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# The effect of dissolved organic carbon on fish foraging

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UNDERC SUMMER 2017

## ABSTRACT

Water browning due to increased dissolved organic carbon from terrestrial runoff affects the foraging of fish. As visibility decreases, fish must adapt in order to sustain themselves. Interspecific competition by juveniles can affect the community structure, thus foraging efficiency is important in early life stages. In this study, fishes from lakes of varying DOC concentrations were placed in both light and dark water treatments to observe zooplankton foraging efficiency. Surprisingly, source lakes had a stronger effect on foraging than experimental light climates, suggesting plasticity in foraging in temporary water conditions as long as strong foraging abilities are established during development.

## INTRODUCTION

As global environmental change progresses, local environments transform in varied ways. Management decisions are based both on local and regional environments. Greenhouse gas emissions and global temperatures are on the rise, causing soil quality to degrade and annual precipitation to increase, leading to increased sediment yields from increased runoff (Wang et al. 2017; Yira et al. 2017). This runoff from terrestrial environments then affects aquatic systems in unique ways. One mechanism by which this happens is dissolved organic carbon, which is increased by precipitation runoff. High dissolved organic carbon (DOC) from the ground can lead to a darkening, or “browning” of lakes (Scharnweber et al. 2016). This darkening of water can affect organisms that live there in a variety of ways, such as methylmercury content, water transparency, and UV penetration. Increases in DOC relates to an

increase in methylmercury content in macroinvertebrates, possibly due to DOC's importance in binding of free MeHg and its transfer to filtering organisms (Rennie et al. 2005). Higher DOC lowers the transparency in freshwater systems due to increased light attenuation (Huenemann et al. 2012; Fee et al. 1996). As browning increases, penetration of UV radiation becomes more limited, though UV radiation still impacts shallow lakes at a greater extent than deep lakes (Arts et al. 2000). As these changes occur, stability of ecosystems decreases due to low nutrient turnover rates (Jones and Lennon 2015), in turn altering plant, plankton, and fish communities in freshwater lakes.

In fish specifically, turbid waters reduce visibility and affects their ability to forage. Murky waters can be used as cover by prey (Ferrari et al. 2013). Therefore, as turbidity increases, consumption rate by predators such as Largemouth Bass decreases, and prey selection is impacted due to the increased difficulty in visually detecting prey (Huenemann et al. 2012; Shoup and Lane 2015). Morphology of fish can also be affected by habitat and foraging behavior, possibly due to prey choice and resource utilization (Bartels et al. 2012; Berchtold et al. 2015; Vila-Gispert et al. 2007). As turbidity influences the morphology and foraging behavior of predators in freshwater systems, the effects of lake browning on these traits must be considered when creating management plans.

In all waterways, competition between fishes varies at different life stages of individuals. The freshwater systems found in North Michigan are north temperate lakes, where Bluegill (*Lepomis macrochirus*) and Largemouth Bass (*Micropterus salmoides*) are common. Bluegill are zooplanktivorous. Largemouth Bass are zooplanktivorous as juveniles and then become piscivorous as adults, developing the ability to feed on

Bluegill (Tsunoda et al. 2015). Thus, as juveniles, these two species of fish compete for the same resources, and as adults, Largemouth Bass prey on Bluegill. Because of the competitive interactions between these two fishes, they make excellent model organisms to explore foraging theory. Understanding Largemouth Bass and Bluegill foraging interactions can also have practical applications to be used by fisheries managers. Due to the competitive foraging interactions that these fishes exhibit, we collected Bluegill and Largemouth Bass in the same life stage to use as our study organisms.

When compared to zooplanktivores, piscivores encounter prey at lower rates, making foraging more difficult (Jonsson et al. 2012). A cruising predator, such as Largemouth Bass, has an advantage in darker waters compared to ambush predators, however they are still negatively affected throughout a darkness gradient as water becomes less clear (VanLandeghem et al. 2011). As both Bluegill and Largemouth Bass are monitored while foraging, we expect that there will be a competitive advantage Bluegill have over Largemouth Bass that arises in varying DOC levels. Demonstrating exploitative competition, juvenile Bluegill, typically the prey of adult Largemouth Bass, can limit the amount of Largemouth Bass that survive to become predators by consuming more zooplankton, a shared common resource, than Largemouth Bass. Though juvenile Largemouth Bass do have the ability to feed on other items, if less zooplankton are available to them, they must depend on other sources of energy that may not be enough for survival. As the abundance of available zooplankton for Largemouth Bass decreases, the survival rate of juvenile Largemouth Bass decreases.

