The effect of dissolved organic carbon on zooplankton communities in freshwater lakes in the Upper Great Lakes region

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Abstract

The increase in precipitation and temperate associated with climate change has been closely linked to an increase dissolved organic matter in fresh-water ecosystems. High levels of dissolved organic matter (DOM) result in high levels of dissolved organic carbon (DOC) in lake systems, leading to an influx of nutrients that could potentially affect different organisms among the lake trophic levels including zooplankton abundance and diversity. Over 10 weeks, we studied six lakes with high or low DOC in an effort to establish how DOC can impact local zooplankton communities. Abundance, Shannon-wiener index and family richness were all found to be correlated to a change in DOC gradient; High DOC lakes showed an overall increase in abundance and family richness while showing a reduced family evenness. We can conclude DOC, which leads to browned waters that can impact nutrient levels, light availability and temperature, is effectively correlated to changes with zooplankton communities which can lead to further implications on the entire food-web of a lake ecosystem.

Keywords: *dissolved organic carbon, zooplankton, copepods, freshwater, biodiversity, abundance*

Introduction

As the earth's climate changes, research on carbon has become more important due to increased carbon sequestration in water bodies. While the increase in ocean carbon- and subsequent effects- has been widely studied, the effect of dissolved carbon on freshwater systems is known to a lesser degree. A primary source of organic carbon in freshwater systems is organic matter dissolved into the water (Cole et al. 2007). There are two pathways by which dissolved organic matter (DOM) can enter a water system. The first pathway is via autochthonous sources, or primary production within the system, and the second pathway is via allochthonous sources, carbon originating from terrestrial stock (Cole et al. 2007). When carbon is introduced into a water system faster than it can be exported or utilized, a flood of effects are observed resulting in a trophic cascade affecting all organisms within the water system- from phytoplankton to fish (Solomon et al. 2015).

Terrestrial carbon in fresh-water systems increases the net flux of organic carbon and often leads to a process known as “browning” (Solomon et al. 2015). DOM absorbs solar radiation at different wavelengths, with higher levels of dissolved organic matter resulting in less permeation of light through the water column, occurring at approximately 5.96 mg/L^{-1} of DOC; this results in warmer surface temperatures as light is more heavily weighted near the top of the water system (Seekell et al. 2015). This difference in light and heat could potentially affect the biodiversity of fresh-water ecosystems (Solomon et al. 2015).

Zooplankton themselves play an integral role in lake ecosystems, both consuming photosynthetic organisms and providing prey to a plethora of consumers that live in the system. Reduced light permeation could result in less photosynthetic activity of phytoplankton and macrophytes, ultimately affecting consumers like zooplankton and fish (Solomon et al. 2015). Phytoplankton are the dietary staple of zooplankton and limited phytoplankton or algal growth reduces the emergence of zooplankton (Robidoux et al. 2015, Seekell et al. 2015). With less zooplankton, turnover of carbon and essential compounds to the higher consumers of the food web is reduced, affecting biodiversity of the water ecosystem (Robidoux et al. 2015).

A reduction in photosynthetic activity due to shading from dissolved carbon also results in reduced turnover of organic carbon into oxygen. This could potentially increase the carbon content and reduce oxygen further in the system, leading to a less sustainable ecosystem or even anoxic conditions (Solomon et al. 2015). The reduction of heat at lower levels of the lake, due to carbon shading, could also drastically influence populations of both producers and consumers (Robidoux et al. 2015).

Following current climate models, dissolved organic carbon (DOC) is predicted to increase as the earth’s climate continues to warm (Solomon et al. 2015). Climate change has been widely proven to cause changes in temperature and precipitation in ecosystems. With increases in temperature and precipitation come changes in primary productivity of terrestrial plants and an increase in organic matter around freshwater systems. Specifically, changes in precipitation following current climate models show direct increases in DOM leading to water browning (Weyhenmeyer et al. 2015). Due to the importance of zooplankton to freshwater lake communities, it has become imperative to study the effect DOC can have on their diversity. Previous studies on DOC have found a correlation between prominence of copepods, compared to cladocerans, and DOC levels (Rodriguez). However, overall family abundance has not been studied over DOC gradients to establish how zooplankton communities change in terms of generic biodiversity (Rodriguez, Kelly et al. 2014) Through studying carbon gradients, I expect zooplankton biodiversity and abundance to decrease with increases in dissolved organic carbon ultimately leading to potential ramifications in reductions in the entire food-web from producers to consumers.

