Dissolved organic carbon and its impact on methane emissions in northern lakes

BIOS 35502: Practicum in Field Environmental Biology

Carolyn A. Hammack

Advisor: Brittni Bertolet

UNDERC East 2016

Abstract

Although most sources of greenhouse gases have been extensively studied, relatively little is understood about methane emissions from lakes. Since methane is a considerably more potent greenhouse gas than carbon dioxide, studies investigating what physical and biological factors control methane emissions in lakes are becoming increasingly important. Recently, dissolved organic carbon (DOC) has been identified as a major driver of ecological change in lakes by altering stratification, thermoclines, phytoplankton communities, and light levels. In this experiment, I investigate methane emissions and phytoplankton biomass in four northern lakes along a DOC gradient to determine how increased DOC concentrations as a result of global change will contribute to atmospheric concentrations of methane. Chlorophyll a and sediment samples were collected from each lake to estimate phytoplankton biomass and to create sediment slurries. Gas was extracted from the slurries every other day for a period of 30 days to obtain levels of methane emitted over time. The final results supported the hypothesis that intermediate DOC lakes would have the highest methane emissions, but did not support the hypothesis that intermediate DOC lakes would have the highest chlorophyll a concentrations. This unexpected trend suggests unforeseen factors are involved in the process of methanogenesis that were beyond the scope of this experiment. Future studies on the relationship between methanogenesis and phytoplankton community structure and function should be conducted to best understand and manage this process.

Introduction

With increasing global temperatures, extreme weather patterns, and shrinking sea ice, the impacts of carbon dioxide as a greenhouse gas have become increasingly apparent over the past few decades. However, many people remain unaware that methane also serves as a greenhouse gas, and its emissions are steadily increasing from both natural and man-made sources. Although there is currently less methane in the atmosphere than carbon dioxide, it is a much more potent greenhouse gas. It is 22x more effective than carbon dioxide at absorbing long wave radiation and has 3.7x more global warming potential per mole, increasing its ability to substantially contribute to global warming (Lashof and Ahuja 1990; Zhuang et al. 2009). Therefore, it is critically important that scientists begin to gain a greater understanding of where methane emissions come from, and how they can be reduced.

One particularly important natural source of methane consists of freshwater lakes and ponds in northern latitudes. Although they are often ignored as substantial sources of methane, studies have shown that these lakes and ponds are actually one of the largest natural methane sources, and contribute two-thirds of all methane emissions in northern regions (Wik et al. 2016). The methane in these lakes is produced as a metabolic byproduct of small archaeans called methanogens that reside in the anoxic and nutrient-limited environments present in the sediments at the bottom of lakes (Deppenmeier 2002). These organisms thrive off of complex organic compounds that consist of either terrestrially derived organic matter or dead and decaying algae that has settled to the bottom of the lake. Increases in these organic compounds have been shown to largely control the structure and function of microbial communities in lakes (Schwartz et al. 2008). Thus, the concentration of dissolved organic carbon (DOC) in the lake basin is an important factor influencing methane emissions from the sediment of lakes.

The complex interaction between DOC and methane emissions is potentially problematic, however, because it poses a threat of a dangerous positive feedback mechanism to global warming. Research has shown that lakes have begun to experience an increase in DOC leaching into the aquatic ecosystem from surrounding soils in recent decades, and this increased organic matter enhances microbial activity and growth (Berggren et al. 2010; Freeman et al. 2004; West et al. 2012). Previous research has attributed this trend to anthropogenic changes in sulfur and sea salt deposition, and also consequences of human-caused global warming such as rising temperatures and increased atmospheric carbon dioxide concentrations (Evans et al. 2006; Freeman et al. 2001; Freeman et al. 2004; Monteith et al. 2007). So, as global warming occurs, methane emissions increase, which progresses global warming even further, resulting in a positive feedback mechanism (Glissmann et al. 2003; Wik et al. 2016). Thus, it is becoming exceedingly important for the scientific community to gain a deeper understanding of aquatic ecosystems and the interactions between methanogens, phytoplankton, and DOC concentrations so this positive feedback mechanism can be adequately managed.

