NEMO TOOLS

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

Deliverable D4.1

A guide to affordable monitoring of biodiversity

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Executive Summary

This deliverable provides a practical guide to affordable and scalable monitoring of marine biodiversity, focusing on coastal and shelf ecosystems. It outlines how Essential Ocean Variables (EOVs) and Essential Biodiversity Variables (EBVs) can be monitored using frugal sampling strategies, low-cost technologies, citizen science, and emerging analytical tools. Emphasis is placed on environmental DNA (eDNA), passive acoustics, automated image analysis, remote sensing and the integration of heterogeneous data streams into coherent monitoring frameworks. By combining established methodologies with innovative, cost-effective tools, this guide supports the implementation of European environmental policies such as the Marine Strategy Framework Directive, while enabling broader spatial and temporal coverage of marine biodiversity assessments under real-world logistical and budgetary constraints.

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1. The need for cost-effective monitoring of coastal seas

Several studies in recent years have described the abrupt changes in the physical, biogeochemical, and biological levels of marine systems, caused by anthropogenic activities. Currently, the planet is experiencing unprecedent warming levels fueled by a strong El Niño – Southern Oscillation (ENSO) event (Lian et al., 2023) and surprising high temperatures records in the North Atlantic Sea surface, reaching > 0.5 oC than the 1991-2020 mean values (Copernicus data). Shifts in marine ecosystems regarding temperature, stratification, oxygen, nutrient content, pH, and other bio-essential elements are ongoing and expected to intensify (Heinze et al., 2021). Scientists are predicting that global temperatures will soon exceed the threshold of 1.5 oC above the pre-industrial mean values, triggering multiple climate tipping points (McKay et al., 2022). Already, extreme climatic events, including prolonged heatwaves and heavy rainfall causing flooding, are impacting coastal areas. To add to the climate pressures, the increased urbanization of coastal regions, the industrialization, and the intensification of agriculture stimulate cultural eutrophication phenomena leading to the prevalence of Harmful Algal Blooms (HABs) and marine ecosystem functioning disruption (Smith, 2003). Ultimately, the newly established abiotic regimes can transform biotic functions and ecological processes, rapidly impacting ecosystem stability in unpredictable ways.

Even though coastal systems are heavily impacted by human activities they are crucial for societal well-being or even survival. Some of the key ecosystem services they provide include food and water security, green energy production, blue bioeconomy goods, and climate change mitigation through carbon pump processes and microorganisms' roles (Duarte et al., 2020). As marine biodiversity enhances resilience, its management and preservation have been prioritized through several policy provisions, such as the Water Framework Directive (WFD, 2000/60/EC) and the Marine Strategy Framework Directive (MSFD, 2008/56/EC), which have set multiple targets for assessing and improving water quality status through European member states. The first step is to, consistently and accurately, monitor and measure essential abiotic and biological variables towards ecological assessment endpoints. The latest technological advances have provided modern state-of-the-art tools to sample, analyze, and interpret numerous variables. For example, high-fidelity instruments to measure key factors, such as temperature, conductivity/salinity, pH, and bio-essential elements, remote sensing tools, and autonomous vehicles are regularly deployed to sample sea water and collect in situ data (Pawlowski et al., 2021). Novel and high-throughput DNA/RNA sequencing technologies are developed and implemented together with classical approaches to measure biodiversity and examine marine biocommunities' responses to environmental change (Bik et al., 2012). Artificial Intelligence and Machine Learning (AI-ML) tools and big data analytics software are used to process the enormous amount of data generated with modern technologies (Beyan et al., 2020).

Often, the operational readiness and the deployment of monitoring systems depend on lengthy administration, complicated logistics, detailed planning and inflexible programming that eventually led to expensive and untimely sampling campaigns. Moreover, data analysis suffers from numerous different output formats for the same variable, and the inconsistent analyses pipelines applied across different laboratories. However, the rapid evolution of anthropogenically-driven environmental and subsequent biological changes calls for immediate actions. Swift, cost-effective, and interdisciplinary solutions are necessary. The ability to organize systematic seawater sampling presupposes adopting frugal, affordable and co-operative strategies; at the same time, the measurement of essential variables benefits from the development of new-generation, cost-effective instrumentation (as per the paradigm by de Vargas et al., 2022) and the inclusion of citizen scientists. These steps are crucial for timely data generation, but developing an integrated, analytical, and user-friendly architecture for shared data analyses tools across scientific fields is the key challenge to characterize biodiversity indicators and predictors linking exogenic pressures and marine systems' responses.

2. Candidate Essential Oceanic Variables

The Global Ocean Observing System (GOOS), affiliated with UNESCO's Intergovernmental Oceanographic Commission (IOC), has proposed that, to project marine forecasting and produce early warning systems and risk assessments to protect marine ecosystem services, the monitoring of Essential Oceanic Variables (EOVs) is crucial (Lindstrom et al., 2012).

Table 1. Candidate physical, biochemical, and cross-disciplinary EOVs for monitoring with frugal sampling strategies. Collection platform/methods, and relevant MSFD descriptors are indicated.

EOV category	EOV description	Collection platform & data generation	Relevant MSFD descriptor(s)
Physics	Sea state	Buoy Drifter	Hydrographical conditions (descriptor 7)
	Sea surface temperature & salinity	Sensor probe CTD* UAV$ ASV$	Hydrographical conditions (7)
	Subsurface temperature, salinity & currents	CTD* AUV$ UUV$	Hydrographical conditions (7)
	Ocean surface heat flux	Satellite	Hydrographical conditions (7)
Biochemistry	Oxygen	Sample analysis CTD Sensor probe	Food webs (4), eutrophication (5)
	Nutrient	Sample analysis Sensor probe	Food webs (4), eutrophication (5), pollution effects (8)
	Inorganic carbon	Sample analysis Sensor probe	Food webs (4), eutrophication (5)
	Transient tracers	Sample analysis Sensor probe	Pollution effects (8)
	Particulate matter	Sample analysis	Pollution effects (8), contamination of seafood (9), marine litter (10)
	Dissolved organic carbon	Sample analysis	Food webs (4), eutrophication (5), contamination of seafood (9)
Cross disciplinary	Ocean colour	UAV$ ASV$ Sample analysis Satellite	Eutrophication (5), pollution effects (8)
	Ocean sound	Hydrophones	Underwater noise (11)
	Marine debris (emerging)	Sample analysis Visual/photographic survey	Marine litter (10)

*CTD: A "conductivity, temperature and depth" device: an array of sensors that detect how conductivity and temperature change in relation to depth deployed vertically through the water column. [$]UAV: Unmanned Aerial Vehicle; ASV: Autonomous Surface Vehicle; AUV: Autonomous Underwater Vehicle; UUV: Unmanned underwater vehicle.

A detailed framework with recommendations on measurement pipelines, observing endpoints and data management guidelines of 31 EOVs was developed (https://goosocean.org/). The role of the proposed EOVs as key indicators of marine ecosystem health is regularly connected to EU policies to protect Europe's seas and coasts by achieving "good environmental status" as described through eleven biotic and abiotic descriptors in the Marine Strategy Framework Directive (MSFD; 2008/56/EC). Candidate physical, biochemical, and cross-disciplinary EOVs to be monitored with cost-effective frugal sampling strategies must comprise a set of parameters easy to measure *in situ* or via remote sensing and sampled with easy-to-transport tools; nevertheless, comprehensive to assess marine status. In this context, frugal collection platforms and data generation methods can include (i) physical/chemical/imaging sensor(s) mounted on platforms (CTD, UAV, ASV, AUV, ROV); (ii) direct measurement via handheld or vessel-based sensor probes; (iii) collection of physical samples for laboratory analysis; (iv) visual or photographic surveys; (v) hydrophone stations for recording of underwater sound; (vi) remote sensing (Table 1). These data collection and generation approaches are appropriate for *in situ* implementation, accompanied by remote monitoring with technologically advanced instrumentation, such as satellite imaging and unmanned vehicles. Even if some EOVs cannot be accurately measured with frugal methods to meet MSFD technical standards, collecting vast amounts of data e.g. via citizen scientists or other innovative versatile methods is very useful to identify hotspots and fill-in undersampled regions (for underwater noise for example).