Methods/Materials

Study Site: This study was performed in the Upper Peninsula of Michigan, Gogebic County, and Vilas County in neighboring Wisconsin. Six lakes found in these counties were sampled: Crampton, Hummingbird, Bay, West Long, East Long, and Morris. All six of these lakes have previous records on dissolved organic carbon which will be taken from Kelly et al. (2014) and Craig et al. (2015). The low DOC lakes of the study include Hummingbird (25.9mgxL^{-1}, depth

7m), East Long (manipulated for high DOC, depth 7.6m) and Morris (17.49mgxL^{-1}, depth 6.7m) while high DOC include Crampton (5.49mgxL^{-1}, depth 15.2m), Bay (5.99mgxL^{-1}, depth 13.7m)and West Long (8.09mgxL^{-1}, depth 7.6m) (Craig et al. 2015, Kelly et al. 2014).

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Methods: Zooplankton samples were taken from tows lowered and raised at a constant rate, approximately 3 seconds per meter, just above the hypolimnion of the lake. For all six lakes, Crampton, Bay, West Long, East Long, Hummingbird and Morris, tows were taken at 12m, 10m, 6m, 6m, 4m and 5m respectively. Three vertical tows with a Nitex mesh net were per sampling date at each lake. Samples were filtered and pooled into a single cup that can be transferred to a petri dish for counting. Each net and sampling cup was rinsed after each use prior to taking new samples. Zooplankton were then preserved in ethanol for future counting (Eckert et al. n.d). A total of three samples were taken per lake per day with four replicates.

From here, sub-samples were taken and specimens identified to approximately the family level, with abundance recorded for each family. Each sample cup was filtered and washed before data was recorded; 100mls of water were added to the sample to standardize volume. Then approximately 300-1000 specimens were pipetted, 5 mls per subset, into a maze counting tray allowing for identification. Identification was done utilizing the Practical Guide to Identifying Freshwater Crustacean Zooplankton (Witty et al. n.d). This methodology is the approved protocol within the Jones lab at the University of Notre Dame Environmental Research Center (UNDERC) for counting and identification of zooplankton. This is the unpublished protocol within the lab, as well as the proper protocol within UNDERC's limnology text and guidelines (Eckert et al. n.d). Overall, this provides us with a representative count of each recorded family to compare generic biodiversity levels among lakes.

Statistics: A Shapiro-Wilks test was performed on all data prior to statistics to test for normalcy. A Shannon-Wiener biodiversity index was run on all zooplankton data for each lake, and utilized for to compare between high and low DOC lakes via a t-test to show the relationship between species abundance and evenness between the relative carbon gradients (Shannon).

$$\text{Shannon- Weiner: } H = -SUM[(pi) * \ln(pi)]$$

Family Richness and overall zooplankton abundance were calculated for each lake and compared using a t-test. Family abundance over DOC gradients were compared using a one-way ANOVA to analyze overall biodiversity at High and Low gradients. As well, multiple t-tests on Shannon-Weiner indexes, Family Richness and overall abundance were compared between East and West Long Lake due to the chances their close proximity (a water gate separating the two sections) has resulted in similar zooplankton counts. A Friedman test was run, due to nonparametric data, comparing DOC against family abundance and lake.

