eDNA Analysis for Non-Invasive Biodiversity Assessment in Offshore Environmental Impact Studies

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SUMMARY

Environmental DNA (eDNA) analysis has emerged as a powerful, non-invasive tool for biodiversity assessment in support of offshore environmental impact studies. In this study, we organized systematic monitoring campaigns, during which water samples were collected and filtered to capture genetic material. Rigorous laboratory protocols ensured contamination-free extraction and amplification of DNA using high-fidelity methods targeting 18S rRNA and COI gene markers. This enabled precise identification of both local and alien species, providing a comprehensive picture of marine biodiversity. Bioinformatic pipelines were applied for sequence clustering and taxonomic annotation, producing operational taxonomic units (OTUs) and enabling alpha and beta diversity analyses. The use of eDNA offered a cost-effective alternative to conventional sampling, minimizing the need for disruptive practices like fishing or physical trapping. By generating high-resolution biodiversity data without extensive direct interference, this methodology supports a conservation-oriented approach to environmental impact assessment. Our approach highlights the value of eDNA in gathering reliable data on species composition and abundance, facilitating more sustainable decision-making in offshore project planning.

INTRODUCTION

Environmental Impact Assessments (EIAs) in offshore marine environments increasingly require innovative, non-invasive methodologies to effectively monitor biodiversity while minimizing disturbance to ecosystems. Environmental DNA (eDNA) analysis has emerged as a powerful tool in this context, enabling the detection of species through genetic material present in water samples, thus offering insights into community composition without the need for direct organismal sampling. Recent studies have demonstrated the efficacy of eDNA in capturing spatial and temporal patterns of marine biodiversity, highlighting its potential for integration into standard monitoring protocols (Valdivia-Carrillo et al., 2024; Bonicalza et al., 2024). The present study applies eDNA metabarcoding across multiple offshore locations within the Mediterranean Sea, providing baseline biodiversity data in support of early-phase environmental assessments for marine infrastructure development.

1. METHODOLOGICAL FRAMEWORK

The methodological workflow applied in this study was designed in line with established international protocols for eDNA-based marine biodiversity monitoring. It reflects recent recommendations for best practices in sample collection, molecular processing, and bioinformatic analysis aimed at minimizing contamination risks and maximizing taxonomic resolution (Deiner et al., 2017; Stat et al., 2019). The process was structured into three main stages: (i) sample collection and DNA extraction, (ii) PCR amplification and library preparation, and (iii) sequencing and bioinformatics analysis.

1.1 SAMPLING AND DNA EXTRACTION

Seawater samples were collected from multiple offshore stations. and for vertical variation. Water was filtered onboard through 0.45 µm nitrocellulose membranes using a peristaltic pump under sterile conditions. Filters were preserved in absolute ethanol and stored at −20°C until processing. DNA was extracted using the Qiagen DNeasy Blood & Tissue Kit, following an optimized protocol that included prolonged Proteinase K digestion (overnight at 56°C) to ensure complete lysis of cellular material. Negative field controls were included to monitor for potential contamination during collection and filtration.

1.2 PCR AMPLIFICATION AND LIBRARY PREPARATION

Two gene markers were targeted to capture complementary aspects of biodiversity: the V4 hypervariable region of the 18S rRNA gene (~450 bp), which is broadly effective for eukaryotes, and a fragment of the mitochondrial Cytochrome Oxidase I (COI) gene (~350 bp), which provides higher resolution for metazoans. Locus-specific primer sets were selected based on performance in marine systems and compatibility with Illumina platforms. PCRs were conducted in triplicate for each sample, pooled, and cleaned using AMPure XP magnetic beads. Libraries were then size-selected via gel electrophoresis to remove non-specific products and adapter dimers prior to indexing.

1.3 SEQUENCING AND BIOINFORMATIC ANALYSIS

Amplicon libraries were sequenced using paired-end reads on an Illumina platform. Raw reads were demultiplexed and subjected to stringent quality filtering using Cutadapt (removal of primers), FLASH (read merging), and fastp (trimming and quality control). VSEARCH was used for chimera detection and clustering of sequences into Operational Taxonomic Units (OTUs) at 97% similarity. Taxonomic assignments were conducted with the SILVA database (v.138) for 18S and the MitoFish reference database for COI. To account for differential amplification success, OTU abundance tables were normalized using cumulative sum scaling. Downstream diversity analyses (Shannon, Simpson, Chao1) and community structure comparisons (NMDS, PCoA, PERMANOVA) were performed in QIIME2 and R (vegan, phyloseq packages).

