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Changes in the Zooplankton Community in Barren River Lake (South Central KY) Between 2008 and 2020

Laurel Philpott

CHANGES IN THE ZOOPLANKTON COMMUNITY IN BARREN RIVER LAKE (SOUTH CENTRAL KY) BETWEEN 2008 AND 2020

A Capstone Experience/Thesis Project Presented in Partial Fulfillment
of the Requirements for the Degree Bachelor of Science
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at Western Kentucky University

By
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ABSTRACT

Zooplankton are small aquatic animals that serve an important role in transferring energy from phytoplankton to higher trophic levels. The zooplankton community composition in Barren River Lake from 2008 and 2020 was compared. A field study was conducted to not only describe the seasonal population dynamics of zooplankton in Barren River Lake, but also to determine if an invasive zooplankton, *Daphnia lumholtzi*, was present. While *D. lumholtzi* was found throughout Barren River Lake in 2008, it was absent from both resampled sites in 2019 and 2020. This, along with erratic patterns of emergence in several other zooplankton species, indicates that species composition in Barren River Lake can be highly variable. *Daphnia lumholtzi* is characterized by a long spine that aids it in avoiding predation from invertebrate predators and small fish. To test if the spines were causing damage to the mouths of small fish, a series of feeding trials were conducted with Bluegill feeding on introduced *D. lumholtzi,* or *Daphnia pulex*, a native species. Juvenile Bluegill from the feeding trials were dissected, and their buccal cavities were observed under a scanning electron microscope (SEM) to check for any spine-inflicted damage. In the feeding trial, Bluegill readily ate both *D. pulex* and *D. lumholtzi.* Examination of the tongues of the fish showed no difference in physical damage (scraping of the epithelium) between the fish consuming *D. lumholtzi* and *D. pulex*. Continued monitoring of the zooplankton community of Barren River Lake is critical to understand the impacts of *D. lumholtzi.*

ACKNOWLEDGEMENTS

Thank you to my mentor, Dr. Philip Lienesch, for your constant support through my undergraduate honors thesis journey. I appreciate all the late-night editing and early morning visits to the lake, and truly feel grateful for your guidance these past two years (and hopefully years to come). I also want to thank Dr. Keith Philips for his great feedback on my paper, as well as Mrs. Leah Thompson for her much-needed grammatical expertise and emotional support! I also want to thank Dr. John Andersland for training me on the SEM and spending hours of his own time to help me succeed. Although the zooplankton made for great company, Alyssa Lowe was a bit more lively, and certainly the best fish-feeding coworker I could ask for. Also, thank you to Nate Mattingly for his help processing many of the samples from 2019 and 2020. A special acknowledgement goes to my friends, Gillie, Amara, Ben, and my beloved cat Gnocchi; thanks for helping me find somewhat of a work-life balance!

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VITA

EDUCATION

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RESEARCH EXPERIENCE

Honors Thesis: Changes in the Zooplankton Community in Barren River Lake (South Central KY) Between 2008 and 2020
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REU, June 2021-August 2021
Advisor: Dr. Wendy Applequist
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Undergraduate Botany Research
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INTRODUCTION

When a species is introduced outside of its native range, it has the potential to become invasive, meaning that it can spread rapidly throughout an area and compete with native organisms for resources (Simberloff 2013). An infamous example of an invasive species found in North America is the zebra mussel, an invertebrate found in such high densities that it can clog pipes and waterways, and can even attach itself to native species of freshwater clam (Woodward and Quinn 2011). Zooplankton communities in aquatic ecosystems have also not escaped the threat of invasion; *Bythotrephes longimanus* is an invasive species that has rapidly spread throughout the Great Lakes and can outcompete small planktivorous fish for prey (Yan et al. 2011). In the 1990s, a new species of zooplankton known as *Daphnia lumholtzi* was introduced to Missouri and Texas (Havel and Hebert 1993), and quickly became established across the Southeastern United States. While *D. lumholtzi* has been verified in Kentucky, the extent of its invasion in Barren River Lake has never been studied. A comprehensive study of *D. lumholtzi* and the overall zooplankton community of Barren River Lake was conducted given the dire consequences of other invertebrate invasions in aquatic systems throughout the United States.

The collective term *zooplankton* is used to describe any microscopic, free-floating consumer that inhabits an aquatic habitat (EPA 2021). In other words, zooplankton are not one individual species, but instead, a variety of taxa living in either marine or freshwater environments. Zooplankton play a crucial role in limnetic systems because they serve as intermediates, transferring energy from phytoplankton to higher trophic levels (Wylie and Curry 1991).

The main groups of zooplankton include protistans, rotifers, copepods, and cladocerans (Pace and Orcutt 1981). Protistans are microscopic, typically unicellular eukaryotes that belong to the kingdom Protista (Finlay 2004). Protistans are oftentimes excluded from zooplankton studies because their microscopic size means they cannot be captured in standard macrozooplankton nets (Pace and Orcutt 1981). This means that while protistans can play an important role in limnetic systems, they are typically studied separately from other zooplankton taxa.

Most zooplankton studies are centered around macrozooplankton, such as rotifers, copepods, and cladocerans. Unlike the protists, the macrozooplankton are a part of kingdom Animalia, meaning they are multicellular eukaryotes that acquire energy by preying upon other organisms. Macrozooplankton are substantially larger than protistans and represent a higher total of the nutrient regeneration, production, and grazing (Pace and Orcutt 1981). Rotifers (*Rotifera*) are macrozooplankton that include about 2200 species; they are known as ‘wheel animals’ because of their characteristic wheel-resembling corona, a feeding device (Segers 2004).

Two abundant genera of rotifers represented in South Central Kentucky lakes are *Asplanchna* and *Keratella*. *Keratella* is an herbivore feeding on various species of algae whereas *Asplanchna* is a predator that feeds on other species of zooplankton.

Copepoda, which is composed of 8,000 to 10,000 species, is a diverse subclass of Crustacea inhabiting both fresh and saltwater ecosystems (Pennak 1989). The two American sub-orders commonly found in Barren River Lake are Calanoida and Cyclopoida, both of which are free-living and elliptic, with a body divided into three sections—a head, thorax, and abdomen (Pennak 1989). Cyclopoid copepods are characterized by a teardrop shaped carapace and antennae that are shorter than those found in Calanoid copepods (Fig. 1).

Fig. 1: A Calanoid Copepod (top) can be seen from a side angle, with its carapace in full view. Two small Cyclopoid Copepods (bottom) are beneath the Calanoid Copepod. Copepods can range in size, meaning Calanoids are not always larger than Cyclopoids.