Results

Sample counts resulted in nine distinct families from which diversity was calculated: *Daphnia, Calanoid copepod, Cyclopoid copepod, Bosmina, Holopedium, Sididae, Chydorus, Chaoborus,* and *Brachionidae.* All data was normally distributed except for zooplankton abundance, requiring a log transformation. A Shannon-wiener index was run on each lake replicate and averaged for overall lake richness/abundance, with high DOC lakes trending greater then 1.0 and low DOC trending below 1.0, excluding East Long (Table.1). Multiple t-tests comparing DOC gradients, High or Low, against Shannon-Wiener index, family richness, and over-all zooplankton abundance showed $p<0.05$ and significant difference (Table.2).

Results of the one-way ANOVA found $p<0.05$ when lake was compared to Shannon-Wiener indices and abundance however resulted in $p>0.05$ comparing family richness to lake. A Tukey post-hoc showed significance ($p<0.05$) between, particularly, Hummingbird and all low DOC lakes in diversity index and abundance (Table 5, Table 6). Friedman analysis of family abundance compared to DOC and Lakes showed no significance ($p>0.05$). Comparing East and West Long lake for differences with a t-test, between their respective Shannon-Wiener indices, abundance and richness, found $p>0.05$.

Discussion

A total of eight zooplankton families were observed during the study and one predominant family of diptera, *Chaoborus* larvae, which is commonly included in the zooplankton food-web and considered in this study as such (Larson). Results of the study indicate that there is enough significance to correlate DOC levels to changes in zooplankton diversity, abundance and family richness ($p<0.05$, Table 2).

Input of DOM, resulting in higher levels of DOC and select nutrients like N and P, has been theorized to provide added nutrients to lake systems and result in higher abundance and diversity within the epilimnion to lower trophic organisms (Hitchcock et. Al 2016). This theory fits results found over the course of this experiment; lakes with higher DOC were found to have higher total zooplankton abundance and family richness (See Fig. 3, Fig. 4). High DOC lakes depicted significantly higher abundances and a broader range of zooplankton families; however high DOC lakes had lower averages of Shannon-wiener indexes meaning their family richness to evenness is lower (Table. 1). Essentially, while high DOC lakes showed high family richness their abundances were uneven in count, with *Brachionidae* composing over 50% of the average zooplankton abundance for all high DOC lakes as compared to low DOC lakes (Fig. 1).

Family composition differed over the gradients considerably, specifically in predominance of *Brachionidae* and type of copepod. *Brachionidae* and *Cyclopoid* copepods dominated zooplankton assemblages in high DOC lakes whereas low DOC lakes were dominated by *Calanoid* copepods (Fig.1). A Friedman test comparing family abundance over the two gradients showed no significance, with $p=0.08>0.05$; however, this p-value is close to our range of significance and with more replicates would likely produce significance. Previous studies have shown a correlation between increased presence of stable $Carbon^{13}$ and Nitrogen isotopes and a decrease of *Calanoid* copepods and increase in *Cyclopoid* copepods (Persaud et al. 2009). This difference suggests a possible difference in diet and temperature tolerance between the two families of copepods that could be affected by lake browning and added nutrients, such as DOC, Phosphorus, and Nitrogen, due to DOM (Persaud et al. 2009, Weidman et al. 2009).

A Tukey post-hoc analysis of diversity indices and abundance showed significance between several DOC gradients but specifically showed trends of significance between Hummingbird and all low DOC lakes (Table 5, Table 6). Hummingbird was statistically different in Shannon index and total abundance between all low DOC lakes and high DOC West Long (See Table 5, Table 6). This difference was noted previously where overall family composition of Hummingbird was dominated by *Brachionidae* (Fig. 1) and is a strong explanation for this result. This high abundance of *Brachionidae*, particularly this phyla *Rotifera*, can be explained by Wilk et. al 2014 which found a positive correlation between rotifers and DOC levels, providing added nutrients for growth and reproduction. Several comparisons between low DOC and high DOC lakes showed p-values close to 0.05 but not considered significant and with increased replication may cross into significant values in the future.