Because the number of juvenile Largemouth Bass that do not survive to become adults increases, Bluegill have less of a predatory pressure.

By attempting to understand the foraging ability of both species at the same life stage, one can better understand trophic cascades in UNDERC lakes and how global climate change influences these cascades at the local level. The goals of this study are to examine competition between juveniles and determine if a competitive advantage is maintained within varying environmental conditions. We conducted a series of comparative laboratory experiments to address the following objectives: (1) Determine if there is a competitive advantage between juvenile Bluegill and Largemouth Bass foraging for zooplankton, (2) Demonstrate how DOC concentrations affect foraging ability of juvenile Bluegill and Largemouth Bass on zooplankton.

## METHODS

The study site was on the border of northern Wisconsin and the Upper Peninsula of Michigan, at the University of Notre Dame's Environmental Research Center (UNDERC), in Land O' Lakes, Vilas County, WI. Crampton and Hummingbird lakes were used as our freshwater study systems. We selected Crampton and Hummingbird lakes due to the vast contrast in DOC content, between 5.3-5.4 mg/L and 19.9-25.9 mg/L respectively (Craig et al. 2015; Kelly et al. 2014).

To examine foraging interactions between Bluegill and Largemouth Bass, fishes in the same life stage (juveniles) were caught and compared to see if there was a difference in quantity of zooplankton eaten while in different DOC concentrations. The DOC in each tank mimicked the naturally occurring DOC contents in Crampton and

Hummingbird lakes. Juvenile Bluegill were collected using Frabill steel mesh minnow traps set for a maximum of twelve hours. Juvenile Largemouth Bass were collected using a mesh fyke net set for a maximum of twelve hours. Once caught, fish were measured to ensure that they were in the juvenile life stage (40-75 mm Bluegill and 70-115 mm Largemouth Bass; unpublished data). All fish were taken to the laboratory, acclimated to the tank temperatures, and placed in 1190 liter cattle tanks. Fish were kept in tanks for 24 hours to ensure that their stomachs were empty before feeding trials began. There were a total of 4 different treatments: bluegill in high and low DOC water, largemouth bass in low DOC water, and both fish species in low DOC water. To achieve desired concentrations of DOC that mimic Crampton and Hummingbird lakes, concentrated terrestrial DOC was added to the tanks a minimum of 24 hours before fish were collected (Super Hume, UAS of America Inc., Lake Panasoffkee, Florida; see Lennon et al. 2013). Desired DOC concentrations were measured at a 35% decrease in light attenuation in the dark water treatment and a 17% decrease in light attenuation in the light water treatment, as reflected in preliminary measurements of light attenuation at a depth of 0.25 meters at each lake (approx. 60 mL and 25 mL per tank, respectively). The dark water treatment consisted of lake water from nearby Tenderfoot lake, darkened using Super Hume (60-70 mL). The light water treatment consisted of tap water, Super Hume (15-25 mL), and fifteen liters of lake water from Tenderfoot lake to allow lake chemical concentrations to be similar. The difference in water sources for tanks is not expected to impact results due to similar results in preliminary water quality testing of general hardness, general alkalinity, pH, nitrite, and nitrate. After fish were fasted for 24 hours, fish were given two hours to forage for zooplankton. Zooplankton

were collected using a plankton tow. Zooplankton tows were collected the day of the trials to ensure that the same species of zooplankton were present in the same proportions. On the day of feeding trials, tows were collected from the deepest point of the lakes that the fish were taken from. The volume of the water used in the zooplankton tows was equal to the volume of water in the tanks, and the tow extended to the hypolimnion to collect zooplankton that were exhibiting diel vertical migration. Each tank for Hummingbird fish received three pulls at 4 m each. Averaged subsamples of Hummingbird tows consisted of 24% *Daphnia*, 8% *Copepoda*, 48% *Holopedium*, 15% *Chaoborus*, <1% *Chydorus*, and 4% unidentifiable zooplankton. Each tank for Crampton fish received one pull at 12 m. Averaged subsamples of Crampton tows consisted of 6% *Daphnia*, 75% *Copepoda*, 8% *Bosmina*, 1% *Holopedium*, 4% *Chaoborus*, and 5% unidentified zooplankton. Feeding trials were conducted in the morning, due to a peak in feeding after dawn (6am-12pm; Vigg et al. 1991). After the two hour feeding trials concluded, the fish were placed in a lethal dose of tricaine methanesulfonate (MS-222) to be used as euthanasia (250 mg/L). The animals were pronounced dead once opercular movement had ceased for a minimum of ten minutes. Further, the GI tract of the fish was taken out of the body, immediately washed out with ethanol to cease digestion and preserve the stomach contents, and the number of zooplankton ingested was quantified. For Crampton fishes, the exact number of zooplankton were quantified. For Hummingbird fish, when there was greater than 150 zooplankton in a stomach, subsamples were used to quantify the number of zooplankton consumed.

