NEMO TOOLS

Next-generation monitoring & mapping tools to assess marine ecosystems & biodiversity

Deliverable D2.1

Innovative Sampling Pipelines

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Executive Summary

This Deliverable 2.1 – Innovative Sampling Pipelines outlines the most frequently used sampling methods of environmental DNA (eDNA) from marine systems. This document summarizes existing knowledge on eDNA sampling techniques, identifies advantages and bottlenecks for each approach, and establishes a framework for cost-effective sampling strategies to implement during the NEMO-Tools project.

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1. Introduction

The use of environmental DNA (eDNA) sequencing has become a very powerful tool that is commonly used to investigate ecological questions in various ecosystems. It has revolutionized the monitoring of aquatic biodiversity, offering critical insights from population dynamics to ecosystem health (Takahashi et al., 2023; Aglieri et al., 2021; Pochon et al., 2017). While substantial efforts have been directed towards optimizing laboratory techniques to address the complexities of biodiversity monitoring through eDNA, the evaluation of capture methods has received comparatively less attention. Despite the increasing need for rigorous, cost-effective, and standardized protocols for sample collection across diverse marine habitats (Patin & Goodwin, 2023), a consensus on best practices for sample collection and processing is yet to be determined. For example, a significant challenge to the global implementation of eDNA monitoring is the necessity to collect substantial volumes of water to enhance detection probabilities (Govindarajan et al., 2022).

Various methods exist for capturing eDNA from aquatic environments, resulting in a lack of uniformity across studies. Techniques such as centrifugation, precipitation using sodium acetate and ethanol, and filtration have been employed in different aquatic settings (Dickie et al., 2018). Centrifugation is one of the earlier methods used for capturing eDNA. This technique involves spinning water samples at high speeds to concentrate eDNA from the water column. The high-speed rotation forces the heavier particles, including eDNA, to settle at the bottom of the centrifuge tube, allowing for their subsequent extraction and analysis. Another method for eDNA capture involves chemical precipitation using sodium acetate and ethanol. This technique relies on the principle that eDNA can be precipitated out of solution by altering the chemical environment. Sodium acetate is added to the water sample to neutralize charges on the DNA molecules, and ethanol is then used to precipitate the DNA. Finally, filtration is perhaps the most common and versatile method for capturing eDNA from aquatic environments (Jerde et al., 2011). This technique involves passing water through a filter membrane that traps eDNA particles. The type of filter, pore size, and material can significantly impact the efficiency of eDNA capture.

This report aims to present innovative eDNA sampling pipelines that address the current gaps and challenges in the field, proposing standardized, efficient, and scalable methods for capturing eDNA in various marine habitats. By focusing on the capture phase of eDNA, this report seeks to discuss the best practices that enhance the reliability and comparability of eDNA-based marine biodiversity assessments globally.

2. Innovative eDNA Sampling Techniques in Marine Systems

2.1. Filtering

Filtering is the most commonly employed method for capturing eDNA from water samples due to its efficiency and ability to process large volumes of water. This technique involves passing water through a porous membrane to concentrate eDNA. Various filtering methods and filter types have been developed to optimize eDNA yield and minimize sample contamination. This step is extremely important because the way and volume of water to be filtered are related to eDNA detection rates and can vary between systems (Capo et al., 2020).

- Pump Filtration

Pump filtration is a widely used technique where water is pumped through a filter unit that can be equipped with different types of membranes, such as cellulose nitrate (CN), mixed cellulose ester (MCE), or glass fiber filters. This method allows for the processing of large water volumes, which is critical for detecting low concentrations of eDNA (Capo et al., 2020). It is a high throughput and efficient strategy in capturing eDNA from large water volumes (e.g., 10 L; Wittwer et al., 2018). It is particularly effective in open ocean environments where large-scale sampling is required. Challenges include filter clogging especially in turbid waters, and that the equipment requires power sources (e.g., water and/or electric pumps) which might limit its use in remote locations.

Different filter materials and pore sizes can significantly impact the efficiency of eDNA capture. Commonly used filters include CN, MCE, glass fiber, and various plastic polymers. CN and MCE filters are known for their high eDNA yield and consistent performance (Hinlo et al., 2017; Djurhuus et al., 2017). However, glass fiber and plastic polymer filters show variable performance depending on water quality and extraction methods. Regarding filter pore sizes, smaller pore sizes (0.2 µm) generally capture higher amounts of eDNA but are more prone to clogging, whereas larger pore sizes (0.45–1.5 µm) may result in lower eDNA yields, especially in low-turbidity waters, and may capture less microbial diversity at the lowest size classes (at the picoplankton level).

- Syringe Filters

Syringe filtration involves manually pushing water through a small-diameter filter (usually 0.45 µm or 0.2 µm pore size) attached to a syringe. This method is suitable for processing smaller volumes of water (1-2 L) and is particularly useful in field conditions where portability is essential. The advantages of the method are portability and ease of use in remote or resource-limited settings, as well as requirements of minimal equipment and no external power source. However, it is limited to smaller volumes of water, which could potentially reduce eDNA yield. Manual operation can be labor-intensive and time-consuming.

- Inline Filters

Inline filters are pre-assembled filter units designed for the collection and preservation of eDNA directly in the field. Water samples are drawn through the inline filter unit using a pump or syringe, and the filter is subsequently sealed for storage and transport. This approach minimizes the risk of contamination and simplifies sample handling. The sealed units preserve eDNA integrity until laboratory processing. On the other hand, these types of filters are of higher cost compared to other filters, and limit to smaller water volumes filtered, compared to pump filtration.

2.2. Passive Samplers

Passive samplers represent an innovative approach to eDNA collection by allowing eDNA to accumulate on a substrate over time, eliminating the need for active filtration.