Given that DOC concentration in lakes have been increasing over the last few decades, and that DOC has profound effects on the structure and function of aquatic ecosystems by altering light levels, nutrient levels, heat distribution, oxygen availability, and predator/prey interactions (Solomon et al. 2015), many studies have been conducted to determine what kinds of changes we can expect in lakes as global change continues to progress. One particular ecological change researchers expect from increasing DOC is in the structure and function of phytoplankton communities which serve as energy sources to methane producing organisms. For example, ecosystems with limited nutrients as a result of very high DOC have been correlated with an increase in the lipid content of phytoplankton communities which makes them a better energy source for methanogens, allowing methane emissions to increase (West et al. 2015). Not only does increased DOC alter phytoplankton communities in a way that may increase methanogen activity, but studies have also indicated that higher levels of DOC can impact stratification in lakes by altering light levels and changing the thermocline. This change in heat distribution can reduce vertical mixing, creating a larger anoxic environment towards the bottom of the lake where methanogens thrive (Solomon et al. 2015).

In this study, I investigated how levels of methane emissions in lakes differed along a gradient of DOC concentration in order to predict how aquatic communities may be altered in the future with global change. Research has suggested that lakes with very low DOC may be detrimental to phytoplankton by exposing them to unhealthy UV levels, while lakes with very high DOC may limit nutrient availability (Carpenter et al. 1998). Conversely, lakes with intermediate DOC concentrations are quite productive because they have high enough levels of DOC to bring in helpful nutrients, but are not high enough to cause severe shading effects (Hansen et al. 2003). So, I hypothesized that lakes with intermediate DOC concentrations would have the highest chlorophyll a and methane levels due to increased phytoplankton biomass, and lakes with the lowest and highest DOC concentrations would have relatively lower chlorophyll a and methane levels due to decreased phytoplankton biomass.

Materials and Methods

Site Selection

This study was conducted at the University of Notre Dame Environmental Research Center (UNDERC) in Land O' Lakes, Wisconsin from May to July of 2016. The property contains over 8000 acres of northern hardwood forest with 30+ lakes and bogs that possess different physical and ecological properties, allowing for comparative studies to be conducted with ease. This study was conducted on four lakes of varying DOC concentrations and other physical characteristics; Crampton, Bay, East Long, and Hummingbird (**Table 1; Figure 1**). DOC concentrations for each lake were obtained from previous research on the area (Godwin et al. 2014).

Chlorophyll a

Chlorophyll a analysis was conducted for each lake to estimate phytoplankton biomass. In order to analyze chlorophyll a levels, water samples from the pooled mixed layer (PML) and the hypolimnion were collected. To obtain the PML sample, a 1000 mL Nalgene bottle was rinsed three times with native water, then filled with equal parts water from the top, middle, and bottom of the epilimnion. The hypolimnion sample was collected using a Van Dorn Sampler and was also placed in a 1000 mL Nalgene bottle. Once both samples were collected, they were returned to the laboratory and filtered through 47 mm GF/F filters using a filter manifold and vacuum pump. Two replicates from both the PML epilimnion and hypolimnion were filtered in this way with 450 mL of sample water each, and then were folded and stored frozen in film canisters until ready for further analysis. This process was repeated three times throughout the course of the study period, resulting in a total of six PML epilimnion replicates and six hypolimnion replicates for each lake. Chlorophyll a was then extracted by submerging the filters in methanol for 24 hours in the dark. The fluorescence of the chlorophyll a in solution was then measured on a Turner Triology Fluorometer and recorded for each replicate from each lake. The concentration of chlorophyll a was calculated using a standard curve from prepared stock standards, and the concentration was amended for the quantity of water filtered through the filter.

Sediment Slurries

In order to measure rates of methanogenesis, sediment slurries were created with hypolimnion water and sediment samples from each of the lakes. Hypolimnion water was collected out of a Van Dorn sampler using syringes to prevent atmospheric air from interacting with the sample and sediment samples were collected with an Ekman Dredge from the deepest point of each lake, as determined by bathymetric maps (USFS 1997). Three samples each of hypolimnion water and sediment were collected from each lake and were returned to the laboratory and refrigerated for 1-2 days.

After the refrigeration period, three replicate sediment slurries were prepared for each lake, resulting in a total of 18 sediment slurries. To prepare these slurries, 30 mL of sediment and 30 mL of hypolimnion water were placed into 100 mL serum bottles. Bottles were capped with rubber septa and were purged with N_2 gas for five minutes, then stored in a dark location at room temperature. After 24 hours, a 10 mL gas sample was extracted from the slurry headspace and injected into a vial for CH_4 analysis on an Agilent 6890 Gas Chromatograph (GC) equipped with a flame-ionizing detector (FID). After analysis on the GC, 10 mL of N_2 gas was then re-added to each slurry to replace the gas that was extracted. This process was repeated every other day over the course of 30 days, and the data collected from the GC was then used to compare the rates of methanogenesis in each lake. Any points for which GC data was severely abnormal were removed.

