Benthic Invertebrate Species Diversity Response to Increased Levels of Dissolved Organic Carbon

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Abstract:

Regional monitoring stations are noting a rise in dissolved organic carbon (DOC) concentrations, and it is expected that DOC levels are increasing globally due to climate and land use change. Higher levels of DOC in aquatic systems cause darker water and are known to reduce the depth of primary production and limit the amount of dissolved oxygen in deep water. These physical changes have the potential to drastically alter the trophic make-up of lakes, rivers, streams, and marine environments. Since the long-term effects of such changes are still largely unknown, this study looked to investigate the effects of current lake DOC concentrations on invertebrate species diversity. Invertebrates provide important ecosystem services in aquatic systems and their abundance, biomass, and species composition can be used as indicators of changing environmental conditions. As such, their composition in lakes sites for this project was crucial to record. We hypothesized that lakes with higher levels of DOC would have less species diversity, and lakes with low DOC levels would exhibit more diverse benthic invertebrate communities. Our study revealed that there was a significant difference between species composition and abundance in high and low DOC lakes. Low DOC lakes exhibited the most diverse benthic communities; whereas, high DOC lakes had the least. In addition, statistical analysis supports the theory that as DOC levels increase, the richness and evenness of invertebrate species will decrease.

Keywords: dissolved organic carbon, benthic diversity, water columns, trophic structure

Introduction:

Dissolved carbon is particularly important to aquatic ecosystems and is a measurement of the amount of organic matter in water. DOC is a known food supplement for invertebrates and supports the growth of microorganisms. It also plays an important role in the global carbon cycle through the microbial loop (Kirchman et al. 1991). A high level of DOC, however, can be detrimental to aquatic systems because it prevents sunlight from penetrating the water column. When light is extinguished rapidly, photosynthesis cannot occur throughout lower levels of the lake. This in turn promotes gradients that are steeper and shallower between oxygenated surface water and cold deep water (Solomon et al. 2015). These combined factors can detrimentally affect metabolic rates of aquatic organisms, biogeochemical processes, and animal habitat (Kirchman et al. 1991). Changes in DOC can also affect surface water pH and acid neutralizing capacity, which has implications for food chain stability. Current global changes, such as global warming and land use changes, are altering the movement of DOC across landscapes. According to Jones and Lennon (2015), dissolved organic carbon that enters aquatic systems is increasing on a global scale and is expected to continue to do so. Some studies, however, report stagnating or negative trends of DOC concentrations (Sucker & Krause 2010). Whether or not there is a widespread positive trend in DOC levels has been the subject of debate. Regardless, there is still little research on the long-term effects of higher DOC concentrations in lake systems and there is still much uncertainty on how an increase will affect local aquatic communities.

Benthic invertebrates are organisms that burrow into lake floor sediments, and include a wide variety of species, such as crayfish, aquatic insect larvae, mollusks, and worms (segmented, round, and flat). They perform important ecosystem services and are known specifically for their abilities to “accelerate detrital decomposition” (Kovich et al. 1999). They also release nutrients into the aquatic system through their feeding activities, excretion, and burrowing into the substrate. Local bacteria, fungi, and algae subsequently take up these nutrients and convert them into energy that promotes their individual growth and ability to reproduce (Kovich et al. 1999). While identifying invertebrates, we were particularly interested in the number of mayflies, caddisflies, and stoneflies present because each of these invertebrates are known as important biological indicators of water quality (McCafferty 1981). Chironomidae larvae (non-biting midges) also play an essential role in aquatic communities. For example, they are important in the production of benthic biomass and are used by limnologists and aquatic ecologists as biotic indicators to classify lakes, determine trophic patterns, and identify current environmental conditions (Bhosale et al 2012). They are used as an indicator species due to the broad ecological tolerance of larval forms to extreme environmental conditions, such as temperature, pH, salinity, depth, and dissolved oxygen (Armitage et al 1995 and Ozkan & Camur 2007).

