Environmental DNA analysis of fish in the Mill Creek

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Introduction

The Mill Creek in the 90's was considered one of the worst urban streams in America. Many different groups have shown great interest in understanding the biodiversity of the stream to document efforts to improve the water quality of the stream such as adding riffles to promote biodiversity. The Mill Creek is affected by impervious surfaces that cause flash floods and scour the stream bed, and many sites where the sewage system overflows and dumps E. coli and raw sewage in the stream. These disturbances make it difficult for many sensitive species to survive in these waters. Both non-government and government groups are interested in quantifying biodiversity to monitor the health of Mill Creek and its tributaries. Environmental DNA could be an effective tool to monitor biodiversity that would be less time and labor intensive than traditional methods.(Shaw et al, 2016). My goal was to assess the ability of eDNA to effectively identify fish from of the Mill Creek Basin. I tested the hypothesis that drainage size was related to species richness.

Figure 1. Jonah eDNA kit for aquatic samples (Left). Orangethroat Darter, one of the many fish found through this method (Right). (Photo from Fish of Ohio)

Methods

In November 2022, DNA samples were collected directly from streams using a 60 ml syringe to push 150-300 ml of water through a 1 um filter, then injected with 0.5M EDTA as a preserving solution. DNA was extracted from the filter using Qiagen blood and tissue kit. A 168bp region of mitochondrial 12S ribosomal RNA (Miya et al, 2015) was amplified (Jonahventures). PCR products were checked on an agarose gel and prepared for sequencing (illumina NovaSeq). Sequences were processed by Jonahventures using vsearch and GenBank (NCBI) reference sequences. I participated in field sampling of larger volumes, as well as DNA extraction, PCR, and gel visualization. I organized the raw sequence data and produced this summary and statistical analysis. Hand sampled and electrofishing results from MBI (Midwest Biodiversityl Institute) surveys in 2021 and 2016 were used to compare to eDNA results.

Results

The 200 different sequences recovered matched known freshwater fish taxa with 98% or greater similarity to known reference sequences. Eleven different families of fish were recovered by eDNA, and 31 species were identified. The true minnows had the most diversity with 86 different sequences followed by suckers with 36 (Fig. 2&3). There is a significant relationship between the drainage area at point of sampling and the number of fish taxa recovered. (Fig. 4)

