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A6.2 Deliverable - Assessment on the use of environmental DNA (eDNA) to supplement traditional monitoring methods

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A6.2 Assessment (and pilot strategy) on the use of environmental DNA (eDNA) to supplement traditional monitoring methods

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1. Introduction

Background

Marine biodiversity is declining rapidly worldwide, highlighting the need for conservation and protection of marine environments (IPBES 2019). This trend is also evident in the Baltic Sea, which is subjected to a wide range of anthropogenic pressures from surrounding countries (Andersen et al. 2015; HELCOM 2018). To address these challenges, the Baltic Marine Environment Protection Commission – Helsinki Commission (HELCOM) was established to promote collaboration among Baltic Sea nations to safeguard and restore the sea's environmental state. As part of its efforts, HELCOM has adopted a Baltic Sea Action Plan (BSAP 2021), a strategic framework aimed at improving the environmental status of the sea through targeted actions. However, the effectiveness of protection strategies, land-use management, and policymaking depends on continuous and systematic monitoring of habitats, species, and communities. Regular assessments are crucial to evaluating the impact of implemented measures and ensuring adaptive management approaches that respond to ongoing environmental changes.

Traditional monitoring methods are based on morphological identification of species, with sampling techniques including visual observations through wading, diving, or underwater cameras, as well as more invasive methods such as trawling, trapping, or grab sampling. These approaches present several challenges and limitations. They often require specialized taxonomic expertise, are time-consuming and costly (Noble-James et al. 2023), and, depending on the method, can also be invasive (Trenkel et al. 2019). Additionally, water conditions and limited visibility can hamper monitoring efforts, as turbidity can limit the

amount of usable imagery data (Noble-James et al. 2023). Therefore, new more cost-beneficial and flexible monitoring methods are needed in the future.

Utilizing environmental DNA (eDNA) is a relatively novel technique for assessing biodiversity in marine environments. eDNA refers to genetic material collected from environmental samples, originating either from DNA shed by organisms or from DNA associated with micro-organisms and their fragments, such as biofilm or plankton samples. It is thus a compilation of different sized particles from multiple origins such as cells or feces.

In aquatic environments, this approach involves collecting samples from water or sediments and extracting DNA from these. The extracted DNA is then amplified and analyzed to identify species presence or community composition by comparing DNA sequences to a reference sequence database of confirmed specimens (Ruppert et al. 2019). Primers are short DNA sequences that bind to specific regions of the genome and are essential for DNA replication, as they allow DNA from targeted groups of organisms to be selectively amplified.

Compared to traditional methods, eDNA sampling provides broader spatial coverage. However, spatial and temporal patterns may be harder to interpret as the DNA could have drifted from the original source. Additionally, the concentration of DNA in the eDNA sample is usually low, and thus the samples are prone to contamination, false negatives, and false positives (Burian et al. 2021; Buxton et al. 2021).

Bulk DNA metabarcoding differs from eDNA sampling, as it is done from samples collected via traditional sampling (e.g., biological monitoring samples such as kicknet samples), samples are homogenized and DNA of organisms in the sample is extracted and then the species are identified in a way similar to eDNA samples (Blackman et al. 2019). It has also proven to be a useful tool in bioassessment (Aylagas et al. 2014; Borja et al. 2024). The Bulk DNA method provides a more accurate view of the species present in a specific location compared to eDNA sampling but does not provide a regional overview of the species present in a certain area. Thus, its spatial coverage is more aligned with traditional sampling.

In Finland, molecular monitoring methods are mostly still in the pilot phase, except for certain game species. On a national level, the adoption of these methods for monitoring biodiversity, invasive species, and threatened species remains limited (Norros et al. 2022).

Objectives

This report provides an overview of the application of eDNA-based monitoring methods in (shallow photic) marine environments mainly focusing on the macrophyte communities and associated invertebrates. It explores the opportunities of eDNA based methods in complementing traditional survey techniques while considering the costs, challenges, and barriers associated with their use in future monitoring efforts.