3. Candidate Essential Biodiversity Variables

Several Essential Biodiversity Variables (EBVs) -connected to the "biology and ecosystems" EOV- has been identified and broadly agreed as the minimum set of biotic parameters to measure towards marine ecosystems monitoring, and biodiversity forecasting and conservation at regional and global scales (Pereira et al., 2013). EBVs can be observed at various spatial or temporal resolutions to characterize the impacts of underlying drivers and pressures, and to detect (Mace and Baillie, 2007) and model (Oliver et al., 2015) biological and ecological responses. Validation of modelling can subsequently feed into policy processes and assessment reports. EBVs are separated into 21 categories that correspond to six classes, namely: genetic composition, species populations, species traits, community composition, ecosystem functioning and ecosystem structure (Pereira et al., 2013). Frugal monitoring of candidate EBVs include (i) samplings, (ii) imaging sensor(s) mounted on platforms (CTD, UAV, ASV, AUV, UUV, buoys); (iii) novel eDNA sequencing technologies; (iv) visual or photographic surveys; (v) passive acoustic methods to document biological sound production (e.g., cetacean vocalizations) or describe soundscapes via acoustic complexity indices; and (vi) remote sensing (Table 2). These tools can characterize most EBV categories connected to at least six MSFD descriptors (Table 2).

Table 2. Candidate biology and ecosystems EOVs with their corresponding EBV categories for monitoring with frugal sampling strategies. Collection platform/methods, and relevant MSFD descriptors are indicated.

Biology and ecosystem EOV	EBV category	Collection platform & data generation	Relevant MSFD descriptor(s)
Phytoplankton biomass & diversity	Species distribution Population abundance Body mass Taxonomic diversity Net primary production	Sample analysis eDNA analysis ASV$ AUV$ Satellite	Marine Biodiversity (descriptor 1), food webs (4), eutrophication (5)
Zooplankton biomass & diversity	Species distribution Population abundance Body mass Taxonomic diversity	Sample analysis eDNA analysis	Marine Biodiversity (1), food webs (4)
Fish abundance & distribution	Species distribution Population abundance Population age/size structure Phenology Body mass Demographic traits Taxonomic diversity	Sample analysis eDNA analysis Visual survey	Marine Biodiversity (1), non-indigenous species (2), commercial fish and shellfish (3), food webs (4)
Marine turtles, birds, mammals	Species distribution Population abundance	Visual survey Photographic survey	Marine Biodiversity (1), non-indigenous

abundance and distribution	Population age/size structure Phenology Body mass Taxonomic diversity Species interactions	Passive acoustics	species (2), food webs (4)
Seagrass cover and composition	Species distribution Phenology Taxonomic diversity Net primary production Disturbance regime Habitat structure Ecosystem extent and fragmentation	Visual survey Photographic survey Sample analysis ROV$ UAV$ ASV$ AUV$	Marine Biodiversity (1), non-indigenous species (2), food webs (4), seabed integrity (6)
Macroalgal canopy cover and composition	Species distribution Population abundance Phenology Taxonomic diversity Net primary production Disturbance regime Habitat structure Ecosystem extent and fragmentation	Visual survey Photographic survey Sample analysis ROV$ ASV$ AUV$	Marine Biodiversity (1), non-indigenous species (2), food webs (4), seabed integrity (6)
Microbe biomass & diversity (emerging)	Population genetic differentiation Species distribution Taxonomic diversity	eDNA analysis	Marine Biodiversity (1)
Invertebrate abundance & distribution (emerging)	Species distribution Population abundance Phenology Taxonomic diversity	Visual survey Photographic survey Sample analysis ROV$	Marine Biodiversity (1), non-indigenous species (2), commercial fish and shellfish (3), food webs (4)

$UAV: Unmanned Aerial Vehicle; ASV: Autonomous Surface Vehicle; AUV: Autonomous Underwater Vehicle; ROV: Remotely Operated Underwater Vehicle.

4. Frugal Tools

Modern marine science requires the cooperation of policy, industry, and society to face the imminent and critical challenges of a changing and multi-stressed environment. The citizens' involvement in any environmental research activity can be key to convey scientific needs, questions, and messages to decision makers; interestingly, citizens are the target recipients of scientific output. Several projects have included the outreach or even participation of citizens in monitoring of the marine environment, including the monitoring of contaminants of emerging concern (Vasantha Raman et al., 2023), the community involvement in marine debris and plastics clean-up initiatives (Purba et al., 2023; Kawabe et al., 2022), the collection of data on marine animals (Merten et al., 2022), and the tracking of marine alien macroalgae (Mannino et al., 2021). Furthermore, state-of-the-art technological advancements, such as smart mobile apps, do-it-yourself (DIY) technologies, drones, and artificial intelligence (AI) services, are increasingly used by citizen end-users navigating through the unlimited potential of novel tools (Garcia-Soto et al., 2021). Passive eDNA samplers have emerged as cost-effective and low-maintenance alternatives to traditional water-collection methods for monitoring aquatic biodiversity. Current active pumps and filtering water which require specialized equipment and biomaterials (e.g., filters), can be replaced by affordable and easy to use materials, capable of collecting eDNA across long temporal windows (e.g., samplers with natural or synthetic materials of high durability). Their simple design should allow the use and deployment in remote or logistically challenging environments, without the need for power, pumps, and/or trained personnel. Passive samplers are extremely well suited for citizen-science initiatives and large-scale schemes that could substantially minimise costs and effort of biomonitoring. Future research should focus on capture efficiency of materials, as well as DNA retrieval protocols to improve quantitative assessments and integrate passive eDNA samplers into standardized monitoring frameworks.

Sampling and in situ measurement strategies in offshore systems often exclude the involvement of non-specialists as they are mostly done on specialized research vessels, with sensitive and expensive equipment. However, the implementation of scientific activities with easy-to-use, accurate, and inexpensive tools for marine systems' monitoring, such as simple instruments and sensors (e.g., secchi disk, miniCTDs), smartphone applications (e.g., Eye-on-water), and easy to build instrumentation (e.g., PlanktoScope; Pollina et al., 2020), while sampling with non-research vessels, such as sailing boats, can maintain the strong analytical capabilities for studying multi-stressed coastal environments, avoiding time-consuming, complex logistics that require inelastic resource management. The recently developed and endorsed by the UN Ocean Decade Actions initiative sailing4science (https://www.sailing4science.org/) is promoting the use of sailing as a tool for educating and connecting citizens with our seas and motivates marine scientists to observe and collect data from the ocean and coastlines onboard sailboats. The link between sailing and science dates to ancient civilizations (e.g., Herodotus, 484 BC), but perhaps the most pronounced sailing campaign for scientific purposes was the trip of HMS Beagle (1831–1836) around the world carrying Charles Darwin, who took advantage of the mobility, accessibility, and isolation offered by the ship, and collected samples and data that inspired him to compose his famous works. Nowadays, sailing is a symbol of sustainable transport, so it is fitting that environmental scientists use this means of transport over the traditionally used diesel-powered heavy research vessels. Sampling and measuring EOVs with frugal means can serve parallel ambitious goals and that is to cost-effectively monitor marine systems and at the same time train citizens in using simple tools to collect quality scientific data when they sail for leisure or business. Sailing for science brings immense potential for continuous data collection at corners of the world that would otherwise be extremely time-consuming and costly for scientists to reach with research vessels on a regular basis.

To prepare for a sampling campaign with frugal tools, the selection of the sampling vessel is key to the success of the campaign and should consider the goals and logistics of the campaign, i.e., how many and which EOVs / EBVs will be sampled and measured, how many researchers will participate, where samplings will take place, the weather and budget constraints. The EOVs / EBVs monitored are related to the equipment required, and the expertise of the people on board. Most of the equipment is easy to handle and the researchers or citizens performing the samplings or measurements can be trained in using them. For example, Niskin water samplers, CTDs or similar multi-instruments, plankton nets, and devices for water filtering for further eDNA and abiotic parameters (temperature, salinity, pH, dissolved oxygen, particles, nutrients) analyses, plankton and eDNA samplings, can be handled with precision by a single person after basic training. Samplings of additional EOVs and EBVs can also be achieved with simple, non-destructive and low-cost approaches, provided that protocols are clearly defined and consistently applied. These include visual census techniques for fish, marine mammals and seabirds, opportunistic photographic documentation of benthic habitats, and the deployment of basic passive samplers for water quality and eDNA collection. In many cases, such activities can be carried out by trained non-specialists, including citizen scientists, under expert supervision. Standardised field protocols and metadata recording are essential to ensure data comparability and to minimise observer bias. By clearly defining the scope of participation and the level of expertise required, frugal sampling strategies can substantially expand spatial and temporal coverage while maintaining scientific robustness (Figuerola-Ferrando et al., 2024). However, expert knowledge is required for operating sensors, such as hydrophones, and automated/unmanned vehicles, such as Unmanned Aerial Vehicles (UAVs), Autonomous Surface Vehicles (ASVs), Autonomous Underwater Vehicles (AUVs) or Remotely Operated Underwater Vehicles (ROVs) (Table 3, Figure 1).

