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### A Reexamination of the Freshwater Mussel Community of the Little Miami River

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### A REEXAMINATION OF THE FRESHWATER MUSSEL COMMUNITY OF THE LITTLE MIAMI RIVER

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8 April 2020

Submitted in partial fulfillment of the requirements for graduation with Honors

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Table of Contents	
Acknowledgements	3
Abstract	4
Introduction	5
Methods	7
Results	10
Population Graphs	12
Biodiversity Graphs	17
Density Graphs	20
Discussion	22
Literature Cited	26
Appendix 1: Mussel Data	28
Appendix 2: Repeated Measures ANOVA Test Results	48

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#### Abstract

Understanding the changes in biodiversity and local freshwater mussel populations is critical to mitigate the risks to these populations. Freshwater ecosystems continue to degrade rapidly. The freshwater mussel community of the Little Miami River is degraded and has been in decline for many years. The biodiversity contained within freshwater ecosystems is lost at unprecedented rates influenced by human activity. Freshwater mussels play a key role in understanding the effects of human activity on freshwater ecosystems as well as maintaining the health of freshwater habitats. However, they are also sensitive to drastic changes, or disturbances, in their ecosystems. This study involved monitoring and recording the presence of freshwater mussel species in the Little Miami River and combining previous and new research to examine the changes in communities and biodiversity over time. Quadrat and general collecting were used to find and record the mussel populations. Overall, we observed a downward trend in the mussel community size. There was a significant decrease in the total number of individuals between 1990 (131.842 individuals) and 2005 (34.842 individuals), F=8.2333, p<0.05). There was also a significant decrease between 1990 and 2019 (41.737 individuals, F=8.2333, p<0.05). When examining the Shannon-Weiner Index, we have seen significant decreases in biodiversity; between 1990 (1.5081) and 2019 (0.945 F=4.5455, p<0.05). These decreases continue as we see a decrease from 2006 (1.1234) and 2019 (F=4.5455, p<0.05). These findings indicate that this community is at an increasing risk of being annihilated which could indicate the same thing of many freshwater ecosystems. If these ecosystems were to be completely destroyed; it would mean a death sentence for all life on earth.

Introduction

Monitoring biodiversity is important to understand changes that occur in populations over time. Biodiversity is defined as organisms within a population, community, or ecosystem and their relationships to each other. Biodiversity is essential to our ecosystems in order to preserve the integrity of the species found in such places, such as freshwater habitats (Shah, 2014). In spite of the critical importance of maintaining biodiversity in ecosystems, there are still many challenges for conservationists. One of the risks to conservation efforts is climate change. Climate change affects species behaviors including migration and distribution, abundance and population dynamics, and functional variation in both aquatic and terrestrial organisms (Ferreira-Rodríguez, et al. 2017). Freshwater ecosystems are also greatly impacted by these global climate changes.

Freshwater ecosystems only account for a small percentage of all of the Earth's ecosystems, however, they are one of the most threatened worldwide (Davidson 2014). Humans are dependent on freshwater ecosystems for many things including drinking water, but our close proximity to these habitats has put them at greater risk. These ecosystems continue to degrade rapidly, and the biodiversity contained within them is lost at unprecedented rates caused by human activity (Davidson 2014). Human activity including channelization, pollution, and habitat modification are just some of the ways that humans have caused disturbances in these ecosystems. Disturbances are events or forces that kill some organisms in an ecosystem, thus changing individual populations and possibly whole ecosystems (Paine, 2012). In order to monitor how our presence in these ecosystems have changed them, we must continue to evaluate populations and communities.

Freshwater species, including freshwater mussels, are one of the many groups that are endangered due to human activity and environmental changes. The worldwide decline of freshwater mussels has been recorded since the beginning of the twentieth century and is one of the major components of freshwater biodiversity loss attributed to global change (Ferreira-Rodríguez, et al. 2017). Freshwater mussels, though rarely the first considered, are one of the taxa that inhabit freshwater ecosystems on nearly every continent, excluding Antarctica or areas within the arctic. Not only are they useful as indicators of water quality, but they play an important role in stream ecosystems. They act to decompose organic matter in streams, recycle nutrients and act as biological water purifiers (Cao, Yong, et al, 2017). Despite how critical the survival of freshwater mussels may be, we are continually seeing their populations decline (Hoggarth and Goodman 2007).

Hoggarth (1992) has spent thirty years studying and recording the freshwater mussel populations of the Little Miami River in South-Western Ohio. During his research in 1990 he was able to provide the first systematic glimpse into the freshwater mussels of the Little Miami River system. He discovered that about a third of the mussels were probably extirpated, a third were in danger of being lost, and the last third appeared to be holding their own. Fifteen years later, Hoggarth and Goodman (2007) documented the replacement of some mussel species in the mainstem of the Little Miami River and East Fork Little Miami River. He also noted the loss of headwater species in the Little Miami River as well as its smaller tributaries. The Little Miami River possesses a large selection of native Ohio species, so monitoring them is important. Using the same methods throughout the years, he has researched what species are present, as well as their abundance and distribution along the river. My study combined the previous data collection with new collections made in the summer of 2019 to more clearly understand how the freshwater mussel populations in the Little Miami River have changed over time. We examined the changes in the populations in this area over time and looked at the changes in biodiversity. By examining these changes, we stand to gain a better understanding of the changes in the population as well as the significance of the biodiversity of the area.

#### Methods

We collected data at sites along the Little Miami River, which is located in southwestern Ohio, from June 2019 through August 2019. We surveyed sites in a linear fashion starting at the headwaters and continuing to the mouth of the river (Figure 1); no more than two sites were sampled per day. General collection at each site was the first thing that we performed. At each location, we measured water quality including turbidity, temperature, pH, and conductivity. During this portion of the study, three collectors searched the area for live, dead, weathered, and subfossil mussels. The live and dead individuals that were collected were later categorized as extant, as there was evidence that they were still present in the stream. The weathered and subfossil individuals found were categorized as extirpated or locally extinct. We performed general collecting for one and a half to two hours at each site. General collecting involved us walking around and attempting to locate any shells or live specimens which would then be counted and classified. All live mussels found at the sites were identified, measured (length, height, and width), aged using the annular ring method, and sexed, if possible. After collecting the data from the individual mussels, we were then able to return all individuals, both live and dead shells, back to the river and proceed to quadrat sampling.

Quadrat sampling involved the use of pre-measured squares which were laid down in the substrate and used to examine a determined amount of space for live individuals. We attempted to perform this in areas where general collecting did not take place in order to reduce any chance of skewing our density calculations. Quarter square meter quadrates were used where two people sampled each quadrat and continued to do so for twenty lengths of the quadrat at each site. This formed a linear sampling area with a total of 40 quadrats examined or ten square meters. We then returned all of the mussels to the river after collecting their individual data. Any specimens of the Asiatic clam (*Corbicula fluminea*) were enumerated along with the mussels. All of the raw data can be found in Appendix 1.

Following the data collection, I performed a statistical analysis of the data to examine different aspects of the biodiversity of the river as well as to compare the changes in the mussel populations over time. To examine the biodiversity of the river, we ran a Shannon-Weiner index using only the extant individuals to analyze the change in biodiversity over time. Both live and dead individuals were used in this analysis because they were extant and therefore still present within the community. The same method was also used to compare the biodiversity across the river. I performed a repeated measures ANOVA to examine the significant differences in biodiversity over time. Along with examining biodiversity, we evaluated the changes in mussel populations over time; this was done in the same methodology as the previous years. I performed ANOVA tests to examine the statistical significance of the changes in the populations. When examining the density of native mussels and *Corbicula fluminea*, a paired t-test was performed as only two years of data were available for comparison.

Graphs were generated using the data collected and the statistical analyses performed. Two styles of graphs are present for each category of data examined. The line graphs display the data along the length of the river. Each data point is a culmination of multiple points along the river, which have remained the same for all three years. The river miles were found by examining maps created by ODNR (River Miles Map of Ohio). The second type of graphs are bar graphs, which display the means of the data with 95% confidence intervals present as error bars. The letters present on the bar graph act to differentiate statistical differences between the years.

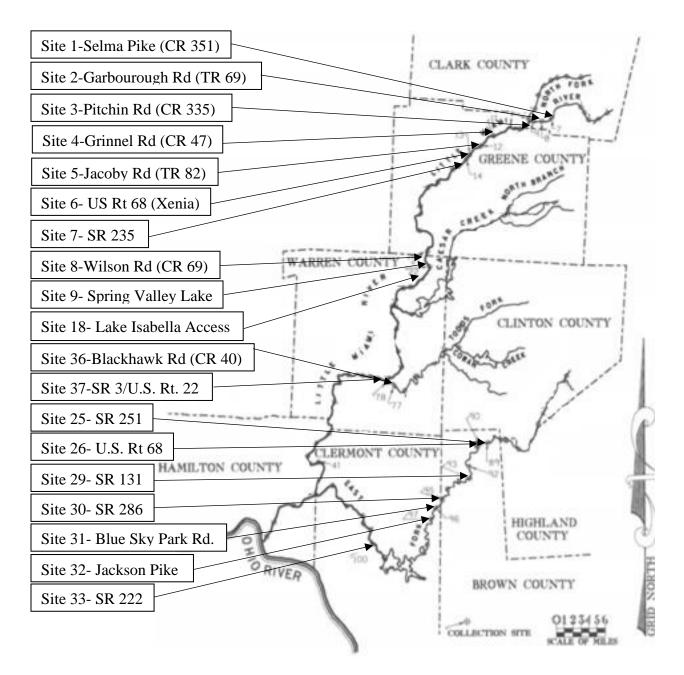


Figure 1. A map of the bridge sites along the Little Miami River that were included in the sampling area

Results

Our study found a continued decline in the number of individuals as well as number of species of mussels in the Little Miami River. Significant differences in the number of individuals, have been found throughout the years (Figure 2). Figure 2 shows the total number of individuals, both extant and extirpated, between 1990, 2006, and 2019. There were significant differences between 1990 with a mean of 131.84, and 2006 with a mean of 34.84 individuals (F=8.233, p<0.05). These findings were the same between 1990 and 2019 with a mean of 41.74 (F=8.233, p<0.05). However, there were no significant differences between 2006 and 2019 (p>0.05, F= 0.1199) in regards to the total number of individuals collected (Figure 3). This trend continued for other categories examined

I examined the total number of extant individuals that were collected during my research and again found significant decreases in the populations (Figure 5). The mean number of extant individuals significantly decreased between 1990 (123.9 individuals) and 2006 (27.4 individuals) (F=9.573, p<0.05). There was a significant decrease in the number of extant individuals between 1990 and 2019 (24.1 individuals) (F=9.573, p<0.05). When examining extirpated individuals (Figure 7), this trend becomes reversed. The highest average number of extirpated individuals was recorded in 2019 with the average being 17.7 extirpated individuals per site. This is significantly more than in 1990 and in 2006 which had averages on 8 and 7.5 respectively (F=5.723, p<0.05). There were no significant changes between 1990 and 2006 (F= 0.0423, p>0.05). On an individual level, there is an overall downward trend, especially between 1990 and 2019.

