Ohio Water Environment Association
Volume 95:1 | Issue 1 2022

Buckeye Bulletin

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Improving Biological Assessments with the Analysis of Environmental DNA

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Activities needed to construct, maintain, and/or improve water infrastructure often require some kind biological and environmental impact assessment prior to implementation. These assessments aid in the protection of vulnerable biodiversity. Traditionally, characterizing biodiversity requires trained field crews with expertise in morphological identification, to physically capture and identify the organisms at a given site. Such surveys can be time-intensive, and some species may be missed due to their cryptic nature (e.g., species that can avoid traditional capture methods or are difficult to morphologically distinguish). As a consequence of these limitations, resource assessment professionals are always looking for more cost effective and accurate means to detect special status species. Recent technology is pushing the limits of what previously was considered science fiction, by seeking to identify aquatic species without the need to capture or even see them within the environment.

Over the past decade, there has been a rise in the use of genetic-based applications for biological assessment purposes. One such method detects environmental DNA (eDNA), which involves collection of genetic material (e.g., feces, urine, mucus, skin cells, or gametes) that an organism leaves behind in the environment (Figure 1). Similar to DNA analysis in criminal forensics, the detection of eDNA can confirm the presence of a species without direct observation of that species. Therefore, eDNA is quickly becoming a powerful survey tool to assess presence of species at low densities or cryptic species, as eDNA sampling can provide higher detection probability for rare organisms (Figure 2). eDNA surveys are less invasive on the environment, and can be less hazardous on the survey staff compared to some traditional methods (e.g., electro-fishing and scuba diving). Additionally, these surveys are often more cost-effective compared to traditional time-intensive surveys.

Figure 1. Environmental DNA (eDNA) involves the collection of genetic material (e.g., feces, urine, mucus, skin cells, or gametes) that an organism leaves behind in the environment.

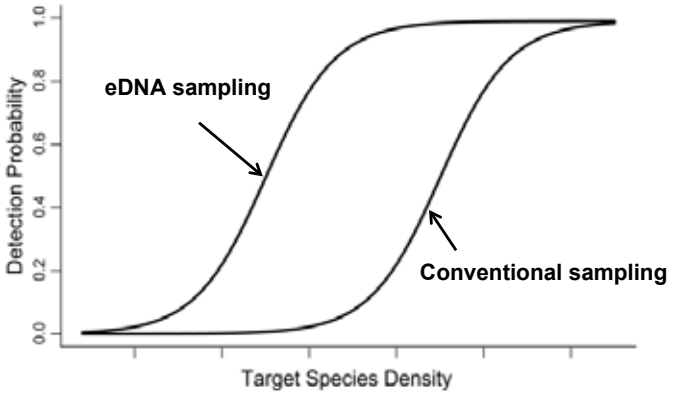

Figure 2. Hypothetical example of increased detection probability with environmental DNA (eDNA) sampling compared to conventional sampling for species at the low abundance or density.

The State of eDNA

eDNA can be collected from the water, soil, and even air, and then preserved for later analysis in the laboratory. Recent innovations (e.g., Smith-Root backpack eDNA sampler) have led to filtration and preservation of samples on-site. The state of eDNA within the environment, and the ability to successfully detect target species, is related to a combination of biotic and abiotic factors that directly influence its release and degradation. The biological and metabolic activity of an organism plays a major role in the amount of eDNA released into the environment. For example, many species display greater eDNA release rates during feeding activity. Additionally, abiotic factors (e.g., temperature and dissolved oxygen) may influence the biological activity of an organism, and thus impact the amount of eDNA released. Environmental factors also play a key role in the persistence of eDNA, with majority of degradation occurring due to the activity of microbes and extracellular enzymes in the water. Therefore, factors that influence microbial activity (e.g., temperature, pH, salinity, and dissolved oxygen) will have direct impacts on eDNA persistence. Acidic warm environments typically have high rates of eDNA degradation. Additionally, ultraviolet light can rapidly degrade DNA, and thus exposure to sunlight can impact eDNA detection. While persistence of eDNA has been found to last for several months within a laboratory setting, the length of persistence within a natural aquatic environment is more likely days to weeks.

Examples of eDNA

Due to the increased sensitivity in organism detection, some of the earliest work with eDNA focused on the detection of aquatic invasive species as they spread into new locations. The most prominent example involves the detection of invasive carps (Bighead and Silver Carp) within the Great Lakes basins, specifically within Illinois waterways and the Chicago Area Waterway System. These invasive carp have been identified as a major threat to the Great Lakes' ecosystem, and in 2009 the U.S. Army Corps of Engineers (USACE) implemented an eDNA monitoring program focused on determining the geographic extent of invasive carp within this system. Invasive carp eDNA monitoring along the Chicago Area Waterway System and throughout the Upper Mississippi River, Ohio River, and Great Lakes continues today, but now samples are processed and analyzed by the Midwest Region of the U.S. Fish and Wildlife Service (USFWS). Results of this monitoring program are made available through the USFWS (*https://www.fws.gov/midwest/Fisheries/eDNA.html*). This early employment of eDNA for the detection of invasive species has been instrumental in the development of eDNA applications for a range of invasives throughout the Great Lakes region (e.g., round goby, Eurasian ruffe, zebra and quagga mussel) and the Ohio River (e.g., snakehead).

