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Integrating environmental DNA-based approaches into fisheries monitoring and sustainable practices

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by

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Preparation of this document

This manual was prepared by the General Fisheries Commission for the Mediterranean (GFCM) of the Food and Agriculture Organization of the United Nations (FAO) to address priorities outlined by Mediterranean and Black Sea countries in international commitments and regional strategies, including the GFCM 2030 Strategy for sustainable fisheries and aquaculture in the Mediterranean and the Black Sea and more particularly its Target 1 “Fisheries and ecosystems: healthy seas and productive fisheries”. In this context, this manual aims to promote the use of alternative, low-impact monitoring methods for marine ecosystems by providing practical guidance on the application of environmental DNA-based approaches, including metaprobe deployment, to improve data completeness, address knowledge gaps and complement traditional monitoring techniques in the Mediterranean and Black Sea region.

This document was prepared under the coordination of Paolo Carpentieri (Fishery Resources Monitoring Officer) and the overall direction of Miguel Bernal (Executive Secretary). The editing and publishing were led by Alexandria Schutte (Publications Specialist) in coordination with Ysé Bendjeddou (Publications Coordinator) and under the supervision of Dominique Bourdenet (Knowledge Management Officer). Koen Ivens performed the graphic design and layout.

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Abstract

Reliable ecological and biological data from marine ecosystems provide crucial insights into fish populations and ecosystem dynamics and form the basis for sound decision-making. Traditionally, these data have been obtained through fishing activities or visual surveys. However, these approaches often involve the physical removal of organisms and can result in data gaps, limiting the complete understanding of ecosystem structures and functions. As a result, there is a growing need for monitoring methods that improve data completeness while reducing ecological impacts.

Environmental DNA (eDNA)-based approaches have emerged as a promising response to these challenges. By detecting genetic material in water, sediments or other substrates, eDNA-based approaches enable the identification of species and communities without direct observation or capture. They are non-invasive, scalable, increasingly cost-effective and have yielded biodiversity estimates that both complement and, in many cases, exceed those derived through conventional techniques. In the Mediterranean and Black Sea region, the integration of eDNA-based approaches into routine monitoring and stock assessment frameworks offers substantial potential to address persistent knowledge gaps.

Environmental DNA can be collected using a range of active and passive approaches, selected according to specific research goals and environmental contexts. Active approaches, which dominate current literature, involve human intervention to concentrate the eDNA present in an aquatic medium. In contrast, passive approaches rely on natural accumulation processes and do not require water filtration. Among these, the recently developed metaprobe is a notable innovation. The metaprobe is a passive eDNA sampler, designed to be used alongside different types of fishing gear. It enables the collection of genetic material from the surrounding environment, allowing local biodiversity to be characterized.

This manual provides a comprehensive look into eDNA-based approaches in the Mediterranean and Black Sea region, with a particular focus on the use of the metaprobe. Chapter 1 positions eDNA within a broader historical context of marine data collection and presents its current applications. Chapter 2 describes the general workflow for eDNA metabarcoding during fishing activities, including field sampling, laboratory procedures for DNA extraction, amplification and sequencing, and subsequent analytical steps. Chapter 3 focuses on the necessary post-processing for eDNA metabarcoding data, while Chapter 4 examines emerging applications for eDNA metabarcoding informed by recent studies. The manual concludes with an appendix that provides a step-by-step protocol for deploying the metaprobe to collect eDNA during fishing activities.

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Abbreviations

COI	cytochrome C oxidase subunit I
eDNA	environmental deoxyribonucleic acid
FAO	Food and Agricultural Organization of the United Nations
g	gravitational force
GFCM	General Fisheries Commission for the Mediterranean
NGS	next-generation sequencing
PCR	polymerase chain reaction
rDNA	ribosomal DNA

1. Introduction

1.1 Background

The marine environment remains largely unexplored, with recent estimates indicating that only 0.001 percent of the deep-sea floor has been observed (Bell *et al.*, 2025). Its vastness and complexity pose challenges for marine exploration. Deep-sea and remote oceanic regions are particularly difficult to access using traditional survey methods, and technical and financial constraints further limit the extent to which marine ecosystems can be surveyed through scientific expeditions.

Historically, fisheries have provided the most direct and practical means of accessing and observing marine biodiversity. Fishing activities have created opportunities to collect ecological and biological data, including species presence, size distribution, reproductive condition and habitat association. Data are also collected through visual surveys conducted by divers, underwater cameras and remotely operated vehicles.

While these approaches provide crucial insights into fish populations and ecosystem dynamics, they have significant limitations. Many rely on the physical removal of organisms from their habitats, potentially causing stress, harm or death. These fishery-dependent data streams are often collected with varying degrees of standardization and completeness, especially in small-scale fisheries, which can limit their reliability and comparability across space and time. Moreover, data derived from physical capture are limited by the selectivity and spatial bias of fishing gear and may miss species that are small, rare, benthic, or that inhabit complex or sensitive environments. This results in data gaps that hinder a complete understanding of the structure and function of marine ecosystems.

To respond to these challenges, there is a strong incentive to expand the use of alternative, low-impact monitoring methods that can increase the extent and frequency of marine ecosystem monitoring while reducing potential negative impacts. In this context, environmental deoxyribonucleic acid (eDNA)-based approaches have emerged as transformative tools. By detecting genetic traces shed by organisms into water, sediments or other substrates, eDNA-based approaches enable the identification of species and communities without direct observation or capture (Sahu *et al.*, 2023). These approaches are non-invasive, scalable, increasingly cost-effective and have yielded biodiversity estimates that both complement and, in many cases, exceed those generated by conventional techniques, such as trawling or visual census (Pawlowski *et al.*, 2018; Westgaard *et al.*, 2024; Zou *et al.*, 2020).

Environmental DNA-based approaches also hold considerable promise for enhancing fisheries monitoring by complementing traditional methods and improving spatio-temporal resolution, especially in data-poor contexts or where existing monitoring frameworks are limited. In the Mediterranean and Black Sea region, characterized by intense anthropogenic pressures, diverse ecosystems, heterogeneous fisheries and the transboundary nature of many stocks, the integration of eDNA-based approaches into routine monitoring and stock assessment efforts could help address critical knowledge gaps, including those related to species distributions, nursery areas, invasive species and ecosystem composition.

To deploy these eDNA sampling protocols at scale, fishing vessels represent a valuable, yet underutilized, platform. Their spatial coverage, frequency of operation and the familiarity of fishers with local environments make them an effective vector for integrating eDNA monitoring into routine maritime activities, thereby improving geographic coverage and data resolution. Empowering fishers to participate in eDNA data collection could also strengthen participatory science efforts, foster greater stakeholder engagement, enhance trust in resulting data streams and pave the way for more robust approaches to leveraging fisheries data and observations (Bradley *et al.*, 2019).

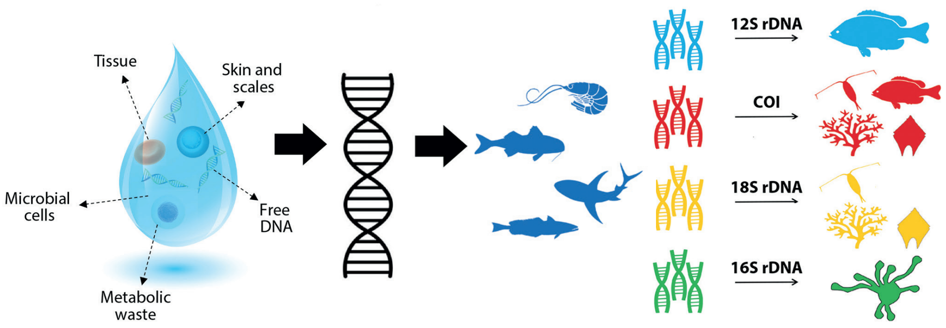
To harness the full potential of eDNA-based approaches in fisheries science and management, there is a pressing need for standardized and internationally recognized protocols that ensure the comparability, reliability and reproducibility of data. Without agreed-upon methodologies for practices ranging from sampling strategies and preservation techniques to laboratory analysis and bioinformatics pipelines, the utility of eDNA-derived data for regional or transboundary management remains limited. There is also a need to address the logistical and financial challenges associated with collecting water samples from marine environments, as DNA is often highly diluted and may require the filtration of large volumes of water.

In response to these issues, researchers have called for the simplification and automation of sampling protocols, including the development of compact devices and passive samplers that can collect genetic material without the need for high-volume filtration (Bessey *et al.*, 2020; Miya, 2022). Promising developments of *in situ* and autonomous eDNA collection technologies such as passive probes (Bessey *et al.*, 2021) are also creating opportunities to integrate these tools into existing maritime operations.

1.2 Definition

Environmental DNA refers to genetic material that organisms shed into the environment through biological processes such as excretion, skin shedding, mucus secretion or decomposition (Figure 1). This extracellular DNA (i.e. DNA contained in cells or cellular debris) becomes suspended in the environment – commonly in water, sediment or soil – and persists for variable periods of time depending on factors such as temperature, exposure to ultraviolet light, microbial activity and water chemistry.

FIGURE 1
Sources of environmental DNA and the main marker genes used for amplification



Note: COI = cytochrome C oxidase subunit I; rDNA = ribosomal DNA.

Source: Adapted from Chavez, F.P., Min, M., Pitz, K., Truelove, N., Baker, J., LaScala-Grunewald, D., Blum, M. *et al.* 2021. Observing Life in the Sea Using Environmental DNA. *Oceanography*, 34(2): 102–119.

The extraction and analysis of eDNA from environmental samples enables the detection of species without the need for physical capture or direct observation. In aquatic environments, eDNA can be collected by filtering water samples and extracting DNA fragments that are then amplified using polymerase chain reaction (PCR) techniques and identified through DNA barcoding or metabarcoding approaches. These molecular techniques can target specific species or entire communities, allowing both targeted monitoring and comprehensive biodiversity assessments.

Environmental DNA-based approaches have gained significant momentum in recent years, owing to their non-invasive, cost-effective and highly sensitive nature. They are capable of detecting a wide range of organisms, from microbenthic to megafauna, including species that are elusive, rare, or present at low abundance and often missed by conventional sampling tools. This makes eDNA particularly powerful in marine contexts, where visibility is limited, habitats are vast or remote, and traditional survey methods are logistically constrained and often invasive. The ability to use a single sampling and processing pipeline across diverse taxa further highlights the value of eDNA-based approaches as a powerful tool for biodiversity monitoring across ecosystems.

The rapid advancement and increasing accessibility of molecular technologies, including high-throughput sequencing and bioinformatics, have significantly enhanced the feasibility and scalability of eDNA-based approaches. These developments have transformed them from niche techniques into widely used approaches for supporting evidence-based management and conservation.

As a result, eDNA is being used in an expanding array of contexts, such as:

- monitoring biodiversity and species presence;
- tracking vulnerable species;
- detecting non-indigenous species;
- monitoring spawning events, larval dispersal or species-specific habitat use; and
- assessing ecosystem health.

These applications highlight the transformative potential of eDNA-based approaches to complement, or in some cases even replace, traditional monitoring techniques, particularly in contexts where time, cost, onboard space, safety considerations and environmental impacts could pose challenges.

1.3 Current research

Recent fisheries research on the use of eDNA has explored applications primarily in marine water – both coastal and offshore – followed by freshwater and, to a lesser extent, transitional water. Table 1 summarizes the key technical aspects derived from 148 scientific papers identified in the Scopus database (Elsevier, 2026), using the keywords “eDNA metabarcoding” and “fisheries”.

Several studies have sampled eDNA at sites associated with human infrastructure, where ongoing activities, such as construction and maintenance, regulatory monitoring or commercial operations, enable consistent access to diverse aquatic environments. These studies highlight the nature and number of complementary data sources that can be used to validate and enhance the findings from eDNA-based approaches, while also underscoring the rapid growth of eDNA.