The downward trend continues when examining the mean percent of species extant (Figure 9). In 1990, 79.9% of the species collected were classified as extant, however there was a

significant decrease to 58.8% in 2006 (F=8.467, p<0.05). By 2019, only there was another significant drop and only 40.9% of species were extant (F=8.467, p<0.05). When examining the ratio of extant to extirpated individuals, this trend once again continues (Figure 11). In 1990, the average ratio was 23.1 but this decreased when compared to 2006, with a mean of 7.9 (F=8.467, p<0.05). 2019, with a mean of 4.6, and 1990 were significantly different as well (F=8.467, p<0.05). However, there was not a significant decrease between 2006 and 2019 (F=0.167, p>0.05).

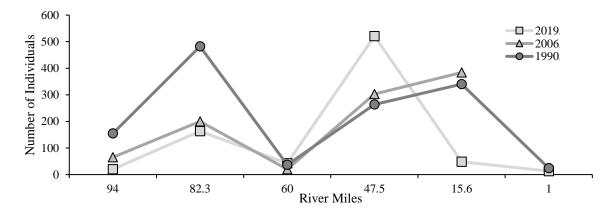


Figure 2. The total number of individuals (extant + extirpated) collected from 6 reaches along the Little Miami River between 1990, 2006, 2019.

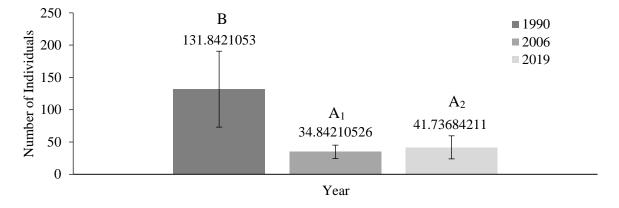


Figure 3. A comparison of the mean total number of individuals (extant + extirpated) collected from the Little Miami River between 1990, 2006, 2019. A and B are statistically different from each other but neither A is statistically different from one another. The total number of mussels collected in 1990 was much greater than collected in 2006 (F=8.2333, p<0.05) and 2019 (F=8.2333, p<0.05) while there was no significant difference in the number of mussels collected in 2006 compared to 2019 (F=0.1199, p>0.05).

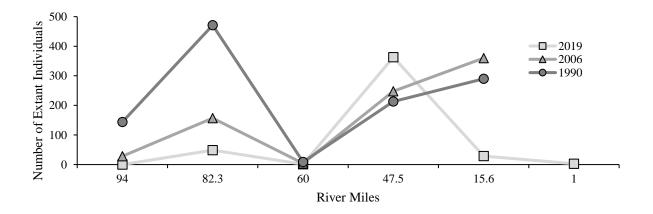


Figure 4. The total number of extant individuals collected from 6 reaches along the Little Miami River between 1990, 2006, 2019.

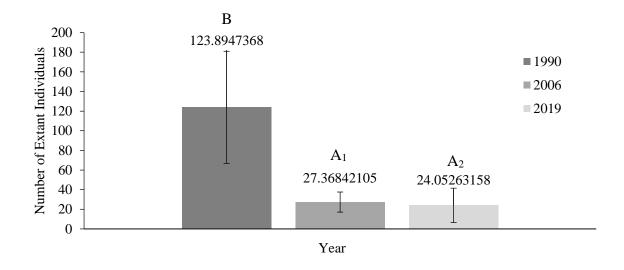


Figure 5. A comparison of the mean total number of extant individuals collected from the Little Miami River between 1990, 2006, 2019. A and B are statistically different from each other but neither A is statistically different from one another. The total number of mussels collected in 1990 was much greater than collected in 2006 (F=9.573, p<0.05) and 2019 (F=9.573, p<0.05), while there was no significant difference in the number of mussels collected in 2006 compared to 2019 (F=0.120, p>0.05).

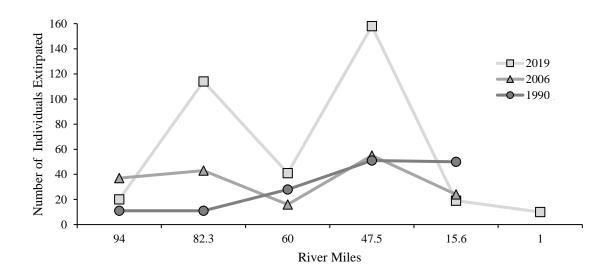


Figure 6. The total number of extirpated individuals collected from 6 reaches along the Little Miami River between 1990, 2006, 2019.

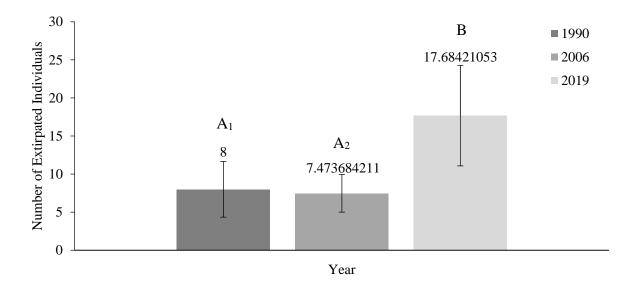


Figure 7. A comparison of the mean number of extirpated individuals collected from the Little Miami River between 1990, 2006, 2019. A and B are statistically different from each other but neither A is statistically different from one another. The total number of extirpated mussels collected in 2019 was significantly greater than in 1990 (F=5.723, p<0.05) and 2006 (F=5.723, p<0.05) while there was no significant difference in the number of mussels collected in 1990 compared to 2006 (F=0.0423, p>0.05).

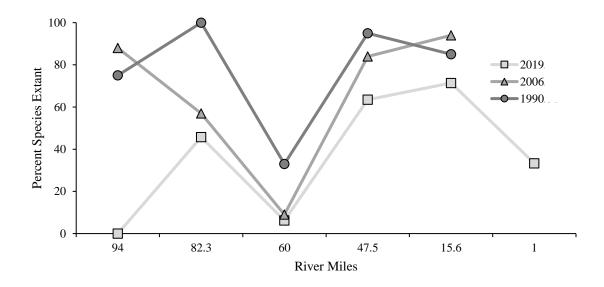


Figure 8. The percent species extant collected from 6 reaches along the Little Miami River between 1990, 2006, 2019.

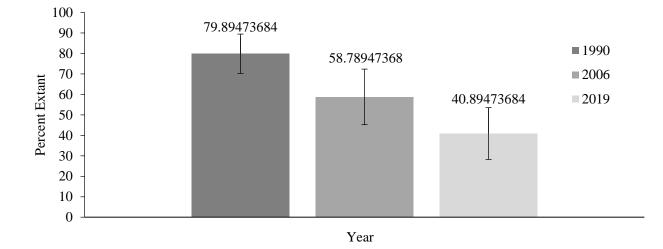


Figure 9. A comparison of the mean percent species extant collected from the Little Miami River between 1990, 2006, 2019. All of the data is significantly different from one another. The percent species of extant mussels collected in 1990 was significantly greater than collected in 2006 (F=8.467, p<0.05) and 2019 (F=8.467, p<0.05). There was a significant decrease in the percent extant species collected in 2006 compared to 2019 (F=8.467, p<0.05).

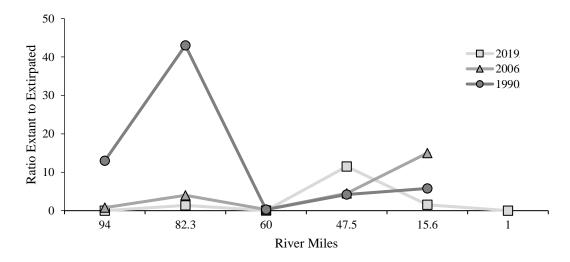


Figure 10. The ratio of extant to extirpated individuals collected from 6 reaches along the Little Miami River between 1990, 2006, 2019.

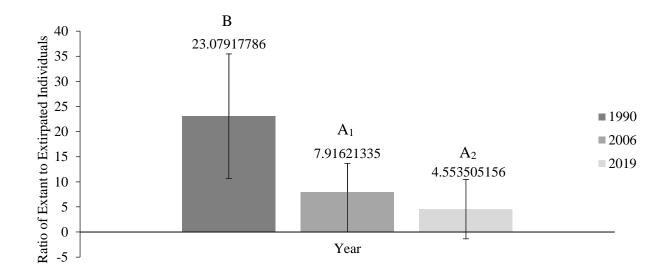


Figure 11. A comparison of the ratio of extant to extirpated individuals collected from the Little Miami River between 1990, 2006, 2019. A and B are statistically different from each other but neither A is statistically different from one another. The ratio of extant to extirpated individuals collected in 1990 was significantly greater than collected in 2006 (F=8.467, p<0.05) and 2019 (F=8.467, p<0.05) while there was no significant difference in the ratio of mussels collected in 2006 compared to 2019 (F=0.167, p>0.05).

#### **Biodiversity Results**

In the second part of the data analysis, I examined changes in biodiversity across the river as well as across the years research was performed. The first set of graphs (Figure 12 and 13) compared the total number of species, or species richness, found at the locations There were no significant changes between 2006 and 2019 which had a mean species richness of 8.14 and 8.15, respectively (F=0.0148, p>0.05). However, there were significant decreases between 1990 and 2006 as well as between 1990 and 2019 (F=8.038, p>0.05). 1990 had an average of 10.3 species collected. This suggests that the species richness has decreased.

