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Novel Monitoring Technologies - an integrated overview of traditional and emerging methods for monitoring marine biodiversity

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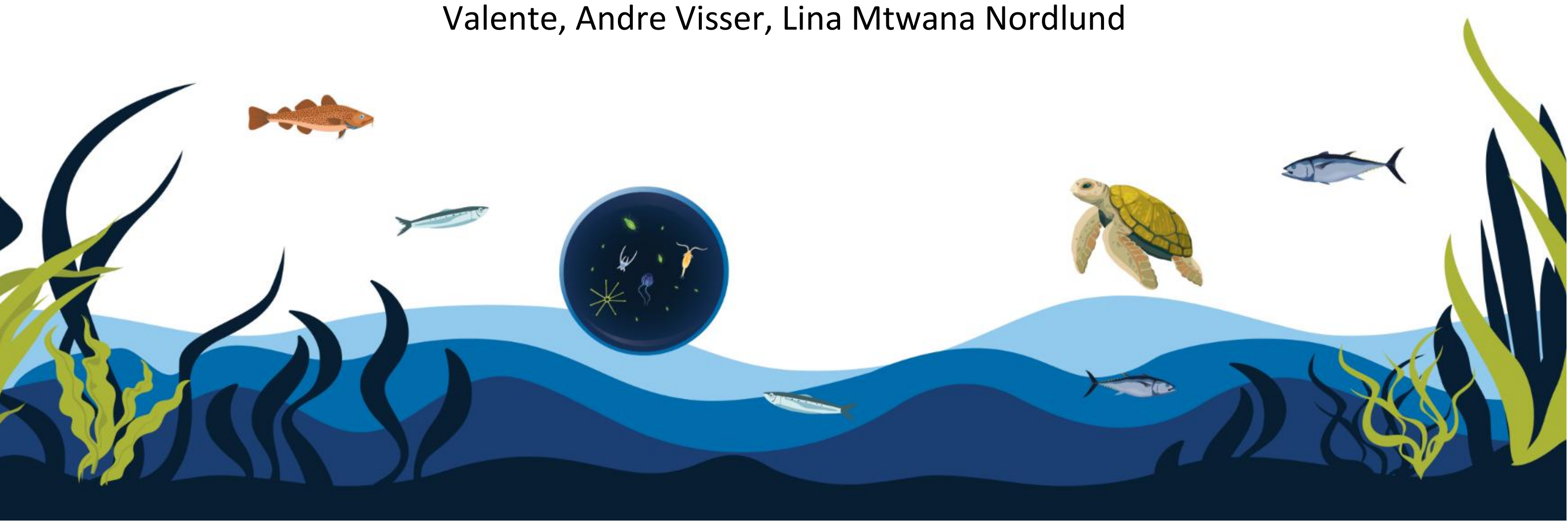

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Executive Summary

This report provides an integrated overview of traditional and emerging methods for monitoring marine biodiversity, with emphasis on Essential Ocean Variables (EOVs). Traditional visual sampling methods such as underwater visual census (UVC), quadrat surveys, and net tows have long formed the backbone of biodiversity monitoring, offering high taxonomic resolution and compatibility with historical data.

Emerging technologies – including environmental DNA (eDNA), imaging combined with Artificial Intelligence (AI) analysis, and remote sensing – promise to boost biodiversity observations by increasing spatial and temporal coverage, automating labour-intensive processes, and reducing environmental impact. Emerging technologies – including environmental DNA (eDNA), imaging combined with Artificial Intelligence (AI) analysis, and remote sensing (satellites and drones) – promise to boost biodiversity observations by increasing spatial and temporal coverage, automating labour-intensive processes, and reducing environmental impact. eDNA enables non-invasive detection of cryptic species, while AI image analysis automates species identification and habitat quantification. Remote platforms such as Unmanned Aerial Vehicles (UAVs) and Remotely Operated Vehicles (ROVs) extend survey capabilities into deep or otherwise inaccessible environments.

Key findings highlight the strengths and limitations of each method. eDNA excels in sensitivity but faces challenges in assigning species to sampling sites and in abundance estimation. AI imaging supports scalable monitoring, but requires large, annotated training sets. Acoustic methods offer large-scale tracking of biomass via proxies, but they still lack taxonomic precision. Hybrid approaches are emerging as best practice: combining traditional methods with novel tools is necessary for cross-validation and to maximize spatial and temporal coverage. Calibration remains essential, as it requires reference libraries and standardized protocols. The combination of traditional and emerging biodiversity observing technologies aligns with ethical research principles, such as the 3Rs (Replacement, Reduction, Refinement), especially by reducing the impact associated with destructive sampling.

We recommend adopting integrated monitoring frameworks that combine *in situ*, genetic, and remote sensing data collection, tailored to the goals and constraints of each project. Supporting policy development and training efforts will be key to mainstreaming these approaches across the EU and global marine monitoring networks.

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Abbreviations

Abbreviation	Definition
AI	Artificial Intelligence
Air Centre	The Atlantic International Research **Centre**
ASV	Amplicon Sequence Variant
AUV	Autonomous Underwater Vehicle
CATAMI	Collaborative and Automated Tools for Analysis of Marine Imagery
CIIMAR	Interdisciplinary Centre of Marine and Environmental Research
CNN	Convolutional Neural Network
CPR	Continuous Plankton Recorder
DSL	Deep Scattering Layer
DVM	Diel vertical migration
eDNA	Environmental DNA
EOV	Essential Ocean Variable
GZ	Gelatinous Zooplankton
mAP	Mean Average Precision
MEDITS	International bottom trawl survey in the Mediterranean
ML	Machine Learning
MOi	Mercator Ocean International
MSFD	Marine Strategy Framework Directive
PAM	Passive Acoustic Monitoring
POC	Particulate Organic Carbon
qPCR	Real-time PCR
RLS	Reef Life Survey
RGB	Red-Green-Blue
ROV	Remotely Operated Vehicle
SDM	Species Distribution Model
SSH	Sea Surface Height
SST	Sea Surface Temperature
UAV	Unmanned Aerial Vehicle
UNIPI	University of Pisa
UU	Uppsala University
UVC	Underwater Visual Census
VHR	Very High-Resolution
VPR	Video Plankton Recorder
YOLO	You Only Look Once

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1. Introduction

Marine biodiversity contributes key ecosystem functions such as nutrient cycling and carbon sequestration and provides critical services for human wellbeing, but it is increasingly threatened by the cumulative effects of climate change and direct anthropogenic pressure (IPBES 2019; IPCC 2021). Evaluating the response of marine biodiversity to these threats is critical to support informed conservation policies and to assess progress towards national and international conservation targets, including the UN Sustainable Development Goals, EU Biodiversity Strategy, Green Deal, and the Kunming-Montreal Global Biodiversity Framework. Monitoring ocean biodiversity is hampered by the difficulty of conducting underwater observations and the myriad of biodiversity indicators proposed to assess environmental health and the multidimensional nature of biodiversity (Chase et al. 2018). A better understanding of biodiversity patterns and their dynamics requires increased spatial and temporal coverage of observations, harmonization of sampling methods, and better coordination of sampling efforts. Improving our capacity to automate data collection and processing will dramatically increase the amount and quality of information and knowledge available to scientists and decision-makers.

The development of new tools such as environmental DNA (eDNA), sensor networks, underwater imaging through cameras and drones, and the use of artificial intelligence (AI) to extract information from expanding data sets are promising technological advancements to improve non-invasive, cost-effective, and high-frequency monitoring of biodiversity. The Essential Ocean Variable (EOV) framework offers a simplified approach to standardize biodiversity observations, reducing the number of measurements to a limited set of socially relevant and feasible variables (Miloslavich et al. 2018; Muller-Karger et al. 2018). For example, EOVs such as coral, mangrove, macroalgal, and seagrass cover and composition can be measured easily and cheaply, informing on habitat state and capturing phenomena such as regime shifts. Microbe, phytoplankton, and zooplankton biomass and diversity provide key links between marine biodiversity and biogeochemical processes, while invertebrate, fish, sea turtle, seabird, and mammal abundance and distribution allow tracking changes in food webs, top predators, and commercial species. The combination of technological innovation and simplified biodiversity frameworks provides an unprecedented momentum to advance monitoring of marine biodiversity globally.

In the BioEcoOcean project, there are several living labs - collaborative platforms - focusing on advancing our understanding of biology and ecosystems in the ocean. Each living lab focuses on a set of EOVs and represents different environments. In this deliverable, we are specifically focusing on the following EOVs; Macroalgal canopy cover and composition, Seagrass cover and composition, Fish abundance and distribution and Zooplankton biomass and diversity.

Promising technologies are still in the early stages of development and need to be calibrated and standardized by directly comparing their outputs with those of traditional methods for monitoring marine biodiversity. Here, we 1) provide an overview of traditional sampling methods for marine biodiversity, with emphasis on the EOVs addressed by BioEcoOcean living labs, 2) introduce eDNA, imaging, remote sensing and AI-based analysis, 3) provide a comparative evaluation of emerging

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technologies and traditional approaches for monitoring biodiversity, 4) discuss hybrid approaches and 5) conclude with some recommendations and way forward. Case studies and applications from living labs are illustrated throughout.

1.1 EOVs in focus

1.1.1 Macroalgal canopy cover and composition

Macroalgal canopies create vital underwater forests on temperate rocky reefs, supporting high biodiversity and providing key ecosystem functions and services such as primary production, nursery habitats, food resources, and coastal protection. These forests face global threats like ocean warming and local pressures such as habitat loss, pollution, eutrophication, and invasive species, which together reduce their resilience and increase the risk of regime shifts and population declines. Such changes often involve replacement by less productive, low-diversity algae or barren areas. Macroalgal forests react quickly to environmental stress, serving as early indicators of change, and their wide range allows monitoring of regional trends and species distributions.

Macroalgal canopy cover and composition is a reliable, easily measured indicators of ecosystem health and environmental change. Monitoring macroalgae allows scientists to track ecological shifts and broader trends. Healthy macroalgae are vital for biodiversity and sustainable coastal economies.

The sub-variables required to assess macroalgal canopy cover and composition include percent cover, stipe density, canopy species diversity and areal extent of occupancy. These sub-variables are indicated in the EOV specification sheet (Macroalgal canopy cover and composition specification sheet), along with ancillary variables and derived products that are necessary to interpret the EOV. Supporting variables include environmental drivers of change such as nutrients, sea surface temperature, salinity, dissolved oxygen and ocean colour, informing on regional productivity. Other EOV related supporting variables include canopy height, photosynthetic biomass plant size classes, photosynthetic efficiency, algal decomposition rate and signs of necrosis on algal thalli. Species composition and abundance of understory assemblages and extent of alternative habitats (e.g., barrens, algal turfs) provide additional measurements to understand the health of macroalgal canopies and the risk of shifting to undesired alternative states.

1.1.2 Seagrass cover and composition

Seagrasses are submerged marine plants that form extensive meadows in shallow coastal waters, serving as foundation species for diverse and productive ecosystems (Unsworth et al. 2022). These habitats provide essential ecosystem services: they stabilize sediments, improve water quality, support fisheries, and offer critical nursery and feeding grounds for marine species (Nordlund et al. 2016). Seagrass meadows are also globally significant carbon sinks, capturing and storing “blue carbon” in

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both biomass and sediments, which helps mitigate climate change (UNEP, 2020). Despite their importance, seagrasses are declining due to coastal development, nutrient pollution, and climate-induced stressors. Monitoring seagrass cover and species composition is thus essential to detect change, assess ecosystem health, and inform conservation and climate strategies.

GOOS defines seagrass cover and composition as an Essential Ocean Variable (EOV), based on three core sub-variables: percent cover, areal extent, and species composition (see Seagrass Cover and Composition EOV Specification Sheet). These are complemented by supporting variables such as water depth, clarity, temperature, salinity, sediment characteristics, and indicators of condition like biomass, productivity, and epiphytic load. Monitoring methods include in situ diver-based surveys, drop cameras, and quadrat sampling for fine-scale data, as well as remote sensing technologies—including drones, satellites, and acoustic tools—for large-scale mapping. New techniques like eDNA are also being explored for species detection.

Derived products include estimates of global and regional distribution, diversity metrics, ecosystem resilience, and carbon sequestration potential. These products support applications across marine spatial planning, biodiversity conservation, climate adaptation, and ecosystem service valuation. Standardised methods and integration into platforms like Ocean Biodiversity Information System (OBIS) and the GOOS BioEco Portal ensure that seagrass data are Findable, Accessible, Interoperable, and Reusable (FAIR).