Despite their advantages, frugal sampling strategies are subject to several practical bottlenecks that must be addressed during planning and implementation. Limited space on small vessels can constrain the number and type of instruments deployed, while sample storage and preservation, particularly for biological and molecular samples, may require access to freezers, liquid nitrogen, or chemical preservatives. Weather conditions and sea state can restrict sampling windows and influence data quality, especially for visual surveys and the deployment of unmanned platforms. Furthermore, the involvement of citizen scientists introduces additional considerations related to training, safety, and data quality assurance. All personal data collected from citizen participants must be handled in full compliance with the General Data Protection Regulation (GDPR), including informed consent, anonymisation, and secure data storage. Effective collaboration with sailing instructors, skippers and amateur sailors is therefore essential to ensure both safe operations and reliable data collection.

Table 3. Frugal sampling and *in situ* monitoring platforms for marine systems' observations: Short descriptions for *in situ* operations, considerations, and targeted EOVs / EBVs.

Workflow	Operations	Considerations	EOVs / EBVs
Sampling preparation	Selection of sailing boat as a sampling vessel	EOVs / EBVs targeted, number of researchers needed onboard, size of equipment, location of sampling, budget.	
	Loading of equipment and consumables	Skills to operate specialized equipment, power requirements, size, and handling	
In situ activities	Water samplings	Possible need for freezer/liquid nitrogen to store samples.	Phyto/Zooplankton counts microbial communities, particles, nutrients and chemicals laboratory analyses
	Water filtration	Onboard power requirements. Sterile conditions.	eDNA, particles, nutrients and chemicals laboratory analyses
	Physics and biochemistry *in situ* measurements	Specific skills for handling certain instruments, such as CTDs, multiparameter portable instruments, sensors.	Temperature, salinity, pH, O_2, nutrients, tracers.
	Deployment of buoys, drifters, and unmanned vehicles	Equipment size. Specific skills for handling certain instruments.	Temperature, salinity, currents, sea colour, phytoplankton/seagrass biomass and cover
	Deployment of hydrophone array	Specific skills for handling hydrophone array.	Marine animals
	Visual/photographic survey	Diving permit for underwater observations	Marine animals, invertebrates, seagrass cover

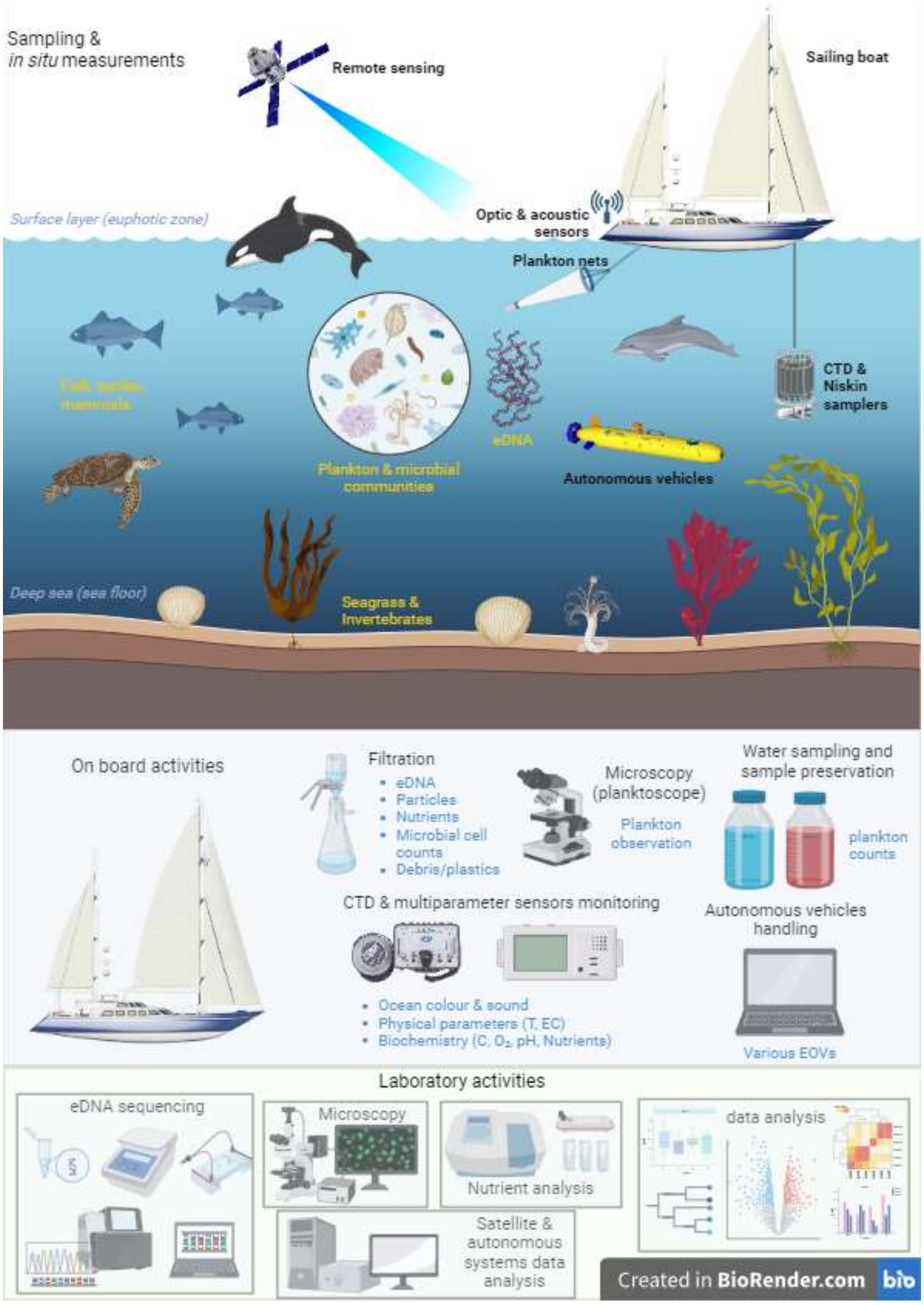

Figure 1. Graphical representation of a comprehensive workflow describing frugal sampling and *in situ* measurement strategies to collect samples and data for estimating key EBVs and EOVs. Yellow fonts: EBVs; bold: sampling and *in situ* measuring platforms, blue: on board operations.

4.1. Novel eDNA sequencing technologies

Environmental DNA (eDNA) collection and analysis is a powerful, high-throughput and emerging approach to assess the deep and overlooked diversity of multiple biocommunities and at multiple levels of focus (e.g., taxonomic, phylogenetic, functional) (Thompson & Thielen, 2023) and can be connected to several EBVs, such as population genetic differentiation, breed and variety diversity, species distribution, and taxonomic diversity. The biocommunities targeted for eDNA analyses range from microbes and phytoplankton to macrofauna categorized in broad, general groups (invertebrates, fish, other vertebrates). The predominate laboratory strategy used is metabarcoding of genetic markers designed to target different communities, although the pipelines employed are adapted to specific case study sites and regions, to laboratory standardized procedures, and to the hypotheses examined (Zhang et al., 2023). The metabarcoding approach currently yields proxies applicable to the EBVs concerning species distributions and taxonomic diversity, but further ecological and statistical tools can be applied to infer trait/functional profiling (e.g., Genitsaris et al., 2022; Djemiel et al., 2022). In recent years, the functional profiling of microbial communities is promoted with metagenomic sequencing as an approach to fill gaps in ecosystem functioning and structure (Lu et al., 2023; Ponsero et al., 2021) – an approach that will also yield EBVs concerning genetic composition. The method relies on sensitive molecular tools like PCR, qPCR, ddPCR, and next-generation sequencing to amplify and identify a range of DNA fragments. Its non-invasive nature, scalability, and ability to detect even low-abundance organisms make eDNA a powerful tool in ecology, conservation, and environmental management. Innovation in eDNA lies in making species and communities detections faster, more accurate, and less labor-intensive. Advances such as portable sequencing devices, sequencing platforms capable of generating large amount of data, as well as standardised bioinformatics pipelines have the ability the technology's reach from laboratory settings to real-time, in-field applications. Such innovations provide comprehensive ecosystem assessments and allow environmental decision-making.