Statistical Analysis

A Shapiro Wilk Test was first run on all data to ensure normality. Where data was not normal, the appropriate nonparametric tests were used. For methane analysis, a large portion of data had to be removed due to GC errors, resulting in only certain days for which data could be used. Because continuous data was not available, Day 21 and Day 24 were chosen to compare absolute value of methane concentration in the sediment slurry headspace, and a significance value of 0.10 was used due to the large amount of noise and machine error. Thus, methane concentration in the headspace of the three replicates was used for each day to run a Kruskal-Wallis Test and determine significant differences in the methane concentrations between the four lakes on the same day. For the chlorophyll a data, a Kruskal-Wallis Test was also used to compare the average concentrations between the lakes.

Results

On both Day 21 and Day 24 of the sediment incubation there were significant differences in the methane concentration between lake sediment slurry headspace (**Figure 2**; $H=6.479$, $d.f.=3$, $p<0.1$). East Long had the highest methane concentration, which ranged from 0.09

umol/L to 0.28 umol/L (mean ± SE; 0.16 ± 0.03), Crampton second highest from 0.08 umol/L to 0.15 umol/L (mean ± SE; 0.12 ± 0.01), Hummingbird third from 0.04 umol/L to 0.19 umol/L (mean ± SE; 0.10 ± 0.02), and Bay fourth from 0.04 umol/L to 0.13 umol/L (mean ± SE; 0.07 ± 0.01).

Furthermore, a Kruskal-Wallis Test was used to compare differences in chlorophyll a concentration among the four lakes and between the epilimnion and hypolimnion of each lake (**Figure 3**; $H=40.798$, d.f.=5, $p<0.05$). Hummingbird had the highest chlorophyll a concentration ranging from 7.8 ug/L to 28.0 ug/L (mean ± SE; 14.9 ± 2.2), followed by Bay from 2.3 ug/L to 20.3 ug/L (mean ± SE; 8.5 ± 1.3), then East Long from 1.55 ug/L to 11.2 ug/L (mean ± SE; 5.4 ± 1.1), then Crampton from 2.7 ug/L to 7.3 ug/L (mean ± SE; 5.0 ± 0.5). All lakes had higher concentrations of chlorophyll in the epilimnion than in the hypolimnion besides Crampton which had the opposite.

Discussion

Ultimately, the hypothesis of this study that lakes with intermediate DOC concentrations would exhibit the highest levels of methane emissions was supported. The results support this conclusion with East Long exhibiting the highest levels of methane and a relatively intermediate DOC concentration of 10.7 mg/L along the study scale of 4.7 mg/L to 20.5 mg/L. While this general trend was certainly followed by the data, there were some discrepancies. For example, the amount of methane in Bay was lower than the amount of methane in Crampton despite Bay having a higher and more intermediate DOC concentration. There are two possible approaches to interpreting this discrepancy. On one hand, the data could still fit into the framework of the original hypothesis because Crampton, a very clear, oligotrophic lake was the only lake in this study with higher chlorophyll a concentrations in the hypolimnion than in the epilimnion. Since phytoplankton need sunlight to survive and sunlight is absent in the hypolimnion, this trend suggests that there are high densities of dead algae drifting down through the water column and finally settling in the sediment on the bottom of Crampton. This presence of many dead phytoplankton fits well with the idea that low DOC lakes can be harmful to phytoplankton communities by exposing them to extremely high UV levels that they are not equipped to handle (Solomon et al. 2015). Thus, if this is the case with Crampton, it would make sense for there to be more methane emissions because high densities of phytoplankton are dying and serving as a food source for methanogens. In contrast, Bay had a relatively normal distribution of phytoplankton with more living in the epilimnion than the hypolimnion, indicating less dead algae. This difference in the biomass of dead algae that acts as a food source between Bay and Crampton is a potential reason for the unanticipated result with Crampton showing higher methane emissions than Bay. Another, more likely explanation is that there were additional unobserved factors at work in these two lakes that were influencing the complex relationships between DOC, phytoplankton, and methanogens. For example, previous studies have suggested that factors such as nutrient concentrations and predator/prey interactions in lakes can have significant impacts on phytoplankton communities in lakes (Alpine and Cloern 1992; Hai et al. 2010). In turn, these changes in phytoplankton communities have been highly correlated with changes in methane emissions (Schulz and Conrad 1995; Wang et al. 2006).