1.1.3 Fish abundance and distribution

Fish play a crucial role in maintaining the balance of marine ecosystems, occupying a wide spectrum of trophic levels. They represent a primary source of food and proteins for humans and support the economic well-being of millions of people, generating a global economic value of approximately $150 billion annually (FAO, 2022). However, climate change, overfishing, and habitat destruction are increasingly threatening these resources. Accurate and standardized monitoring of the status and dynamics of fish populations is therefore necessary to guide the sustainable management of these resources and ensure the health of marine ecosystems, food security, and economic stability globally.

Fish abundance, length distribution, biomass, and species composition represent the four sub-variables proposed by GOOS to estimate this EOV (see Fish abundance and distribution EOV specification sheet). Furthermore, supporting-variables are important to describe the environmental context (e.g., seawater temperature, salinity, habitat and depth), the management of the area, for example the presence of an MPA, and the sampling method and design. Other EOV-related supporting variables include among the others, fish life history traits (such as reproductive strategy), trophic ecology, movement, and behavior.

By combining this information, it is possible to derive key indicators of the status of fish communities. These products include abundance, size, and biomass indices, taxonomic and functional diversity, indicators of community structure, foodweb indicators (e.g., the proportion of predatory fish or mean trophic level), and measures of fish production (e.g., spawning biomass or recruitment).

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1.1.4 Zooplankton biomass and diversity

Zooplankton, drifting animal plankton ranging from microscopic copepods to gelatinous organisms, are foundational to marine ecosystems worldwide. They provide a key node in marine food webs by linking primary producers (phytoplankton) to higher trophic levels, thus directly sustaining fisheries and supporting vulnerable marine megafauna. Meso-zooplankton (~ 0.02–2 cm) have a central role in the marine ecosystems, as prey of all larger species and contributor to the “biological pump” through the sinking of particulate organic carbon (POC), diel vertical migrations (DVM) – and seasonal migration to mesopelagic layers, all mechanism resulting in the storage of CO_2 in the deep ocean. There is also a renewed interest in the exploitation of some lipid-rich species, *e.g.*, *Calanus finmarchicus* in the sea of Norway (Wiborg 1976; Aasum et al. 2025). Rapid changes in the marine environment linked to ocean warming, deoxygenation, and ocean acidification are impacting the zooplankton species communities and dynamics, with cascading effects through all the upper trophic levels. Marine ecosystem models, including these zooplankton species or groups of species, are facing a serious lack of comprehensive datasets to properly calibrate model parameters and estimate their biomass and composition. Monitoring zooplankton is essential to detect phenological shifts, range expansions, and changes in community structure driven by warming, acidification, and deoxygenation.

GOOS recognises zooplankton biomass and diversity as an Essential Ocean Variable (EOV), consisting of three sub-variables: biomass, abundance, and species composition (see Zooplankton Biomass and Diversity EOV Specification Sheet). These are ideally measured at depth-resolved vertical profiles and expressed in units such as mgC/m^3 or $individuals/m^3$. Supporting variables include temperature, salinity, oxygen, and phytoplankton biomass, as well as biological traits such as body size, life stage, and biochemical composition.

Sampling methods range from net-based collections (e.g. Bongo nets, MOCNESS), Continuous Plankton Recorder (CPR) surveys, and ship-based optical systems, to advanced tools like autonomous gliders, acoustic profilers, and holographic imaging systems. Emerging molecular techniques like eDNA also enhance taxonomic resolution and monitoring capabilities.

From these observations, a range of derived products can be generated, including diversity indices, size structure metrics, functional group indicators, and estimates of secondary production. These support ecosystem assessments, fisheries management, and integration with climate models and biodiversity indicators (EBVs, ECVs). To maximise utility, zooplankton data must be shared through global platforms (e.g., OBIS, COPEPOD) and align with FAIR principles.

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2. Methodological overview

2.1 Traditional Sampling Methods

Traditional visual sampling methods have long been the cornerstone of marine biodiversity monitoring and ecological assessments across European coastal ecosystems. These methods are typically low-tech, widely standardized, and offer compatibility with historical data series — making them particularly valuable for long-term monitoring efforts. This section provides an overview of the classical methodologies used to survey macroalgae, zooplankton, and fish communities, as well as seagrass meadows. It also outlines their main strengths and limitations.

2.1.1 Macroalgal canopy cover and composition

For benthic macroalgae, visual quadrat sampling is the method of choice (Sutherland 2006). Quadrats, typically 50 x 50 or 100 x 100 cm in size are placed at intervals along transects, allowing divers to estimate key sub-variables of the EOV, including percent cover, species diversity, stipe density (see Macroalgal canopy cover and composition specification sheets). This approach is especially effective in intertidal and shallow subtidal areas dominated by canopy-forming macroalgae such as Fucales and Laminariales. It allows researchers to document spatial heterogeneity and canopy structure, which are often related to ecosystem resilience and biodiversity patterns.

In recent years, the use of photographic quadrats has increased, particularly in subtidal environments where diving time is limited. High-resolution images taken *in situ* are later processed and annotated in the laboratory using specialized software, such as CPCe, PhotoQuad, or CoralNet. This method enhances reproducibility, facilitates long-term archiving, and allows for retrospective analyses of macroalgal canopy dynamics or benthic invertebrate colonization. However, photographic methods require high image quality and are often less effective at detecting small, cryptic, or understory species. As a result, they are frequently used in tandem with traditional visual assessments.

The visual assessment of sessile organism percent cover typically involves two main methods: the Random Point Quadrat and visual estimation. The latter is generally preferred, as it tends to yield greater precision among observers and is more sensitive to the detection of rare species (Dethier et al. 1993; Benedetti-Cecchi et al. 1996). Moreover, variability among observers with different levels of expertise tends to decrease with continued sampling, and initial training can further reduce this variability. Finally, although visual methods may be limited in their ability to achieve fine-scale taxonomic identification, organisms can be grouped into broader morphological categories to maximize the ecological information obtained. This approach is supported by standardized systems such as the Collaborative and Automated Tools for Analysis of Marine Imagery (CATAMI) classification scheme, which provides a hierarchical vocabulary that facilitates consistent annotation across studies and platforms (Althaus et al. 2015).

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Complementing these traditional approaches is a range of other visual tools. Towed video and drop-camera systems enable rapid assessment over larger spatial scales. They are instrumental in soft-bottom or low-complexity habitats where diver-based surveys are less effective. Stereophotogrammetry, although historically used for reef profiling through diver sketching, has been revitalized through modern 3D imaging technologies, allowing for precise estimates of habitat complexity and structural change. Permanent photo stations and fixed markers have also become a mainstay in long-term monitoring programs, allowing for consistent documentation of benthic habitat dynamics and recovery following disturbances.

Ultimately, the choice of visual census method depends on the biological system under investigation, the spatial and temporal resolution required, and the logistical constraints of the survey. While traditional visual surveys offer high-resolution and taxonomically detailed data, newer image-based approaches provide scalability and reproducibility. When combined thoughtfully, these techniques form a powerful toolkit for the integrated assessment of marine biodiversity and ecosystem health.

2.1.2. Seagrass cover and composition

Seagrass monitoring methods share several similarities with those used for macroalgae, relying heavily on visual assessments, permanent plots, and shoot density counts within quadrats (commonly 20 × 20 or 50 × 50 cm). Additional descriptors such as leaf length, epiphyte load, and percent cover are routinely recorded. Where water clarity permits, snorkel-based line transects or diver counts are used to map meadow extent and patch structure. In some programs, semi-destructive techniques, such as rhizome collection and morphometric analysis, are applied to study growth and demographic parameters, although their use is generally limited to research settings due to their impact on the habitat.

In traditional monitoring programs, the core variables observed include seagrass percent cover and species composition, typically measured *in situ* via quadrat-based surveys, drop cameras, and/or diver observations. Cover estimates are often quantified using percent cover or Braun-Blanquet scale categories, while species composition is recorded to the lowest taxonomic level possible. Supporting variables – such as shoot density, canopy height, and above- and below-ground biomass – provide additional ecological context and are frequently integrated into long-term ecological research programs.

Mapping of the areal extent has traditionally relied on GPS-enabled point surveys (*e.g.,* swimming, boating, or walking along meadow boundaries) and diver-based video transects, though remote sensing techniques (*e.g.,* aerial photography and satellite imagery) are increasingly adopted. Standardised protocols (*e.g.,* Kenworthy et al. 1993; MarineGEO 2020, SeagrassNet, Seagrass Watch) are commonly used to ensure consistency in data collection across spatial and temporal scales. The GOOS Seagrass Essential Ocean Variables (EOVs) (GOOS, 2025) framework aims to globally coordinate the seagrass monitoring to improve tracking seagrass habitat trends, detecting shifts in species composition, and estimating carbon storage and ecosystem health. Through the development of tools

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such as the Seagrass cover and composition EOV specification sheet (Seagrass cover and composition specification sheet), GOOS provides guidance to collect standardised observations that can be comparable nationally, regionally, and globally.

2.1.3 Fish abundance and distribution

The most widespread observing approaches for reef fish communities use visual surveys carried out by divers or using video systems. These approaches allow for the monitoring of key sub-variables that are fish abundance (number of individuals), fish length, fish biomass, and species composition.

Underwater Visual Census (UVC) is the standard non-destructive technique used to characterize fish assemblages in shallow waters and Marine Protected Areas. A widely used method involves divers conducting surveys along predefined linear transects – for example, 25 × 5 m – in which species identity, abundance, and estimated size classes (*i.e.*, small, medium, large) are recorded (Harmelin-Vivien & Harmelin 1975). This technique is highly effective in structured habitats, such as shallow rocky reefs or seagrass beds, where complex three-dimensional structures provide habitat and shelter for diverse taxa. The method is well-integrated within established regional and international protocols, including the Reef Life Survey program, and supports compliance with the EU Water Framework and Marine Strategy Framework Directives.

Reef Life Survey (RLS) is a global citizen-science program designed to maximize comparability between surveys conducted around the world (Edgar and Stuart-Smith 2014; Edgar et al. 2020). The RLS protocol includes three distinct sampling methods to collect abundance data on reef fishes, mobile invertebrates, and sessile organisms such as corals and macroalgae. For reef fish, two separate methods are used at each site (Fig. 1). The first involves recording the number and size of each fish species observed along two adjacent 50 × 5 m transects. The second method focuses on cryptobenthic fishes, which are often overlooked by the first method. In this case, the diver swims close to the bottom and records benthic species along two paired 1 m-wide transects placed along the same line used in the first method. In both methods, total fish length is noted using increasing size intervals of 2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 20.0, 25.0, 30.0, 35.0, 40.0, 50.0, 62.5 cm, and above.

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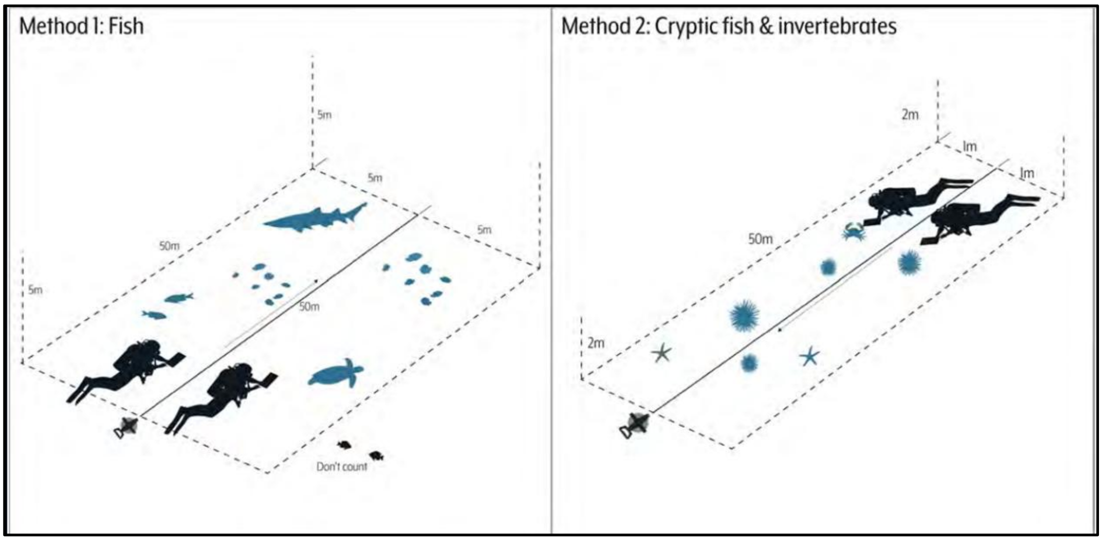

Figure 1: Layout of the two methods for fish survey and the blocks underwater (adapted from ReefLifeSurvey)

UVC allows real-time observations and taxonomic identification, but it is sensitive to observer bias and environmental conditions like turbidity and water movement, which can limit visibility and dive time. Moreover, UVC methods tend to underestimate elusive and highly mobile species, while favoring those that are either highly visible or attracted to divers (Kulbicki, 1998; Watson and Harvey, 2007).

