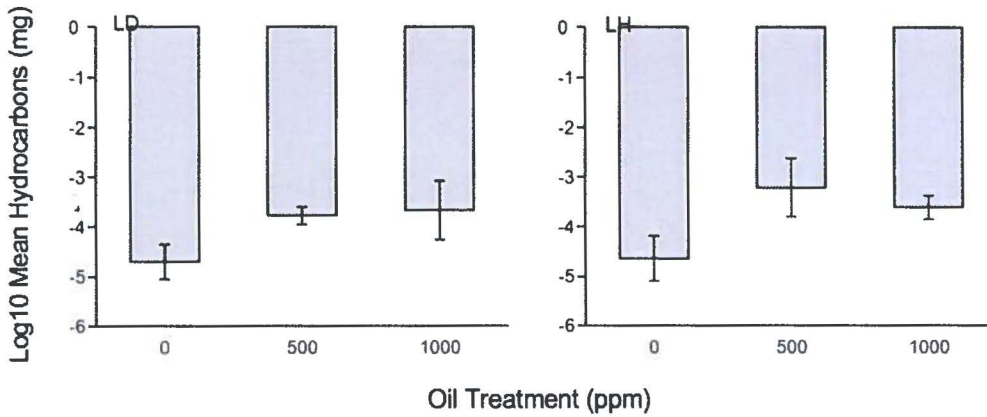


Figure 3. Box plots comparing the mean natural log of hydrocarbons (y-axis) found in the control and two experimental treatments (x-axis). Douglas Lake (LD) is on the left and Lake Huron (LH) on the right. Standard error whiskers are shown for each treatment.

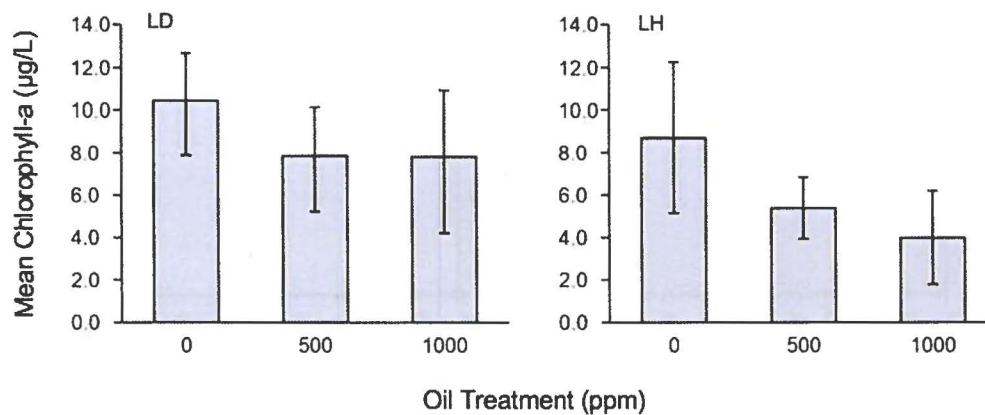


Figure 4. Box plots comparing the mean natural log of chlorophyll (y-axis) found in the control and two experimental treatments (x-axis). Douglas Lake (LD) is on the left and Lake Huron (LH) on the right. Standard error whiskers are shown for each treatment.

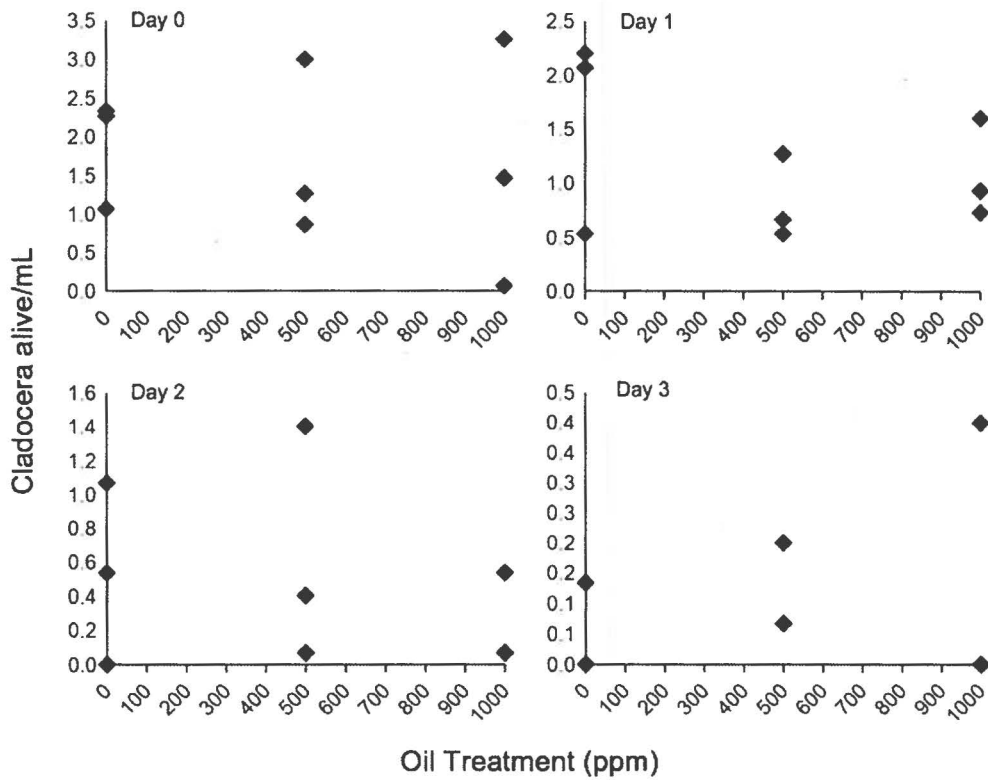


Figure 5. Regression from second run of the experiment (Cheboygan Harbor) of living *Cladocera* in each oil exposure, 0ppm, 500ppm, and 1000ppm. Black squares represent the amount of living *Cladocera* (y-axis) to the oil exposure amounts (x-axis) for each tank per day.