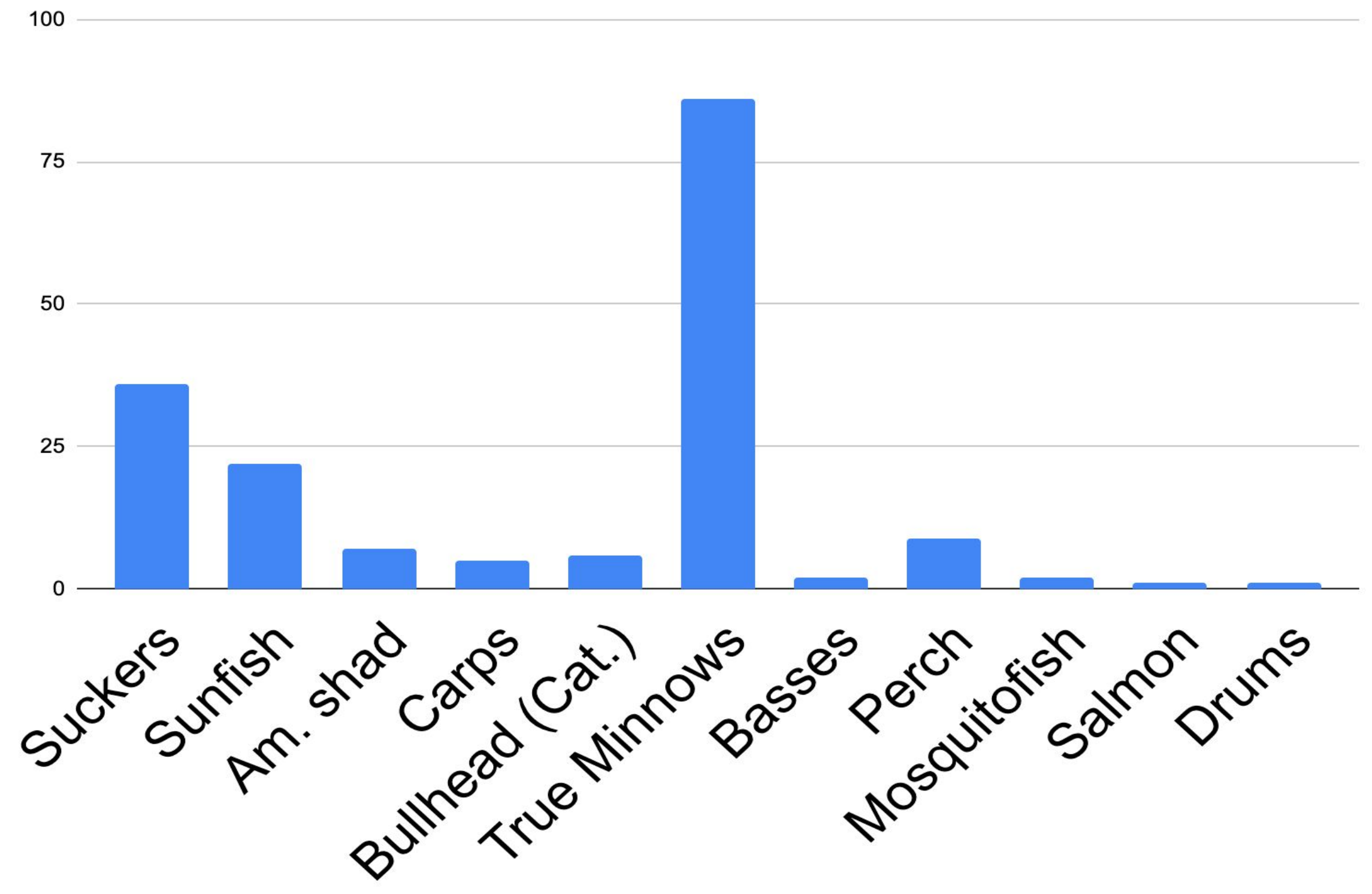

Figure 2. Amount of individuals found in all 11 different families.