2. Potential of eDNA in marine monitoring

eDNA based monitoring methods offer several advantages to marine monitoring. Collecting water samples is faster and requires less field time than traditional methods. Additionally, sampling does not require taxonomic expertise, and it is possible to identify fragmented specimens. This allows for more cost-effective sampling. Collecting eDNA from water and sediment is also non-invasive, as there is no harm caused to marine species or ecosystems.

A meta-analysis of several studies comparing the accuracy and detectability of eDNA and traditional survey methods found that eDNA detected more species than traditional approaches (Fediajevaite et al. 2021). eDNA could be a solution in areas where turbidity and low visibility are known limiting factors for traditional sampling. Additionally, eDNA methods are better at detecting rare (Schmelzle and Kinziger 2016), cryptic (Allen et al. 2023), and early-stage organisms that may lack morphological features crucial for traditional species identification.

Research on how well eDNA performs in detecting rare or endangered aquatic plants in marine environments is still lacking. However, the few studies available from freshwater systems show promising results, demonstrating that eDNA can successfully detect rare aquatic plants that are difficult to find by using traditional methods (e.g., Tsukamoto et al. 2021). Much of the existing literature on the usage of eDNA in aquatic plant monitoring focuses mainly on the detection of invasive species (Prieto et al. 2023), as these methods offer important benefits for early detection (Flitcroft et al. 2025). With eDNA, it is possible to detect invasive species more rapidly and at lower population densities, including at early life stages. This can significantly aid management efforts, as removal is more achievable before the species has established well sustaining populations (Castro et al. 2021; Flitcroft et al. 2025). The spatial coverage of eDNA sampling is generally larger than that of traditional

monitoring, as DNA can disperse through the water column. This characteristic can be both an advantage and a limitation, as the broader coverage increases the likelihood of detecting rare species or those present at low abundances, but it also makes it more difficult to determine the precise location of the organisms.

Beyond plant monitoring, eDNA could also help improve our understanding of mobile species in marine environments. Efficient monitoring of mobile species is one of the five main gaps in the current monitoring of the Baltic Sea (Emmerson et al. 2019; Kahlert et al. 2020). These mobile species may modify their behavior during traditional sampling and thus may be underrepresented in the data. For example, traditional fish monitoring methods, such as electrofishing and netting, are time-consuming, require specialized skills, and are inefficient for community assessment, while also causing stress and harm to the fish (Snyder 2003). With eDNA methods, it is possible to monitor these species in a more efficient way and thus offer more accurate estimates of overall biodiversity and state of the environment. eDNA could also help to monitor infaunal species, which may easily get undetected by divers (Staehr et al. 2022).

The cost of eDNA sampling and following analyses varies widely, as the field is still developing, and new sampling methods and service providers continue to emerge. There is a rough estimate that the cost of metabarcoding and associated laboratory work could decrease to around € 20 per sample within the next five years, which is considerably less than the cost of processing traditional samples (prof. Florian Leese 2021, personal communication in Norros et al. 2022). However, it is important to prioritize the quality of analyses and laboratory work over cost alone, as methodological standards are still under development, and high-quality workflows are essential for reliable results. In addition to potential cost reductions, eDNA methods offer improved reliability compared to traditional approaches, as species identification does not depend on individual taxonomic expertise and is less vulnerable to human error or fatigue (Norros et al. 2022).

3. Challenges and limitations of eDNA in monitoring

Although eDNA based monitoring offers highly promising new methods, there are some limitations in using it as is routinely in marine monitoring. Limitations noted include incomplete reference databases (Zaiko et al. 2018; Duarte et al. 2021; Espinosa Prieto et al. 2024), primer bias (Aylagas et al. 2016; Duarte et al. 2021), environmental challenges

such as DNA transportation, dilution and degradation (Foote et al. 2012), and difficulties in quantification (abundance metrics) (Prieto et al. 2023). It may also be impossible to obtain detailed population information, such as age or size structure or indicators of individual health, using eDNA methods. This is because the data are based solely on DNA captured from the environment. Additionally, while eDNA sampling is relatively effortless, the steps that follow the sampling are more complicated and involve various technical choices, which need specific expertise on DNA extraction, PCR amplification, sequencing and bioinformatics (Norros et al. 2022), and ill-advised decisions can lead to misleading results.

