

Handbook Monitoring marine macro-fauna using ferries/large vessels as multidisciplinary research platforms



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Informazioni legali

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These Guidelines were developed within the framework of major European collaborative initiatives, including the LIFE Programme project *Conceptu Maris*, and the subsequent After-LIFE roadmap, the *Fixed Line Transect -Mediterranean Monitoring Network* and the IMPEL *FLT-Maritime Transborder Transects* networking initiatives. All projects were implemented with the financial support of the European Union and were aimed at strengthening transnational cooperation, environmental governance, and the exchange of scientific and operational best practices in the field of marine biodiversity monitoring, specifically for cetacean and turtles and some of their main pressures such as floating plastics and maritime traffic. In particular, the initiatives contributed to the advancement of harmonized approaches supporting compliance with European Union environmental legislation and policy frameworks, including requirements related to marine environmental protection, monitoring, reporting, and cross-border cooperation.

The development of these Guidelines therefore reflects a shared commitment to promoting consistent methodologies, improving data comparability, and facilitating the effective implementation and enforcement of EU environmental standards across European and Mediterranean marine contexts.

Le presenti Linee Guida sono state sviluppate nell'ambito di importanti iniziative collaborative europee, tra cui il progetto del Programma LIFE *Conceptu Maris* e la sua successiva roadmap per l'After-LIFE, nonché le iniziative di networking *Fixed Line Transect - Mediterranean Monitoring Network* e IMPEL *FLT-Maritime Transborder Transects*. Tali progetti sono stati realizzati con il sostegno finanziario dell'Unione Europea e sono stati finalizzati al rafforzamento della cooperazione transnazionale, della governance ambientale e dello scambio di buone pratiche scientifiche e operative nel campo del monitoraggio della biodiversità marina, nello specifico di cetacei e tartarughe ed di alcune delle loro principali pressioni (plastiche galleggianti e traffico marittimo). In particolare, le iniziative hanno contribuito allo sviluppo di approcci armonizzati a supporto della conformità alla legislazione e ai quadri strategici ambientali dell'Unione Europea, inclusi gli obblighi relativi alla protezione dell'ambiente marino, al monitoraggio, alla rendicontazione e alla cooperazione transfrontaliera.

Lo sviluppo delle presenti Linee Guida riflette pertanto un impegno condiviso volto a promuovere metodologie coerenti, migliorare la comparabilità dei dati e facilitare l'efficace attuazione e applicazione degli standard ambientali dell'UE nei contesti marini europei e mediterranei.

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Introduction

The conservation of cetaceans and sea turtles (CEPTU) is mandated by key global, European, and regional legislation, including the Kunming-Montreal Global Biodiversity Framework (KM GBF), the EU Habitats Directive (HD), the Marine Strategy Framework Directive (MSFD), and the Regional Sea Conventions (i.e. UNEP/MAP-Barcelona Convention for the Mediterranean Region), which specifically requires the protection and monitoring of marine biodiversity. However, the high mobility, extensive ranges, and frequent presence in remote or hard-to-survey areas of CEPTU result in limited data, and a conservation status (CS) often considered unknown in many Member States. In addition, their vulnerability to anthropogenic threats that may affect populations or habitats makes fine-scale data essential for identifying conservation priorities, supporting spatial planning and detecting early changes that require targeted mitigation measures.

Three main projects, (i.e. IMPEL FLT; IMPEL MTT, LIFE Conceptu Maris) drove the harmonisation of protocols across the three main network collecting data from large commercial vessels from EU waters creating a unified standard of marine surveillance (FLT Med Net, ORCA, CETUS).

The LIFE project Conceptu Maris (2022-2026, LIFE20 NAT/IT/00137), builds on the long-term experience of the Fixed Line Transect Mediterranean monitoring Network (FLT Med Net), both coordinated by ISPRA, was designed to fulfil the HD requirements for systematic surveillance (Article 11) and six-yearly reporting (Article 17) on species' CS. This status is evaluated through four mandatory parameters: Range, Population, Habitat for the species, and Future Prospects. To address the challenges of monitoring vast offshore areas, the project adopted a multidisciplinary and integrated approach, using large vessels such as passenger ferries operating along Fixed Line Transects (FLT) that are representative of the region's ecological variability. This design enables cost-efficient, systematic sampling and ensures data representativeness, reproducibility, and temporal consistency. At European larger scale two IMPEL projects (FLT Europe 2020-2021 and Europe MTT 2022-2024) further drove the harmonisation of protocols across the three main network collecting data from large commercial vessels from EU waters creating a unified standard of marine surveillance (FLT Med Net in the Mediterranean, ORCA in the North Sea and Atlantic, CETUS in the Macaronesia regions).

