Figure 1: Environmental DNA (eDNA) can be recovered directly from water samples without disturbing the organisms and their habitat. The use of eDNA in environmental biomonitoring studies can improve accuracy of surveys, reduce cost and provide more time-effective workflows.

EDNA WILL ENHANCE ENVIRONMENTAL SURVEYS FOR 500 CANADIAN LAKES

By **Tzitziki Loeza-Quintana** and **Gordon Wichert**

All living organisms release genetic material into the environment as they move and interact in their habitat and as a result of their biological processes (e.g., mucous, gametes, shed skin, feces, etc.). When released in the environment, this genetic material can be retrieved and used to detect the organisms without having to trap or visually count them.

Genetic material recovered from the environment is called environmental DNA (eDNA). It can be recovered from different environmental sources such as water, sediments, soil or even air, and has been used successfully to reveal species-at-risk, invasive species and pathogens.

HOW ARE EDNA SAMPLES COLLECTED AND INTERPRETED?

From the first study using eDNA to detect an invasive species in 2008 (Ficetola et al., 2008), development of methods and technology to detect and interpret eDNA have increased steadily.

Overall, there are three main steps to analyze eDNA: Sample collection, extraction and purification of the DNA, and molecular detection and identification of the species.

Sample collection includes the collection of an environmental sample, i.e., water, soil, sediment, surface swabs, feces, or air. The next step is the extraction and purification of the DNA contained in the environmental sample. Lastly, there are two main molecular approaches for species detection using eDNA: targeted detection and metabarcoding.

DNA from a single target species is detected using a molecular method called polymerase chain reaction (PCR). In this process, billions of copies of a target sequence are replicated exponentially to increase likelihood of detection. PCR amplification indicates whether a targeted species is present, and the results require relatively little processing. This process can be run in realtime using quantitative PCR (qPCR) via fluorescent signal of the target DNA.

In recent years, scientists have also used a more precise and sensitive technique called digital droplet polymerase chain reaction (ddPCR) for targeted detection of a single species eDNA. Alternatively, detection could be done via next generation sequencing, which consists of

amplifying and sequencing all the DNA in the sample. This approach is called DNA metabarcoding and involves comparing all the amplified DNA sequences against a database of know sequences to identify which species are present in the environmental sample.

There are advantages and disadvantages for each approach and the most suitable would depend on the goal of the survey. If the goal is to focus on a single or a limited number of species, the targeted detection approach is quicker and cheaper than DNA metabarcoding. Targeted detection is also more sensitive, so if a species is present, you might be more likely to detect it. It can be used to estimate relative abundance based on the concentration of DNA in a sample.

In comparison, DNA metabarcoding can be quicker and more cost-efficient for detecting many species at once, which is advantageous for assessing biodiversity. However, species at very low abundance might be missed when using DNA metabarcoding. Processing the results can also take longer as metabarcoding sequence datafiles are usually very large and powerful bioinformatic tools are necessary for data analyses.

Methods are rapidly evolving and some limitations from both approaches may be overcome with future developments.

Conventionally, molecular protocols to analyze eDNA are run in specialized laboratories, but recent technological advances are making it possible to run molecular tests directly in the field, accelerating the process of eDNA surveys.

THE UTILITY OF EDNA

Using eDNA in environmental biomonitoring studies can improve accuracy of surveys, reduce costs and provide more time-effective workflows. The potential utility of this molecular approach can have great implications in many fields and industries. Being able to rapidly and accurately detect the presence of a target species through eDNA and without direct observation has opened the doors in ecology studies.

eDNA detection has been demonstrated as more sensitive than conventional surveys such as electrofishing (e.g., Evans et al., 2017; Wilcox et al., 2016). eDNA surveys also offer a particularly valuable advantage in the early detection of invasive species (e.g., Balasingham et al., 2018; Carim et al., 2019; Mychek-Londer et al., 2019; Thomas et al., 2019) and for monitoring of endangered species (e.g. Currier et al., 2018; Mychek-Londer et al., 2019). Further research and advances in technology can expand the applications for eDNA surveys.

Figure 2: With current technological advances, the extraction and purification of environmental DNA can be performed in the field in just a few minutes, accelerating the process of eDNA surveys.

eDNA DETECTION VS. CONVENTIONAL SAMPLING METHODS

Conventional biomonitoring surveys involve observation or direct capture and additional extensive documentation. This can be difficult and labour intensive, especially if the species of interest is rare, very small, cryptic or difficult to identify. Additionally, conventional surveys can cause stress and other risks to the organisms and damage sensitive habitats. These challenges tend to restrict the frequency and scale of biomonitoring surveys, limiting the available information to environmental managers.

Biomonitoring surveys could be improved by the use of eDNA. For example, a conventional study to identify brook trout using electrofishing methods showed that about 20 person hours were required to detect, or fail to detect, brook trout at 10 sampling locations. This is approximately two person-hours per site (Evans et al., 2017). Alternatively, in the same study, approximately 6.8 person-hours, or approximately 40 minutes per site, were required to detect the species using eDNA (Evans et al., 2017).

eDNA surveys appear suitable for exploratory investigations in advance of more detailed environmental assessments. Rapid screening can identify presence and absence of aquatic communities, while providing evidence of rare species or early stage invasive species. This screening level information can support proponents during project feasibility stages, early indications for permitting and approvals, and also inform appropriate levels of effort for more intensive studies for environmental effects assessment.

