OIL SPILL CONCENTRATION EFFECTS ON ZOOPLANKTON MORTALITY, HYDROCARBON CONTENT, AND CHLOROPHYLL CONTENT

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Enbridge Line 5 is an oil pipeline that threatens the Great Lakes ecosystem. Zooplankton are at the heart of this ecosystem, as they are an important food source for many fish and are primary grazers that prevent harmful algal blooms. We simulated the effects of an oil spill on zooplankton from Douglas Lake and the Straits of Mackinac. We measured mortality of one order of zooplankton, as well as hydrocarbons and chlorophyll ingested by zooplankton in the tanks after four days. We hypothesized that an increase in oil concentration would be associated with an increase in hydrocarbons and chlorophyll, as well as an increase in zooplankton mortality. We found no significant difference in mortality of zooplankton or ingested chlorophyll, but hydrocarbon content significantly increased in zooplankton subjected to oil treatments, suggesting that zooplankton were ingesting the oil.

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Abstract

Enbridge Line 5 is an oil pipeline that threatens the Great Lakes ecosystem. Zooplankton are at the heart of this ecosystem, as they are an important food source for many fish and are primary grazers that prevent harmful algal blooms. We simulated the effects of an oil spill on zooplankton from Douglas Lake and the Straits of Mackinac. We measured mortality of one order of zooplankton, as well as hydrocarbons and chlorophyll ingested by zooplankton in the tanks after four days. We hypothesized that an increase in oil concentration would be associated with an increase in hydrocarbons and chlorophyll, as well as an increase in zooplankton mortality. We found no significant difference in mortality of zooplankton or ingested chlorophyll, but hydrocarbon content significantly increased in zooplankton subjected to oil treatments, suggesting that zooplankton were ingesting the oil.

Introduction

The devastating effects of oil spills on the biodiversity of aquatic and terrestrial systems have been the subject of numerous studies (Bence *et al.,* 1996) (Jung *et al.*, 2010). These spills give rise to high mortality rates across many different species including birds, mammals, and fishes (Bence *et al.,* 1996). Oil initially stays on the surface of bodies of water, but due to its density, will often migrate downward, and thus, may affect many organisms throughout the water column (Bence *et al.,* 1996). Since most oil spills have occurred in marine ecosystems, there is a significant amount of research on the effects of oil on salt water ecosystems (Bence *et al.,* 1996) (Jung *et al.*, 2010). While there is some research that examines the toxicity of oil on freshwater organisms, there is limited data on the impact of oil in these systems. There are many oil pipelines over freshwater ecosystems that could potentially devastate these ecosystems (Bhattacharyya *et al.*, 2002).

The Great Lakes region is an important economic resource for the midwestern United States secondary to tourism, fisheries, and other recreational activities (Talhelm, 1988). Enbridge Line 5 is an oil pipeline over the Straits of Mackinac, which connect Lake Huron and Lake Michigan. This pipeline contains synthetic crude oil that is composed of bitumens, which are heavy oil particles that are particularly difficult to clean up in oil spills (Enbridge Pipelines Inc. 2011) (Fingas *et al.,* 1999). According to a worst case scenario spill prediction, oil spilled from Pipeline 5 in the Straits of Mackinac could spread through the connected system of the Great Lakes. This could affect up to 700 miles of coast, impacting birds, mammals, and other wildlife in addition to fish and aquatic species (Schwab, 2014). If the pipeline were to rupture, predicting how oil might affect organisms living in these lakes would be challenging due to the limited available data on freshwater ecosystems. To better understand the potential effects of an oil spill on the Great Lakes, species that are crucial to these ecosystems must be studied.

Zooplankton are low on the food chain, and thus may have bottom-up effects on ecosystems, and thus were chosen as the subject of this study. Many lentic organism diets consist of zooplankton. They are primary grazers that prevent harmful algal blooms; thus, they are essential to the biodiversity of lake systems (Brett *et al*., 2007). *Cladocera* were the most commonly found zooplankton found in both trials of our study, were the focus of this experiment. Because zooplankton are a keystone species in lentic systems like the Great Lakes, it is important to understand how they would be affected by an oil spill.