- Artificial Sponges

Artificial sponges mimic natural filter feeders and have been shown to be effective passive samplers for eDNA. Sponges are deployed in the water for a specific duration, allowing eDNA to accumulate on their surfaces. It is a simple approach involving deployment and retrieval, with reduced labor and equipment costs, thus increasing the ability to capture eDNA over extended periods. However, the amount of eDNA captured can vary depending on water flow and deployment duration. In addition, effective deployment in dynamic marine environments requires careful consideration of hydrodynamic conditions.

- Natural Sponges

Marine sponges filter thousands of liters of water in a single day (Gökalp et al., 2020), therefore they can exceed the volume that could be collected with artificial devices for aquatic eDNA metabarcoding (Harper et al., 2023). DNA particles are trapped and concentrated in sponge tissue until digestion and/or excretion, therefore a sponge biopsy could provide DNA for a subsequent metabarcoding analysis (Mariani et al., 2019). Sponges are commonly found in various aquatic habitats, whilst biopsies have minimal environmental impact and are not destructive when conducted carefully as sponges can regenerate quickly (Harper et al., 2023).

- Settlement Plate Arrays

Settlement plates provide surfaces for eDNA to adhere to and can be deployed in various marine habitats. These plates are made of materials like plastic or clay and are submerged in the water, allowing eDNA to settle on them over time. They offer long-term monitoring capacity and are suitable for various marine environments. They can be easily deployed and retrieved with minimal disturbance to the habitat. The variability in eDNA capture efficiency depends on water movement and biofouling, while plates may need regular maintenance and cleaning.

- Underwater automated samplers

The logistical challenges of eDNA collection can be reduced by using newly designed automated eDNA samplers that can operate without the need for a researcher on-site (Herfort et al., 2016). High-tech robotics can be used to sample volumes of water for aquatic eDNA. Automated samplers overcome the difficulties of eDNA sampling in the field, allowing a fine-scale spatial and temporal sampling of eDNA in various aquatic environments (Formel et al., 2021). They also reduce the need for highly trained personnel to carry out deployment and recovery. Additionally, some automated eDNA samplers can be mounted on different underwater machinery, such as autonomous underwater vehicles, allowing for sampling over highly resolved spatial scales in marine systems. Peristaltic pumps are mainly used to sample water that is then filtered on different types of filters according to the project's requirements (see above for filter types). Despite the advantages of their application, their high build costs (from 6,000 to 100,000 Euros) are a financial obstacle. Attempts to create low-cost samplers are currently being made (Formel et al., 2021).

- Custom-made plastic probes

Up to date, one probe design (metaprobe) is used to our knowledge for biodiversity mapping in aquatic ecosystems (Maiello et al., 2022). This is a bespoke 3D-printed hollow perforated plastic spherical probe of extremely low-cost that works as a container that contains rolls of gauze capable to capture DNA from the surrounding environment during sampling. The probe can be placed inside the trawl net, attached to other underwater vessels and divers and can absorb traces of DNA that are found in the water column. This method is low-cost, easy to use, with no requirements for prior expertise knowledge on handling, and it produces similar results to previous methods (see above).

2.3. Continuous Low-Level Aquatic Monitoring (C.L.A.M.) System

The C.L.A.M. system provides time-integrated sampling by continuously filtering water over extended periods. The system consists of a filter unit that is towed or allowed to drift, capturing eDNA continuously over time. The advantages of the approach include the ability to capture spatial and temporal variation in eDNA, providing a comprehensive picture of biodiversity, and there is a reduced need for frequent sample collection. However, the initial setup costs, the possible equipment loss or damage in harsh marine environments, and the requirement for careful calibration and maintenance are potential limitations of the approach.

2.4. Modified Plankton Samplers

Plankton samplers adapted for eDNA collection can filter large volumes of water while minimizing cross-contamination. The water is drawn through the sampler using a pump, with the eDNA captured on filters within the device. This is a high-volume sampling suitable for detecting low concentrations of eDNA, and it is designed to reduce contamination between samples. The set up comprises of complex deployment of equipment which is limited in mobility due to the need for controlled towing speeds.

3. Conclusions

Innovative eDNA sampling techniques offer significant advancements in the ability to monitor marine biodiversity. Filtering methods, including pump filtration, syringe filters, inline filters, and various filter materials and pore sizes, provide robust options for capturing eDNA across different water conditions. Passive samplers, such as artificial sponges, custom made probes, settlement plate arrays, the C.L.A.M. system, and modified plankton samplers, present promising alternatives that simplify field operations and reduce contamination risks. Each method has its unique advantages and challenges, and the choice of technique depends on specific research objectives, environmental conditions, and logistical constraints. By optimizing and standardizing these innovative eDNA sampling pipelines, researchers can enhance the accuracy, efficiency, and scalability of marine biodiversity assessments, contributing to more effective conservation and management efforts in marine ecosystems

Among these eDNA sampling techniques, vacuum pump filtering, inline filtering, and passive samplers stand out for their complementary strengths in different research contexts. Vacuum pump filtering offers unmatched efficiency for processing large water volumes, crucial for capturing eDNA in diverse and expansive marine environments. Its ability to handle substantial sample sizes enhances detection probabilities, making it ideal for comprehensive biodiversity assessments. Inline filtering, on the other hand, provides a streamlined and contamination-resistant option for fieldwork, preserving eDNA integrity from collection to laboratory analysis. Its portability and ease of use make it a practical choice for studies conducted in remote or resource-limited settings. Lastly, passive sponge samplers introduce a low-cost, low-labor alternative that captures eDNA over time, offering valuable temporal resolution and reducing the need for extensive field equipment. Together, these three methods provide a versatile toolkit that can be tailored to various marine research needs and will be applied in the context of the NEMO-Tools project.

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