The standard approach integrates all phases of the information chain, from data collection to data management and data analysis. To ensure the scientific robustness, comparability, and policy relevance, these **harmonized guidelines** are structured across **four key components**.

- **Data Collection:** the guidelines provide detailed protocols for systematic and multidisciplinary data acquisition. This includes visual monitoring of CEPTU and recording of major anthropogenic stressors (maritime traffic, marine litter). Innovative molecular techniques, such as Environmental DNA (eDNA) analysis and Stable Isotope Analysis (SIA), are also collected at fixed sampling stations (FSSs) to provide complementary insights into species presence and ecological baselines.
- **Data Management:** recognizing that the methodology produces long-term, fine-scale data, the project established guidelines for data harmonization, quality control, and storage. New data could be integrated with current LIFE Conceptu Maris and historical FLT Med Net data (since 2008) and stored in the shared geodatabase developed within the National Biodiversity Network (NNB ISPRA), ensuring consistency and accessibility for large-scale analyses and long-term trend detection.

-
- **Data Analysis and Parameter Assessment:** the guidelines suggest a set of methodologies for analysing this extensive dataset, in line with the requirements for accurately populating the four HD parameters: Range, Population, Habitat for the species, and Future Prospects. Distance sampling techniques are applied to estimate *population abundance and trends*, while Kernel Density Estimators (KDE) and Species Distribution Models (SDMs) map *species range and suitable habitats*. *Future Prospects* are evaluated by combining population and habitat trends with current and projected pressures, including ship strikes, marine litter, and climate change. **To prioritize conservation actions**, the guidelines suggest an approach to integrate multiple ecological indicators, species richness, abundance, juvenile presence, and rare species occurrence, into a composite *Conservation Priority Index*. Furthermore, the guidelines include a *Spatial Risk Exposure Analysis (REA)* that combines species sighting rates (SPUE or ER) with quantified human pressures (FMML and maritime traffic density) to identify high-risk areas requiring mitigation. *In-situ* observation of *Near Miss Events (NME)*, where unaware cetaceans are sighted in close proximity to vessels, serves as a direct proxy for collision risk and is used alongside REA outputs to validate risk maps and support evidence-based management decisions.
 - **Data Integration and Decision Support:** the guidelines culminate in the development of a Decision Support System (DSS), a digital tool that integrates the validated *in-situ* data, open-source datasets (oceanographic, model-based, maritime traffic), and the results of the statistical analyses. The DSS provides a synoptic and interactive representation, allowing managers and decision-makers to overlay species distribution, important key areas with anthropogenic pressures and existing conservation measures. This functionality is designed to identify potential protection gaps, define targeted improvement actions, and produce georeferenced outputs for official reporting, thereby bridging the gap between scientific data and the implementation of effective conservation policies.

By linking consistent data acquisition, standardized data management, and essential analytical methods, these integrated guidelines provide a comprehensive and replicable framework that supports research institutions, environmental agencies, and NGOs in establishing new monitoring programmes in previously unsurveyed areas, thereby expanding scientific knowledge and enabling the long-term assessment of species' Conservation Status. The Guidelines could help Member States meet both the broad mandates of the HD and the indicator-driven requirements of the MSFD (also fulfilling, by extension, the Ecosystem Approach requirements of the Barcelona Convention). Acting as an ecological "*diagnostic laboratory*", the multidisciplinary approach, combining visual surveys, SDMs, eDNA, and SIA, offers a holistic understanding of cetacean and sea turtle distribution, ecological dynamics, and exposure to anthropogenic pressures. This enables conservation managers not only to determine *where* species occur, but also to understand *why*, facilitating timely and targeted management and mitigation measures across the region.