Although the data supports the hypothesis that intermediate DOC concentrations will have the highest methane emissions, the hypothesis that intermediate DOC concentrations will have the highest phytoplankton biomass was unsupported. Rather than following the expected pattern, the lake with the highest DOC concentration, Hummingbird, had the highest chlorophyll a concentration followed by Bay, East Long, and finally Crampton. In fact, the most apparent trend in the data from this study seems to indicate that lakes with higher methane emissions tend to have relatively lower chlorophyll a concentrations and vice versa. Unfortunately, this finding is in direct contrast with the results of many previous studies that show a highly significant positive correlation between phytoplankton biomass and methane emissions (Schulz and Conrad 1995; Wang et al. 2006). Thus, it is most logical to assume that, again, there were some underlying factors at play that were beyond the scope of this experiment that were altering either the phytoplankton communities or the methanogen activity.

Additionally, it is important to consider the possibility that these unexpected trends could be due to error in the experimental design. Over the course of this study, many problems were encountered with the Gas Chromatograph which may have potentially skewed the data and given inaccurate methane values. Additionally, due to time limitations, this study included a relatively low amount of replication that makes it difficult to properly determine significant relationships between the variables that were measured. This experiment could certainly be improved, but regardless, the results still hold much importance because they highlight the incredibly complex nature of the interactions between DOC, phytoplankton communities, and methane emissions in northern lakes. In order to properly understand these complex interactions, it is necessary for further experimentation to be conducted on this topic. For example, a study including many more lakes with a broader DOC gradient would be beneficial, and a study including additional measurements such as nutrient concentrations and quantification of phytoplankton predator abundance would help narrow down the specific factors that control phytoplankton communities and thus impact methanogenesis. Furthermore, it would be interesting to conduct a similar study over a longer period of time that involves different periods of algal blooms and die offs to determine the exact extent to which phytoplankton populations influence methane emissions. Studying the structure and function of the methanogen communities directly would also be an interesting alternative that may provide a more direct avenue to understanding methane emissions from lakes.

Ultimately, studies such as this one and those outlined above are becoming critically important as greenhouse gas emissions continue to flood our atmosphere and threaten the future and wellbeing of natural ecosystems. A deeper and more intricate understanding of the highly complex interactions that lead to natural greenhouse gas emissions such as the relationship between DOC, phytoplankton, and methanogens in lakes can help scientists to figure out how we can better manage global warming, and can give them the necessary knowledge to work with local, state, and federal governments to in creating policies that ensure a healthier world for generations to come.

Acknowledgements

First and foremost, I would like to extend my gratitude to Brittni Bertolet for all her help this summer, from the early mornings out in the field to the late nights in the lab running samples. I could not have done this project without her endless support, guidance, patience, and assistance every day. Additionally, I would like to thank Dr. Gary Belovsky, Dr. Michael Cramer, Hannah Madson, the teaching assistants Kristen Bahleda and Catherine McQuestion, my field partner Chelsey Fattal, and other members of the UNDERC East class of 2016 for all of their teaching, editing, advice, and field assistance. Most especially, I would like to thank the University of Notre Dame Environmental Research Center and the Bernard J. Hank Family

Endowment for providing me with this opportunity and the financial means for me to conduct this research and live and work on the UNDERC property this summer.

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<u>Tables and Figures</u>

Table 1. Lakes included in this study with their respective DOC concentrations in mg/L.