Data Interpretation:

Multiple different factors influence the amount of eDNA in the water and the joint effects of these can be hard to interpret (Collins et al. 2018; Saito and Doi 2021). The main factors are the amount of initial DNA shed by organisms, and the degradation rate, which is dependent on multiple environmental parameters (e.g., temperature, salinity, and UV-exposure). Different organisms shed DNA in different ways, depending on the physical characteristics and level of activity

In marine environments, eDNA transport and distribution mechanisms are not yet fully understood, which may complicate the interpretation of its origin and the spatial coverage of the samples (Bruce et al. 2021). Distribution of DNA by air or water may cause indirect observation, which means it is harder to interpret the location of the species in the sample. In shallow marine areas, eDNA is primarily dispersed by horizontal advection driven by currents and changing sea level, while vertical transport is usually limited by water column stratification (Andruszkiewicz et al. 2019). eDNA can drift up to 35 km from its original source within five days (Andruszkiewicz et al. 2019). However, the actual transport distance also depends on the degradation rate of DNA particles. In warmer conditions, where degradation is faster, the dispersal range is shorter, whereas in colder, low-energy environments, eDNA can persist and travel farther (Murakami et al. 2019). In marine environments it is also important to consider the depth gradients as the vertical mixing of water column may be more important factor for eDNA transportation in shallow water than in deeper areas, where eDNA is detected only relatively close to the depth where it has been (Allan et al. 2021). Salt acts as a preservative for DNA and higher salinity leads to slower degradation rates (Collins et al. 2018; Saito and Doi 2021). The conditions in the Baltic Sea are somewhat different than in other sea-areas as the salinity in much lower and there is a gradient in

salinity from south to north, which can influence the degradation rate of eDNA, and thus influence the interpretation of the origin of the eDNA.

Even though the overall number of species detected by eDNA methods is higher than in traditional methods, some taxa are still harder to detect. For example, Staehr et al. (2022) found significantly fewer macroalgae species using eDNA compared to diving. This may be due to selected primers or limitations in reference libraries. Additionally, the amount of DNA shed by organisms varies between taxa. For example, taxa with a hard shell (e.g, crustaceans) have been shown to shed less DNA into the water and thus they are harder to detect by eDNA sampling (Andruszkiewicz Allan et al. 2021; Crane et al. 2021). Therefore, it is important to consider the sampling season when planning monitoring programs. Taking samples in the mating season could help to enhance the detection possibilities, as crustaceans shed more DNA into water due to increased adult activity, and presence of eggs and sperm and molting of juveniles increases the amount of DNA in the water. This also applies to plant monitoring, since the concentration of DNA from various plant species in the water has been found to increase during the life cycle and peaking during the senescence (d'Auriac et al. 2019; Prieto et al. 2023), or during growing season (Matsuhashi et al. 2019).

Technical Issues:

While eDNA methods have been well studied across multiple taxa, and there are promising results in monitoring species such as fish (Staehr et al. 2022), the study of higher plants still lags behind (Banerjee et al. 2022; Prieto et al. 2023). One of the main reasons behind the paucity of using eDNA methods in detecting plant species could be the absence of one specific universal barcode targeting all plant taxa, which increases both the labor time and the overall costs of the analyses (Prieto et al. 2023). Although a single universal barcode marker for plants is not available, several markers (such as *mat*K, *rbc*L, ITS2, ITS1, and *trn*L) are commonly used, each targeting different regions of the plant genome, and the complementary use of these are recommended (Prieto et al. 2024). The quality of the results with eDNA sampling relies also heavily on the performance of primers that are applied to identify species (Prieto et al. 2024; Chen et al. 2025). Thus, it is important to consider which primers are used in the planning stage of monitoring program.

