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MARCO-BOLO

STRENGTHENING BIODIVERSITY OBSERVATION IN SUPPORT OF DECISION MAKING

Deliverable 2.4

Final report on the definition of eDNA-based EBVs with associated eDNA-based EBV datasets and efficiency of eDNA for detecting/quantifying taxa/species of interest.

Version 1.0

29/11/2025

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Executive summary

Work Package 2 (WP2) aims to enable environmental DNA (eDNA)-based methods for standardized biodiversity monitoring. This report (D2.4) presents the outcomes of Task 2.4, which focused on defining eDNA-based Essential Variables – namely Essential Ocean Variables (EOVs) and Essential Biodiversity Variables (EBVs) – and evaluating their utility for European policy frameworks, including the Marine Strategy Framework Directive (MSFD), the Water Framework Directive (WFD), and the Habitats Directive.

Building on other WP2 deliverables (D2.1, D2.2, and D2.3), we identified and generated a suite of eDNA-based EOVs from multiple case-study datasets, including a global survey (*Tara Oceans*), three coastal time-series (SOMLIT-Astan, LTER-MC/NEREA, the EMO BON UiT genomic observatory), and a dedicated cetacean dataset from the Portuguese coast (CIIMAR). This report demonstrates that EOVs are currently the most operational product directly derivable from eDNA data. The candidate eDNA-based EOVs presented here were formatted to Darwin Core standards and will be deposited in public repositories, providing the foundation for fully functional eDNA-based EOVs. These EOVs serve as standardized building blocks and EBV candidates that can, with expanded sampling and modelling, be transformed into full EBVs. These essential variables (EOVs/EBVs) can work as biological indicators aligned with European frameworks, such as the WFD and the MSFD, and regional conventions, such as OSPAR (Northeast Atlantic), or UNEP-MAP (Mediterranean Sea).

Across the case studies, we demonstrate the usefulness of eDNA for generating EOVs and detecting policy-relevant taxa and species:

- **Phytoplankton & Harmful algal bloom (HABs) events:** eDNA metabarcoding from time-series data successfully captured seasonal phytoplankton dynamics and HABs events. Generated EOVs are relevant for assessing eutrophication and ecosystem function in marine environments.
- **Fish communities:** eDNA metabarcoding revealed seasonal patterns in fish diversity and community composition, while targeted species-specific digital droplet PCR (ddPCR) assays provided quantitative information for sentinel species such as the European anchovy, directly supporting regional indicator frameworks.
- **Marine mammals:** eDNA detected multiple cetacean species, including those protected under the EU Habitats Directive, with seasonal occurrences of species validated against visual surveys.
- **Invasive Alien Species (IAS):** eDNA expanded the known distribution of several OSPAR-listed invasive species, demonstrating strong potential for early detection in data-deficient areas.
- **Species of conservation concern:** eDNA detected several fish species listed as threatened, near-threatened, or vulnerable on the IUCN Red List, highlighting its value for monitoring vulnerable species.

Since dedicated performance-evaluation experiments were not available, the efficiency (or usefulness) of eDNA-derived variables was evaluated by comparing them with expected biodiversity signals from conventional observations, and global biodiversity databases (GBIF, OBIS, iNaturalist). These comparisons showed that eDNA patterns were largely congruent with standard methods, while also providing additional insight into taxonomic diversity and species distributions.

In conclusion, this deliverable provides a practical scheme and a set of validated case studies showing that eDNA-based EOVs offer a powerful and scalable tool for producing policy-relevant biodiversity indicators. These eDNA-based EOVs are a stepwise improvement from simple raw eDNA observations of individual species and fit the information needs of European and regional environmental directives, paving the way for more comprehensive, standardized, and efficient biodiversity monitoring.

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Contents

1. Objectives

Work Package 2 (WP2) of the MARCO-BOLO project aims *"to enable eDNA-based approaches (i.e. single-species detection, DNA metabarcoding and metagenomics) for biodiversity monitoring across trophic and functional groups—from microbes to vertebrates—and across marine, terrestrial and freshwater systems."* Within this framework, Deliverable 2.4 presents the outcomes of Task 2.4: Defining eDNA-based variables for the EV, MSFD and WFD frameworks (lead: CNRS; contributors: UiT, MBA, VLIZ, SU, MS, SZN, UNESCO). This report presents Deliverable 2.4: "*Report on the definition of eDNA-based EBVs with associated eDNA-based EBVs datasets and efficiency of eDNA for detecting/quantifying taxa/species of interest"*.

Although WP2 as a whole covers eDNA applications across marine, freshwater, and terrestrial systems, the scope of Deliverable 2.4 is intentionally restricted to the marine domain. This is because (i) the case studies available for Tasks 2.2–2.4 are predominantly marine, (ii) the relevant European policy frameworks considered here (MSFD, WFD coastal components, Habitats Directive for marine species, UNEP-MAP, OSPAR) are primarily marine-focused, and (iii) existing GOOS BioEco EOVs developments provide the most mature reference structures for aligning eDNA-derived variables.

The general objective of Task 2.4 is therefore determining how eDNA-derived biodiversity information can be translated into Essential Variables (EOVs and EBVs) that are relevant to the European policy frameworks. Building on the eDNA suitability assessment from Task 2.1 (D2.1), part of the datasets assembled in Task 2.2 (D2.2), and the eDNA–microscopy comparisons from Task 2.3 (D2.3), this deliverable (D2.4) specifically aims to:

- Identify and process eDNA-derived biodiversity variables that can be expressed as Essential Variables (EOVs/EBVs), and to format these outputs in accordance with global observation frameworks such as GOOS BioEco and GEO BON.
- Generate eDNA-based EOVs from multiple case-study datasets—covering microbes, phytoplankton, zooplankton, fish, and marine mammals—and to assess which of these variables can function as EBV candidates.
- Evaluate the capacity of eDNA-based methods (metabarcoding, metagenomics, and single-species targeted assays) to retrieve species and higher taxonomic groups prioritised in European and international policy frameworks, including the MSFD, WFD, Habitats Directive, OSPAR, UNEP-MAP, and the IUCN Red List.
- Assess the efficiency or usefulness of eDNA for detecting and quantifying taxa or species of interest across case studies—phytoplankton (including HAB species), fish, cetaceans, and invasive alien species—by comparing eDNA-derived variables with temporal, spatial, and taxonomic biodiversity signals derived from traditional monitoring.

Through these objectives, Deliverable 2.4 demonstrates how eDNA-derived biodiversity observations can be standardised into EOVs, developed into EBV candidates, and ultimately used to support policy-relevant indicators under European and regional environmental directives.

2. Background

In the last couple of decades, the need for continuous and standardized collection of biodiversity data has become increasingly evident as we are faced with dramatic environmental changes. Biomonitoring data provide essential information on where species occur in time and space, how communities change over time, and how ecosystems respond to natural variability and anthropogenic pressures. These data underpin conservation strategies, environmental assessments, and evidence-based policy making. Yet, it is increasingly clear that the current demand for biodiversity information cannot be met by sole reliance on conventional approaches that require labour-intensive procedures and are typically limited to geographically restricted sampling sites. New scalable, standardized, technology-enabled observation systems, including environmental DNA (eDNA), may support effective biodiversity monitoring and ecosystem management at regional to global scales.

The Group on Earth Observations Biodiversity Observation Network (GEO BON) has developed the concept of Essential Biodiversity Variables (EBVs) to capture biodiversity beyond just individual species detections. EBVs define a minimum set of harmonized biodiversity metrics that can be consistently measured, compared, and integrated across monitoring programmes. They provide a bridging framework between raw biodiversity observations and high-level indicators used to assess progress towards international targets such as the Kunming-Montreal Global Biodiversity Framework. Within GEO BON, the Marine Biodiversity Observation Network (MBON) focuses specifically on coordinating and advancing biodiversity observations in the marine realm, ensuring that EBVs are tailored to and applicable across ocean ecosystems.

In the marine domain, the Global Ocean Observing System (GOOS) plays a parallel role by defining Essential Ocean Variables (EOVs). EOVs represent priority physical, biogeochemical, and biological measurements required to describe ocean state and change. The biological and ecosystem (BioEco) EOVs define a shared foundation for observing marine life, supporting interoperability across national and regional monitoring systems and ensuring that collected data are suitable for long-term assessments, modelling, and forecasting. These BioEco EOVs are complemented by the EBVs defined by GEO BON: EOVs supply the fundamental observations of key parameters or processes, while EBVs represent derived time series of biodiversity patterns across genes, populations, communities, or ecosystems. In this framework, BioEco EOVs, alone or combined with other GOOS EOVs, serve as building blocks for EBVs, which can be synthesised as local time series or as gridded or modelled biodiversity products (Muller-Karger et al., 2018; Bax et al., 2019; Jetz et al., 2019).

