eDNA Topic

eDNA Sampling: Collect Representative eDNA Samples

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Introduction

Collecting representative environmental samples is essential to ensuring the accuracy and reliability of an environmental DNA (eDNA) study. General considerations for eDNA sampling, regardless of the technique or material, include strict measures to prevent cross-contamination and the use of negative controls. Cross-contamination between samples can lead to misleading results, so it is important to use sterilized equipment, wear protective gear when handling samples, and sterilize collection tools between samples. However, residual bleach used for sterilization can degrade DNA in subsequent samples, so collection tools should be thoroughly rinsed and dried. Alternatively, single-use collection systems can be used to avoid the need for bleach entirely.

Additionally, negative controls—samples processed without any environmental material—should be incorporated into many study designs. These controls help detect and measure background contamination, ensuring that any detected DNA comes from the target environment, not external sources or sampling biases. These practices collectively improve the credibility and accuracy of eDNA studies.

Further considerations are necessary when sampling from different media. The following sections outline best practices for collecting eDNA from aquatic environments, air, soil, sediment, and general surfaces.

Aquatic eDNA Collection

When collecting eDNA from aquatic environments, both active and passive filtration methods can be employed. Each method is suitable for different water conditions and research purposes.

Active Filtration

Active filtration involves the intentional collection of water to capture eDNA through filtration. There are two main approaches: direct filtration from the water body and filtration of water from containers that have transported the water from the collection site.

Direct filtration

Direct filtration happens in the field, and water is draw directly from the source using a portable filtration system. This method minimizes the risk of DNA degradation, which can occur if samples are stored before processing. It is also efficient for processing large volumes of water on-site. Syringes are commonly used to collect water for active filtration via positive pressure, while portable vacuum or peristaltic pumps can be used for on-site filtration via negative pressure. However, direct filtration can be relatively slow, as samples are typically collected and processed in sequence, meaning that no new samples are collected until the previous one has been filtered.

When water is collected into containers, researchers can either filter the samples on-site or transport them to a controlled environment—typically while chilled—where filtration can be conducted under regulated conditions using either positive or negative pressure systems. Studies show no significant difference in results between filtration using positive or negative pressure, and the magnitude of the applied pressure does not seem to affect the outcomes.

The filters used in active eDNA sampling vary widely in design and materials, which can significantly influence their performance. Factors such as surface area, pore size, membrane type, and filter configuration all impact filtration efficiency and DNA capture.

- **Surface Area:** Larger surface areas allow more water to be processed quickly. However, this may not always be ideal, depending on the study. Large filters may be less efficient for DNA extraction because they often need to be divided for downstream processing, complicating the extraction process.
- **Pore Size:** Smaller pore sizes (<1 µm) are more effective at capturing smaller particles, but larger-pore filters can still capture some smaller particles through various mechanisms. Filters are typically rated by the size of the largest pore, not the average or smallest size, which means a filter with a larger-rated pore size might still capture a wide range of particles.
- **Membrane Type:** The choice of membrane—whether cellulose, glass fiber, or polymer-based—can affect DNA capture efficiency and the ease of extraction. However, studies show minimal differences in results across membrane types, so the choice often depends on other factors like cost or availability.
- **Filter Configuration:** The configuration of the filter is also important. Capsule filters generally have more surface area than disc filters, allowing for the filtration of more water. However, they also require more preservative fluid, which can complicate downstream processing. Additionally, large surface area filters may not always be efficiently extracted since only a portion of the filter is processed. This reduces the advantage of filtering larger volumes of water.
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Passive Filtration

Passive filtration collects eDNA without the need to actively push or pull water through a filter. In this method, material is placed in the water, and DNA is passively captured onto the filter, often aided by natural water currents. Research indicates that passive filtration can be as effective as active filtration, though it generally takes longer to capture an equivalent amount of DNA. The material used for passive filtration can vary, from simple disc filters to cellulose sponges, each with its own advantages depending on the water conditions and research goals.

Airborne eDNA Collection

Similar to water sampling, collecting airborne environmental DNA (eDNA) involves pulling air through a filter to capture genetic material. The effectiveness of this method depends on several factors:

- **Fan Power:** A powerful fan increases the volume of air processed, improving eDNA capture, especially in large or open areas. For example, a typical 10-cm fan can filter 200 m^3 of air per hour, even with a filter attached. Over a few hours, hundreds of cubic meters of air can be filtered, often yielding ecologically relevant amounts of airborne eDNA.
- **Filter Surface Area and Type:** The size of the filter determines how much air can be sampled. Larger filters increase eDNA collection by allowing more air to pass through. However, large filters may not always be fully extracted for analysis. HEPA filters are not necessary for air eDNA collection; a standard MERV 13 filter is effective at trapping fine particles that contain eDNA.
- **Duration of Filtration:** The duration of filtration varies based on the air volume to be sampled and the fan power. However, ecologically relevant amounts of airborne eDNA can typically be collected in as little as one hour of filtration.

