

# **A Case Study of Zooplankton Response to Oil Contamination as a Predicted Consequence of Pipeline 5 Failure in the Straits of Mackinac, Michigan**

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## **Abstract**

*Oil spills are known to harm aquatic environments, and the potential failure of an underwater oil pipeline, Line 5, threatens the Great Lakes. The objective of this study was increased understanding of freshwater oil exposure, looking at (a) zooplankton survivability; (b) zooplankton oil bioaccumulation; and (c) chlorophyll concentrations as phytoplankton health indicators. Two study sites were selected near Line 5 (Douglas Lake-Pellston and Lake Huron-Cheboygan). Water and organism samples were collected at each site and exposed for 3 days to 0 ppm, 500 ppm, and 1000 ppm of oil (in 3 oxygenated tanks per treatment per site). Zooplankton survival assessment (live counting) was done daily. Biomass filtering was done on the last day to assess zooplankton oil bioaccumulation and chlorophyll concentrations. Results showed significant correlations for zooplankton oil bioaccumulation at both sites, almost significant chlorophyll correlations at Lake Huron, and no significant correlations for zooplankton survivability at either site, perhaps due to sampling errors, insufficient exposure duration, and insufficient natural conditions in the laboratory. Results suggest that a Line 5 oil spill will negatively impact the Great Lakes, likely inhibiting phytoplankton and dependent trophic levels and facilitating oil bioaccumulation up the food chain.*

**Keywords:** *freshwater zooplankton, phytoplankton, oil spill, Great Lakes, Line 5.*

## Introduction

Oil spills are recognized ecological disasters, particularly in aquatic environments (Perhar & Arhonditsis, 2014). Many believe that the waters of the Great Lakes are threatened by the potential failure of an aging oil pipeline, known as Line 5, in northern Michigan, US (Groundwork Center for Resilient Communities, 2018). Line 5 is actually a set of underwater oil pipelines that lie exposed on the Great Lakes lake bed in the Straits of Mackinac. The pipelines are owned by a Canadian energy company, Enbridge Inc., that pumps nearly 23 million gallons of synthetic crude oil (SCO) through the pipelines and through the Great Lakes in one of the world's most ecologically sensitive areas between Michigan's upper and lower peninsulas (Groundwork Center for Resilient Communities, 2018). With increased awareness of the deteriorating state of the pipelines, there are growing concerns about an oil spill due to a pipe leak or break and associated consequences on the Great Lakes and its biological communities.

While real estate, tourism, industry, and commercial fishing are expected to be casualties of an oil spill (Groundwork Center for Resilient Communities, 2018), less is known about how the greater ecosystem would be affected when the communities at the bottom of the ecosystem food chain are impacted. Inhibitory and toxic effects of oil contaminants on aquatic systems, including phytoplankton and zooplankton, have been studied. Zooplankton largely comprise the base of the food chain and are prey to small fish, aquatic insects, and other zooplankton (New Zealand Government, 2018). Their health and fitness are vital to the health of an ecosystem, making them a strong representative for assessing the effects of contaminants on the surrounding community.

The objective in this study was to further examine the changes in freshwater zooplankton survival as a function of varying levels of oil contamination that might occur in the Great Lakes

with a Line 5 failure. We focused on examining zooplankton survival while also looking at zooplankton oil sequestration and chlorophyll concentrations as an indicator of phytoplankton health. Past research suggests that increased oil concentrations leads to: (1) decreased chlorophyll production/concentrations in terrestrial plants (Baruah *et al.*, 2014); (2) increased mortality of zooplankton (Almeda *et al.*, 2013); (3) bioaccumulation of many hydrocarbons in zooplankton, which then biomagnifies through the food chain (Almeda *et al.*, 2013); and (4) significant adverse effects when algal and copepod communities are subjected to oil concentrations at or above 1,000 ppm (Jung *et al.*, 2010). Based on the results of past research, it was hypothesized that increased oil concentrations would decrease the health of aquatic communities at two representative sites in the Great Lakes region near Line 5. We specifically predicted that oil contamination of freshwater samples taken from these two sites would (1) reduce the productivity of phytoplankton and chlorophyll concentrations in the samples; (2) increase zooplankton mortality and (3) increase bio-concentrations of hydrocarbons in zooplankton.