Within Europe, biodiversity and ecosystem monitoring is guided by major environmental directives. The Water Framework Directive (WFD) establishes the legislative basis for achieving “good ecological status” in inland and coastal waters, which is assessed through Biological Quality Elements (BQEs) such as phytoplankton, phytobenthos, benthic invertebrates, macrophytes, and fish. The Marine Strategy Framework Directive (MSFD) extends this objective to the wider marine environment by defining “good environmental status” across descriptors including biodiversity, food webs, and seafloor integrity. Several regional conventions further coordinate monitoring and assessment in specific European basins, such as OSPAR for the Northeast Atlantic and UNEP-MAP for the Mediterranean Sea, each providing regionally tailored monitoring strategies and indicator frameworks.

In this context, eDNA-based essential variables (EOVs, EBVs) have the potential to enhance these monitoring frameworks by enabling additional sensitive detection and quantification of taxa and species of interest, increasing spatial and temporal coverage to improve comparability across regions. They can also act as standardized intermediate products between raw observations and indicators defined by regional directives. This report contributes to this vision by defining eDNA-based EOVs/EBVs, assembling associated datasets, and evaluating the efficiency of eDNA for biodiversity detection and quantification across marine ecosystems. As part of this deliverable, we provide eDNA-based EOV datasets as case studies, demonstrating how eDNA-derived metrics can be implemented within global and European observation frameworks and how they can contribute to policy-relevant ecological assessments.

3. Selected eDNA-based Essential Variables (EOVs, EBVs)

Based on the suitability assessment provided in the D2.1 report (appendix IV), we selected a set of EOVs and EBVs that could be robustly calculated from eDNA data. These variables were extracted from several representative case-study datasets, namely: *Tara Oceans*, three coastal time-series (SOMLIT-Astan, LTER-MC/NEREA, EMO BON UiT genomic observatory), and a cetacean eDNA dataset (CIIMAR, Portugal).

Following internal evaluations and discussions with stakeholders during the Biodiversa+ Conference (Barcelona, May 2025) and the 3rd MARCO-BOLO co-creation workshop on eDNA-based EBVs held on the 27th of June 2025 (MARCO-BOLO 2025), we concluded that EOVs are currently more readily generated from eDNA compared to EBVs in the marine realm. EOVs correspond to well-defined observational sub-variables that can be directly deposited—with appropriate metadata—into repositories such as Zenodo and OBIS, following Darwin Core biodiversity data standards (Figure 1).

In contrast, EBVs typically require spatially and/or temporally comprehensive datasets to support modelling workflows, and are generally delivered as model products with a Network Common Data Form (NetCDF) through the EBV Data Portal: https://portal.geobon.org/datasets. While our case-study eDNA datasets include global surveys and multi-year time series, they do not provide the level of coverage needed to support the modelling workflows required for EBV generation. Therefore, for purposes of this deliverable, the selected EOVs should be considered as EBV candidates: if expanded sampling or long-term datasets were available, these EOVs could be directly transformed into EBVs through appropriate modelling (Figure 1). As an illustrative exception, we highlight an existing model product of plankton distributions, based on the Coupled Model Intercomparison Project Phase 5 (CMIP5) (Bopp et al., 2013), generated from the *Tara Oceans* observations (Ibarbalz et al. 2019), which meets EBV requirements and will be deposited in the EBV Data Portal as part of this deliverable.

Finally, several of the selected EOVs also correspond to policy-relevant biological indicators used in regional frameworks such as OSPAR and the Mediterranean Action Plan (UNEP-MAP) (Figure 1), further reinforcing their utility as intermediate products between raw eDNA observations and operational biodiversity indicators.

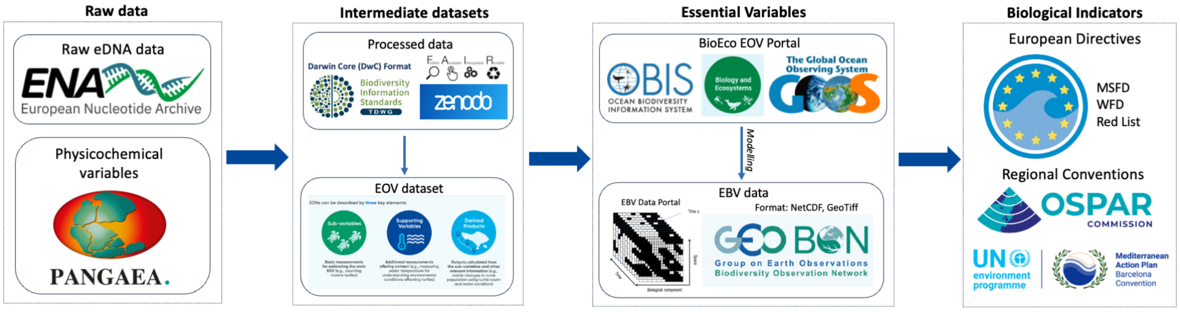

Figure 1. Workflow for generating eDNA-based EOVs and their links to EBVs and policy-relevant indicators. Conceptual workflow implemented in this report illustrating how raw eDNA data and associated physicochemical measurements are processed to produce Darwin Core–formatted EOV datasets for deposition in Zenodo, which connect to the GOOS BioEco EOV portal hosted by OBIS. These EOVs function as standardized intermediate products that can be further modelled into EBV data products (e.g., NetCDF, GeoTIFF) for the EBV Data Portal. The diagram also shows how both EOVs and EBVs can serve as essential variables feeding into biological indicators defined under European directives (e.g., MSFD, WFD, EU Red List of Habitats) and regional conventions (e.g., OSPAR, UNEP-MAP).

4. Case studies of eDNA-based EOVs

In this report, we generated eDNA-based GOOS BioEco EOVs as practical examples spanning multiple trophic and taxonomic levels—from microbes, phytoplankton and zooplankton to fish and marine mammals. The subsections below describe each case-study dataset, with corresponding links to public repositories (Appendix I and II). For each case study, we also identify the EBVs that are related to, or could be derived from, the presented EOV sub-variables.

4.1 Zooplankton, phytoplankton, and microbial EOVs

Within the *Tara Oceans* project, numerous plankton and microbial variables (e.g., diversity, richness, community composition in relative abundance) generated from eDNA have already been published, and many are conceptually aligned with the EOV framework. However, these outputs were not available in GOOS-compliant EOV formats. In this deliverable, we curated and formatted these variables as EOV sub-variables following the Darwin Core standard. All derived EOVs from the *Tara Oceans* dataset will be deposited in Zenodo together with links to their corresponding raw and processed data and associated publications (Appendix I), and in the GOOS BioEco EOV metadata portal, currently under development (https://bioeco.goosocean.org/). These examples illustrate Zooplankton, Phytoplankton, and Microbial EOVs that could, with appropriate modelling, be used to generate EBVs (Appendix I).

In addition to this global spatial dataset, we also produced phytoplankton EOVs from two eDNA time-series (SOMLIT-Astan and LTER-MC/NEREA) presented in D2.2. Time-series are particularly valuable, as many EOVs and EBVs depend on long-term observations. To support reproducibility, two GitHub repositories containing the full R workflows were created:

- **Extraction of phytoplankton reads** from total 18S metabarcoding reads based on PR2 (v5.1.0) taxonomy and literature review: https://github.com/pcjunger/phyto-tax-pr2
- **Extraction of harmful algal bloom (HAB) reads** based on the HAB species list in PR2 (v5.1.0): https://github.com/pcjunger/eDNA-EOVs-HABs

Phytoplankton relative abundances derived from 18S-V4 eDNA metabarcoding demonstrated clear seasonal dynamics over eight years (2008–2016) at SOMLIT-Astan, with significantly higher relative abundances during spring and summer (Figure 2). This highlights the potential of eDNA to monitor phytoplankton relative abundance as a proxy of the “Phytoplankton Biomass (concentration)” EOV sub-variable. This EOV also aligns with the “Ecosystem Function” EBV class (generic name: Ecosystem Phenology), for which marine spring phytoplankton bloom phenology has been presented as an operational EBV example by EUROPABON. The same pipeline will be applied to the LTER-MC/NEREA 18S-V9 eDNA metabarcoding dataset, and the associated EOV datasets will be deposited in a Zenodo repository (Appendix II), and in the GOOS BioEco EOV metadata portal, currently under development (https://bioeco.goosocean.org/).