When further examining the biodiversity of the Little Miami River, a Shannon-Weiner Index was calculated for all of the sites examined. The following set of graphs (Figure 14 and 15) use the number of species found at each location to perform a Shannon-Weiner Index which enabled me to better understand the biodiversity of the river as a whole. The Shannon-Weiner Index examines the abundance and evenness of species present in the community and uses this relationship to generate a H' value. I was able to perform a data analysis of these generated H' values using Repeated Measures ANOVA tests. There were no significant differences for the H' values calculated between 1990 and 2006 which had means of 1.5 and 1.12 respectively (F=0.0021, p>0.05). However, there were significant differences between 1990 and 2019, which had a mean H' value of 0.945 (F=4.55, p<0.05). This trend can also be seen when comparing 2006 and 2019 which showed a significant decrease in the H' values (F=4.55, p<0.05).

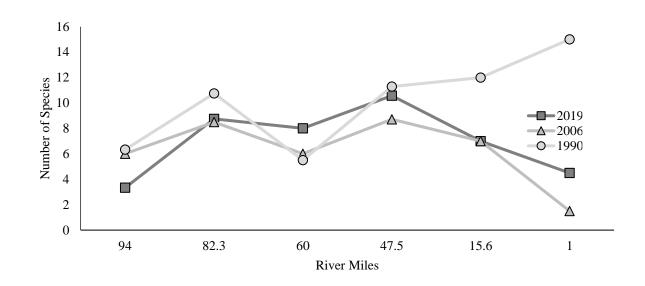


Figure 12. The total number of extirpated individuals collected from 6 reaches along the Little Miami River between 1990, 2006, 2019

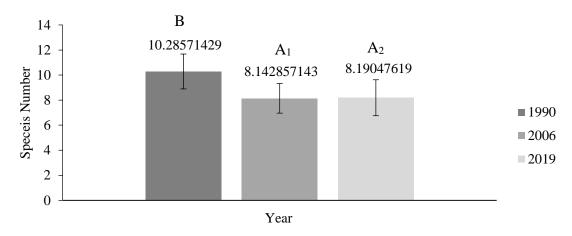


Figure 13. A comparison of the total number of species collected from the Little Miami River between 1990, 2006, 2019. A and B are statistically different from each other but neither A is statistically different from one another. The number of mussel species collected in 1990 was significantly different from 2006 (F=8.038, p<0.05) and 2019 (F=8.038, p<0.05). There were no significant changes in the number of species collected in 2006 compared to 2019 (F=0.0148, p>0.05).

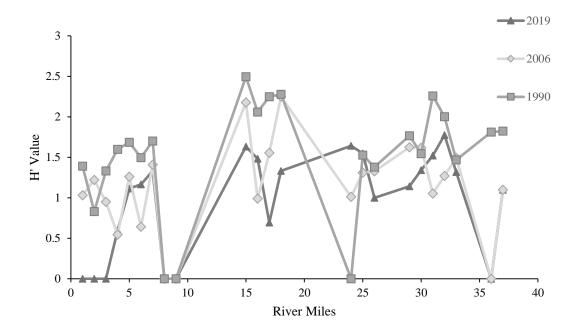


Figure 14. The total number of extirpated individuals collected from 6 reaches along the Little Miami River between 1990, 2006, 2019

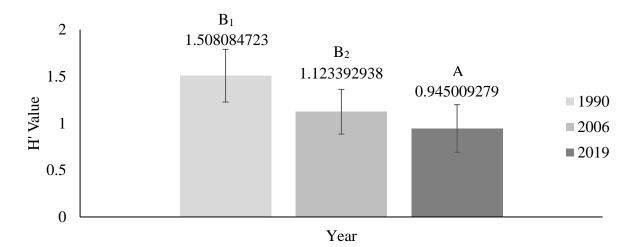


Figure 15. A comparison of the Shannon-Weiner Index analyzed for the Little Miami River between 1990, 2006, 2019. A and B are statistically different from each other but neither A is statistically different from one another. The H' value analyzed for 1990 was significantly greater than that for 2019 (F=4.5455, p<0.05). The H' value for 2019 was also significantly less than in 2006 (F=4.5455, p<0.05). There was no significant difference in the H' value found for 1990 compared to 2006 (F=0.0021, p>0.05).

#### Density Graphs

The following density graphs were generated by determining the density of both native freshwater mussels as well as the invasive *Corbicula fluminea*. Using previous data, I was able to perform an analysis, using the quadrat sampling data, to find the density of the mussels along the river (Hoggarth 1992; Hoggarth and Goodman 2007). The exact density values can be found in Appendix 1. Using a 95% confidence interval along with a t-test, I was able to determine the significant differences found between the densities. I was only able to perform this analysis with data collected in 2006 and 2019 as I was unable to find the same from the report generated in 1991. After performing a data analysis of the densities, there were no significant differences of the freshwater mussel densities between 2006 and 2019 with means of 0.162 and 0.358 respectively (T=-0.745, p>0.05) (Figure 16). However, I found a significant decrease in the density of *Corbicula fluminea* between the 2006 and 2019 with means of 29.1 and 5.3 respectively (T=3.57, p<0.05) (Figure 17).

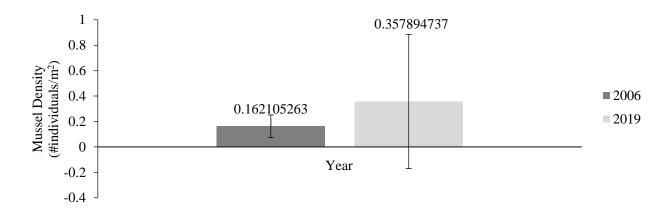


Figure 16. A comparison of the mean mussel densities (excluding *Corbicula fluminea*) collected from the Little Miami River between 2006 and 2019. There were no significant differences between the two years where data was analyzed (T=-0.745, p>0.05).

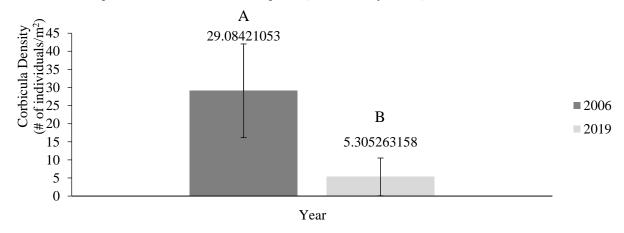


Figure 17. A comparison of the mean *Corbicula fluminea* densities collected from the Little Miami River between 2006 and 2019. There was a significant decrease in the density of *Corbicula flumiea* calculated in 2006 compared to 2019 (T=3.5667, p<0.05).

Discussion

The findings of this study show significant decreases in both population numbers and biodiversity which may be used to analyze the quality of the river system. Mussel populations are good indicators of water quality, and this study supports the findings that the river system is being negatively affected (Ferreira-Rodríguez, et al. 2017). The reasoning for this decrease in population numbers may be explained by disturbances in the ecosystem which are most likely due to human presence (Paine, 2012). An increase in human presence, and their effect of the river system, can be explained by an increase in urbanization and development. Over the course of thirty years, the area surrounding the Little Miami River has rapidly developed which may contribute to this disturbance.

Along with the increase in human presence surrounding the Little Miami River, the introduction of invasive species may also be to blame for some of these findings. Competition between invasive Asian Clams and native species could cause the decline of the native mussels in the Little Miami basin . Another possibility is that native mussels may be declining due to water pollution which enables invasive, and more pollution-tolerant invaders to flourish. (Hedeen, 2008). Asian Clams are the primary invasive mussel species in the Little Miami River system. However, my research found a decrease in the Asian Clam density when comparing 2006 and 2019. This could potentially indicate that the invasive species are now also succumbing to pollutants or other environmental factors. Since we are not currently seeing a rise in native freshwater mussel populations, these findings could indicate that the river conditions are slowly becoming uninhabitable to all mussel species.

An increase in urbanization can be detrimental to an area that used to be wild. Deforestation tends to be one of the first things discussed when urbanization comes up, and it does play a large role in the terrestrial ecosystem. Urbanization leads to an increase in runoff due to the presence of more roadways, concrete, and housing. These high flow rates indirectly and directly affect many aquatic invertebrates, including freshwater mussels (Death, 2008). This increases the stress on the stream bed and forces organisms to relocate as they get swept up as the substrate itself moves (Death, 2008). This runoff also degrades the river banks and causes dirt and other pollutants to flow into the river. Many studies have found that floods reduce the abundance and biodiversity of many invertebrate communities (Death, 2008). These "minifloods" are increasing due to the decrease in erosion-resistant surfaces, such as trees, brush, and undeveloped ground. During the research period, we noted that the banks had been eroded drastically which prevented us from accessing certain sites without a canoe. This erosion may just be one of the reasons that populations have been negatively impacted.

Many of the areas surrounding the Little Miami River are dedicated to agriculture which could lead to an increase in pollutants running off into the river. The agricultural development has led to streams being polluted with a variety of deadly chemicals including herbicides, insecticides, and fungicides (Hedeen, 2008). In the East Fork of the Little Miami River, around forty-two pesticide compounds have been discovered (Hedeen, 2008). It has been found in previous studies that freshwater mussels are some of the most sensitive aquatic organisms (Connors, 2004). Common lawn chemicals and agricultural chemicals may cause DNA damage which could be used to better evaluate the impact of these chemicals on freshwater mussels, as well as other freshwater organisms (Connors, 2004). Overall, we are unsure as to the exact cause that is leading to the decimation of the freshwater mussel populations, but it seems reasonable that this is the result of human and environmental factors. Using the data that we have collected

may provide further insight into what the causes are and what the future holds for this community.

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#### Appendix 1:

**2020 Site # 1**: Little Miami River at CR 351 (Selma Pike) bridge, Green Twp., Clark Co., Ohio. 1990 Site # 7: 2006 Site # 1. 39°48'36.4"N 83°44'21.7"W. River Kilometer 153 (RM 95.0). 39.810113° -83.739359°. South Charleston 7.5 Quad.