## **Methods and Materials**

### ***Oil Type***

Used motor oil was used in this study as a facsimile for the SCO that is pumped through Line 5 and transported to refineries for further processing into usable petroleum products (Petropedia Inc., 2018). The SCO in Line 5 is a mixture of complex hydrocarbons (known as bitumen) that are extracted from heavy oil and tar sands. Due to the unavailability of SCO for this study, alternatives were explored. It was concluded—following mass spectrometry analysis—that used motor oil was the best facsimile for SCO as a readily accessible and heavy

compound similar to SCO. Compared to other unused oil compounds, used motor oil consists of larger hydrocarbons, formed during the repetitive heating of the oil, which are more similar to the compounds in SCO. It should be noted that larger hydrocarbons have generally been shown to be less toxic to aquatic life than shorter hydrocarbons (Klerks *et al.*, 2003).

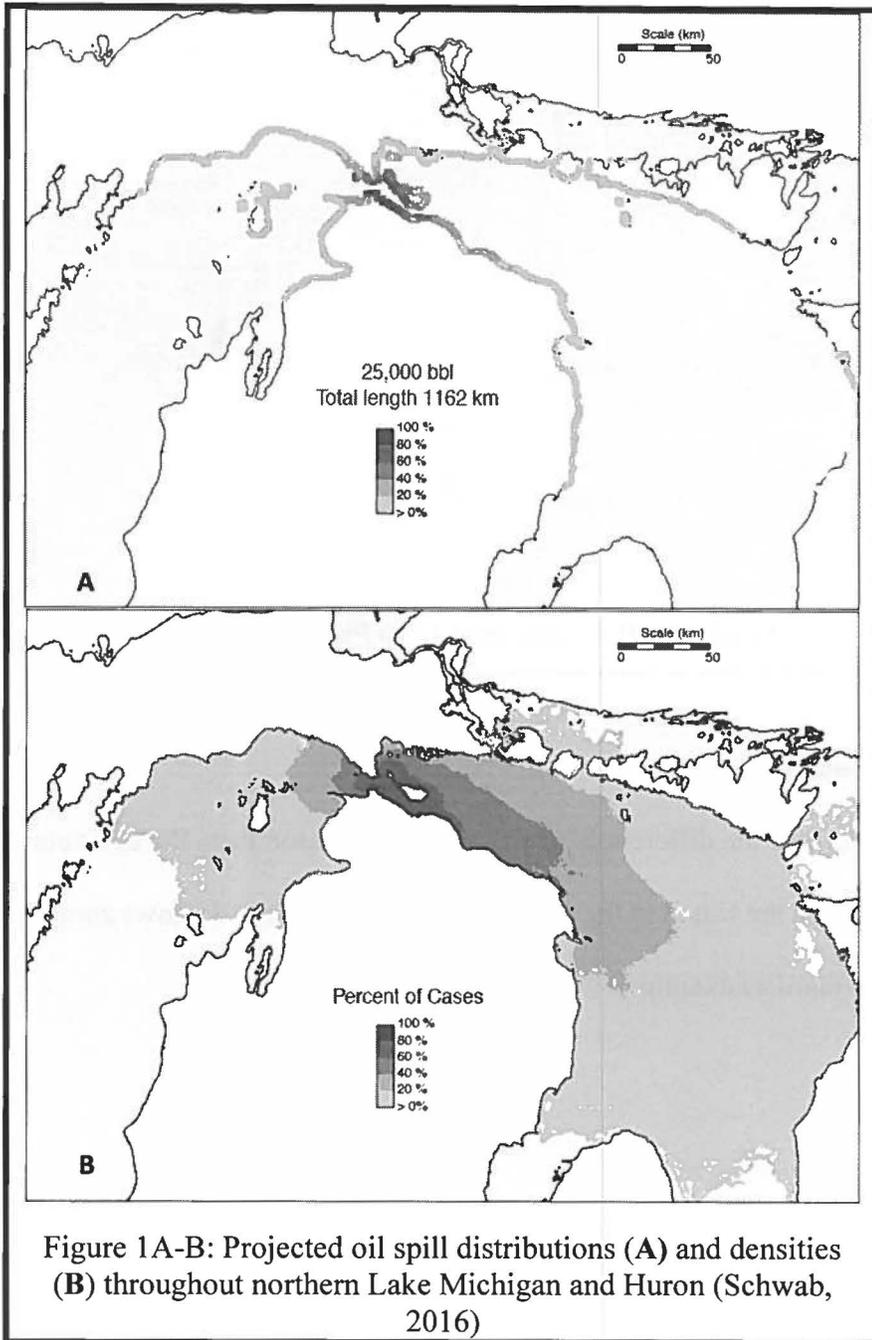
### ***Study Sites***

In this study, two northern Michigan sites were used for water sampling and phytoplankton and zooplankton collection: a representative Great Lakes site on Lake Huron near Cheboygan (near the Cheboygan pier) and a Douglas Lake site at the University of Michigan Biological Station (UMBS) near Pellston.