TABLE 1

Summary of environmental DNA metabarcoding and fisheries studies, 2016–2025

Reference	Approach	Method of sample collection	Mean volume (L)	Filter size (µm)	Freshwater		Transitional water	Marine water		Infrastructure				Land	Accessory data source	Primer				PCR	qPCR	Reference database	Quantitative correlation
					Lake	River	Estuary and gulf	Coastal area	Off shore	Port	Offshore oil and gas platform	Farm	Market			12S	16S	18S	COI				
(Afzali *et al.*, 2021)	A	WF	2	1.2			√								TS	√				√		BOLD	Yes
(Aglieri *et al.*, 2023)	A	WF	2	0.45						√						√			√	√		GENBANK	No
(Ahn *et al.*, 2020)	A	WF	0.6	0.45			√									√				√		GENBANK	No
(Albonetti *et al.*, 2023)	A/P	SLU/ME		0.2					√						CC	√				√		GENBANK	No
(Alexander *et al.*, 2022)	A	WF	1.7	0.22							√				ROV		√			√		GENBANK	No
(Alexander *et al.*, 2023)	A	WF	1.7	0.22							√				BF + SE		√			√		GENBANK	No
(An, Mun and Kim, 2023)	A	WF	10	0.45		√										√				√		MitoFish	No
(Andres *et al.*, 2023)	A	WF	1.75	1	√										CBS	√				√		GENBANK	No
(Antognazza *et al.*, 2021)	A	WF	1	0.45		√									CBS	√				√		GENBANK	Yes
(Bakker *et al.*, 2017)	A	WF	4	0.45					√										√	√		Cust	No
(Bessey *et al.*, 2020)	A	WF	0.14	0.45					√								√			√		Cust	No
(Bessey *et al.*, 2021)	P	MM	1	0.45					√						WF		√			√	√	Cust	No
(Burian *et al.*, 2023)	A	WF	1.4	0.8				√								√				√		GENBANK	No
(Cavallo *et al.*, 2020)	A	FP												√			√			√		GENBANK	Yes
(Cicala *et al.*, 2024a)	A	SLU							√						TS	√			√	√		GENBANK	No

Reference	Approach	Method of sample collection	Mean volume (L)	Filter size (µm)	Freshwater		Transitional water	Marine water		Infrastructure				Land	Accessory data source	Primer				PCR	qPCR	Reference database	Quantitative correlation
					Lake	River	Estuary and gulf	Coastal area	Off shore	Port	Offshore oil and gas platform	Farm	Market			12S	16S	18S	COI				
(Closek *et al.*, 2019)	A	WF	1	0.22					√						TS	√				√		GENBANK	No
(Cole *et al.*, 2022)	A	WF	1	0.45	√										BRUV	√	√			√		GENBANK	No
(Cortez *et al.*, 2025)	A	WF	1	0.22		√										√	√		√	√		GENBANK	No
(Cote *et al.*, 2023)	A	WF	1.5	0.22					√						AS	√		√	√	√		GENBANK	No
(Deeg *et al.*, 2025)	A	WF	2	0.22		√		√	√							√			√	√	√	GENBANK	No
(Di Muri *et al.*, 2020)	A	WF	2	0.45	√										CBS	√				√		GENBANK	Yes
(Dugal *et al.*, 2024)	A	WF	1	0.45				√							BRUV		√	√		√	√	GENBANK	No
(Euclide *et al.*, 2021)	A	WF	0.5			√									CBS	√				√		GENBANK	No
(Fernandez *et al.*, 2024)	A	WF	2	0.2					√									√	√	√		GENBANK	No
(Fontes *et al.*, 2024)																√	√			√		GENBANK	No
(Fraija-Fernández *et al.*, 2020)	A	WF	5	6					√						TS	√			√	√		GENBANK	No
(Frühe *et al.*, 2021)	A	SE												√			√	√		√		GENBANK	No
(Garcia-Vazquez *et al.*, 2021)	A	WF	6	0.2					√									√	√	√		GENBANK	No
(Gehri *et al.*, 2021)	A	WF	1	0.45	√										CBS	√	√			√		GENBANK	No
(Gibson *et al.*, 2024)	A	WF	2	0.22			√								TS	√				√		GENBANK	No

Reference	Approach	Method of sample collection	Mean volume (L)	Filter size (µm)	Freshwater		Transitional water	Marine water		Infrastructure				Land	Accessory data source	Primer				PCR	qPCR	Reference database	Quantitative correlation
					Lake	River	Estuary and gulf	Coastal area	Off shore	Port	Offshore oil and gas platform	Farm	Market			12S	16S	18S	COI				
(Gold *et al.*, 2021)	A	WF	4	0.22	√										UVC	√				√		Cust	No
(Gold *et al.*, 2024)	A	FP		0.22				√							CBS	√				√		Cust	Yes
(Good *et al.*, 2022)	A	SE						√										√	√	√		GENBANK	No
(Green *et al.*, 2024)	A	WF	1						√								√			√		GENBANK	No
(Guimarães-Costa *et al.*, 2020)	A	TI						√							CC				√	√		GENBANK	No
(He *et al.*, 2023a)	A	WF	1	0.22				√	√						TS	√	√	√	√	√		GENBANK	No
(He *et al.*, 2023b)	A	WF		0.3					√							√				√		GENBANK	No
(Ip *et al.*, 2024)	A	WF	2	0.45			√								CC	√				√		GENBANK	No
(Jeunen *et al.*, 2024)	P	MS						√							CBS		√			√	√	Cust	No
(Jiang *et al.*, 2023)	A	WF	15	0.45			√								TS	√				√		Cust	No
(Karahan *et al.*, 2017)	A	TI					√								CBS				√	√		BOLD	No
(Kasmi *et al.*, 2024)	A	WF	5	0.45					√						TS	√				√		MARE-MAGE	No
(Lee, Liao and Hsu, 2021)	A	WF	1	0.45									√			√			√	√		MitoFish	No
(Li *et al.*, 2023)	A	TI					√												√	√		BOLD	No
(Li *et al.*, 2022)	A	WF	0.8	0.45		√									CBS + HS	√				√		GENBANK	No
(Liu *et al.*, 2019)	A	WF	1	0.45			√									√				√		GENBANK	No

Reference	Approach	Method of sample collection	Mean volume (L)	Filter size (µm)	Freshwater		Transitional water	Marine water		Infrastructure				Land	Accessory data source	Primer				PCR	qPCR	Reference database	Quantitative correlation
					Lake	River	Estuary and gulf	Coastal area	Off shore	Port	Offshore oil and gas platform	Farm	Market			12S	16S	18S	COI				
(Liu *et al.*, 2022)	A	WF	2	250				√							TS	√				√		GENBANK	No
(Madduppa *et al.*, 2022)	A	WF	3	0.45				√									√			√		GENBANK	No
(Maggini *et al.*, 2024)	A	CW	0.05	0.22				√								√	√		√	√		Cust	No
(Maggio *et al.*, 2023)	A	WF	2	0.45				√											√	√		GENBANK	No
(Mahon, Grey and Jerde, 2023)	A	WF			√											√	√		√	√		Cust	No
(Maiello *et al.*, 2024)	P	ME							√							√				√		GENBANK	No
(Maiello *et al.*, 2023)	A/P	SLU/ME							√						CC	√			√	√		GENBANK	No
(Manel *et al.*, 2024)	A	WF	30	0.2						√						√				√		Cust	No
(Maracle *et al.*, 2024)	A	WF	1	1.0		√									CBS	√				√		Cust	No
(Mathon *et al.*, 2022)	A	WF	16	0.2			√								UVC	√				√		ENA	No
(McClenaghan *et al.*, 2020)	A	WF	1.5	0.2				√							CBS	√		√	√	√		GENBANK	No
(Meulenbroek *et al.*, 2022)	A	WF	22	0.45	√											√				√		Cust	No
(Miya, 2022)	A	WF	1	0.45												√				√		GENBANK	No
(Pukk *et al.*, 2021)	A	WF	1	0.45	√										CBS	√	√			√		Cust	No
(Qiu *et al.*, 2023)	A	WF	1	0.45											CBS	√				√		GENBANK	No

Reference	Approach	Method of sample collection	Mean volume (L)	Filter size (µm)	Freshwater		Transitional water	Marine water		Infrastructure				Land	Accessory data source	Primer				PCR	qPCR	Reference database	Quantitative correlation
					Lake	River	Estuary and gulf	Coastal area	Off shore	Port	Offshore oil and gas platform	Farm	Market			12S	16S	18S	COI				
(Rahuman *et al.*, 2024)	A	WF	0.5	0.22	√													√		√		Silva 1	No
(Rehill *et al.*, 2024)	A	WF	5	0.2				√							TS	√				√		GENBANK	No
(Rey *et al.*, 2023)	A	WF	2	0.22			√								UVC	√	√			√		GENBANK	No
(Richards *et al.*, 2022)	A	WF	1	0.5									√		PS		√			√		MitoFish	No
(Russo *et al.*, 2020)	A	SLU							√						TS	√			√	√		GENBANK	No
(Saiperakiet *al.*, 2024)	A	TI				√									CBS				√	√		GENBANK	No
(Sard *et al.*, 2019)	A	WF	1	0.45	√										CBS	√	√			√	√	Cust	No
(Sato *et al.*, 2021)	A	WF	10	0.45				√								√				√	√	GENBANK	No
(Sbrana *et al.*, 2024)	A/P	SLU/ ME						√								√			√	√		GENBANK	No
(Schiøtt *et al.*, 2023)	A	WF	2					√							SIA + LK	√				√		GENBANK	No
(Seemani *et al.*, 2025)	A	WF	1	0.45		√										√				√		MitoFish	No
(Seymour *et al.*, 2020)	A	WF	1	0.22	√											√		√	√	√		GENBANK	No
(Shaw *et al.*, 2019)	A	SE								√								√		√		Cust	No
(Shen *et al.*, 2023)	A	WF	6	0.45		√									CBS	√				√		GENBANK	No
(Shen *et al.*, 2022)	A	WF	1	0.45		√									CBS	√				√		MitoFish	No
(Simões *et al.*, 2025)	A	WF	2						√							√				√		GENBANK	No

Reference	Approach	Method of sample collection	Mean volume (L)	Filter size (µm)	Freshwater		Transitional water	Marine water		Infrastructure				Land	Accessory data source	Primer				PCR	qPCR	Reference database	Quantitative correlation
					Lake	River	Estuary and gulf	Coastal area	Off shore	Port	Offshore oil and gas platform	Farm	Market			12S	16S	18S	COI				
(Son Hwa-Seong *et al.*, 2023)	A	WF	4				√												√	√		GENBANK	No
(Stat *et al.*, 2019)	A	WF	0.5	0.45				√							BRUV		√			√		GENBANK	No
(Stepien, Snyder and Elz, 2019)	A	TI				√													√	√		GENBANK	No
(Stoeckle *et al.*, 2021)	A	WF	1	0.45				√							TS	√				√		Cust	No
(Suter *et al.*, 2023)	A	WF	1	5					√								√			√	√	GENBANK	Yes
(Teed *et al.*, 2024)	A	WF	1	0.8			√								UVC				√	√	√	GENBANK	No
(Thomsen *et al.*, 2016)	A	WF	2	0.45				√							TS	√				√		GENBANK	Yes
(Urban *et al.*, 2024)	A	WF	0.045						√						CBS	√					√	Cust	Yes
(Ushio *et al.*, 2023)	A	WF	8					√								√				√	√	Cust	No
(Valdivia-Carrilloet *al.*, 2021)	A	WF	1	0.44			√								UVC	√				√		GENBANK	No
(Valsecchi *et al.*, 2021)	A	WF	13	0.45					√							√	√			√		GENBANK	No
(Westgaard *et al.*, 2024)	A	WF	5	0.22					√						TS	√				√		GENBANK	No
(Willette *et al.*, 2021)	A	SLU							√						CC	√				√		CRUX	No

Reference	Approach	Method of sample collection	Mean volume (L)	Filter size (µm)	Freshwater		Transitional water	Marine water		Infrastructure				Land	Accessory data source	Primer				PCR	qPCR	Reference database	Quantitative correlation
					Lake	River	Estuary and gulf	Coastal area	Off shore	Port	Offshore oil and gas platform	Farm	Market			12S	16S	18S	COI				
(Wu *et al.*, 2022)	A	WF	1.5	0.7					√							√				√	√	GENBANK	Yes
(Wu *et al.*, 2024)	A	WF	1	0.45	√											√				√		GENBANK	No
(Xiao, Li and Li, 2022)	A	WF	1	0.45	√										CBS	√			√	√		Cust	No
(Yamamoto *et al.*, 2017)	A	WF	1	0.7				√								√				√		MitoFish	No
(Yang and Zhang, 2020)	A	WF	2	0.45		√										√				√		GENBANK	No
(Yang *et al.*, 2023)	A	WF	1	0.45	√											√			√	√		GENBANK	No
(Yang and Zhang, 2020)	A	ZO	20		√														√	√		GENBANK	No
(Zhang *et al.*, 2025)	A	WF	1	0.2			√									√				√		GENBANK	No
(Zhang *et al.*, 2023)	A	WF	1	0.45		√										√				√		GENBANK	No
(Zhou *et al.*, 2022)	A	WF	1	0.45				√							TS	√				√		GENBANK	No
(Zhu *et al.*, 2023)	A	WF	12				√								TS + SE + CBS	√				√		MitoFish	No
(Zou *et al.*, 2020)	A	WF	20	0.8			√								TS	√				√		GENBANK	No

Notes: A = active, AS = acoustic survey, BF = biofoul, BRUV = baited remote underwater video, CBS = catch biological sampling, CC = commercial catch, CW = catch water, HS = hydroacoustic surveys, FP = faecal pellet, LK = local knowledge, ME = metaprobe, MM = membranes, MS = marine sponges, P = passive, PCR = polymerase chain reaction, qPCR = quantitative polymerase chain reaction, PS = photographic surveys, ROV = remotely operated vehicle, SE = Sediment, SIA = stable isotope analysis, SLU = slush, TI = tissues, TS = trawling survey, UVC = underwater camera, WF = water filtration, ZO = Zooplankton.