Video techniques have been increasingly employed in the last two decades. These techniques include remote underwater video, either baited or not, diver-operated video, and towed video (Mallet and Pelletier, 2014). Remote videos can be obtained by cameras deployed at fixed stations (*e.g.*, tripods on the seafloor, moorings) or mounted on Remote Operated Vehicles (ROVs). They allow for long-term monitoring, behavioral studies, and exploration of deep habitats (Gilby et al. 2018; Jones et al. 2021). Underwater videos can be analyzed manually or through AI tools (as described comprehensively in section: Imaging & AI-based Analysis). Despite the growing use of video-based techniques, driven by technological progress and improved cost-efficiency, there is still a lack of standardized sampling protocols, which are essential to ensure data comparability among studies (Nalmpanti et al. 2023).

2.1.4 Zooplankton biomass and diversity

Net trawling is the traditional approach to estimate the Zooplankton biomass and diversity EOV and associated sub-variables, including biomass, abundance, and diversity (see Zooplankton biomass and diversity specification sheet). This is a costly method that requires considerable sampling effort to cope with the patchy distribution of these organisms. Nevertheless, long-term time series with regular measures of abundance in a few fixed oceanographic stations, *e.g.*, off the coast of Hawaii (Hawaii Ocean Time-series (HOT) station) or in the Sargasso Sea (Bermuda Atlantic Time Series (BATS) station), are essential and precious information to calibrate models and analyze long-term changes. Consistent use of the same sampling gear (the Bongo net) and standardized method is central to providing reliable abundance estimates. A Bongo net is a twin circular frame in metal, each of 60 cm in diameter (though

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sizes may vary) to which are attached two plankton nets made of fine mesh (commonly 200 µm, but can range from 64–500 µm depending on target organisms). Each net ends in a detachable collection cup (cod end) where the sample is concentrated. The sampling gear is towed obliquely or vertically from a defined depth. For a quantitative estimate, it is important to measure the volume of water filtered, either using a flowmeter on the net or starting the vertical tow from a measured maximum depth. For oblique tow, the hauling speed is usually 1–2 knots.

The Continuous Plankton Recorder (CPR) is another traditional approach designed in the 1930s by Alister Hardy in Lovestoft laboratory (UK). It is a robust mechanical sampling device designed to collect plankton over long distances while being towed behind ships at high speeds. Plankton is collected on a slowly moving band of silk mesh, which is later preserved and analysed in laboratories. The CPR captures organisms from the upper layer of the ocean (typically ~10 m depth). It provides only relative abundance, but the interest is its use over very long-term periods and large areas, *e.g.*, over 90 years in the North Atlantic, providing amongst the longest and most consistent biological datasets in marine science. Other traditional approaches include bottle sampling (*e.g.*, Niskin), allowing the collection of zooplankton at discrete depths along vertical profiles, and sediment traps deployed at specific depths to collect sinking particles, including dead or vertically migrating zooplankton. These latter methods are of particular interest to study vertical migration and carbon flux. Pump sampling systems onboard research vessels with filtering and collectors allow efficient, continuous, or discrete sampling from specific depths. The sampled water can also be directed into analytical instruments such as the Video Plankton Recorder (VPR) or the ZooScan (Table 1).

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Table 1. Historical developments of zooplankton observation systems.

Period	System	Comment
Late 1800s – Early 1900s	**Net Sampling (*e.g.*, Ring net, Bongo net, WP2)**	First standardised plankton nets
1931	**Continuous Plankton Recorder (CPR)**	Sir Alister Hardy (UK) https://www.cprsurvey.org/
1950s–1970s	**Bottle Sampling (Nansen, Niskin, Van Dorn)**	Used for collecting water at discrete depths; often paired with fine sieves for zooplankton.
1960s–1980s	**Pump Sampling Systems**	
1980s	**Video Plankton Recorder (VPR)**	Woods Hole Oceanographic Institution (WHOI); early prototypes for laboratory analyses.
1990	**Laser Optical Plankton Counter (LOPC)**	Brook Ocean Technology (Canada); first commercial version developed. High-frequency sampling of the size spectrum of particles. No species identification.
Mid-1990s	**Video Plankton Recorder (VPR)** (Operational)	WHOI; used for real-time, high-res imaging of plankton *in situ*.
1996–2000	**ZooScan** (Lab-based imaging)	French CNRS laboratory allows semi-automated analysis of net/pump samples.
2003–2005	**Underwater Vision Profiler (UVP5)**	Developed by Hydroptic (France). High-resolution imaging system. Archiving photos for further identification and counting. Later, coupled with AI imaging systems.
2010s	**Integration with Autonomous Platforms**	Gliders, AUVs, and profiling floats begin to incorporate VPR, LOPC, and UVP.
2020s–present	**AI and Deep Learning for Image Classification**	Automated species-level classification of plankton from imaging systems (e.g., EcoTaxa, PlanktonNet).

2.2 Strengths & Limitations for Traditional Sampling Methods

2.2.1 Benthos – marine life found on the bottom – diver-based underwater visual census

Strengths

- **Non-invasive and cost-effective**: Ideal for long-term ecological monitoring without damaging habitats.

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- **High taxonomic resolution (UVC and quadrats)**: Allows direct identification and size/abundance estimation, especially for fish, macroalgae, and visible invertebrates.
- **Standardized protocols**: Widely used across Europe (e.g., MSFD), facilitating cross-study comparisons.
- **Useful in structured habitats**: Effective in areas like rocky reefs and seagrass beds where habitat complexity supports diverse communities.
- **Photographic methods enable archiving**: Images allow retrospective analysis and reduce observer bias during post-processing.
- **Applicable across biological systems**: Adaptable to fish, macroalgae, benthic invertebrates, and seagrasses.

Limitations

- **Observer bias and training dependency**: Accuracy in UVC and quadrat methods depends heavily on diver expertise and consistency.
- **Limited by environmental conditions**: Turbidity and swell can reduce visibility and survey accuracy.
- **Photographic methods may miss cryptic/understory species**: Lower resolution for small or hidden taxa compared to *in situ* identification.
- **Spatial and temporal resolution vary**: Not all methods capture fine-scale or rapid ecological changes (*e.g.*, seasonality).
- **Limited depth coverage**: SCUBA-based methods are constrained to depths reachable by divers (typically <30 m).
- **Image analysis is time-consuming**: Manual annotation of images requires significant post-processing effort.

2.2.2 Reef fish non-destructive sampling methods

Strengths

- **UVC is non-destructive:** appropriate for the monitoring of MPAs.
- **Availability of standardized protocols for UVC** (e.g., RLS) **and capture-based surveys** (e.g., ICES 2022).
- **Video techniques increase capabilities:** enabling long-term, continuous monitoring and deep-habitat exploration.
- **Availability of standardized video methods**: harmonized protocols for baited video surveys have become available recently, improving data comparability (Langlois et al. 2020).

Limitations

- **Observer bias and skill dependency:** UVC accuracy may be influenced by diver expertise and

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inter-observer variability.

- **Frequent underestimation of elusive/mobile species**: Species that avoid divers or move rapidly are often missed or miscounted.
- **Depth limitations for UVC**: SCUBA-based surveys are generally restricted to shallow habitats (<30 m).
- **Manual video analysis is time-consuming:** Without automated tools, processing large video datasets is labor-intensive.

2.2.3 Net sampling of zooplankton

Strengths

- **Cost-efficient and accessible**: Relatively simple and inexpensive equipment requiring a minimum of infrastructure in the field (in some cases).
- **Standardised and widely used**: Established protocols allow for easy comparison across studies and periods.
- **Efficient for filtering large volumes**: Can sample large volumes of water quickly, which increases the likelihood of capturing rare or patchy species.
- **Adaptability to environmental requirements**: Can be used in various marine settings.
- **Collection of physical specimens**: Samples can be preserved for detailed laboratory analyses.
- **Assessing trophic community structure**: Enables studies on community composition, diversity, and food web dynamics.

Limitations

- **Mesh size bias**: Selective for organisms larger than the mesh size (smaller taxa pass through undetected).
- **Sample damage**: Fragile organisms can be destroyed or deformed beyond recognition during towing, especially at high speed.
- **Net avoidance behaviour**: Some zooplankton (mostly macrozooplankton) can detect and avoid approaching nets, leading to an underrepresentation of certain species.
- **Quantification limitation**: Accurately estimating sampled water volume can be difficult, especially in open-ocean and turbulent settings.
- **Labour-intensive identification**: Requires experienced taxonomists to identify organisms, especially for diverse assemblages or cryptic species.
- **Not-real time**: Net sampling does not provide real-time data.
- **Potential contamination**: Cross-contamination between samples or improper cleaning of gear can affect data quality.

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2.3 Emerging Methods

2.3.1 Environmental DNA (eDNA)

Environmental DNA (eDNA) enables the detection of DNA fragments originating from organisms in aquatic environments. These DNA fragments are typically released into the environment through natural processes such as cell sloughing, the shedding of reproductive structures, or the degradation of tissue (Thomsen and Willerslev 2015; Deiner et al. 2017). By detecting these fragments, eDNA methods provide a highly sensitive tool for identifying the presence of macroalgal, fish, seagrass, and plankton communities without the need for direct visual observation or physical collection of the organisms. This approach offers significant advantages for monitoring hard-to-access or visually cryptic species within aquatic ecosystems.

Sampling (water collection, filtration, DNA extraction, primers, sequencing)

The eDNA workflow begins with the collection of water samples from environments of interest. The volume of water required for detection is based on the target organism of interest, with rarer or pelagic species (*e.g.*, fish or cephalopods) often requiring greater volumes to be collected. The water is ideally filtered directly in the field, or, if unable to do so, into sterile containers which are then filtered at a later time point (Goldberg et al. 2016). It must be noted that eDNA can also be sampled from sediment, which follows a similar procedure except that sediment replaces the water filters. Following collection, DNA is extracted from the collected filters using specialized extraction kits, such as PowerSoil® Pro or PowerWater® kits (Qiagen), which are selected depending on sample type and desired downstream applications. After extraction, specific primers designed to amplify DNA sequences of organisms of interest, targeting regions such as the rbcL or Cytochrome *c* oxidase subunit I (COI) genes, are used (see Table 2; Stat et al. 2017). Amplified DNA products are then sequenced using high-throughput sequencing platforms (*e.g.*, Illumina MiSeq), providing detailed information about the diversity and relative abundance of taxa present in the sampled environment.

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Table 2. Common primer pairs used to target different organisms of interest. COI indicates the cytochrome-c-oxidase subunit 1 within the mitochondrial electron transport chain.