Data management processes for eDNA ensure that the results which include samples, sequences, and analysis steps, are reliable and reproducible. They follow a standardised workflow from field collection to long-term data archiving. Data include metadata (location, time, environmental variables, sampling method, filtration details, and staff), laboratory records (extraction methods, PCR conditions, reagents, controls, contamination checks, are documented in lab notebooks), sequence data (raw reads are stored and processed for quality control, filtering, denoising, chimera removal, and taxonomic assignment), and data storage (raw and final sequence files, metadata, and scripts are deposited in secure servers, cloud platforms, and public repositories such as NCBI SRA, ENA, Dryad, GitHub, to support transparency and reuse). The eDNA data can be efficiently integrated into existing environmental, industrial, and monitoring infrastructures. The addition of innovative and simple sampling methods and standardized analysis to current workflows are easy to be applied. Existing laboratories (molecular, chemical, microbial testing) can incorporate eDNA extraction and sequencing with upgrades. The eDNA method can be integrated into existing environmental monitoring programs, fisheries and aquaculture operations, management of protected areas, water processing infrastructures. Data generated from eDNA can be merged into biodiversity databases and surveys, invasive species detection, and endangered species monitoring, public health systems, stock assessment models, or environmental impact frameworks. Therefore, the integration of eDNA could enhance sensitivity, enable early detection of species or pathogens, and improve ecosystem understanding, with the use and modifications of existing infrastructures.

Thus, eDNA has become a powerful tool with rapidly expanding applications, yet it faces multiple challenges. Many have been or are currently being subjected to evaluation. Briefly, these are the following:

Technical: DNA degradation, sample transport in sterile conditions, contamination from other samples (during sampling, DNA extractions, library preparation), incomplete online databases for taxonomic assignment, and detection biases (depending on primers used).

Financial: requirements for molecular laboratory setup (PCR machines, clean hoods, sequencing), high quality consumables, trained personnel, building reference databases.

Environmental: highly depends on environmental variables (e.g., pH, salinity, UV exposure, and temperature), and challenges interpreting results in dynamic systems.

Legal: requirements for permits during samples, governing genetic resource regulations, many countries do not have standardized policy for incorporating both eDNA metabarcoding and data in management decisions.

Ethical: potential presence of human DNA and use of such data, sensitive species data (e.g., endangered species). Future directions focus on improving accuracy, scalability, and real-world applicability.

Advances in sequencing particularly long-read sequencing, and the standardization of protocols will improve both species identification resolution, as well as data reliability. Additionally, emerging AI-driven analysis and the use of portable sequencing platforms will allow faster and real-time biodiversity assessments. The optimization of genetic reference databases and the use of existing tools, such as traditional surveys, hydrological models, and multi-omics approaches, will deepen ecological insight and broaden use in diverse habitats, including remote or extreme environments. Additionally, the use of simple and low-cost passive samples will allow the participation of citizen science elevating biodiversity monitoring at unprecedented scales. Finally, new developing methods that will link eDNA data to species abundance or biomass are expected to be implemented in species assessments (e.g., endangered, alien), environmental impact statements, conservation planning, and fisheries and water quality monitoring.

4.2. Remotely Operated Underwater Vehicles (ROVs)

Within NEMO-Tools, an acoustic–optical protocol was developed that combines high-frequency side-scan sonar (SSS) mapping with targeted ROV surveys, quantitative image analysis and, when needed, 3D photogrammetry. The workflow is designed for the mesophotic zone (ca. 25–60 m), where SCUBA-based monitoring becomes difficult or unsafe and where many coralligenous and gorgonian assemblages remain virtually unmapped. In the Tokmakia Natura 2000 site (NE Aegean), SSS was first used to map seabed morphology and identify key habitats along a ~3 km rocky drop-off; SSS images were then used to guide ROV dives for (i) quantitative assessment of coralligenous formations and gorgonian populations of Eunicella cavolini and Paramuricea clavata, (ii) marine litter assessment surveys, and (iii) incidental detection and mapping of a dense black seabream (Spondyliosoma cantharus) nesting aggregation. (Sini et al., 2025; Pistevos et al., 2025). Scientifically, the protocol addresses multiple gaps. Coralligenous bioconstructions host around 10% of known Mediterranean marine species and include several Red List taxa, yet NE Aegean formations have been poorly studied and rarely monitored beyond the SCUBA depth range (Sini et al., 2019; Sini et al., 2025). Standard SSS surveys offer spatial broad coverage but cannot reliably resolve community composition, while ROV campaigns are often descriptive and spatially restricted. The NEMO-Tools work explicitly couples SSS-based habitat mapping with ROV dives to apply distance and photoquadrat image sampling, in order to assess the health status of coralligenous formations and gorgonian populations, and assess the impact of marine litter, thereby, producing spatially explicit baselines for habitats (coralligenous reefs, gorgonian forests), pressures (lost fishing gear and other marine litter) and key ecological functions (reproductive aggregations) in an MPA that still lacks an operational management plan (Sini et al., 2025). The same protocol directly serves cross-disciplinary EOVs (e.g. “marine debris”, “ocean colour / seafloor reflectivity”) and several biology and ecosystems EBVs, including invertebrate abundance and distribution, habitat structure, and fish abundance and distribution. In policy terms, it delivers evidence for MSFD descriptors on biodiversity, food webs, seabed integrity and marine litter in a single integrated field operation (Sini et al., 2025).

Technologically, the tool rests on five core components: (i) a high-frequency SSS system (325 kHz CM2 C-MAX) and processing chain in SonarWiz for seabed mosaicking and habitat interpretation; (ii) an observation-class ROV (FIFISH E-MASTER) capable of 4K video, dual laser scaling and stable flight; (iii) a photogrammetry workflow (Metashape) to generate 3D models and orthomosaics for selected areas; (iv) an analytical backbone combining Fiji/ImageJ measurements with distance sampling (DS) in DISTANCE 7.5 (Sini et al., 2025), and (v) AI-assisted semi-automated image analysis of photoquadrat images through the CoralNet software (see also T3.1). What is genuinely new is not the individual instruments but their integration and the way they are used:

- SSS is used not just for “background mapping” but to design and optimise ROV effort, focusing dives on coralligenous rims, drop-offs and mixed hard-soft interfaces. The Tokmakia survey covered ~4.2 km² with high line overlap, and the acoustic interpretation directly determined where ML and gorgonian transects were placed. (Sini et al 2025)
- ROV imagery is fed into a formal distance-sampling framework. DS models detection as a function of perpendicular distance and colony size, rather than simply counting colonies per unit area, providing statistically robust density estimates with quantified uncertainty for E. cavolini and P. clavata. (Sini et al 2025)
- ROV photoquadrat image samples are processed using an AI-assisted semi-automatic image analysis tool, that enables the rapid and consistent analysis of extensive benthic image collections, overcoming the limitations of labor-intensive manual method for the assessment of the coralligenous habitat.
- Marine litter is treated as a structured dataset: every item is classified by type, interaction mode, biofouling stage (as a proxy for residence time) and removal feasibility, and linked to the presence of vulnerable erect taxa such as gorgonians and sponges. (Sini et al., 2025; Constanzo et al 2020).
- In the black seabream case, SSS and ROV-based photogrammetry are explicitly linked: seabed features visible in both datasets are used to scale and position the orthomosaic of the nest field within the broader mapped corridor, demonstrating that the same platform can capture fine-scale reproductive structures within a mesophotic seascape. (Pistevos et al., 2025)

Relative to multibeam/AUV solutions, this is deliberately built around relatively affordable, commercially available hardware and open-source or widely used software, making it suitable for institutes with limited capital equipment budgets but access to a coastal vessel.