The studies presented were identified on 9 March 2025 through the Scopus platform using a search of the keywords “eDNA metabarcoding” and “fisheries”.

Source: Elsevier. 2026. Sources. In: *Scopus*. Amsterdam. [Cited 27 February 2026]. www.scopus.com/sources

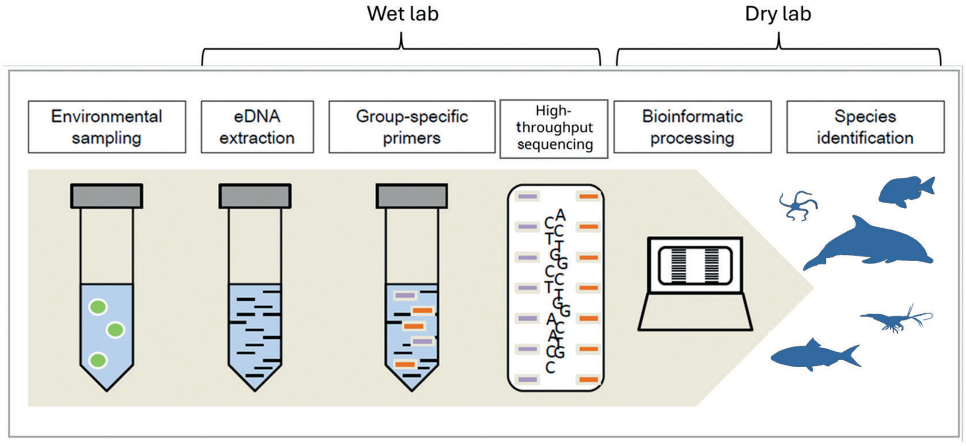
2. General workflow for environmental DNA metabarcoding during fishing activities

The eDNA metabarcoding workflow begins with the development, validation and sharing of a general protocol, which can be broken down into three key stages (Figure 2):

- field sampling, storing and pre-processing;
- wet lab (DNA extraction, amplification and sequencing); and
- dry lab (bioinformatics and data analysis).

FIGURE 2

Environmental DNA metabarcoding workflow



Source: Adapted from INRAE. 2024. Métabarcoding. In: *Diagnopict*. Paris. [Cited 5 June 2026]. https://diagnopict.hub.inrae.fr/techniques/metabarcoding

2.1 Field sampling

Environmental DNA can be collected using a variety of active and passive approaches, each suited to specific research goals and environmental contexts. An approach is considered “active” when human intervention is required to concentrate the eDNA present in an aquatic medium. Active approaches typically involve direct sampling strategies, such as collecting water, which is then filtered – using vacuums and peristaltic pumps or manual syringes – to concentrate suspended genetic material. They also include the retrieval of biofilms, plankton or sediments, which contain mixtures of DNA from various organisms associated with the habitat in question. In contrast, an approach is considered “passive” when it is based on the deployment of structures and surfaces that autonomously accumulate or absorb eDNA without the need for active water processing (Bessey *et al.*, 2021; Jeunen *et al.*, 2024; Maiello *et al.*, 2022). These include membrane-based samplers deployed for extended periods, metaprobes (specially designed spherical devices with porous surfaces that capture genetic material) and slush water (i.e. water drained from fishing nets and trawls), which contains eDNA released by organisms during fishing operations. The retrieval of DNA fragments naturally trapped in the tissues of filter-feeding organisms is a passive approach that has been gaining traction. This method has shown the greatest success when using marine sponges (Cai *et al.*, 2024; Mariani *et al.*, 2019; Neave *et al.*, 2023) and, in some cases, has proven successful in association with fishing activities (Jeunen *et al.*, 2024). The collection of eDNA from catch waters, defined as the waters of the tanks containing caught animals, biofilms and scats, although sharing some characteristics with passive sampling, is generally considered active since a filtration step is required.

Collectively, active and passive approaches provide a wide range of tools that can be applied to various scenarios, thereby enhancing the spatial and temporal resolution of biodiversity assessments in aquatic ecosystems. Although most studies rely on active eDNA collection approaches, passive approaches have produced largely comparable results. This imbalance likely reflects the greater familiarity with and standardization of active techniques, while also suggesting a potential for further expansion and innovation of passive eDNA sampling strategies.

2.1.1 Active approaches

Active sampling approaches provide researchers with a high level of control over the sampling process, allowing them to precisely define the sampling location, volume and timing, thereby improving data consistency and reproducibility. They tend to yield high concentrations of eDNA, particularly when conducted shortly after an organism is present. However, active approaches can be labour-intensive and logistically demanding, requiring specialized equipment, personnel and sometimes dedicated vessels or field stations. Their effectiveness can be limited in remote or extreme environments, and repeated sampling may introduce sampling bias or disturb the ecosystem. Despite these limitations, active approaches are the backbone of most eDNA monitoring efforts due to their robustness and reliability.

The amount of eDNA in aquatic environments varies significantly (Chavez *et al.*, 2021), depending on the number of organisms present, the rate of tissue and DNA degradation, as well as several environmental factors, including currents and vertical motion (Collins *et al.*, 2018). For this reason, active approaches almost always require an initial concentration step. However, this step is not needed when researchers use fishing techniques to artificially concentrate eDNA. When many organisms are confined in a small volume, as in the case of capture waters (Maggini *et al.*, 2024), or are confined and subjected to stress, as inside a fishing net, the concentration of eDNA can increase. For example, the dense water draining from fishing nets just after the end of hauling operations, also known as “slush” (Russo *et al.*, 2020), contains a high concentration of eDNA and can be used to collect representative catch samples (Plate 1) (Maggini *et al.*, 2024; Russo *et al.*, 2020).

PLATE 1
Researchers filtering water samples (left) and collecting slush from a trawl net (right)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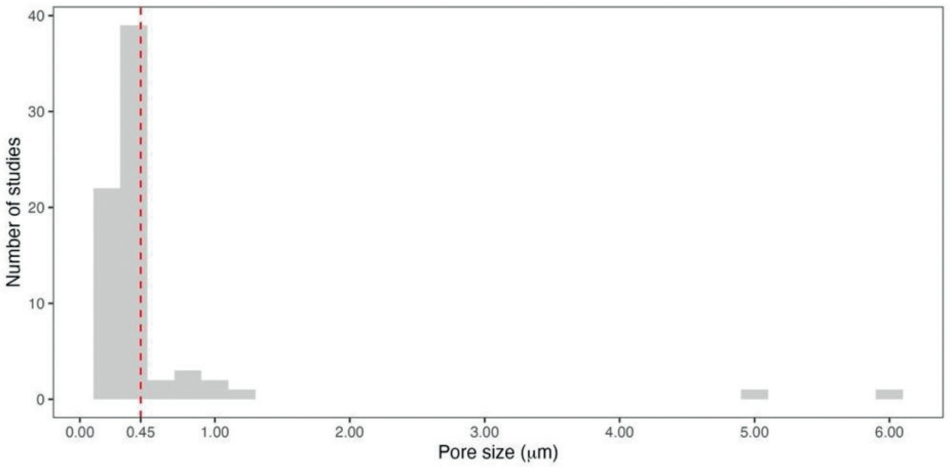
The volume of water required to extract eDNA varies greatly depending on the type of aquatic matrix. Larger volumes are typically needed for natural environments, such as seas, rivers and lakes, whereas smaller volumes suffice for concentrated sources, such as catchment tanks or slush. In shallow or surface waters, water collection is relatively simple and can be carried out using pumps. However, sampling becomes more challenging in less accessible areas, such as deep waters, where collection is generally limited to the use of Niskin bottles, which restrict the volume that can be obtained.

Once the water has been collected, it must be filtered to further concentrate the eDNA that is present. While molecular techniques, such as PCR and sequencing, are used to concentrate eDNA segments, they are based on processing small samples, on the order of millilitres. In contrast, filtration enables the concentration of DNA from larger volumes of water, thereby enhancing the detection of rare species (Deiner *et al.*, 2015; Shu, Ludwig and Peng, 2020). Filtration can be conducted in a laboratory setting or during field sampling using enclosed filters and pumps or syringes to drive water through a membrane (Schumer *et al.*, 2019; Thomas *et al.*, 2018). However, in certain coastal habitats where water is eutrophic and rich in sediment and plankton, filters may clog rapidly, limiting efficiency and requiring larger volumes of water. After use, the filters must be safely frozen or stored in a preservative, and great care should be taken to avoid proximity with spaces or equipment that have been in contact with fish or other animal tissues, to avoid DNA contamination.

Most studies that have been recently conducted on eDNA employed filters with pore sizes of around 0.45 µm (Figure 3), reflecting a general preference for fine filtration to maximize eDNA capture efficiency. Only a few studies utilize larger pore sizes, highlighting their limited application.

FIGURE 3
Pore sizes of the nitrocellulose filters used during environmental DNA metabarcoding and fisheries studies, 2016–2025



Note: The studies presented were identified on 9 March 2025 through the Scopus platform using a search of the keywords "eDNA metabarcoding" and "fisheries".

Source: Elsevier. 2026. Sources. In: *Scopus*. [Cited 27 February 2026]. www.scopus.com/sources

2.1.2 Passive approaches

Passive sampling approaches eliminate the need for water filtration and require only the deployment and retrieval of the sampling device. The inspiration for passive sampling came from natural organisms that, based on their ecology and physiology, collect eDNA present in an aquatic environment. Marine sponges (phylum Porifera) have been used for this purpose and are a potential source of information (Cai *et al.*, 2024; Harper *et al.*, 2023; Jeunen *et al.*, 2024; Mariani *et al.*, 2019). Based on this concept, filter membranes (non-charged cellulose ester and positively charged nylon) have been successfully deployed by researchers in the water column to passively capture eDNA (Bessey *et al.*, 2021).

With the support of the GFCM, the University of Rome Tor Vergata has developed and applied a new tool for passive eDNA collection that can be deployed during fishing operations. This tool, called a metaprobe (Plate 2), is a three-dimensionally printed, hollow, perforated sphere made of polylactide (a biodegradable plastic material) that contains sterile gauze to adsorb eDNA from the surrounding environment (Maiello *et al.*, 2022). It can be placed inside a fishing net or tied to an otter board of a trawl net to efficiently collect eDNA and provide a broad view of local biodiversity (Cicala *et al.*, 2024; Maiello *et al.*, 2022, 2023, 2024). Its use integrates with fishing activities without adding undue impacts or complicating monitoring activities during scientific surveys. When deployed during trawl fishing, the metaprobe is placed inside the codend before setting the gear and can be easily retrieved during catch sorting (Plate 3). It has also been successfully used in conjunction with various other fishing gear (Figure 4), including longliners (Spiga *et al.*, 2026). A detailed protocol for the use of metaprobes to sample eDNA during fishing activities is presented in the Appendix.