Target organism	Gene region	Gene	Primer name		Sequence (5'→3')	Reference
Universal metazoan	COI	cox1	mlCOIintF	F	GGWACWGGWTGAACWGTWTAYCCYCC	Leray et al 2014
			jgHCO2198	R	TAIACYTCIGGRTGICCRAARAAYCA	Geller et al 2014
Macroalgae	COI	cox1	cox1-117F	F	TTTCHACNAAYCAYAAAGATAT	Bittner et al., 2008
			cox1-784R	R	ACTTCDGGRTGDCCAAAAAACCA	
			cox1-789F	F	TNTAYCARCATTTATTTTGGTT	Silberfield et al 2010
			cox1-1378R	R	TCYGGNATACGNCGNGGCATACC	
			gazF2	F	CCAACCAYAAAGATATWGGTAC	Lane et al., 2007
			gazR2	R	GGATGACCAAARAACCAAAA	
		cox3	cox3-44F	F	CAACGNCAYCCWTTTCATTT	Silberfield et al 2010
			cox3-739R	R	CATCNACAAAATGCCAATACCA	
	Nadh	nad1	nad1-113F	F	CGWAAAATTATGGCNGGTATTCA	Silberfield et al 2010
			nad1-895R	R	AGNGGYAARAAACAYTTCCAACC	
		nad4	nad4-242F	F	TTYTNGGKGTNGATGGTATNTC	Silberfield et al 2010
			nad4-985R	R	ACAAAMCCATGRCTTAANCTTTG	
	Chloroplast	rbcL	rbcL-708R	R	TTAAGNTAWGAACCYTTAACTTC	Bittner et al., 2008
			rbcL-543F	F	CCWAAATTAGGTCTTTCWGGWAAAAA	
		psbA	psbA-F	F	ATGACTGCTACTTTAGAAAGACG	Yoon et al., 2002
			psbA-R2	R	TCATGCATWACTTCCATACCTA	
Plankton	COI		(Plank)Minibar-Mod	F	TCCACTAATCACAAAGAYATYGGYAC	
				R	AGAAAATCATAATRAANGCRTGNGC	Berry et al. 2015
Cephalopods	16S rRNA		S_Cephalopoda_F	F	GCTRGAATGAATGGTTTGAC	Peters et al. 2014
				R	TCAWTAGGGTCTTCTCGTCC	
			Ceph16S1	F	GACGAGAAGACCCTADTGAGC	Berry et al 2017
				R	CCAACATCGAGGTCGCAATC	
Crustaceans			Crust16S	R	GGGACGATAAGACCCTATA	Berry et al 2017
				F	ATTACGCTGTTATCCCTAAAG	
Mammals			16Smam1	F	CGGTTGGGGTGACCTCGGA	Taylor 1996
				R	GCTGTTATCCCTAGGGTAACT	

Data output: presence/absence, abundance proxies, and phenology

The primary output from eDNA analysis is the detection of species presence or absence, offering information on whether particular taxa are present within the sampled area (Kelly et al. 2014).

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Abundance proxies can also be inferred based on the quantity of DNA sequences obtained for each taxon. Furthermore, incorporating qPCR assays with sequencing data can give clearer information on the 'absolute' read numbers of genes of interest (Nappi et al. 2022). While these abundance estimates provide valuable ecological insights, it is important to note that they are influenced by factors such as DNA degradation rates, shedding rates, and various environmental conditions and may not accurately represent the actual biomass of the target organism (Yates et al. 2019). Moreover, eDNA methodologies can capture temporal patterns in species occurrence, including reproductive events. For instance, DNA fragments associated with reproductive phases, such as spore release or gamete production, can be detected, thus providing insights into the phenology of macroalgal species.

2.3.2 Imaging & AI-based analysis

Imaging and artificial intelligence-based analysis, particularly those leveraging Machine Learning (ML) techniques like Convolutional Neural Network (CNN), are increasingly demonstrating their abilities and applications in ecological research (Piechaud et al. 2019; Rubbens et al. 2023). These techniques are being used for tasks such as segmentation and classification, which enable the identification of species from images and videos. Such applications enhance the scalability, efficiency, and accuracy of biodiversity monitoring, species recognition analysis, animal behavior tracking, and predictive ecological modelling (Piechaud and Howell 2022; Rubbens et al. 2023). However, these techniques rely on access to sufficient, high-quality datasets to perform effectively. This underscores the need to deploy appropriate data collection protocols or platforms, like underwater cameras, remotely operated vehicles (ROVs), and diver-operated systems for gathering images and other outputs to train algorithms for species detection, pattern recognition, and ecological change monitoring (Bridges et al. 2025).

Imaging platforms: underwater cameras, Remotely Operated Vehicles (ROVs), diver-operated systems

Accurate image-based ecological analysis begins with the deployment of effective imaging platforms capable of capturing high-resolution visual data in underwater environments (Moghimi and Mohanna 2021; Nawaz et al. 2025). The most common tools include underwater cameras, remotely operated vehicles (ROVs), and diver-operated systems. Each platform offers unique advantages depending on the depth, habitat type, species behavior, and operational conditions.

- **Underwater cameras** are widely used for monitoring benthic habitats, including macroalgal communities and seagrass meadows, as well as fish populations. These cameras can be deployed on tripods, floats, autonomous underwater vehicles (AUVs), or moorings. They are particularly valuable for time-lapse studies, long-term observations, and monitoring the diurnal/nocturnal behavior of marine species. For seagrass meadows, monitoring fish assemblages and the frequency of grazing events within the habitat can provide valuable insights into ecosystem dynamics, trophic interactions, and overall meadow health (Gilby et

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al. 2018; Jones et al. 2021).

- **ROVs** are tethered robotic systems operated from the surface and equipped with high-definition cameras and sensors. They allow access to deep or hazardous environments without risking diver safety. ROVs are ideal for systematic transect imaging, habitat mapping, and exploration of deeper reef systems (Perkins et al. 2022). Their integration with real-time data transmission makes them effective for adaptive data collection and remote monitoring missions.
- **Diver-operated systems** remain crucial in shallow water surveys where precision and flexibility are required. In diver-operated systems, divers are equipped with handheld or body-mounted cameras, allowing for close-range, targeted data collection. These systems are particularly useful for capturing images of targeted species and fine-scale ecological interactions.

Leveraging Convolutional Neural Network (CNN) as a Machine Learning (ML) technique

Machine Learning (ML) is a subset of Artificial Intelligence, which involves the use of algorithms that are capable of understanding patterns within inputted data and automatically improving from experience without the need for explicit programming (Sarker 2021). However, within ML, there is a more advanced technique known as **deep learning,** which leverages artificial neural networks to model complex, non-linear relationships in data (enabling tasks like image recognition, natural language processing, etc.). This application is useful when working with unstructured data like images and videos. In ecological research, Convolutional Neural Networks (CNNs) are increasingly applied to automate the process of species identification, coverage estimation through image segmentation, and to monitor behavioral patterns from large volumes of visual data (Mac Aodha et al. 2018; Pichler and Hartig 2023). CNN, as a deep learning technique, is structured to automatically detect spatial hierarchies in images by learning to extract features such as edges, textures, and complex shapes through layers of convolutional filters. Popular CNN model architectures include **AlexNet**, **VGGNet**, **ResNet**, and **YOLO,** each optimized with different algorithms for different image analysis tasks. Once trained on annotated datasets, these models can generalize well to new, unseen data, making them ideal for large-scale ecological monitoring across space and time. Before CNN can effectively recognize patterns or features in images, it must be trained using annotated data/labelled images.

Image annotation

Annotation helps define the “ground truth” that CNNs use to learn. This process requires drawing bounding boxes around individual species or objects of interest in the image. These labeled datasets serve as supervised learning targets, enabling CNNs to associate specific visual patterns within the species of interest. There are several software/tools used for image annotation or labelling, most of which are open source (LabelImg, CVAT, LabelMe, Roboflow). CNNs work by learning patterns in visual data, but they require labeled/annotated examples of the data to perform effectively. The annotation serves as supervised learning targets, enabling the network to understand what it should learn from each image to achieve fine-grained classification. These annotated images allow CNNs to learn what

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visual characteristics are associated with specific ecological categories or classes (Nawaz et al. 2025). As CNNs learn by recognizing patterns in annotated examples, high-quality annotation is essential because errors or inconsistencies in labeling can lead to poor model performance or misclassification (Moghimi and Mohanna 2021; Perkins et al. 2022).

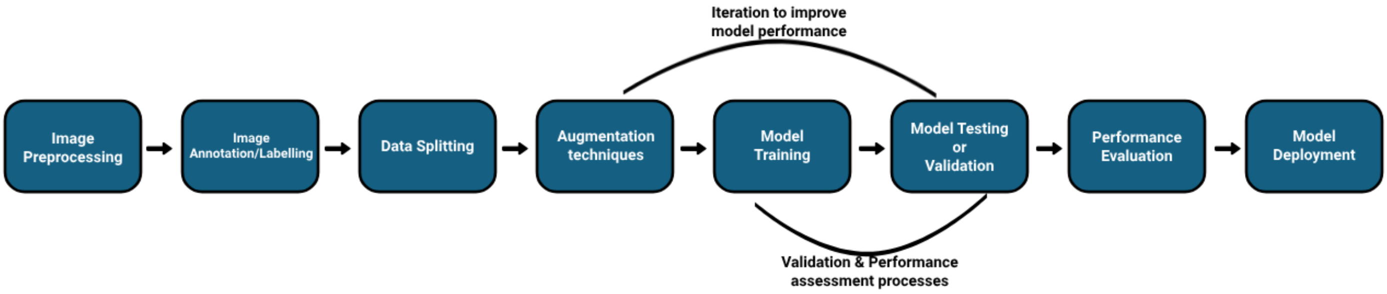

Figure 12. A simple workflow/pipeline for an AI-based species recognition model.

Model training

To properly train a model, it is essential to select the best model based on the application or use case, because the model can range from segmentation to classification. Therefore, once the dataset has been prepared through image annotation, the CNN model is ready for training (Fig. 2). This is the process where the network learns to identify features in each dataset. During training, CNN makes predictions and compares them to the correct species labels (Fig. 2). Once the training is complete, the model is evaluated using the test dataset split. The model is ready for deployment if high accuracy is achieved (a common method of training is the transfer learning method). To better understand the performance of the model, they are usually evaluated using performance metrics (confusion matrix, precision, recall, F1-score, etc.) (Fig. 3). For instance, the confusion matrix reveals misclassifications and highlights areas needing improvement. These metrics can be combined to further provide a balanced indicator of the overall model's effectiveness.

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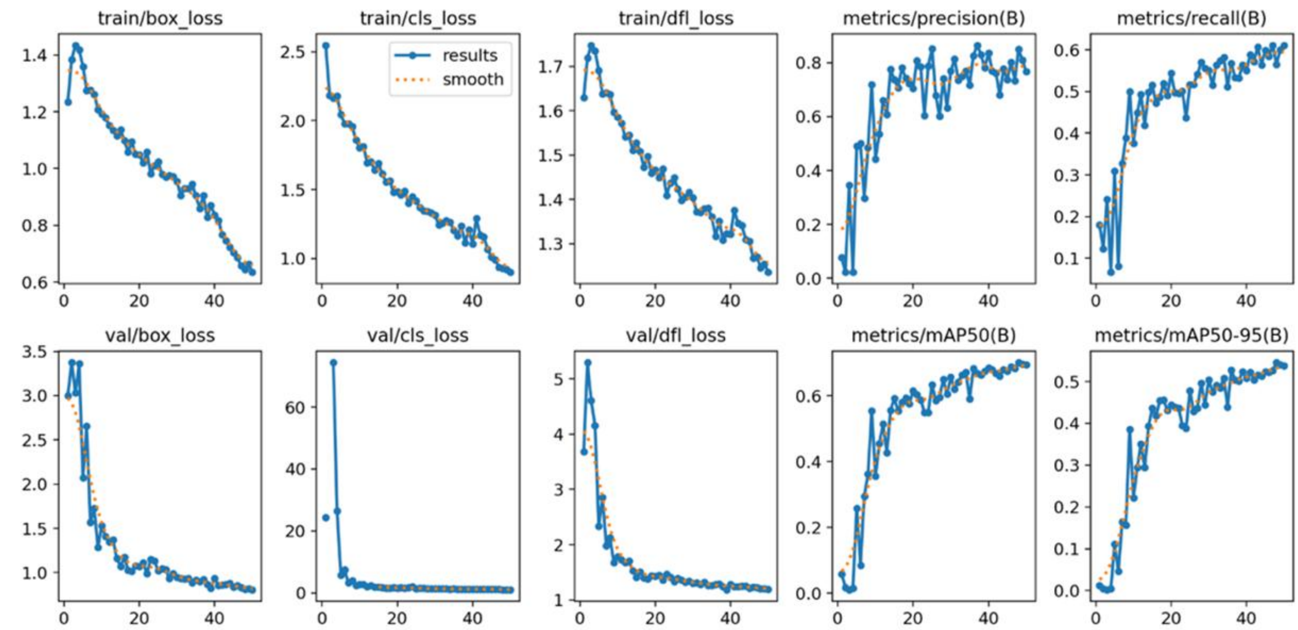

Figure 3. YOLO training summary showing loss, precision, recall, and mAP metrics over epochs.