LAKE	**DOC (mg/L)**
Crampton	4.7
Bay	6.3
East Long	10.5
Hummingbird	20.5

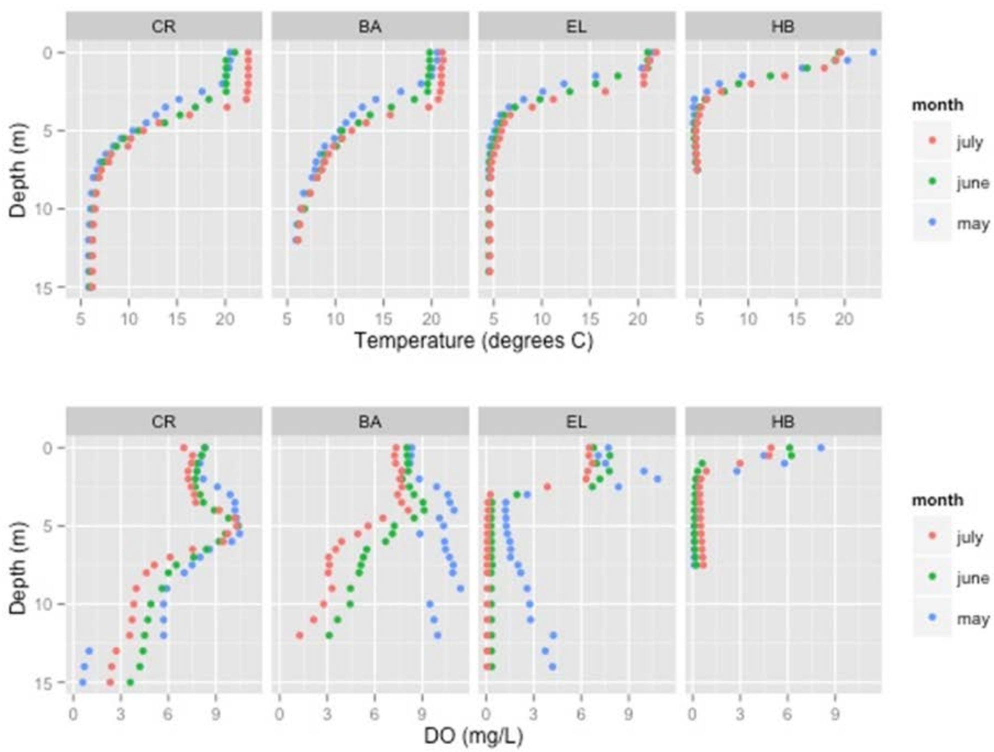

Figure 1. Temperature and Dissolved Oxygen Profiles for Four Lakes. These figures depict the differences in temperature and dissolved oxygen among the four lakes used in this study. Lakes with lower DOC exhibit more stratification and higher levels of DO at greater depths while lakes with higher DOC have less stratification and larger anoxic environments.