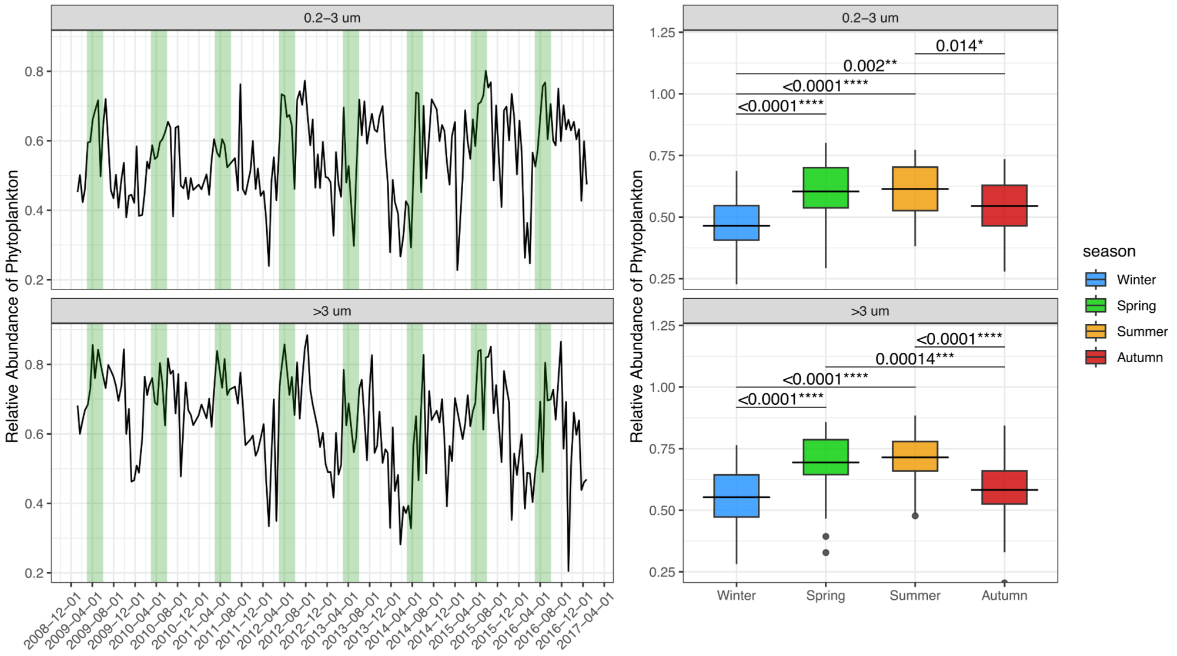

Figure 2. Phytoplankton relative abundance time series at SOMLIT-Astan (2008–2016). Monthly trajectories (left panels) of phytoplankton relative abundance for the 0.2–3 µm and >3 µm fractions from 18S-V4 eDNA metabarcoding at SOMLIT-Astan. The green bars depict the spring period. Boxplots (right panels) show significantly higher relative abundances in spring and summer, with significance levels (T-tests) indicated above comparisons. This figure demonstrates the capacity of eDNA time series to capture phytoplankton seasonality, supporting the “Phytoplankton Biomass (concentration)” EOV sub-variable and related EBV phenology products.

Additionally, we demonstrate the ability of eDNA metabarcoding to record harmful algal bloom events (Figure 3), characterize HAB community composition, and track species-level seasonal dynamics (Figure 4). These observations represent the “Phytoplankton Biomass and Diversity” EOV sub-variable “Number of HAB Events” and can be used to generate derived products such as HAB-related indices. These EOVs correspond to the “Ecosystem Function” EBV class (generic name: Ecosystem Disturbances), where marine HABs have been identified by EUROPABON as an operational EBV example. The same pipeline will be applied to the LTER-MC/NEREA 18S-V9 eDNA metabarcoding dataset, and the associated EOV datasets will be deposited in a Zenodo repository (Appendix II), and in the GOOS BioEco EOV metadata portal, currently under development (https://bioeco.goosocean.org/).

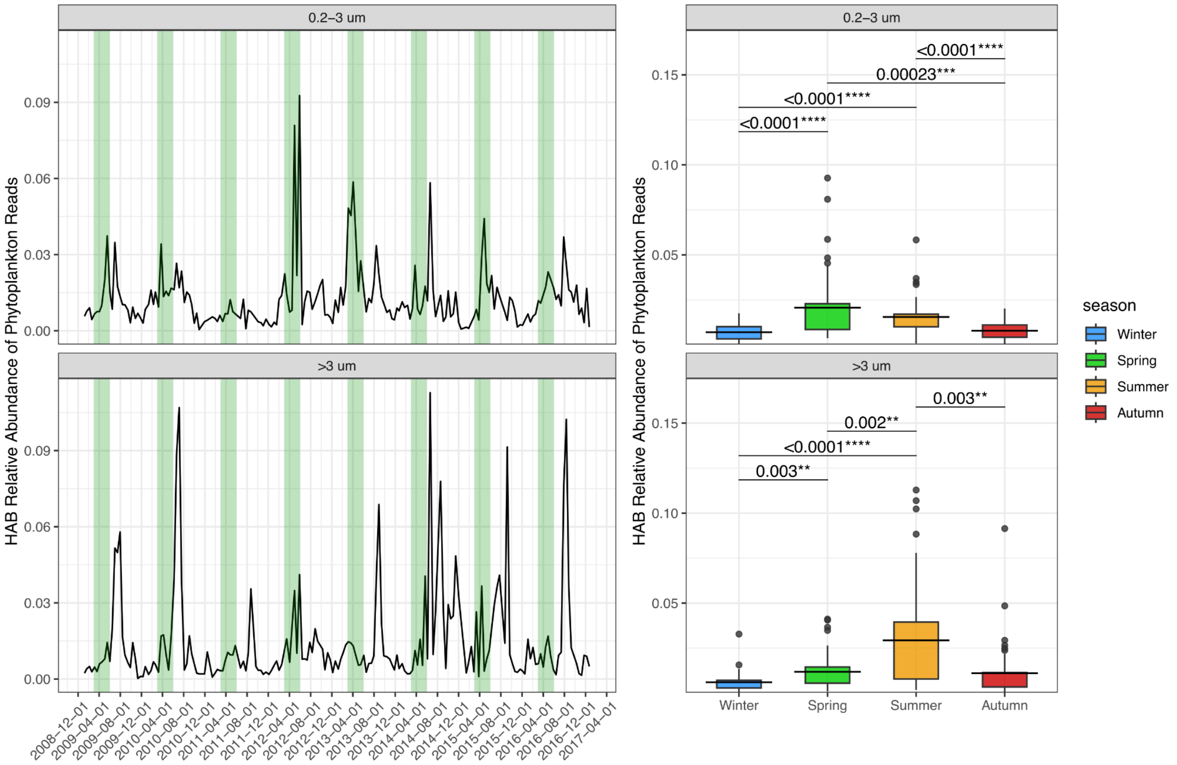

Figure 3. Harmful algal bloom (HAB) detections over time at SOMLIT-Astan (2008–2016). Time series (left panel) of HAB-related reads detected by 18S-V4 eDNA metabarcoding at SOMLIT-Astan, with green bars indicating the spring period. Boxplots (right panel) summarize the seasonal distribution of HAB occurrences, showing higher detection frequencies during spring and summer; significance levels (T-tests) are indicated above comparisons. This figure illustrates the capacity of eDNA time series to record HAB events, supporting the "Phytoplankton Biomass and Diversity" EOV sub-variable and derived HAB-related EBV products.