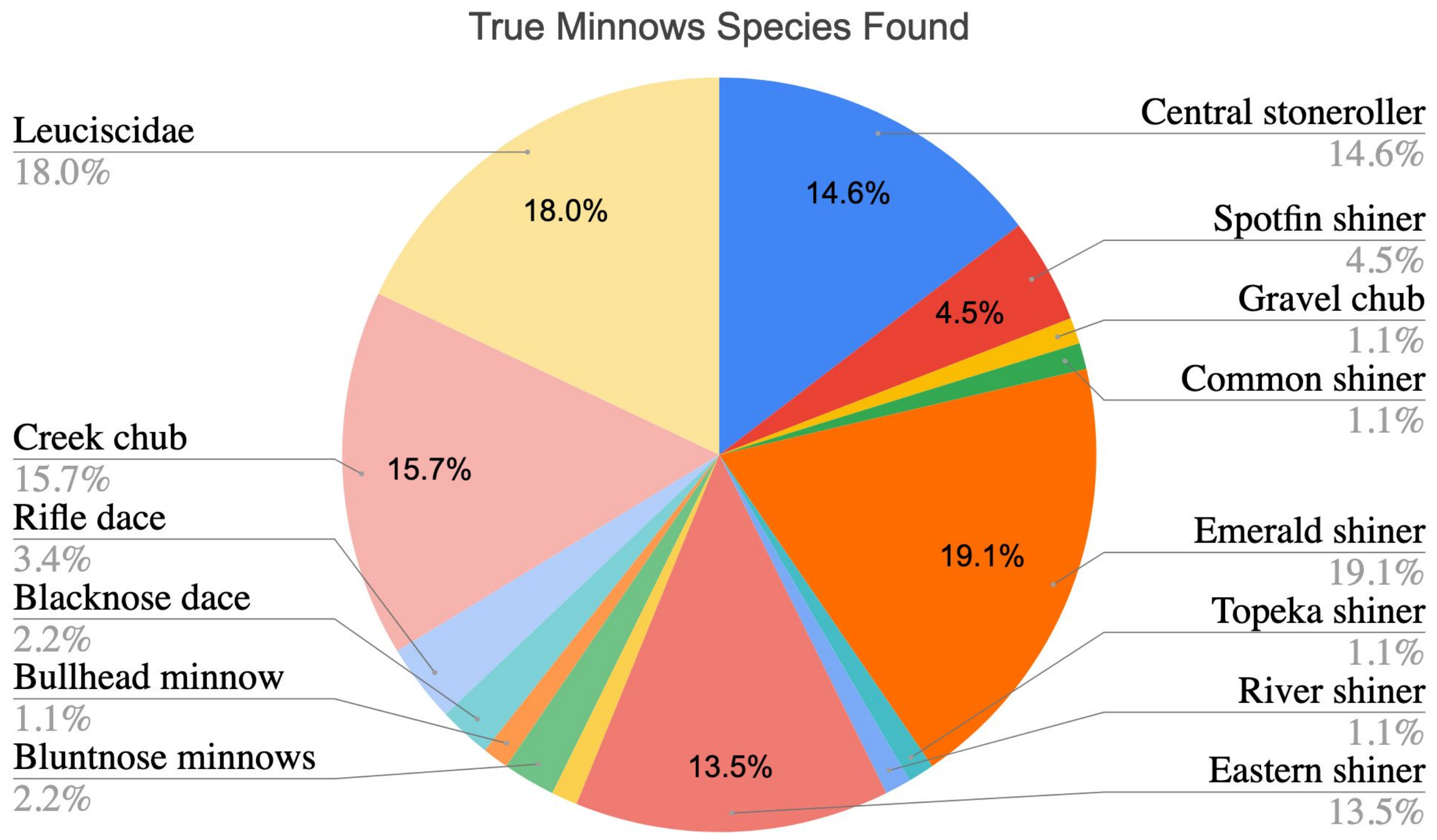

Figure 3. Minnows were the largest family of fish in the Mill Creek. Both Common and endangered species were found.

