Environmental DNA:
A sensitive tool for species detection

Agency Fact Sheet

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Environmental DNA (eDNA) has become an important tool for tracking the potential occurrence of species that may otherwise be hard to find, such as invasive species during early stages of arrival and expansion or after control or eradication efforts. Similarly, eDNA is increasingly being used to locate rare or endangered species that may be elusive or scarce enough that regular sampling or monitoring is unlikely to find them, or that might be harmed by conventional sampling methods such as netting, traps or electrofishing.

What is environmental DNA?

eDNA is genetic material that's been shed by living or dead organisms into the surrounding environment. Living organisms continuously shed living and dead cells into the environment via skin, feces, blood and urine, as well as eggs and sperm at spawning times. All of these contain DNA, which can be used as a species detection or tracking tool, just as DNA is used for forensics cases.

eDNA can be detected by sampling the habitats that species live in, such as soil or water. It's not necessary to directly encounter or catch the species themselves, since detections are focused on the trace DNA left in the environment. If DNA from the species of interest is detected at a particular location, it may indicate that the species is present, even if it has not been detected by other sampling methods. By looking for these DNA traces, eDNA testing acts as a sensitive early-warning system that identifies areas or habitats that may merit a closer look. Comparison studies have shown that eDNA sampling is more sensitive than electrofishing and can be effective at detecting species that are difficult to capture by netting. Repeated spatial or temporal sampling can also be used to assess the effectiveness of species control or eradication efforts, as well as identifying seasonal habitat use or movements by migratory or mobile species.

How is eDNA testing done?

Collecting environmental DNA samples is straightforward: water samples are collected according to a sampling design appropriate to the question(s) being asked, and the DNA present in the water samples is concentrated by filtration or centrifuging so that it can be purified and tested. Field sampling should include stringent precautions against contamination at every step of the process. To test for eDNA from a particular species of interest ("is Species X present?"), scientists develop genetic markers or assays that target species-specific DNA sequences and enable their detection.

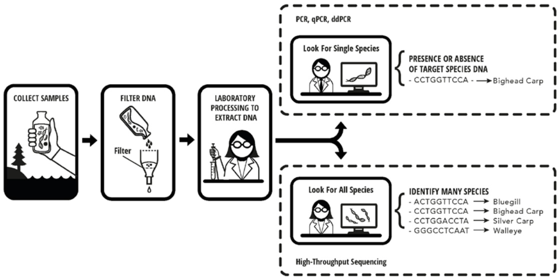

Figure 1: Process for collecting and analyzing eDNA.

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eDNA markers are now available for a growing list of aquatic and terrestrial species. eDNA tests for high-profile species such as invasive carp are increasingly being standardized among labs, so that each lab's results are readily interpretable and repeatable by other labs and agencies. Most eDNA testing is done for particular species of interest, such as invasive carp in the Great Lakes, but new research is looking at different ways to use eDNA to identify the full set of species present within sampled habitats.

Because eDNA testing methods are very sensitive and can pick up very faint traces of DNA in water or soil samples, stringent quality control safeguards and testing are required at all stages to prevent samples from becoming contaminated. This may involve bleaching and thorough rinsing of all field and lab equipment to get rid of any potential contaminant DNA that could be mistaken for a positive detection (species presence). Lab personnel follow sterile procedures with detailed protocols and rely on 'clean room' techniques to prevent carry-over or contamination between samples. Since eDNA testing includes risks of false positive and false negative results similar to medical diagnostic tests, testing labs go to great lengths to minimize these potential errors and adhere to stringent quality standards to make sure that positive or negative eDNA results are real. Controls are included at all stages to guard against false results; for example, “blank (known negative) samples are included at all stages (field sampling, DNA capture and extraction, and assay tests) to detect contamination. Positive controls with known target DNA are included during testing to guard against false negative results.

eDNA detections are influenced by the numbers, size, and biomass of the species of interest in or near a given location, as well as how close (or far away) the species is from where water samples are collected. Species movements (preferred habitats and environmental conditions), water movements (depth, flow, wind, and water currents) are all important considerations for planning sampling efforts. This is particularly important in flowing or wind-swept systems, since a positive detection may be caused by the species being upstream / upwind, rather than where the water sample was collected. Sampling strategies therefore take habitat information (waterbody size, depth, flow if appropriate, and likely/preferred habitat for the species of interest) into consideration to optimize the probability of detecting species if they are present.

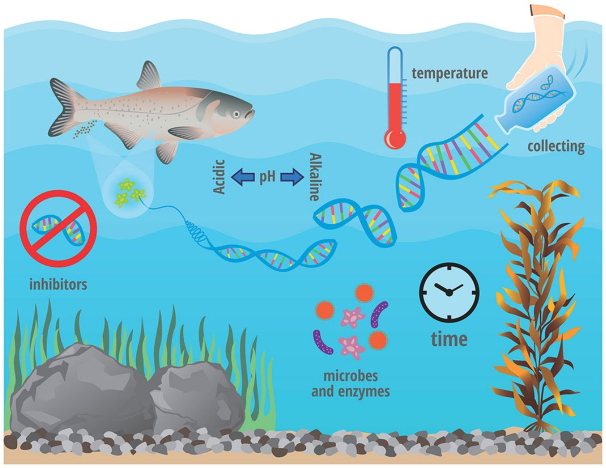

Figure 2: Factors that can influence eDNA degradation in the environment.