Advantages of the method

Using fixed-transect sampling offers a sustainable, cost-effective approach that enables **year-round, repeatable monitoring over large spatial scales**, including offshore and high-seas areas that are hard to access with traditional research vessels. The method **increases precision and statistical power**, ensures seasonal coverage, and supports the detection of long-term trends and rare species or events thanks to frequent and consistent sampling.

Fixed transects also support **robust ecological analyses**, since repeated surveys over the same route distributed to be representative of the ecological variability reduce spatial bias and enable the investigation of relationships between species occurrence and environmental or anthropogenic variables. The approach promotes **multidisciplinary data collection** (e.g., oceanographic, biological, anthropogenic parameters) and strengthens **collaboration** among research institutions, environmental agencies, ferry companies, and other stakeholders. Finally, the visibility and continuity of the platform enhance opportunities for **public awareness and communication** on marine conservation.

1. Preparatory phase

In this section we will detail the steps needed to define the adequate framework for the establishment of the CEPTU monitoring program.

1.1 Identification and selection of fixed transect routes for sampling

Depending on the study area, the first step is to **select fixed transects** along the available vessel routes to enable repeated monitoring over time, ensuring representativeness of the study area. Transects should be selected to cover key environmental gradients (e.g., depth, slope, temperature, mixed-layer depth) and important habitats for target species. Specifically, the design should ensure:

1. *Coverage of different habitat types*, including both coastal and offshore areas, and overlapping continental shelf, topographically complex areas (e.g., submarine canyon, seamounts), slope, and bathyal zones.
2. *Sampling of environmental gradients*, ideally placing transects perpendicular to bathymetric lines or migration path and, where possible, to oceanographic structures such as seasonal or permanent fronts and sea surface eddies.
3. *Balanced spatial coverage*, encompassing both frequently traversed routes and under-monitored regions to maximize ecological representativeness.

Logistic aspect: sampled routes shall be checked also considering the schedule of the identified ships, considering daylight-time navigation, as well as seasonality (especially for passenger vessels). A list of the main ports in the area is needed to determine the types of maritime traffic, distinguishing between tourist ports and marinas, commercial ports, and military ports. If no existing information on the main maritime traffic corridors is available, relevant data can be obtained from maritime traffic sources, such as:

- www.marinetraffic.com Provides real-time information on vessel presence and distribution in the area. It is possible to filter vessels by type; by selecting cargo and passenger vessels, an immediate overview of ships currently in the area can be obtained.
- <https://emodnet.ec.europa.eu/geoviewer/> Offers maps of maritime traffic density generated from AIS data. Users can access traffic density maps computed at different temporal scales (yearly, seasonal, monthly) and for different vessel categories. These maps help identify main navigation corridors.
- <https://globalfishingwatch.org/> Provides maps of maritime traffic density derived from various data sources, including AIS. The maps can be viewed by different vessel categories and temporal scales.

Once the potential transects have been identified, information on the companies and type of the main vessel types (e.g., cargos, ferries, cruise ships) navigating in the area can be found in the same websites.

1.2 Platform Selection

The approach makes use of regular commercial vessels (e.g., ferries and cargos) that follow consistent routes across the study area. Depending on the identified routes, slightly different types of vessels can be present in the area. When assessing potential ferries to be used for monitoring, the following features should be considered:

- *Height of observation point:* Prefer the highest accessible location on the vessel suitable for Marine Mammal Observers (MMOs), typically command bridge at the bow of the ship, considering agreements with the vessel operator.
- *Cruise speed:* Ensure that speed allows effective observation and data collection (exclude fast ferries travelling >30kts).
- *Engine and machinery layout:* consider vessels safely supporting trained observers and, if applicable, staff and small sampling equipment for complementary techniques requiring water samples (e.g., eDNA, Stable Isotope Analysis).

Important: These features should not exclude vessels; they are primarily relevant for post-collection data analysis and interpretation. It is always valuable to explore different types of monitoring platforms to maximize coverage and sampling opportunities.