Our aim was to simulate a rupture of Enbridge Line 5 and determine the impact on zooplankton. We compared hydrocarbons of used motor oil to synthetic crude oil, which is found in Pipeline 5, and determined that it was a suitable substitute for synthetic crude oil. The purpose of this study was to understand how are zooplankton are affected by different concentrations of oil in terms of death rate and hydrocarbons ingested. To test this, we measured the amount of hydrocarbons and chlorophyll content of filtered zooplankton and the amount of zooplankton alive. We expected that as oil concentration increases, both ingestion of oil by zooplankton and death rate would increase.

Methods

Study Design

The study consisted of two trials. The first trial of the experiment, zooplankton and tank water were collected from Douglas Lake (Trial 1)and for the second, the water was collected from the Straits of Mackinac (Trial 2). Zooplankton were collected using a plankton net with a Nalgene™ bottle or vial attached. Plankton nets were cast 3 times to fill each bottle and we collected 9 L of concentrated zooplankton water. Lake water, which was later used for filling the water tanks with zooplankton. was collected in buckets. Using data from past oil spills (Anderson *et al.*, 1974), we decided on two experimental treatments: 500 ppm and 1000 ppm of oil, in addition to a control treatment of 0 ppm.

Glass tanks (9) with a 10-gallon capacity were used and each treatment (500 ppm, 1000 ppm, and negative control) was performed in triplicate. The glass tanks were filled with 14 L of lake water and 1 L of concentrated zooplankton water caught from the nets. Aquarium air pumps (9) were used to oxygenate the water. The experiment ran for 4 days. Each day, zooplankton were identified by morphological characteristics and counted in petri dishes at approximately the same time every day. On Day 0, zooplankton were counted before any oil was added to the tank to get a baseline amount of zooplankton alive. Organisms were classified as *Cladocera*, *Copepoda*, *Rotifera*, and other. Algae were also counted, but not classified. In Trial 1, 3 ml per tank were counted per day and in Trial 2, 15 ml were counted per tank per day. At the end of each run, we filtered and analyzed the samples for hydrocarbon and chlorophyll content of the zooplankton using gas chromatography.

Statistics

Statistics were run on the two trials separately. Each treatment was performed in triplicate, so we averaged the number of *Cladocera* alive per day per treatment. We then performed a linear regression on the average amount of *Cladocera* alive in each treatment per day for both Trial 1 and Trial 2. One way ANOVAs were performed on the gas chromatography results for the average amount of hydrocarbons and chlorophyll across each treatment to see if there was a significant difference between the means. For hydrocarbons, we ran a Tukey Post Hoc to determine if the control treatment was different from each of the two experimental treatments.

Results

Trial 1

Every day we counted zooplankton, there was a general negative trend in the amount of oil per tank and the amount of *Cladocera* found alive per mL on every day except for day 3 (Fig. 1). This trend was not significant for day 0, day 1, day 2, or day 3 ($p=0.45$, $p=0.43$, $p=0.17$, $p=0.34$, respectively). The negative correlation between the amount of oil and *Cladocera* alive was most significant for day 2. There was little to no observable trend in the amount of chlorophyll found in the *Daphnia* with increased oil content (Fig. 2) ($p=0.41$). The Tukey Post Hoc found that the levels of chlorophyll per treatment are nearly the same between the control and the experimental treatments of 500 ppm and 1000 ppm ($p=0.47$, $p=0.48$) and that there was no significant difference between the experimental 500 ppm and 1000 ppm treatments themselves ($p=1.00$). There was a significant correlation between the amount of hydrocarbons found in filtered zooplankton and oil content (Fig. 3) ($p=0.023$). There was a significant difference increase from the amount of hydrocarbons in the control to the two experimental treatments of 500 ppm and 1000 ppm ($p=.044$, $p=.028$) but there was no difference between the two experimental treatments themselves as found by the Tukey Post Hoc ($p=.926$).