The protocol offers several practical capabilities that are directly relevant to affordable biodiversity monitoring. First, it couples broad spatial coverage with high ecological resolution. SSS provides full-coverage acoustic mapping over kilometres, distinguishing soft sediments, rocky outcrops, biogenic mounds and seagrass patches, while ROV dives supply benthic assessment of coralligenous formations, colony-level data on gorgonian density, size structure and injury, alongside detailed records of marine litter (Sini et al., 2025). This explicitly targets the "spatial–temporal resolution gap" between sparse dive surveys and coarse remote sensing. Second, it generates quantitative indicators rather than just descriptions. For coralligenous formations, it provides data on substratum slope, basal living cover, coralline algae cover (indirect indicator of biogenic reef), sedimentation levels. For gorgonians, outputs include density (colonies m^{-2}), height distributions and injury categories, with detection bias accounted for via DS. For marine litter, it provides densities, composition, biofouling stages and interaction types with structuring species. For fish, the same imaging system captured the extent and density of S. cantharus nests in a previously unknown breeding aggregation (Sini et al., 2025; Pistevos et al., 2025). Third, the protocol is explicitly designed to feed management decisions. The combined acoustic–optical products identify coralligenous drop-offs and gorgonian forests that are strong candidates for no-take / no-trawl zoning, pinpoint litter entanglement hotspots where active removal is feasible, and reveal critical breeding grounds that may require seasonal fisheries restrictions (Sini et al., 2025; Pistevos et al., 2025).

Operationally, the protocol follows a simple but strict sequence: (i) SSS survey and interpretation, (ii) ROV ground-truthing and quantitative surveys, (iii) optional ROV photogrammetry for specific features, and (iv) image-based analysis and modelling. The SSS stage requires a vessel capable of towing the towfish at 3–4 knots and maintaining stable headings; a 325 kHz CM2 system was used at Tokmakia, with ~80% line overlap to ensure complete coverage. SSS data were processed in SonarWiz for mosaicking, texture-based classification and manual habitat mapping where automatic classification failed due to complex relief (Sini et al 2025). The ROV stage uses an observation-class vehicle with 4K video, dual lasers, and adequate thrust to hold position along steep slopes. For ML surveys, the ROV was flown at ~1.5 m above the seabed along random paths across the drop-off; for distance and photoquadrat sampling, altitude was reduced to 0.5–1 m with the cameraoriented perpendicular to the substrate and laser points kept visible. Vessel GPS at deployment and retrieval was used to approximate dive length, given the absence of underwater tracking (Sini et al 2025). Personnel needs are modest but non-trivial: a skipper and deckhand for safe SSS/ROV operations; an acoustic operator/geomorphologist for SSS survey design, analysis and interpretation; an ROV pilot; and one or two benthic ecologists that are comfortable with Fiji/ImageJ, CoralNet, and DISTANCE workflows or equivalent software. This is not a citizen-science tool; it is an “affordable professional” solution, where the main cost drivers are vessel, time, and staff, not bespoke high-end hardware.

The methodological approach generates several data streams: raw SSS files and processed mosaics; ROV video (4K, 30 fps) and extracted still frames; photogrammetric products (dense clouds, meshes, DEMs, orthomosaics); and tabular measurements (perpendicular distances, colony sizes, litter attributes, nest metrics) (Sini et al., 2025; Pistevos et al., 2025). Input data are handled with standard software that is already widely used in the marine community: SonarWiz for SSS; Fiji/ImageJ for image calibration and measurements; CoralNet for photoquadrat image analysis; DISTANCE 7.5 for DS; and Metashape for photogrammetry. Outputs can be exported as GIS-ready layers (habitat polygons, gorgonian density rasters, ML density layers, nest-field outlines) and as analysis-ready tables for further analysis (Sini et al., 2025; Pistevos et al., 2025). Quality control is built into the workflow. SSS classification is cross-checked against ROV ground-truthing; where automatic texture classification fails, manual mapping is used and this subjectivity is explicitly acknowledged. ROV measurements are repeatedly calibrated using the fixed 10 cm laser spacing, and right-truncation plus AIC-based multi-model inference are applied in distance sampling to maintain acceptable detection probabilities and avoid over-fitting.

The protocol is deliberately designed to sit on top of existing national infrastructure: a coastal research vessel (or equivalent working boat) able to tow an SSS and deploy an ROV; standard commercial software licences; and off-the-shelf observation-class ROVs. In governance terms, it is already embedded in the management framework of a Natura 2000 SAC and directly supports implementation of the MSFD for seabed integrity and marine litter descriptors (Angiolillo et al 2023; Sini et al 2025; Pistevos et 2025). The same design could be integrated into larger multi-platform observatories, where satellite and aerial data provide surface and shallow-water context, multibeam/AUV platforms contribute high-resolution bathymetry, and the ROV+SSS tool supplies the “fine-scale ecological and impact layer” for mesophotic habitats.

The protocol is powerful, but there are clear constraints that matter if it is to be scaled as a “frugal” tool. Technically, the main bottleneck is navigation. Without USBL/DVL tracking, ROV positions are approximated from surface GPS and cable geometry, which is adequate for transect-based density estimates but introduces uncertainty in fine-scale mapping and in ML density per unit area. The SSS classification step is also not fully automated: texture-based classification failed in steep, heterogeneous sectors of the Tokmakia drop-off and had to be replaced by manual mapping. Operationally, the method is sensitive to sea state, currents and visibility. Rough conditions and strong currents limit SSS towing and safe ROV flight, particularly along high-relief coralligenous rims. There is also a non-negligible risk of physical disturbance to erect fauna if tether management or piloting is poor (Angiolillo et al 2023; Sini et al 2025). Additionally there is added risk if the ROV is deployed in areas with substantive marine litter such as fishing lines and nets as the risk of entanglement is high leading to damage to the ROV and even potential loss of equipment if it is deployed too deep for diver retrieval. Financially, this is cheaper than multibeam/AUV options but not “ultra-frugal”: it still requires a dedicated vessel, SSS and ROV systems, and trained staff. Scaling from a single site to a national network will require sustained funding for vessel time, hardware maintenance and data analysis capacity (Angiolillo et al 2023; Sini et al 2025). Legally and ethically, work inside MPAs demands permitting and coordination with competent authorities. Sensitive outputs—such as the exact location and extent of the S. cantharus nest field—are deliberately not disclosed publicly to avoid increased fishing pressure, which means that data-sharing must balance transparency with conservation risk. The method itself is non-invasive (fish are only observed by ROV), but repeated surveys in breeding seasons could still cause behavioural disturbance if not carefully managed. (Sini et al 2025; Pistevos et al 2025)

In the short to medium term, development priorities are clear. Integrating low-cost acoustic tracking (USBL or similar) would dramatically improve ROV georeferencing and thereby strengthen density estimates, ML mapping and change detection in repeated surveys. Applying machine-learning approaches to coupled SSS–ROV datasets could reduce reliance on manual classification for both habitat and litter mapping. Regular temporal replication of the protocol at Tokmakia and comparable sites would turn one-off baselines into true monitoring time series for gorgonian condition, litter accumulation and reproductive habitat persistence. (Angiolillo et al 2023; Sini et al 2025). Over a 5–10 year horizon, the realistic vision is a mesophotic observatory network in which this ROV+SSS protocol is deployed across multiple Natura 2000 sites and prospective Fisheries Restricted Areas, delivering harmonised indicators for coralligenous status, gorgonian populations, marine litter impacts and key fish reproductive habitats. In a Global Ocean Observing System (GOOS) context, such a network would provide cross-disciplinary EOV and EBV data streams that are currently missing for deeper coastal habitats, and inform both MSFD reporting and fisheries management (e.g. spatial or seasonal protection of spawning aggregations) (Angiolillo et al 2023; Sini et al 2025; Pistevos et al., 2025).

From a D4.1 perspective, the key point is that NEMO-Tools has pushed ROV work away from ad hoc “video tours” and towards a structured, statistically robust, still relatively affordable protocol that can be replicated, scaled and integrated with other frugal platforms in a wider biodiversity observing system.

4.3. Automated image analyses of macroalgal communities

The primary tool assessed within NEMO-Tools is CoralNet (Version 1.0), a free, open-source, web-based repository and collaboration platform for benthic image analysis (Beijbom et al., 2015). It employs artificial intelligence (AI), specifically deep neural networks, to perform fully or semi-automated point-based annotation of benthic images. The platform was developed to address the growing need for efficient monitoring of marine ecosystems initially for coral reefs, but in our research, we used it to assess rocky reefs and coralligenous formation (See previous paragarph on acoustic–optical ROV-based protocol for mesophotic coralligenous habitats), which are among the most diverse and biologically productive coastal ecosystems (Sala & Giakoumi, 2018; Bevilacqua et al., 2021). The development of such tools is driven by the need to assess the ecological status of habitats that are increasingly degraded by cumulative human pressures (Lejeusne et al., 2010; Micheli et al., 2013; Dailianis et al., 2018). While macroalgal communities are reliable indicators for ecosystem health (Thibaut et al., 2017; EU, 2000; EU, 2008; Ballesteros et al., 2007; Orfanidis et al., 2011; D’Archino & Piazzi, 2021), traditional manual annotation of photoquadrats is labour-intensive and time-consuming, which increases costs and delays results (Rivera-Sosa et al., 2025). The innovative aspect of this work lies in validating AI-assisted annotation for regional-scale assessments. Recent cost-benefit analyses estimate that expert manual annotation costs approximately US$5.41 per image, compared with US$0.07 using machine learning—roughly 1.3% of the manual cost—while processing images orders of magnitude faster (González-Rivero et al., 2020). This study confirms that AI-derived assessments using the reef-EBQI index (Thibaut et al., 2017) can achieve high agreement (87%) with manual methods, proving effective for scalable monitoring (Zotou et al., 2025).