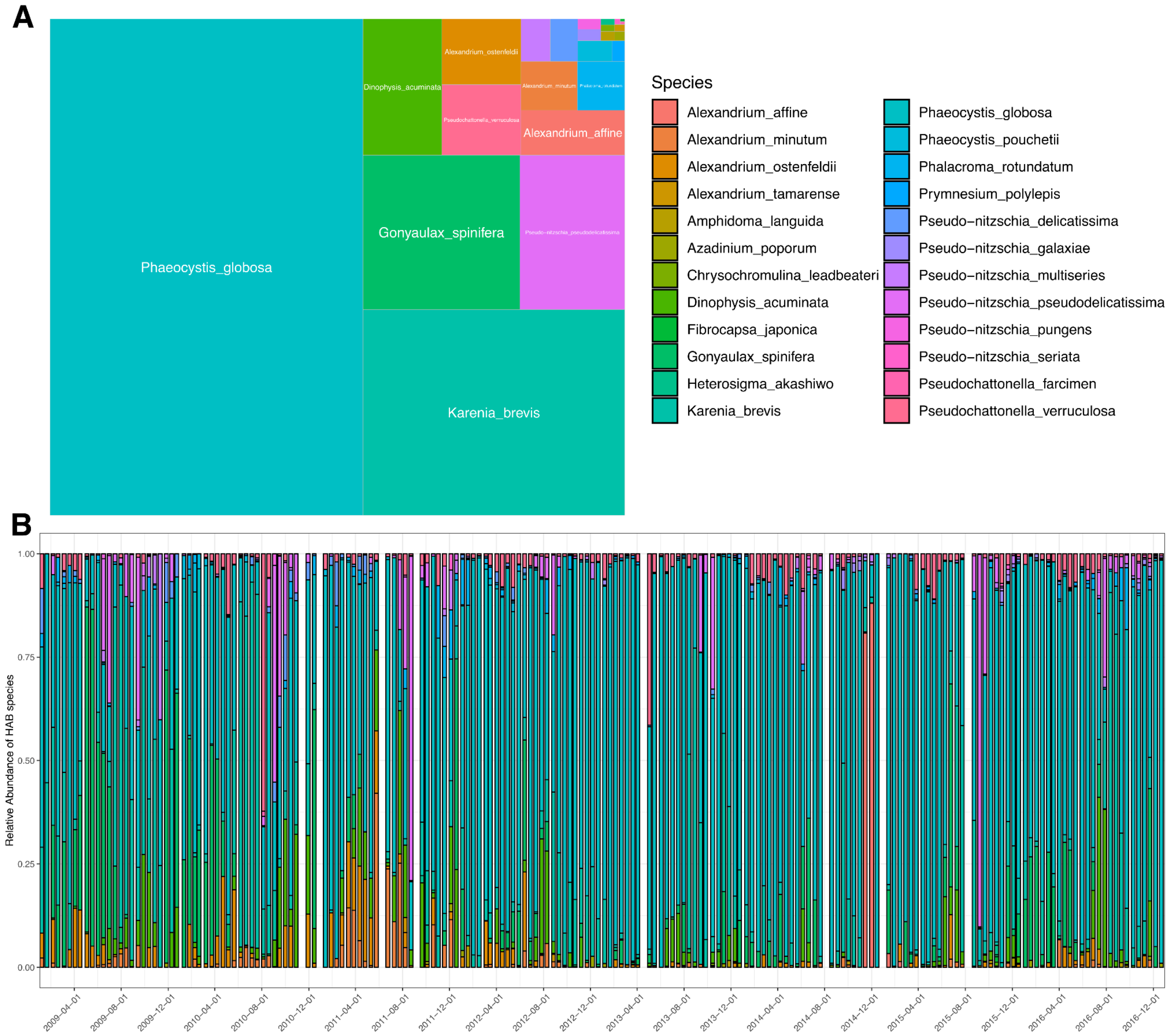

Figure 4. Harmful algae bloom (HAB) community composition and species dynamics at SOMLIT-Astan. (A) Treemap showing the HAB community composition considering the complete SOMLIT-Astan time-series; (B) Monthly relative abundance of HABs species throughout the sampling period (2008–2016). Seasonal changes and species-level dynamics are evident, illustrating how eDNA can characterise HAB community structure and temporal shifts. This information is going to be used to derive EOV sub-variables such as “Number of HAB Events” and HAB composition.

4.2 Fish EOVs

For fish, we used the EMO BON UiT genomic observatory 12S time-series dataset presented in D2.2. This dataset reveals strong seasonal patterns in fish diversity, with higher Shannon diversity during late spring, summer, and autumn, and lower values in winter (Figure 5A). Species richness shows a more moderate seasonal signal, with the lowest estimates in December (Figure 5B). The underlying fish community composition table corresponds to the EOV sub-variable “Fish species composition”, from which the derived EOV product “Fish species diversity indices” was calculated. These EOVs correspond to the EBV class "Species Populations" (generic name: Species Distribution), for which marine fish distributions have been proposed as a practical EBV example by EUROPABON.

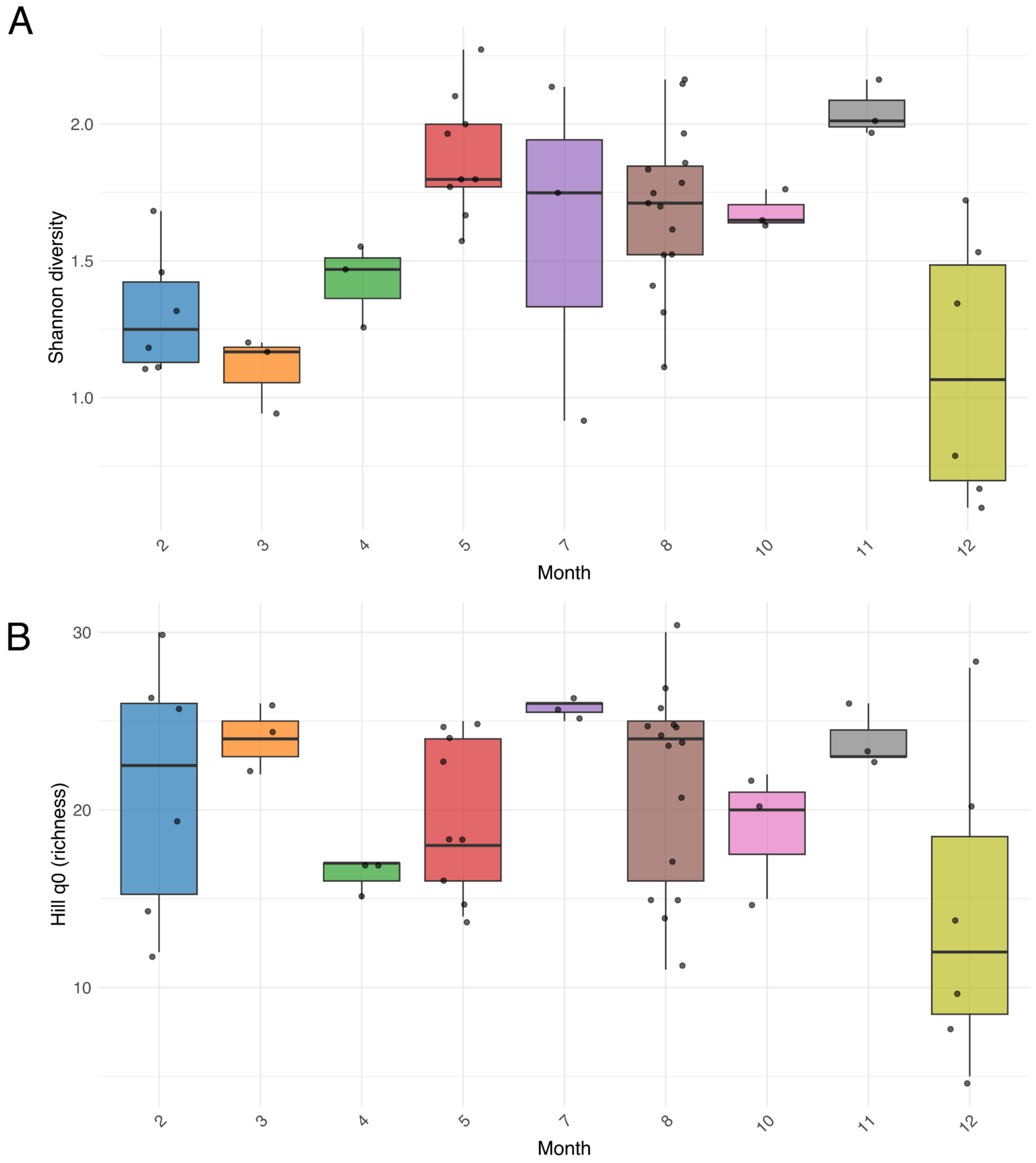

Figure 5. Seasonal patterns of fish diversity and richness from the EMO-BON UiT 12S eDNA time series. Boxplots indicating (A) Shannon diversity index and (B) species richness by month. These panels illustrate the "Fish species composition" EOV sub-variable and derived diversity indices used to inform EBVs in the Species Populations class.

In addition, here we present droplet digital PCR (ddPCR) quantification as an eDNA-based quantitative method to monitor *Engraulis encrasicolus* (European anchovy) in the Mediterranean Sea, which is an ecosystem health indicator in the UNEP-MAP convention (Figure 6). The highest abundance values in the summer are coherent with the historical fish stock record of the species (Bonanno et al. 2016). Quantitative detection corresponds to the "Fish Abundance" EOV sub-variables, which can be further used to derive EBVs within the "Species Populations" class (generic name: *Species Abundances*), with species abundances of marine commercial fish species as an operational example provided by EUROPABON. This case illustrates how EOVs and EBVs can act as intermediate products linking raw eDNA data to the biological indicators used by regional conventions.