Trial 2

There was an observable downward trend of *Cladocera* alive per mL and the amount of oil added to each tank for days 1 and 2, but there was a positive trend on day 3 and a barely observable downward trend on day 0. (Fig. 4). However, the results for day 0, day 1, day 2, and day 3 cannot be described as a correlation ($p=0.76$, $p=0.37$, $p=0.48$, $p=0.45$ respectively). A negative trend in the amount of chlorophyll per treatment was observable (Fig. 5), but once again this was not a correlation ($p=.093$). This lack of significant data was confirmed by a Tukey Post Hoc. The difference in chlorophyll between the experimental 1000 ppm and the control was nearly significant ($p=.088$), but the difference between the 500 ppm treatment and the control ($p=.235$) and the difference between the two experimental chlorophyll amounts ($p=.731$) were very insignificant. While there was no trend found with the amount of oil added and hydrocarbons found in the filtered zooplankton through gas chromatography, the experimental treatments of 500 ppm and 1000 ppm of oil were significantly higher in hydrocarbons than the control treatment ($p<0.05$). This difference between the two experimental treatments (500 ppm, 1000 ppm) compared to the control was confirmed by a Tukey Post Hoc ($p=.010$, $p=.042$), but no difference was found between the 500 ppm and 1000 ppm treatments themselves ($p=.471$).

Discussion

While the water from Lake Douglas was much more concentrated in zooplankton than the water from the Straits of Mackinac, the results from Trials 1 and 2 did not differ greatly from one another. Most of our results were insignificant and could be due to chance. For day 0 for both Trial 1 and Trial 2, *Cladocera* found alive was lower in the two experimental treatments than in the control which was notable because oil had not been added to the tank yet. However, this trend had very weak significance and likely did not affect the data collected. On days 1 and 2 of the experiment, there was a negative trend between *Cladocera* found alive and oil concentration in the tank, which if significant, would indicate that more *Cladocera* die as oil concentration increases. However, on day 3 for both Trial 1 and Trial 2, *Cladocera* found alive increased with oil concentration. These unanticipated trends may be explained by the human error of counting zooplankton under the microscope, or that zooplankton may be more prevalent in specific portions of the water column. The R^2 values were relatively low for both trials, meaning there was a lot of variation in our values and the data are not well described by the line of best fit. We did not find significant evidence to suggest that *Cladocera* die at higher rates when more oil is present, and thus we fail to reject the null hypothesis.

In both Trial 1, the amount of chlorophyll found compared to the amount of oil in the sample had no obvious trend or correlation. However, in Trial 2, there was a nearly significant (p=.093) negative trend of chlorophyll found as oil concentration increased. This could possibly be explained by zooplankton consumption decreasing as oil increased. Zooplankton have been observed to vary in feeding rate under certain conditions such as high intraspecific competition and exposure to algal toxins, and may be able to change feeding rate when exposed to oil as well (Helgen, 1987) (Carmichael *et al.,* 1991). This could be the subject of a further research-whether zooplankton feeding rates decrease with increased oil concentration. However, the results from this study were insignificant at the 95% confidence level and thus, we cannot conclude whether zooplankton chlorophyll intake is affected by oil concentration.

For both Trial 1 and 2, the hydrocarbon content of the filtered zooplankton significantly increased in the experimental treatments compared to the control treatments. This means that the zooplankton exposed to oil treatments likely ingested it. There was no significant evidence, however, to support an increase in oil ingestion as oil concentration increased, as the 500 ppm and 1000 ppm treatments did not differ from one another consistently between the trials. A future study could examine whether increased oil concentration increases zooplankton oil ingestion with more narrow intervals of oil concentration.

Though most of our results were insignificant, the trends we found indicate that zooplankton are adversely affected by oil. Thus, a spill from Enbridge Line 5 into the Straits of Mackinac could have catastrophic bottom-up consequences on the ecosystem. It is imperative that we continue to study the effects of oil on freshwater systems to preserve the biodiversity of the Great Lakes.