The Cheboygan site was chosen as a location that is predicted to be affected by a Line 5 failure. In the event of an oil spill in the Straits of Mackinac, it is estimated that 5,000 to 25,000 barrels of oil (bbl) would be released into the Great Lakes, impacting up to 700 miles of Lake Michigan and Lake Huron shoreline. According to the University of Michigan Water Center, the greatest impact and highest oil concentrations are projected to be in and around Mackinac Island, Bois Blanc Island, Mackinaw City, Cheboygan, Beaver Island, Cross Village, Harbor Springs, and other areas of the Lake Huron-Michigan shoreline (Figure 1a-b), (Schwab, 2016). Our selected site near the Cheboygan shoreline is therefore representative of an area that would experience oil contamination with a Line 5 failure.

Due to the oligotrophic nature of the Lake Huron exposure at the Cheboygan site, we selected a site at Douglas Lake (near Lakeside Lab) as a second sampling location (Figure 2). In comparison to Lake Huron, Douglas Lake has higher concentrations of phytoplankton and zooplankton and higher water temperatures. Despite the environmental and community

differences, the Douglas site was also reasonably representative of the aquatic microflora and microfauna in the Great Lakes and offered more accessibility to on-site researchers and opportunity for experimental adjustments if needed.





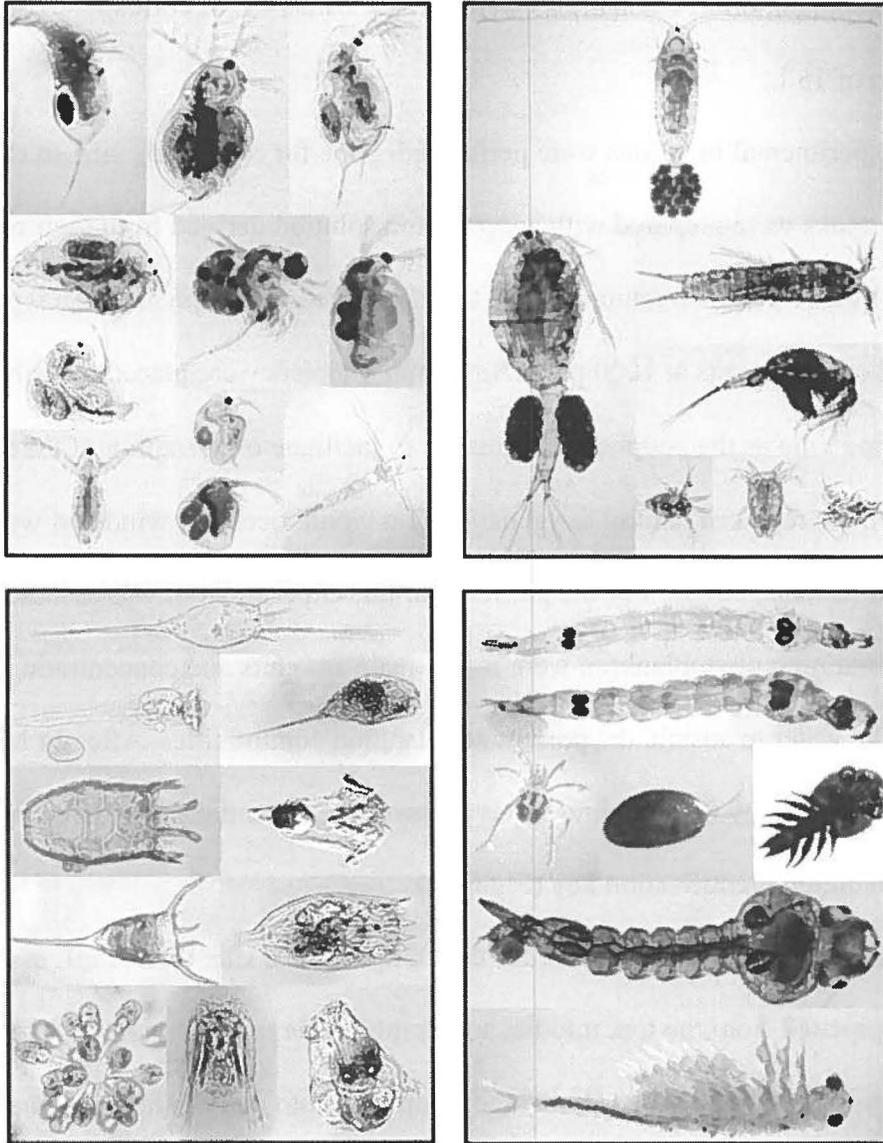


Figure 3: Zooplankton identification key with *Cladocera* (upper left), *Copepoda* (upper right), *Rotifera* (bottom left), and other Arthropods (bottom right) (Haney *et al.*, 2013)

### ***Experimental Setup and Design***

Water samples and zooplankton samples were collected from the two study sites at Douglas Lake and Lake Huron. Zooplankton samples were collected using plankton nets at night when zooplankton populations are at higher density near the surface (Zaret & Suffern, 1976).