In the past decade, eDNA projects continued to advance and now its applications are recognized as a complementary survey tool to aid in water resource management projects. Several projects have employed eDNA sampling to evaluate the success of restoring connections in riverine habitats. Following the removal of two dams on the Elwha River in Washington State, eDNA successfully documented the re-colonization of 11 fish upstream of previous dam sites, providing direct evidence of the migratory barrier elimination (*https://doi.org/10.1002/edn3.134*). In this case eDNA outperformed traditional methods which required site specific observations of the target species. Additionally, eDNA provided evidence of fish usage within a newly constructed channel passage to reduce ecological impacts of the Itaipu Hydroelectric Power Plant (2nd largest in the world) along the Paraná River between Paraguay and Brazil (*https://doi.org/10.1038/s41598-021-02593-5*). As riverine habitat restoration and monitoring becomes more common practice, the use of eDNA will no doubt be

a valuable survey alternative. eDNA has additionally been utilized to help determine species presence associated with water intake construction projects and even dam decommissioning projects. The removal of the Six Mile Dam on the Walhonding River in Coshocton County near Warsaw, Ohio, included a biological assessment of the freshwater mussel community. In tandem with a mussel conventional survey, eDNA samples were collected to assess the presence of threatened and endangered mussels (*https://www.stantec.com/en/projects/united-states-projects/s/six-mile-dam-removal-edna-native-freshwater-mussel*). eDNA analysis provided similar results to traditional methods for describing community composition along the Walhonding River, including detecting two federally-listed mussels (Figure 3), with only a fraction of the search effort. eDNA provides opportunities to expand temporal and spatial surveys beyond many of the constraints associated with conventional methods.

While not necessarily falling directly under the definition of "eDNA", the collection of water and