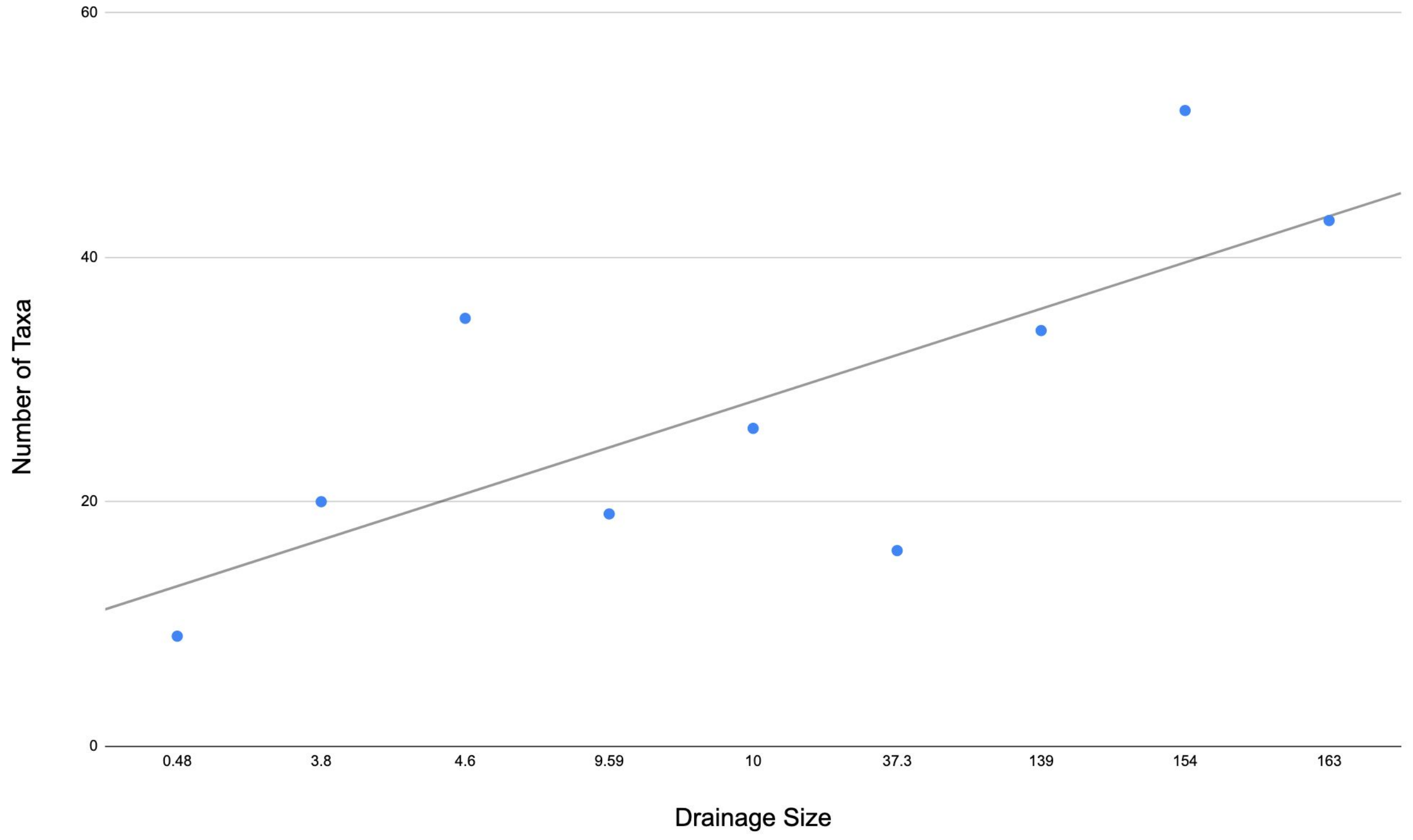

Figure 4: Correlation of drainage size versus number of taxa. Higher square mileage correlates to higher number of taxa. (*P* < 0.018. *df*= 9).

