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54° Congresso SIBM: Biodiversità e funzionamento attraverso approcci di analisi di DNA ambientale (eDNA)

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Viewpoint

BIODIVERSITY AND FUNCTIONING THROUGH ENVIRONMENTAL DNA ANALYSIS

BIODIVERSITÀ E FUNZIONAMENTO ATTRAVERSO APPROCCI DI ANALISI DI DNA AMBIENTALE

Abstract – *The eDNA is an emerging tool for evaluating biodiversity and understanding models, as well as spatial and temporal patterns of communities, by directly sequencing DNA samples. The topic 'Biodiversity and Functioning Through Environmental DNA Analysis', introduced at the 54th Conference of Italian Marine Biology Society, aims to illustrate how the continuously developing metabarcoding approach of eDNA can revolutionise current biodiversity assessment methodologies to evaluate short- and long-term ecosystem changes, as well as the impacts of pollution and climate change on populations and communities.*

Keywords: *Diversity, eDNA (environmental DNA), Field samples, HTS (high-throughput sequencing), Metabarcoding.*

Introduction – The advent of high-throughput sequencing technologies has created a paradigm shift in the field of molecular taxonomy, while morpho-taxonomic inventories remain indispensable as complementary and integrative approaches. The use of DNA-based methodologies is based on the assumption that taxa can be consistently identified on the basis of a particular fragment of its genome. This fragment is referred to as a "DNA barcode" region. The term "metabarcoding" is employed to denote the process of utilising High-Throughput Sequencing (HTS) of a DNA barcode region for the parallel identification of numerous species from complex samples. Bulk metabarcoding is the analysis of samples consisting of mixed collections of organisms. In contrast, eDNA metabarcoding refers to the analysis of environmental samples and it is defined as 'a complex mixture of genomic DNA from many different organisms found in an environmental sample' (Taberlet *et al.*, 2012). In consideration of the encouraging indications of its potential, the application has been demonstrated to be effective in a variety of domains, including also, the monitoring of biodiversity, and the assessment of air, soil, and water quality, the analysis of trophic interactions, the paleogenomics, and the response to pollution (Pawlowski *et al.,* 2012; Sahu *et al.,* 2025). The technique has been shown to allow the survey of an ecosystem in a quick and effective way, and it is continuously benefitting from the development and improvement of HTS, which allows for the rapid and simultaneous identification of a huge quantity of sequences and, consequently, of organisms. Now, the integration of eDNA, metabarcoding, and HTS has emerged as a potent instrument within the domain of environmental science (Fig. 1). As demonstrated in the literature (Pietramellara *et al*., 2009), DNA fragments, both intracellular and extracellular, are present in all environmental matrices. These fragments can be analysed to obtain a inclusive picture of habitats from a small amount of sediment (Marinchel *et al.,* 2024), water, litter (Casabianca *et al.,* 2025) or other sources without stressing the target environment. Nevertheless, the methodology is not entirely immune to technical biases that may originate in the various steps of the experimental workflow, including sample collection and DNA extraction protocols, the choice of barcode genes and primers (e.g., preferential amplification of some taxa, gene copy number variation, etc.), the use of sub-optimal bioinformatic pipelines and incomplete or inaccurate reference sequence databases (Ruppert *et al.,* 2019; Ficetola and Taberlet, 2023). The aforementioned shortcomings may result in a number of potential outcomes, including false negatives (i.e., the failure to detect some taxa), false positives (due to misclassification or contamination), or biased relative abundances of sequences. Furthermore, the absence of consensus on approaches is evidenced by the utilisation of different protocols and instruments. This results in a heterogeneous set of biological information that precludes the reaching of generalised, shared conclusions. The primary challenge arises in the integration of the distinct characteristics of each target ecosystem, whilst concomitantly ensuring the necessity for a shared and standardised experimental design. This is crucial to ensure the realization of accurate, replicable, and reliable results, reinforced by robust statistical support. In addition, during the experimental set up, contamination with exogenous DNA is a potential issue that can occur, thus requiring the application of severe experimental protocols and procedures.

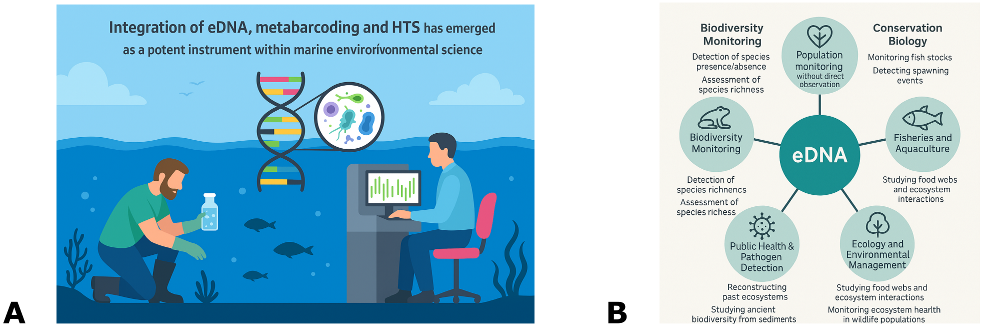

Fig. 1. – A) Integration of eDNA, metabarcoding, and HTS as an emerging tool in marine environmental science; B) Different applications of eDNA analysis in environment, health, and fishery production fields.
A) Integrazione di eDNA, metabarcoding e HTS come strumento emergente nella scienza dell'ambiente marino; B) Diverse applicazioni dell'analisi di eDNA in campo ambientale, della salute e delle produzioni ittiche.

However, in recent years, eDNA metabarcoding has emerged as a powerful tool to achieve an integrative assessment of the environmental health status, through a broad, relatively rapid, and cost-effective taxonomic monitoring of biodiversity at different spatial scales. The eDNA has been successfully used to prioritise fish macrofauna (Tsuji *et al.,* 2019) and support conventional monitoring approaches in species surveys of aquatic environments, providing more detailed information on species distribution over time and space (Stoeckle *et al.,* 2017; Aglieri *et al.,* 2021). Furthermore, numerous studies on eDNA focus on various aquatic ecosystems, such as coastal waters, lagoons and the open sea (Cananzi *et al.,* 2022; Tagliabue *et al.,* 2025). This is largely because these environments play a direct economic role in intensive aquaculture and fisheries, and are affected by water pollution, climate change and the introduction of alien species, which makes their protection essential (Solidoro *et al.,* 2010; Occhipinti-Ambrogi *et al.,* 2011).

Conclusions - The eDNA is a transformative tool for collecting ecological data and for monitoring habitat biodiversity (Thomsen *et al.,* 2015). It offers potentially species detection and reduces the biases inherent to traditional methods. The eDNA technology has released up new paths for ecological research and habitat management, providing major future opportunities. The rapid advancements of molecular techniques, particularly in DNA sequencing technologies, can likely improve metagenomic approaches in the future, improving biodiversity assessment and ecosystem management. As methodologies involving the use of environmental DNA (eDNA) gain popularity, several strategic developments are necessary to maximise their effectiveness in conservation, and these must be implemented as soon as possible. To make eDNA studies more versatile and affordable several major developments are required, such as standardization of protocols and reference libraries or repository, development and miniaturization of portable instruments, cost-effectiveness of techniques, integration with traditional methods, advancements in detection techniques and mobile application.

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