Many of the macrozooplankton, such as *Bosmina, Diaphanosoma*, and *Daphnia* are included in the order Cladocera. *Bosmina* (Fig. 2) is a widely distributed group of Cladocerans that are characterized by a sensory bristle found in between the eye and the tip of the rostrum (Pennak 1989). While they can range in size considerably, the *Bosmina* are small, ranging from 0.3 to 0.5 mm in length. *Diaphanosoma* are much longer than *Bosmina*, ranging from about 0.8 to 1.2 mm (Pennak 1989). Unlike some other Cladocerans, *Diaphanosoma* does not have spines on its post abdominal surface. *Diaphanosoma* is a common taxon and is found globally (Pennak 1989).

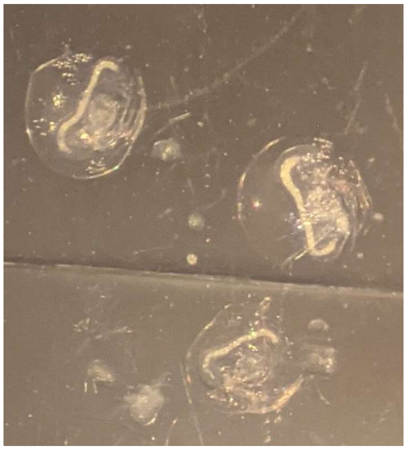


Fig. 2: A group of three *Bosmina*; note the distinctive rostrums projecting from their heads.

The dominant group of cladocerans are the *Daphnia*, which are commonly referred to as “water fleas.” They, like all Cladocerans, are a group of crustaceans that range from 0.2 to 6.0 mm in length and are found globally in both marine and freshwater habitats (Forro et al. 2008). The method by which they reproduce is unique, as they use cyclical parthenogenesis, which is an alternation between phases of sexual and asexual reproduction. In favorable environmental conditions, the cladocerans use amictic parthenogenesis to reproduce, meaning that females produce offspring that are clones of themselves. When conditions change and it is more beneficial to have a diverse population, males are produced and sexual reproduction commences (Decaestecker et al. 2009). Once eggs are fertilized, they are encased in a hard covering to produce an ephippia, which protects the embryo until the harsh conditions improve (Schultz 1977).

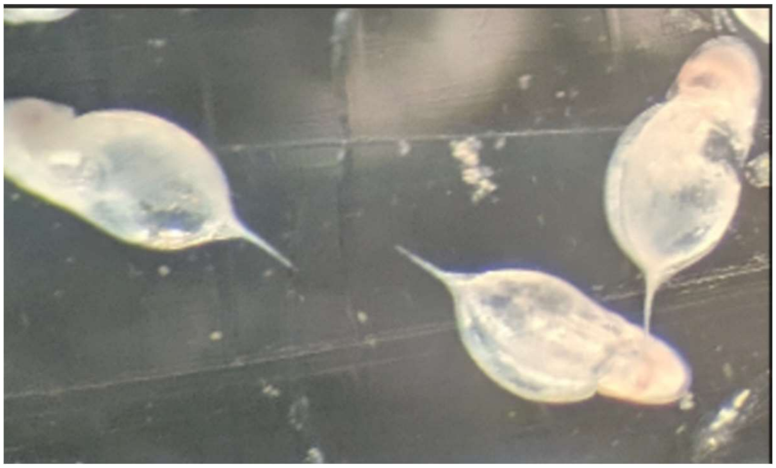


Fig. 3: Three native *Daphnia* exuviae that have been preserved in formalin since 2008. Note that the native *Daphnia* have spines, but they are only about a fourth of the length of the body.

While some water flea species are native to Kentucky (*Daphnia longispina, Daphnia pulex*), others are introduced non-native species. *Daphnia lumholtzi* (Fig. 4) is a non-native species in North America that has spread from tropical lakes in Asia, Africa, and Australia (Havel and Hebert 1993). The main dispersal mechanization of *D. lumholtzi* within the United States is likely recreational boating (Dzialowski et al. 2000).

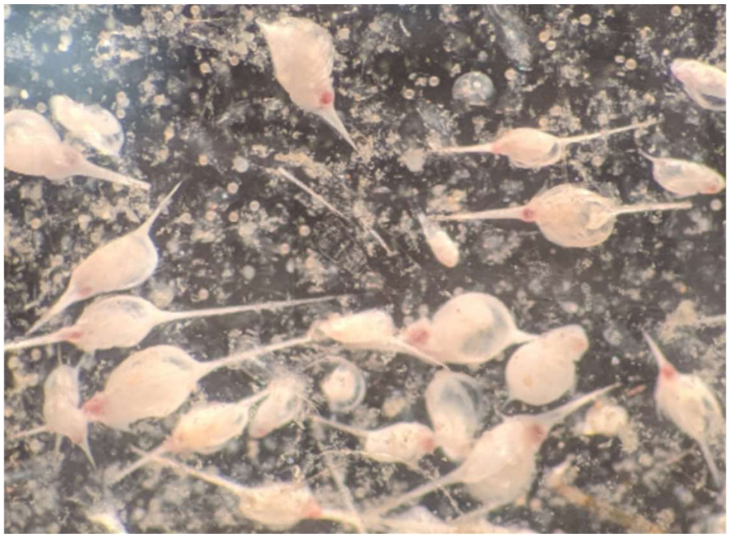


Fig. 4: A swarm of *D. lumholtzi*, which are characterized by long spines that are up to three times the size of the carapace.

While *D. lumholtzi* is commonly referred to as an invasive organism, the extent of its damage on south central Kentucky lakes is unknown. *Daphnia lumholtzi* would be inflicting harm upon the environment if it was outcompeting with native species or was using resources and is not susceptible by zooplanktivorous predators.

Existing ecological theory is in place to explain the competition that exists between introduced and native species. The Enemy Release Hypothesis asserts that because non-native zooplankton are not as susceptible to predation by local predators and parasites as native zooplankton are, the non-native zooplankton are at an advantage and subsequently have higher success rates (Liu and Stiling 2006). The Evolution of Increased Competitive Ability (EICA) Theory expands upon this, arguing that because non-native species do not have to allocate substantial energy towards defense, they can instead use their energy to grow and reproduce, which heightens their competitive edge further (Blossey and Notzold 1995). While the full impacts of *D. lumholtzi* on limnetic communities have not yet been discovered, researchers fear that because of its long spine, tendency to swarm, and ability to survive in warmer temperatures, *D. lumholtzi* may serve as a potential threat to native species (Stoeckel and Charlebois 1999). However, this assumption cannot be confidently asserted without experimental support.