					2019/20 data							
	live	dead	wea	subf	live	dead	wea	subf	live	dead	wea	subf
1. Anodontoides ferrusacianus	10	2	1	0	0	1	3	0	0	0	0	1
2. Strophitus undulatus	1	1	0	0								
3. Alasmidonta viridis	5	6	0	0	0	3	6	0	0	0	1	0
4. Lasmigona compressa	4	0	0	0	0	1	2	0	0	0	0	1
5. Toxolasma parvus	0	0	1	0	0	0	1	0				
6. Lampsilis siliquoidea	3	0	0	0	4	4	0	0				
Total	23	9	2	0	4	9	12	0	0	0	1	2
Total species	6				5				3			
Total live + dead species	5				4				0			
Total live + dead individuals	32				13				0			
Data												
Date sampled	30 Ju	ly 1990			16 Ju	ne 2006			23 Ju	ly 2019		
Flow	Not a	vailable			80 cfs	s @ oldt	own ga	ige	75 cfs	s @ oldt	own ga	ge
Other (2019): Conductivity – 696	,		-7.3 M	NTU, Ten	nperature	- 19.2°	С, рН -	- 7.5				
Unionid density from Transect: 0/	10  m2 = 10  m	0.0/m2										
Corbicula fluminea density $= 331$	/10m2 = 3	33.1/m2										

**2020 Site # 2**: Little Miami River at TR 69 (Garbough Road) bridge, Green Twp., Clark Co., Ohio. 1990 Site # 8. 2006 Site # 2. 39°48'20.2"N 83°45'49.8"W. River Kilometer 151 (RM 93.4). 39.805612° -83.763842°. Clifton 7.5 Quad.

		1990/	91 data		2006/7 data				2019/20 data			
	live	dead	wea	subf	live	dead	wea	subf	live	dead	wea	subf
1. Pyganodon grandis	0	0	1	0								
2. Anodontoides ferrusacianus	6	7	0	0	0	0	5	0	0	0	1	0
3. Strophitus undulatus	0	0	1	0					0	0	3	0
4. Alasmidonta viridis	22	18	0	0	0	5	9	0	0	0	1	2
5. Lasmigona compressa	5	1	2	0	0	2	2	0	0	0	0	1
6. Toxolasma parvus	0	0	1	1	0	1	0	0				

7. Lampsilis siliquoidea					0	2	2	0				
Total	33	26	5	1	0	10	18	0	0	0	5	3
Total species	6				5				5			
Total live + dead species	3				4				0			
Total live + dead individuals	59				10				0			
Data												
Date sampled	30 Jul	y 1990			16 Jun	ne 2006			23 July	y 2019		
Flow	Not av	ailable			80 cfs	@ oldt	own gag	ge	75 cfs	@ oldto	own gag	e
Other (2019): Conductivity – 70	0 uS/cm, Tu	urbidity	– 6.9 N	TU, Ten	nperature	$-20.5^{\circ}$	С, рН –	8.3				
Unionid density from Transect:	0/10  m2 = 0	).0/m2										
Corbicula fluminea density $= 41$	8/10m2 = 4	1.8/m2										

**2020 Site # 3**: Little Miami River at CR 335 (Pitchin Road) bridge, Green Twp., Clark Co., Ohio. 1990 Site # 9. 2006 Site # 3. 39°48'21.1"N 83°46'53.0"W. River Kilometer 150 (RM 92.2). 39.805858° -83.781469°. Clifton 7.5 Quad.

			2006/7 data					2019/	20 data	L		
	live	dead	wea	subf	live	dead	wea	subf	live	dead	wea	subf
1. Utterbackia imbecillis	0	1	0	0								
2. Anodontoides ferrusacianus	7	0	1	0	0	1	1	0				
3. Strophitus undulatus	5	4	0	0	0	0	1	0				
4. Alasmidonta viridis	19	10	0	0	0	0	1	0	0	0	0	6
5. Lasmigona compressa	4	0	1	0	0	0	2	0	0	0	0	2
6. Elliptio dilatata					3	0	0	0				
7. Ptychobranchus fasciolaris					1	0	0	0				
8. Toxolasma parvus	0	0	1	0	0	0	1	0				
9. Lampsilis siliquoidea	3	0	0	0	0	0	1	0	0	0	1	0
Total	38	15	3	0	4	1	7	0	0	0	1	8
Total species	7				8				3			
Total live + dead species	6				3				0			
Total live + dead individuals	53				5				0			
Data												
Date sampled	4 Aug	gust 199	0		17 Ju	ne 2006			23 Ju	ly 2019		
Flow	Not a	vailable			75 cfs	s @ oldt	own ga	lge	75 cfs	s @ oldt	own ga	ıge

Other (2019): Conductivity – 696 uS/cm, Turbidity – 5.1 NTU, Temperature – 20.8°C, pH – 8.3 Unionid density from Transect: 0/10 m2 = 0.0/m2Corbicula fluminea density = 76/10m2 = 7.6/m2

**2020 Site # 4**: Little Miami River at CR 27 (Grinnel Road) bridge, Miami Twp., Greene Co., Ohio. 1990 Site # 11. 2006 Site # 4. 39°46'57.9"N 83°52'31.8"W. River Kilometer 139 (RM 85.5). 39.782762° -83.875535°. Yellow Springs 7.5 Quad.

	1990/91 data live dead wea subf li						7 data		(		20 data	
	live	dead	wea	subf	live	dead	wea	subf	live	dead	wea	subf
1. Pyganodon grandis	1	0	2	0								
2. Anodontoides ferrusacianus					0	0	1	0				
3. Strophitus undulatus	1	1	0	1	0	0	1	0				
4. Alasmidonta viridis	2	4	1	0	0	0	4	0	0	0	1	0
5. Lasmigona costata					0	0	1	0				
6. Lasmigona compressa					0	0	2	0				
7. Amblema plicata	2	1	0	0								
8. Quadrula pustulosa	0	1	0	0								
9. Fusconaia flava	4	6	0	0					0	0	1	0
10. Elliptio dilatata	57	17	0	0	30	0	1	0	10	0	0	0
11. Ptychobranchus fasciolaris	11	12	0	0	3	0	5	0	1	3	0	0
12. Toxolasma parvus					0	0	1	0				
13. Villosa iris	0	0	1	0								
14. Lampsilis siliquoidea	7	4	0	0	1	0	2	0	0	0	1	0
15. Lampsilis cardium	3	4	0	0	1	0	0	0	0	0	1	0
16. Lampsilis fasciola	2	1	0	0	0	0	1	0				
Total	90	51	4	1	35	0	19	0	11	3	4	0
Total species	12				11				6			
Total live + dead species	11				4				2			
Total live + dead individuals	141				35				14			
Data												
Date sampled		gust 199				ne 2006				ly 2019		
Flow		vailable				s @ oldt	0	0	50 cfs	s @ oldt	own ga	ıge
Other (2019): Conductivity – 650		-	v - 3.3 I	NTU, Ten	nperature	$-23.0^{\circ}$	С, рН -	- 8.2				
Unionid density from Transect: 0/	10  m2 =	0.0/m2										

#### Corbicula fluminea density = 0/10m2 = 0.0/m2

2020 Site # 5: Little Miami River at TR 82 (.	Jacoby Road) access, Xenia T	wp., Greene Co., Ohio. 1990 S	Site # 12. 2006 Site # 5.
39°45'51.4"N 83°54'10.3"W. River Kilomet	er 136 (RM 83.2). 39.764337	<sup>o</sup> -83.902815 <sup>o</sup> . Yellow Springs	7.5 Quad.

				2006/	7 data	1 0		2019/20 data		ι		
	live	dead	wea	subf	live	dead	wea	subf	live	dead	wea	subf
1. Pyganodon grandis	0	0	1	0								
2. Strophitus undulatus	0	1	0	0					0	0	0	1
3. Alasmidonta viridis	0	7	0	0	0	5	0	0	0	0	0	1
4. Lasmigona costata	0	1	0	0								
5. Fusconaia flava					0	1	1	0	0	0	0	1
6. Elliptio dilatata	23	6	0	0	14	7	2	0	4	3	7	0
7. Ptychobranchus fasciolaris	3	6	0	0	2	2	1	0	0	1	2	0
8. Villosa iris	1	1	0	0					0	2	0	0
9. Lampsilis siliquoidea	0	7	0	0	0	0	1	0	1	0	2	0
10. Lampsilis cardium	2	2	0	0	0	2	0	0	0	0	0	1
11. Lampsilis fasciola	1	2	0	0	2	0	0	1				
Total	30	33	1	0	18	17	5	1	5	6	11	4
Total species	10				7				8			
Total live + dead species	9				6				4			
Total live + dead individuals	63				35				11			
Data												
Date sampled	11 Au	igust 19	90		25 Ju	ne 2006	/5 Augi	ust 2006	30 Ju	ly 2019		
Flow	Not a	vailable			60/25	cfs@d	oldtown	gage	50 cfs	s @ oldt	own ga	ige
Other (2019): Conductivity $-6$	577 uS/cm, T	urbidity	r – 3.9 ľ	NTU, Ten	nperature	$-21.4^{\circ}$	С, рН –	- 7.8				
Unionid density from Transect	: 3/10 m2 =	0.3/m2										
Corbicula fluminea density $= 3$	30/10m2 = 3	.0/m2										

**2020 Site # 6**: Little Miami River at USR 68 bridge, Xenia Twp., Greene Co., Ohio. 1990 Site # 13. 2006 Site # 6. 39°44'54.0"N 83°55'51.0"W. River Kilometer 131 (RM 80.6). 39.748126° -83.931082°. Xenia 7.5 Quad.