Minitab 17.3.1 was the statistical program used to analyze the results. Using a two-way ANOVA, we examined the effects of the lake Bluegill were collected from and

the DOC concentration on the number of zooplankton eaten, using length of fish as a covariant. A one-way ANOVA was used to examine the number of zooplankton eaten by Largemouth Bass and Bluegill from Crampton in the light water treatment.

## RESULTS

196 fish were taken into the laboratory for trials (14 Largemouth Bass from Crampton, 112 Bluegill from Crampton, and 70 Bluegill from Hummingbird). Of the fish taken into the laboratory, there was a 21% mortality of Largemouth Bass, 88% mortality of Bluegill from Crampton, and 47% mortality of Bluegill from Hummingbird.

There were seven species of zooplankton eaten by Bluegill and four species of zooplankton eaten by Largemouth Bass (Figure 1). Largemouth Bass greatly preferred *Daphnia*, then *Chaoborus*, and ate less than 10% *Holopedium* and *Bosmina*. Oppositely, Bluegill preferred *Holopedium*, then *Copepoda*, *Chaoborus*, and *Daphnia*, with under 10% *Bosmina* and *Chydorus*. The maximum proportion of consumed zooplankton from the amount provided per tank was 46%, suggesting that zooplankton was not a limiting factor in feeding trials.

There was a significant difference in zooplankton eaten by Bluegill with respect to the lake the fish were taken from ( $p < 0.0001$ ;  $F = 18.58$ ;  $DF = 1$ ; Figure 2). There was no significant difference in zooplankton eaten by bluegill with respect to the DOC concentration replicated ( $p = 0.434$ ;  $F = 0.62$ ;  $DF = 1$ ; Figure 2). Similarly, covariance in zooplankton eaten with respect to the length of fish was not statistically impacted ( $p = 0.089$ ;  $F = 3.03$ ;  $DF = 1$ ).

Further, there was no significant difference in zooplankton eaten in the light water treatments with respect to the species of fish, Bluegill and Largemouth Bass ( $p=0.210$ ;  $F=1.68$ ;  $DF=1$ ; Figure 3).

## DISCUSSION

Though hypothesized that there would be a competitive advantage between juvenile Bluegill and Largemouth Bass, there was no observation to support that conclusion. Similarly, DOC concentrations did not affect the foraging ability of fishes. There was, however, a significant difference in the number of zooplankton eaten due to the lake that the fish were taken from. This could be attributed to morphological differences in fish from those habitats that support an increased ability to forage. Due to murky visual conditions, increased eye size can be exhibited as DOC concentrations increase (Bartels et al. 2016). Though not examined further, Hummingbird fish may have had a larger eye, contributing to their increased ability to forage in both light and dark water treatments. Another possibility that explains the increased ability for Hummingbird fish to forage could be their behavior. It was noted that survival and adaptability in Hummingbird fish was much greater than those of Crampton. Hummingbird fish exhibited more exploratory behavior, a more observable foraging performance, and exhibited schooling, while for the most part Crampton fish did not. Though this could be due to differences in personality of the fish, the stark differences in overall observable behavior could be attributed to source lakes.

It was not exhibited that light climate affects the quantity of zooplankton eaten, even though it was expected that as reductions in visibility occurred, detection of prey

would decrease as well (Higham et al. 2015). Though not examined statistically, a trend in zooplankton preferences have been exhibited. Only 18% of Largemouth Bass in this experiment could successfully forage for zooplankton. Among those individuals, a clear preference for large zooplankton (i.e. *Daphnia*, *Chaoborus*, and *Holopedium*) was exhibited. This preference was especially robust when compared with Bluegill feeding, in which the largest zooplankton were only eaten 62% of the time. Aksnes and Giske (1993) developed a model explaining that as light and size of prey increase, visual range increases. Therefore, since smaller zooplankton require a stronger ability of fish to forage, it is possible that Bluegill do display a competitive advantage. Since larger food items provide a greater amount of sustainable energy for the consumer, if Bluegill can consume a large quantity of larger zooplankton, success of Largemouth Bass will still be limited.