Concentrated zooplankton in 1 L samples were diluted with 14 L of corresponding lake water for a total solution of 15 L.

Two experimental iterations were performed—one for each study site. In each iteration, nine aquarium tanks were prepared with zooplankton solution derived from their respective study site. Three tanks received no treatment, three tanks received oil treatments at 500 ppm, and three tanks received oil treatments at 1000 ppm. Aquarium bubblers were placed in each of the nine tanks at the same time as the zooplankton solution to facilitate oxygenation of the system. Aquarium bubblers replaced natural oxygenation that would occur by wind and wave activity in a larger system (Likens, 2010). For the purposes of this experiment, it was assumed that chlorophyll-containing phytoplankton were in adequate amounts and concentrations in the undisturbed lake water to sustain the present zooplankton communities. After 24 h of acclimation to the mesocosms, on Day 0, a baseline survival assessment was done for each of the aquariums, using the zooplankton identification key (Figure 3).

In the first experimental iteration, for the Douglas Lake site, three 1 mL assessment samples were pipetted from the top, middle, and bottom layers of each tank with zooplankton solution. The pipette contents were discharged, dropwise, into petri dishes and surviving zooplankton within the drops were examined and counted with dissecting microscopes.

In the second experimental iteration, for the Lake Huron site, each tank was homogenized prior to assessment sampling, and one 15 mL sample was pipetted from each tank and discharged into a petri dish with surviving zooplankton counted systematically in a grid pattern under microscopy. It was determined that a larger sample volume was necessary for the Lake Huron sampling because the zooplankton density was much lower in Lake Huron for the same volume of concentrate at Douglas Lake.

After the initial baseline survival assessment in both experimental iterations, used motor oil was weighed and added to six of the nine tanks, resulting in oil concentrations of 0 ppm in three tanks, 500 ppm in three tanks, and 1000 ppm in three tanks. The mesocosms in each tank were then examined daily. Daily community survival counts were taken using the assessment procedure described above for three days (Day 1-3). On the final day of experimentation (Day 3), after the final survival count, two additional zooplankton samples were taken from each tank for each site: (1) 1 L sample (in a Nalgene bottle) for zooplankton oil ingestion assessment and (1) 250 mL sample (in a Nalgene bottle) for chlorophyll content assessment. Due to the higher zooplankton concentration at Douglas Lake, only 200 mL samples were able to be filtered out of the 1 L sample for the zooplankton oil bioaccumulation assessment. Each sample was then vacuum filtered onto glass fiber filters and freeze dried to obtain a mass of the filtered contents for incorporation in the density calculations. Chemical analyses were run using gas chromatography, providing chlorophyll concentrations and oil bioaccumulation concentrations.