While the presence of *D. lumholtzi* has been noted in nearby Kentucky Lake (Yurista et al. 2000) and Nolin River Lake (Beaver et al. 2017), this invasive species has not been identified in Barren River Lake. Therefore, the first question addressed is whether *Daphnia lumholtzi* occurs in Barren River Lake, a 10,000-ha reservoir in south central Kentucky, and if so, is it abundant compared to native zooplankton. Despite the importance of zooplankton to the balance of aquatic ecosystems, the current literature on the freshwater zooplankton community in Barren River Lake is limited. One of the last large-scale studies published on seasonal changes in zooplankton in the Barren River Lake region was conducted by Novotny and Hoyt (1982). They conducted their survey before the accidental introduction of *D. lumholtzi* to North America in 1990 (Sorenson and Sterner 1992). A study analyzing the changes in zooplankton composition in the last decade will provide insight into overall community health and if known zooplankton patterns have shifted because of altered abiotic or biotic conditions.

The second question addressed is whether juvenile Bluegill (*Lepomis macrochirus*) feeding on spiny *D. lumholtzi* receive more damage to their mouths than when they feed on non-spiny native *Daphnia*. Previous studies have shown that the spines of *D. lumholtzi* inhibit predation by small fish (Swaffar and O’Brien 1996, Lienesch and Gophen 2005). Here we look for direct evidence that the spines of *D. lumholtzi* are causing damage in the mouths of small planktivorous fishes. Juvenile Bluegills were fed either *D. lumholtzi* or the native species *D. pulex*, and the number of zooplankton consumed in a 10-minute period was observed. We then examined the tissue in the buccal cavity of the bluegill with an SEM to determine if there was a difference in the amount of physical damage caused by the two species of *Daphnia.*

MATERIALS AND METHODS

Field Study

Barren River Lake is a 10,000-acre flood-control reservoir in south-central Kentucky. The impoundment was created in 1964 by the U. S. Army Corps of Engineers and drains 940 square miles, mainly in Barren, Allen, and Monroe counties. Zooplankton samples were collected monthly from May to November 2008 at seven sites: Austin, Barren River Mouth (BRM), Bridge, Peninsula, Port Oliver Yacht Club (POYC), Skaggs, and WKU (Fig. 5). In addition to zooplankton samples, water quality parameters were measured throughout the water column at each site. Five of the sites were selected along the old channel of the Barren River, the additional two sites (WKU and Skaggs) were in the Skaggs Creek tributary arm of the reservoir (Fig. 5). Two of the sites (POYC and Skaggs Creek) were resampled once a month as part of a project in 2019-2020 to look for changes in the zooplankton community over the last decade. The 2019 and 2020 samples used the same techniques as the 2008 data set.

Fig. 5: Seven sites sampled in 2008 at Barren River Lake in Kentucky. Skaggs and POYC sites were re-sampled as part of another project in 2019 and 2020.

The zooplankton collecting technique was modified from the EPA's guidelines for Standard Operating Procedure for Zooplankton Sample Collection and Preservation (2013). Zooplankton sampling was performed by slowly dropping a Wildco (86475 Gene Lasserre Blvd. Yulee, FL 32097) Wisconsin zooplankton net (80 um mesh) five meters and slowly pulling the net back toward the surface. The net filtered 56.5 liters of lake water while collecting the zooplankton. If the depth of the lake was less than five meters, then the net was dropped to the lake bottom. Zooplankton were deposited into a 200 ml glass jar and the net was rinsed twice into the collection jar. The sample was preserved with 10% formalin.

In the lab, three 9.0 mL subsamples from each jar were examined using a zooplankton wheel, and the quantity of zooplankton taxa in each subsample was recorded. The quantity of each taxa across all three sub-samples was used to estimate the number present in the entire sample. The density of each taxa (# per liter) was calculated by dividing the total abundance from each sample by the 56.5 liters of water sampled by the Wisconsin net. Zooplankton taxa were as follows: Rotifers, Copepod nauplii, Calanoid copepods, Cyclopoid copepods, *Bosmina*, *Ceriodaphnia*, *Diaphanosoma*, native *Daphnia spp.*, and *Daphnia lumholtzi*.

Water quality parameters were measured with a Hydrolab Quanta multimeter. Measurements recorded included temperature, specific conductance, pH, dissolved oxygen, salinity, % dissolved oxygen, oxidation reduction potential, and turbidity. Readings were taken at the surface, 1 m, 3 m, 5 m, 7 m, 9 m, 11 m, and 13 m. In the lab, the depth of the epilimnion (upper well mixed layer of a stratified lake) was determined by identifying the thermocline, which is the point in which water temperature drops significantly and marks the threshold to the bottom layer of the lake, the hypolimnion (Denys 2009). The average of each water quality parameter in the epilimnion was calculated. If the lake was not stratified, readings from the entire water column were used to characterize the environmental conditions.

One of the main questions of the study was to identify if *D. lumholtzi* was present in Barren River Lake in 2008, and if it was, to examine how its seasonal distribution compared to that of native species of *Daphnia*. When *D. lumholtzi* was present, time series graphs were constructed to compare the abundance of *D. lumholtzi* and native *Daphnia* for each month at the seven sites in 2008.

Non-metric multidimensional scaling (NMDS) graphs characterized the monthly samples from each site, which helped examine how the zooplankton community changed over the growing season. The NMDS graphs arrange taxa by their abundance from May to November at each of the seven sites in 2008. Taxa that were most abundant at similar times were more closely associated on the graph. The environmental data was then added to the graphs as a series of vectors. The longer an environmental vector is represented on the graph, the more closely associated the vector is with either the corresponding month or taxa.

The trends in community composition in 2008 were compared to those from 2019 and 2020. Four additional NMDS graphs were created using the Skaggs 2019 and 2020 data, as well as the POYC 2019 and 2020 data. Although the 2019 and 2020 sampling included winter months (December, January, February), only data from May through November is included to make the dataset comparable to that from 2008.

Laboratory Experiment

A feeding experiment was conducted to test if small zooplanktivorous fish are damaged by the long spines of *D. lumholtzi.* One of the most notable features of *D. lumholtzi* is its long spines. Although it makes sense that the spine would be an advantage because it could ward off predators, tests were needed to confirm that native fish were inhibited from preying on this species.