Experimental trials consisted of only Bluegill, only Largemouth Bass, and both Largemouth Bass and Bluegill. Interestingly, every tank that had both Bluegill and Largemouth Bass had a 100% mortality rate of Bluegill. To that effect, every trial that used Largemouth Bass used bass only and every trial that consisted of Bluegill used Bluegill only. The high mortality rate could be attributed to stress on Bluegill from the chemical cues of Largemouth Bass, due to a high sensitivity to learned predator kairomones (Miyai et al. 2016). In future studies, using naïve laboratory-reared Bluegill in the presence of juvenile Largemouth Bass may counteract the learned predatory threat that the wild-caught individuals exhibited (Martin 2014).

As with any study, an increase in sample size could change the results of the experiment. Under 32% of total fish brought into the laboratory survived the 24 hours

until trials, which caused significant concern. Water quality in the form of general hardness, alkalinity, pH, nitrite, and nitrate were measured with quantities seemingly normal between tanks. A copper test was also conducted, with a value above 1000  $\mu\text{g/L}$ . 1100  $\mu\text{g Cu/L}$  has been determined to be the median tolerance limit for juvenile Bluegill (Benoit 2011). Further, Brix et al. (2001) mentions that nearly 90% of species are affected at a dissolved copper concentration of 1000  $\mu\text{g/L}$ , with a species mean acute value at 1203.5  $\mu\text{g/L}$ . Nearing, and possibly going above the threshold for tolerable copper concentrations in juvenile Bluegill may have influenced the results in this experiment. Additional measures should be taken to counteract or prevent copper effects if fish are to be brought back to the laboratory in future studies.

In summary, this study demonstrates that the DOC concentrations that fish populations are reared in has a greater effect on foraging efficiency than temporary habitat conditions. This may point towards plasticity in environmental conditions so long as strong foraging abilities are developed. Variances in morphological features as well as personality and adaptability traits could contribute to more successful foraging efficiency at the individual and population level. There was not a strong difference in interspecific ability to forage for zooplankton, though preferences may have been exhibited. Overall, relationships between rearing environment and foraging efficiency can be examined further to understand ecological processes occurring within fish populations.

## ACKNOWLEDGEMENTS

I'd like to thank my mentor, Colin Dassow, for his guidance throughout each step of this project. Gratitude is owed to Emily Miller, Emily Ramirez, and Mariah Lighthall for their hard work and help in trapping fish. Patrick Larson and Michael Cramer deserve a warm thank you as well for help and guidance, especially with equipment. A generous thank you is also extended to the Bernard J. Hank Family Endowment for funding my research and lodging at the UNDERC property. I'd also like to give a thank you to all faculty, staff, and students at UNDERC for continued support throughout my experiment.