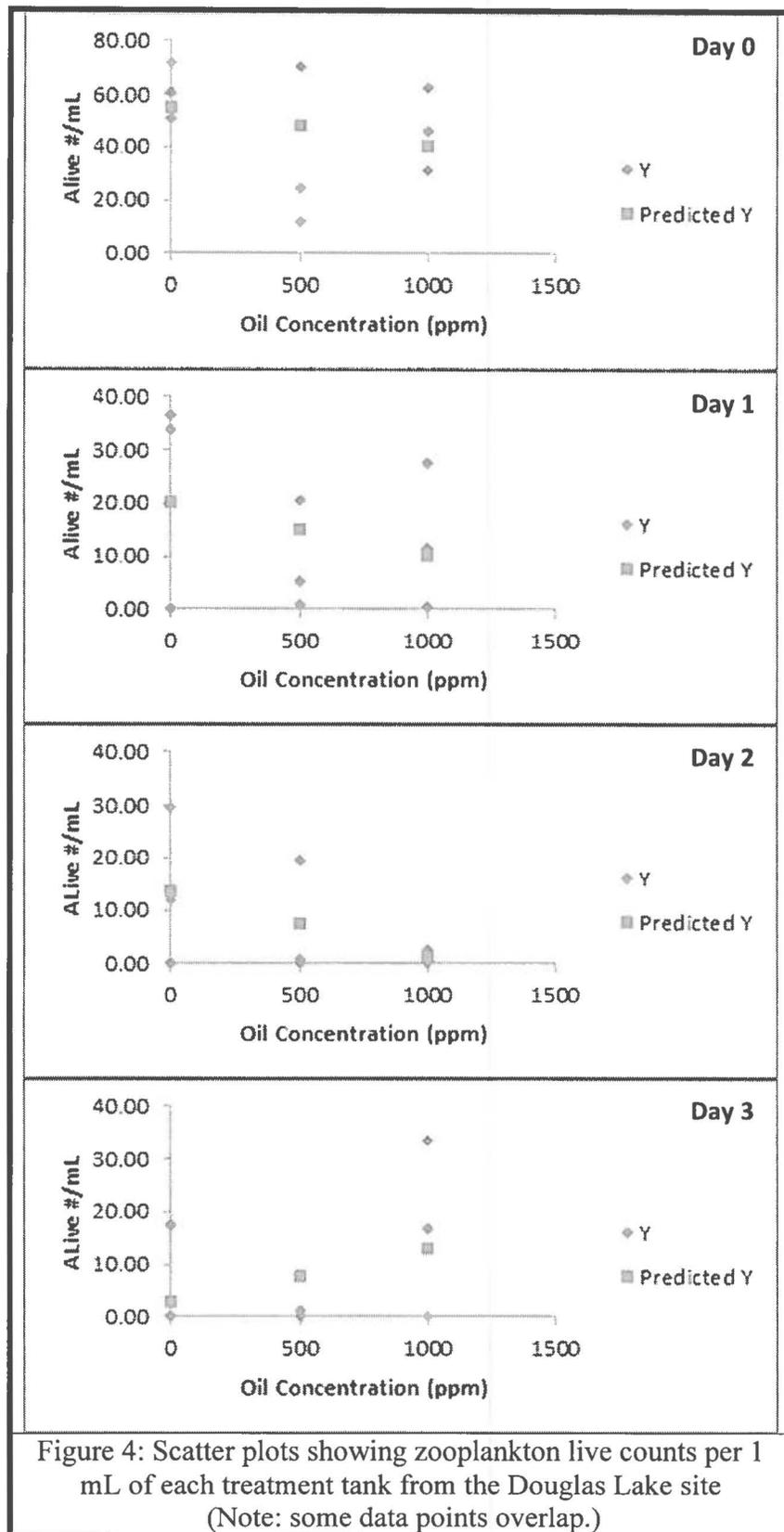
### ***Data Analysis and Statistics***

Using the survival data, scatter plots were established for the different treatment groups, illustrating the change in alive *Cladocera* per equal volume (1 mL) with increasing oil concentration. A linear regression was applied to each scatter plot to assess statistical correlation. ANOVAs were run, testing the difference in the mean concentrations of zooplankton oil bioaccumulation between treatment groups, as well as the difference in mean chlorophyll concentrations between treatment groups at each study site.

## Results

### *Zooplankton Mortality Rates*

Of the four freshwater zooplankton families studied--*Cladocera*, *Copepoda*, *Rotifera*, and other Anthropods—only *Cladocera* demonstrated sufficient live counts for statistical analysis. Linear regressions—run between treatment groups for each day measured—did not show significant trends that would support a relationship between increased oil concentrations and decreased alive counts or *Cladocera* ( $p > 0.05$ ). Figure 4 and Figure 5 show the linear regression between *Cladocera* alive counts and oil concentration for both study sites and between daily measurements.