PLATE 2
Printed metaprobe

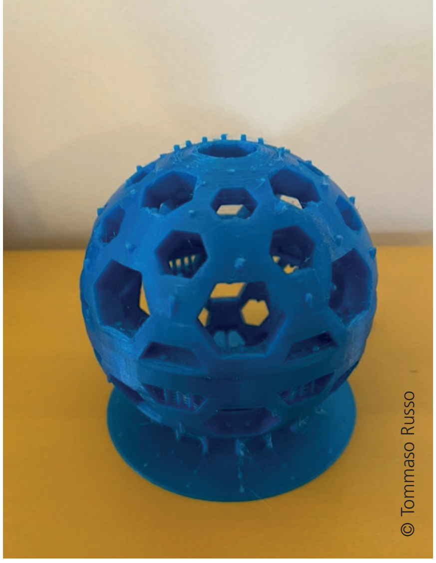


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PLATE 3
Metaprobe inside a trawl net (top left), tied to the otter board of a trawl net (top right) and on deck during catch sorting (bottom)

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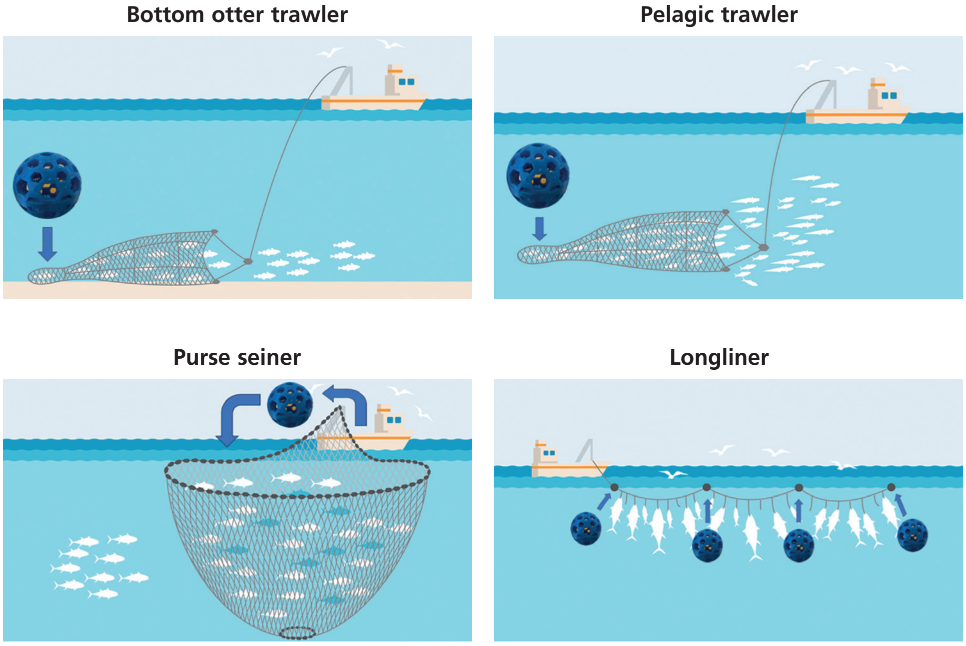
FIGURE 4
Metaprobe placement by type of fishing gear



Bottom otter trawler

Pelagic trawler

Purse seiner

Longliner

Source: Adapted from Marine Stewardship Council. 2026. Fishing methods and gear types. In: *MSC*. [Cited 12 March 2026]. https://www.msc.org/what-we-are-doing/our-approach/fishing-methods-and-gear-types

Metaprobes are an effective, convenient, inexpensive and accessible tool. The raw materials required for their production – biodegradable polylactide plastic and sterile gauze – are widely available and inexpensive, and the three-dimensional printer needed to produce metaprobes does not require any particular specifications. At the laboratories of the Department of Biology of the University of Rome Tor Vergata, a Prusa i3 MK3S+ model is used, which requires approximately 12 hours to print a metaprobe of average quality (Plate 4).[1]

Metaprobes are quick and easy to assemble, and ready-to-use kits can be prepared and packaged to facilitate their deployment, such as the kit shown in Plate 5. The kit contains a ready-made metaprobe with two gauze rolls and two test tubes for holding and storing the gauze after use. Common fixatives, such as ethanol, or inexpensive materials, such as silica gel, can be used to preserve the gauze containing captured eDNA inside the test tubes. The samples should then be stored on ice until they are transferred to a laboratory for analysis where they should be frozen at −20 °C. Each metaprobe is reusable countless times, after appropriate sterilization.

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[1] The specifications of the project are publicly available in a dedicated GitHub repository (Bezombes *et al.*, 2022).

PLATE 4
Metaprobe printing process (left) and the final product (right)

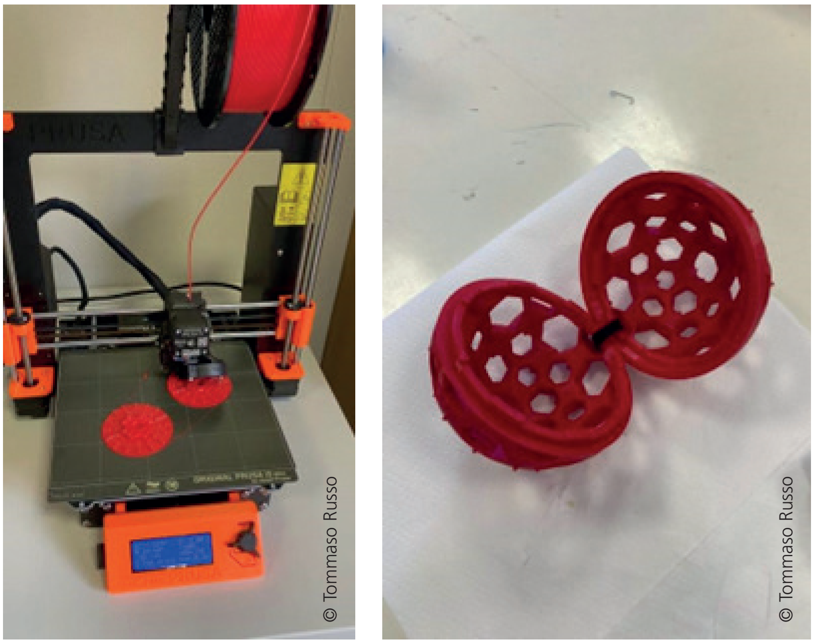
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PLATE 5
Sampling kit containing a ready-made metaprobe and two test tubes


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2.2 Wet lab: extraction, amplification and sequencing

Following sample collection and appropriate storage, eDNA must be extracted, amplified and sequenced. Although a range of bespoke and modular DNA isolation methods are available (Dabney and Meyer, 2019; Sellers *et al.*, 2018), extraction is normally conducted using the commercial kits most suitable for the matrices from which DNA will be extracted, as these provide the quickest and most standardized option.

To minimize the risk of external contamination during sample processing, DNA extraction and PCR amplification are carried out using full coverall suits, masks and hoods in a dedicated eDNA laboratory, where materials are sterilized using 10 percent bleach, rinsed with 70 percent ethanol and subsequently irradiated with ultraviolet light. Throughout the entire laboratory workflow, filter pipette tips and clean gloves are used (Champlot *et al.*, 2010; Goldberg, Strickler and Pilliod, 2015). Replicates (Ficetola, Taberlet and Coissac, 2016), as well as negative controls (i.e. only reagents with no biological sample) and positive controls (i.e. known DNA composition) should be included to monitor for possible contamination of reagents, cross-contamination between samples or the accidental transfer of a sample-specific barcode tag from one sample to another (i.e. tag switching) (Deiner *et al.*, 2017a; Ficetola, Taberlet and Coissac, 2016).

Depending on the type of sample, extraction protocols can be slightly modified, including additional steps for sample preparation prior to extraction. For active approaches, water leaking from the net codend (i.e. slush) or contained in the catch hold is usually collected directly into sterile tubes. Once in the laboratory, samples are processed using one of two main methods: i) tubes are centrifuged to obtain a pellet from which DNA is directly extracted, as described by Russo *et al.* (2020); or ii) water is filtered with a vacuum pump through DNA-cellulose filters (0.2 µm) to concentrate the DNA before extraction, following the procedure outlined by Maiello *et al.* (2023). For passive approaches, sterile gauze from sampling tools, such as the metaprobe, is collected and immediately placed in sterile tubes containing ethanol or silica gel for DNA preservation. The DNA is then extracted directly from the gauze by cutting small pieces off various parts of the rolls using sterile scissors and forceps. The amount of gauze used for DNA extraction is usually adjusted to fit into a 1.5 ml Eppendorf tube. If samples are stored in ethanol, the gauze pieces are dried before extraction, either using blotting paper or by letting the ethanol evaporate under a clean hood. The full extraction procedure from metaprobe gauze rolls is described by Maiello *et al.* (2022).

Once the DNA has been extracted, various molecular techniques can be employed to screen it, depending on the purpose of the study. These techniques fall into two main categories: i) narrow-focus analyses aimed at detecting a single species; and ii) broad-spectrum analyses aimed at characterizing a multitude of taxa simultaneously (Table 2).

TABLE 2

Molecular techniques for environmental DNA analysis

Category	Technique	Description	Output
Single-species analysis	Real-time quantitative PCR	Uses highly specific primers to amplify and quantify DNA from a target species or taxon. The DNA is extracted, amplified and compared to standard curves.	Provides DNA copies per microlitre of the target species or taxonomic group in the sample.
	Digital droplet PCR	Sample is partitioned into thousands of droplets, each undergoing PCR amplification to estimate target DNA presence and abundance.	Gives absolute DNA concentration estimation without needing standard curves.
Multi-species analysis	Metabarcoding	Uses high-throughput sequencing of PCR-amplified DNA with primers targeting specific organism groups.	Produces millions of sequence reads representing multiple variants of the target marker gene, which should reflect the taxon assemblage under study.
	Metagenomics	Direct sequencing of all extracted DNA without PCR amplification, followed by bioinformatic analysis.	Reflects relative organism abundance more realistically but may be biased towards bacterial DNA and degraded fragments.

This protocol focuses on the use of the metabarcoding technique as it allows for the detection of multiple taxa and can provide a comprehensive biodiversity assessment. For eDNA metabarcoding studies, the selection of appropriate PCR primers and metabarcodes is critical. A perfect metabarcode should aim to maximize: i) amplification universality, i.e. the scenario whereby a primer pair would be able to equally amplify any existing member of a target taxon; ii) amplification specificity, i.e. the ability of a primer pair to exclusively amplify the members of a taxon; iii) taxonomic discrimination, i.e. the capacity of a metabarcode to correctly distinguish sequences belonging to different species; and iv) representativeness in reference databases, i.e. the scenario where all members of a target taxon have a verified, publicly accessible reference sequence for in silico identification (Collins *et al.*, 2018; Miya, Gotoh and Sado, 2020).

Mitochondrial genes are used as standard markers for metabarcoding because of their taxonomic species-discrimination power and the greater abundance of their gene copies. Multiple primer pairs have been designed for DNA metabarcoding analysis across a wide range of taxonomic groups, such as bacteria, arthropods, fishes, mammals, vertebrates and metazoans (Taberlet *et al.*, 2018). In particular, the 12S ribosomal DNA (rDNA) gene (e.g. MiFish primers) is commonly used for vertebrate detection, the cytochrome c oxidase subunit I gene (COI) (e.g. Leray/Folmer primers) targets a wide range of metazoans, the 18S rDNA gene primarily captures eukaryotic diversity, and the 16S rDNA gene (V2-V3, V3-V4, V5-V6 regions [Bukin *et al.*, 2019; Leoni *et al.*, 2020]) is used for profiling bacterial and archaeal communities.

Genes and primers can vary in their coverage, resolution and bias toward certain taxa. Indeed, primer selection may bias results by preferentially amplifying certain target sequences more than others or by amplifying non-target groups (Cristescu, 2014). An approach to address this issue involves the use of multiple primer sets, potentially coupling broad-spectrum primers with more specific ones (Ficetola and Taberlet, 2023). The number of replicates is also important for PCR amplification, as multiple PCR replicates increase species detection and reduces the occurrence of false negatives (Alberdi *et al.*, 2018; Ficetola, Taberlet and Coissac, 2016). However, PCR replicates are not a substitute for biological replicates; both are essential to ensure robust and comprehensive results (Goldberg *et al.*, 2016).