The tool relies on Computer Vision and Machine Learning (CV/ML) technologies. CoralNet utilises a convolutional neural network (CNN) that is trained iteratively on confirmed point annotations to classify benthic substrate categories. It operates as a cloud-based service, enabling classifier training without specialised local hardware. The system provides confidence scores (posterior probabilities) for its predictions, allowing users to verify uncertain labels while automating high-confidence ones (Beijbom et al., 2015). This study introduces a novel integration workflow by developing a custom "bridge" between desktop manual annotation software and cloud AI. A custom MATLAB script was created to batch-parse layer files from photoQuad (Trygonis & Sini, 2012) and automatically generate CoralNet-ready CSV files. This allows for the utilisation of pre-existing datasets and expert annotations to train the AI tool. Furthermore, the study demonstrates that using broad morphofunctional groups (Littler & Littler, 1980; Steneck & Dethier, 1994) —such as "shrubby algae" or "turf"—rather than species-level identification is a viable strategy for training classifiers that yield robust ecological status assessments.

A key capability of the tool is its potential to standardise and accelerate biodiversity assessments. Automated tools can complete in two days what would otherwise require two months of manual effort (Hermanto et al., 2023). This efficiency enables broader spatial-temporal resolution, facilitating the analysis of extensive datasets across large geographic areas, such as the 89 sites surveyed across the North and South Aegean Sea, a broad study area with a variety of both biotic and abiotic factors affecting the image dataset. Accordingly, with the existing trained classifier of CoralNet, we can identify and estimate the cover percentages of morphofunctional groups in any other photographic dataset of the region. By employing consistent algorithms, the tool helps mitigate observer bias, which is often compounded when multiple human annotators are involved (Beijbom et al., 2015; Curtis et al., 2024). The use of standardised morphofunctional categories further enhances the comparability of data across different regions and monitoring programs.

The fieldwork methodology is built upon standard non-destructive sampling methods, specifically photoquadrat sampling, which is preferred for its low cost and minimal environmental impact. Operationally, the workflow requires a set of underwater benthic images, using a frame of reference. If the dataset is already annotated in another software, a “bridge” is required to acquire a CSV file with pixel coordinates and the label/ annotation for each point, to be used to train the classifier. In case of raw photographic data (images without annotations), then experts in identifying marine sessile organisms should be included in the training of CoralNet’s classifier. In our case study (Zotou et al., 2025), half of the pre-annotated image dataset was used to iteratively train the CoralNet classifier. The platform employs an "unconfirmed" status for new images, where suggested labels are presented to the user, who must verify them until the classifier reaches a desired confidence threshold. This function allows for fewer hours and personnel requirements in analysing large image datasets. The primary inputs are high-resolution benthic images and corresponding annotation files (CSVs) containing pixel coordinates and labels. Outputs include estimated benthic cover, which allows calculating ecological indices like the reef-EBQI (Thibaut et al., 2017). Quality control is managed through the platform's built-in validation framework; a portion of the confirmed dataset (e.g., 1/8th) is automatically set aside to test classifier accuracy.

The tool integrates well with existing monitoring frameworks, such as the Marine Strategy Framework Directive (MSFD) (EU, 2008). It complements standard field protocols (D'Archino & Piazzi, 2021) and desktop software like photoQuad (Trygonis & Sini, 2012) through custom data bridging scripts. This flexibility supports the "hybrid" use of AI, where automated tools are used for screening-level assessments or to reduce post-processing bottlenecks in large-scale monitoring programs or citizen science initiatives (Li et al., 2025).

Technical and environmental constraints include:

- Image Quality: Annotation accuracy is heavily dependent on image quality. Issues such as illumination, colour aberration, and turbidity can hinder classifier performance (Beijbom et al., 2015; Zhou et al., 2024; Li et al., 2025).
- Habitat Complexity: Complex benthic structures and three-dimensional growth forms (e.g. dense canopies) can obscure underlying layers, making 2D image analysis challenging (Hermanto et al., 2023).
- Class Imbalance: Classifiers tend to perform poorly on rare taxa. In this study, canopy-forming macroalgae (key indicator) were underrepresented, leading to potential misclassification (Li et al., 2025).
- Label Ambiguity: Morphologically variable groups, particularly "turf" algae, are prone to error in both manual and automated classification (Hermanto et al., 2023; Williams et al., 2019).
- While operational costs are lower, there is a significant upfront requirement for expert time to create high-quality training datasets. Additionally, taxonomic uncertainty and inconsistency among analysts remain sources of error that AI mimics if trained on biased data (Curtis et al., 2024).

Future efforts should focus on improving label designs to reduce variability and testing weighted training strategies to mitigate class imbalance for rare but ecologically important groups (Althaus et al., 2015). There is also a need to incorporate explicit image-quality metadata to better understand how environmental factors (like turbidity) influence classifier performance (Hermanto et al., 2023). The long-term vision involves fully integrating AI-assisted tools into standardised monitoring protocols to enable cost-effective, high-throughput biodiversity assessments (Williams et al., 2019). Over the next decade, these tools are expected to support large-scale citizen science initiatives and provide the quantitative data necessary for implementing marine restoration agendas and management objectives (Gerovasileiou et al., 2019; Fraschetti et al., 2022).

4.4. Hydrophones

Passive acoustics is a key, non-invasive tool for measuring, monitoring, and identifying the sources of sound in underwater environments (Van Parijs et al., 2009; Merchant et al., 2015; Montgomery & Radford, 2017). It enables scientists to study the acoustic behaviour and ecology of marine animals (Tyack & Clark, 2000; Ladich, 2019), the levels of anthropogenic noise (Hildebrand, 2009) and its impacts on marine biota (Slabbekoorn et al., 2010; Erbe et al., 2019), and the geophysical or atmospheric processes that introduce acoustic energy underwater (Wenz, 1962). By utilizing passive acoustic monitoring (PAM) tools over large spatiotemporal scales or in short-term studies at specific sites, researchers can monitor marine animals and their habitats and provide evidence-based assessments to policymakers and marine protected area (MPA) managers (Haren, 2007; Williams et al., 2015). Some PAM applications are particularly demanding in equipment specifications, data storage needs, calibration accuracy, deployment duration, and data collection protocols. Such applications include long-term underwater noise monitoring (Picciulin et al., 2023; Fuentes Rivera Escalante et al., 2025), deep water high frequency autonomous hydrophones (Wiggins et al., 2007), in situ validation of shipping noise models (Putland et al., 2022), or source level measurements of animals (Trygonis et al., 2013) and anthropogenic sound sources (Scrimger & Heitmeyer, 1991). However, many other passive acoustic studies can be implemented without high-end costly equipment, e.g. acoustic detection of animals in the wild (Romero Vivas & López, 2010; Charrier et al., 2023), portable audio-video underwater platforms (Mouy et al., 2023), monitoring aquaculture facilities (Kyriakou et al., 2019), and speedboat traffic assessment at coastal sites or MPAs (Corrias et al., 2023). Indeed, the current PAM literature highlights both a strong call for and a clear lack of detailed, multi-context evaluations of low-cost hydrophone devices against research-grade commercial systems, in order to facilitate affordable research, education, and citizen science initiatives (Lamont, 2022; De Marco et al., 2023; Chapuis et al., 2025). To this end, the NEMO-Tools project designed, constructed and evaluated under different usage scenarios a low-cost hydrophone toolkit, targeting both research and citizen science applications (Galanos et al., 2025a). The custom-made, do-it-yourself (DIY) hydrophone was built from off-the-shelf components at a cost of 20 €, and was designed for ease of use when paired with a common cell phone, or for providing a complete PAM toolkit priced at ~50 € when paired with a commercially available handheld audio recorder.