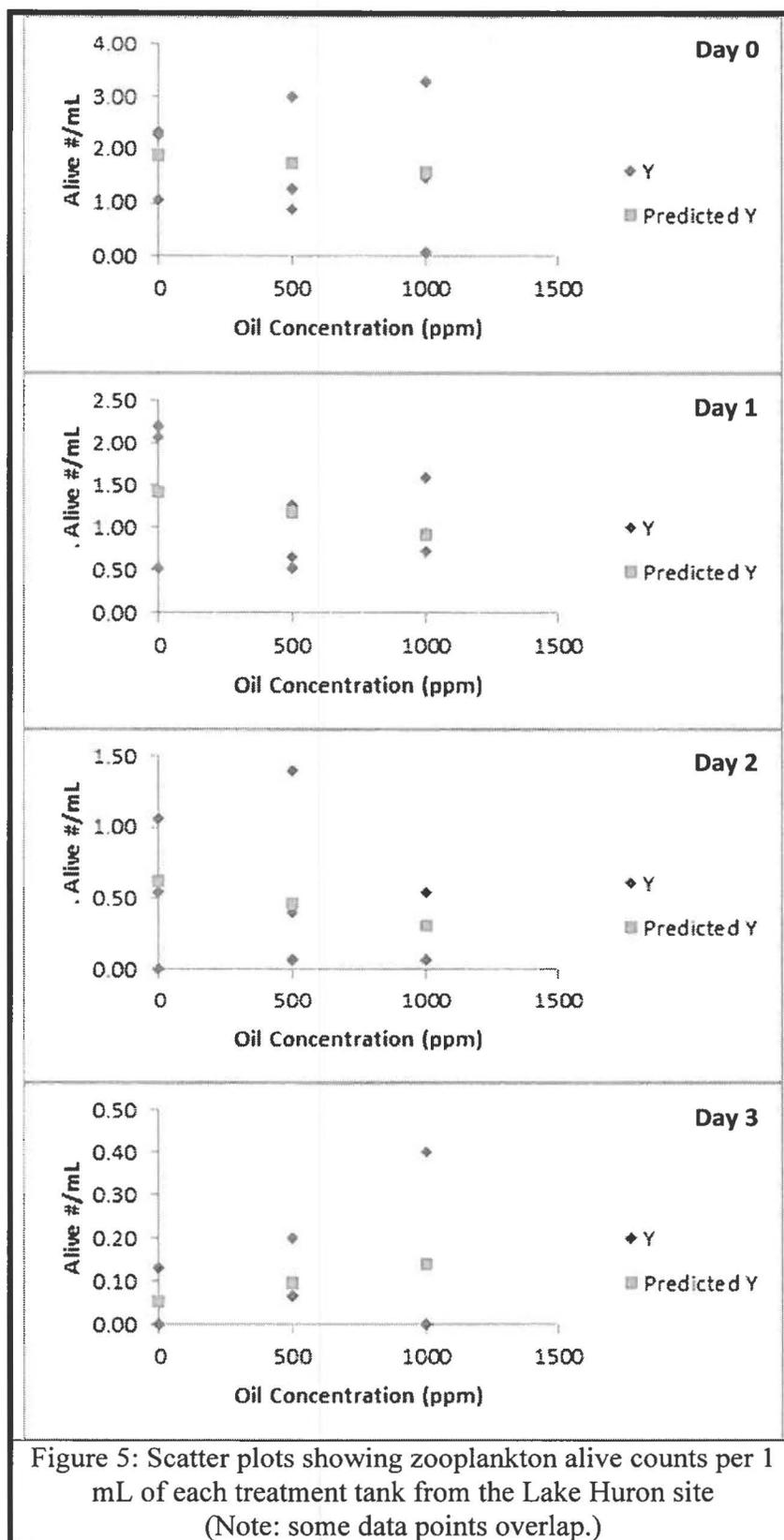


Figure 5: Scatter plots showing zooplankton alive counts per 1 mL of each treatment tank from the Lake Huron site (Note: some data points overlap.)

### *Zooplankton Hydrocarbon Contents*

Table 1 shows the statistical analyses for the differences in mean hydrocarbon contents between the three treatments. Control treatments exhibited significantly lower hydrocarbon contents when compared with the 500 ppm and 1,000 ppm treatments (Figure 6). The difference between the 500 ppm and 1,000 ppm treatments were statistically insignificant.