During PCR amplification for next-generation sequencing (NGS), primers are tagged with short nucleotide sequences, commonly known as indices or barcodes, to uniquely identify their source in a process referred to as multiplexing. The primer sets most commonly adopted in fisheries studies, and more generally for the characterization of fish communities and assemblages, are reported in Table 3. All NGS platforms perform the sequencing of millions of small DNA fragments in parallel. Although several NGS platforms using different sequencing technologies are available, Illumina sequencing currently outperforms other NGS platforms in terms of depth and accuracy (Deiner *et al.*, 2017b).

TABLE 3

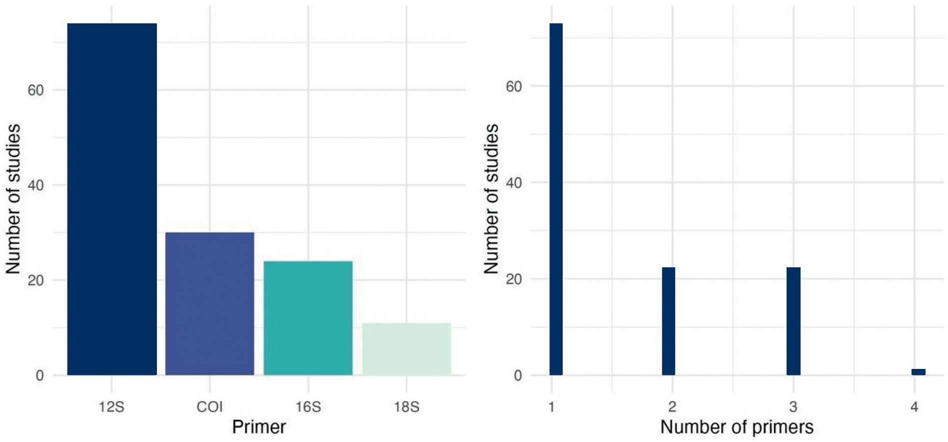
Commonly used primer pairs for fish community biodiversity assessments

Barcode region	Primer name	Reference
12S	MiFish U/E	Miya *et al.*, 2015
12S	Tele02	Taberlet *et al.*, 2018
12S	Elas02	Taberlet *et al.*, 2018
12S	L1848/H1913	Valentini *et al.*, 2016
12S	12S-V5	Riaz *et al.*, 2011
COI	mlCOlintF/jgHCO2198	Leray *et al.*, 2013
COI	FishF1/R1	Ward *et al.*, 2005
16S	Fish16sF/D	Berry *et al.*, 2017
16S	L2513/H2714	Kitano *et al.*, 2007
cytb	L14912-CYB	Minamoto *et al.*, 2012

Recent studies conducted on the use of eDNA metabarcoding in fisheries (see Table 1) show a predominant use of 12S primers, followed by COI, 16S and 18S in similar proportions (Figure 5). The majority of these studies used a single primer set.

FIGURE 5

Type and quantity of primers used during environmental DNA metabarcoding and fisheries studies, 2016–2025



Note: The studies presented were identified on 9 March 2025 through the Scopus platform using a search of the keywords “eDNA metabarcoding” and “fisheries”.

Source: Adapted from Elsevier. 2026. Sources. In: *Scopus*. [Cited 27 February 2026]. www.scopus.com/sources

2.3 Dry lab: bioinformatics and data analysis

After sequencing, raw sequencing data undergo bioinformatic processing using dedicated software and pipelines to retrieve the nucleotide sequences that can be assigned to species or haplotypes. A key challenge at this stage is to filter out molecular artefacts from the sequencing process to produce a final, reliable taxonomic output. Several packages and platforms have been developed for NGS data analysis, such as PhyloPythia, Mothur, Usearch, QIIME, OBITools, Cutadapt and various R software packages and tools (Mathon *et al.*, 2022; Sigsgaard *et al.*, 2017; Zhang *et al.*, 2017). The main bioinformatic steps are:

- demultiplexing – separating sequences belonging to different samples that were sequenced together;
- quality filtering – removing reads with low-quality bases;
- primer removal – trimming primer sequences from the sequenced reads, so they only contain taxonomically relevant information;
- pair merging – alignment and merging of the two paired-end reads to retrieve the sequences;
- dereplication – removing duplicate sequences;
- chimera removal – removing artefact sequences deriving from the amplification of fragments that used DNA templates belonging to different species;
- clustering/denoising – selecting real haplotypes and grouping them into taxonomic units; and
- taxonomic assignment – matching sequences to the most likely taxa using reference databases.

Taxonomic assignment is a critical step in metabarcoding data analysis. It can be performed by comparing the sequences obtained with those deposited in public and specific reference databases, such as MIDORI2, MitoFish, Mare-MAGE, GenBank, the European Molecular Biology Laboratory, the Barcode of Life Data System, SILVA and the European Nucleotide Archive (Table 4).

TABLE 4

Main databases for sourcing reference data to identify environmental DNA-derived sequence reads

Database	Target gene	Target organisms	Link
BOLD	COI	Universal	https://boldsystems.org
EMBL			http://ebi.ac.uk/embl
ENA		Universal	https://ebi.ac.uk/ena/browser/home
GENBANK		Universal	https://GENBANK.nlm.nih.gov/genbank
Mare-MAGE	12S and COI	Fish	https://mare-mage.weebly.com
MIDORI2		Universal	https://reference-midori.info
MITOFISH	12S	Fish	https://mitofish.aori.u-tokyo.ac.jp
SILVA	16S, 18S, 23S and 28S	Bacteria, Archea and Eukarya	https://arb-silva.de
Sequence reads archive		Universal	https://ncbi.nlm.nih.gov/sra

Several studies have shown that comprehensive, curated and well-annotated reference databases are critical for rigorous taxonomic assignment (Gold *et al.*, 2021; Hleap *et al.*, 2021; Leray, Knowlton and Machida, 2022). Furthermore, the accurate identification of species is strongly dependent on the breadth of sequence data available in reference databases, as incomplete and inaccurate reference sequences can lead to false negatives (i.e. the non-detection of species that are present in a given sample or area) and false positives (i.e. the detection of species that are not present in a given sample or area) (Miya, Gotoh and Sado, 2020; Watts *et al.*, 2019). The current taxonomic coverage of publicly available reference databases remains inadequate for accurate taxonomic assignments, given the enormous diversity of fish, with more than 32 000 known species in aquatic environments worldwide. Enhancing these databases requires international effort and collaboration (Miya, 2022; Nelson, Grande and Wilson, 2016).

For studies focusing on specific genetic markers and geographical regions, the creation of custom-curated reference databases based on user-specific parameters is strongly recommended. Several software and packages have been developed to extract specific amplicon sequences through in silico PCR, local alignments or profile-hidden Markov models. Software, packages and pipelines available to generate custom-reference databases include: Creating Reference databases for Amplicon-Based Sequencing (Jeunen *et al.*, 2023), Meta-Fish-Lib (Collins *et al.*, 2021), ecoPCR in OBITools (Boyer *et al.*, 2016), RESCRIPt (Robeson *et al.*, 2021) and MetaCurator (Richardson *et al.*, 2020).

2.4 Statistical analyses and post-processing

Once the taxonomy is assigned to the sequences, a frequency table is generated showing the number of reads for each sequence. The read count corresponds to the number of times a given sequence is detected within the dataset and can be influenced by several technical aspects (Miya, 2022; Van Der Loos and Nijland, 2021). For this reason, while eDNA metabarcoding is increasingly employed as a primary source of biological information for ecology, conservation and management, including fisheries, the quantitative interpretation of sequence read proportions remains debated. As such, depending on the purpose of the study, metabarcoding data can be treated in two ways:

- presence/absence – presence/absence binary data are used when the aim is to detect non-indigenous, rare or cryptic species, as it is sufficient in such cases to confirm whether the species is present or not; and
- semi-quantitative – within semi-quantitative data processing, the number of reads can be converted into relative proportions or transformed using different methods such as Hellinger, square root and fourth-root; it is more suitable for biodiversity monitoring.

When working with eDNA-metabarcoding data derived from fisheries, molecular data can complement information obtained from the fishing activity, providing insights not only into the species caught by the fishing net but also into the taxa that are present in the surrounding environment (Maiello *et al.*, 2022). If catch information (i.e. the number of individuals and biomass of each species) is accurately reported, technical studies can be conducted to explore the quantitative potential of this method. This represents a significant step toward upscaling marine monitoring efforts and has direct relevance for fisheries management applications.

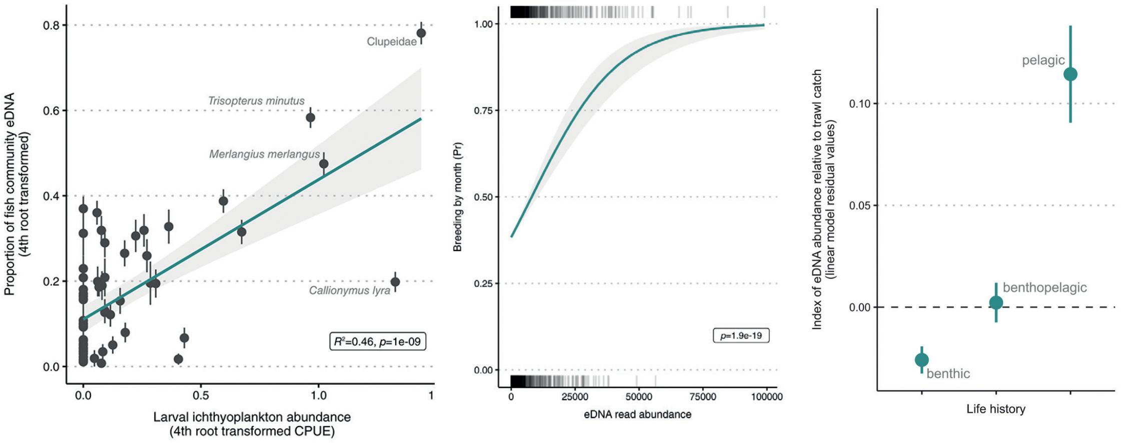
3. Applications

3.1. Monitoring the influence of reproductive and life history traits

At present, eDNA analysis is unable to produce estimates of the sex, size or physiological state of the species detected. However, the mixing of cellular material and DNA particles in the water column is such that when large amounts of gametes or larvae are present, strong eDNA signals should be expected. Collins *et al.* (2022) showed that the reproductive period of marine species is a good predictor of proportional abundance of eDNA reads within the samples. The authors studied the same area for over a year, using trawling, ichthyoplankton surveys and eDNA metabarcoding, and observed that proportional abundance of eDNA in samples was particularly sensitive to the reproductive period of a species. The abundance of species at larval stages in the water column was correlated with their eDNA abundance, and, when a sample was taken during a period when certain species were spawning, there was a greater probability of detecting eDNA peaks for those species (Figure 6). The pelagic or benthic habits of a species were also linked to differences in the proportional abundance of eDNA, with eDNA from pelagic species being relatively more common in water samples than would be expected based on the composition of benthic trawl catches, likely because pelagic species have a low likelihood of capture in bottom trawls (Figure 6). These findings open opportunities for monitoring life-history fluctuations of commercially important species, in space and time, using non-invasive molecular methods.