Central to the NEMO-Tools hydrophone design is a thin piezoelectric (PZ) disk 35 mm in diameter, chosen for its low cost and availability in the market, identical to the PZ elements commonly used in gift cards and electronic buzzers. After soldering it to an off-the-shelf audio cable, the PZ disk is glued onto a plexiglass disk of 4 mm thickness. The latter is then superglued to a PVC threaded pipe fitting and the overall enclosure is waterproofed using a sealant paste that is commonly used in marine applications (Figure 2). What is genuinely new in this effort is not the individual components (as, by design, the hydrophone was built from readily available off-the-shelf materials), but their integration into a standalone, robust unit that can be easily constructed in large production batches

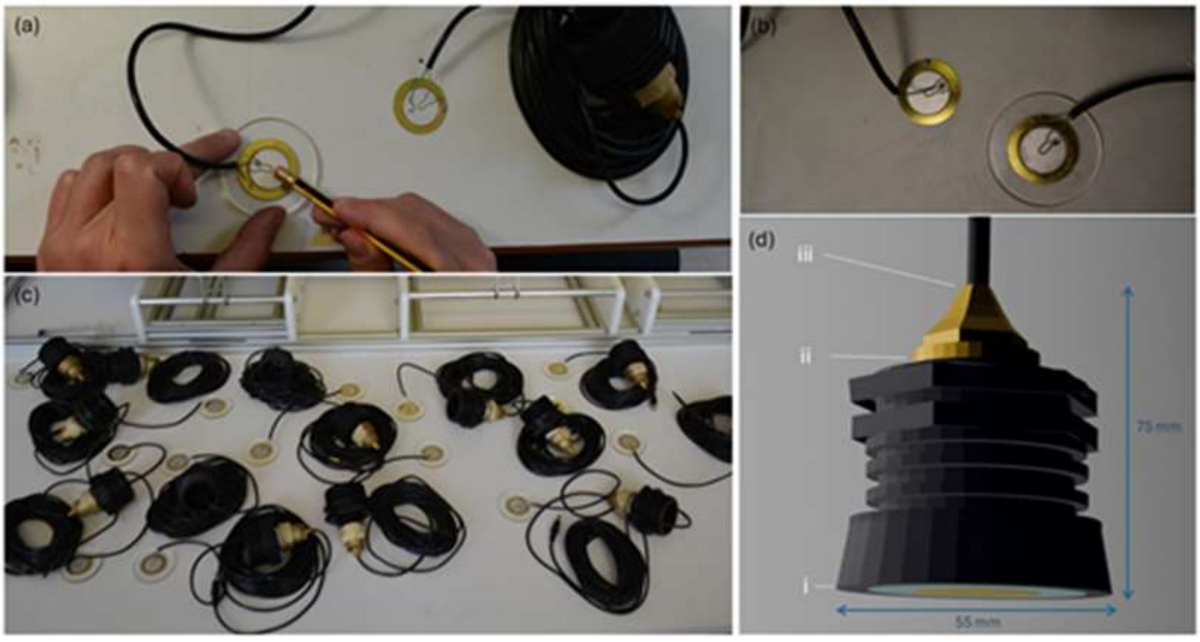

Figure 2. Construction steps of the low-cost hydrophones developed in the NEMO-Tools project. (a) Coupling of the piezoelectric element onto the plexiglass disk using cyanoacrylate glue; (b) The resulting acoustic element after the glueing process; (c) A hydrophone production batch before water sealing; (d) 3D model of the low-cost hydrophone, rendered in the open-source Blender software; the annotations (i, ii, iii) mark points where water sealants were applied.

The NEMO-Tools DIY hydrophone operates as any other passive acoustic sensor for obtaining short- or long-term underwater recordings. However, the work conducted in the NEMO-Tools project fills the recognised gap for a specific, easily reproducible toolkit, and does so across multiple use-cases, i.e. controlled tank tests, small-boat traffic in an MPA, port noise, coastal soundscapes and fish sounds (Galanos et al., 2025a), all conducted in direct comparisons with a calibrated reference hydrophone and a widely used commercial unit. The generalizable contribution of this work therefore lies in: (i) quantifying, across a broad range of realistic acoustic scenes, where and by how much a very low-cost device diverges from reference systems in terms of spectra, sound pressure level metrics and detection of key features; (ii) explicitly linking these deviations to design features (e.g. resonance behaviour and dynamic range) that are shared by many piezo-based, low-budget hydrophones; and (iii) translating these findings into concrete recommendations about which classes of ecological and regulatory questions (e.g. relative comparisons of boat noise, presence/absence and temporal patterns of fish choruses, citizen-science soundscape recording) can be addressed reliably with such a toolkit, and where calibrated, higher-end instruments remain indispensable (e.g. for absolute compliance monitoring under MSFD Descriptor 11). The combination of systematic comparative testing, transparent description of performance limitations, and explicit discussion of fit-for-purpose applications constitutes both a substantive and broadly applicable scientific and operational advance in the context of ongoing efforts to democratise PAM for conservation and policy support. The fieldwork methodology and operational needs are identical to any other standard uses of portable ("drop-off") hydrophones. Field and laboratory tests (Galanos et al., 2025a; 2025b) showed that the NEMO-Tools hydrophones can be used for soundscape monitoring, speedboat traffic assessment, marine mammal detections and qualitative noise monitoring, registering most sounds detected by their scientific counterpart, apart from some low-intensity or low-frequency events.

The raw data obtained by the NEMO-Tools hydrophones are 16-bit uncompressed audio WAV files, i.e. industry-standard audio files used in any other passive acoustic monitoring applications. Therefore, their integration with existing infrastructure and processing pipelines is seamless.

Challenges include:

- **Technical constraints:** Field evaluations showed performance limitations of the NEMO-Tools hydrophones for low-frequency events. Low intensity fish sounds that are typically below 1 kHz were not always recorded, while all shipping noise metrics below 200 Hz were underestimated because of the poor sensitivity of the PZ sensor at low frequencies.

Another downside of the PZ sensor used was its strong resonant frequency at 3 kHz, which saturates spectrograms around 2.6 to 3.1 kHz and introduces systematic bias in most spectral descriptors and noise metrics in this frequency band. Lastly, the single disk-shaped acoustic sensor used renders the NEMO-Tools hydrophones quite directional, i.e. they perform better when directed towards the sound source.

- **Citizen scientist data management constraints:** As mentioned above, accepting audio samples uploaded by citizen scientists requires a dedicated, user-friendly online platform and pre-processing quality control.

Future upgrades of the NEMO-Tool hydrophones will focus on mitigating the aforementioned technical issues and enhancing operational capabilities. We plan to experiment with multiple piezoelectric elements of different diameters in the sensor design to improve low-frequency response and reduce blind spots attributed to directionality, starting by employing larger in diameter PZ disks. The current design was kept simple (relying on the recorder's built-in gain options), but incorporating a dedicated inexpensive preamplifier circuit could significantly boost the signal-to-noise ratio. A compensating filter could also help mitigate mid-frequency resonance peaks. Additionally, a small low-cost array could enable rudimentary localization of sound sources (through time-of-arrival differences and a multi-channel recorder) or improve detection of specific events through cross-correlation of signals. Despite limitations, the advantage of an easily replaceable inexpensive device is that researchers can afford to deploy many units simultaneously, covering multiple sites or larger areas. This trade-off between quality and quantity could actually be an advantage in certain scenarios; for example large-scale, lower-resolution datasets could be highly effective for detecting broad spatial and temporal patterns of underwater noise that might otherwise go unnoticed in smaller, high-precision studies; while even if lower in resolution, could allow researchers to compare seasonal trends in human uses across different areas and regions. In conclusion, this NEMO-Tools effort demonstrated that a carefully designed, inexpensive hydrophone toolkit can greatly augment passive acoustic monitoring. By empowering citizen scientists and resource-limited programs, a far greater volume of data on underwater soundscapes can be collected, ultimately supporting better-informed management of noise in marine protected areas and beyond.