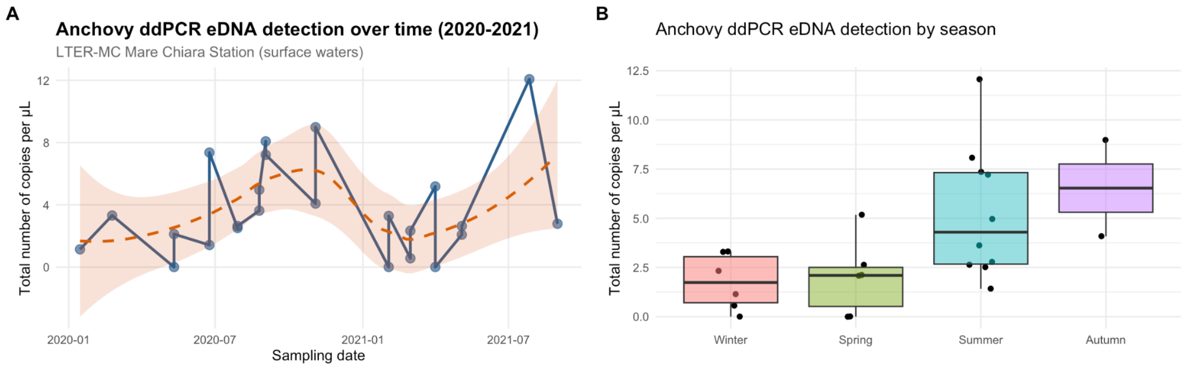

Figure 6. Droplet digital PCR (ddPCR) quantification of *Engraulis encrasicolus* (European anchovy). Quantitative eDNA monitoring results for *Engraulis encrasicolus* using ddPCR assays in the LTER Mare Chiara (SZN) in the Mediterranean Sea. (A) Copies per reaction (calibrated abundance proxy) over time and (B) between seasons. The figure demonstrates how targeted ddPCR yields EOV sub-variables for "Fish abundance", which can feed into species-level EBVs and inform policy-relevant indicators.

4.3 Marine mammals EOVs

For marine mammals, we used a 16S rRNA metabarcoding dataset collected along the Portuguese coast (Appendix IV) during summer and winter surveys, from 2021 to 2024 (Figure 7). Seasonal occurrences of *Delphinus delphis* (common dolphin), *Grampus griseus* (Risso's dolphin), *Tursiops truncatus* (common bottlenose), *Balaenoptera physalus* (fin whale), and *Phocoena phocoena* (harbour porpoise) were detected, revealing clear contrasts between summer and winter species assemblages. These detections were validated using visual observations conducted along transects. The presence/absence records correspond to a "Marine Mammal Abundance and Distribution" EOV sub-variable, which can support derived products such as habitat maps, home range estimates, and migration pathways. This EOV aligns with the EBV class "Species Populations", for which marine mammal species distributions represent another EUROPABON example. Furthermore, the fin whale is considered a globally vulnerable species in the IUCN Red list.

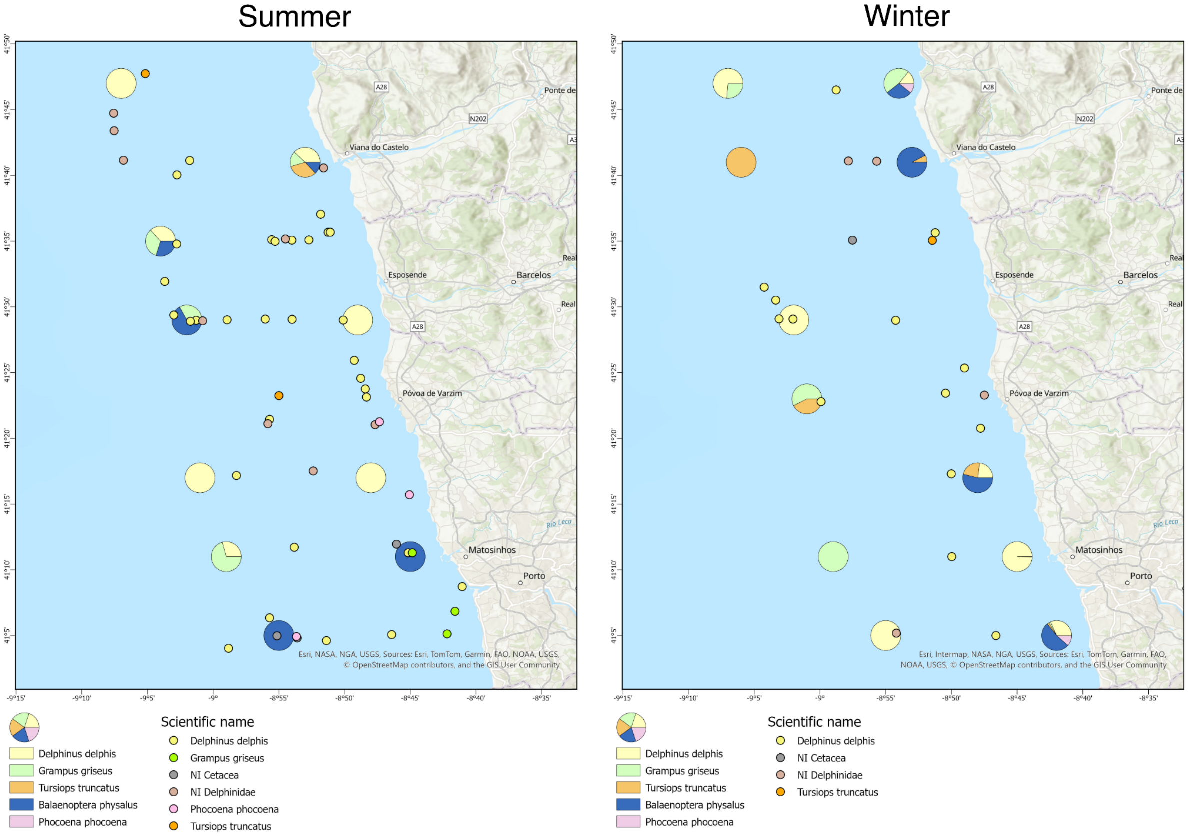

Figure 7. Cetacean occurrence from 16S eDNA metabarcoding (Portuguese coast). Detections of cetacean species from 16S eDNA metabarcoding surveys along the Portuguese coast (summer and winter campaigns). Pie charts indicate the relative abundance of reads and occurrence records for *Delphinus delphis*, *Grampus griseus*, *Tursiops truncatus*, *Balaenoptera physalus*, and *Phocoena phocoena*. Visual transect observations (dots) used for cross-validation are shown. These presence/absence records form a "Marine Mammal Abundance and Distribution" EOV sub-variable and support EBV-level distribution products.

4.4 Invasive alien species

The ability of eDNA to detect invasive alien species (IAS) was demonstrated using cytochrome oxidase unit I (COI) metabarcoding data from the EMO BON UiT genomic observatory (Figures 8, 9, and 10). Three species listed as "of concern" in the OSPAR IAS list were detected: the bryozoan *Schizoporella japonica* (Figure 8), the clinging jellyfish *Gonionemus vertens* (Figure 9) and the skeleton shrimp *Caprella mutica* (Figure 10). When compared with GBIF and iNaturalist records, the eDNA detections broadly matched areas where these species are already known to occur, while also revealing a small number of putative new occurrences. For example, *S. japonica* was detected in a sample collected further north than existing GBIF/iNaturalist records indicate, suggesting a possible range extension. However, this detection was based on only three reads in a single sample, and no subsequent samples showed a sustained signal. Such low-read occurrences should therefore be interpreted with caution, as they may represent background noise, transient eDNA, or early, low-abundance presence rather than a confirmed establishment. This example highlights the importance of replicated sampling and the need for meaningful biological thresholds when interpreting metabarcoding data for IAS monitoring. Overall, the patterns observed with eDNA are consistent with known distributions while also pointing to potential new areas that warrant further investigation. These occurrences correspond to "Species Distribution" EOVs and fall under the EBV class "Species Populations", for which distributions of invasive marine taxa have been identified by EUROPABON as an operational EBV example.