This study aimed to investigate the effects of high and low DOC concentrations on benthic species diversity of several lakes. We hypothesized that lakes with high levels of DOC will have lower species diversity and that lakes with low levels of DOC will have higher species diversity. We also were interested in observing the relative abundance between species and lakes.

Materials and Methods:

Study sites were located at the University of Notre Dame Environmental Research Center (UNDERC) in Land O' Lakes, Wisconsin. The property contains lakes, streams, bogs, and is comprised of nearly 3,035 hectares of northern hardwood forest. In order to study the differences between benthic communities found in lakes of differing levels of dissolved organic carbon (DOC), samples were collected from the following four lakes: Crampton, Bay, Hummingbird, and Morris. These lakes were chosen because they have well documented DOC concentrations, with Crampton and Bay having the lowest recorded amounts and Hummingbird and Morris with the highest (Table 1). Sampling was conducted over a period of two weeks throughout late May and early June.

At each lake, three sediment samples were taken at three different depths: the epilimnion, metalimnion, and hypolimnion. This accounted for a total of nine samples per lake. These depths were obtained by observing historical dissolved oxygen extinction graphs that are available for lakes on UNDERC property, as demonstrated in Figure 1. An Ekman dredge and core sampler were used to sample the benthic community of each lake. At sites deeper than one meter, an Ekman dredge was used to sample the sediment with three replicates per bucket, and a total of 3.5 liters of sediment per replicate. At depths of one meter or less, core sampling was used (Jones 2016). Due to a smaller sampled volume per replicate (1 liter), five replicate cores were then taken at each site and pooled into one container. Prior to returning to the laboratory the samples were sieved with 250 mL sieves and placed in labeled buckets for transport.

Once samples had been collected and returned to the laboratory, invertebrates were hand picked from the substrate and preserved in 70% ethanol to be later identified. For the purpose of this research project, organisms were identified to the level of family. The family Chironomidae was further identified to genus. They received additional identification because they are typically the most abundant insects found in freshwater and the species composition of chironomid assemblages can differ qualitatively and quantitatively among microhabitats (Pinder 1983 and Maasri et al 2008). For example, a total of 284 chironomids were identified in Bay, making them the most plentiful invertebrates in the water. By separating chironomids into different morphotypes and extending their identification to genus I was able to generate a better model of species diversity (Figure 2). Classifying the chironomids was done by mounting prominent morphotype heads and using the *Chironomidae of the Holarctic Region, Part 1: Larvae* text as a baseline for identification.

A Simpson index was used to calculate benthic diversity between lakes (Craig et al 2015). For the purpose of this research project, the Chironomidae genera identified was included with other invertebrate families when the Simpson index was calculated. Additionally, dissolved organic carbon levels in this set of lakes is well characterized, so I used published UNDERC records to perform a regression analysis to determine if there was a relationship between DOC levels and the calculated average values for Simpson's diversity. Lastly, a Kruskal-Wallis test was conducted to determine if there was any variation of benthic diversity between the four lakes. All analyses were conducted in *R* (R Studio 2015).

Results:

The Simpson diversity index indicated that Bay and Crampton lakes have higher levels of species diversity relative to Morris and Hummingbird (Table 2). Crampton ranked the highest with a value of 0.6937 on a scale of 0-1, while Hummingbird had the lowest value of 0.4144. The linear regression of Simpson diversity averages also resulted in a strong correlation coefficient value of 0.77138 between dissolved organic carbon levels and calculated species diversity (Figure 3, $F = 6.748$, $p = 0.1217$, $df = 1, 2$). A second linear regression compared two lake layers; the epilimnion ($F = 3.364$, $p = 0.2081$, $df = 1, 2$) and hypolimnion ($F = 2.592$, $p = 0.2487$). Both regressions resulted in correlation coefficients values of 0.6272 (epilimnion) and 0.5645 (hypolimnion) (Figure 4). Data was not normally distributed and so a Kruskal-Wallis test was run in lieu of a one-way ANOVA. The Kruskal resulted in significance between lake DOC and benthic invertebrate diversity ($p < 0.0001$, Table 3). Since the non-parametric test discovered significance the Holm-Bonferroni method was used as the p-value adjustment. A Wilcoxon signed-rank test was then performed as the post hoc test (Table 4). The test resulted in values that demonstrated the difference in benthic variation between lakes. The low DOC lakes, Crampton and Bay, were not significantly different from one another ($p = 0.4435$). High DOC lakes, Hummingbird and Morris, were not significantly different from one another as well ($p = 0.661$). High to low DOC lake comparisons resulted in significant difference from each other.

Discussion:

Relative to Hummingbird and Morris, Crampton and Bay lakes had the largest Simpson diversity values as indicated by an increase in species richness and evenness. A large Simpson value suggests the Crampton and Bay have the most diverse invertebrate communities. However, when Crampton and Bay values are observed individually the strength of their diversity indices is much lower, with 0.6937 and 0.5792 respectively on a scale to 1. The first regression analyses resulted in a strong average R^2 value of 0.77138 between dissolved organic carbon levels and calculated species diversity. Between lake light levels, the R^2 values were weaker at 0.6272 and 0.5645, but still remained above 0.5. The Kruskal-Wallis test produced a strong $p < 0.0001$. Further testing with the Holm-Bonferroni method and Wilcoxon signed-rank test produced results that indicate there is significant benthic variation between high and low level DOC lakes (Table 4). Bay and Crampton lakes had non-significant p-values, as did Hummingbird and Morris. This is expected because the paired lakes have low DOC and high DOC concentrations, respectively. When Bay and Crampton were compared individually to Hummingbird and Morris p-values revealed high levels of significance, all values < 0.05. This is again expected because the lakes contrast one another by being clear or dark. These results combined suggest that there is a correlation between high DOC levels and invertebrate diversity, and further support my hypothesis.

My project findings agree with what others have theorized. Unfortunately, most researchers are trying to discover why and where DOC levels are increasing, if at all, and not if those DOC increases will affect invertebrate communities. Projects exploring the long-term effects of DOC concentrations on aquatic systems have so far been restricted to zooplankton. Research on zoobenthos suggests that ongoing terrestrial carbon inputs will negatively affect the productivity of aquatic food webs (Kelly et al 2014). Further research investigating the links between zoobenthos and fish indicate that high DOC levels detrimentally affect fish production by limiting their zoobenthic prey sources. The lower zooplankton populations are attributed to poor light conditions and less opportunities to photosynthesize in “dark” water (Craig et al 2015). Zoobenthos research further supports the idea of trophic change due to DOC increases and is the essential first step in recognizing how changes in water systems can affect entire aquatic communities. My research has demonstrated that there are changes in aquatic systems due to differing DOC levels; whether or not those changes are detrimental have yet to be proven. Thus far, I can confidently agree with other scientists in the field that long-term DOC increases may have wide-ranging impacts on freshwater biota, drinking water quality, coastal marine ecosystems and upland carbon balances (Evans et al 2005).

Time constraints and other commitments limited the number of samples collected, and so future research should be much more extensive in nature. Additionally, lake size is another variable that could have affected the diversity and abundance of invertebrates sampled. For example, Crampton Lake has an area of 26 hectares and a maximum depth of 15 meters (UNDERC “Crampton” 1995). Hummingbird on the other hand, is significantly smaller with an area of 0.8 hectares and a maximum depth of 8 meters (UNDERC “Hummingbird” 1997). Further studies would require sampling of several clear and dark lakes of different sizes and DOC concentrations in order to generate more accurate results. Chironomid diversity was interesting to observe throughout this project’s completion. A chironomid index for each lake that includes species present and their abundance would be noteworthy to observe as a future project.