4.5. Satellite imaging of macroalgal forests

Remote sensing provides a valuable tool to monitor seagrass distribution, health, and changes over time. Satellite imagery, particularly from Sentinel-2 and other commercial providers, offers multispectral data that can be used to map seagrass meadows, detect changes in their extent, and assess their condition. Hyperspectral sensors, deployed on satellites or drones, can capture the specific wavelengths of light reflected by seagrass, allowing for detailed classification and health monitoring but the scalability is not yet mature. For example, by analyzing light absorption patterns, it's possible to detect signs of stress in seagrass beds, such as discoloration due to water pollution or increased turbidity. Aerial drones are also becoming increasingly popular in seagrass monitoring. With high-resolution cameras and the ability to fly at low altitudes, drones can provide detailed images of seagrass beds, enabling researchers to observe small-scale changes such as the encroachment of invasive species or the impact of human activities from uncontrolled anchorage and small-scale coastal developments. Furthermore, drones can be equipped with LiDAR (Light Detection and Ranging) to provide 3D mapping of seafloor topography, which can help assess habitat structure and changes in sediment dynamics. Brown algae forests, particularly those dominated by Cystoseira species, are another key component of the Mediterranean's submerged habitats. These algae form dense underwater forests that provide important ecosystem services, such as habitat for marine species, protection against coastal erosion, and carbon sequestration. However, brown algae forests are increasingly threatened by pollution, overfishing, and habitat degradation. Remote sensing technologies can be used in monitoring the extent and health of brown algae forests to a certain extent. In addition to spatial mapping, remote sensing can help detect changes in algae health, such as loss of biomass, which are indicative of environmental stressors or the dynamics of the habitat. In case of availability of airborne-based hyperspectral data, these can be used to distinguish between different species of algae based on their unique spectral signatures, allowing for more accurate assessments of biodiversity and habitat quality. Despite the promising benefits, remote sensing of seagrass and brown algae habitats faces several challenges. One of the primary limitations is water turbidity, which can reduce the effectiveness of satellite imagery and drone-based observations, particularly in areas with high levels of sediment or pollution. Additionally, the temporal resolution of remote sensing platforms and the limited spectral resolution of the high-resolution commercial platforms can be a constraint; while satellites provide frequent observations, the data may not be sufficiently detailed or timely to detect small-scale or rapidly evolving changes in these ecosystems. Moreover, the complex nature of these submerged habitats—such as the intricate structural variations in algae forests or seagrass meadows—means that remote sensing data alone cannot fully capture the biological and ecological processes. Integration with in-situ surveys, genetic monitoring, and other technologies is often necessary for a comprehensive understanding.

5. Laboratory Analysis

Samples and data collected during frugal sampling campaigns require appropriate laboratory processing to transform raw material into quantitative and interpretable indicators of ecosystem status. Laboratory analyses remain a critical component of cost-effective marine monitoring frameworks, as they provide validated, reproducible and policy-relevant measurements for a wide range of Essential Ocean Variables (EOVs) and Essential Biodiversity Variables (EBVs). When combined with standardised field protocols and emerging analytical tools, laboratory-based approaches enable robust assessments of both abiotic conditions and biological communities across spatial and temporal scales.

For several physical and biogeochemical EOVs, laboratory methodologies are well established and widely standardised. Nutrient analyses (e.g. nitrate, nitrite, ammonium, phosphate and silicate) are routinely conducted using colorimetric, fluorometric or automated analyser techniques, many of which have been in use for several decades and are supported by extensive intercalibration exercises and quality assurance protocols. These measurements are fundamental for assessing eutrophication, biogeochemical cycling and food-web dynamics, and they directly support reporting obligations under the Water Framework Directive and the Marine Strategy Framework Directive. Similarly, laboratory determination of dissolved and particulate organic carbon, suspended particulate matter and transient tracers complements in situ sensor measurements and provides higher analytical precision for calibration and validation purposes.

Samples and data collected from the sampling campaign can be further processed using wet lab procedures and (bio)informatics pipelines towards a comprehensive architecture of the ecosystem's state. For several EOVs / EBVs, laboratory analyses have been standardized and many research teams have optimized protocols to quantify abiotic parameters and to describe diversity estimators for multiple biocommunities. The first seawater nutrient measurement protocols have been already published more than 50 years ago, and several comprehensive guides have been developed since then (for an extensive review see Daniel et al., 2020). Plankton microorganisms are typically identified, and their population abundances quantified through microscopy, which allows the taxonomic characterization (Lund et al., 1958;

Edler et al., 2010) the accurate estimation of various community metrics, such as population biomass (Hildebrand et al., 1999), Chl a content (Álvarez et al., 2017), and the description of ecological interactions. Additionally, satellite colour pattern sensors have been launched and put into operation to estimate Chl a concentrations in large spatial scales to observe phytoplankton distribution and algal blooms (Kemp & Villareal, 2013). Cutting-edge, high-throughput eDNA sequencing technologies have complemented laborious and time-consuming classical approaches to facilitate biodiversity assessments across multiple biocommunities and aquatic systems (Bunholi et al., 2023).

From an operational perspective, cost-effective laboratory monitoring relies on optimised workflows, shared infrastructure and standard operating procedures. Many analyses can be conducted in existing chemical, biological or molecular laboratories with modest upgrades, allowing institutions to leverage current capacity rather than invest in dedicated facilities. Inter-laboratory calibration, the use of reference materials, and participation in proficiency testing schemes are essential to ensure data comparability across regions and time. Moreover, careful planning of sample preservation, storage and transport is necessary to minimise degradation and contamination, especially in frugal or remote sampling campaigns. Finally, laboratory-generated data must be tightly integrated with field observations and broader data management frameworks. Metadata describing sampling conditions, analytical methods, detection limits and uncertainty should accompany all laboratory results to support transparency and reuse. When embedded within integrated data handling pipelines, laboratory analyses contribute directly to the generation of operational indicators for ecosystem status, trends and pressures. In this way, laboratory analysis remains a cornerstone of affordable, scalable and policy-relevant marine biodiversity monitoring, underpinning both scientific understanding and effective environmental management.

6. Integration of Data Handling

The need for cost-effective coastal and marine ecosystem mapping and monitoring approaches has never been greater, particularly in the context of accelerating environmental change and the rapid expansion of heterogeneous data streams generated by modern observing systems. Contemporary marine monitoring increasingly relies on a wide array of data sources, including molecular and genetic information, in situ physicochemical measurements, remote sensing products, autonomous and semi-autonomous platforms, citizen science observations, and long-established survey and sampling methodologies. While each of these data streams provides valuable and often complementary insights into ecosystem structure and functioning, their integration into a coherent analytical framework remains a major scientific, technical and organisational challenge.

These datasets differ substantially in format, spatial and temporal resolution, taxonomic scope, measurement units, sampling effort and associated uncertainty. For example, eDNA metabarcoding data provide highly sensitive presence information across broad taxonomic groups, but are often temporally discrete and influenced by environmental conditions, while satellite-derived products offer synoptic spatial coverage but with limited taxonomic or vertical resolution. Citizen science observations can greatly enhance spatial coverage and frequency of data collection, yet they often require additional validation and standardisation steps. As a result, significant preprocessing, harmonisation and quality control are required before joint analyses can be conducted, increasing analytical complexity and computational demands.

Effective data handling frameworks must therefore prioritise interoperability, scalability and transparency from the outset. This requires the systematic adoption of the FAIR data principles (Findable, Accessible, Interoperable and Reusable) to ensure the long-term value, traceability and reusability of monitoring outputs (Wilkinson et al., 2016). Practical implementation of FAIR principles involves the use of standardised metadata schemas, controlled vocabularies and ontologies, persistent digital identifiers, and deposition of datasets and workflows in open and trusted data repositories. Such practices not only facilitate data reuse and synthesis across projects and regions, but also support reproducibility, auditability and compliance with national and European data management policies.

Artificial Intelligence and Machine Learning (AI-ML) approaches are increasingly recognised as essential components of modern marine data infrastructures. These tools offer powerful capabilities for harmonising large and complex datasets, automating quality control and anomaly detection, extracting patterns from high-dimensional data, and linking environmental drivers to biological and ecological responses across multiple spatial and temporal scales. AI-ML methods can also support data fusion, enabling the combined use of remote sensing, in situ observations and biological data to improve predictive modelling, early-warning systems and scenario analyses.

Ultimately, integrated data handling pipelines should enable the synthesis of multiple Essential Ocean Variables (EOVs) and Essential Biodiversity Variables (EBVs) into robust, transparent and operational indicators. Such indicators must be directly interpretable and usable by policymakers, environmental managers and stakeholders, supporting evidence-based decision-making, adaptive management and effective communication of ecosystem status and trends (Beierkuhnlein et al., 2025). Developing and maintaining these pipelines within cost-effective monitoring frameworks will significantly enhance the capacity of national and regional programmes to deliver timely, policy-relevant assessments of marine ecosystem status, contributing directly to the implementation of the Marine Strategy Framework Directive and related European and international strategies for marine conservation and sustainable use.

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