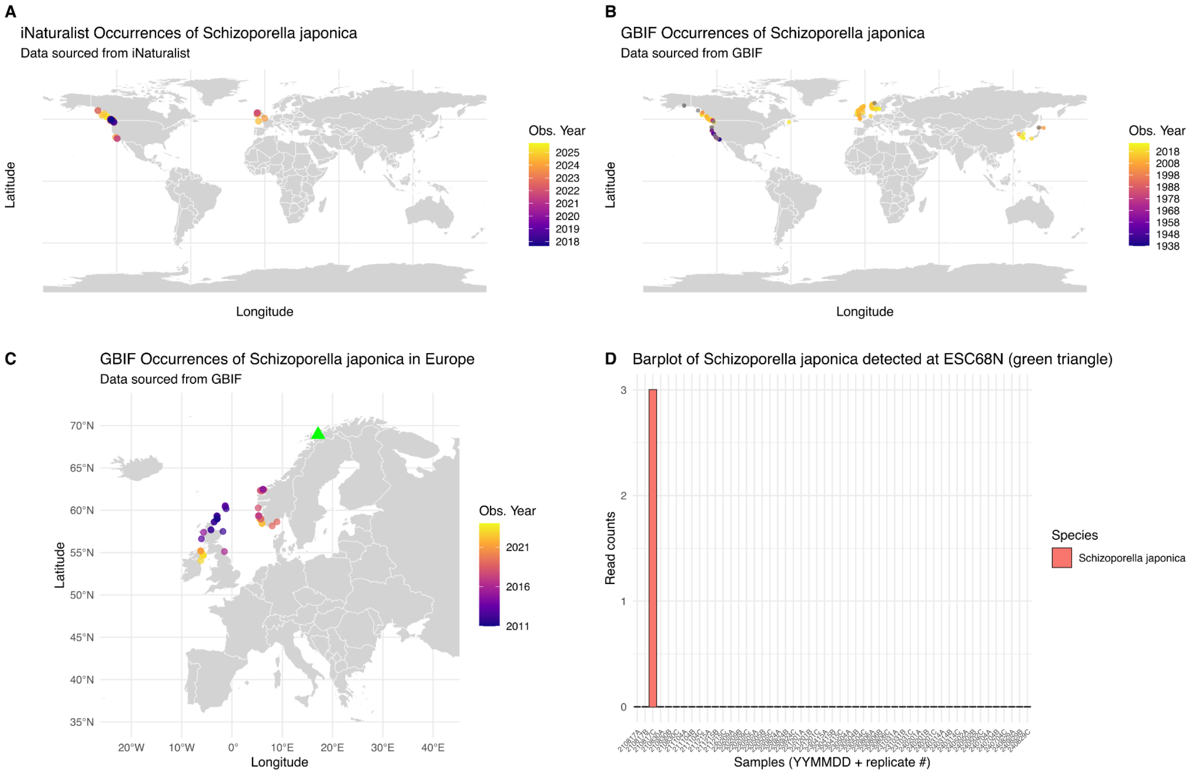

Figure 8. Detection of the invasive bryozoan *Schizoporella japonica* from COI metabarcoding (EMO-BON UiT). Temporal records of IAS detected via cytochrome oxidase unit I (COI) metabarcoding at the EMO-BON UiT genomic observatory (ESC68N, green triangle). The maps show locations with detected occurrences of *Schizoporella japonica* from (A) iNaturalist and (B,C) GBIF, while the barplot (D) depicts the read counts of this OSPAR-listed IAS in the ESC68N samples. These occurrences correspond to "Species Distribution" EOVs and relate to EBVs under the Species Populations class for invasive taxa of European concern.

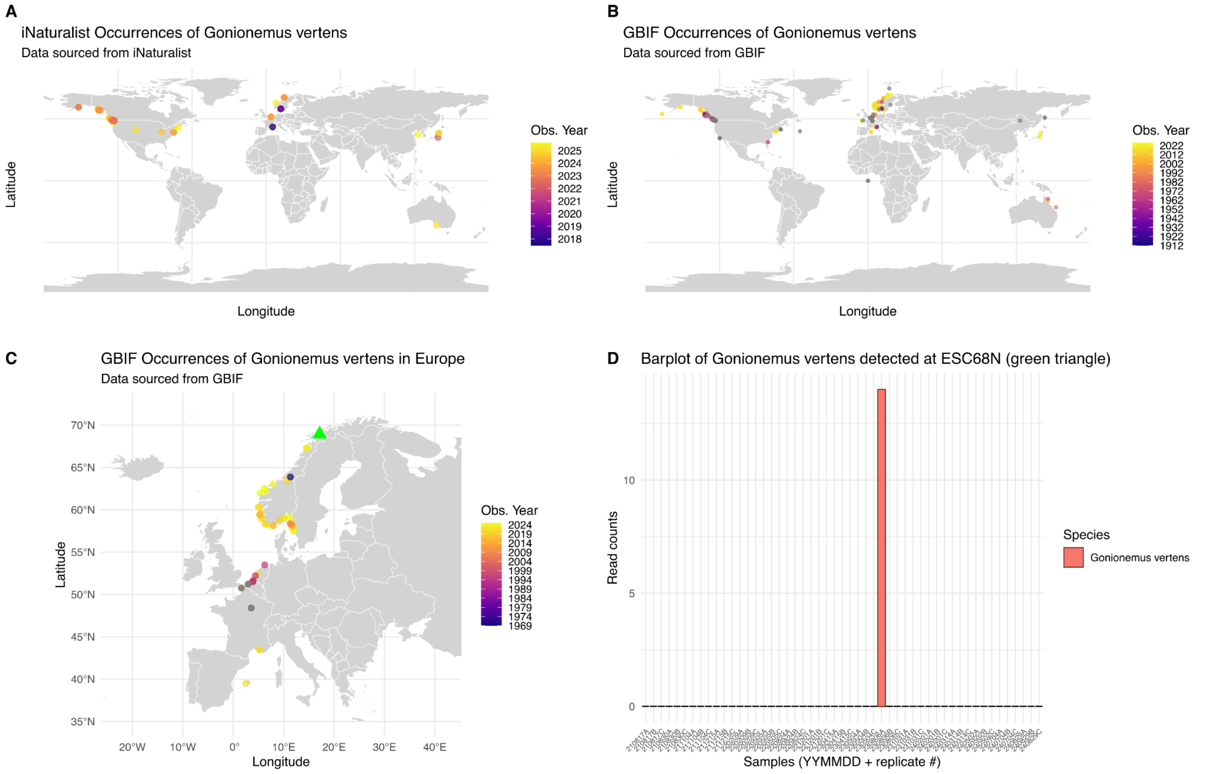

Figure 9. Detection of the invasive clinging jellyfish *Gonionemus vertens* from COI metabarcoding (EMO-BON UiT). Temporal records of IAS detected via cytochrome oxidase unit I (COI) metabarcoding at the EMO-BON UiT genomic observatory (ESC68N, green triangle). The maps show locations with detected occurrences of *Gonionemus vertens* from (A) iNaturalist and (B,C) GBIF, while the barplot (D) depicts the read counts of this OSPAR-listed IAS in the ESC68N samples. These occurrences correspond to “Species Distribution” EOVs and relate to EBVs under the Species Populations class for invasive taxa of European concern.