1.3 Agreements with company

Before embarking researchers onboard, the following points should be clarified, discussed, and agreed upon with the company:

- *Role onboard:* depending on company policy, researchers may be embarked either as passengers (with a named ticket) or as crew members (listed in the official crew or additional crew list). This distinction must be clarified for both cost and insurance purposes.
- *Costs:* costs are generally involved only when researchers are embarked with a named ticket. The company may cover the full or partial ticket cost. Additional potential expenses include meals and accommodation onboard, as well as harbour taxes. Clarify these details in advance to ensure an accurate budget estimate for the monitoring campaigns.
- *Insurance:* some companies may require additional insurance coverage, especially if researchers are embarked as crew members. Ensure that your institution's insurance covers researchers, interns, students, and volunteers as needed.
- *Placement onboard:* while the highest point of the ship in the common bridge is the ideal location, it might not always be available. Ensure that the company communicates with the captain and officers on duty to explain the role of the researchers onboard and their requirements.

2. Data collection

2.1 Objectives

The monitoring program is designed as a multi-target, multi-taxon survey (Table 2.1). It should be conducted synoptically, with all targets surveyed together to maximize the integrated, multidisciplinary effort. This synoptic approach must, however, consider the specific operational limits and environmental constraints of each protocol. Visual surveys for cetaceans, sea turtles, other species, and maritime traffic are always conducted jointly. When litter monitoring is included, it is carried out together with these surveys. Likewise, when eDNA or SIA activities are added, they are performed simultaneously as well with additional teams. The following table summarizes the main objectives and key parameters associated with each specific target.

Tab 2.1 - Summary of the approaches, target and key parameters monitored

| Technique | Target | Purpose | Key Parameters |
|-----------|--|---|--|
| Visual | Marine Megafauna (Cetaceans, Sea Turtles, Other Macrofauna) | Assess spatial distribution, abundance/density, and habitat use at a large scale. Provide information on animal's behaviour also in relation to the ship and frequency and dynamic of Near Miss and collision events. | Species identification, GPS position, group size, behaviour, swim direction, response to vessel, collision/near miss events. |
| Visual | Maritime Traffic | Provide real-time information on distribution, abundance, and characteristics of maritime traffic; investigate relationship with megafauna sightings. | Vessel type, size class (<5 m, 5-20 m, >20 m), distance (<2 nm / >2 nm), frequency. |
| Visual | Floating Marine Macro Litter (FMML) | Evaluate spatial distribution, quantity, composition, sources, and pathways. | Item size (>20 cm), material, buoyancy, source, category, GPS position. |
| eDNA | Whole Biological Community (Cetaceans, Sea Turtles, Fish, Invertebrates) | Detect the species that characterize the sampled marine area, even during nocturnal hours, subsequently allowing for the reconstruction of local food webs and the identification of keystone species. | Trophic relationships, diel variation in biodiversity composition, detection of elusive species. |
| SIA | Suspended Particulate Organic Matter (POM) | Understand marine food web structure, ecosystem functioning, and trophic dynamics; create spatial maps of isotopic values ("isoscares") to reveal information on primary production and nutrient availability. | $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, C/N ratios |

2.2 Visual monitoring

This section provides clear, step-by-step technical instructions for the systematic visual monitoring of marine megafauna (CEPTU and other macrofauna) and key anthropogenic pressures, specifically maritime traffic (including near-collision events) and floating marine macro litter (FMML), along fixed routes using large vessels such as ferries or cargo ships as observation platforms. This methodology enables sustainable, long-term monitoring of extensive offshore areas and, through a harmonized data collection approach, ensures the production of comparable, high-quality data across different geographical and spatial scales. The instructions presented here are based on the methodological framework developed within the FLT MED NET and further refined by the LIFE project Conceptu Maris, also networking with the IMPEL project Marine Transborder Transect-MTT).

2.2.1 Sampling design

The *spatial sampling design* is based on fixed transects along regular commercial vessel routes, enabling repeated, systematic monitoring over time. Transects are defined to capture key environmental and anthropogenic gradients (see Paragraph 1.1 *Identification and selection of fixed transect routes for sampling*). To validate representativeness, the sampling coverage should be evaluated against the environmental gradients of the study area to ensure that the range of key environmental variables are adequately sampled. The choice of transect routes should be adjusted if important habitats or gradients are underrepresented, guaranteeing that the monitoring network provides robust, representative, and reproducible data suitable for ecological assessments and long-term trend analysis.

