

Environmental DNA (eDNA) Sampling and How it Helps us Understand the Ecosystem ChatGPT – 3/2/2023

“I” = Interviewer, “G” = ChatGPT AI

I: Hello G., let's talk about the relatively new science of environmental DNA (eDNA) as a tool to survey aquatic species.

G: Certainly! Environmental DNA (eDNA) is a rapidly developing field in biology and ecology that allows scientists to detect and monitor species in aquatic environments without the need for direct observation or capture. eDNA refers to the DNA that is released into the environment by living organisms in the form of shed skin cells, feces, urine, or other bodily fluids.

By collecting water samples from aquatic environments and analyzing the eDNA present in those samples, scientists can identify the presence of various species in that environment. This can be a valuable tool for monitoring the distribution and abundance of aquatic species, especially those that are difficult to observe or capture using traditional survey methods.

eDNA has already been used to detect a wide range of aquatic species, including fish, amphibians, crustaceans, and mollusks. It has also been used to monitor the spread of invasive species, track the recovery of endangered species, and assess the impact of human activities on aquatic ecosystems.

However, eDNA technology is still developing, and there are still some limitations to its use. For example, eDNA sampling may not be effective in detecting species that do not shed much DNA or those that have low population densities. In addition, there may be challenges in interpreting eDNA data, as DNA from multiple species can be present in a single sample, and some species may leave more DNA in the environment than others.

Overall, though, eDNA has great potential as a tool for surveying aquatic species and monitoring the health of aquatic ecosystems.

I: Can you tell me something about how eDNA is used in rivers and what limitations or complexities river present?

G: Sure! eDNA can be used in rivers to detect and monitor the presence of aquatic species such as fish, amphibians, and invertebrates. However, there are some particular limitations and complexities associated with using eDNA in river systems.

One of the main challenges in using eDNA in rivers is the potential for the DNA to be dispersed downstream, which can make it difficult to determine the exact location of the source organism. This can be particularly challenging in fast-moving rivers, where the DNA can be quickly carried away from its source.

Another challenge in using eDNA in rivers is the potential for degradation of the DNA. In moving water, DNA can be exposed to a range of environmental stressors, such as UV radiation and microbial activity, which can break down the DNA and reduce the amount of detectable DNA in the water sample.

Additionally, rivers can present challenges in terms of the spatial and temporal heterogeneity of the environment. Different river sections or different times of year can have different environmental conditions that can affect the detectability of eDNA. For example, water temperature, flow rate, and sedimentation can all impact the distribution and persistence of eDNA in a river.

To address some of these challenges, scientists may use multiple sampling locations and time points to increase the chances of detecting eDNA. They may also use different analytical methods to maximize the amount of DNA that can be extracted and amplified from the water samples.

Despite these challenges, eDNA remains a promising tool for monitoring aquatic species in rivers and other freshwater ecosystems. By combining eDNA with other survey methods, such as traditional sampling and acoustic monitoring, scientists can gain a more comprehensive understanding of the health and diversity of these ecosystems.

I: What about false positives or false negatives? In other words, how reliable are the results of typical riverine eDNA surveys?

G: The reliability of eDNA survey results can be influenced by a number of factors, including the sensitivity and specificity of the eDNA assay used, the amount of DNA shed by the target species, and the quality of the environmental sample collected.

False positives, where eDNA is detected for a species that is not actually present, can occur if DNA from a related species or a species that shares genetic similarity with the target species is present in the sample. To address this issue, scientists can use more specific assays that target genetic markers unique to the target species, or they can use multiple assays to increase the likelihood of accurately detecting the target species.

False negatives, where eDNA is not detected for a species that is present, can occur if the DNA has degraded or has been dispersed too far from its source to be detected. Factors that can influence the reliability of eDNA surveys include the type of water sample collected, the volume of water sampled, and the timing and frequency of sampling.

Overall, eDNA surveys can be highly reliable when conducted using rigorous sampling and laboratory protocols, and when used in combination with traditional survey methods to confirm the presence or absence of a species. However, the reliability of eDNA surveys can vary depending on the specific system being studied and the factors influencing the detection of eDNA in that system. It is important for scientists to carefully consider these factors when interpreting eDNA survey results.