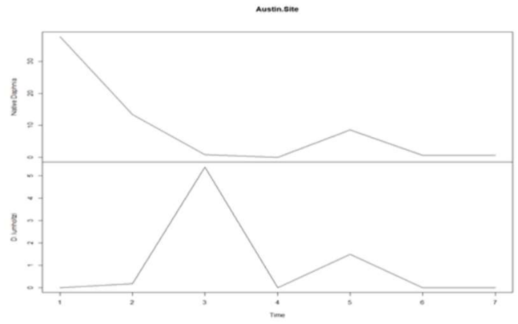
In my feeding trials, juvenile Bluegill from the Western Kentucky University Green River Biopreserve were caught and transported to the lab. The fish were kept in a 40-gallon aquarium under a 12:12 photoperiod until they were used in a feeding trial. *Daphnia lumholtzi* were collected from the epilimnion of Rough River Reservoir with a Wildco (86475 Gene Lasserre Blvd. Yulee, FL 32097) Wisconsin zooplankton net (153 um mesh). *Daphnia pulex*, a species native to Barren River Lake, was purchased from Carolina Biological Supply Company (Burlington, NC). All zooplankton were used within 5 days of being brought into the lab. Bluegill of approximately the same length (48 - 63 mm) were chosen one at a time and put into a separate tank with approximately 50 individuals of either *D. lumholtzi* or the native *Daphnia pulex*. The number of attacks each fish made in a span of 10 minutes was recorded, as well as behavioral observations such as the number of times a fish attacked, and then rejected, a prey item. In total, 10 trials with 10 individual fish were conducted; five fish ate *D. pulex* and five ate *D. lumholtzi*. Prior to the experiment, the fish were fed bloodworms so as not to interfere with their preferences.

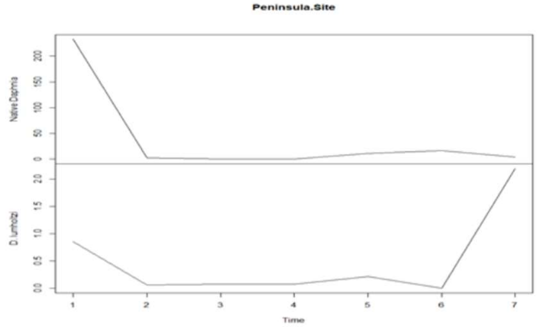
Following the 10-minute observation period, the fish were euthanized using MS-222. These fish were preserved in 10% formalin for use in the last part of the study. The fish's lower jaws and tongues were examined with a scanning electron microscope (SEM) to help determine if the spines of *D. lumholtzi* caused any physical damage to the mouth lining. Once dissected, the jaws were washed in water for three days to remove the formaldehyde. The jaws were then transferred to 70% ethanol for one day and then 100%

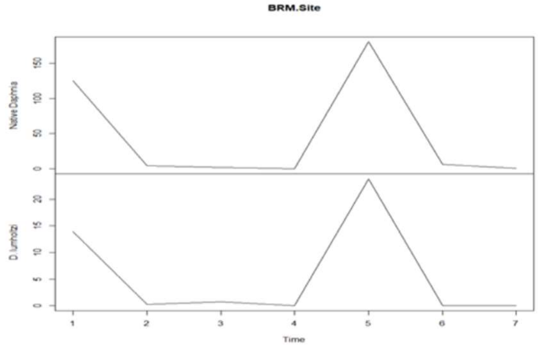
ethanol for one day before undergoing critical point drying with liquid CO_2 and sputter coated with gold.

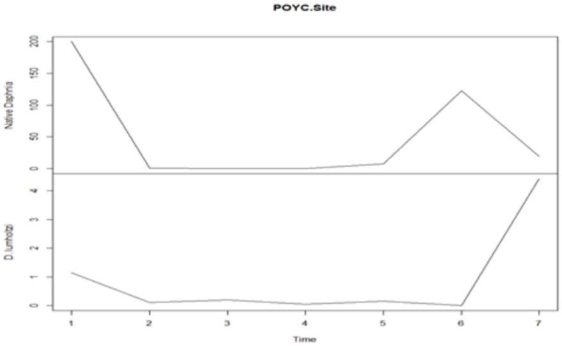
RESULTS

Both *D. lumholtzi* and the native *Daphnia* sp. were present in Barren River Lake in 2008 at all seven sites. *Daphnia lumholtzi* reached its maximum abundance at a different time than the native *Daphnia* at six of the seven sites (Fig. 6). The native *Daphnia* peaked in abundance during May and June, and then sometimes reappeared for a smaller peak in September and October. *Daphnia lumholtzi* is a species that emerges in the summer months, around June through August (Fig. 6, see sites Austin, Bridge, BRM, Skaggs, and WKU). At the Peninsula and POYC sites, however, *D. lumholtzi* reached maximum abundance in October instead of summer. It is important to note that the peaks at the Peninsula and POYC are lower than the other sites; only two individuals were present in the water column at the Peninsula site in October, and five were found in October at the POYC site. 20 native *Daphnia* were found at POYC and the Peninsula sites in October, so even though *D. lumholtzi* was reaching its annual maximum, it was not the dominant Daphnid present in the community.