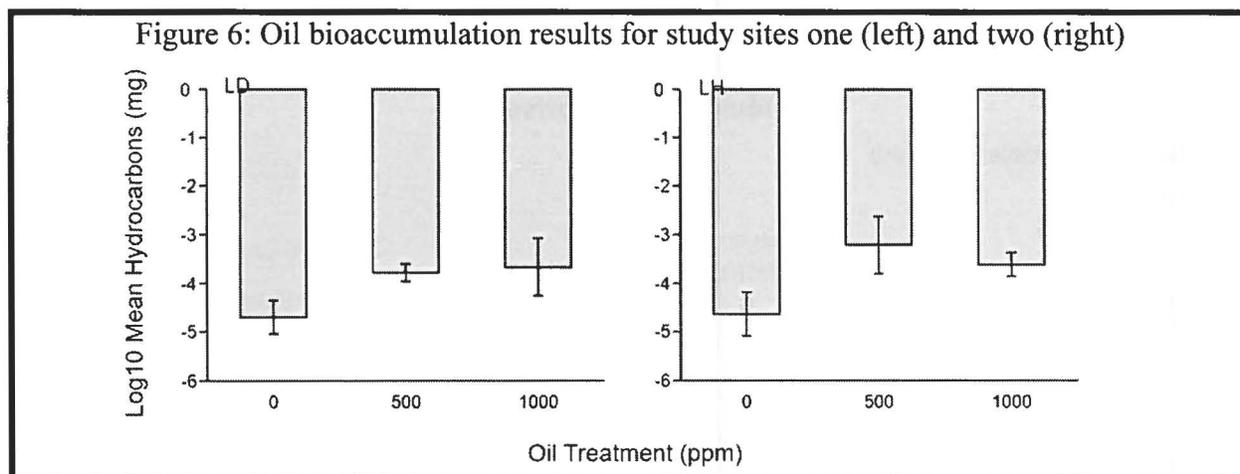


Table 1: Statistical analysis of oil content differences between treatments for the Douglas Lake site.						
<b>ANOVA</b>						
Log_mg						
	Sum of Squares	df	Mean Square	F	Sig.	
Between Groups	1.920	2	.960	7.593	.023	
Within Groups	.759	6	.126			
Total	2.679	8				
<b>Multiple Comparisons</b>						
Dependent Variable: Log_mg						
Tukey HSD						
(I) Sample_ID	(J) Sample_ID	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-.92052*	.29034	.044	-1.8114	-.0297
	3	-1.03000*	.29034	.028	-1.9208	-.1392
2	1	.92052*	.29034	.044	.0297	1.8114
	3	-.10948	.29034	.926	-1.0003	.7814
3	1	1.03000*	.29034	.028	.1392	1.9208
	2	.10948	.29034	.926	-.7814	1.0003

\*. The mean difference is significant at the 0.05 level.

Table 2 shows the statistical significance of the differences in mean hydrocarbon contents between the three treatments in Exposure 2. Like Exposure 1, the control treatments illustrated significantly lower concentrations of oil than the 500 ppm and 1,000 ppm treatments. Zooplankton oil concentrations were not significantly different between the 500 ppm and 1,000 ppm treatments.

Table 2: Statistical significance of oil content differences between treatments for the Lake Huron site

<b>ANOVA</b>						
Log_mg						
	Sum of Squares	df	Mean Square	F	Sig.	
Between Groups	3.235	2	1.618	10.592	.011	
Within Groups	.916	6	.153			
Total	4.152	8				

<b>Multiple Comparisons</b>						
Dependent Variable: Log_mg						
Tukey HSD						
(I) Sample_ID	(J) Sample_ID	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-1.42333*	.31909	.010	-2.4024	-.4443
	3	-1.02518*	.31909	.042	-2.0042	-.0461
2	1	1.42333*	.31909	.010	.4443	2.4024
	3	.39814	.31909	.471	-.5809	1.3772
3	1	1.02518*	.31909	.042	.0461	2.0042
	2	-.39814	.31909	.471	-1.3772	.5809

\*. The mean difference is significant at the 0.05 level.

### ***Chlorophyll Concentrations***

The mean concentrations of chlorophyll were statistically insignificant for the Douglas site experimental iteration. Figure 7 shows the mean chlorophyll concentrations for both study sites.

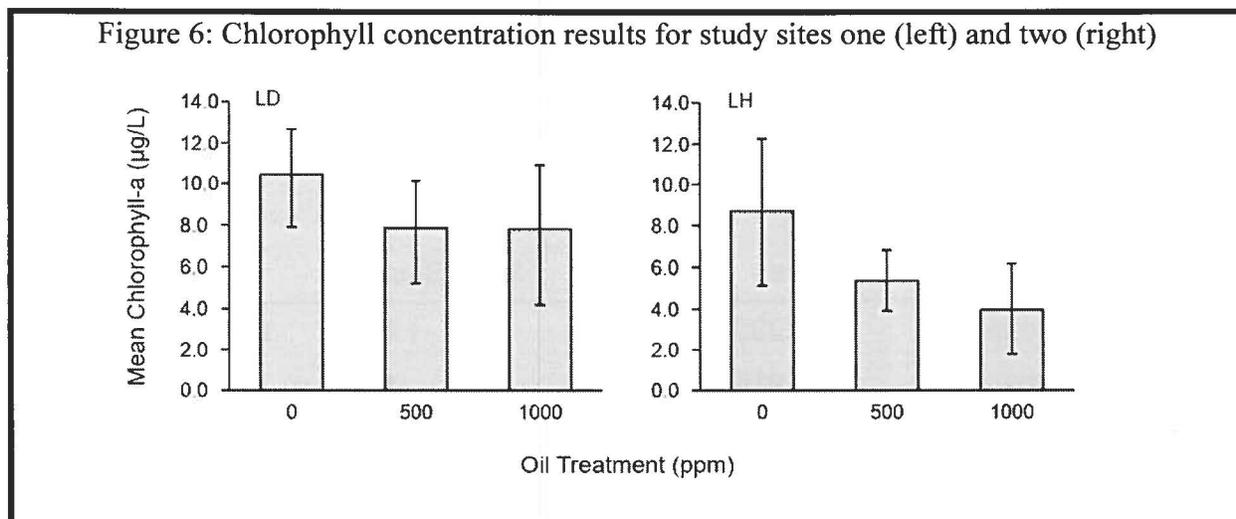


Table 3: Statistical analysis of chlorophyll content differences between treatments for the Douglas Lake site