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APPENDIX

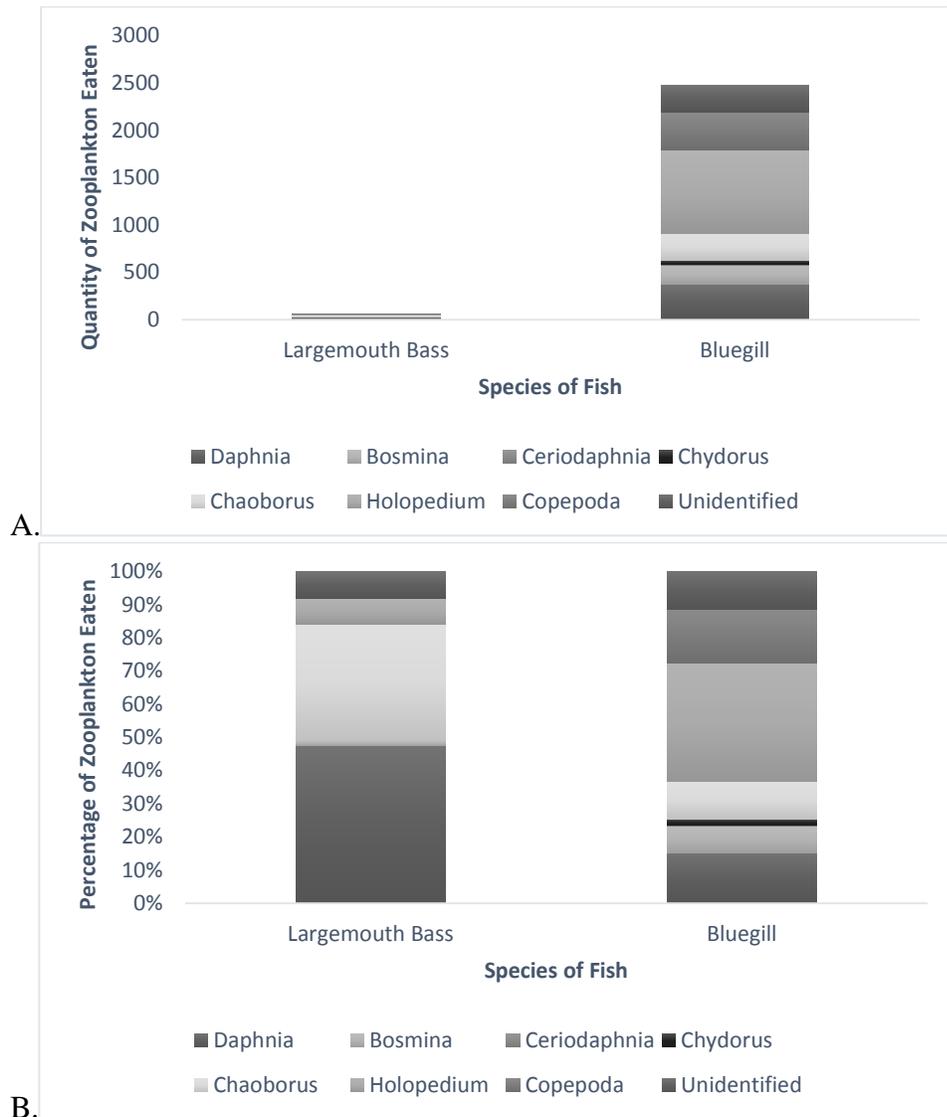


Figure 1. A. The quantity of total zooplankton eaten by Largemouth Bass and Bluegill.  
 B. The observed percentage of zooplankton species eaten by Largemouth Bass and Bluegill.