Austin.Site


Peninsula.Site


BRM.Site


POYC.Site

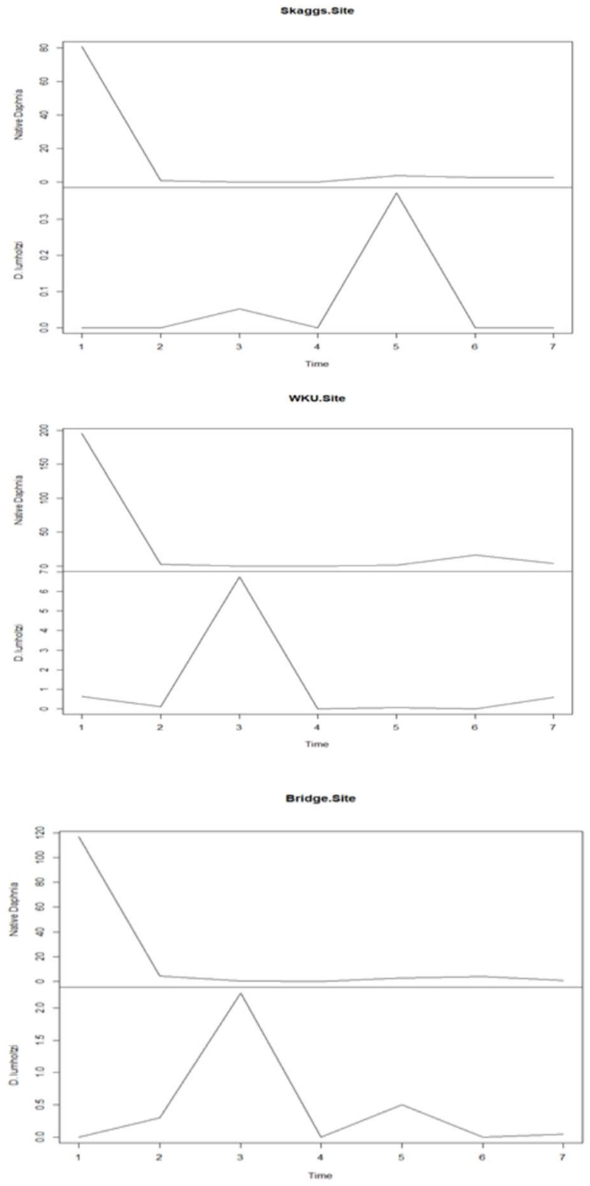


Fig. 6: Time series graphs showing *D. lumholtzi* vs. native *Daphnia* in 2008. Note that the scales for each species are different, as the graphs emphasize when the peak of abundance occurs, not the relative abundance between taxa.

NMDS analysis confirms that at the seven sites in 2008, *D. lumholtzi* was primarily a summer species, whereas the native *Daphnia* peaked in the spring and fall (Fig. 7, Table 1). The NMDS graphs also offer insight into the overall seasonal trends of the other zooplankton within the community in 2008. The NMDS graph for the Austin site is presented (Fig. 7) as a typical site. There was a suite of species (Rotifers, *Bosmina*, *Ceriodaphnia* and native species of *Daphnia*) that were strongly associated with spring (May sample) and a smaller peak associated with the fall (October and November: Fig. 7, Table 1).

Copepods, nauplii, *Diaphanosoma*, and *Daphnia lumholtzi* were associated with the summer samples (June through Sept). Calanoid Copepods in 2008 were associated with the summer at every site except for Austin (Fig. 7) and Bridge, where the taxon reached a maximum in November and October, respectively. Similarly, the cyclopoid Copepods were consistently found in greatest abundance during the summer (Table 1), except for in one instance where it was found in November 2008 at the WKU site.

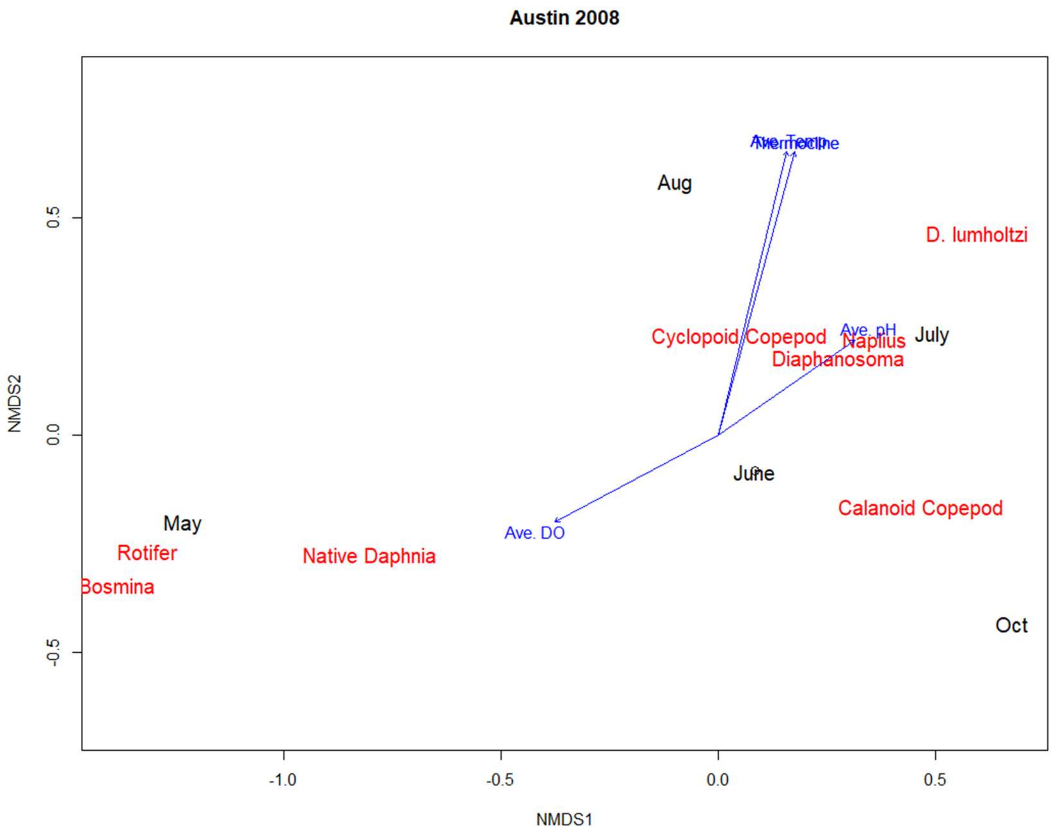
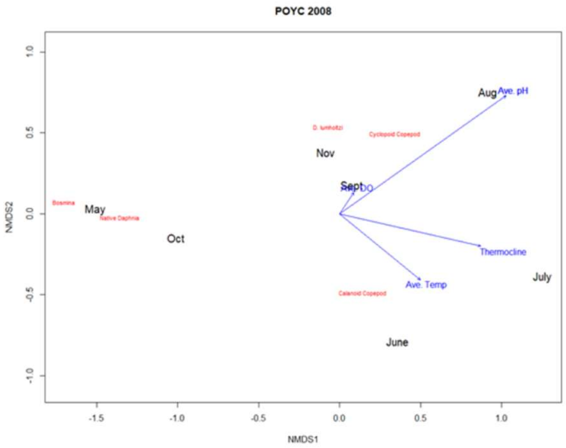
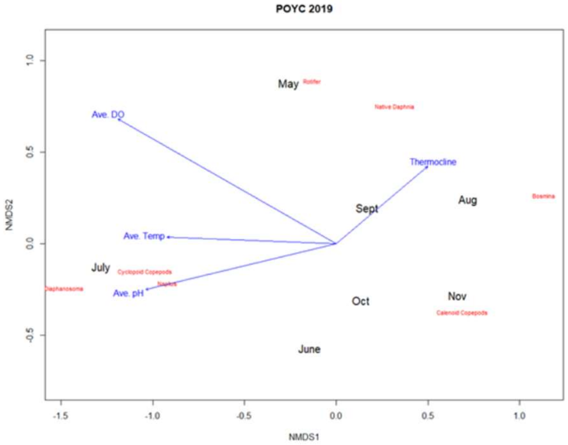
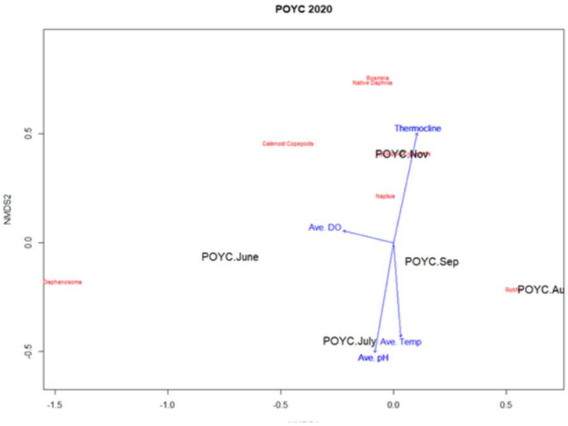