**ANOVA**

Chlorophyll_A_ugL	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	13.777	2	6.889	1.039	.410
Within Groups	39.795	6	6.633		
Total	53.573	8			

In the second experimental iteration at the Lake Huron site, there was a weak correlation between increased oil concentrations and decreased chlorophyll content ( $p = 0.093$ ). The control and 1,000 ppm treatments exhibited the most correlation ( $p = 0.088$ ). There were no conclusive statistical results within the 95% confidence interval for statistical significance.

Table 4: Statistical analysis of chlorophyll content differences between treatments for the Lake Huron site

<b>ANOVA</b>						
Chlorophyll_A_ugL						
	Sum of Squares	df	Mean Square	F	Sig.	
Between Groups	35.340	2	17.670	3.610	.093	
Within Groups	29.369	6	4.895			
Total	64.709	8				

<b>Multiple Comparisons</b>						
Dependent Variable: Chlorophyll_A_ugL						
Tukey HSD						
(I) Sample_ID	(J) Sample_ID	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	3.32667	1.80645	.235	-2.2160	8.8693
	3	4.72433	1.80645	.088	- .8183	10.2670
2	1	-3.32667	1.80645	.235	-8.8693	2.2160
	3	1.39767	1.80645	.731	-4.1450	6.9403
3	1	-4.72433	1.80645	.088	-10.2670	.8183
	2	-1.39767	1.80645	.731	-6.9403	4.1450

## Discussion

In this study, we hypothesized that there would be significant relationships between increased oil concentration and zooplankton survival, zooplankton oil bioaccumulation, and chlorophyll concentrations (as an indicator of phytoplankton health). Our data results only partially supported our hypotheses. We found significant differences in zooplankton oil bioaccumulation (at both sites) with positive correlations between increased oil concentrations and increased zooplankton oil bioaccumulation, these results support other research that found significant zooplankton oil ingestion when zooplankton were exposed to oil contaminants

(Almeda *et al.*, 2013). Ecologically, this poses a significant threat to organisms in higher trophic levels as it suggests that oil contamination can lead to biomagnification of hydrocarbons in communities like the Great Lakes.

We found a weak, but statistically insignificant ( $p = 0.09$ ), correlation between increased oil concentrations and decreased chlorophyll concentrations at the Lake Huron site and no correlation at the Douglas Lake site. Experimental error at the Douglas Lake site likely impacted expected chlorophyll reductions. For instance, a lack of homogenization of the concentrated zooplankton solution during the experimental setup likely skewed initial chlorophyll concentrations, as evidenced by a significant difference in green treatment coloration. Inadequate homogenization may also have been a factor for the Lake Huron zooplankton solution, reducing the significance of those results as well. Results at the Lake Huron site were almost significant, however, and other researchers have found significant negative correlations between oil exposure and phytoplankton and chlorophyll concentrations (Baruah *et al.*, 2014) such that it is likely that phytoplankton would be inhibited to some degree by oil exposure in the Great Lakes. It should be noted that some researchers have found that oil toxicity to phytoplankton is affected by oil dispersion time as well, which was not a factor in our study (and some phytoplankton have exhibited greater toxic responses to oil dispersants than to oil) (Ozhan *et al.*, 2014). No significant correlations were observed between oil concentration and zooplankton survival at either site. There are several possible explanations for why we did not obtain the full results expected, however. Comparable studies suggest that our exposure time (3 days) was too short to detect any delayed or longer-term effects of the oil contamination. In the study by (Federle *et al.*, 1979), significant reductions in zooplankton survival only occurred after four days of exposure. Another found that natural sun exposure increased the toxicity of oil on zooplankton, perhaps

indicating the need for better emulation of natural conditions in future experimentation (Almeda *et al.*, 2013).

Overall, our study indicated that an oil spill in the Great Lakes would likely have negative effects, likely inhibiting phytoplankton and dependent trophic levels and facilitating oil bioaccumulation up the food chain. Concerns therefore appear justified over the potential failure of Line 5, the underwater oil pipeline in the Great Lakes.

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