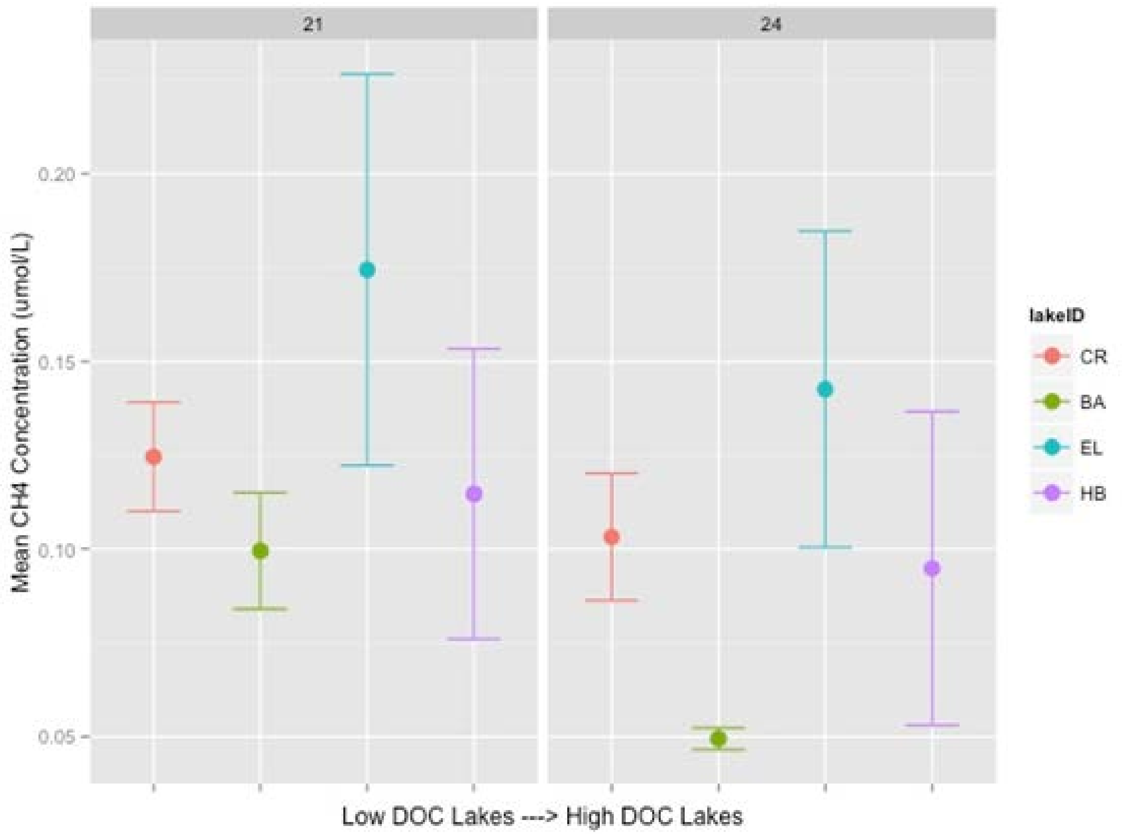

Figure 2. Mean Methane Emissions on Days 21 and 24. Comparisons of methane levels in each of the four lakes for days 21 and 24. East Long had the highest methane followed by Crampton, Hummingbird, and Bay ($H=6.479$, d.f.=3, $p<0.1$).

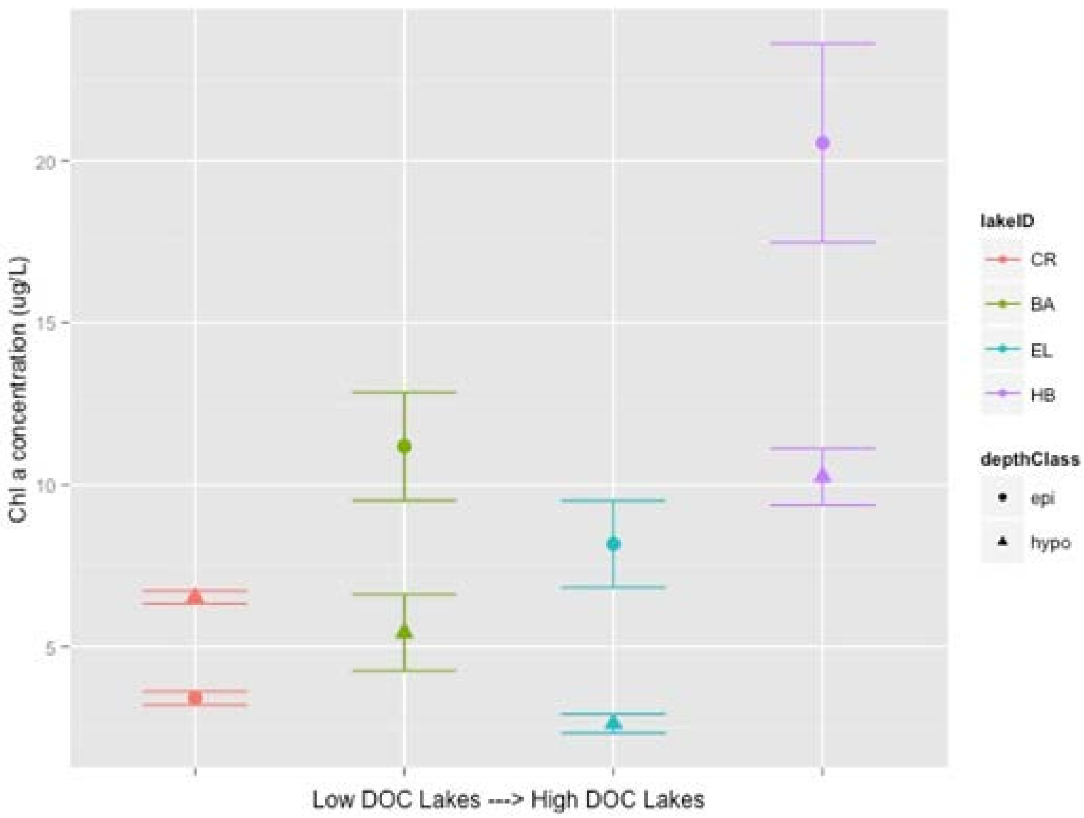

Figure 3. Mean Chlorophyll a Concentrations. Results of a Kruskal-Wallis Test on mean chlorophyll a concentrations across the four lakes. Hummingbird Lake exhibited the highest levels of chlorophyll ($H=40.798$, d.f.=5, $p<0.05$).