As the eDNA method is an indirect way of observing species in the environment, false positives (contamination) and false negatives (incomplete detection) are also aspects of eDNA sampling to be considered (Buxton et al. 2021). These errors can happen in the field sampling phase, in the laboratory, or in the bioinformatics phase. The best way to ensure accurate results is to plan the sampling and laboratory work carefully before conducting the work. There are a few methodological guides and workflow summaries available (e.g., Bruce et al. 2021; Banerjee et al. 2022), and planning of eDNA pilots and research should be based on these.

Standardization Issues:

Despite the few existing methodological guidelines, another main challenge hindering the broader implementation of eDNA methods in monitoring is the lack of standardization and unified protocols for sample collection and analysis (Laamanen et al. 2025). There are several standards under preparation and a standard for collecting, capturing and preserving eDNA samples from water (`CEN 17805:2023`) already exists, but it is relatively new, and the methodologies have not been in use for long. Variability in methodology across studies makes comparisons difficult, as there is significant variation in sample volume, replication and quality control measures across studies (e.g, Lacoursière-Roussel et al. 2018; Suarez-Menendez et al. 2020; Corral-Lou et al. 2025). Additionally, method optimization, improved quantitative estimates, and the development of comprehensive reference libraries are needed for larger implementation of eDNA based monitoring methods (Laamanen et al. 2025).

eDNA based monitoring methods are currently the most studied and the readiest for use in biomonitoring in aquatic ecosystems compared with terrestrial systems. Macroinvertebrates and fish are the most extensively investigated organism groups, whereas aquatic plants have received less attention in previous studies (Prieto et al. 2023; Laamanen et al. 2025). This disparity can affect the accuracy of monitoring depending on the target species.

4. Complementing traditional methods with eDNA

Studies have shown that eDNA methods can detect more taxa than traditional methods but there are several taxa missed by eDNA methods but detected by traditional surveys (e.g, Staehr et al. 2022), while on the other hand traditional methods require intensive sampling

effort to detect rare species, and identification of juvenile specimens and cryptic species may be impossible. There are also distinct advantages and disadvantages to both traditional and eDNA methods (Table 1). This emphasis the need for complementary use of both traditional and eDNA methods in monitoring. Traditional marine monitoring in the Baltic Sea could benefit from complementary use of eDNA methods by broadening species detection (eg., Jerney et al. 2022; Preston et al. 2024; Chevrinais et al. 2025).

Table 1. Comparison of eDNA methods and traditional monitoring methods (FOEN 2020).

	eDNA	Traditional monitoring methods
Sampling time	Faster (compared to traditional methods)	Slower (compared to eDNA methods)
Cost	Sampling is fixed but for the analyses cost decreases with larger number of samples.	Fixed
Sensitivity	High (does not require large sampling effort to detect multiple species, can detect juvenile species)	Low (need for larger sampling efforts to detect more species, may not be able to detect juveniles)
Taxonomic range	Broad, one sample contains information on multiple taxa	Narrow. May need different sampling methods for different taxa. Limited to the morphological identification.
Detectability	High, can detect rare and invasive species with relatively low sampling effort. However, detectability depends on taxa!	Lower, more intensive sampling effort needed for detecting rare species
Sampling	Non-invasive (except for bulk samples)	Invasive (except for visual observations)
Field observations	Require equipment, such as portable PCR (e.g., Nanopore MinION)	Possible
Sample processing	Require specific expertise, can be automated	Simple, cannot be automated
Contamination	High risk	Low risk
Infrastructure	Molecular laboratory	Simple equipment