Automated identification of macroalgae and percentage (%) cover calculation

Once trained, CNNs can process large volumes of visual data collected by underwater cameras or remotely operated vehicles (ROVs), enabling rapid and accurate analysis of habitat composition. This includes the identification and quantification of key benthic marine life such as macroalgae species, seagrasses, corals, etc. All of which are components of Essential Ocean Variables (EOVs). By automating these processes, CNNs significantly reduce the time, labor, and cost associated with traditional methods of surveys, which are often resource-intensive, requiring significant time, manual effort, and financial investment, which limits their scalability and broader application (Giménez-Romero et al. 2024; Nawaz et al. 2025). Techniques such as image segmentation and masking may be used to quantify percentage coverage. Through segmentation and masking of the area of interest in an image (Fig. 4) and comparing the difference to the total area of the image, the percentage coverage of this isolated area can be calculated.

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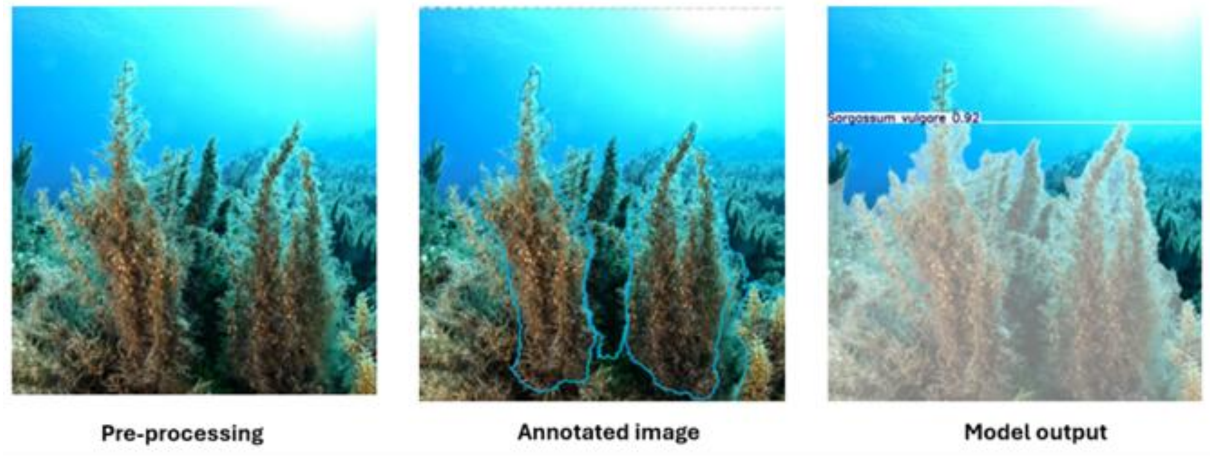

Figure 4. A depiction of an annotated image and a segmented model prediction output

Integrated approaches to zooplankton monitoring

Integrating the Underwater Vision Profilers (UVPs) with autonomous platforms and automatic image recognition using AI is a promising avenue for the coming years that should allow the accumulation of very large quantities of data to monitor zooplankton in 3 dimensions and over time. Already, using a few thousand profiles from multiple oceanographic campaigns, global mean biomasses of various groups of mesozooplankton built using AI techniques have been published (Drago et al. 2022; Soviadan et al. 2024). In addition, this technique could be combined with automatic eDNA sampling in the future.

2.3.3 Remotely operable platforms and remote sensing

Satellite remote sensing provides unique capabilities for monitoring marine biodiversity. Remote sensing enables regular, synoptic observations across a wide range of spatial and temporal scales from a few meters to ocean basins, and from days to decades. Among available techniques, optical remote sensing in the visible range remains the primary method for biological and biodiversity observations. Although the technology itself is well established, recent advances in spatial and spectral resolutions and data accessibility are expanding its applications.

Phytoplankton monitoring has long been a central application of optical remote sensing. Sensors such as NASA's MODIS and SeaWiFS, and Copernicus Sentinel-3 OLCI, with spatial resolutions between 300 m and 1 km, provide a nearly 30-year continuous global record of ocean color data from which the maps of chlorophyll-a concentration, the proxy for phytoplankton biomass, are produced. Newer hyperspectral missions, such as the recent NASA PACE and the upcoming Copernicus CHIME and NASA SBG, will provide an enhanced spectral resolution, which is widely anticipated to enable new capabilities for observing phytoplankton communities, including their composition and functional types (Groom et al. 2019).

Optical satellite data are also instrumental in monitoring foundation species such as submerged and floating macroalgae (*e.g.*, giant kelp, pelagic Sargassum), seagrass meadows, and coral reefs. Mapping these species has relied on medium-resolution data from Landsat (30 m, since 1982) or very high-

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resolution (VHR; ~1 m) imagery from commercial providers (*e.g.*, MAXAR's WorldView). Since 2017, the availability of Copernicus Sentinel-2 imagery, with free open-access data at up to 10 m spatial resolution, has expanded monitoring potential. Future similar missions (*e.g.*, Sentinel-2 NG and Landsat Next) are expected to ensure the continuity of these open-source high-resolution datasets into the 2030s. In parallel, new hyperspectral missions with high spatial resolutions (*e.g.*, EnMAP with 30 m ground resolution) and the growing accessibility of commercial VHR imagery from emerging national constellations and lower-cost providers are likely to further increase the volume and variety of satellite data available for scientific and operational monitoring.

For higher trophic levels such as fish, sea turtles, and marine mammals, direct detection with optical data is occasionally feasible, for example, identifying whales in VHR images. However, due to the high cost and limited spatial coverage of such imagery, indirect approaches are more commonly employed. For instance, species distribution models (SDMs) estimate species abundance based on satellite-derived environmental variables such as sea surface temperature (SST), sea surface height (SSH), and chlorophyll-a, as well as derived dynamic features as eddies, fronts, and upwelling zones.

While passive optical remote sensing remains a key technique for biological and biodiversity observation from space, other technologies are emerging. Spaceborne LiDAR, capable of penetrating the upper tens of meters of the water column, offers complementary subsurface information. Notably, LiDAR instruments like the previous CALIOP mission have revealed global-scale patterns of the diel vertically migrating ocean animals, and it is envisaged that current missions (*e.g.*, NASA's ICESat-2) and proposed future missions (*e.g.*, CALIGOLA) can support a novel three-dimensional ocean ecosystem observation from space (Behrenfeld et al. 2023).

2.3.4 Mapping intertidal macroalgae and canopy floating kelp

In recent years, the application of Unmanned Aerial Vehicles (UAVs) as a low-cost yet efficient monitoring solution has increased in several contexts. New sensors that can be mounted on UAVs have been developed, providing new tools for research implementation. Such is the case with multispectral and hyperspectral cameras, although the latest have been less used due to their cost and the need for high computational capabilities. In the coastal area, several UAV-based multispectral methodologies and workflows, combined with Red-Green-Blue (RGB) or satellite data, have been proposed for mapping intertidal reefs (Borges et al. 2021; Collin et al. 2019) and macroalgae/seaweed in particular (van der Wal et al. 2014; Brodie et al. 2018; Taddia et al. 2020; Tait et al. 2019; Rossiter et al. 2020a; Borges et al. 2023).

Traditionally, the mapping of intertidal macroalgae cover on the rocky reefs involves the estimation of the beds' length and width using measuring tapes or circling the beds by foot for establishing their perimeter using a hand-held GPS with a fixed recording rate. Such approaches are time-consuming, labour-intensive, and with low accuracy, covering only a small area of the rocky reef under study at each low tide period. With the advance of high-precision multispectral UAVs, applying vegetation indexes using the camera bands, it is possible to estimate the macroalgae coverage area with good

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accuracy for larger areas in reduced time, taking the most of the low tide period. Furthermore, when integrated into workflows combining multispectral satellite data estimation, the coverage area can be expanded by several kilometers (see Borges et al. 2025, for details).

On the contrary, hyperspectral sensors (such as UAV-mounted cameras combined or not with *in situ* spectroradiometer data) have been applied for improving species discrimination, relative to multispectral data. The first sensors allow a better acquisition of the spectral properties of observed surfaces by the acquisition of a large number of contiguous and narrow spectral bands, which provide a more accurate identification of the observed objects (Douay et al. 2022; Rossiter et al. 2020b). Indeed, macroalgae species discrimination with high accuracy has been reported in some studies (*e.g.*, Diruit et al. 2022; Rossiter et al. 2020b), promising to be a valuable tool for future monitoring assessments.

For Giant Kelp (*Macrocystis pyrifera*) with a floating canopy, workflows including the calculation of simple vegetation indices from RGB imagery, have been developed to classify kelp canopy as a binary presence/absence layer, and to discriminate these organisms from the surrounding background (Bell et al. 2020; Cavanaugh et al. 2021; Saccomanno et al. 2022).

2.3.5 Passive acoustics for assessing marine forest health and diversity

Passive acoustic monitoring (PAM) has rapidly emerged as a valuable approach for assessing marine ecosystems, including complex habitats such as marine forests and seagrass meadows (Gibb et al. 2019; Gottesman et al. 2020; Pieretti et al. 2020; Mooney et al. 2020). This technique involves the deployment of hydrophones that continuously record the ambient soundscape, capturing a variety of biological, physical, and anthropogenic sounds (Lindseth and Lobel 2018; Muñoz-Duque 2024). The resulting acoustic data provide a unique, non-invasive window into the ecological processes and biodiversity of marine forests, complementing traditional visual and physical survey methods (Van Parijs et al. 2009; Bertucci et al. 2016).

Marine forests, being dynamic environments, support high levels of biodiversity and productivity, and are critical habitats for fish, invertebrates, and marine mammals (Graham et al. 2007; Wernberg 2019). Many of these organisms produce species-specific sounds during activities such as feeding, courtship, and territorial defence (Radford et al. 2010; Bertucci et al. 2016). By capturing these acoustic signals, PAM enables the detection of the presence and relative abundance patterns of soniferous species, including those that are cryptic, nocturnal, or otherwise difficult to observe directly (Gibb et al. 2019; Mooney et al. 2020). Beyond biological sounds, the soundscape of marine forests also includes abiotic components such as wave action and substrate movement, as well as anthropogenic noise from vessels and coastal development (Merchant et al. 2015). Analysing changes in the overall biotic and abiotic acoustic environment can provide early warning signs of ecosystem stress, degradation, or recovery (Sueur et al. 2014; Pieretti et al. 2020; La Manna et al. 2024). For example, a decline in the intensity or diversity of fish choruses has been linked to habitat degradation in seagrass meadows (La Manna et al. 2024).

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Recent advances in acoustic technology, data storage, and automated sound analysis have greatly expanded the potential of PAM for long-term, large-scale ecological monitoring (Lindseth and Lobel 2018; Gibb et al. 2019). Acoustic indices and machine learning algorithms now allow for the rapid assessment of biodiversity and ecosystem health from complex sound recordings (Sueur et al. 2014; Williams et al. 2022; La Manna et al. 2024). As a result, passive acoustics is increasingly recognised as a critical tool for marine conservation, restoration, and management, especially in habitats where traditional survey methods are logistically challenging or potentially disruptive (Van Parijs et al. 2009; Muñoz-Duque et al. 2024).

2.3.6 Active acoustics and the deep scattering layer

For nearly a century, scientists have used sound waves reflected back from underwater organisms - measured by acoustic equipment on ships - to map how marine life is distributed at different ocean depths. Areas where sound reflects strongly, known as the deep scattering layer (DSL), are frequently associated with a diverse community of epipelagic and mesopelagic organisms (*e.g.*, zooplankton, krill, fish), oftentimes engaged in vertical migrations spanning 10s to 100s of meters. The vertical dance of this diverse community, sometimes referred to as Vinogradov's ladder, has been shown to have important consequences for the biological carbon pump. It has been estimated that up to 60% of the biological carbon pump reservoir (about 2000 PgC) has been processed by these vertical migrants before its eventual sequestration in the deep ocean (Pinti et al. 2023).

2.4 Strengths & Limitations for Emerging Methods

2.4.1 eDNA

Strengths

- **Non-invasive**: species presence can be detected without physically disturbing the organisms or their habitat (Barnes and Turner 2016).
- **Rapid turnaround and time efficiency**: eDNA analysis can significantly reduce the time required for biodiversity assessments compared to traditional methods like microscopy or manual sorting, especially for cryptic taxa such as zooplankton (Porter and Hajibabaei 2018).
- **Species-specific**: eDNA analysis is highly species-specific when primers are carefully designed to target unique genetic markers. This enables the precise identification of species, even when they are present at very low abundances (Rees et al. 2014).
- **Cryptic species detection**: Another notable strength is the method's effectiveness in detecting cryptic, elusive, or rare taxa that may otherwise be missed by traditional survey methods, thereby enhancing biodiversity assessments and monitoring efforts.
- **Reduced labour and increased area coverage**: eDNA methods require less labour and can cover

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larger areas more efficiently, especially in inaccessible or sensitive habitats (Moore et al. 2025).