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Trial 1 (Lake Douglas)

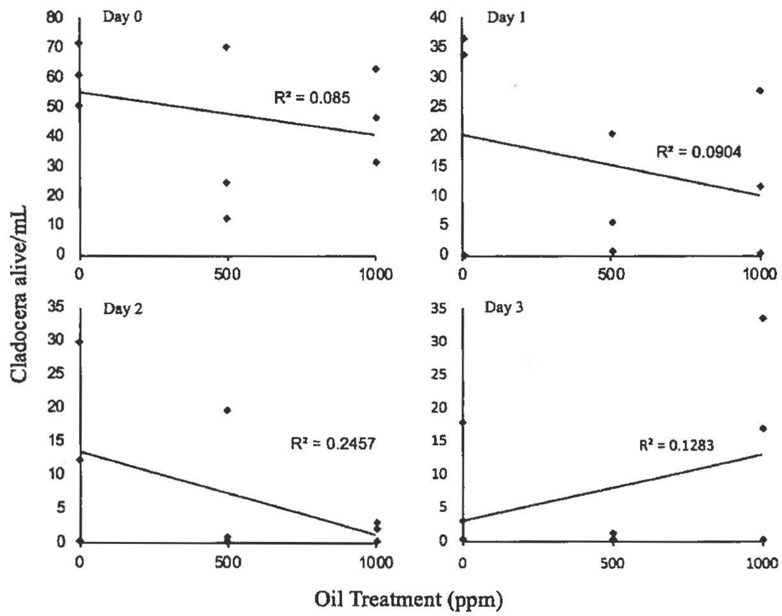

Figure 1: The amount of living *Cladocera* counted per mL divided into 3 oil concentration treatments, 0 ppm (control), 500 ppm, and 1000 ppm. Each treatment was done in triplicate. Each graph represents a different day of Trial 1. This trial (Trial 1) was completed with water from Lake Douglas. Day 0: p=0.45,Day 1: p=0.43, Day 2:p=0.17, Day 3:p=0.34

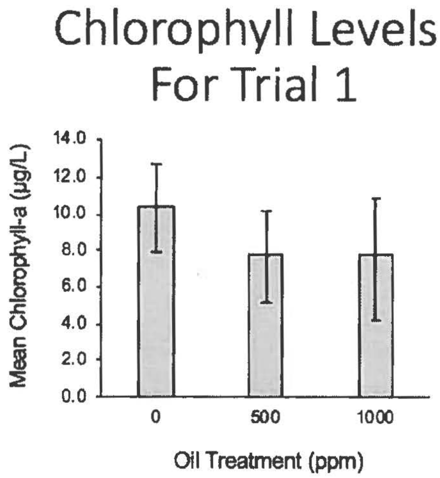

Figure 2: The average amount of chlorophyll (µg/L) found in filtered zooplankton from tanks containing 0ppm, 500ppm, and 1000ppm of motor oil after three days (p=0.41) This trial (Trial 1) was completed with water from Lake Douglas

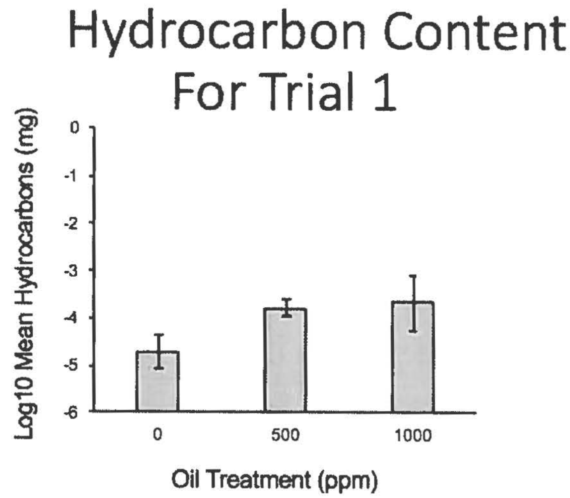

Figure 3: The average amount of hydrocarbons (mg) found in filtered zooplankton from tanks containing 0ppm, 500ppm, and 1000ppm of motor oil after three days (p=0.023) This trial (Trial 1) was completed with water from Douglas Lake.