I: I assume that rivers produce more false negatives because a particular species may not be present in the reach of river being sampled, while they may be present just downstream or too far upstream to be detected by eDNA.

G: Yes, that's a valid assumption. The presence or absence of a species in a river can be influenced by several factors, including habitat suitability, migration patterns, and barriers to movement, such as dams or other human-made structures.

If a species is present upstream or downstream of the sampled reach of a river, but not within the reach being sampled, then eDNA surveys may produce false negatives. Similarly, if a species is present in low densities, or if its DNA degrades quickly in the river environment, eDNA surveys may also produce false negatives.

To minimize the risk of false negatives, scientists may use multiple sampling locations along the length of the river or at different depths in the water column. They may also use multiple assays or different primers to target different genetic markers to increase the sensitivity of the survey.

In addition, scientists may also use eDNA surveys in combination with other survey methods, such as electrofishing or visual surveys, to confirm the presence or absence of a species in a particular reach of the river.

Overall, while rivers can present challenges for eDNA surveys, careful study design and analysis can help to minimize the risk of false negatives and improve the reliability of the survey results.

I: Let's talk about cost. eDNA can be an expensive tool to use, but might other sampling methods actually cost more than eDNA?

G: The cost of eDNA surveys can vary depending on a number of factors, such as the number of samples collected, the complexity of the assays used, and the cost of laboratory analysis. While eDNA surveys can be expensive, they can also be a cost-effective alternative to other survey methods, especially in cases where traditional sampling methods would be impractical or time-consuming.

For example, traditional survey methods such as electrofishing or visual surveys can be time-consuming, require specialized equipment, and can be limited by the habitat and environmental conditions of the river. In contrast, eDNA surveys can be conducted using a relatively simple water sample collection kit, and can cover a larger area of the river in a shorter amount of time.

In some cases, eDNA surveys may also be more cost-effective than traditional survey methods when considering the cost per individual surveyed. This is because eDNA surveys can detect the presence of multiple species in a single water sample, whereas traditional survey methods may only detect a limited number of species.

Overall, while eDNA surveys can be expensive, they can also be a cost-effective alternative to traditional survey methods, especially in cases where the habitat and environmental conditions of the river make it difficult to conduct traditional surveys. The cost-effectiveness of eDNA surveys should

be evaluated on a case-by-case basis, considering the specific objectives of the study and the available resources.

I: What other advantages are there to using eDNA sampling?

G: There are several advantages to using eDNA sampling for aquatic species surveys:

1. **Non-invasive:** eDNA sampling is a non-invasive method, which means that it does not require physical contact with the target species. This can be particularly beneficial for rare or endangered species, where minimizing disturbance is important.
2. **Increased sensitivity:** eDNA sampling can detect the presence of species even when they are present at low densities or are difficult to detect using traditional survey methods.
3. **Comprehensive:** eDNA sampling can detect the presence of multiple species in a single water sample, making it a more efficient method than traditional survey methods.
4. **Rapid results:** eDNA sampling can provide results quickly, often within days of sample collection, compared to traditional survey methods, which can take weeks or months.
5. **Cost-effective:** as I mentioned earlier, eDNA sampling can be a cost-effective alternative to traditional survey methods, especially when considering the cost per individual surveyed.
6. **Potential for new discoveries:** eDNA sampling can provide information on the presence of cryptic or elusive species, which may not have been detected using traditional survey methods.

Overall, eDNA sampling has several advantages over traditional survey methods, including its non-invasive nature, increased sensitivity, efficiency, and cost-effectiveness. These benefits make it an increasingly popular tool for aquatic species surveys.

I: OK, that's very informative. Since this chat will be used to educate people about eDNA, can you help us understand what the lab does when it gets the eDNA samples to identify the target species?

Sure, I'd be happy to explain the process.