Limitations

- **Spatially implicit**: One major limitation is the spatially implicit nature of eDNA detection. Because DNA can be transported by water currents, the presence of a species' DNA in a water sample does not necessarily reflect its exact location or spatial distribution in the environment (Jane et al. 2015; Shogren et al. 2017).
- **Challenges in quantifying cover and biomass**: Quantifying the cover or biomass of macroalgal species remains challenging with eDNA, as DNA concentration does not directly correlate with abundance or physical biomass.
- **Variation in eDNA shedding rates**: Variations in shedding rates, environmental degradation, and other ecological factors can all influence DNA signal strength (Barnes and Turner 2016).
- **Detection bias**: Detection of DNA does not necessarily indicate the viability or reproductive capacity of the organism, as DNA from dead, degraded, or senescent tissues may persist in the environment and be detected through eDNA methods (Dejean et al. 2012).
- **Cost**: eDNA has higher upfront laboratory costs, but may be more cost-effective in the long term, especially for large-scale or repeated monitoring.
- **Reliance on reference databases**: eDNA can identify species with high precision if a comprehensive genetic reference library exists. Its accuracy diminishes for poorly represented taxa.

2.4.2 Imaging & AI techniques

Strengths

- **Non-invasive and scalable**: Imaging technologies (*e.g.*, drones, satellites, camera traps) enable biodiversity assessments over large spatial scales without physically disturbing habitats or organisms (Turner et al. 2003; Koh and Wich 2012).
- **Automated species identification**: AI-powered image recognition tools can automate species identification with high accuracy, reducing the need for manual taxonomic expertise and accelerating data processing (Norouzzadeh et al. 2018).
- **Real-time or near-real-time monitoring**: Some systems can provide near-real-time updates on species presence, movement, or habitat change, enabling rapid conservation response and decision-making (Hodgson et al. 2016).
- **Detection of behavioural patterns**: Camera traps and video monitoring combined with AI can capture and analyze species behavior, interactions, and activity patterns over time (Beery et al. 2020).
- **Broad habitat applicability**: Imaging systems can be adapted to various environments, including terrestrial, freshwater, and marine ecosystems, offering flexibility across biodiversity studies (Hodgson et al. 2019).

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- **High-resolution habitat mapping**: Remote sensing and AI can produce detailed land cover maps and detect subtle changes in habitat structure, aiding in ecosystem health assessments (Pettorelli et al. 2014).

Limitations

- **Algorithm bias and training limitations**: AI models require large, well-annotated datasets for training. Biases in training data can lead to misidentification or under-detection of rare or cryptic species (Tabak et al. 2019).
- **Limited taxonomic coverage**: While image-based systems excel at detecting large or visually distinctive organisms (*e.g.*, mammals, birds), they are less effective for small, cryptic, or morphologically similar taxa (Weinstein 2018).
- **High initial cost and technical requirements**: Developing and deploying AI-enabled imaging systems requires significant investment in hardware, software, and technical expertise (Hodgson et al. 2016).
- **Environmental constraints**: Weather, lighting, vegetation cover, and terrain can all impact the quality and consistency of image capture, particularly for aerial or drone-based surveys (Duffy et al. 2018).
- **Detection limitations in complex habitats**: Dense Forest canopies, underwater turbidity, or cluttered environments can obscure visibility, leading to under-sampling or false negatives (Christie et al. 2016).
- **Data storage and management challenges**: High-resolution imagery and video data generate large volumes of information, necessitating robust storage, processing, and data management infrastructure.

2.4.3 Satellite remote sensing

Strengths

- **Frequent, wide-scale observations**: Satellite data provides regular, synoptic coverage, ideal for monitoring dynamic coastal and marine ecosystems.
- **Technologically prone to rapid advances**: Recent advances, such as open-source 10 m spatial resolution from Sentinel-2, hyperspectral data from PACE, and LiDAR technologies, can greatly improve detection and monitoring capabilities.
- **Data accessibility and operational use**: Cloud platforms and open-access policies rapidly enable monitoring efforts.
- **Broader range of ecological indicators**: Allows co-detection of biological and physical oceanographic features relevant to biodiversity assessment.

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Limitations

- **Reduced accuracy in optically complex waters**: Turbid and productive coastal zones, and water bodies with limited exchange (*e.g.*, Baltic Sea) present bio-optical challenges, lowering the reliability of algorithms.
- **Limited species-level resolution**: Species discrimination is challenging, and satellite observations are often restricted to the areal extent of the dominant species in an area.
- **Atmospheric and depth constraints**: Cloud cover and limited light penetration reduce the effectiveness of optical sensors for subsurface and benthic monitoring.
- **Cost barriers for satellite imagery**: Access to very-high resolution (~1 m) optical data from commercial providers is are expensive, limiting widespread use in biodiversity applications and regular monitoring.

2.4.4 UAV-based mapping

Strengths

- **High-resolution data collection**: UAVs provide ultra-fine spatial resolution, making them particularly well-suited for detecting small-scale heterogeneity in coastal and intertidal habitats (Cavanaugh et al. 2021).
- **Site-specific adaptability**: UAVs can be deployed with tailored flight paths and sensors (*e.g.*, RGB, multispectral, hyperspectral) to target specific habitats or ecological features with great precision.
- **Rapid, repeatable monitoring**: UAVs allow for fast and frequent surveys over dynamic coastal environments, enabling detection of temporal changes such as tidal cycles, algal blooms, or seasonal shifts in community structure.
- **Non-invasive mapping**: Surveys are conducted from the air, minimizing disturbance to fragile or sensitive ecosystems like rocky shores or floating kelp canopies.

Limitations

- **Weather and lighting constraints**: UAV-based surveys require stable weather, absence of rain, and minimal cloud cover—especially for hyperspectral and multispectral imaging—to avoid light distortion and shadowing (Cavanaugh et al., 2021).
- **Limited area coverage**: Compared to airplanes or satellites, UAVs can cover only small spatial extents per flight, limiting their scalability for large-scale coastal mapping.
- **Topographic shadowing**: The rugged nature of rocky intertidal zones introduces complex shadows in imagery. Survey timing (ideally at solar zenith) must be carefully planned to minimize distortion in orthomosaics.
- **Sun glint and water surface distortion**: For canopy-floating kelp surveys, sun glint and wave reflections can degrade image quality and introduce errors into classification or pixel-based analysis, requiring post-processing corrections.
- **Hardware limitations**: UAVs may be constrained by battery life, payload capacity, and wind resistance, particularly in coastal environments that often experience high wind conditions.

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- **Need for site-specific protocols**: Methodologies must often be adapted to specific environments or objectives, making it harder to standardize UAV workflows across diverse coastal ecosystems.

2.4.5 Passive acoustic monitoring

Strengths

- **Non-invasive monitoring:** Hydrophones record underwater sounds without disturbing marine life or habitats, making them ideal for sensitive or protected areas (Gibb et al. 2019; Mooney et al. 2020; La Manna et al. 2024; Muñoz-Duque 2024).
- **Continuous, long-term data collection:** PAM enables round-the-clock monitoring, capturing diel, seasonal, and annual trends that periodic surveys may overlook (Van Parijs et al. 2009; Lindseth and Lobel~~,~~ 2018).
- **Detection of cryptic and nocturnal species:** Many marine organisms are difficult to observe visually, but produce distinctive sounds, allowing detection of species that are hidden, nocturnal, or inhabit deeper waters (Bertucci et al. 2016; Mooney et al. 2020). This strength is particularly valuable in environments with high water turbidity, where traditional visual surveys are limited or ineffective; hydrophones can reliably capture biological sounds regardless of water clarity (Bertucci et al. 2016; Gibb et al. 2019; Mooney et al. 2020).
- **Broad spatial coverage:** Sound travels efficiently underwater, so a single hydrophone can monitor a relatively large area, an advantage in complex habitats like kelp forests (Radford et al. 2010; Dziak et al.-2023).
- **Cost-effective for extended studies:** Once deployed, hydrophones require less human intervention than repeated diver or ROV surveys, reducing operational costs for long-term projects (Van Parijs et al. 2009; Gibb et al.-2019).
- **Sensitive to ecosystem changes:** Shifts in the acoustic community, such as changes in fish chorusing or increases in anthropogenic noise, can indicate alterations in ecosystem health or biodiversity (Merchant et al. 2015; Bertucci et al. 2016).

Limitations

- **Species identification constraints:** Not all marine species produce sounds, and some acoustic signals are difficult to attribute to specific taxa without supporting visual or genetic data (Gibb et al. 2019; Mooney et al. 2020). To ameliorate this, integrated cameras can be useful.
- **Acoustic masking:** Dominant sounds (*e.g.*, snapping shrimp, waves, boat engines) can mask quieter biological signals, complicating data interpretation and potentially underestimating diversity (Merchant et al. 2015; Mooney et al. 2020).
- **Environmental and technical biases:** Sound propagation varies with water depth, temperature, substrate, and hydrophone placement, which can bias recordings and complicate comparisons across sites (Radford et al. 2010; Dziak et al. 2023).

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- **Large data management needs:** PAM generates substantial audio data, requiring significant storage, processing power, and expertise for effective analysis (Lindseth and Lobel 2018; Gibb et al. 2019).
- **Limited to soniferous species:** Species that are silent or rarely vocalise remain undetected, potentially biasing biodiversity assessments toward more vocal taxa (Bertucci et al. 2016; Mooney et al. 2020).
- **Need for validation:** Acoustic patterns and indices often require validation with traditional survey methods (*e.g.*, visual censuses, net sampling) to ensure ecological relevance and accuracy (Gibb et al.-2019; Van Parijs et al. 2009).

2.4.6 Active acoustic

Strengths

- **High technological readiness**: Acoustic backscatter technology is already highly developed and routinely used on research vessels, with increasing availability on autonomous and remote platforms (Haëntjens et al. 2020).
- **Established global data pool**: A substantial global archive of acoustic backscatter data already exists and continues to grow through ongoing oceanographic research, offering a strong foundation for long-term biodiversity and ecosystem monitoring.
- **Scalable data integration**: Creating pipelines to compile and integrate new observations into global databases would be relatively straightforward, enabling large-scale and consistent analysis across regions.
- **Functional diversity and ecosystem relevance**: Acoustic data can inform understanding of functional diversity and key ecosystem processes, such as the biological carbon pump, linking biodiversity to climate-relevant functions.
- **Broad application potential**: While current uses may be focused on a subset of ecological functions, acoustic backscatter has promising potential for broader applications across biodiversity assessment and marine ecosystem monitoring.

Limitations

- **Proxy-based, not taxon-specific**: Acoustic backscatter primarily reflects biomass but not species identity. Differences in organism morphology and material properties mean acoustic signatures vary, leading to uncertainty in species composition.
- **Inability to resolve community structure alone**: Without additional data sources (*e.g.*, eDNA, visual imaging), acoustic backscatter cannot resolve fine-scale taxonomic or trait-based diversity, limiting its effectiveness as a standalone biodiversity tool.
- **Need for technological and methodological integration**: Advancements such as frequency-modulated acoustics, improved classification algorithms, and integration with trait-based ecological models are needed to improve accuracy and ecological relevance.

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3. Case studies

3.1 eDNA sampling versus traditional fish surveys in the Baltic Sea

In a case study led by Uppsala University (UU) in the Baltic Sea Living Lab, traditional coastal monitoring nets (modified Nordic coastal survey net) were compared with eDNA methods to monitor fish communities in seagrass-dominated ecosystems around the island of Gotland, Sweden. The study, currently being prepared for scientific publication, was conducted at six locations: Ajkesviken, Fårösund, Vägumeviken, Lausviken, Burgsvik, and Klintehamn. At each site, eDNA samples were collected from the same locations where nets were deployed, allowing for direct comparison between the two approaches (Fig. 5).