		1990/9	91 data			2006/			20019/20 data				
	live	dead	wea	subf	live	dead	wea	subf	live	dead	wea	subf	
1. Pyganodon grandis	0	0	0	1					0	0	2	0	

2. Strophitus undulatus	0	0	1	0								
3. Alasmidonta viridis	0	3	0	0	1	0	2	0	0	0	4	9
4. Lasmigona costata	2	1	0	0	1	0	0	0	0	2	3	1
5. Amblema plicata	0	1	0	0					0	0	3	0
6. Fusconaia flava	19	6	0	0	2	0	0	0	0	1	6	0
7. Pleurobema clava					0	0	0	1	0	0	0	3
8. Elliptio dilatata	73	21	0	0	45	1	2	0	7	4	3	0
9. Ptychobranchus fasciolaris	49	11	0	0	1	0	0	0	0	1	0	0
10. Truncilla donaciformis	0	1	0	0								
11. Villosa iris	0	2	0	0								
12. Lampsilis siliquoidea	12	3	0	0	3	0	2	2	0	1	5	5
13. Lampsilis cardium	2	0	0	0	0	0	1	0	3	0	3	1
14. Lampsilis fasciola	2	2	0	0					0	0	1	0
Total	159	51	1	1	53	1	7	3	10	9	30	19
Total species	13				8				11			
Total live + dead species	11				6				6			
Total live + dead individuals	210				54				19			
Data												
Date sampled	11 Au	gust 199	90		25 Jun	e 2006			6 Augi	ıst 201	9	
Flow	Not av	ailable			60 cfs	@ oldto	own gag	ge	40 cfs	@ oldt	own gag	ge
Other (2019): Conductivity – 6	62/682 uS/ci	n, Turbi	dity – 4	.6/5.0 N	TU, Temp	erature	-21.5°	C, pH –	8.0 - at the	e bridge	e and da	am
Unionid density from Transect:	0/10  m2 = 0	).0/m2										
Corbicula fluminea densit	y = 0/10m2 =	$= 0.0/m^{2}$	2									

## **2020 Site # 7**: Little Miami River at SR 235 bridge, Xenia Twp., Greene Co., Ohio. 1990 Site # 14. 2006 Site # 7. 39°44'27.1"N 83°56'11.9"W. River Kilometer 130 (RM 79.9). 39.740867° -83.936656°. Xenia 7.5 Quad.

		1990/	91 data			2006/		2019/20 data				
	live	dead	wea	subf	live	dead	wea	subf	live	dead	wea	subf
1. Alasmidonta viridis	1	2	0	0	0	1	2	0	0	0	1	0
2. Lasmigona costata	0	1	0	0	0	1	0	0	0	0	1	0
3. Fusconaia flava	2	1	0	0	0	3	1	0	0	1	0	0
4. Pleurobema clava									0	0	1	0
5. Elliptio dilatata	17	6	1	0	2	16	0	0	1	1	6	0

6. Ptychobranchus fasciolaris	5	2	0	0	1	4	3	0	0	1	2	0
7. Villosa fabalis					0	0	1	0				
8. Villosa iris									0	0	1	0
9. Lampsilis siliquoidea	3	5	0	0	0	4	2	0	0	0	0	2
10. Lampsilis cardium	2	3	2	0	0	1	0	0	0	1	2	0
11. Lampsilis fasciola	1	2	0	0					0	0	0	1
Total	31	22	3	0	3	30	9	0	1	4	14	3
Total species	8				8				10			
Total live + dead species	8				7				4			
Total live + dead individuals	53				33				5			
Data												
Date sampled	11 Au	gust 199	90		25 Jun	e 2006			6 Augi	ıst 201	9	
Flow	Not av	ailable			60 cfs	@ oldto	own gag	ge	40 cfs	@ oldt	own gag	ge
Other (2019): Conductivity $-67$	76 uS/cm, Tu	urbidity	– 5.7 N	TU, Ter	nperature -	– 22.9°C	C, pH –	8.3				
Unionid density from Transect:	3/10  m2 = 0	).3/m2										
Corbicula fluminea density = $1/$	10m2 = 0.1m	m2										

**2020 Site # 8**: Little Miami River at CR 61 (Roxanna-New Burlington Road) bridge, Spring Valley Twp., Greene Co., Ohio. 1990 Site # 21. 2006 Site # 8. 39°35'00.2"N 84°01'46.7"W. River Kilometer 100 (RM 60.8). 39.583443° -84.029596°. Waynesville 7.5 Quad.

	01 1017	1990/91 data				2006/	7 data	0.102/07	2019/20 data			
	live	dead	wea	subf	live	dead	wea	subf	live	dead	wea	subf
1. Lasmigona costata									0	0	1	0
2. Lasmigona complanata	0	1	0	0					1	0	0	0
3. Amblema plicata					0	0	1	0	0	0	1	6
4. Quadrula quadrula					0	0	1	0	0	0	0	1
5. Fusconaia flava					0	0	1	0				
6. Elliptio dilatata									0	0	0	1
7. Leptodea fragilis	0	0	2	0	0	0	1	0				
8. Potamilus alatus	0	0	1	0	0	2	0	0				
9. Lampsilis cardium									0	0	0	1
10. Lampsilis fasciola									0	0	0	1
11. Epioblasma triquetra									0	0	1	0
Total	0	1	3	0	0	2	4	0	1	0	3	10

Total species	3	5	8
Total live + dead species	1	1	1
Total live + dead individuals	1	2	1
Data			
Date sampled	19 August 1990	5 August 2006	7 August 2019
Flow	Not available	25 cfs @ oldtown gage	40 cfs @ oldtown gage
Other (2019): Conductivity – 8	60 uS/cm, Turbidity -	- 9.8 NTU, Temperature – 21.2°C, pH – 8.0	
Unionid density from Transect	0/10  m2 = 0.0/m2		
Corbicula fluminea density $= 0$	/10m2 = 0.0/m2		

**2020 Site # 9**: Little Miami River at Spring Valley Lake access, Wayne Twp., Warren Co., Ohio. 1990 Site # 22. 2006 Site # 9, 39°33'48.9"N 84°01'16.0"W. River Kilometer 94 (RM 59.1). 39.563612° -84.021111°. Waynesville 7.5 Quad.

	1990/91 data				2006/7 data				2019/20 data				
	live	dead	wea	subf	live	dead	wea	subf	live	dead	wea	subf	
1. Alasmidonta marginata					0	0	1	0					
2. Lasmigona costata	0	0	0	1	0	0	1	0					
3. Lasmigona complanata	0	0	5	0					0	0	1	0	
4. Amblema plicata	0	0	3	0	0	0	2	0	0	0	13	0	
5. Quadrula quadrula	0	0	0	1									
6. Fusconaia flava	0	0	2	0	0	0	1	0	0	0	0	5	
7. Pleurobema clava	0	1	3	0	0	0	1	0					
8. Elliptio dilatata	0	0	0	1	0	0	1	0	0	0	1	0	
9. Ptychobranchus fasciolaris									0	0	0	1	
10. Obovaria subrotunda									0	0	0	1	
11. Lampsilis siliquoidea	0	0	4	0	0	0	1	0	0	0	1	3	
12. Lampsilis cardium									0	0	1	1	
Total	0	1	17	3	0	0	8	0	0	0	17	11	
Total species	8				7				8				
Total live + dead species	1				0				0				
Total live + dead individuals	1				0				0				
Data													
Date sampled	19 August 1990				6 August 2006				6 August 2019				
Flow	Not available				0				40 cfs	40 cfs @ oldtown gage			

Other (2019): Conductivity – 860 uS/cm, Turbidity – 8.5 NTU, Temperature –21.2°C, pH – 8.5 Unionid density from Transect: 0/10 m2 = 0.0/m2Corbicula fluminea density = 0/10m2 = 0.0/m2

**2020 Site # 15**: Little Miami River at island upstream of Stubbs Mills Rd. bridge, Union/Hamilton Twp., Warren Co., Ohio. 27 July 2007. 1990 Site # 32. 2007 Site # 30. 39°21'50.2"N 84°09'45.3"W. River Kilometer 60 (RM 36). 39.363924° -84.162594°. South Lebanon 7.5 Quad.

	1990/91 data					2006/7 data				2019/20 data				
	live	dead	wea	subf	live	dead	wea	subf	live	dead	wea	subf		
1. Anodonta suborbiculata	0	1	0	0										
2. Pyganodon grandis	1	2	2	0	4	0	0	0						
3. Strophitus undulatus									0	0	0	1		
4. Alasmidonta viridis	0	1	0	0										
5. Alasmidonta marginata	1	1	1	0										
6. Lasmigona costata	0	2	3	0	4	0	0	0	1	0	0	0		
7. Lasmigona complanata	0	2	0	0					0	1	1	0		
8. Amblema plicata	0	1	1	0	6	0	0	0	1	0	1	1		
9. Tritogonia verrucosa	0	2	0	0	17	0	0	0	3	1	1	0		
10. Quadrula quadrula					11	0	0	0	0	1	1	0		
11. Cyclonaias pustulosa					1	0	0	0						
12. Fusconaia flava	0	0	1	0	4	0	0	0	0	0	2	0		
13. Leptodea fragilis	1	1	1	0	8	0	0	0	2	4	0	0		
14. Potamilus ohiensis	0	2	1	0										
15. Potamilus alatus	0	5	0	0	11	0	0	0	4	8	1	0		
16. Truncilla truncata					1	0	0	0						
17. Truncilla donaciformis	0	1	0	0										
18. Obliquaria reflexa					1	0	0	0						
19. Lampsilis siliquoidea	1	1	0	0	1	0	0	0	0	0	0	3		
20. Lampsilis cardium	3	2	1	0	1	0	0	0	1	0	2	0		
21. Epioblasma triquetra	0	0	2	0					0	0	1	0		
Total	3	34	12	0	70	0	0	0	12	15	10	5		
Total species	14				13				12					
Total live + dead species	12				13				8					

Total live + dead individuals	37	70	27
Data			
Date sampled	29 September 1990	27 July 2007	19 August 2019
Flow	Not available	60 cfs @ oldtown gage	35 cfs @ oldtown gage
Other (2019): Conductivity – 705	uS/cm, Turbidity - 11.4 NTU	, Temperature $-27.9^{\circ}$ C, pH $-8.3$	
Unionid density from Transect: 4/	10  m2 = 0.4/m2		
Corbicula fluminea density = $0/10$	0m2 = 0.0/m2		

**2002 Site # 16**: Little Miami River from broken dam at Middletown Junction to below SR 150 (Grandin Road) bridge, Deer Field/Hamilton Twp., Warren Co., Ohio. 24 July 2007. 1992 = Site # 35. 2007 Site # 31. 39°21'42.5"N 84°14'27.5"W. River Kilometer 50 (RM 30.8). 39.361793° -84.240994°. South Lebanon 7.5 Quad.