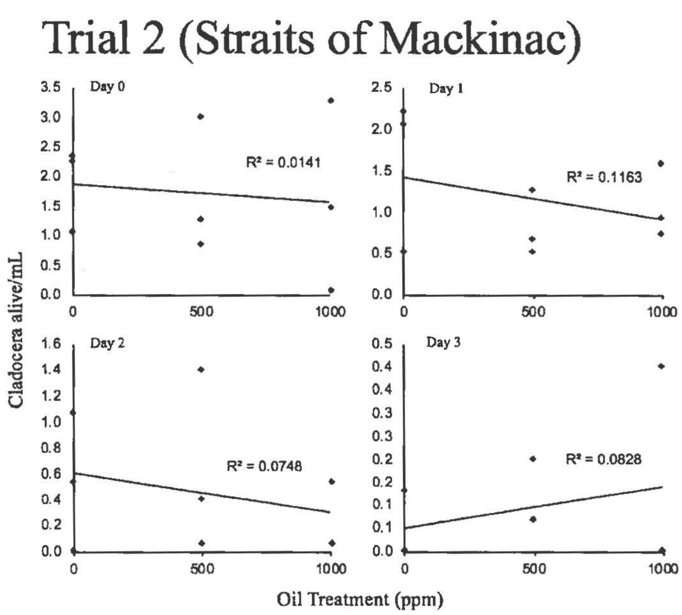

Figure 4: The amount of living *Cladocera* counted per mL divided into 3 oil concentration treatments, 0 ppm (control), 500 ppm, and 1000 ppm. Each treatment was done in triplicate. Each graph represents a different day of Trial 1. This trial (Trial 2) was completed with water from the Straits of Mackinac. Day 0: p=0.76, Day 1: p=0.37, Day 2: p=0.48, Day 3: p=0.45

Chlorophyll Levels For Trial 2

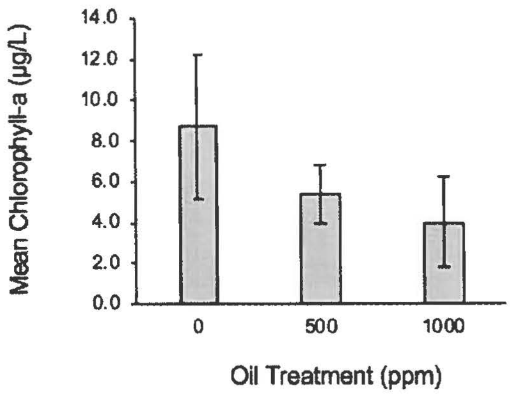

Figure 5: The average amount of chlorophyll (µg/L) found in filtered zooplankton from tanks containing 0ppm, 500ppm, and 1000ppm of motor oil after three days (p=.093) This trial (Trial 2) was completed with water from the Straits of Mackinac.

Hydrocarbon Content for Trial 2

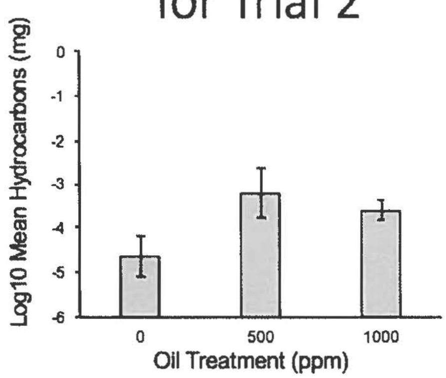

Figure 6: The average amount of hydrocarbons (mg) found in filtered zooplankton from tanks containing 0ppm, 500ppm, and 1000ppm of motor oil after three days (p=.011) This trial (Trial 2) was completed with water from the Straits of Mackinac.