In conclusion, while this experiment cannot definitively determine whether or not high levels of DOC will always detrimentally affect invertebrate diversity in freshwater systems, it certainly has given assurance that changes in DOC levels will affect the composition of communities over time. Full understanding of the significance that DOC changes pose requires further knowledge of the extent of natural long-term variability, and of the natural “reference” state of these systems. As climate change becomes more of a global concern I envision the importance of DOC research expanding as well.

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Tables:

Table 1: Dissolved organic carbon levels in sampled lakes (Craig et al 2015).

	DOC (mg/L)
Crampton	5.3
Bay	6.1
Morris	15.7
Hummingbird	19.9

Table 2: Simpson diversity index values were calculated for each lake and then averaged. The higher the value, more diverse the sample is considered (0-1).

Lakes:	**Simpson diversity index averages:**
Crampton	0.693655729
Bay	0.57918587
Morris	0.45492345
Hummingbird	0.41435039

Table 3: A Kruskal-Wallis test was run in place of a one-way ANOVA. The test resulted in a strong p-value that supports the hypothesis of benthic variation between DOC levels.

Kruskal-Wallis	Chi-Square (x^2)	df	p-value
	20.566	3	0.0001295

Table 4: A Wilcoxon signed-rank test was performed after the Holm-Bonferroni method was used to correct for p-value adjustment. Values are not significant between Bay and Crampton (0.4435), and Hummingbird and Morris (0.661). However, significant values can be found between high and low level DOC lakes.

Wilcox signed-rank:	**Bay**	**Crampton**	**Hummingbird**
Crampton	0.4435	---	---
Hummingbird	0.0339	0.0022	---
Morris	0.0383	0.0022	0.661

Figures:

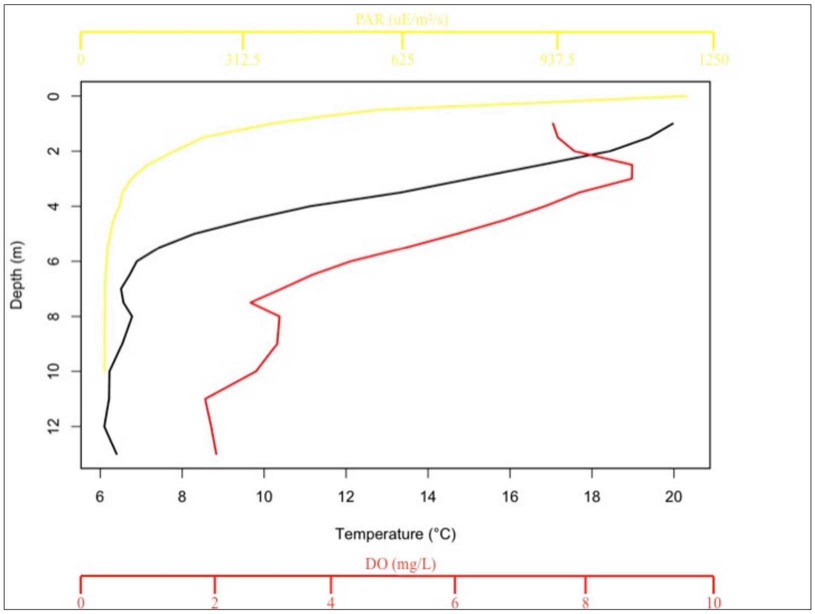


Figure 1: Epilimnion, thermocline, and hypolimnion depths were obtained by observing dissolved oxygen extinction graphs. The first spike in dissolved oxygen (DO) where it increases on the right hand side is the end of epilimnion (2 m.) and the beginning of the thermocline (4 m.). Where the DO drops off again is the beginning of the hypolimnion (8 m.). This process was completed for each lake (UNDERC "Appendix II" 1997).