Newly-developed eDNA detection assays undergo rigorous testing and validation, ideally by more than one lab. This testing includes determining the specificity of the detection assay to ensure that positive detections are specific to the species of interest, as well as the test's sensitivity or limits of detection. It is important to recognize that positive and negative eDNA results reflect the assay that is used, as well as the potential presence or absence of the species itself. Accurate eDNA results rely on both an appropriate sampling design and a well-validated assay: together, these enable stronger interpretation and greater confidence in the resulting data. As described earlier, results will also be strengthened by repeated spatial or temporal sampling as appropriate.

Assessing the composition of aquatic communities using eDNA metabarcoding has additional considerations. In contrast to targeted species detection, eDNA metabarcoding assays are intended to detect all species within a taxonomic group that are present, and should ideally be non-selective in their amplification efficiency. Not all assays amplify all species equally well, however, so pilot or prior testing should investigate and report on potential species selectivity, preferential detection, or detection bias. Field sampling design and number of replicates per site also have substantial effects on the likelihood of species' DNA being detected, as do species ecology and abundance. Robust sampling designs are therefore essential for achieving study objectives. Results from the same sample can vary among test replicates, so reporting testing conditions and numbers of replicates tested per site and sample are critical to evaluate how representative test results are with respect to the source communities. The reliability of interpretations from eDNA metabarcoding for species identification and abundance estimation is highly dependent on the robustness, accuracy and completeness of reference databases, as the assignment of sequences to putative species is limited to what baseline sequence data are contained within the databases being searched. How reference searches are conducted can also affect interpretation of metabarcoding data, as assignment methods may identify closest fit (greatest similarity) for sequences rather than full species concordance, potentially enabling misleading interpretations. Users should also avoid interpreting detection strength (number of sequences amplified for a given species) as an indicator of species abundance, as other variables may also influence amplification success (e.g., primer bias, proximity to or distance from sources, species activity levels, temperature, flow).

What do positive and negative detections mean?

A positive detection is a good indication that the DNA from the species of interest is likely present at the sampling location. This doesn't necessarily mean that there's an established population, since the eDNA test doesn't distinguish between live and dead sources, or stationary versus mobile ("just passing through") ones. eDNA at a site can also result from water or wind currents, boats, or other sources, although these latter sources are less likely than a local biological source. Repeating sampling efforts at areas with positive detections can help resolve these uncertainties, since the signal from dead or transient sources will disappear within days to weeks. Conversely, spatially clustered positive detections can increase confidence of the source being present. Positive eDNA results are useful for informing conventional field sampling efforts by identifying candidate areas for a closer look or more intensive sampling.[1]

With careful planning, false positive and false negative eDNA results may still occur at very low frequencies but can be guarded against and minimized by careful planning and testing. False positive detections can happen as a result of sample contamination during field sampling or lab testing, but can be minimized and identified by including negative controls at all stages of the process, from sample collection to testing. Negative results may not necessarily mean that a species is not present: detection failures can happen as a result of insufficient sampling effort, inappropriate temporal or spatial sampling design, environmental chemicals ('inhibitors') that interfere with DNA detection, or too little DNA being present. These risks can be minimized by appropriate field sampling design and quality control testing during eDNA analysis. Repeated

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[1] See the "Management Support Tree for the Interpretation of Positive Laboratory Results" for further guidance on the interpretation of positive results in a management context.

sampling can increase both power and confidence of eDNA findings, with the qualifier that target species may move between sampling events.

Like any other tool, eDNA testing has its limits. Over time and distance, the ability to detect species with eDNA declines as DNA breaks down or is dispersed in the environment. A positive eDNA result only indicates that DNA from the species of interest was present when and where the sample was collected. Short turnaround times (rapid processing and testing) are therefore important for eDNA surveillance to be useful. Research is ongoing to quantify the relationship between DNA detection strength and numbers or biomass of source organisms, but this relationship can be complex and location specific, since many other variables are involved (shedding rate, time, distance, flow, dilution, and rate of DNA degradation, among others). Conversely, if a species is so uncommon that very little DNA is shed into the surrounding environment, it may not be possible to detect.

Take home messages

As a way to collect information that is otherwise hard to obtain, eDNA has already demonstrated its value as a species detection tool that augments and complements conventional sampling methods. Adding eDNA to the information toolkit available to management agencies dealing with invasive and endangered species is helping to support timely responses and the sustainable management of aquatic resources. Management applications of eDNA tools should be aware of the technology's limitations as well as its strengths, and it is highly advisable to consult with an eDNA expert on sampling design and effort, assay sensitivity and specificity, and interpreting potential results before initiating monitoring or surveillance efforts. Management agencies and university scientists are actively pursuing research on eDNA methods, sampling design, and analyses, and finding new uses and applications for eDNA data such as documenting seasonal habitat use, rapid testing for species presence in understudied systems, follow-up testing after eradication or control efforts, characterizing species assemblages present at sampling locations, and diet analysis. As eDNA science and its applications continue to grow, this sensitive tool for detecting species occurrences will likely find more applications for species and ecosystem management.