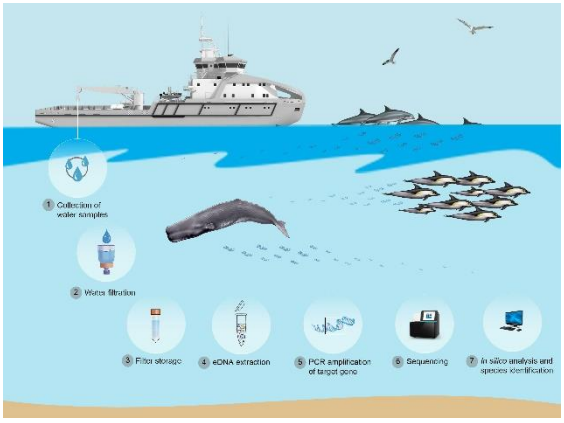

Figure 1: Application of eDNA methodology in the sea (Adapted from Suarez-Bregua et al., 2022).

1. MONITORING APPROACH

This study was conducted in the southern portion of the Mediterranean Sea, a region of growing strategic interest for the development of marine infrastructure and renewable energy projects. The selected sites are representative of typical ecological and oceanographic conditions encountered in Mediterranean offshore environments, encompassing a range of habitats from continental shelf zones to deeper benthopelagic transition areas.

Sampling stations were identified based on geophysical, bathymetric, and ecological criteria, with the goal of capturing both natural variability and potential anthropogenic gradients relevant to environmental planning. Each station was assigned a role within broader survey objectives, such as characterizing baseline biodiversity conditions in areas of future intervention.

All samples were filtered and processed, resulting in high-throughput sequencing datasets targeting both the 18S rRNA gene for broad eukaryotic coverage and the COI gene for improved taxonomic resolution among metazoans. Results revealed consistently high levels of biodiversity across the surveyed area, with 18S data detecting over 300 unique OTUs and COI data contributing an additional 200+ OTUs. Dominant taxonomic groups included annelids, arthropods, mollusks, and teleost fishes. Notably, some stations showed higher richness in benthic invertebrate taxa, while others were characterized by a prevalence of pelagic organisms.

2. RESULTS

The application of eDNA metabarcoding techniques in the southern Mediterranean Sea yielded comprehensive insights into marine biodiversity patterns. The analyses encompassed sequencing metrics, taxonomic composition, and diversity indices, providing a multifaceted understanding of the ecological status of the surveyed areas.

2.1 SEQUENCING METRICS

The sequencing workflow yielded high-throughput data with excellent quality across both gene markers. For the 18S rRNA gene, the number of raw paired-end reads per sample ranged from approximately 40,000 to 100,000, with a post-filtering average read length of ~420 bp. After quality control and chimera removal, over 95% of sequences were retained, indicating effective primer performance and minimal degradation. For the COI marker, a slightly higher level of amplification variability was observed across replicates, particularly in deeper or turbid stations, but overall library quality remained high. The cumulative analysis resulted in the identification of 738 OTUs from 18S sequences and 212 OTUs from COI, distributed across hundreds of taxonomic units. Negative controls yielded negligible or zero reads, confirming the absence of cross-contamination. Overall, the sequencing effort provided a robust dataset suitable for biodiversity characterization and multivariate ecological analysis.

2.2 TAXONOMIC COMPOSITION

Environmental DNA analysis revealed a diverse array of marine eukaryotic organisms across all sampled offshore areas. The 18S marker captured a broad phylogenetic spectrum, encompassing protists, macroinvertebrates, and metazoans. Taxa from the phyla Annelida, Arthropoda, and Mollusca were consistently abundant across locations, reflecting typical benthic and planktonic communities in Mediterranean offshore habitats. The COI marker, with a stronger affinity for metazoans, provided higher-resolution taxonomic identification of vertebrates, particularly teleost fishes and invertebrates with mitochondrial references.

Functional diversity was also apparent: suspension feeders (e.g., bivalves), detritivores (e.g., polychaetes), benthic grazers (e.g., gastropods), and predators (e.g., decapods and demersal fishes) were all detected. Several taxa of ecological relevance—either due to their role as bioindicators or their conservation status—were identified in multiple locations. Additionally, sequences attributable to non-indigenous or cryptogenic species were detected at low frequencies, underlining the importance of eDNA for early warning in environmental monitoring.