Species identification	Requires reference databases	Based on taxonomic expertise
Qualitative data	List of species (and operational taxonomic units, OTUs)	List of species, population structure, can detect age and size structures
Quantitative data	Relative abundance of reads or DNA quantification (PCR)	Absolute species abundance
Data analysis	Special bioinformatic analyses	Relatively simple statistical analyses
Data interpretation	There are technical issues that one needs to consider and method specific issues (e.g., DNA degradation, transportation, contamination, live vs. dead organisms)	Personal expertise and ecological knowledge
Standardization	Work in progress	Exists

Possible complementary uses of eDNA in marine monitoring include hybrid approaches in which eDNA methods are used for initial assessments, followed by targeted traditional surveys. eDNA could also serve as a rapid screening tool to prioritize areas for more intensive monitoring. Implementing molecular methods for critical, rare, or endangered species, such as species mentioned in HELCOM Red List of the Baltic Sea (HELCOM 2025), could further improve conservation status assessments. Water-based eDNA sampling can also broaden species detection, provide early warnings of invasive species, and improve monitoring in habitats that are difficult to access. In single-species detection, it is possible to screen certain endangered or invasive species. In the Baltic Sea, these could be, for example, invasive species *Elodea canadensis* which has been successfully detected in freshwater environments (d'Auriac et al. 2019). With metabarcoding methods, it is also possible to get information on the whole community. This could be used to detect whole communities, for example benthic and epifaunal communities in *Fucus vesiculosus* habitats (Preston et al. 2024). However, for plant monitoring, this approach may require the development of more specific barcodes and improvements to reference libraries, as research on plant eDNA is still limited.

5.1 eDNA pilot in shallow photic marine environments

To enable reliable eDNA monitoring of aquatic plants, endangered species, and epifaunal communities in marine lagoons, a pilot study should be conducted to refine the sampling design and assess the capability of existing reference libraries and genetic markers to detect Baltic Sea species. More information on best practices for monitoring aquatic plants and associated epifauna in marine environments is also needed, as the current literature remains limited.

A pilot study that complements traditional monitoring methods with eDNA metabarcoding would help bridge this knowledge gap while allowing methodological refinement. Water and sediment samples could be collected alongside traditional surveys to enhance species detection. Taking multiple samples distributed throughout each lagoon would provide improved information on the spatial distribution of species (following the approach of Bruce et al. 2021). Such a pilot should be conducted across different seasons before routine implementation (e.g., Jerney et al. 2022) to determine the optimal sampling period for specific species. However, this seasonal replication would increase the overall cost of monitoring.

Bulk DNA metabarcoding can further improve the identification of macroinvertebrates (Elbrecht et al. 2017), enhancing our understanding of epifaunal community structure, particularly for taxa that are difficult to identify morphologically. In *Fucus vesiculosus* epifaunal communities, for instance, some species (e.g., amphipods) are challenging to identify at the species level using traditional methods and are often classified only at the genus level. During the eDNA pilot study, bulk epifaunal samples could be collected and analyzed using molecular methods to evaluate how well traditional and molecular identifications correspond.

6. Conclusions

eDNA monitoring methods could enhance species detection and provide added information to biodiversity assessments. However, there are some concerns hindering broader use of these methods in routine monitoring shallow photic marine environments. The main concern is the limited amount of supporting research currently available. While the use of eDNA has been well studied for detecting the presence of various fauna, the application of eDNA

methods in assessing plant diversity remains notably less studied. However, it is important to note that research on this front has been accumulating over the last couple of years, and eDNA has been successfully used in detecting invasive, rare and endangered plants, and whole plant communities (Banerjee et al. 2022; Prieto et al. 2023). Key bottlenecks limiting the implementation of eDNA methods for marine vegetation biomonitoring include challenges with measuring abundance, lack of universal primers, shortages in reference libraries, and lack of standardization. Also, the uncertainty of spatiotemporal dynamics in eDNA can hinder the usability of the method in biomonitoring. Thus, there is an urgent need for testing and developing primers suitable for aquatic plants as well as more comprehensive reference libraries. Additionally, we need to pilot already existing methods in the Baltic Sea environment to see how well these methods perform in this context.

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