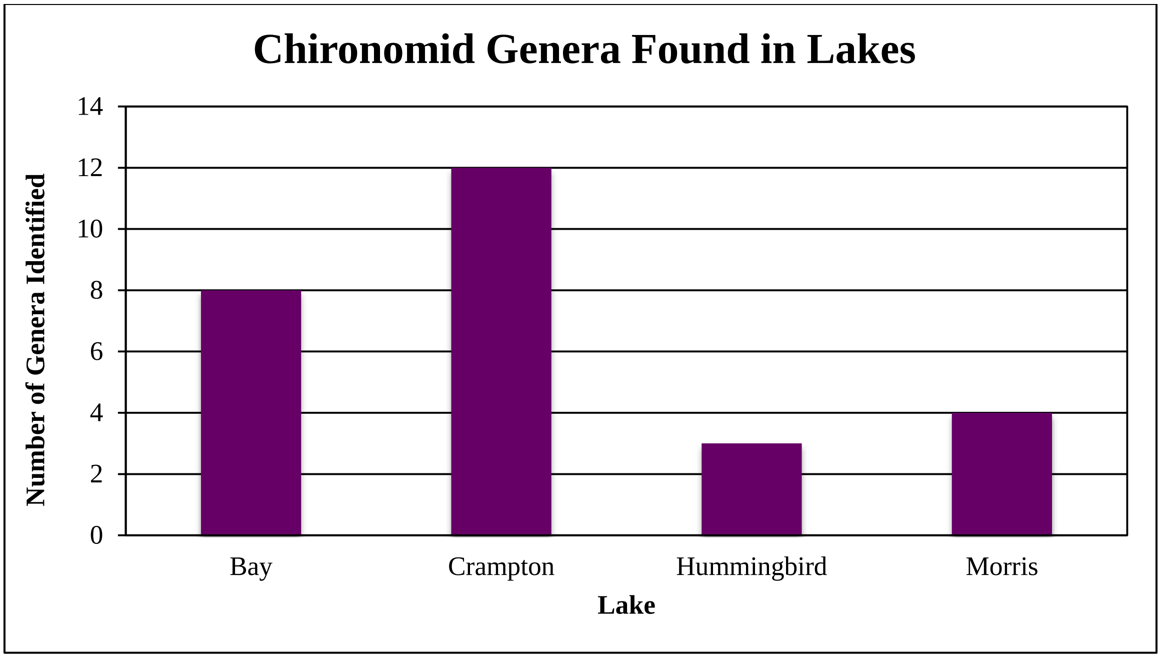


Figure 2: Crampton and Bay, low DOC lakes, had the largest number of chironomid genera identified, with 12 and 8 morphotypes. Hummingbird and Morris had significantly lower genera identified.