Traditional coastal nets (Nordic coastal survey net), the established monitoring method in the region, offer valuable data continuity and allow for physical measurements such as fish length, weight, and age (through otolith collection). In this study, nets captured 19 species in total, with perch and herring being the most common. However, this method is labour-intensive, requiring two boat trips (at dawn and dusk) to each location, as well as substantial time for net handling and fish processing. Moreover, it is inherently invasive: a significant number of fish are killed during the process.

eDNA, by contrast, offers a non-invasive, efficient, and highly sensitive alternative. Across the six sites, eDNA identified more than twice as many fish species, including small-bodied and cryptic species that are typically underrepresented in net catches. Sampling is rapid and minimally disruptive, requiring only a single boat trip per location and no handling of fish. Although eDNA does not provide physical measurements, abundance estimates, and currently lacks historical time series in this region, its ability to expand species detection while reducing impact on wildlife makes it a valuable tool. The main limitations are the relatively high cost and the time required for laboratory analysis.

Discussions with local authorities are ongoing, with a strong interest in integrating eDNA into the monitoring framework. A combined approach – reducing the number of nets while incorporating eDNA – is emerging as a promising path forward, enhancing both ecological insight and ethical standards in long-term fish monitoring.

Figure 5. Fieldwork around Gotland, looking for suitable fishing grounds and testing environmental DNA (eDNA) sampling protocol (left), Seagrass-dominated fishing ground (middle), Fish catch being measured in the lab (right).

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3.2 eDNA versus traditional macroalgal sampling in the Tuscan Archipelago living lab

Calibration of emerging technologies such as environmental DNA (eDNA) with traditional visual sampling methods for monitoring the macroalgal canopy cover and composition EOV, is one of the main tasks of the Tuscan Archipelago living lab, led by the University of Pisa (UNIPI). UNIPI has conducted a series of field campaigns to evaluate the efficacy of eDNA sampling compared to traditional methods (photo quadrats) for assessing macroalgal cover and composition in Tuscan Archipelago living lab. During the summer of 2024, surveys were carried out at three islands: Capraia, Pianosa, and Giannutri. A total of 72 1L eDNA samples and 58 tissue samples were collected using a sampling design that matches photoquadrats with water samples for eDNA at multiple sites (Fig. 6) to a sequencing failure, only 32 samples passed quality control, and the results should therefore be considered preliminary. Despite this limitation, the findings are promising. eDNA data showed a positive correlation between algal cover of *Cystoseira foeniculacea* and the relative abundance of its corresponding Amplicon Sequence Variant (ASV) (β = 0.327, t = 3.62, R^2 = 0.56, p < 0.01). (Fig. 7). Moreover, on average, eDNA detected approximately 45% more canopy-forming species than traditional visual methods. To address the technical setbacks encountered in 2024, UNIPI is planning an additional series of field campaigns in 2025, with the goal of expanding the barcode reference library and improving sequencing success rates. Furthermore, the detection range of eDNA shall be assessed using the sampling design depicted in Figure 6. Together, these preliminary results highlight the potential of eDNA to detect rare canopy-forming species and to quantify the more abundant ones.

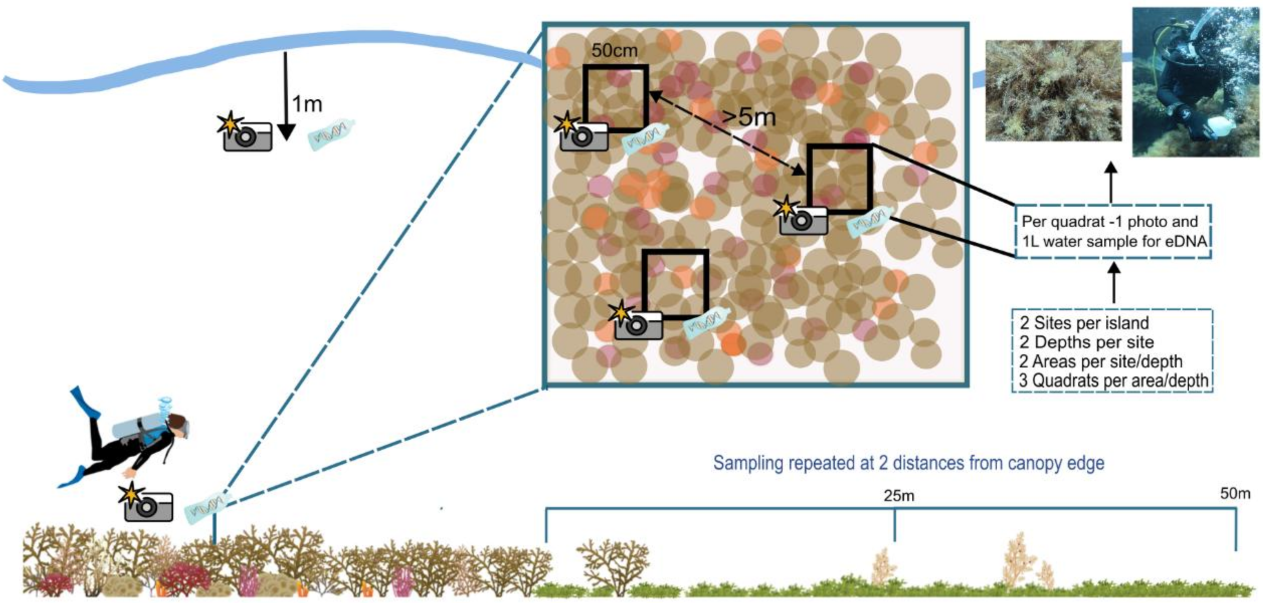

Figure 6. Sampling design for the 2025 DNA field campaign. Water samples shall are collected within the macroalgal canopy and at replicate distances from the canopy edge to assess the distance eDNA can be detected from remnant canopies.

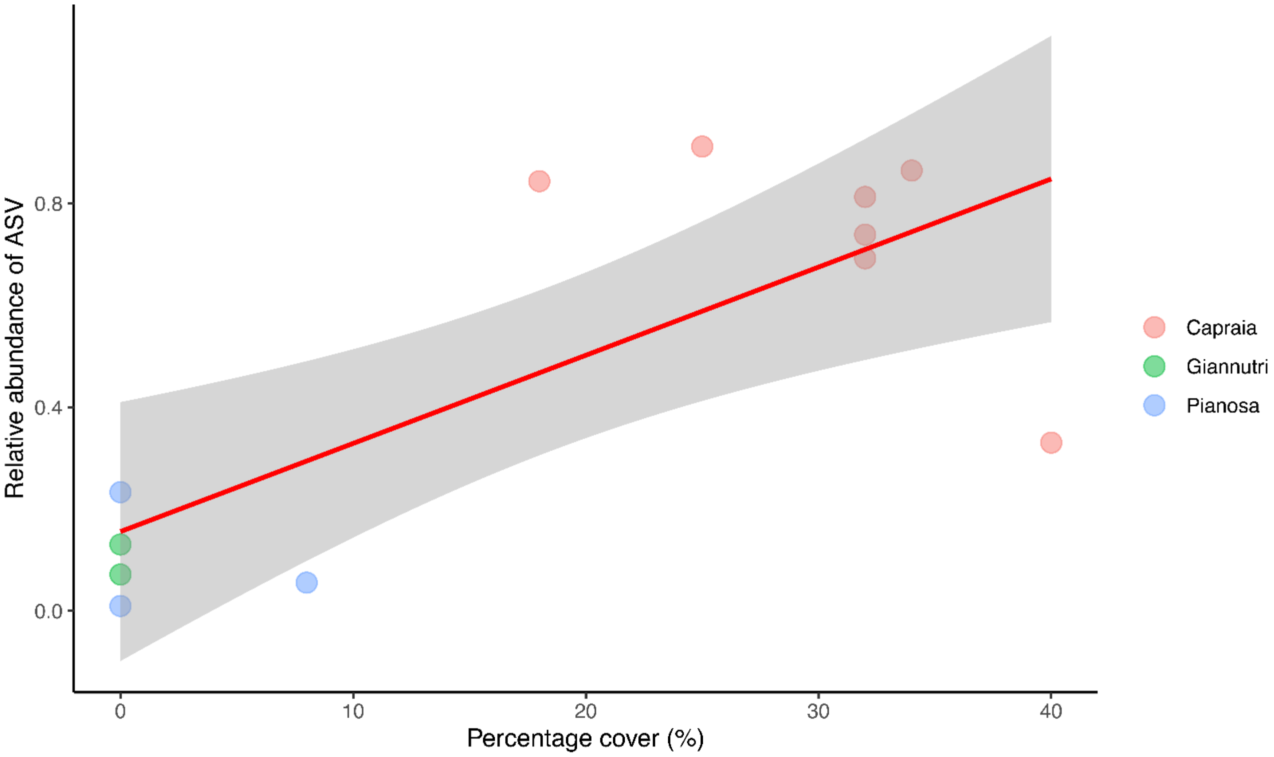

Figure 7. Relationship between the relative abundance of Amplicon Sequence Variants (ASVs) and the corresponding percentage cover of Cystoseira foeniculacea across islands (color-coded) in the Tuscan Archipelago Living Lab.

3.3 AI-based imaging for species recognition in the Tuscan Archipelago living lab

The University of Pisa (UNIPI) is currently developing a YOLO architecture based on deep learning to support automated assessment of the macroalgal and seagrass canopy cover and composition EOVs in Tuscan Archipelago Living Lab. The model also enables quantitative assessments such as percentage coverage estimation. By developing an automated AI-based biodiversity monitoring technique with considerably high species identification accuracy, this could impact scalable solutions for monitoring diverse coastal ecosystems. Beyond species identification, the project also aims to integrate a percentage cover estimation workflow by combining instance segmentation techniques with pixel-based analysis. This will enable the quantification of species-specific spatial distribution and coverage, providing valuable metrics for monitoring changes in cover of seagrass and macroalgal meadows over time. The development of this AI-powered biodiversity monitoring tool holds the potential to significantly improve the scalability, frequency, and objectivity of coastal habitat assessments, compared to traditional manual surveys. This novel method could offer reliable insights and a cost-effective way for tracking habitat changes, detecting early signs of ecological shifts, and informing conservation strategies across the Tuscan Archipelago and similar Mediterranean coastal ecosystems.

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3.4 Using video to estimate jellyfish abundance in the Baltic Sea

In a recent study led by UU (Lüskow et al. 2025) in the Baltic Sea Living Lab, existing video material, which was originally collected for the study of seagrass-associated fish in the coastal waters of Gotland, was used opportunistically to estimate the abundance of jellyfish. This "by-product" use of the recordings of a GoPro HERO 5 Black camera that showed a considerable number of more than 4000 *Aurelia aurita* jellyfish, underlines the enormous potential of novel technologies in marine research. In the central Baltic Sea region, where quantitative long-term monitoring programmes for gelatinous zooplankton (GZ) are almost completely missing, innovative approaches such as camera transects offer a valuable, non-invasive, and time-efficient alternative. They enable us to overcome financial and logistical restrictions and historical sampling difficulties. Although the primary collection of data (seagrass-associated fish fauna) served a different purpose, it provided first-time insights into *A. aurita* aggregation patterns over seagrass-dominated habitats with salinities between 6 and 8. The orientation and inclusion of such new technologies are, therefore, crucial to effectively improve our understanding of long-term pelagic community changes and to enable more comprehensive monitoring of GZ populations.

3.5 Assessing Vertical Migrant Communities Using Acoustic Backscatter Data

To assess the feasibility of acoustic backscattering data (historical and current) to quantify the state and functional diversity of the vertical migrant community and its impact on the biological carbon pump. A, database was compiled of recent acoustic profiles (2006 to 2023) covering a combined sampling track of over 800,000 km. To streamline this initial data assembly, only observations collected at a 38 kHz frequency were used. Data was pre-processed to remove noise, deal with missing data, and collate observations into 10 km segments. Depth bins were then classified into background and DSL bins using a column-wise contextual filter. This allowed DLS metrics to be extracted – (a) weighted mean depth, (b) depth and strength of the strongest DLS, (c) depth and strength of all DLSs.