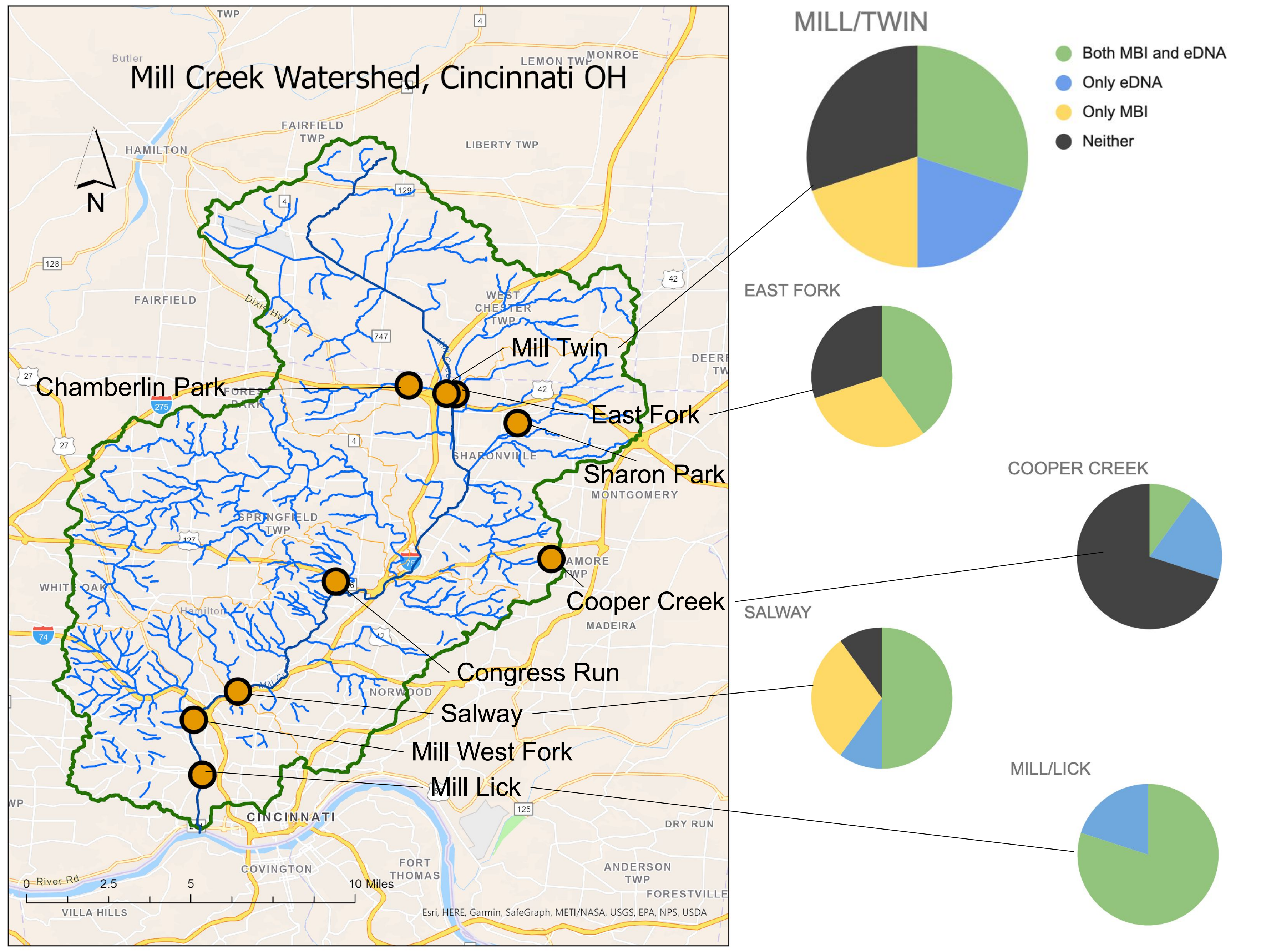

Figure 5. Map of nine sampling locations in the Mill Creek Basin. Pie charts for five locations compare eDNA and hand-sampled biodiversity: Green indicates proportion of taxa found in both eDNA and hand samples, Blue are taxa only found by our eDNA methods, yellow are taxa found by electrofishing but not eDNA, and black are taxa not found by either method at that site,

Discussion

Using eDNA is an efficient way to identify taxa in streams and in some cases found fish that were not recovered in previous sampling studies such as MBI electrofishing. Some of the fish found such as the Topeka Shiner (Mill/West), River Shiner, and Gravel Chub shows the progress of this stream over the past 30 years going from one of the worst rated streams to holding endangered species that require sufficient water quality- even though it was done on an off season. Further studies are needed to see if more sampling can detect the species recovered using field capture methods, or whether some species are harder to detect using eDNA. eDNA holds promise to expand the scope of surveillance across the Mill Creek and elsewhere, as long as costs can be kept reasonable for more general use.

References

Midwest Biodiversity Institute (MBI). 2021. Biological and Water Quality Assessment of Mill Creek 2021. Hamilton County, Ohio. Technical Report MBI/2017-6-8. Columbus, OH 43221-0561. 74 pp. + appendices. http://www.msdgc.org/initiatives/water_quality/index.html.

Miya, M., et al. "MiFish, a Set of Universal PCR Primers for Metabarcoding Environmental DNA from Fishes: Detection of More than 230 Subtropical Marine Species." *Royal Society Open Science*, vol. 2, no. 7, 2015, p. 150088., https://doi.org/10.1098/rsos.150088.

Shaw, J. L. A., Clarke, L. J., Wedderburn, S. D., Barnes, T. C., Weyrich, L. S., & Cooper, A. (2016). Comparison of environmental DNA metabarcoding and conventional fish survey methods in a river system. *Biological Conservation*, *197*, 131–138. https://doi.org/10.1016/j.biocon.2016.03.010