Fig. 7: NMDS graph showing the distribution of zooplankton from May to November 2008 at the Austin site in Barren River Lake, KY. This graph is typical of the overall trends seen in 2008 for the five sites in the main channel.

NMDS graphs visualizing changes in the community composition for both sites between 2008, 2019, and 2020 can be found in Fig. 8. One of the primary differences between the datasets was that *D. lumholtzi* was absent from both sampled sites (POYC and Skaggs) in 2019. Additionally, some zooplankton taxa tended to reach points of maximum abundance at later times than expected based upon the 2008 data. For instance, while native *Daphnia* peaked in May 2019 at the POYC site as expected, at Skaggs 2019 the peak was closely associated with July and August (Fig. 8, Table 1). The Copepods also saw variation from their 2008 trends. In 2020 at Skaggs, both calanoid and cyclopoid Copepods peaked in October (Fig. 8, Table 1). The 2019 POYC cyclopoid Copepods were also closely associated with October. Likewise, the calanoid Copepods also peaked in November in 2019 and 2020 at POYC (Fig. 8, Table 1).


POYC 2008
1.0
0.5
0.0
-0.5
-1.0
NMDS2
-1.5
-1.0
-0.5
0.0
0.5
1.0
NMDS1
Aug
Ave. pH
D. lumholtzi
Cyclopoid Copepod
Nov
Sept
Ave. DO
Bosmina
May
Native Daphnia
Oct
Thermocline
Ave. Temp
July
Calanoid Copepod
June


POYC 2019
1.0
0.5
0.0
-0.5
NMDS2
-1.5
-1.0
-0.5
0.0
0.5
1.0
NMDS1
May
Rotifer
Native Daphnia
Ave. DO
Thermocline
Aug
Bosmina
Sept
Ave. Temp
July
Cyclopoid Copepods
Naplius
Daphanosoma
Ave. pH
Oct
Nov
Calenoid Copepods
June


POYC 2020
0.5
0.0
-0.5
NMDS2
-1.5
-1.0
-0.5
0.0
0.5
Bosmina
Native Daphnia
Thermocline
Calenoid Copepods
POYC.Nov
Naplius
Ave. DO
POYC.June
POYC.Sep
Daphanosoma
POYC.Au
POYC.July
Ave. Temp
Ave. pH

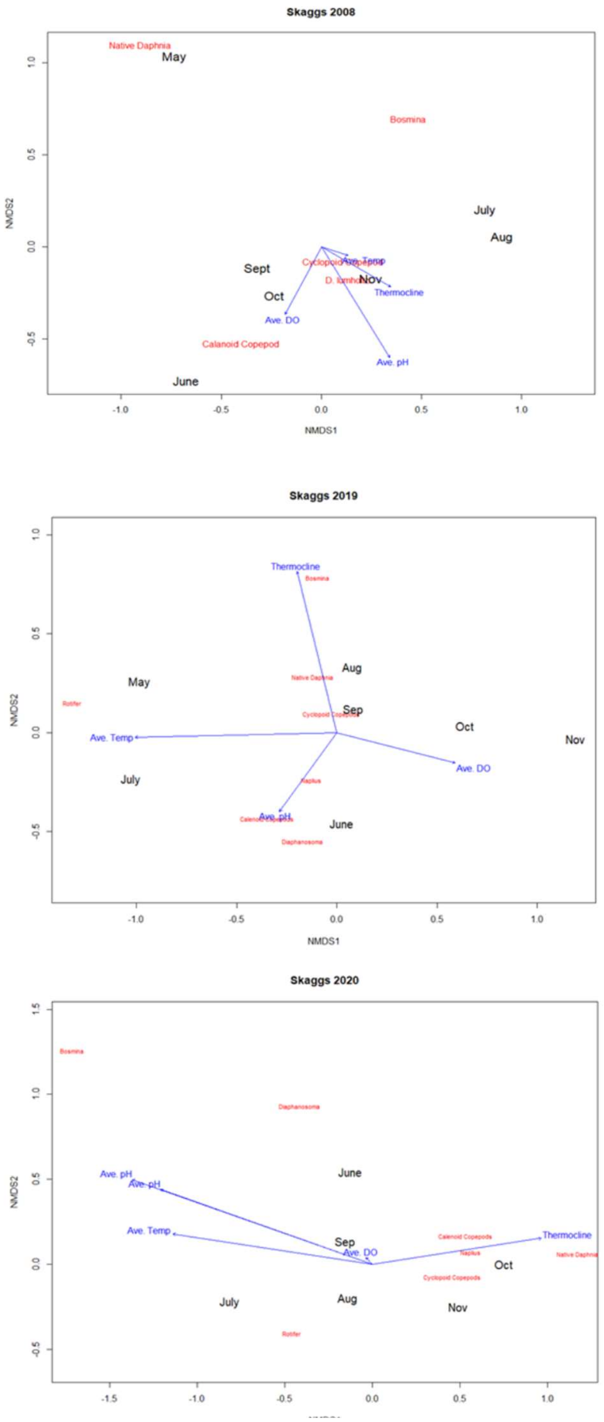


Fig. 8: Changes in POYC and Skaggs between 2008, 2019, and 2020.