It is interesting to note that Crampton and Bay Lakes held significant difference between their total zooplankton abundance, even though both were low DOC lakes (Table 6). Future studies should analyze factors that could attribute to these differences, before drawing conclusions from our study, such as the presence/absence of zooplankton predators between the two lakes since there is a studied negative correlation of presence of predators and presence of zooplankton in lakes (Romare et al. 2016). This can ultimately affect the temporal trends of the zooplankton from early morning to late evening, known as diurnal vertical migration, where zooplankton stay near the hypolimnion in the early morning to avoid predators and rise into the epilimnion when fish are not present (De los Rios et al. 2015). Ultimately both of these factors should be considered, and taken into account in future correlative studies between zooplankton and DOC gradients, as they can have complex interactions with differing DOC levels.

High dissolved organic carbon has been correlated to large/shallow and eutrophic lakes with low water residence times compared to deep lakes with long water residence (Toming et al. 2016). An influx of DOC would lead to a stark change in nutrients available for the zooplankton community and could be plausible explanation for the differences in copepods found between high DOC and low DOC lakes, along with potentially influencing freshwater eutrophication. While we cannot distinguish from our data that our lakes were eutrophic, it is important to note that copepods can be used as biological indicators for eutrophic lakes and changes in nutrients; *Cyclopoid* copepods are particularly insensitive to eutrophication of lakes and are found in higher abundance (Perbiche et al. 2016). This trend fits out data, where *Cyclopoid* copepods were found in higher abundance in high DOC lakes. Future studies analyzing the diets of *Calanoid* and *Cyclopoid* copepods should be established to distinguish the difference in family abundance between the two and their respective DOC gradients, as well as how DOC can influence eutrophication within lakes.

While our Friedman Test showed no correlation between DOC and family abundance, further replication and larger samples could resolve this issue and should be performed. Ultimately, we can begin drawing conclusions on the correlation of DOC gradients on the diverse zooplankton community. DOC results in browned waters, increased nutrients and extreme temperatures between the epilimnion and lower lake depths and has been shown within this study to have a correlation between abundance, diversity, and overall family richness between high and low gradients (Solomon et al. 2015). Our initial hypothesis stated that diversity and abundance should decrease with higher DOC gradients due to water browning; however, our research found abundance and family richness increased while evenness decreased with DOC, likely due to the influx of added nutrients. We can effectively conclude that DOC influenced both the diversity and abundance of zooplankton within lakes and showed DOC is resulting in changes within our freshwater ecosystems and the communities that reside within them.

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Tables and Figures

Table. 1: Shannon-Wiener indices for all lakes shown against their DOC gradient; df=69, n=72. Group mean for High DOC = 0.958, group mean for Low DOC = 1.194 (Standard Error High DOC = 0.0753, Low DOC= 0.0643)

	Crampton	Bay	Morris	Hummingbird	West Long	East Long
Shannon-Wiener	1.052	1.238	0.997	0.739	1.293	1.137
DOC	Low	Low	High	High	Low	High

Table. 2: Comparison of DOC gradient to Shannon-Wiener, Family Richness, and Total Abundance; abundance log transformed to fit data parametrically. Df=69, n=72. Standard Error: Shannon-Wiener High DOC = 0.0753, Low DOC= 0.0643, Family Richness High DOC= 0.218, Low DOC= 0.240, Abundance High DOC = 19.924, Low DOC= 10.245.

	High DOC Mean	Low DOC Mean	p-value	Test statistic
Shannon-Wiener	0.96	1.19	0.00087	-3.48
Family Richness	5.63	5.06	P=0.0036	3.01
Total Abundance	4.513	4.064	P= 0.008	2.73

Table. 3: One-way ANOVA output of Shannon-Wiener diversity indexes compared by lake. F and P values indicate significance, but not which groups exhibit significance between each other (see

Zooplankton Shannon-wiener indexes	Sum of Squares	Df	Mean Square	F	P-value
Between Groups	2.364	5	0.473	7.011	<<0.05
Within Groups	4.451	66	0.0674		

Table. 4: One-way ANOVA output of zooplankton total abundance compared by lake. F and P values indicate significance, but not which groups exhibit significance between each other (see

Zooplankton Abundance	Sum of Squares	Df	Mean Square	F	P-value
Between Groups	14.18	5	2.835	7.885	<<0.01
Within Groups	23.73	66	0.360		

Table. 5: Tukey Multiple Comparisons output showing significant comparisons for Shannon-wiener index between all six lakes.