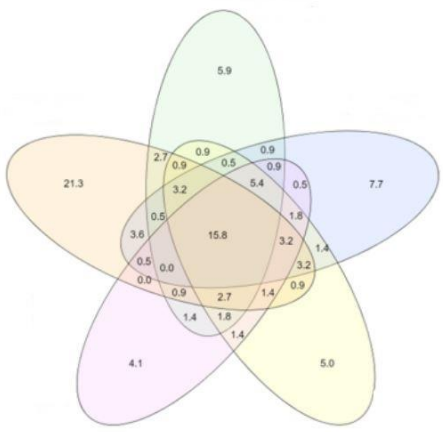

Figure 2: Venn diagram for the eukaryotic community (18S)

2.3 DIVERSITY ANALYSIS

Alpha diversity indices varied across stations and between the two gene markers. The 18S marker showed consistently higher OTU richness and evenness, reflecting its broader taxonomic scope. Shannon and Simpson indices suggested moderately to highly diverse communities, with some offshore stations showing particularly even assemblages. COI-based alpha diversity was lower in absolute terms, as expected, but still captured distinct components of community structure—especially in relation to nektonic and benthic metazoans.

Chao1 estimates highlighted the potential presence of rare or under-sampled taxa, particularly in offshore areas with deeper profiles or more dynamic hydrological conditions. Beta diversity analysis using Bray-Curtis dissimilarity revealed clear separation between groups of samples, suggesting ecological differentiation likely linked to depth, hydrodynamics, and substrate variation. Ordination techniques (e.g., NMDS and PCoA) showed clustering by functional zone (e.g., infrastructure corridors vs general offshore background), indicating that eDNA data are sensitive to both natural gradients and early anthropogenic signatures.

3. DISCUSSION

The application of eDNA metabarcoding in this offshore Mediterranean context demonstrated remarkable efficiency in detecting a broad spectrum of marine taxa with minimal field effort and without the need for destructive or invasive sampling. Across the surveyed stations, distinct community compositions were revealed, confirming the ability of eDNA to capture spatial heterogeneity in marine ecosystems. These findings highlight the potential of this approach not only to provide baseline biodiversity data but also to support early detection of non-indigenous species and evaluate ecological quality at fine spatial resolution.

However, the implementation of eDNA-based assessments is not without challenges. Variability in amplification success, particularly for COI markers in certain replicates, was observed and attributed to a combination of environmental factors (e.g., turbidity, inhibitory substances) and primer-template mismatches. In one case, failed amplification prompted an in-depth quality control step that confirmed the integrity of the field and extraction protocols, ultimately leading to protocol refinement for future campaigns. Rather than representing a weakness, this episode exemplifies the iterative potential of eDNA workflows, where anomalies can serve as diagnostic tools to improve methodological robustness.

Another important consideration concerns the accuracy of taxonomic assignment. During the bioinformatic phase, several OTUs were initially matched to unlikely or ecologically incongruent taxa—such as freshwater or non-Mediterranean species. These cases were investigated manually and found to result from sequence similarity to incomplete or misannotated database entries. The re-evaluation of these sequences using alternative databases or cross-validation against known species distributions allowed us to correct or reinterpret the findings. This underlines a key point: while eDNA offers extraordinary resolution, its reliability is strongly linked to the quality of reference databases and the ecological validation of taxonomic outputs.

Moreover, the resolution power of the method varies depending on the genetic marker. The 18S gene offers broader detection of eukaryotic diversity, including protists and invertebrates, but tends to underresolve closely related metazoans. In contrast, COI provides higher taxonomic precision among metazoans, particularly fish, but is more sensitive to PCR biases and more vulnerable to incomplete database coverage. These characteristics must be accounted for when interpreting results, and they further reinforce the importance of multimarker strategies.

The study confirms that eDNA metabarcoding holds exceptional promise for marine biodiversity assessments in support of environmental impact evaluations. When coupled with rigorous quality control and ecological validation, the method provides high-resolution, replicable, and scalable biodiversity data. The minor limitations observed are manageable within a robust workflow, and in fact, they offer opportunities to refine protocols over time.

4. CONCLUSION

Environmental DNA (eDNA) analysis proved to be a valuable, scalable, and non-invasive tool for offshore biodiversity monitoring, offering concrete support to Environmental Impact Assessments (EIAs). The method demonstrated strong potential to streamline baseline ecological surveys by providing detailed, replicable data on species composition across multiple trophic and functional levels (Stat et al., 2017; Pawlowski et al., 2018). Its capacity to detect elusive, rare, or cryptogenic taxa enhances the depth and resolution of biodiversity assessments, while the limited physical footprint of sampling aligns well with conservation goals.

The ability to generate timely, site-specific biodiversity data supports evidence-based planning and contributes to more transparent, accountable environmental management processes (Hering et al., 2018). These features make eDNA particularly suited for informing conservation-oriented design of offshore infrastructure and guiding early-stage screening for potential environmental sensitivities.

Looking ahead, future efforts should focus on further integrating eDNA outputs with conventional biological surveys, oceanographic variables, and habitat data to enable more holistic ecosystem assessments. As the technique continues to mature, eDNA is poised to become a foundational tool in the marine environmental assessment toolkit.