	1990/91 data					2006/	7 data		2019/20 data				
	live	dead	wea	subf	live	dead	wea	subf	live	dead	wea	subf	
1. Anodonta suborbiculata	0	1	0	0									
2. Pyganodon grandis	0	3	3	0	1	0	0	0					
3. Alasmidonta marginata	0	5	0	0									
4. Lasmigona costata	0	1	2	0					0	0	0	1	
5. Lasmigona complanata					3	0	0	0	1	1	3	0	
6. Tritogonia verucossa					23	0	0	0	3	2	3	0	
7. Quadrula quadrula	0	3	1	0	1	0	0	0	0	0	1	0	
8. Pleurobema sintoxia									0	0	0	1	
9. Leptodea fragilis	0	2	0	0	3	0	0	0	0	5	0	0	
10. Potamilus ohiensis	0	1	0	0									
11. Potamilus alatus	0	3	1	0	53	0	0	0	7	13	0	0	
12. Truncilla truncata									0	2	0	0	
13. Truncilla donaciformis									0	1	0	0	
14. Ligumia recta									0	1	0	0	
15. Villosa fabalis									0	0	0	1	
16. Lampsilis siliquoidea									0	0	1	0	
17. Lampsilis cardium	0	2	0	0					1	0	0	1	
Total	0	21	7	0	84	0	0	0	12	25	8	4	
Total species	9				6				13				
Total live + dead species	9				6				8				

Total live + dead individuals	21	84	37
Data			
Date sampled	1 October 1990	24 July 2007	19 August 2019/
			26-27 September 2019
Flow	Not available	60 cfs @ oldtown gage	30-35 cfs @ oldtown gage
Other (2019): Conductivity – 72	6-857 uS/cm, Turbidity – 7.	9-15.2 NTU, Temperature – 19.3-27	7.6°C, pH – 7.8-8.2
Unionid density from Transect:	0/10  m2 = 0.0/m2		
Corbicula fluminea density $= 0/1$	10m2 = 0.0/m2		

**2002 Site # 17**: Little Miami River at Glenn Canoe launch near the US Rt. 22/SR 3 bridge, Deer Field/Hamilton Twp., Warren Co., Ohio. 27 July 2007. 1992 = Site # 36. 2007 Site # 32. 39°19'10.6"N 84°15'08.9"W. River Kilometer 45 (RM 23.1). 39.319698° - 84.252504°. Mason 7.5 Quad.

	1990/91 data					2006/	7 data		2019/20 data				
	live	dead	wea	subf	live	dead	wea	subf	live	dead	wea	subf	
1. Pyganodon grandis	0	3	1	0									
2. Alasmidonta marginata	0	2	0	0									
3. Lasmigona costata	0	1	0	0									
4. Lasmigona complanata	0	5	1	0	0	1	0	0					
5. Amblema plicata					0	1	0	0					
6. Tritogonia verrucosa	0	0	1	0	0	0	1	0	1	0	2	0	
7. Quadrula quadrula	0	8	2	0	0	2	0	0					
8. Actinonaias ligamentina					1	0	1	0					
9. Leptodea fragilis	2	0	2	0	1	0	1	0					
10. Potamilus ohiensis	0	1	0	0	0	1	0	0					
11. Potamilus alatus	0	3	2	0	0	0	2	0	0	1	4	0	
12. Truncilla truncata	0	2	0	0	0	2	0	0					
13. Truncilla donaciformis	0	1	0	0									
14. Ligumia recta					0	0	1	0					
15. Lampsilis siliquoidea	0	2	1	0					0	0	1	0	
16. Lampsilis cardium	1	6	0	0					0	0	0	1	
17. Epioblasma triquetra	0	0	2	0									
Total	3	34	12	0	2	7	6	0	1	1	7	1	
Total species	14				10				4				

Total live + dead species	12	7	2
Total live + dead individuals	37	9	2
Data			
Date sampled	21 July 1990	25 August 2007	19 August 2019
Flow	Not available	60 cfs @ oldtown gage	35 cfs @ oldtown gage
Other (2019): Conductivity –	711 uS/cm, Turbidity - 21.8 M	NTU, Temperature $-24.6^{\circ}$ C, pH $-8.1$	
Unionid density from Transec	et: $0/10 \text{ m}2 = 0.0/\text{m}2$		

Corbicula fluminea density = 0/10m2 = 0.0/m2

**2020 Site # 18**: Little Miami River at Lake Isabella access, Symmes/Miami Twp., Hamilton/Clermont Co., Ohio. 25 August 2007. 1990 Site # 38. 2006 Site # 33. 39°14'30.3"N 84°17'47.5"W. River Kilometer 33 (RM 20.8). 39.241828° -84.296517°. Madeira 7.5 Quad.

	1990/91 data					2006/	7 data		2019/20 data				
	live	dead	wea	subf	live	dead	wea	subf	live	dead	wea	subf	
1. Pyganodon grandis	2	4	1	0	2	0	0	0					
2. Alasmidonta marginata	0	5	1	0	2	0	0	0					
3. Lasmigona costata	0	4	0	0	1	0	0	0	0	0	1	0	
4. Lasmigona complanata	0	2	0	0	3	0	0	0	0	1	0	0	
5. Amblema plicata	0	1	1	0					0	0	0	3	
6. Tritogonia verucossa					4	0	0	0	0	0	0	1	
7. Quadrula quadrula	1	3	2	0	1	0	0	0	0	0	0	1	
8. Cyclonaias pustulosa	0	0	1	0									
9. Leptodea fragilis	0	9	0	0	5	0	0	0	0	2	8	0	
10. Potamilus ohiensis	0	9	0	0	2	0	0	0					
11. Potamilus alatus	0	5	1	0	12	0	0	0	0	0	6	0	
12. Truncilla truncata	1	16	0	0	7	0	0	0	0	1	7	0	
13. Truncilla donaciformis	0	16	0	0	4	0	0	0	0	0	3	0	
14. Obliquaria reflexa	0	1	0	0					0	0	1	0	
15. Lampsilis siliquoidea	0	1	0	0	1	0	0	0					
16. Lampsilis cardium	0	1	1	0	1	0	0	0	0	1	0	1	
Total	4	77	9	0	45	0	0	0	0	5	26	6	
Total species	15				14				11				
Total live + dead species	14				14				4				
Total live + dead individuals	81				45				5				

## Data

Date sampled	21 July 1990	25 August 2007	19 August 2019
Flow	Not available	60 cfs @ oldtown gage	35 cfs @ oldtown gage
Other (2019): Conductivity -	– 717 uS/cm, Turbidity – 17.1 N	TU, Temperature $-28.7^{\circ}$ C, pH $-7.5$	
Unionid density from Transe	ect: $0/10 \text{ m}2 = 0.0/\text{m}2$		
Corbicula fluminea density =	= 0/10m2 = 0.0/m2		

**2020 Site # 24**. Little Miami River upstream of SR 125/32 bridge, Hamilton/Clermont Co., Ohio. 4 October 2007. 1990 Site # 45. 2006 Site # 36. 39° 06' 33.5"N, 84° 24' 07.8"W. River Kilometer 6 (RM 3.5). 39.109323° -84.402155°. Newport KY 7.5 Quad.

	1990 data				,	2019/20 data						
	live	dead	wea	subf	live	dead	wea	subf	live	dead	wea	subf
1. Lasmigona complanata					1	2	0	0	0	1	0	0
2. Amblema plicata					1	0	1	0				
3. Quzdrula quadrula									1	0	0	0
4. Leptodea fragilis									0	4	1	0
5. Potamilus ohiensis									0	3	0	0
6. Potamilus alatus									2	5	2	0
7. Truncilla truncata									0	0	2	0
8. Truncilla donaciformis									0	1	0	0
9. Ligumia recta									0	1	0	1
10. Villosa fabalis									0	0	0	1
11. Lampsilis cardium					1	1	0	0	0	0	1	0
Total	0	0	0	0	3	3	1	0	3	15	6	2
Total species	0				3				10			
Total live + dead species	0				3				7			
Total live + dead individuals	0				6				18			
Data												
Date sampled	22 Ju	ly 1991			4 Oct	ober 20	07		15-17	August	2019	
Flow	Not a	vailable			25 cfs	s @ oldt	own ga	ıge	35 cfs	s @ oldt	own ga	ige
Other (2019): Conductivity –	493-542 uS/	/cm, Tui	bidity -	- 19.3-2	2.8 NTU,	Tempe	rature -	-23.9-24.7	<sup>7</sup> °С, рН –	7.6-7.7		
Unionid density from Transec	et: $0/10 \text{ m2} =$	0.0/m2										
Corbicula fluminea density =	0/10m2 = 0.0	/m2										

	1990/91 data					2006/	7 data		2019/20 data				
	live	dead	wea	subf	live	dead	wea	subf	live	dead	wea	subf	
1. Pyganodon grandis	1	0	3	0	1	0	0	0	0	1	0	0	
2. Anodontoides ferrusacianus	2	2	0	0									
3. Strophitus undulatus	10	2	0	0	2	1	0	0	0	0	0	1	
4. Alasmidonta viridis	7	6	9	0	0	0	1	0	0	0	0	1	
5. Lasmigona costata	0	2	0	0	1	0	0	0					
6. Lasmigona complanata	1	0	0	0					0	1	0	0	
7. Amblema plicata	39	3	4	0	2	1	3	0	0	2	5	0	
8. Quadrula quadrula	1	0	2	0									
9. Fusconaia flava	23	6	2	0	0	0	1	0	0	0	1	0	
10. Villosa lienosa	0	2	0	0									
11. Lampsilis siliquoidea	139	7	4	0	7	5	1	0	1	1	1	41	
12. Lampsilis cardium	19	4	1	0	1	0	0	0	1	0	0	2	
Total	242	34	25	0	14	7	6	0	2	5	7	45	
Total species	12				8				8				
Total live + dead species	12				6				5				
Total live + dead individuals	276				21				7				
Data													
Date sampled		ne 1990			-	tember				ne 2019			
Flow		vailable				vailable			35 cfs @	Willia	nsburg	gage	
Other (2019): Conductivity – 559		•	v - 9.4 l	NTU, Temp	perature	-22.0 °	°C, pH	-7.6					
Unionid density from Transect: 2/													
Corbicula fluminea density = $2/10$	m2 = 0.2	/m2											

**2020 Site # 25**: East Fork Little Miami River at SR 251 bridge, Perry Twp., Brown Co., Ohio. 1990 Site # 89. 2006 Site # 19. 39°13'31.2"N 83°53'43.5"W. River Kilometer 77 (RM 63.3). 39.225340° -83.895429°. Mount Orab 7.5 Quad.