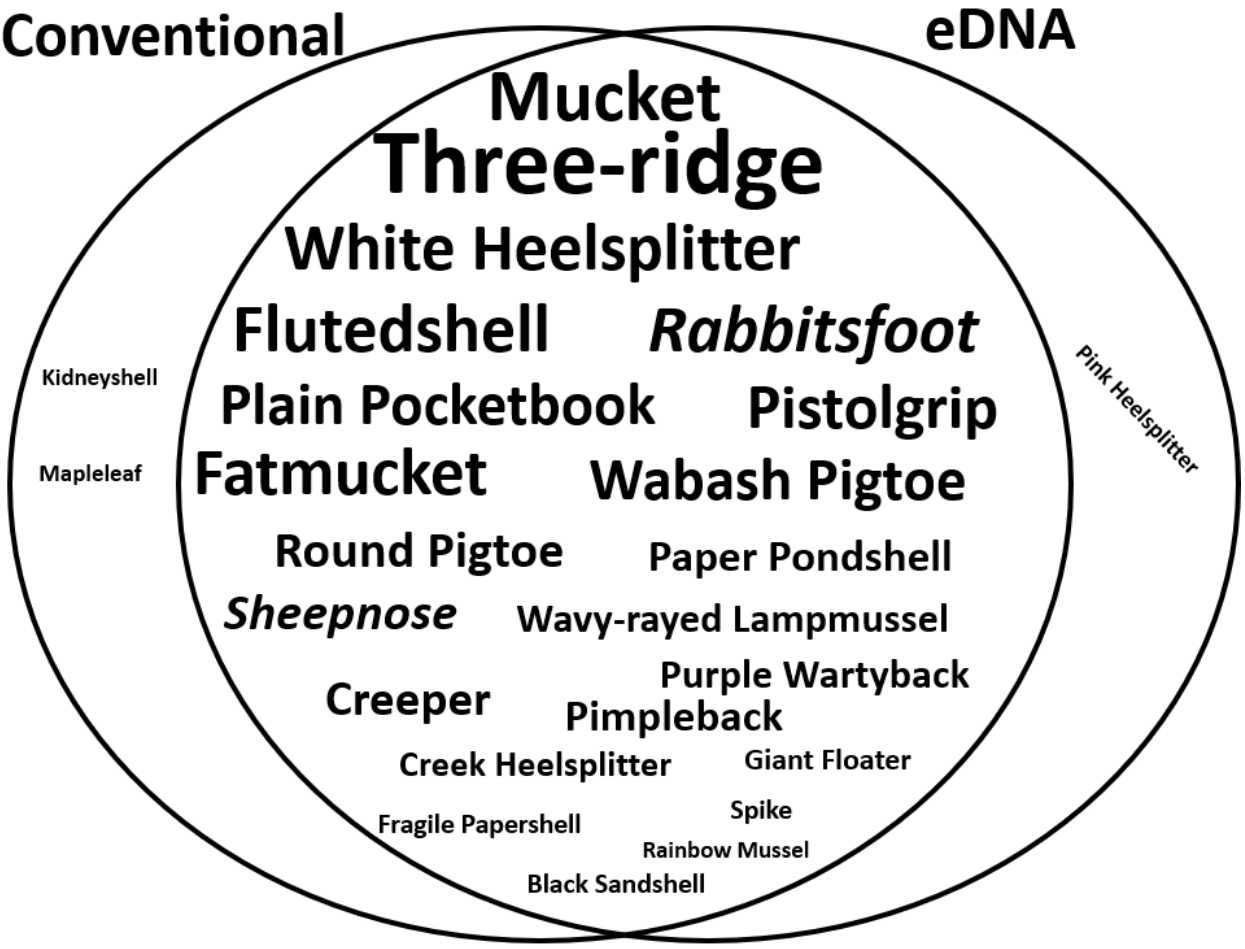

Figure 3. Detection of freshwater mussel species at the Six Mile Dam on the Walhonding River in Coshocton County near Warsaw, Ohio, with eDNA detecting two federally-listed mussels (Rabbitsfoot and Sheepnose) plus several others that were collected as single animals. Font size represents relative mussel abundance of each species found with a conventional survey.

subsequent genetic analysis provides an opportunity to detect the presence of harmful or nuisance microbial and algal communities. Anthropogenic activities and climate change have largely altered ecological communities worldwide. Eutrophication of freshwater systems by humans often leads to formation of harmful algae blooms (HABs), along with decreases in water quality, reductions in biodiversity, and increasing threats to human health. HABs throughout the western basin of Lake Erie caused by toxic *Microcystis* blooms have presented unique challenges for water intake plants. DNA analysis has become an instrumental monitoring tool for tracking and assessing the prevalence of *Microcystis* throughout spring and summer months within Lake Erie (*https://www.glerl.noaa.gov/res/HABs_and_Hypoxia/habTracker.html*). Furthermore, fecal contamination and consequently its associated pathogens is a major environmental and public health problem that is correlated with increases in waterborne disease. Identification of fecal contamination and the associated pathogens through DNA analysis provides critical information for water managers, river authorities, and public health officials. In a similar fashion, monitoring municipal wastewater for the DNA/RNA of a virus, for example COVID-19, allows for the detection of the specific virus within a population and can provide an early warning of positive and asymptomatic cases. DNA analysis of water samples is not restricted to macro-organisms, but can also provide detailed information about the microbial community.

Current Limitations of eDNA

While there are many advantages to eDNA applications, there are a few important considerations that must be recognized to effectively implement an eDNA survey. For example, eDNA can be shed at different rates across different organisms, and thus understanding the biology of the target species is crucial for appropriate implementation strategies. Additionally, eDNA might have different shedding rates across life-stages of an organism (e.g., reproduction versus hibernation), and thus eDNA concentrations are likely to vary seasonally. As mentioned previously, eDNA degradation is largely dependent upon environment-specific factors, and eDNA persistence will vary across environments. Careful consideration of these factors can help determine how recently a species was present in the environment, to avoid detecting potential "legacy eDNA". The persistence and detectability of eDNA will also be affected by the hydrology of the system, with potential downstream transport occurring in streams and rivers. Many researchers are currently focused on addressing issues related to eDNA transport and how to appropriately interpret eDNA detections. Proper sampling design, with the help of an experienced professional, can limit uncertainty regarding certain environmental variables, such as the potential transport of genetic material. Lastly, conventional biological surveys often include population and density estimates based on the abundance of an organism collected. While the quantification of eDNA has shown some promise in establishing abundance or biomass estimates, many factors influence the concentration of eDNA within the environment, and the current state of the science does not allow for reliable direct estimates.

In conclusion, eDNA is an emerging approach for detecting aquatic species (from macro to micro-organisms) and is beginning to advance management and monitoring practices. The examples provided here only begin to scratch the surface for the potential of eDNA as a survey tool. From describing community composition, to detection of federally-listed or invasive species, eDNA methodology provides complementary tools to conventional methods. As eDNA transitions from the realm of pure research to widespread application, much

work remains to optimize field and laboratory techniques. Nevertheless, the potential for eDNA to improve the characterization of aquatic biodiversity is clear, and it can considerably improve the monitoring and management of aquatic ecosystems moving towards the future.

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An environmental scientist, Nate has experience developing and implementing environmental DNA (eDNA) methodology for improving biological assessments. His enthusiasm for innovation and biodiversity is contagious—he is always searching for new ways to assess biological communities through a collaborative approach.

Nate has a masters from the University of Texas where he aided in unionid freshwater mussel conservation by studying the interaction between host fish and unionid mussel larvae. Nate obtained his PhD from the University of Toledo, where he developed eDNA methods for the early detection of invasive dreissenids (zebra and quagga mussels) and implemented eDNA metabarcoding for the description of entire aquatic macroinvertebrate communities.

When Nate is not working, he enjoys walks with his two dogs—a husky named Yeti and a lab mix named Miggy.

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