Group Comparison (Shannon-W. Index)	Difference	95% CI Lower Bound	95% CI Upper Bound	P-Value
Humm-Bay	-0.498	-0.809	-0.187	<<0.01
Humm-Crampton	-0.312	-0.624	-0.001	0.048
Humm-East	-0.398	-0.709	-0.086	0.004

West-Humm	0.553	0.242	0.864	<<0.01

Table. 6: Tukey Multiple Comparisons output showing significant comparisons of total abundance (log transformed) between lakes.

Group Comparison (Abundance)	Difference	95% CI Lower Bound	95% CI Upper Bound	P-Value
Crampton-Bay	<<0.01	0.215	1.652	0.004
Humm-Bay	<<0.01	0.229	1.666	0.003
Morris-Bay	<<0.01	0.029	1.736	0.001
West-Crampton	<<0.01	-1.652	-0.215	0.004
West-Humm	<<0.01	-1.666	-0.229	0.003
West-Morris	<<0.01	-1.736	-0.299	0.001

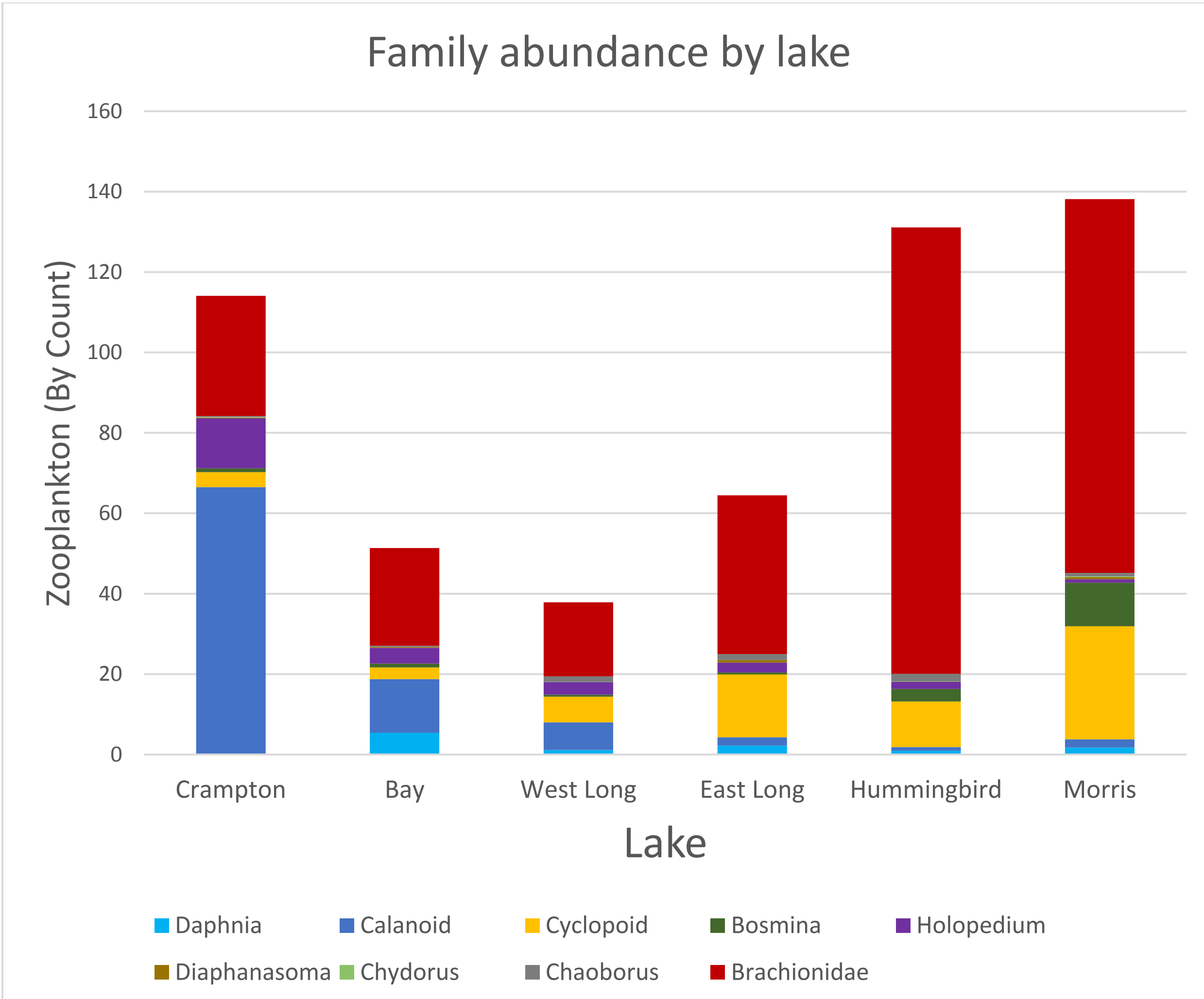

Fig. 1: Depiction of average family abundance out of total mean zooplankton abundance by lake. High DOC lakes were East Long, Hummingbird, and Morris and Low DOC lakes were Crampton, Bay and West Long.