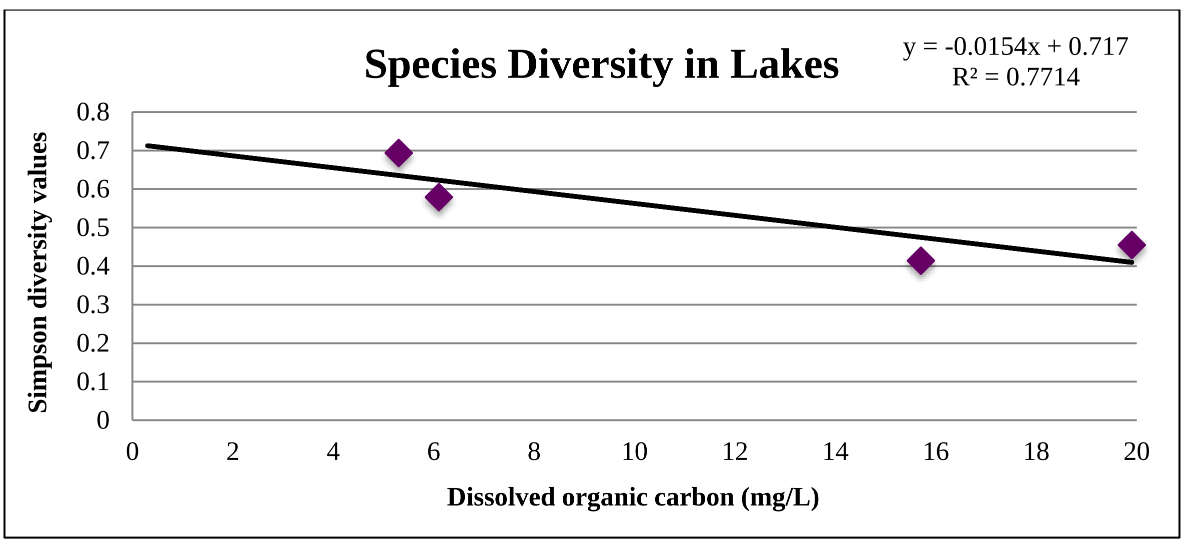
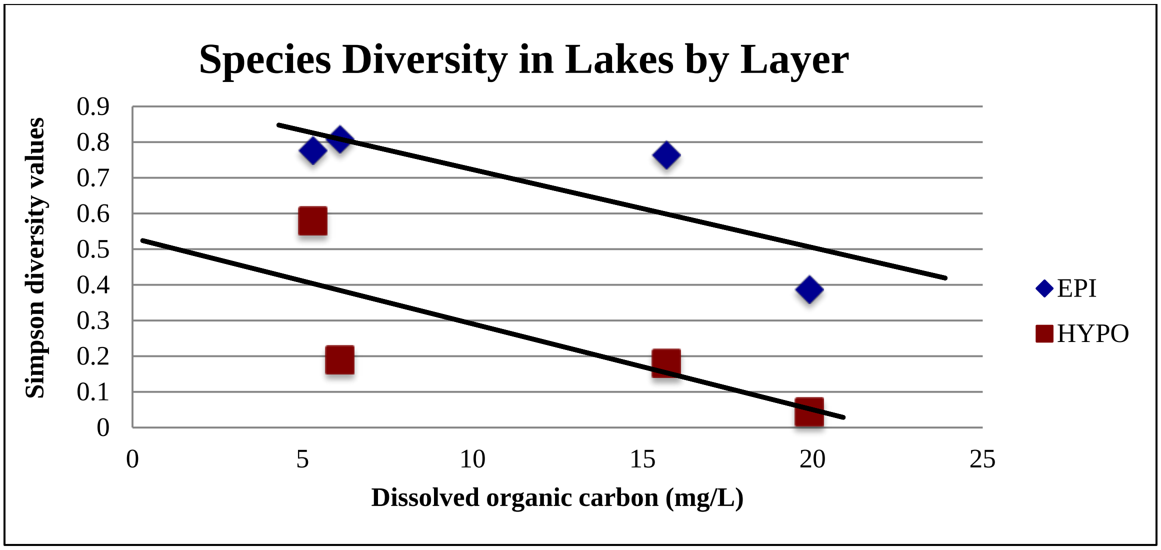


Figure 3: A linear regression was conducted in order to determine if there was a relationship between the concentration of DOC in lakes and respective diversity rankings. The two points farthest to the right represent Crampton and Bay lakes; whereas, Hummingbird and Morris lakes are located on the left hand side. The regression resulted in a strong R^2 value of 0.77138.



EPI	HYPO
R^2 = 0.62716	R^2 = 0.56445
y = -0.0219x + 0.9415	y = -0.0241x + 0.5313

Figure 4: A second linear regression looked at the relationship between lake layers and their respective DOC concentrations and Simpson diversity rankings. The epilimnion regression resulted in an R^2 value of 0.62716, while the hypolimnion had a value of 0.56445. These values are relatively strong and continue to support the theory that species diversity decreases as DOC levels rise.