LIMITS AND CHALLENGES OF EDNA DETECTION

There are some important considerations when using eDNA, especially when attempting to advance the level of data interpretation from presence/absence to abundance estimations. Species biology and ecology (preferred habitat), and water movement (flow, depth, water current) are some factors to be considered when planning eDNA surveys to maximize the probability of species detection.

Furthermore, different species shed different amounts of genetic material at different rates, as do individuals at different life stages within a given species (e.g., Jo et al., 2020; Klymus et al., 2015). The source of detected eDNA may be ambiguous. For example, did the genetic material come from one or a few individuals nearby, or from a group of organisms further away?

The degradation rate of DNA is also highly dependent on environmental variables (temperature, pH), microbial activity and the type of substrate (water, soil, ice) (Strickler et al., 2015). An organism

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may occur in a locality but go undetected because of the high degradation rate of its genetic material. These, and other factors, can make it difficult to accurately estimate species density or how long ago an organism was present in the environment.

However, further investigation of shedding rates, environment-specific factors influencing degradation rate, and robust molecular testing can overcome these challenges in the future.

Currently, there is some information that eDNA cannot provide. For example, it cannot necessarily tell us the size, sex, developmental stage, or the health of individuals. Conventional biomonitoring techniques are still necessary to obtain that level of detail about the individuals within populations. Therefore, environmental managers need to carefully consider their questions before deciding whether or not to employ eDNA.

Increased diversity in methodological approaches for the analysis of eDNA have led to the lack of standards among eDNA practitioners. Regulatory agencies must fully accept the results obtained from eDNA surveys in decision making, before the environmental private sector can fully use the approach as part of their regular operations.

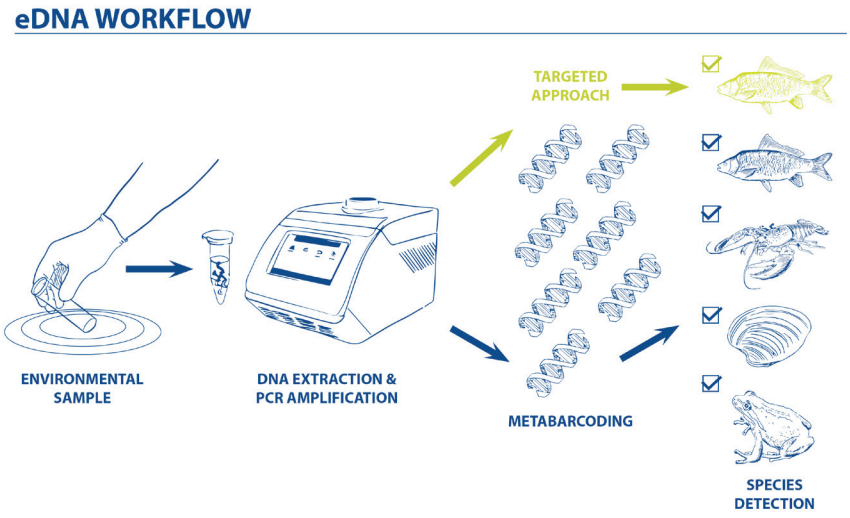

Figure 3: A flowchart showcasing the difference between traditional targeted sampling approach and the eDNA approach to sampling.

POTENTIAL PROJECT APPLICATIONS AND FUTURE WORK

The University of Guelph and SLR Consulting are collaborating on applied research opportunities to explore practical application of eDNA to augment, or in some cases be a substitute for, present practice. For instance, recruitment of groundwater sensitive species such as brook trout is essential to maintain sustainable populations but can be affected negatively by groundwater extraction.

Visual spawning surveys can produce variable results. Increased confidence in monitoring results and more effective environmental management may be possible if more accurate spawning and recruitment data were available. eDNA approaches may represent an opportunity to increase confidence in assessment of spawning and recruitment of sensitive species.

The collaboration between the University of Guelph and SLR Consulting is also part of the overarching project Genomic Network for Fish Identification, Stress and Health (GEN-FISH) funded by Genome Canada and the Ontario Genomic Institute. GEN-FISH is a nation-wide project that aims to improve sampling techniques to determine the distribution and abundance of all Canadian freshwater fish species and how they respond to anthropological stressors.

One of the GEN-FISH goals is to develop, test and validate a rapid and reliable eDNA field-based sampling methodology that will be used on 500 lakes across Canada to answer questions such as: Which fish species are present in this waterbody? How many of each species are there? What are they eating? How stable is the population within the food web? This will be the largest aquatic eDNA survey ever performed in Canada.

ADVANCEMENT OF KNOWLEDGE

Continuous collaboration among academia, regulatory agencies and industry is necessary to advance the use of eDNA and promote standardization of methods. With that in mind, the upcoming international conference Pathway to Increase Standards and Competency of eDNA Surveys (PISCeS) aims to bring together academics, regulators and industry to engage in discussions and further advance the standards of environmental DNA for biomonitoring. ■

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