FIGURE 6

Relationship between root-transformed environmental DNA reads and larval taxon abundance (left); logistic regression of environmental DNA read abundance probability per reproductive month (middle); and residual values of environmental DNA abundance regressed against trawl catch per life history group (right)



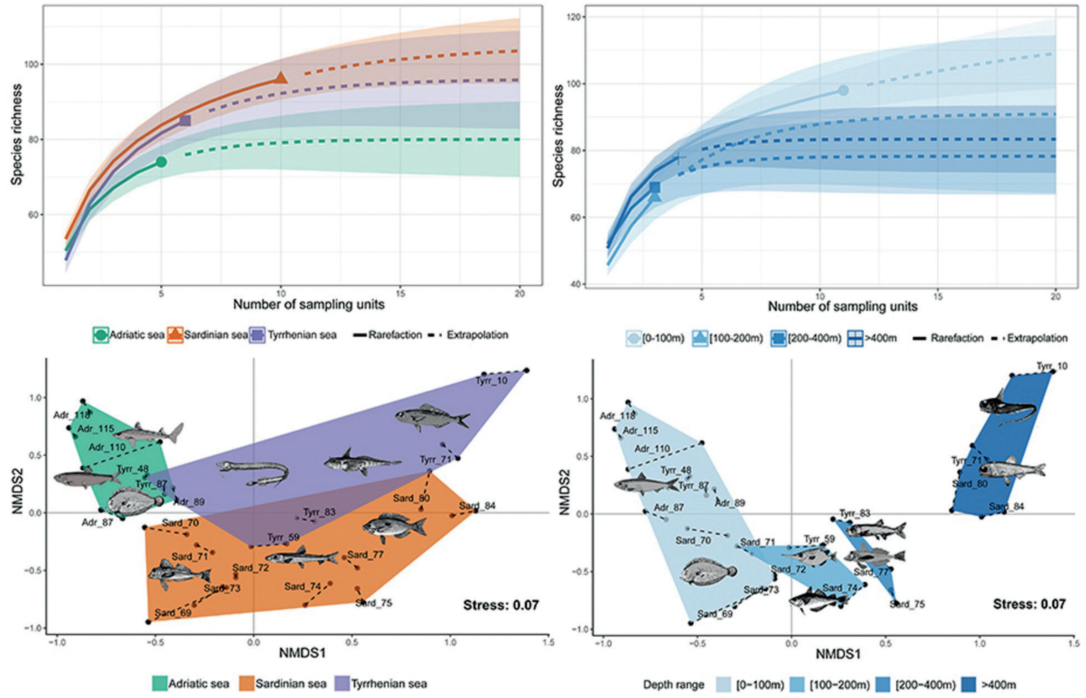
Source: Adapted from Collins, R.A., Baillie, C., Halliday, N.C., Rainbird, S., Sims, D.W., Mariani, S. & Genner, M.J. 2022. Reproduction influences seasonal eDNA variation in a temperate marine fish community. *Limnology and Oceanography Letters*, 7: 443–449.

3.2. Characterizing trawl-associated fish assemblages

Maiello *et al.* (2024) implemented an eDNA-based approach using metaprobes by collecting samples on board trawling survey vessels operating in three around surrounding Italy, namely the northern Adriatic Sea, the Ligurian and Tyrrhenian Seas, and the waters off the coast of Sardinia, Italy. The resulting data identified a total of 108 fish species. Of these, more than 60 percent overlapped with those caught by the trawl net, and eDNA data consistently revealed the presence of additional species, including pelagic taxa that were not sampled using bottom trawls but that were present in the surrounding environment, such as endangered elasmobranchs. The eDNA data allowed for an accurate reconstruction of the fish community composition within the studied areas, revealing differences in species assemblages linked with geographic area and depth (Figure 7). The spatial characterization of the assemblages was more clearly delineated and more robust using data derived from the eDNA-based approach than relying on solely trawl data. The study highlighted the utility of the metaprobe as a versatile and inexpensive tool that can be used in conjunction with pre-existing vessel surveys to increase the range of marine data collection, including for rare and endangered species, such as elasmobranchs.

FIGURE 7

Rarefaction curves of the total taxa detected through environmental DNA metabarcoding, by sampling area (top left) and depth range (top right), and distribution of species assemblages across sampling sites, by sampling area (bottom left) and depth range (bottom right)

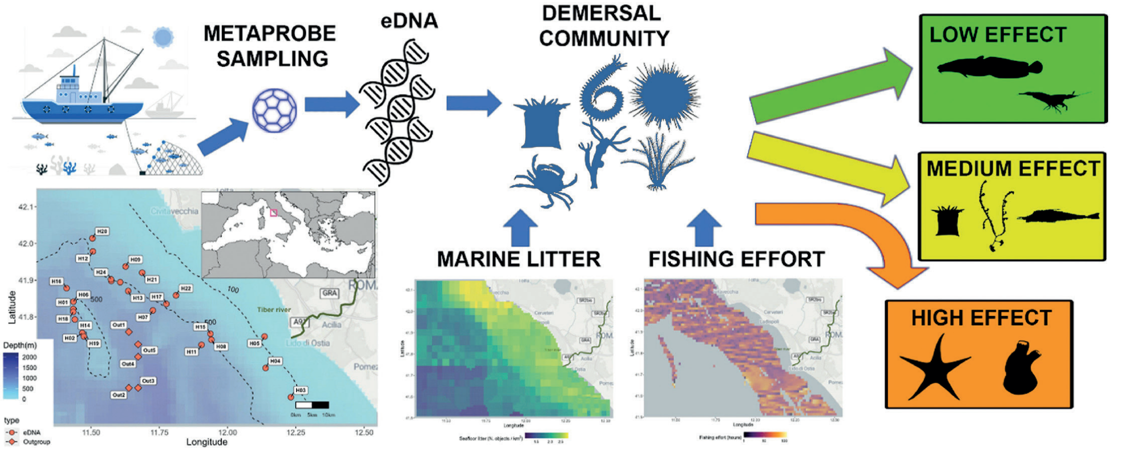


Source: Maiello, G., Bellodi, A., Cariani, A., Carpentieri, P., Carugati, L., Cicala, D., Ferrari, A. *et al.* 2024. Fishing in the gene-pool: implementing trawl-associated eDNA metaprobe for large scale monitoring of fish assemblages. *Reviews in Fish Biology and Fisheries*, 34: 1293–1307.

3.3. Determining the effects of seafloor litter and fishing effort on marine biodiversity

Sbrana *et al.* (2024) studied the relationships between demersal community species composition, environmental features and anthropogenic impacts, including fishing effort and seafloor litter, based on eDNA data collected from the central Tyrrhenian Sea (Figure 8). The application of this method, in combination with statistical analysis (i.e. random forest, cluster analysis and indicator species analysis), enabled the identification of differences in species composition depending on the level of anthropogenic pressure. In particular, it showed that fishing effort and seafloor litter influence species composition and diversity. The study highlighted the use of eDNA-based approaches to assess anthropogenic impacts during scientific surveys, thereby improving the capacity to monitor marine ecosystems and evaluate the effects of pollution.

FIGURE 8
Environmental DNA metabarcoding workflow to determine the effects of seafloor litter and trawling on marine biodiversity



Note: Refer to the disclaimer on page ii for the names and boundaries used in this map.

Source: Adapted from Sbrana, A., Maiello, G., Gravina, M.F., Cicala, D., Galli, S., Stefani, M. & Russo, T. 2024. Environmental DNA metabarcoding reveals the effects of seafloor litter and trawling on marine biodiversity. *Marine Environmental Research,* 106415.

4. Conclusion

Environmental DNA has rapidly evolved into one of the most transformative tools for marine biodiversity assessment and fisheries monitoring. Although scientific literature contains numerous studies demonstrating the potential of this approach for collecting biodiversity data and, potentially, for the sustainable management of human activities at sea, there remains a lag between scientific developments in eDNA-based approaches and their applications for fisheries management. According to Baetscher *et al.* (2025), this lag is partly due to the lack of affordable guidelines and templates for sample collection, processing and data analysis.

The methodology reviewed in this protocol demonstrates that eDNA-based approaches can substantially expand the spatial and temporal coverage of marine monitoring while reducing sampling impacts and operational costs. Their non-invasive nature, scalability and sensitivity position them as complementary, rather than substitute, tools alongside traditional fishery-dependent and fishery-independent surveys. In the Mediterranean and Black Sea region, where ecological complexity, heterogeneous fleets and transboundary stock distributions challenge conventional monitoring frameworks, the integration of eDNA approaches offers a strategic opportunity to fill long-standing information gaps related to species occurrence, community composition and habitat use.

Several methodological, technological and institutional challenges continue to constrain the operational deployment of eDNA approaches at management-relevant scales. Major barriers include the absence of standardized sampling and laboratory protocols, the need for robust and curated reference databases, uncertainties associated with DNA shedding, transport, degradation and logistical constraints related to collecting sufficiently large or replicated water samples in marine environments. Overcoming false positives, false negatives and variability in detection probability requires coordinated improvements in sampling design, replication, sequencing depth and bioinformatic processing, as well as integrating eDNA with traditional survey methods for cross-validation (Baetscher *et al.*, 2025). The remaining obstacles to operationalization are often less technical than institutional, with the most significant barrier being the lack of interdisciplinary teams able to jointly design, interpret and implement eDNA surveys in fisheries-related contexts.

Despite these limitations, there are documented cases, such as the integration of eDNA-based indices of abundance into the age-structured stock assessment of Pacific hake (*Merluccius productus*), that demonstrate the potential of eDNA approaches (Johnson *et al.*, 2025). Pilot experiences suggest that eDNA-based indices of abundance show increasing convergence with established acoustic or trawl indices, especially when sampling is paired and spatially explicit. Available evidence demonstrates that eDNA surveys can quantify observation error, characterize fine-scale variability and, under controlled designs, produce biomass-related indices with uncertainty comparable to conventional monitoring methods. As a result, eDNA is increasingly viewed as a viable data stream for informing stock assessments (Baetscher *et al.*, 2025).

Several lines of evidence support the use of fisheries vessels, including commercial and small-scale fishing fleets, as platforms for large-scale eDNA sampling. Their spatial coverage, operational regularity and the ecological knowledge of fishers make these vessels ideal candidates for participatory monitoring, particularly when equipped with simplified or automated sampling devices, such as passive samplers or metaprobes. Such integration increases data collection efficiency, enhances stakeholder engagement and fosters trust in molecular monitoring approaches. In the United States of America, for instance, several agencies are already using eDNA-based approaches, and discussions are under way regarding a national strategy for its use in marine resource management (Kelly *et al.*, 2023).

Looking ahead, the convergence of high-throughput sequencing, long-read technologies, improved taxonomic reference databases and advanced modelling tools, such as occupancy and process-based models, will continue to strengthen the reliability and interpretability of eDNA data for fisheries applications.

In conclusion, eDNA has the potential to play an increasingly central role in modern fisheries science and ecosystem-based management. Realizing this potential will require investment in standardized and interoperable protocols, expansion of reference libraries, interdisciplinary collaboration and long-term commitments to maintaining eDNA time series. When embedded within integrated monitoring frameworks, including those supported by the GFCM, eDNA-based approaches provide a pathway toward more sustainable, transparent and data-rich fisheries management that can meet the growing demands for ecological information.

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Appendix

Protocol for the use of metaprobes to sample environmental DNA during fishing activities

This protocol outlines an ideal operational workflow for a fisher participating in monitoring and research activities within a programme designed to collect environmental DNA (eDNA) samples and related metadata. It is divided into four segments: i) materials; ii) field sampling; iii) wet lab; and iv) dry lab. The protocol is based on the methodologies developed by Albonetti *et al.* (2023) and Maiello *et al.* (2022, 2023, 2024).

Materials

The following materials are required:

- a metaprobe
- sterile gauze
- plastic zip ties or cable ties
- sterile 50-ml Falcon tubes
- 99 percent ethanol or silica gel
- sterile gloves, scissors and forceps
- freezer for storage at –20 °C
- resealable, sterile plastic bags

Field sampling

Field sampling consists of preparatory activities, sampling at sea and the return to port. The following steps are designed to be implemented with or without the presence of observers, such as researchers on board the fishing vessel as part of monitoring programmes.