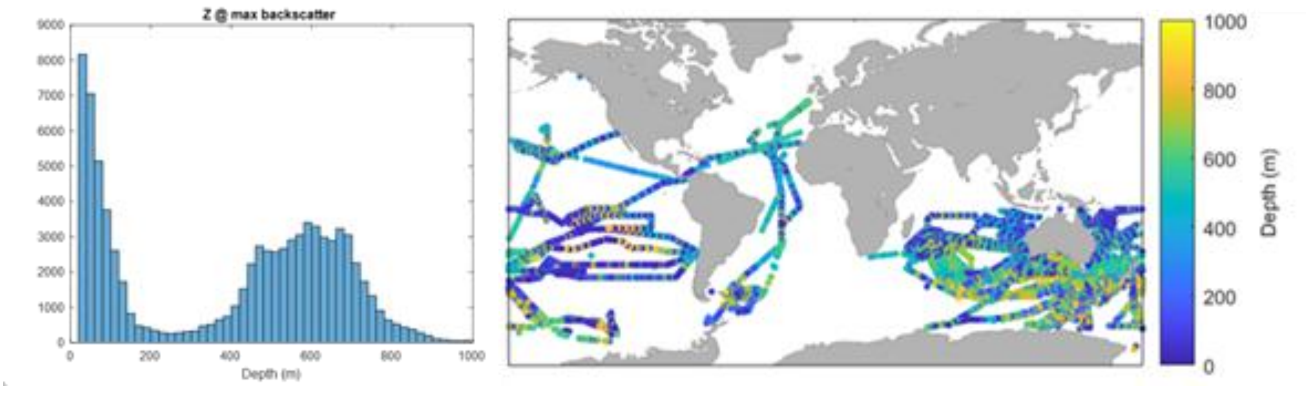

Figure 8. The frequency distribution and spatial distribution of the principal DSL.

The principal (*i.e.*, strongest) DSL shows a bimodal frequency distribution (Fig. 8), some in the surface layer (0–200 m), but also a surprisingly large fraction (more than 50%) in the mesopelagic zone (400–800 m).

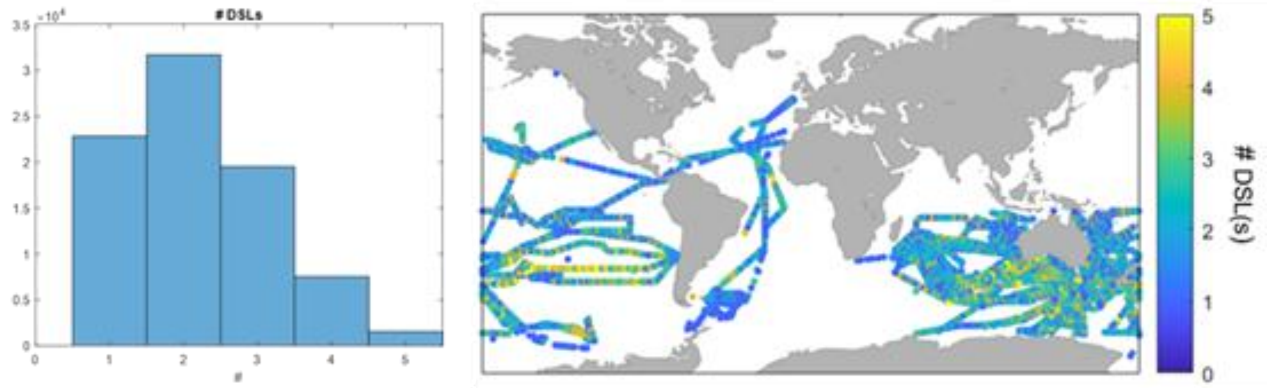

Figure 9. The frequency distribution and spatial distribution of the multiple DLSs.

In addition to the principal DSL, there are often multiple additional DLSs, usually representing other taxa in the mesopelagic community (Fig. 9).

4. Comparative evaluation

4.1. Summary of strengths and limitations of different methods

Here, we provide a summary of the relative strengths, limitations, and logistical considerations of traditional and innovative approaches for biodiversity monitoring, focusing on their utility for Essential Ocean Variable (EOV) assessment (Table 3). Traditional methods, while historically rich and taxonomically precise, are often invasive and resource intensive. In contrast, newer technologies such as environmental eDNA, imaging with AI, and remote sensing offer scalable, non-invasive alternatives, though they still require calibration and careful validation. Furthermore, emerging methods offer key advantages for remote or inaccessible environments. For example, eDNA sampling and satellite or UAV imaging can be deployed without requiring divers or vessels, reducing logistical barriers. Imaging systems and eDNA enable rapid assessments, while traditional methods remain superior for high-resolution long-term monitoring. Species-specific detection is enhanced by eDNA and AI, whereas habitat-level metrics are best captured by UAVs or remote sensing platforms. The choice of method depends on monitoring goals, spatial scale, and environmental constraints. A hybrid approach that leverages the strengths of each method is increasingly seen as best practice for robust, cost-effective, and ethical monitoring of marine biodiversity.

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Table 3. Comparative evaluation of traditional and emerging methods for marine biodiversity monitoring.

Criterion	Traditional Methods	eDNA	Imaging / AI	Acoustics / Remote Sensing
Taxonomic resolution	High (if expert available)	High (if reference barcodes exist)	Medium to high (depends on image quality and training data)	Low (typically functional groups or biomass proxies only)
Invasiveness	Often invasive (e.g., nets, trawls)	Non-invasive	Non-invasive	Non-invasive
Abundance estimation	Direct (counts, biomass)	Indirect (sequence counts as proxies)	Possible (via segmentation and pixel quantification)	Proxy-based (backscatter strength)
Detection of rare/cryptic	Variable; often missed	Excellent (if DNA shed is present)	Moderate (depends on training and resolution)	Poor (limited to reflective organisms)
Spatial resolution	High (fine-scale site-based)	Low–medium (due to DNA dispersion)	Medium–high (depending on sensor resolution and positioning)	High (satellites/UAVs), but lower in species-level detail
Temporal resolution	Low (periodic surveys)	Medium (repeated water sampling possible)	High (e.g., continuous camera deployments)	High (real-time or repeated satellite/floats)
Automation potential	Low	Medium (automated lab pipelines exist)	High (image processing and ML automation)	Medium–high (pipelineable via remote platforms)
Logistical needs	Diver teams, vessels, and lab processing	Field kits + lab sequencing	Cameras, ROVs/AUVs, annotation tools	Ship acoustics, UAVs, satellites, or autonomous floats
Calibration required	Standardized protocols exist	Calibration to biomass needed	Ground-truthing against manual ID needed	Cross-validation with other datasets is essential
Cost (setup & running)	High (staff-intensive, ship time)	Medium (lab-based, but scalable)	Medium–high (hardware + training costs)	Variable (low for satellites, higher for acoustics/UAVs)
Key strengths	Detailed ID, long-term comparability	Cryptic taxa, early detection	Scalable, fast, and objective once trained	Broad coverage, functional/biomass data
Key limitations	Time-intensive, invasive	Spatially implicit, not quantitative for abundance	Requires large labeled datasets, species bias in training	Low taxonomic resolution, signal interference, needs modeling
Rapid versus long-term use	Excellent for long-term datasets	Suitable for repeated, short-term surveys	Scalable for high-frequency use	Excellent for repeated, real-time monitoring
Species versus habitat coverage	Good for both, species-specific (if an expert is available)	High for species, low for habitat structure	High for both, especially with AI segmentation	Primarily habitat-level or biomass patterns
Remote area accessibility	Limited (requires divers, ships)	High (lightweight field kits)	Medium–high (depends on platform)	High (satellites, UAVs, Argo floats)

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5. Advancing underwater monitoring in line with the 3s

In line with the principles of the 3Rs – Replacement, Reduction, and Refinement – there is a need to transition from traditional fish monitoring methods, such as trawling and netting, to more ethical and innovative approaches. Conventional sampling techniques often result in high levels of bycatch and mortality, including the unintended killing of non-target species, juveniles, and vulnerable populations. These methods conflict with contemporary efforts to minimise animal suffering and are increasingly difficult to justify in the context of both legal obligations and societal expectations regarding animal welfare. Novel, emerging, non-invasive technologies such as environmental DNA (eDNA) sampling, underwater video systems, and acoustic monitoring offer viable alternatives that align closely with the 3Rs. These tools can replace or significantly reduce the need to capture or kill animals by enabling effective biodiversity assessments and fish population monitoring without physical harm. They also contribute to refinement by eliminating or reducing the distress and mortality associated with traditional methods. Adopting these approaches supports a more sustainable and humane model for aquatic research and conservation, consistent with the goals of EU animal welfare legislation and scientific best practice.

This is directly relevant to Directive 2010/63/EU of the European Parliament and of the Council, which mandates the implementation of the 3Rs in all scientific procedures involving animals, including those involving fish. However, in certain cases – such as when assessing, for example, fish fitness, analysing bone structure, or examining otoliths for age and growth studies – the physical specimens themselves are still required. In such instances, combining modern non-invasive techniques with targeted, minimally harmful traditional sampling can offer a balanced approach that upholds the 3Rs while meeting essential scientific objectives.

6. Integration and hybrid approaches

The integration of diverse monitoring methods represents a significant advancement for marine biodiversity assessments. Hybrid approaches leverage the complementary strengths of various techniques, significantly enhancing both data quality and ecological understanding. For example, combining eDNA with imaging methods, such as underwater photography or video analysis, provides a more comprehensive representation of biodiversity. eDNA captures genetic signatures of organisms, including elusive or cryptic species that might otherwise remain undetected visually, whereas imagery allows direct observation of small-scale variations in species abundance (Alfaro-Cordova et al. 2022). A study conducted in the Gulf of California demonstrated that eDNA and underwater visual census (UVC) each detected distinct subsets of species, and their integration nearly doubled the total number of species detected (Valdivia-Carrillo et al. 2021).

Similarly, the combination of drone-based remote sensing and traditional *in situ* sampling techniques creates opportunities for scaling monitoring efforts while maintaining data accuracy. Drones facilitate rapid, large-scale mapping and assessment, providing spatially extensive data that can guide targeted

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in situ sampling (Ventura et al. 2023). Ground-based sampling then validates and refines remote assessments, ensuring accurate interpretation and ecological relevance.

A key aspect of successful hybrid approaches is robust calibration, wherein traditional monitoring methods are employed to validate data obtained from eDNA analyses, automated processes, AI-driven approaches, or remote sensing techniques. This step is crucial for ensuring data quality, accuracy, and reliability, facilitating iterative improvement and refinement of machine learning algorithms and predictive models (Chen 2021). Integrating machine learning with eDNA methods, for instance, can effectively detect environmental change patterns at landscape scales (Keck et al. 2023). Consequently, calibrated hybrid approaches significantly enhance the consistency, comparability, and scalability of biodiversity monitoring across various spatial and temporal scales. For long-term monitoring programs, maintaining continuity in traditional methods may be necessary to avoid disrupting valuable time series; however, introducing new technologies as early as possible—ideally with overlapping implementation—can build the foundation for eventual transitions and potentially replace traditional methods in the future.

The growing development and usage of portable field-based sequencing platforms, such as Oxford Nanopore's MinION, and their integration with drone-based imagery, field-based imagery, and AI-driven recognition, promise substantial improvements in biodiversity monitoring. Portable sequencing platforms enable rapid, low-cost, and scalable sequencing even in field conditions, making them highly suitable for integration into automated and remote monitoring systems (Srivathsan et al. 2021). Similarly, drones possess considerable yet largely untapped potential for field research, especially when combined with artificial intelligence and additional data sources, greatly enhancing the scope and scale of biodiversity assessments (Surasinghe et al. 2025). Strengthening these integrative approaches would facilitate the establishment of continuous monitoring systems, enabling real-time biodiversity assessments and rapid detection of ecological changes.

7. Recommendations

- **Combine methods for complementarity**. Use hybrid monitoring frameworks that integrate eDNA, AI-based image analysis, and traditional sampling to maximize biodiversity detection and monitoring accuracy.
- **Prioritize calibration and benchmarking**. Side-by-side comparisons are essential to ensure consistency and comparability between emerging and traditional methods.
- **Build shared, open-access resources.** Develop and maintain open-access barcode libraries, annotated image databases, and cloud-based analytical tools to support data standardization and model training. All resources should adhere to FAIR principles (Findable, Accessible, Interoperable, and Reusable) to maximize transparency, usability, and integration across platforms and research communities.
- **Support capacity building and engagement.** Promote training in AI and molecular techniques across institutions and involve citizen scientists using robust and scalable protocols (*e.g.*, RLS).

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- **Align with policy and ethical standards**. Ensure that monitoring practices comply with EU directives and reduce unnecessary animal harm via non-invasive techniques (*e.g.* 3R principle).
- **Advance automation and real-time monitoring.** Develop modular monitoring platforms (*e.g.*, drones + MinION sequencing + AI) to boost *in situ*, high-frequency, and scalable biodiversity observations.
- **Synthesize data streams in simple and meaningful biodiversity indicators.** Employ the EOV framework to systematically select key indicators that leverage newly generated data, enabling comprehensive assessment of the status and trends of marine biodiversity and providing insights into underlying driving processes.

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