Temporal coverage and frequency of survey: surveys should be conducted year-round (depending on vessel schedule), with repeated monitoring of the same transects, to capture seasonal and interannual variability and enable robust temporal trend analysis (Table 2.2). Although the number of surveys may vary by route, five surveys per season are recommended to guarantee adequate seasonal representation and support robust statistical analyses, with a minimum of one survey per month. For FMML monitoring, a minimum coverage of 25 km² per season is required, which should be increased to 31–40 km² in areas of low litter density. For the purposes of the protocol, a “season” is defined as a complete three-month period (e.g., Summer: from the beginning of July to the end of September - “meteorological season”).

Sampling method: Several protocols are implemented in parallel, listed below and detailed in the next paragraphs (Figure 2.1, Table 2.2).

1. Standard line transect in passing mode

- a. **Cetaceans, sea turtles and other megafauna records** (including two birds species of conservation interest *C.diomedea* and *P.yelkouan*) are collected using the standard line transect method in “passing mode” (continuous search effort, with schools or animals not being approached) scanning the entire sea surface from the vessel to the horizon;

2. Point count transect

- a. **Maritime traffic *in situ* data** are collected following a ‘random/control’ approach, counting all vessels by type within a systematic scan sampling of 360° of the horizon at regular random points, on an hourly basis, along the transect (in absence of sightings) and at each cetacean sighting (point-count transect). Reports of collision or near collision events are also standardized.

3. Fixed strip-width transect

- a. **Floating Marine Macro Litter (FMML)** data are collected using the fixed strip-width transect method with a 50 m strip width, based on the MedSealitter and JRC protocol;
- b. **Sea Turtles records** are also collected under the fixed strip-width transect monitoring of Floating Marine Macro Litter (see below) to consider sea turtle detectability.

Tab 2.2 - Sampling method and temporal coverage and frequency of the survey

| Target | Sampling method | Minimum Requirement per route | Recommended Value per route | Notes |
|--|-------------------------------------|--------------------------------|--|--|
| Marine Megafauna (CEPTU, Other Macrofauna) | Fixed line transect in passing mode | At least 1 survey per month | 5 per season | Ensure seasonal representativeness and statistical robustness. |
| Maritime traffic | Point count transect | Together with Marine megafauna | | |
| FMML | Fixed strip-width transect (50m) | 25 km ² per season | Increase to 31–40 km ² in low-density areas | Coverage required to detect seasonal variability. |

Season Definition : 3-month period. Example: July–September. Applies to all monitoring protocols