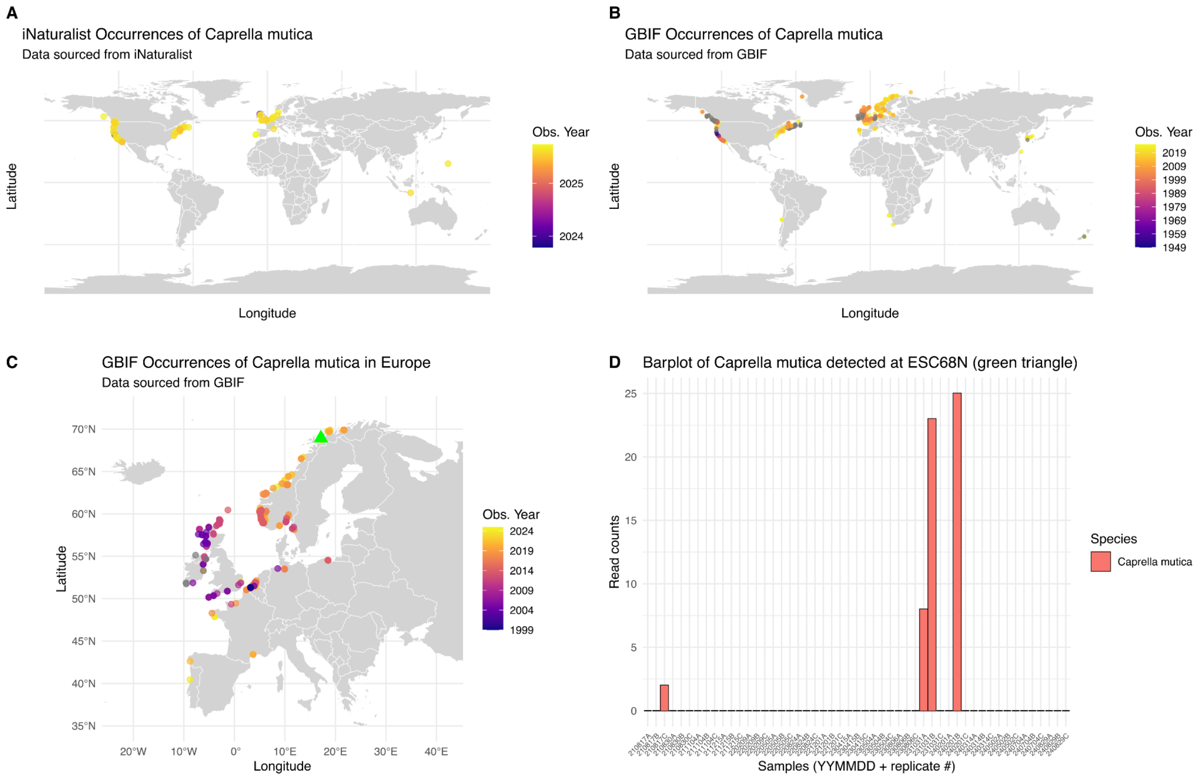

Figure 10. Detection of the invasive skeleton shrimp *Caprella mutica* from COI metabarcoding (EMO-BON UiT). Temporal records of IAS detected via cytochrome oxidase unit I (COI) metabarcoding at the EMO-BON UiT genomic observatory (ESC68N, green triangle). The maps show locations with detected occurrences of *Caprella mutica* from (A) iNaturalist and (B,C) GBIF, while the barplot (D) depicts the read counts of this OSPAR-listed IAS in the ESC68N samples. These occurrences correspond to “Species Distribution” EOVs and relate to EBVs under the Species Populations class for invasive taxa of European concern.

4.5 Fish species of conservation concern (IUCN Red List)

The usefulness of eDNA metabarcoding for detecting species of conservation concern was evaluated using the 12S dataset from the EMO BON UiT genomic observatory. Among the taxa recorded, six marine fish species listed as Near Threatened (NT), Vulnerable (VU), or Endangered (EN) on the IUCN Red List were detected: *Hippoglossus hippoglossus* (VU), *Chimaera monstrosa* (NT), *Coryphaenoides rupestris* (EN; tentative ID due to limited reference material), *Cyclopterus lumpus* (NT), *Somniosus microcephalus* (NT), and *Reinhardtius hippoglossoides* (NT). These species are ecologically and socio-economically relevant in northern marine ecosystems, are regularly observed in the region, yet are often difficult to monitor through conventional methods because of low detectability, deep-sea habitats, or cryptic behaviour.

The eDNA detections capture seasonal and interannual variation in the presence of these threatened or near-threatened species (Figure 11). Although the lack of concurrent traditional fish surveys in this area limits our ability to evaluate potential false negatives, when cross-checked with existing records from OBIS and GBIF, the eDNA occurrences broadly align with known distribution ranges, providing additional resolution in understudied periods or locations. For five of the six detected species—commonly occurring in the region despite declining population trends—the eDNA results are therefore consistent with expectations. The detection of *Coryphaenoides rupestris*, a deep-living species, is more surprising; however, this identification remains tentative due to limited COI reference material. This case study demonstrates again how eDNA time-series can contribute to “Species Distribution” EOVs and to the Species Populations EBV class, for which distributions of threatened marine taxa can be particularly useful for strengthening biodiversity assessments under policy frameworks that prioritise the monitoring of red-listed species.

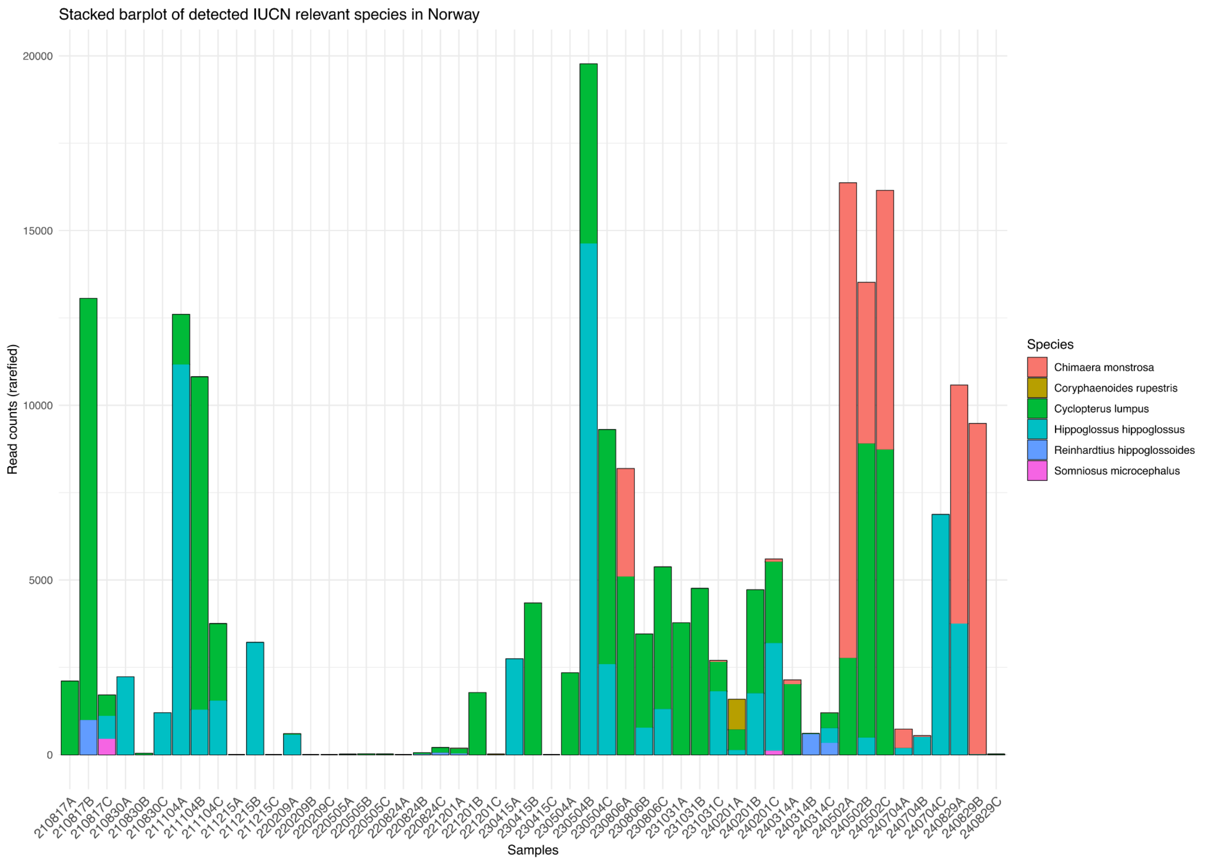

Figure 11. Detection of IUCN Red List species using 12S eDNA metabarcoding (EMO BON UiT). Temporal detections of six fish species listed as Endangered (EN), Vulnerable (VU), or Near Threatened (NT) in the IUCN Red List, recorded from 12S eDNA metabarcoding at the EMO BON UiT genomic observatory. Stacked barplots show occurrences of *Hippoglossus hippoglossus* (Atlantic halibut, status = VU), *Chimaera monstrosa* (Rabbitfish, status = NT), *Coryphaenoides rupestris* (tentative ID; Roundnose grenadier, status = EN), *Cyclopterus lumpus* (Lumpfish, status = NT), *Somniosus microcephalus* (Greenland shark, status = NT), and *Reinhardtius hippoglossoides* (Greenland halibut, status = NT) across sampling dates. The occurrences are coherent with distribution records from OBIS/GBIF/iNaturalist.

5. Monitoring species and taxa of interest with eDNA-based EOVs

The case studies presented in this report demonstrate how eDNA-derived EOVs can be generated across diverse taxonomic groups. Building on these examples, this section examines how such variables can be mobilised for monitoring species and taxa of interest under international, European, and regional frameworks.

5.1 Water Framework Directive (WFD)

Under the WFD, Biological Quality Elements (BQEs) include phytoplankton composition and blooms, phytobenthos such as diatoms, benthic invertebrate communities, and fish fauna. The SOMLIT-Astan case study illustrates how eDNA metabarcoding can describe seasonal phytoplankton dynamics and detect periods with elevated relative abundances of HAB occurrences. While HAB monitoring currently relies on microscopy and toxin measurements from shellfish flesh, eDNA could act as an early-warning indicator by signalling when additional, established HAB monitoring practices should be triggered. Likewise, the EMO BON case study demonstrates that eDNA metabarcoding can reveal temporal variation in fish community diversity and composition. Together, these examples evidence how eDNA-derived EOVs can contribute directly to WFD assessments of ecological status.