Taxa	**Average Trend in 2008 Across 7 sites**	**2019 Skaggs**	**2020 Skaggs**	**2019 POYC**	**2020 POYC**
Bosmina	**Spring/Fall**	**Summer**	**NA**	**Summer**	**Fall**
Native Daphnia	**Spring/Fall**	**Summer**	**Fall**	**Spring/Fall**	**Fall**
D. lumholtzi	**Summer**	**Absent**	**Absent**	**Absent**	**Absent**
Cyclopoid Copepods	**Summer**	**Summer**	**Fall**	**Summer**	**Fall**
Calanoid Copepods	**Summer**	**Summer**	**Fall**	**Fall**	**Fall**

Table 1: Summary of when peak abundance occurred for selected zooplankton at all sites in 2008, and at the Skaggs and POYC sites for 2019 and 2020 in Barren River Lake, KY.

Laboratory Experiment

A laboratory experiment was conducted to look for evidence of physical damage in juvenile Bluegill that might be associated with feeding on *Daphnia pulex* or spiny *Daphnia lumholtzi.* There was not a significant difference between the number of attacks when juvenile Bluegill were feeding on *D. pulex* (M = 58.6) and when feeding on *D. lumholtzi* (M= 35.2); t(4)= -1.0702, p-value = 0.337.

The SEM photographs did not show any more damage in the mouths of the fish that fed on *D. lumholtzi* compared to those that fed on *D. pulex*. Of the five lower jaws that were examined from Bluegill feeding on *D. lumholtzi*, one showed potential evidence of scraping consistent with that expected from spine damage (Fig. 9). Similarly, one lower jaw from the five fish that fed on *D. pulex* showed evidence of scraping (Fig. 10).

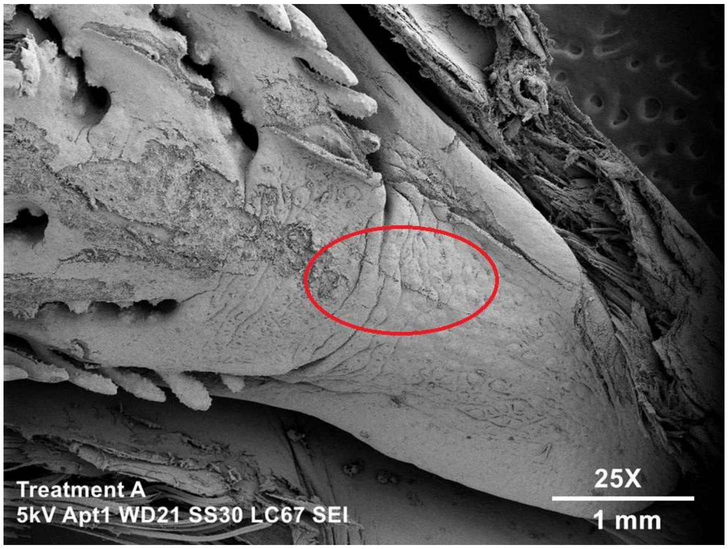


Fig. 9: Scanning Electron Micrograph from a Bluegill that was feeding on *Daphnia lumholtzi.*

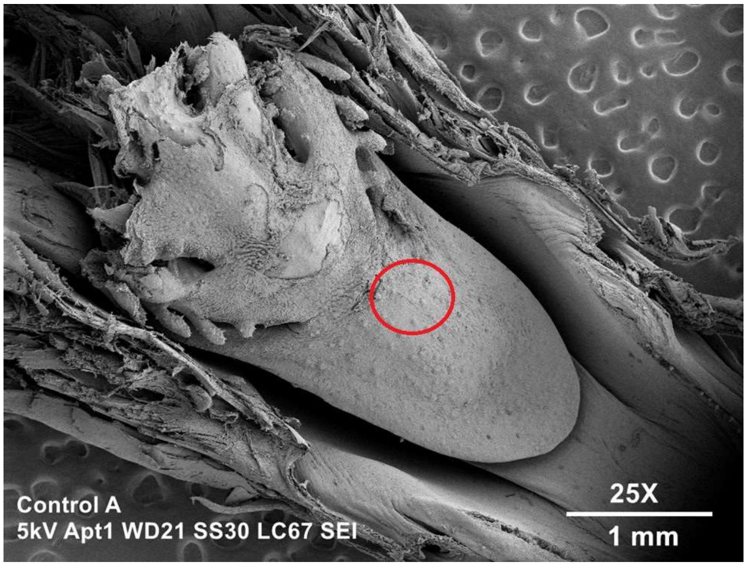


Fig. 10: Scanning Electron Micrograph from a Bluegill that was feeding on *Daphnia pulex.*

DISCUSSION

Field Experiment

The first major finding of the study is that *D. lumholtzi* was present at all seven sites in Barren River Lake in 2008 but was absent based on Skaggs and POYC in 2019 and 2020. Of the seven sites observed in 2008, there is a cluster of five sites in the main channel and two outliers (Skaggs and POYC) that are more upstream and downstream (Fig. 5). Although *D. lumholtzi* numbers were high in the middle five sites in 2008, they were low at the most downstream (POYC) and most upstream (Skaggs). It therefore seems reasonable that no *D. lumholtzi* were found at either site in 2019-2020, as the *D. lumholtzi* population was less successful at POYC and Skaggs in 2008. The Skaggs site is located up a small tributary arm of the reservoir where flow from the creek would be able to flush *D. lumholtzi* downstream in the reservoir. POYC is the most downstream site where the reservoir is deep, wide, and there is very slow flow as water approaches the dam. From our data, it appears that *D. lumholtzi* does best in areas of the reservoir where there would be intermediate amounts of flow. Follow-up studies should sample the other five sites to determine if *D. lumholtzi* is truly absent in Barren River Lake today. This is especially crucial considering that *D. lumholtzi* was collected in 2021 from similar nearby reservoirs (Nolin River Lake, Rough River Lake, and Green River Lake) and would be presumed to still be in Barren River Lake. Still, previous studies confirm that *D.*

lumholtzi has been known to fail to re-establish itself from year to year (Finn et al. 2012), so the variability in presence is not unprecedented.

The BRM site shows that even when the two populations co-occur, the density of native *Daphnia* was seven times higher than that of *D. lumholtzi* (Fig. 6). When *D. lumholtzi* and native *Daphnia* co-exist, the *D. lumholtzi* numbers are so small that they do not pose much competition to the native species.

The 2008 NMDS graphs for each of the sites show that there is a tendency for taxa to either peak in the spring (May) and fall (October and November), or to be closely associated with the summer months. This trend matches the well-established seasonal cycles for zooplankton (Horne and Goldman 1994, Wetzel 1983, Yurista et al. 2000). This pattern of seasonal variation amongst zooplankton success may be explained by seasonal nutrient cycling between upper and lower depths of the lake.