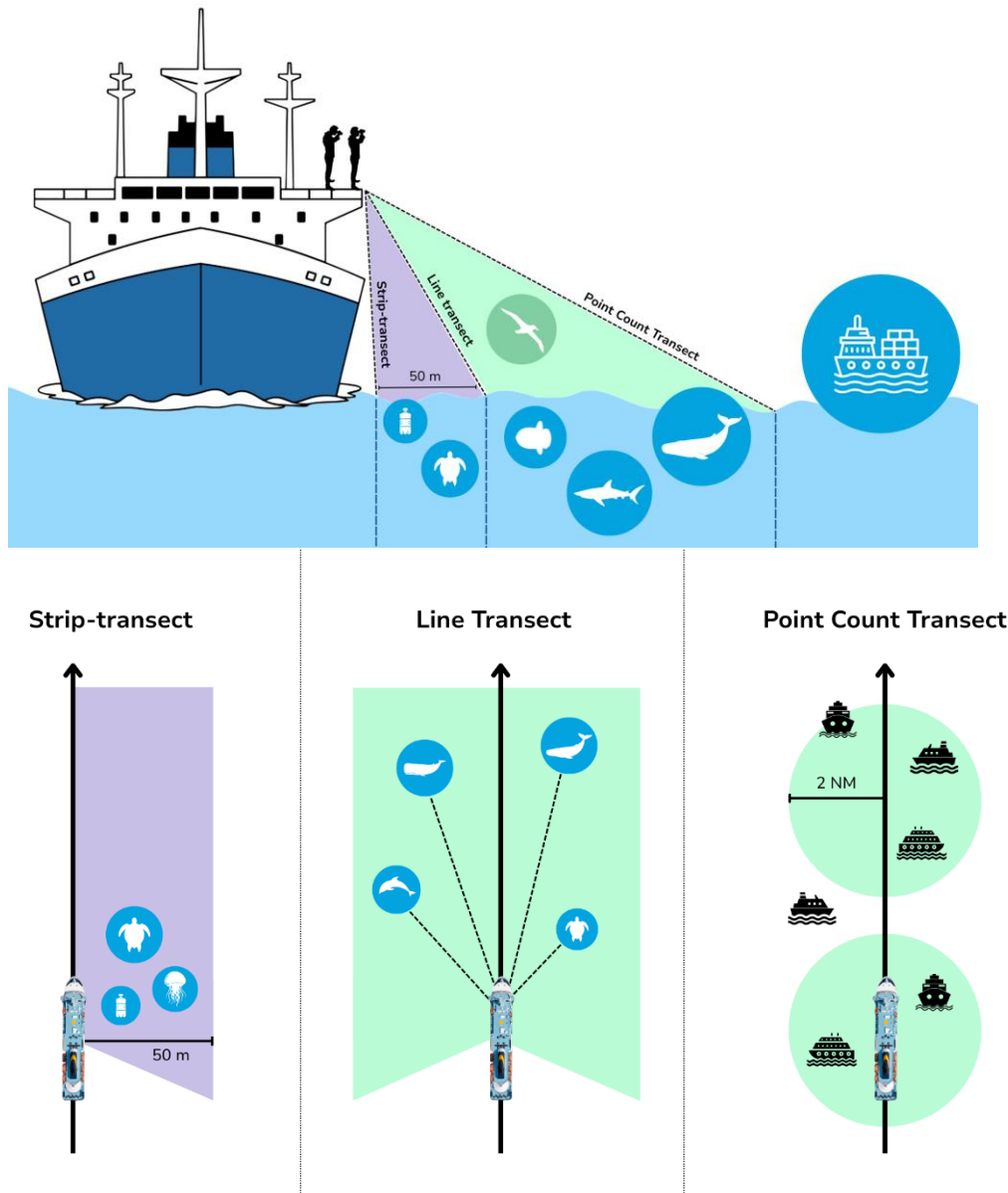


Fig 2.1 - Survey design for macrofauna, maritime traffic and floating marine macro litter

The survey design based on representative fixed transect year-round provides robust, reproducible data for assessing species distribution, abundance, and exposure to anthropogenic pressures.

2.2.2 Team

The number of observers required for a multi-target, multi-taxon survey may vary depending on factors such as route duration, vessel characteristics, and the specific monitoring targets. Ideally, observer teams conducting marine macrofauna and FMML monitoring should consist of a minimum of four Dedicated Observers (DOs). Observers are classified into three experience levels, senior, experienced, and in training (Table 2.3), based on a combination of personal aptitude, prior fieldwork experience (particularly on ferries or large vessels), and proficiency in species identification under diverse environmental conditions. Each team must include at least one Senior Observer, who is responsible for assessing and validating the experience level of the other team members, and no more than one observer in training.

Tab 2.3 - Observer experience

| Dedicated Observer (DO) | Profile | Role |
|-------------------------|--|--|
| Senior | Extensive field experience (typically multiple years) in marine megafauna monitoring, distance sampling, and standardized protocols. Proficiency in species identification, data recording, and use of data. Capable of training and supervising other team members. Responsible for data quality assurance and decision-making. Experience coordinating with ferry crews and managing staff relations and operational issues. | Leads monitoring teams. Ensures correct application of protocols. Validates observations and manages complex or uncertain identifications. Ensure respectful relationships with the crew. |
| Experienced | Solid background in marine surveys with enough experience to work independently. Reliable in species identification and data recording. Requires limited oversight from senior personnel. Able to coordinate with ferry crews and manage staff relations and operational issues. | Conducts routine transect observations. Supports data collection, navigation, and equipment handling. Assists seniors in training and field logistics. |
| In training | New or early-career participant with limited or no previous marine monitoring experience. Has received introductory training in protocols, species identification, and safety procedures but still requires guidance. | Participates under direct supervision of senior or experienced personnel. Focuses on learning identification skills, data entry, and field procedures. Does not perform independent critical tasks (e.g., confirming uncertain sightings). |