5.2 Marine Strategy Framework Directive (MSFD)

The MSFD utilizes several descriptors that rely on detecting and quantifying specific taxa or functional groups. Descriptor D1 (Marine Biodiversity) requires distribution and abundance information for plankton, fish, marine mammals, and benthic species. Descriptor D2 focuses on numbers of newly arrived non-indigenous species (NIS), while D4 (Food webs) and D5 (Eutrophication) rely on sentinel species or indicator phytoplankton groups.

The eDNA-derived EOVs presented here—including phytoplankton phenology, HAB events, fish species composition, IAS/NIS, and cetacean detections—illustrate how eDNA can supply standardized essential variables that align with MSFD requirements. In addition, the MARCO-BOLO Task 5.2 develops a workflow to calculate the MSFD Pelagic Habitat Indicators (e.g. Lifeform ratios-PH1, Diversity Indices-PH3) using the validated WEC L4 eDNA dataset from D2.3. This provides a concrete example of how eDNA can support MSFD indicator development through the EOV frameworks.

5.3 EU Habitat Directive and European Red List of Marine Habitats

Although the EU Habitats Directive is habitat-centred, it also lists species of community interest in its Annexes, including several marine mammals. The cetacean case study presented here detected five species—*Delphinus delphis*, *Grampus griseus*, *Tursiops truncatus*, *Phocoena phocoena*, and *Balaenoptera physalus*—all of which are protected under Annex IV, with *Tursiops truncatus* and *Phocoena phocoena* additionally listed under Annex II, requiring the designation and monitoring of Special Areas of Conservation (SACs). These detections illustrate how eDNA-derived EOVs can provide occurrence and distribution information for strictly protected marine species in coastal and offshore waters, complementing conventional survey methods and supporting Natura 2000 conservation objectives.

EOVs based on eDNA are also relevant for supporting assessments under the European Red List of Marine Habitats, which identifies Europe's marine environments as among the most threatened. According to the latest assessment, 31% of marine habitat types are classified as threatened, while 36% are listed as "Data Deficient", particularly deep-sea and offshore areas where traditional monitoring remains sparse. Key pressures include bottom trawling, eutrophication, pollution, and coastal development, all of which influence pelagic ecosystem structure and functioning.

Although the case studies presented in this deliverable focus on pelagic systems rather than benthic habitat types, they nonetheless address several of the knowledge gaps highlighted by the Red List:

- Eutrophication and algal bloom dynamics: eDNA-derived EOVs such as phytoplankton phenology and HAB occurrences provide indicators of nutrient pressures affecting coastal habitats including seagrass beds, lagoons, and shallow shelf communities.
- Species distributions in poorly sampled regions or "Data Deficient" areas: eDNA-based EOVs (particularly species-distribution variables) can efficiently scale up biodiversity observations and extend known species ranges into regions that are classified as "Data Deficient".

5.4 Regional conventions

Species of interest are often defined regionally. The eDNA-based EOVs fit particularly well within regional conventions such as OSPAR, UNEP-MAP, and HELCOM, which maintain regional indicator lists for eutrophication, invasive alien species (IAS), and ecosystem health. More precisely:

- OSPAR (Northeast Atlantic): The IAS case study (detection of *Schizoporella japonica, Gonionemus vertens,* and *Caprella mutica*) shows that eDNA can detect priority invasive taxa and extend known distributions, especially in transition zones between the North Atlantic and Arctic Ocean.
- UNEP-MAP (Mediterranean Sea) relies on sentinel fish species for ecosystem assessments; the anchovy ddPCR case study illustrates how eDNA-based EOVs related to fish abundance can support such regional indicators.
- HELCOM (Baltic Sea) uses diatoms and dinoflagellates as eutrophication indicators. Although our case studies do not include Baltic sites, both the *Tara Oceans* and the time-series (SOMLIT-Astan and LTER-MC/NEREA) case-studies, and the associated EOV datasets, demonstrate that these phytoplankton-related EOVs can be calculated from eDNA-derived data elsewhere and therefore, in principle, could feed directly into HELCOM indicator workflows.

5.5 IUCN Red List

Beyond European legislation, eDNA also contributes to global conservation frameworks such as the IUCN Red List, which relies on robust spatial and temporal records for assessing species extinction risk. In our marine case studies, eDNA metabarcoding recovered multiple taxa of conservation concern. The cetacean dataset confirmed the presence of the fin whale (*Balaenoptera physalus*), listed globally as Vulnerable. Importantly, it also detected the harbour porpoise (*Phocoena phocoena*), which is categorised as Least Concern at the global scale but corresponds in our study area to a genetically isolated and regionally threatened population, classified as Critically Endangered in the Portuguese Red List of Mammals (2023). Similarly, the EMO BON fish dataset detected six species classified as Endangered, Vulnerable, or Near Threatened, including Atlantic halibut (*Hippoglossus hippoglossus*), rabbitfish (*Chimaera monstrosa*), and Greenland shark (*Somniosus microcephalus*). These examples demonstrate that eDNA can capture the distribution of threatened species and complement biodiversity databases such as OBIS, GBIF, and national monitoring programmes.

6. Conclusions

The case studies presented in this deliverable show that eDNA-based observations can recover a wide range of policy-relevant taxa, including phytoplankton, HAB species, zooplankton, fish, cetaceans, invasive species, and threatened species. These observations can be translated into EOVs, or EBV candidates, which are the building blocks of indicators. Therefore, eDNA-based EOVs provide a scalable and harmonised approach to monitoring species and taxa of interest across European and international frameworks.

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Appendix I. *Tara Oceans* eDNA-based EOVs and EBVs

Table 1. List of eDNA-based EOVs generated from Tara Oceans datasets: link to raw sequences, processed data, Zenodo repositories, and GOOS EOV metadata (to be deposited by the end of the project in December 2026): https://github.com/pcjunger/MBO-eDNA-EOVs-EBVs

Table 2. List of eDNA-based candidate variables that could be used to generate EBVs from Tara Oceans datasets: https://github.com/pcjunger/MBO-eDNA-EOVs-EBVs

Appendix II. eDNA-based EOVs from MBO D2.4 case studies

Table 3. List of EOVs datasets generated with the case-studies described in this report: link to Zenodo repositories, and GOOS EOV metadata (to be deposited by the end of the project in December 2026): https://github.com/pcjunger/MBO-eDNA-EOVs-EBVs

Appendix III. GitHub repositories and links

Codes

R scripts to extract phytoplankton reads from total 18S metabarcoding reads based on PR2 (v5.1.0) taxonomy and literature review: https://github.com/pcjunger/phyto-tax-pr2

R script to extract harmful algal bloom (HAB) reads from eDNA metabarcoding datasets based on the HAB species list in PR2 (v5.1.0): https://github.com/pcjunger/eDNA-EBVs-HABs

Appendix IV. eDNA methods

eDNA time-series datasets

The methods used to generate the three time-series used in this report (EMO BON UiT genomic observatory, SOMLIT-Astan, LTER-MC/NEREA) are described in D2.2.

Cetacean 16S eDNA dataset

Environmental DNA (eDNA) surveys were conducted along the north coast of Continental Portugal. Six seasonal monitoring campaigns were performed between the summer of 2021 and the winter of 2023/2024. Samples were collected at predetermined stations located at the vertices of the campaign transect (2 and 12 nautical miles from the shore) using a bucket and rope. Between 1 and 3 liters of water were immediately filtered on board through 0.22 µm Sterivex units using a peristaltic pump. Following filtration, samples were transported to the laboratory and stored at -80°C. A total of 96 samples were collected. Total eDNA was extracted using the DNeasy® PowerWater® Sterivex™ Kit (QIAGEN). Sequencing was carried out on an Illumina MiSeq300 platform, employing MarVer3(A) primers (Valsecchi et al., 2020) and Superfi II Polymerase (Invitrogen™) in a two-step PCR amplification protocol (Afonso et al., 2024; Álverez-González et al., 2023). After sequencing, adapters were removed using Cutadapt 3.5.0 (Martin, 2011), and raw reads were filtered, with Amplicon Sequence Variants (ASVs) inferred through DADA2 (Callahan et al., 2016). Taxonomic assignment of each ASV was performed by comparing its representative sequence against a local database of cetacean mitochondrial genomes retrieved from NCBI's Nucleotide database (last updated 09/10/2024) using BLASTn v2.13.0+ (Camacho et al., 2009), with parameters set to 99% minimum percent identity, an e-value threshold of 1e-05, and a minimum hit coverage of 80%. In cases where a single ASV matched multiple species, the top hit with 100% identity was used for taxonomic assignment.

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MARCO-BOLO

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