**2020 Site # 26**: East Fork Little Miami River at USR 68 bridge, Perry Twp., Brown Co., Ohio. 1990 Site # 90. 2006 Site # 20. 39°13'33.5"N 83°54'39.0"W. River Kilometer 75 (RM 62.0). 39.225862° -83.910658°. Mount Orab 7.5 Quad.

	1990/91 data					2006/	7 data		2019/20 data				
	live	dead	wea	subf	live	dead	wea	subf	live	dead	wea	subf	
1. Pyganodon grandis	0	0	4	0	0	0	2	0					
2. Anodontoides ferrusacianus	0	0	4	0									

3. Strophitus undulatus	17	1	1	0					2	0	4	0
4. Alasmidonta viridis	0	6	0	0	0	0	3	0	$\frac{2}{0}$	Ő	4	0
5. Lasmigona costata	ů 0	0 0	1	0 0	2	ů 0	0	0 0	ů 0	Ő	2	Ő
6. Lasmigona complanata					$\overline{0}$	2	0 0	0 0	ů 0	Ő	0	2
7. Amblema plicata	131	12	2	0	14	2	3	0 0	15	21	11	0
8. Tritogonia verucossa					0	1	0	0	0	1	0	0
9. Quadrula quadrula	1	0	0	1					0	2	0	0
10. Fusconaia flava	35	1	6	0	5	4	1	0	0	$\frac{2}{3}$	5	0
11. Villosa lienosa	1	0	2	0					0	0	0	1
12. Truncilla donaciformis	1	0	2	0	0	0		0	0	0	0	1
13. Lampsilis siliquoidea	140	9	5	0	22	3	3	0	0		6	0
			5 1			3	3	0		2	0	-
14. Lampsilis cardium	23	2	1	0					0	2	1	0
Total	348	31	26	1	43	12	13	0	17	31	33	3
Total species	11				9				11			
Total live + dead species	8				6				7			
Total live + dead individuals	379				55				48			
Data												
Date sampled	1 July	1990			9 Sept	ember 2	2006		15 Jul	y 2019		
Flow	Not av	ailable			-	ailable			25 cfs @		nsburg s	gage
Other (2019): Conductivity – 5				NTU Te				-82				56-
Unionid density from Transect:		-	15.11	, 10, 10	mperature	25.0	с, рп	0.2				
•												
Corbicula fluminea density $= 1$	$1/10m^2 = 1.$	$1/m^2$										

## **2020 Site # 29**: East Fork Little Miami River at SR 131 bridge, Perry Twp., Brown Co., Ohio. 1990 Site # 93. 2006 Site # 23. 39°09'56.8"N 83°56'27.7"W. River Kilometer 65 (RM 54.4). 39.165780° -83.941044°. Mount Orab 7.5 Quad.

				2006/	7 data		2019/20 data					
	live	dead	wea	subf	live	dead	wea	subf	live	dead	wea	subf
1. Pyganodon grandis	0	0	1	0	0	0	1	0	0	0	0	1
2. Strophitus undulatus	2	1	0	0	1	0	1	0				
3. Alasmidonta viridis	2	1	0	0	0	0	1	0	0	0	0	1
4. Lasmigona costata					4	1	0	0	0	1	0	2
5. Lasmigona complanata					1	0	1	0	2	5	1	0
6. Amblema plicata	7	0	0	0	9	5	0	0	6	4	0	7

7. Tritogonia verucossa									3	0	0	0
8. Quadrula quadrula	1	0	1	0	1	0	0	0				
9. Fusconaia flava	2	3	0	0	1	4	0	0	0	0	2	0
10. Villosa lienosa		1	0	0	0	0	1	0				
11. Lampsilis siliquoidea	13	2	0	0	11	4	0	0	0	0	4	0
12. Lampsilis cardium	2	3	0	0	1	1	0	0	0	0	2	0
Total	29	11	2	0	29	15	5	0	11	10	9	11
Total species	9				11				9			
Total live + dead species	8				8				4			
Total live + dead individuals	40				44				21			
Data												
Date sampled	1 July	1990			10 Sep	otember	2006		15 Jul	y 2019		
Flow	Not av	vailable			Not av	ailable			25 cfs @	Willian	nsburg	gage
Other (2019): Conductivity – 4	412 uS/cm, T	urbidity	-144 N	VTU, Te	mperature	$-28.2^{\circ}$	С, рН –	8.6				
Unionid density from Transect	t: 0/10  m2 = 0	).0/m2										
Corbicula fluminea density = 0	0/10m2 = 0.0/	′m2										

**2020 Site # 30**: East Fork Little Miami River at SR 286 bridge, Perry/Jackson Twp., Brown/Clermont Co., Ohio. 1990 Site # 95. 2006 Site # 24. 39°08'16.8"N 84°00'10.1"W. River Kilometer 55 (RM 46.9)1. 39.138054° -84.002795°. Newtonville 7.5 Quad.

		1990/	91 data	,		2006/	7 data			2019/2	20 data	L
	live	dead	wea	subf	live	dead	wea	subf	live	dead	wea	subf
1. Pyganodon grandis	0	0	1	0					0	2	0	0
2. Anodontoides ferrusacianus	0	2	0	0								
3. Strophitus undulatus	4	4	0	0	0	0	1	0	0	3	0	0
4. Alasmidonta viridis	0	6	0	0					0	0	4	0
5. Lasmigona costata	6	0	1	0	5	1	0	0	2	6	0	0
6. Lasmigona complanata	5	0	0	0	7	0	0	0	4	5	0	0
7. Amblema plicata	100	3	0	0	34	5	0	0	40	41	0	0
8. Tritogonia verrucosa					1	1	0	0	8	7	0	0
9. Quadrula quadrula	7	2	1	0	1	0	1	0	0	0	4	0
10. Fusconaia flava	15	4	0	0	2	2	0	0	1	5	0	0
11. Pleurobema sintoxia					1	0	0	0				
12. Villosa fabalis	0	1	0	0								

13. Villosa lienosa	0	4	0	0					0	0	0	1
14. Lampsilis siliquoidea	0	5	0	0	12	1	0	0	2	2	0	0
15. Lampsilis cardium	0	4	0	0	7	0	1	0	0	1	1	0
Total	137	35	3	0	70	10	3	0	57	72	9	1
Total species	13				10				12			
Total live + dead species	12				9				9			
Total live + dead individuals	172				80				129			
Data												
Date sampled	7 July	1990			12/28	August	2006		16 Jul	y 2019		
Flow	Not av	vailable			Not av	vailable			22 cfs @	Willian	nsburg g	gage
Other (2019): Conductivity $-40$	4 uS/cm, T	urbidity	- 14.8	NTU, Te	emperature	e – 26.1	°C, pH	- 8.3				
Unionid density from Transect:	5/10  m2 = 0	).5/m2										
Corbicula fluminea density $= 0/$	10m2 = 0.0/	′m2										

**2020 Site** # **31**: East Fork Little Miami River at Blue Sky Park Road bridge, Jackson Twp., Clermont Co., Ohio. 1990 Site # 96. 2006 Site # 25. 39°06'53.9"N 84°01'29.5"W. River Kilometer 53 (RM 44.2). 39.114993° -84.024850°. Williamsburg 7.5 Quad.

		1990/	91 data	,		2006/	7 data		U	2019/2	20 data	L
	live	dead	wea	subf	live	dead	wea	subf	live	dead	wea	subf
1. Pyganodon grandis	0	1	0	0	1	0	0	0	0	1	0	0
2. Anodontoides ferrusacianus	0	2	0	0								
3. Strophitus undulatus	0	4	0	0					3	2	0	0
4. Alasmidonta viridis	0	2	1	0					0	0	1	0
5. Lasmigona costata	0	2	0	0	3	0	0	0	4	6	0	0
6. Lasmigona complanata					3	0	0	0	0	15	0	0
7. Amblema plicata	0	4	0	0	37	0	0	0	1	15	0	0
8. Tritogonia verucossa									45	19	0	0
9. Quadrula quadrula	0	2	1	0	2	1	0	0	0	0	0	1
10. Fusconaia flava	0	11	0	0	1	0	2	0	1	0	0	0
11. Villosa fabalis	0	1	0	0								
12. Villosa lienosa	0	4	0	0								
13. Lampsilis siliquoidea	0	5	0	0	3	0	0	0	0	3	0	0
14. Lampsilis cardium	0	4	0	0					6	0	3	0
Total	0	42	2	0	50	1	2	0	60	61	4	1

Total species	12	7	11
Total live + dead species	12	7	9
Total live + dead individuals	42	51	121
Data			
Date sampled	7 July 1990	27 August 2006	16 July 2019
Flow	Not available	Not available	22 cfs @ Williamsburg gage
Other (2019): Conductivity -	- 392 uS/cm, Turbidity – 13	5.5 NTU, Temperature – 27.7 °C, pH – 8.7	
Unionid density from Transe	ect: $53/10 \text{ m2} = 5.3/\text{m2}$		
Corbicula fluminea density =	= 133/10m2 = 13.3/m2		

**2020 Site # 32**: East Fork Little Miami River at Jackson Pike bridge, Jackson Twp., Clermont Co., Ohio. 1990 Site # 97. 2006 Site # 26. 39°05'47.3"N 84°02'26.6"W. River Kilometer 50 (RM 41.1). 39.096468° -84.040722°.