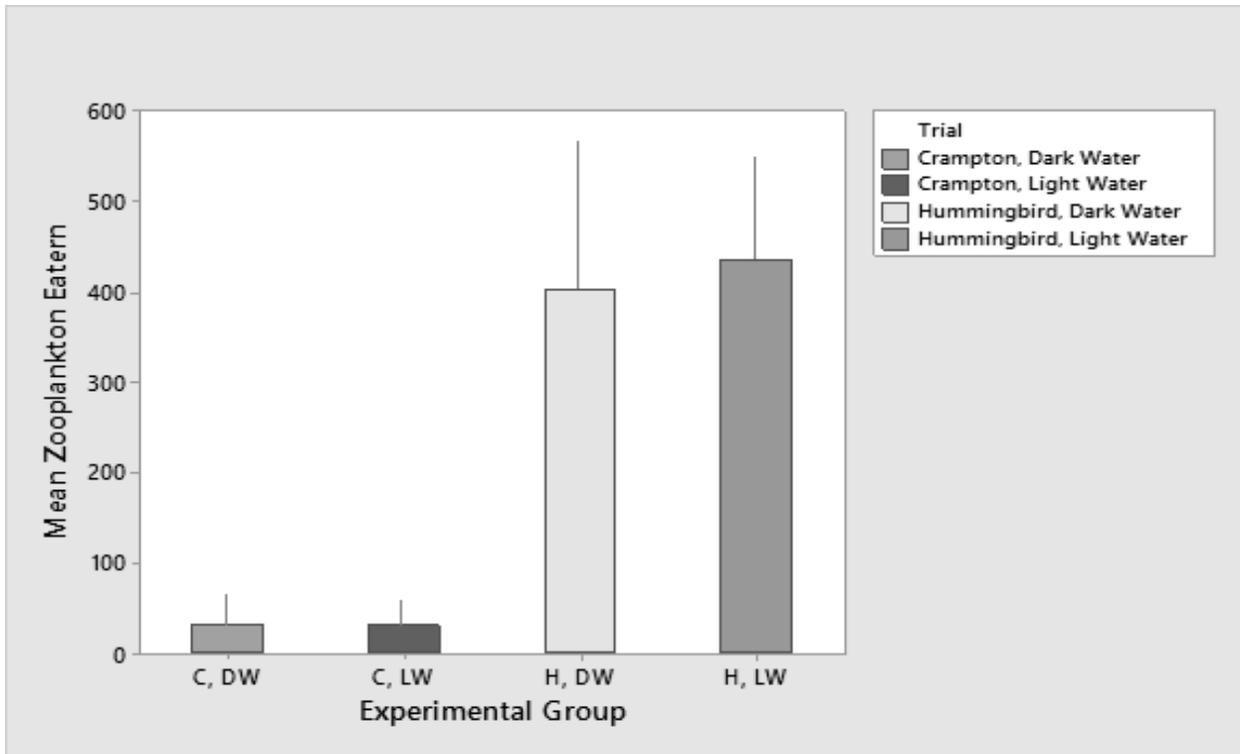


Figure 2. The mean zooplankton eaten in each experimental group: fish taken from Crampton lake and placed in a dark water treatment, fish taken from Crampton lake and placed in a light water treatment, fish taken from Hummingbird lake placed in a dark water treatment, and fish taken from Hummingbird lake placed in a light water treatment. Error bars represent standard deviations. With respect to the lake taken from,  $p < 0.0001$ ,  $F = 18.58$ ,  $DF = 1$ . With respect to DOC concentration in water treatments,  $p = 0.434$ ,  $F = 0.62$ ,  $DF = 1$ .

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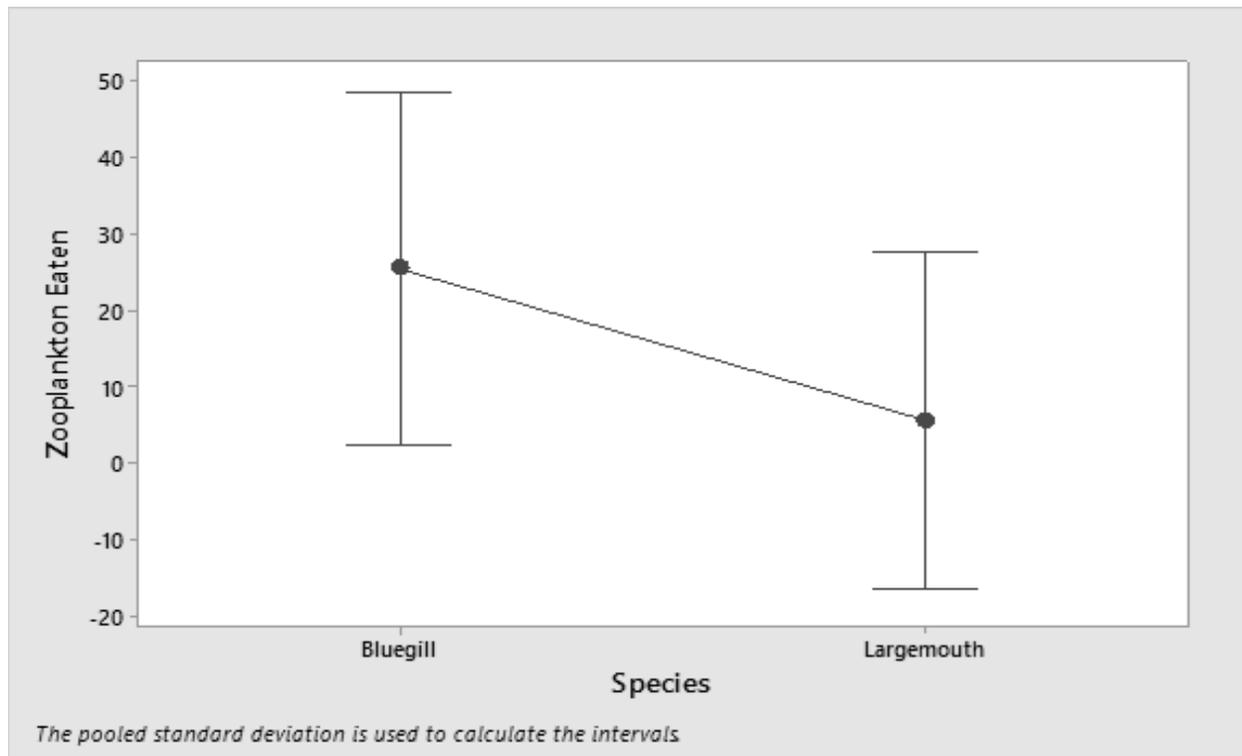


Figure 3. An interval plot of mean zooplankton eaten by Bluegill and Largemouth Bass from Crampton in the light water treatment. Error bars represent standard deviations. This had a 95% confidence interval about the mean.  $P=0.210$ ;  $F=1.68$ ;  $DF=1$ .