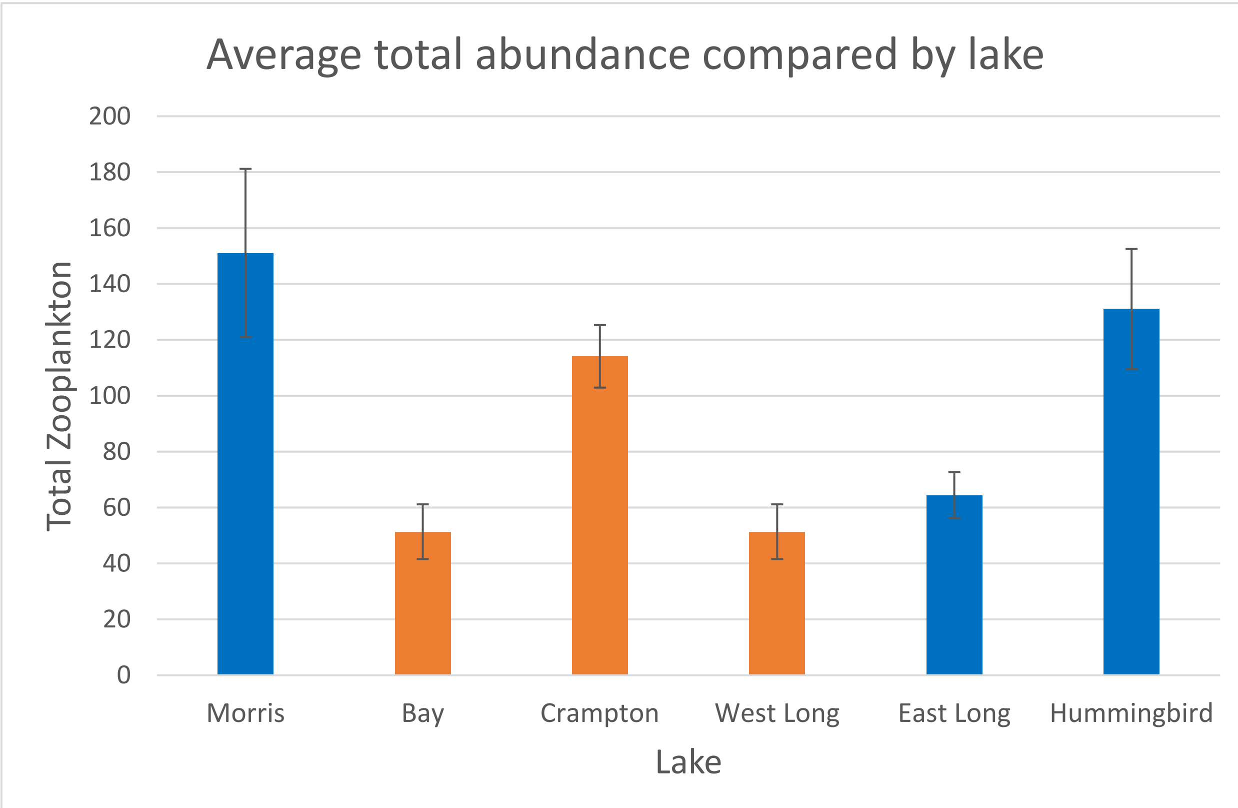

Fig. 2: Average total abundance compared by lake with standard error bars; Standard error of the mean by lake respectively: Morris = 30.103, Bay = 9.780, Crampton= 11.176, West=9.780, East= 8.238, Hummingbird= 21.431.