20. 57 65 17.5 11 61 62 20.6 11.1	1990/91 data			2006/	7 data		2019/20 data					
	live	dead	wea	subf	live	dead	wea	subf	live	dead	wea	subf
1. Utterbackia imbecillis									0	1	0	0
2. Pyganodon grandis	0	1	0	0					0	1	0	0
3. Strophitus undulatus	0	3	0	0					0	3	0	0
4. Alasmidonta viridis	0	7	0	0	0	0	1	0	0	0	1	0
5. Lasmigona costata					1	0	0	0	1	3	0	0
6. Lasmigona complanata					2	1	0	0	0	4	0	0
7. Amblema plicata	0	3	0	0	1	2	0	0	0	0	4	0
8. Tritogonia verucossa									7	7	0	0
9. Quadrula quadrula	0	2	1	0	0	0	1	0	0	0	0	1
10. Fusconaia flava	0	4	0	0					0	1	1	0
11. Villosa fabalis	0	1	0	0								
12. Villosa lienosa	0	0	1	0								
13. Lampsilis siliquoidea	0	4	0	0	7	0	1	0	0	2	0	0
14. Lampsilis cardium	0	1	0	0	12	3	0	0	1	1	0	0
Total	0	26	2	0	23	6	3	0	9	23	6	1
Total species	10				7				12			
Total live + dead species	9				5				9			
Total live + dead individuals	26				29				32			
Data												

Date sampled7 July 199027 August 200629 July 2019FlowNot availableNot available20 cfs @ Williamsburg gageOther (2019): Conductivity - 367 uS/cm, Turbidity - 14.6 NTU, Temperature - 25.3 °C, pH - 8.0Unionid density from Transect: 0/10 m2 = 0.0/m2Corbicula fluminea density = 6/10m2 = 0.6/m2Corbicula fluminea density = 6/10m2 = 0.6/m2

**2020 Site # 33**: East Fork Little Miami River at SR 222 bridge, Batavia Twp., Clermont Co., Ohio. 1990 Site # 100. 2006 Site # 27. 39°03'35.9"N 84°10'44.7"W. River Kilometer 24 (RM 15.6). 39.059993° -84.179073°. Batavia 7.5 Quad.

	1990/91 data		2006/7 data				2019/20 data					
	live	dead	wea	subf	live	dead	wea	subf	live	dead	wea	subf
1. Utterbackia imbecillis	0	1	0	0								
2. Pyganodon grandis	2	0	1	0								
3. Simpsonaias ambigua	0	0	1	0								
4. Lasmigona costata	50	3	0	0	1	0	2	0	0	0	0	2
5. Lasmigona complanata	2	0	1	0					0	3	0	0
6. Amblema plicata	2	0	0	0	2	0	2	0	1	0	2	0
7. Tritogonia verrucosa	50	3	1	0	0	0	1	0				
8. Fusconaia flava	100	1	2	0	1	0	2	0	6	6	5	0
9. Elliptio dilatata	100	10	0	0	0	0	2	0	6	4	7	0
10. Lampsilis siliquoidea	100	4	0	0	1	0	2	0	2	1	2	0
11. Lampsilis cardium	50	1	0	0	3	0	3	0	0	0	1	0
12. Lampsilis fasciola	0	0	1	0								
Total	456	23	7	0	8	0	14	0	15	14	16	2
Total species	12				7				7			
Total live + dead species	11				5				5			
Total live + dead individuals	479				8				29			
Data												
Date sampled	8 July	/ 1990			10 Se	ptember	2006		29 Ju	ly 2019		
Flow	Not a	vailable			Not a	vailable			20 cfs @	Willia	nsburg	, gage
Other (2019): Conductivity $-23$	54 uS/cm, T	urbidity	v - 12.7	NTU, Te	emperatur	re – 25.2	°C, pH	[-8.0]				
Unionid density from Transect:	0/10  m2 =	0.0/m2										
Corbicula fluminea density $= 0/$	10m2 = 0.0	/m2										

2000 Site # 17: 37 20 45.7 10 04 00	11.7		91 data		.0). 57.5	2006/			isunt i fun	-	20 data	
	live	dead	wea	subf	live	dead	wea	subf	live	dead	wea	subf
1. Utterbackia imbecillis	1	0	0	0								
2. Pyganodon grandis	5	1	7	0	0	0	0	1				
3. Strophitus undulatus	0	0	1	0								
4. Alasmidonta viridis	0	8	1	0					0	0	0	1
5. Alasmidonta marginata	1	0	1	0								
6. Lasmigona costata	1	2	1	0								
7. Lasmigona complanata	0	0	2	0								
8. Fusconaia flava					0	0	0	1				
9. Leptodea fragilis	7	8	2	0					0	0	2	0
10. Potamilus ohiensis	0	0	1	0					0	0	2	0
11. Potamilus alatus	13	4	0	0								
12. Truncilla truncata	0	1	0	0								
13. Truncilla donaciformis	0	1	1	0								
14. Obliquaria reflexa	0	0	1	0								
15. Lampsilis siliquoidea	29	14	0	0	0	0	0	1	0	0	3	0
16. Lampsilis cardium	16	5	0	0					0	0	1	0
Total	73	44	18	0	0	0	0	3	0	0	8	1
Total species	15				3				5			
Total live + dead species	11				0				0			
Total live + dead individuals	117				0				0			
Data												
Date sampled	10 Se	ptember	: 1991		13 Aı	igust 20	06		29 Ju	ly 2019		
Flow		vailable				vailable						
Other (2019): Conductivity – 469			r - 5.4 l	NTU, Ten	nperature	-27.3 °	°C, pH	- 8.6				
Unionid density from Transect: 0												
Corbicula fluminea density = $0/1$	0m2 = 0.0	)/m2										

**2020 Site # 36**: Todds Fork Little Miami River at CR 30 (Blackhawk Road) bridge, Salem Twp., Warren Co., Ohio. 1990 Site # 77. 2006 Site # 17. 39°20'45.7"N 84°06'41.7"W. River Kilometer 3 (RM 1.8). 39.346032° -84.111562°. Pleasant Plain 7.5 Quad.

**2020 Site # 37**: Todds Fork Little Miami River at SR 3/USR 22 bridge, Salem Twp., Warren Co., Ohio. 1990 Site # 78. 2006 Site # 18. 39°21'14.7"N 84°07'47.8"W. River Kilometer 1 (RM 0.2). 39.354079° -84.129934°. Morrow 7.5 Quad.

		1990/	91 data			2006/	7 data			2019/2	20 data	l
	live	dead	wea	subf	live	dead	wea	subf	live	dead	wea	subf
1. Pyganodon grandis	0	9	1	0								
2. Anodontoides ferrusacianus	0	0	1	0								
3. Strophitus undulatus	3	0	0	0								
4. Alasmidonta viridis	5	1	5	0								
5. Alasmidonta marginata	2	2	0	0								
6. Lasmigona costata	1	1	1	0								
7. Lasmigona complanata	0	0	0	1								
8. Quadrula quadrula	1	0	0	0								
9. Leptodea fragilis	19	6	2	0					0	1	0	0
10. Potamilus alatus	8	2	0	0					0	0	1	0
11. Truncilla truncata	0	0	1	0								
12. Truncilla donaciformis	1	1	0	0								
13. Lampsilis siliquoidea	40	12	0	0					0	1	0	0
14. Lampsilis cardium	15	0	0	0					1	0	0	0
Total	95	34	11	1	0	0	0	0	1	2	1	0
Total species	15				0				4			
Total live + dead species	11				0				3			
Total live + dead individuals	129				0				3			
Data												
Date sampled	10 Se	ptember	: 1991		13 Au	ugust 20	06		29 Ju	ly 2019		
Flow	Not a	vailable			Not a	vailable						
Other (2019): Conductivity – 412		•	v - 5.4 N	NTU, Ten	nperature	– 29.9 °	°C, pH	- 8.7				
Unionid density from Transect: 0												
Corbicula fluminea density = $0/1$	0m2 = 0.0	/m2										

## **Appendix 2. Statistical Analysis Values**

Year 1	Year 2	F Statistic	p value
1990	2006	8.2333	0.005
1990	2019	8.2333	0.006
2006	2019	0.1199	0.301

Table 1. Total number of individuals comparison for 1990, 2006 and 2019 for the mussels of the Little Miami River.

Table 2. Number of extant individuals' comparison for 1990, 2006 and 2019 for the mussels of the Little Miami River.

Year 1	Year 2	F Statistic	p value
1990	2006	9.573	0.004
1990	2019	9.573	0.004
2006	2019	0.120	0.616

Table 3. Number of extirpated individuals' comparison for 1990, 2006 and 2019 for the mussels of the Little Miami River.

Year 1	Year 2	F Statistic	p value
1990	2006	0.0423	0.820
1990	2019	5.723	0.005
2006	2019	5.723	0.012

Year 1	Year 2	F Statistic	p value
1990	2006	8.467	0.029
1990	2019	8.467	0.000
2006	2019	8.467	0.000

Table 4. Percent of species extant comparison for 1990, 2006 and 2019 for the mussels of the Little Miami River.

Table 5. Ratio extant to extirpated individuals' comparison for 1990, 2006 and 2019 for the mussels of the Little Miami River.

Year 1	Year 2	F Statistic	p value
1990	2006	8.467	0.035
1990	2019	8.467	0.014
2006	2019	0.167	0.366

Table 6. Total number of species comparison for 1990, 2006 and 2019 for the mussels of the Little Miami River.

Year 1	Year 2	F Statistic	p value
1990	2006	8.038	0.005
1990	2019	8.038	0.025
2006	2019	0.0148	0.953

Table 7. Shannon-Weiner Index comparison for 1990, 2006 and 2019 for the mussels of the Little Miami River.

Year 1	Year 2	F Statistic	p value
1990	2006	0.0021	0.131
1990	2019	4.5455	0.001
2006	2019	4.5455	0.005

Table 8. Mussel Density comparison for 1990, 2006 and 2019 for the mussels of the Little Miami River.

Year 1	Year 2	T Statistic	p value
2006	2019	-0.745	0.2332

Table 9. *Corbicula fluminea* Density comparison for 1990, 2006 and 2019 for the mussels of the Little Miami River.

Year 1	Year 2	T Statistic	p value
2006	2019	3.5667	0.0012