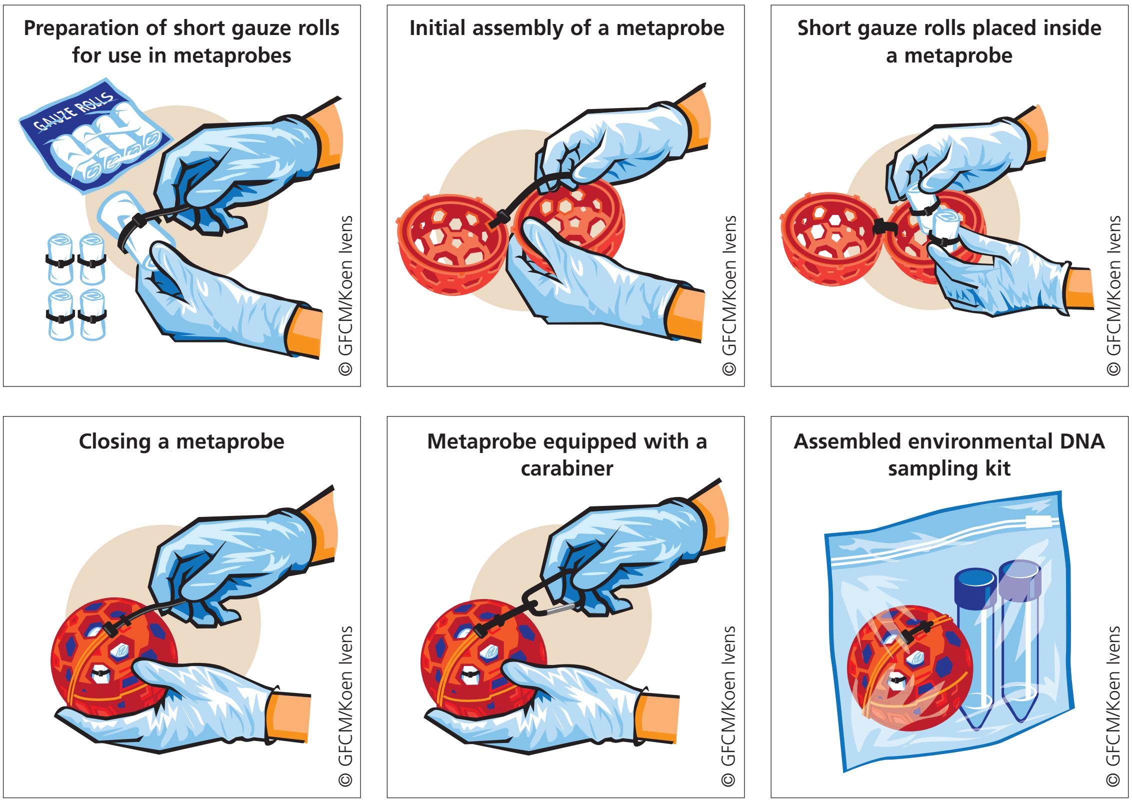
Preparing the sampling kits

Prior to going out to sea, sampling kits must be prepared. The number of kits that should be prepared depends on the quantity of samples to be collected and on the methodological aspects defined in the monitoring programme. The kits must be prepared in a sterile environment according to the following procedure (Figure A1):

1. Wearing new, clean, disposable latex gloves, prepare rolls of sterile gauze and secure each roll with a plastic zip tie or cable tie. Long rolls are available commercially and can each be cut into 4–5 shorter rolls. The number of gauze rolls to be prepared depends on the number of sites to be sampled and the intended number of replicates.
2. Fasten the two halves of the sterilized metaprobe together using a plastic zip tie or cable tie.
3. Place the gauze rolls inside the metaprobe. Although multiple eDNA samples can be obtained from a single gauze roll, it is preferable to place as many gauze rolls inside the metaprobe as there are replicates to be collected per haul.
4. Collect two additional gauze rolls as field blanks, which should be later processed along with the other gauze samples.

5. Snap the two halves of the metaprobe shut and secure with a second plastic zip tie or cable tie placed opposite the first.
6. If needed, equip the metaprobe with a carabiner to allow it to be attached to various vessel structures, such as lead lines, float lines, divergents or cables.
7. Place the prepared metaprobe, together with one 50-ml Falcon tube per gauze roll, inside a resealable, sterile plastic bag. No special precautions for the kits are necessary during transport or before use.

FIGURE A1
Preparation of an environmental DNA sampling kit



Preparation of short gauze rolls for use in metaprobes © GFCM/Koen Ivens

Initial assembly of a metaprobe © GFCM/Koen Ivens

Short gauze rolls placed inside a metaprobe © GFCM/Koen Ivens

Closing a metaprobe © GFCM/Koen Ivens

Metaprobe equipped with a carabiner © GFCM/Koen Ivens

Assembled environmental DNA sampling kit © GFCM/Koen Ivens

Solutions to preserve the gauze rolls inside the Falcon tubes must also be gathered before sampling begins. Typically, either a 99 percent ethanol solution or silica gel is used. The former is flammable and more expensive. Silica gel is almost as effective, inexpensive and safer.

Since it is essential to identify each metaprobe and eDNA sample, identification labels must be prepared and included in each kit. The labels should be completed during or after sample collection. A practical solution is to use a small, portable printer that can be connected to a smartphone (Figure A2), allowing the operator to print a QR code that links to a document containing the following information: identification of the fishing vessel (e.g. Maritime Mobile Service Identity code, International Maritime Organization number, where applicable, or national vessel registration number), geographic coordinates of fishing operations and the fishing gear used. Additional information may also be included on the water depth (particularly for fishing gear operating on the seabed), operating depth of the fishing gear (for fishing gear operating in the water column), weather and sea conditions, target species, time and duration of each fishing operation, as well as any other information considered relevant by the fisher.

FIGURE A2
Portable printer for an identification label with information related to a collected environmental DNA sample

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Sampling at sea

Once at sea, fishing operations should proceed according to routine practices. Generally, the metaprobe is used for the duration of a single fishing operation and recovered during catch sorting after the fishing gear has been retrieved.

The metaprobe should be deployed, recovered and treated as quickly as possible after recovery, following these steps:

1. Attach the prepared metaprobe to the fishing gear. For trawls, place the metaprobe inside the trawl net, near the codend; for purse seines, launch the metaprobe once the net is partially closed; for longlines, tie the metaprobe to one of the bait hooks.
2. Conduct the fishing activity as usual.
3. Record global positioning system data, depth, duration and environmental variables for each haul and complete the document linked to the identification label that is included in the sampling kit.
4. Recover the metaprobe once the net or fishing gear is hauled on board and process it as soon as possible.
5. Wearing new, clean disposable latex gloves and using sterilized scissors, cut the plastic zip ties or cables ties that secure the two halves of the metaprobe.
6. Remove the gauze rolls and place each roll in a sterile Falcon tube.
7. Fill each Falcon tube with a 99 percent ethanol solution or silica gel, close the tubes and place them back inside the sampling kit. Follow the necessary safety procedures when handling ethanol and preservatives.
8. If not already completed during sampling, finalize the document linked to the identification label and add it to the sampling kit.
9. Store all samples on ice, away from light, until transfer to the laboratory.
10. Sterilize all tools with bleach before conducting subsequent sampling.
11. After returning to port, transfer the sampling kits to the laboratory and freeze the samples at –20 °C.

Wet lab

Samples must be processed in specialized facilities for DNA extraction, with strict separation between pre-PCR and post-PCR procedures to minimize the risk of contamination. Upon arrival at the laboratory, the samples must be stored at –20 °C until molecular analyses are performed. Before extraction, the gauze rolls must be removed from the Falcon tubes, and two to five pieces must be cut from each roll, each measuring approximately 2 cm x 2 cm and weighing between 0.2 g and 0.4 g. These pieces must then be transferred individually into 1.5-ml Eppendorf tubes. The Eppendorf tubes should be placed open under a biological hood for 30 minutes to dry the gauze pieces and prevent PCR inhibition associated with ethanol preservation. The eDNA from each piece of gauze must then be extracted.

For this purpose, a commercial kit can be used. The following procedure describes the use of the DNeasy Blood and Tissue Kit from QIAGEN, with modifications (Neave, Mariani and Meek, 2023; QIAGEN, 2020):

1. Warm the Buffer ATL and Buffer AL to 56 °C to fully dissolve any precipitates that may have formed during storage.
2. Add 720 µl of Buffer ATL and 80 µl of Proteinase K to each Eppendorf tube containing a gauze piece. This solution can be premixed according to the number of samples.
3. Mix thoroughly by pulse-vortexing for 5–10 seconds and incubate at 56 °C in a thermomixer overnight (approximately 16 hours).
4. Using a pipette, transfer the supernatant to a sterile 1.5-ml Eppendorf tube.
5. Measure the volume of the supernatant in the Eppendorf tube and add the same volume of Buffer AL to the sample and mix thoroughly by pulse-vortexing. Add an equal volume of 100 percent ethanol and mix again by pulse-vortexing. For example, 600 µl of supernatant requires the addition of 600 µl of Buffer AL and 600 µl of ethanol.
6. Pipette the mixture from the Eppendorf tube into the DNeasy Mini spin column placed in a 2-ml collection tube and centrifuge at a speed of at least 6 000 x g (8 000 rotations per minute [rpm]) for 1 minute.
7. Empty the collection tube and repeat step 7 until all the mixture has been passed through the spin column. Replace the collection tube with a fresh tube.
8. Add 500 µl of Buffer AW1 to the spin column and centrifuge for 1 minute at a speed of at least 6 000 x g (8 000 rpm). Discard the flow-through and collection tube.
9. Add 500 µl of Buffer AW2 and centrifuge for 3 minutes at 20 000 x g (14 000 rpm) to dry the DNeasy membrane. Discard the flow-through and collection tube.
10. Place the DNeasy Mini spin column in a clean 1.5-ml or 2-ml Eppendorf tube and pipette 50 µl of Buffer AE directly onto the DNeasy membrane.
11. Incubate at room temperature for 1 minute, then centrifuge for 1 minute at a speed of at least 6 000 x g (8 000 rpm) to elute.
12. Pipette the same 50-µl Buffer AE from step 10 back onto the membrane and repeat step 11 to increase the final DNA concentration in the eluate.

Once the eDNA is extracted, it must be amplified and sequenced according to the following procedure:

1. Amplify the targeted barcode region using the chosen primer pair, such as mitochondrial 12S rRNA fragments using Tele02 and Elas02 primers or cytochrome C oxidase subunit I (COI) for broader metazoan coverage. To amplify the COI barcode region, follow a thermal cycle consisting of polymerase activation at 95 °C for 10 minutes followed by 35 cycles of 94 °C for 1 minute, 45 °C for 1 minute, 72 °C for 1 minute and a final elongation of 72 °C for 5 minutes.

2. Visualize each PCR run on a 1.5-percent agarose gel stained with a gel stain, such as SYBR Safe DNA Gel Stain from Invitrogen, to check for the successful amplification of target fragments.
3. Perform the PCR in triplicate and pool together using, for example, the QIAquick PCR Purification Kit from QIAGEN.
4. Purify the PCR products with magnetic beads, such as the Mag-Bind TotalPureNGS magnetic beads from Omega Bio-tek, using 30 µL of PCR products and a 0.8 ratio of magnetic beads.
5. Quantify the DNA using a fluorometer and assay kit, such as the Qubit Flex 4.0 fluorometer and Qubit dsDNA HS Assay Kit from Invitrogen. Based on the total DNA concentration, normalize and pool samples in equimolar concentrations.
6. Prepare the libraries according to the manufacturer's protocol using a kit, such as the NEXTFLEX Rapid DNA-Seq Kit 2.0 for Illumina platforms from PerkinElmer, and quantify them using quantitative PCR with a PCR cycler, such as the Rotor-Gene Q from QIAGEN, and a library quantification kit, such as the NEBNext Library Quant Kit for Illumina from New England Biolabs.
7. Dilute the COI libraries to 4 nM and quantify all final libraries and PhiX Control using quantitative PCR before sequencing.
8. Pool the COI libraries in equimolar concentrations and sequence together at 12.5 pM with a 10 percent spike in of a control library, such as PhiX Control, using V3 chemistry (2 × 250 base pair paired-end) on a sequencing platform, such as the Illumina MiSeq platform 3.0.