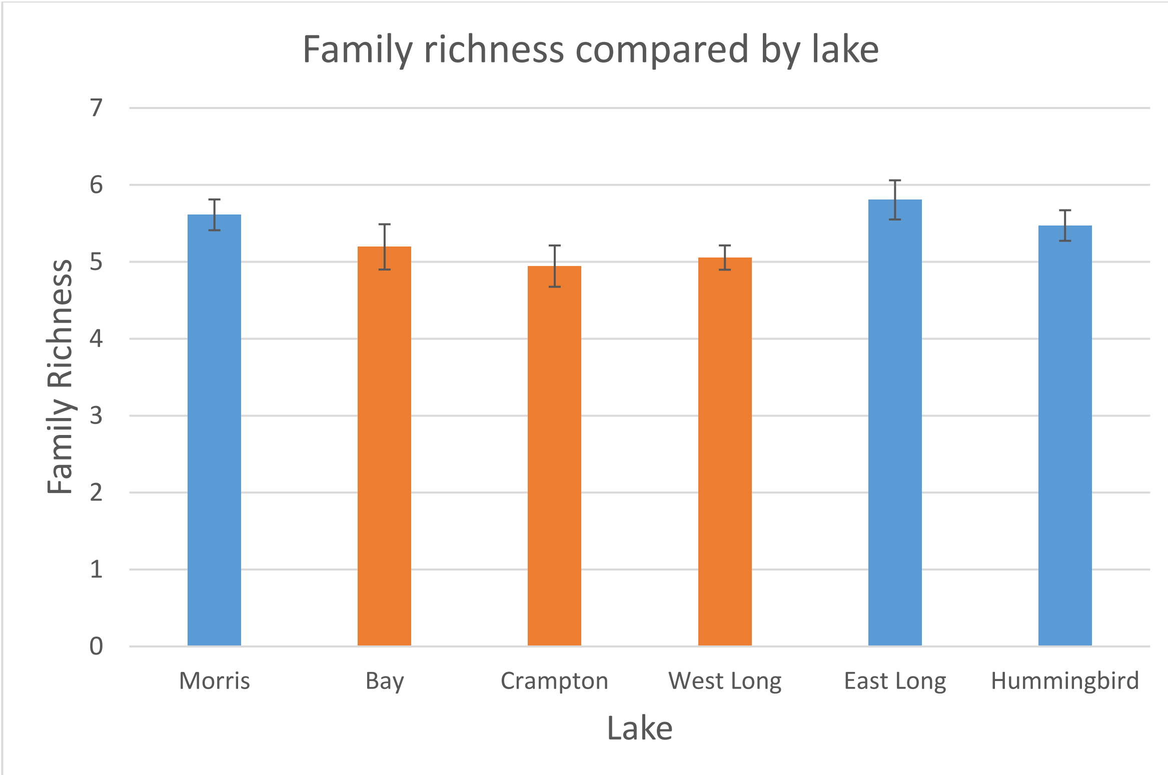

Fig. 3: Average family richness, out of nine families observed, per lake with standard error bars; Standard error of the mean by lake respectively for family richness: Morris=0.200, Bay=0.294, Crampton=0.268, West=0.158, East=0.254, Hummingbird=0.199.