Soil and Sediment eDNA Collection

Collecting soil or sediment for environmental DNA (eDNA) analysis involves careful consideration of several factors to ensure the accuracy and consistency of the data:

- **Amount of Material:** Typically, a standardized quantity of soil or sediment—often a few grams—is collected to maintain consistency across samples. This ensures that each sample provides comparable data during analysis.
- **Sampling Location:** The selection of sampling locations should strategically represent the study area to capture a comprehensive genetic snapshot. Careful planning of sample sites can help ensure that the biodiversity of the area is accurately reflected.

- **Pre-Treatments:** Pre-treatment steps, such as sieving, are essential for removing larger debris and homogenizing the sample. These steps help to reduce variability and improve the efficiency of eDNA extraction and analysis, leading to more reliable data.

General Surface eDNA Collection

Collecting surface eDNA typically involves using a simple cotton swab, which is run along a surface to capture eDNA. For dry surfaces, the swab is often moistened with DNA-free water to enhance DNA collection. After swabbing, the tip is usually clipped to fit into a smaller vial for storage. In some cases, researchers have used paint rollers to collect eDNA from larger surfaces, such as cave ceilings. The eDNA is then rinsed from the roller and collected onto a filter for further analysis.

Example Protocol: Coastal sampling for aquatic biodiversity

1. Equipment Preparation

Gather sterile 60 mL syringes, 25-mm 1µm luer lock syringe filters, and labeled bags for storing the filters. Ensure all equipment remains sterile to prevent contamination. Wear gloves and use sterile techniques throughout the entire sampling process to maintain the integrity of the eDNA samples.

2. Sample Collection

Approach the water body carefully to minimize disturbance, as this could affect the distribution of eDNA in the water. Submerge the tip of the syringe into the water, positioned upcurrent of the collector, ideally at a depth of 10 cm below the surface to avoid collecting excessive surface debris. Pull the plunger to draw water into the syringe, making sure to fill it completely to ensure you have enough volume for effective filtration.

3. Filtration

Attach the syringe filter securely to the tip of the syringe to prevent any leaks. Push the plunger to force water through the filter and into the collection vial, applying steady pressure to maintain a consistent flow. If many samples are being processed, using a caulk gun or modified bar clamp can help ease the task. Repeat the process as needed to collect the required volume of filtered water or until the filter becomes clogged.

Frequently Asked Questions

1. Do I need to collect 1 liter of water for a sample?

   While collecting 1 liter of water is common in many eDNA studies, the volume needed can vary based on the required detection sensitivity, the amount of particulates in the water, and the anticipated abundance of the target species. For species that are less abundant or when higher sensitivity is required, collecting larger volumes of water may improve detection. If uncertain, it's best to continue filtering water until the filter becomes clogged, ensuring maximum eDNA capture.

2. What is the minimum amount of water I need to collect?

   The minimum volume of water required typically depends on the detection method and the concentration of eDNA in the water. Highly sensitive detection methods may allow for the use of volumes as small as 15-50 mL, but most studies opt for at least 500 mL to 1 liter to improve the likelihood of detection. Filtering until the filter becomes clogged and collecting replicate samples is always a reliable strategy to maximize eDNA capture and ensure robust results.

3. If I use a larger filter, is detection better?

   Using a larger filter or one with a higher capacity can enhance detection by allowing you to process more water and capture more eDNA. However, the choice of filter should also take into account the particulate content of the water, as filters can clog more quickly in turbid or debris-filled environments. Balancing filter size and capacity with the likelihood of clogging is essential for optimizing eDNA collection.

4. Should I collect water from the surface, or at depth?

   The decision to collect water from the surface or at depth depends on the habitat preferences of the target species and the degree of water stratification. If the target species is known to inhabit deeper areas, or if the water body is stratified, sampling at depth may provide more relevant eDNA data. Tailoring the sampling depth to the species' behavior and the water's physical characteristics improves the likelihood of detecting the target species.

5. What types of species can I detect from air?

   Airborne eDNA can be used to detect a variety of species, including plants, fungi, birds, mammals, and insects. The eDNA in the air can originate from sources such as pollen,

spores, skin cells, feathers, and hair, making it a valuable tool for monitoring biodiversity across multiple taxa.

6. Can DNA found in water/soil/air be from species that are no longer present at my site?

   Yes, DNA found in environmental samples such as water, soil, or air may come from species that were recently present but are no longer at the site. eDNA can be transported by water flow, wind, or animal movement, and can persist in the environment for varying durations depending on the conditions. This can sometimes result in the detection of "ghost" DNA from species no longer actively present in the area. To address this, some researchers target environmental RNA (eRNA) instead of DNA, as RNA degrades more quickly in the environment, providing a more current snapshot of species presence.

Further reading

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