Dry lab

Following the sequencing process, a FASTQ file is generated. At this stage, the data must be processed using a bioinformatic pipeline. To do so, the following commands should be run on a Linux system after having installed the OBITools package and its dependencies. This pipeline must be run for demultiplexed raw reads. Within the command lines, comments are indicated using the hash symbol (#).

```
# Activate the OBITools environment

$ conda activate obi

# Annotate R1 and R2 reads and add a label with the sample name

$ for file in reads/*_R1_001.fastq; do sample=${file#*dir/};
sample=${sample%_R1_001.fastq}; obiannotate -S sample:${sample}
${sample}_R1_001.fastq > ${sample}_R1_annotated.fastq; done
$ for file in reads/*_R2_001.fastq; do sample=${file#*dir/};
sample=${sample%_R2_001.fastq}; obiannotate -S sample:${sample}
${sample}_R2_001.fastq > ${sample}_R2_annotated.fastq; done

# Merge all annotated reads into a single file

$ cat *_R1_annotated.fastq >> COI_TK_R1.fastq

$ cat *_R2_annotated.fastq >> COI_TK_R2.fastq

# Remove the forward primer sequence and the reverse complement of
the reverse primer for R1 and the reverse primer sequence and the reverse
complement of the forward primer for R2. The sequence reported here
belongs to mICOIintF/jgHCO2198 primer pair (Leray et al., 2018)

$ cutadapt -a ^GGWACWRGWTGRACWNTNTAYCCYCC...
TGRTTYTTYGGNCAYCCNGARGTNTA -A ^TANACYTCNGGRTGNCCRAARAAYCA...
GGRGGRTANANWGTYCAWCYWGTWCC --discard-untrimmed -o cut_COI_TK_
R1.fastq -p cut_COI_TK_R2.fastq COI_TK_R1.fastq COI_TK_R2.fastq
```

```
# Trim sequences with the desired length based on FASTQC output. These
values are case-sensitive

$ obicut -e 274 cut_COI_TK_R1.fastq > cut_COI_TK_R1_274.fastq
$ obicut -e 228 cut_COI_TK_R2.fastq > cut_COI_TK_R2_228.fastq

# Tag sequences based on merging score and discard those that are of low
quality

$ illuminapairedend -r cut_COI_TK_R2_228.fastq cut_COI_TK_R1_274.fastq |
obiannotate -S goodali:'"Good_COI_TK" if score>40.00 else "Bad_COI_TK"' |
obisplit -t goodali

# Count the number of sequences

$ obicount -s Good_COI_TK.fastq

# Cluster identical sequences and track their abundance

$ obiuniq -m sample Good_COI_TK.fastq > COI_TK_dereplicated.fasta

# Label each sequence with the attribute "seq-length". The length of the
amplicons depends on the primers used

$ obiannotate --length COI_TK_dereplicated.fasta > COI_TK_dereplicated_
length.fasta
$ obistat -c seq_length COI_TK_dereplicated_length.fasta
$ obigrep -p 'seq_length>290' -p 'seq_length<340' -s '^[ACGT]+$' COI_TK_
dereplicated_length.fasta > COI_TK_dereplicated_length_filt.fasta

# Manipulate the data

$ obiannotate --seq-rank COI_TK_dereplicated_length_filt.fasta | obiannotate
--set-identifier '"MCOI_%09d"%seq_rank' > COI_TK_dereplicated_short.fasta

# Generate a new attribute in the header of the sequence based on its
position in the file using --seq-rank and rename the sequence based on that
number using --set-identifier. This is needed for owi_scripts

$ obicount -s COI_TK_dereplicated_short.fasta
$ obitab -o COI_TK_dereplicated_short.fasta > COI_TK_dereplicated.tab

# Generate a tab file that contains the sequence identifier and the read
abundance in every sample

# Remove chimeras. The folder found at the following link should be
downloaded: https://github.com/metabarpark/R_scripts_metabarpark

$ Rscript metabarpark/owi_obifasta2vsearch -i COI_TK_dereplicated_short.
fasta -o COI_TK_vsearch.fasta

# Create a vsearch input .fasta file (i.e. >seqID;size=x)

$ sed -i -e 's/[[:space:]].*;size/;size/g' COI_TK_vsearch.fasta

# Remove extra space by replacing ' ;size' with ';size' to create a swarm/
vsearch imput file

$ vsearch --uchime_denovo COI_TK_vsearch.fasta --sizeout --minh 0.90
--nonchimeras COI_TK_nochim.fasta --chimeras COI_TK_chim.fasta
--uchimeout COI_TK.uchimeout.txt

# Perform de novo clustering of chimera-filtered amplicon sequence variants
into operational taxonomic units. The d parameter depends on the primer
pair used
```

```
$ swarm -d 13 -z -t 2 -o COI_TK_swarm3_out -s COI_TK_swarm3_stats -w COI_TK_swarm3_seeds.fasta COI_TK_nochim.fasta
$ Rscript metabarpark/owi_recount_swarm COI_TK_swarm3_out COI_TK_dereplicated.tab
# Generate a file called COI_TK.swarm3_out.counts.csv
# Remove singletons
$ sed -i 's/;size=/; size=/g' COI_TK_swarm3_seeds.fasta
$ obigrep -p 'size>1' COI_TK_swarm3_seeds.fasta > COI_TK_swarm3_seeds_nosingl.fasta
# Create a database in a format requested by BLAST from a .fasta file
$ makeblastdb -in MIDORI2_UNIQ_NUC_GB267_CO1_BLAST.fasta -dbtype nucl -out MIDORI2_UNIQ_NUC_GB267_CO1_BLAST
$ blastn -task blastn -num_threads 4 -evalue 1e-50 -word_size 7 -max_target_seqs 10 -db  database/MIDORI2_UNIQ_NUC_GB267_CO1_BLAST -outfmt "6 qseqid sseqid pident length evalue bitscore stitle" -out COI_TK_blast.out -query COI_TK_swarm3_seeds_nosingl.fasta
$ echo -e 'id\tblastDbid\tblastPident\tblastLength\tblastEvalue\tblastBitscore' > headers
$ cat headers COI_TK_blast.out > COI_TK_blast.tsv
```

At this stage, the resulting TSV and the count.csv file should be imported into RStudio.

```
# Load data
blast <- read.csv("COI_TK_blast.tsv", sep = " ", header = F)
abund <- read.csv("COI_TK_swarm3_out.counts.csv", sep = ";")
# Blast
bn1 <- blast %>% slice(-1) %>% rename (id = V1, blastDbid = V2, blastPident = V3, blastLength = V4, blastEvalue = V5, blastBitscore = V6) %>% unnest(blastDbid) %>% separate(blastDbid, into = c("accession","taxa"), sep = ";", convert = TRUE, extra = "merge")
bn2 <- bn1 %>% select(id, accession, taxa, blastPident, blastLength, blastEvalue, blastBitscore) %>% unnest(taxa) %>% separate(taxa, into = c("kingdom", "phylum", "class", "order", "family", "genus", "species"), sep = ";", convert = TRUE, extra = "merge")
bn3 <- bn2
bn3$genusBlast<-gsub("g:","",as.character(bn3$genus))
bn3$species1<-gsub("s:","",as.character(bn3$species))
bn3$speciesBlast<-gsub("_"," ",as.character(bn3$species1))
bnAll <- subset(bn3, select= -c(genus, species, species1))
bnAll <- bnAll %>% select(id, accession, blastPident, blastLength, blastEvalue, blastBitscore, kingdom, phylum, class, order, family, genusBlast, speciesBlast) %>% unnest(id) %>% separate(id, into = c("id", "count"), sep = ";", convert = TRUE, extra = "merge")
bnAll <- subset(bnAll, select= -c(count))
```

```
# Filter blast output by keeping the maximum percent identities for each
motu or all percent identities that are 100

bnident <- bnAll %>% mutate(blastPident = as.numeric(blastPident)) %>%
group_by(id) %>% filter(blastPident == max(blastPident) | blastPident == 100)

# Keep all unique motu and species combinations

bntopscore <-bnident %>% group_by(id, speciesBlast) %>% slice_
max(blastBitscore, n = 1, with_ties = FALSE)

# Keep the species if there is only one match, assign to a genus if there are
multiple matches

bngroup <- bntopscore %>% group_by(id) %>% count(id)
bntaxa <- merge(bngroup, bntopscore, by.x = "id")
bntaxa$taxaBlast <- ifelse(bntaxa$n==1, bntaxa$speciesBlast,
bntaxa$genusBlast)

# Remove duplicates based on id

bn_clean <- bntaxa[!duplicated(bntaxa$id), ]

# Clean up columns that no longer have context

bn_clean <- subset(bn_clean, select = -c(accession, genusBlast, speciesBlast))
bn_filt <- bn_clean %>% filter(blastPident >= 97)

# Merge all taxonomy and counts

# Remove columns with unnecessary variables

abund <- subset(abund, select = -c(definition, ali_length,count, direction,
goodali, mode, score,        score_norm, seq_a_deletion, seq_a_insertion,
seq_a_mismatch, seq_a_single, seq_ab_match, seq_b_deletion, seq_b_
insertion, seq_b_mismatch, seq_b_single, seq_length, seq_rank, subsequence,
cluster_weight))
ab_tab <- left_join(bn_filt, abund, by = "id")

# Join the dataframes to make raw motu table and put all data frames into a
list

df_list <- list(bn_filt, abund)
# Merge all data frames into a list
motu_all <- df_list %>% reduce(full_join, by="id")
motu_all <- motu_all %>% drop_na(sequence)

# Group the negatives and blanks so the algorithm can decontaminate
depending on what is found in the samples

motu_decon <- motu_all %>% select(id, sample.Gismondi78, c(13:27))
motu_res <- decon(motu_decon, numb.blanks = 1, numb.ind = 15, taxa = F,
thresh = 1, prop.thresh = 0)
motu_clean <- motu_res$decon.table %>% select(-sample.Gismondi78)
%>% left_join(motu_all %>% select(c(1:12, total_reads, sequence))) %>%
mutate(total_reads = rowSums(across(starts_with("sample")), na.rm = TRUE))
%>% filter(total_reads > 0)
taxa_fin <- motu_clean %>% select(-c(id, sequence)) %>% group_
by(taxaBlast) %>% summarise(across(starts_with("sample."), ~ sum(.x, na.rm
= TRUE)), total_reads = sum(total_reads, na.rm = TRUE), .groups = "drop")
%>% slice(1:(n() - 1)) %>% mutate(taxid = str_extract(taxaBlast, "[0-9]+$"),
```

```
taxon = str_remove(taxaBlast, "[0-9]+$") %>%       str_replace("_$", ""))
%>% mutate(taxid = as.numeric(taxid)) %>% mutate(phylum = NA, class =
NA, order = NA, family = NA) %>% select(phylum, class, order, family, taxon,
taxid, total_reads, everything()) %>% select(-taxaBlast)
taxonomy_list <- classification(taxa_fin$taxid, db = "ncbi")
for(i in 1:nrow(taxa_fin)) {
  temp_df <- taxonomy_list[as.character(taxa_fin$taxid[i])]
  if (!is.null(temp_df) && nrow(temp_df[[1]]) > 0) {
    # Try extracting each rank, if missing assign NA
    taxa_fin$phylum[i] <- ifelse(any(temp_df[[1]]$rank == "phylum"),
    temp_df[[1]] %>% filter(rank == "phylum") %>% pull(name),
    NA)
    taxa_fin$class[i] <- ifelse(any(temp_df[[1]]$rank == "class"),
    temp_df[[1]] %>% filter(rank == "class") %>% pull(name),
    NA)
   taxa_fin$order[i] <- ifelse(any(temp_df[[1]]$rank == "order"),
    temp_df[[1]] %>% filter(rank == "order") %>% pull(name),
    NA)
    taxa_fin$family[i] <- ifelse(any(temp_df[[1]]$rank == "family"),
    temp_df[[1]] %>% filter(rank == "family") %>% pull(name),
   NA)
  } else {
    # Assign NA if no classification is found for the taxid
    taxa_fin$phylum[i] <- NA
    taxa_fin$class[i] <- NA
    taxa_fin$order[i] <- NA
    taxa_fin$family[i] <- NA
  }
}
taxa_fin_bin <- taxa_fin %>% mutate(taxon = str_extract(taxon, "^\\S+(\\s+\\
S+)?")) %>%
  select(-taxid) %>% group_by(taxon) %>% summarise(phylum =
first(phylum), class = first(class),   order = first(order), family = first(family),
across(where(is.numeric), sum, na.rm = TRUE), .groups = "drop") %>%
select(phylum, class, order, family, taxon, total_reads, everything())
write.csv(taxa_fin_bin, "COI_TK_blast.csv")
```

Finally, a site x species table containing the number of reads of the taxa amplified in each sample is generated.

Reliable ecological and biological data are crucial to understanding marine ecosystems and informing effective fisheries management. However, traditional sampling approaches are often invasive and can leave critical gaps in knowledge, limiting the comprehensive understanding of ecosystem structure and function. In the Mediterranean and Black Sea region, there is an increasing need for monitoring methods that enhance data quality while minimizing ecological impacts. Environmental DNA (eDNA)-based approaches offer a compelling response to these challenges. By detecting genetic material in water, sediments and other substrates, eDNA-based approaches enable the identification of species and communities without direct observation or capture. Among emerging methods, the metaprobe – a three-dimensionally printed, hollow, perforated sphere containing sterile gauze to passively adsorb eDNA from the surrounding environment – is a notable innovation. When deployed on fishing gear, it enables the collection of genetic material from the surrounding environment and the characterization of local biodiversity. This manual provides a focused and practical overview of eDNA-based approaches, with particular emphasis on metaprobes. It outlines the complete workflow for eDNA metabarcoding during fishing activities and reviews emerging applications. Designed to support implementation in real-world settings, the manual also includes clear step-by-step protocols for deploying a metaprobe in the field.

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