Lakes are composed of three primary layers; the warmest, uppermost layer known as the epilimnion, the metalimnion (includes the thermocline), and the lower hypolimnion (Denys 2009). The density difference between the warm epilimnion and the cold hypolimnion inhibits mixing of the layer in summer and effectively separates the two strata of the lake.

Lake stratification is dynamic, meaning that the water can move between layers at different seasonal points because changing atmospheric temperatures can alter the density of water (Denys 2009). In the fall, when the epilimnion begins to cool, the density of the water increases, causing the water to sink, and fall turnover occurs. The water from the hypolimnion then mixes with the epilimnion, bringing with it a collection of nutrients and ensuring an even distribution of oxygen (Denys 2009). The water remains mixed all winter (unless freezing occurs) and then it stratifies again in the spring (by May in Barren River Lake). The spring has algal and phytoplankton blooms that can sustain increased numbers of zooplankton. As the population of herbivorous zooplankton peaks, phytoplankton density declines and the ecosystem enters the “clear water” phase when many native zooplankton reach their peak densities. There is also an increase in many native zooplankton after fall turnover when increased nutrients in the epilimnion lead to increasing phytoplankton density and eventually zooplankton density.

Perhaps the summer zooplankton taxa who do not correlate with the spring bloom and fall turnovers are well adapted to warmer conditions. Many native zooplankton begin to decline when water temperatures exceed 25 C (Moore et al. 1996). In Barren River Lake, temperatures approach or exceed 25 C from June into September, which results in native taxa decreasing in abundance during the summer months. *Daphnia lumholtzi*, however, has a higher physiological tolerance to heat (Work and Gophen 2001, Lennon et al. 2001) and can therefore exist in the warm summer water. Invasive species do particularly well in ecosystems that are either depauperate of species or have been disturbed leading to low species abundance (Simberloff, 2013). In other words, Barren River Lake may be particularly susceptible to invasive species from June to September because many native zooplankton are not able to occupy the epilimnion during summer stratification. Reservoirs, as artificially created lakes, are disturbed river sections which makes them disturbed habitats. The unusual hydroperiod and flow patterns in reservoirs result in unique habitats that did not exist prior to the last century. These unusual conditions may help *D. lumholtzi* prosper in North American reservoirs in summer, as they are avoiding the increased levels of competition present in the spring (Work and Gophen 1995, 2001). Our findings that *D. lumholtzi* peaks in summer agrees with many other studies of southern reservoirs (Lienesch and Gophen, 2001).

In 2019 and 2020, the emergence of taxa is more variable compared to the trends seen in both 2008 and the literature. Something to note is that in 2020 there was a flood that rendered sampling impossible in May. Because of this, the *Bosmina* appear to be in uncharacteristically low numbers. In reality, the *Bosmina* were likely at their peak in May, but without the samples to support this, it looks as if they are highly disassociated from the rest of the community in the 2020 graphs of Fig. 8. The ranges of the other taxa are highly variable, however. Perhaps as climate change causes an influx in erratic weather conditions, there will be an increase in inconsistent zooplankton cycles. It is important to acknowledge the limitations of this trend, as it is only shown at two sites, over the course of two years. Continued monitoring is essential for confirmation. Still, recognizing this trend is important because it sheds light on how changing environmental conditions can alter when zooplankton emerge.

Laboratory Experiment

The SEM micrographs were inconclusive in proving that the spines were causing major damage to the buccal cavities of juvenile Bluegills. It was expected that a spine would leave a long scratch along the surface of the tongue epithelial tissue, and while such a mark was apparent in the micrographs for one fish that ate *D. lumholtzi*, the other four did not show signs of this damage. Indeed, the only other fish that had a similar scratch was one that was not exposed to *D. lumholtzi*, but instead, to *D. pulex*.

Most of the samples had symmetrical tears that were likely caused by the tissues expanding and cracking as osmotic conditions changed. In order to decrease the chances of osmotic conditions leading to fractures, follow-up studies should submerge the samples in gradually increasing ethanol content (70%, 80%, 90%) instead of putting the samples in 70% directly into 100% ethanol. It would also be interesting to compare Bluegill caught at the same site before and after *D. lumholtzi* emerged for the season. This experiment only exposed fish to the two prey types for 10 minutes, and there were fewer than 50 prey captures during that time. Fish that have been feeding on spiny prey for many days should show signs of accumulated damage if any such damage does occur.

Swafford and O'Brien (1996) reported higher rates of rejection when Bluegill fed on spiny *D. lumholtzi* versus native *Daphnia*. From this we anticipated that the spines would be physically impaling the tissues of the mouth and inhibiting ingestion following capture. The length of the Daphnia were not measured, and perhaps they were too small to cause problems during ingestion. Both Swaffar and O'Brien (1996) and Lienesch and Gophen (2005) reported that larger zooplanktivorous fish readily consume *D. lumholtzi*, despite the long spines. This topic deserves a more indepth experiment involving predators and prey of various sizes to elucidate how the spines of *D. lumholtzi* inhibit predation by zooplanktivorous fish. This is particularly true given the small sample size of this study; follow up experiments should examine more fish to produce more confident results.

CONCLUSION

A major goal of this study was to analyze field samples to determine if *D. lumholtzi* was present in Barren River Lake. Ultimately, *D. lumholtzi* was found at all seven sampled sites in 2008 but was absent from both of the sites resampled in 2019 and 2020. Future studies should continue to monitor when and where *D. lumholtzi* is abundant in Barren River Lake in order to understand what factors contribute to the invasive species maintaining itself each year. Additionally, this study aimed to compare the seasonal trends of the zooplankton community between 2008 and 2020. The zooplankton within the 2008 samples closely followed seasonal trends found in previous literature; *Bosmina* and native *Daphnia* reached maximum abundance in May with a smaller peak in the fall, while the Copepods and native *Daphnia* peaked in the summer. In 2019 and 2020, the zooplankton diverged from their expected seasonal trends, as their patterns of emergence became highly variable from both site to site and year to year. A laboratory study was conducted to examine if Bluegill sustained injuries from the long spines of *D. lumholtzi*. In the end, examination of fish buccal cavities under the SEM did not indicate that the spines of *D. lumholtzi* were causing significant damage. Further analysis should look at fish that were exposed to the spines at higher rates for longer periods of time, while also taking extra precautions to minimize handling damage.

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