G: After eDNA samples are collected from the field, they are transported to a laboratory for analysis. The following is a general overview of the steps that a lab might take to analyze eDNA samples:

1. **Extraction:** The first step in eDNA analysis is to extract DNA from the water sample. This is typically done using a filtration or centrifugation method, where the DNA is separated from other material in the sample, such as sediment or organic matter.
2. **Amplification:** Once the DNA is extracted, it is amplified using polymerase chain reaction (PCR). PCR is a technique that produces multiple copies of a specific DNA sequence, making it easier to detect and identify the target species.
3. **Sequencing:** After amplification, the DNA is sequenced using high-throughput sequencing technology. This generates millions of short DNA sequences, which are then assembled into longer DNA fragments.
4. **Analysis:** The assembled DNA sequences are compared to DNA reference databases, such as GenBank or BOLD, to identify the species present in the water sample. This is typically done

using bioinformatics tools that compare the DNA sequences to known sequences in the database.

5. **Verification:** To verify the accuracy of the results, labs may use additional methods, such as cloning or digital PCR, to confirm the presence of the target species and rule out the possibility of false positives.

Once the analysis is complete, the lab will provide a report detailing the species detected in the water sample. This report may also include information on the concentration of DNA, which can be used to estimate the abundance of the target species in the water sample.

It's important to note that the specific methods used for eDNA analysis may vary depending on the lab and the target species. Additionally, the analysis may require specialized expertise and equipment, which can impact the cost and turnaround time for the analysis.

I: That's a nice explanation. Finally, one limitation to all eDNA studies is the availability of DNA "markers" for the species we want to survey. What can you tell us about this problem, and how we might minimize it?

G: You're right, one limitation of eDNA studies is the availability of DNA markers for the target species. DNA markers are specific regions of DNA that can be used to identify a particular species. The availability of these markers can vary depending on the species and may not exist for some species, particularly those that have not been well-studied.

To minimize the impact of this limitation, researchers can use a combination of approaches:

1. **Reference databases:** As more species are sequenced and their DNA is added to reference databases such as GenBank or BOLD, the chances of finding a match for an unknown DNA sequence increase. Researchers can use these databases to search for DNA sequences that match the target species.
2. **Universal markers:** Researchers can use universal markers, which are DNA sequences that are conserved across multiple species, to detect the presence of a broad range of taxa in a water sample. This approach can be particularly useful for surveys targeting diverse communities or species that do not have specific DNA markers.
3. **Development of new markers:** Researchers can develop new DNA markers specific to the target species through a process called marker development. This involves sequencing the genome of the target species to identify unique regions of DNA that can be used as markers.
4. **Collaboration:** Collaboration between researchers can help to overcome the limitations of eDNA surveys by sharing resources and expertise. For example, researchers can collaborate to develop new DNA markers or to share information on the DNA markers that have been successfully used in previous studies.

In summary, the availability of DNA markers can be a limitation of eDNA studies, but researchers can use a combination of approaches to overcome this limitation, including reference databases, universal markers, development of new markers, and collaboration with other researchers.

I: Researchers might collaborate by sharing the cost of marker development, or by conducting assays that benefit several projects.

G: Absolutely, collaboration can take many forms and sharing the cost of marker development or conducting assays that benefit multiple projects are excellent examples. Collaborative efforts can not only help to overcome the limitations of eDNA studies but can also promote the sharing of data, resources, and expertise, ultimately leading to better scientific outcomes. By working together, researchers can advance the field of eDNA and contribute to a better understanding of aquatic ecosystems and the species that inhabit them.

I: Thanks very much for a great discussion, G. Let me ask one more question, and could you please be brief? The question is, what can we expect the cost of eDNA sampling to do as time goes on?

G: As technology continues to improve and become more accessible, we can expect the cost of eDNA sampling to decrease. For example, the cost of DNA sequencing has been steadily decreasing over the past decade, and this trend is expected to continue. Additionally, the development of more efficient and cost-effective DNA extraction and processing methods could also contribute to a decrease in the cost of eDNA sampling.

Furthermore, as eDNA becomes more widely accepted as a standard tool for aquatic biodiversity assessment, there may be an increase in funding for eDNA research and applications. This could lead to increased economies of scale and lower costs for eDNA sampling.

Overall, while eDNA sampling can currently be an expensive tool, we can expect the cost to decrease over time as technology and funding continue to advance.

I: Thanks, G.

G: You're welcome! If you have any more questions, feel free to ask.