During surveys, two DOs are positioned on each side of the command deck to monitor cetaceans and other megafauna using the standard line transect method, while a third observer takes note of all the data (secretary). A fourth observer, with specific technical training in addition to that required for megafauna monitoring, implements the fixed strip-width protocol to monitor FMML and sea turtles on one side of the ferry. The four observers rotate duty each hour, ensuring sustained attention and maintaining high data quality throughout the survey. This structured team composition enhances consistency, reduces observational bias, and supports standardized, high-quality data collection across different marine regions. **Note:** Multidisciplinary monitoring implementing eDNA and SIA requires additional personnel beyond visual observers (see 2.3.2).

2.2.3 Equipment

The following table summarises the minimum set of tools required to conduct the monitoring activities (Table 2.4).

Tab 2.4 – Equipment

| Category | Tool | Target | Purpose |
|-----------------------|---|--------------------------------------|---|
| Data Recording | GPS (handheld) / Tablet | Megafauna/FMML/ Maritime Traffic | To record the transect track, mark the position of sightings (waypoints), and record effort data. The same device is used to record information on fauna and FMML. |
| | Data Sheets/Clipboards/APP | Megafauna/FMML/ Maritime Traffic | To record observation characteristics. Dedicated sheets are needed for CEPTU/Other Sightings, FMML, Weather Conditions, and Maritime Traffic. |
| Detection/ID | Binoculars (7x50 magnification) | Megafauna/ FMML/ Maritime Traffic | Binoculars with reticle rangefinders are recommended. Binoculars are used to periodically scan the horizon and to confirm species and group size. |
| | Species Identification Sheets/Photo-catalogues | Megafauna | To support species identification. |
| | Litter photo catalogue | FMML | To help with litter items identification. |
| | Photographic Cameras | Megafauna/ FMML/ Marine Traffic | Useful to confirm sightings and assess species/group size <i>a posteriori</i> . It is highly recommended to use a zoom lens with a maximum focal length of no less than 300 mm. |
| Measurement | Goniometer / Compass | Megafauna | Used to measure the angle between the sighted target and the ship's bow. |
| | Clinometer | Megafauna/ FMML | Used to measure the angle for distance calculation and to fix the width of the FMML monitoring strip. |
| | Rangefinder /Stick/ Measuring Stick | Megafauna/ FMML/ Marine Traffic | Used in absence of binoculars with reticle rangefinders to measure radial distance to the target immediately. |
| Communication | Walkie talkie | Megafauna | It is important to maintain communication between the observers positioned on opposite sides of the command bridge and the secretary. |

2.2.4 Getting Ready for Monitoring Activities

Before starting a multi-target, multi-taxa monitoring survey, thorough preparation is essential to ensure efficiency and data quality. The Senior Dedicated Observer must:

- prepare and check all necessary materials (listed above) before departure,
- secure all necessary documentation, including identification documents or a passport, especially for international travel,
- ensure that GPS batteries are fully charged and spare batteries/charger are available. Synchronize all clocks (GPS, phone, cameras). Set GPS to continuously record track data at maximum possible resolution (e.g., 15-30 seconds for a 10-hour activity). Set coordinates to “decimal degree” format with more than 3 decimal values, DATUM WGS84,
- if binoculars without a graduated grid are used, ensure that each DO has a personal Range Stick tailored to their eye height, arm length, and the observation platform height,
- conduct a quick briefing before departure to test team familiarity with datasheets, codes, and recording procedures. Organize the rotation schedule for DO positions.

Important: Team members must pack appropriate clothing, and sufficient personal food and water supplies (if not provided by the company onboard) for the entire trip considering limited storage and the absence of refrigeration in cabins.

Important: at the embarkment the team must go to the reception to 1) inform them they are onboard, 2) ask permission and indications to reach the command deck, and 3) thank the whole crew and inform them about disembarking.

2.2.5 Procedures

Metadata Recording (Marine Megafauna/Maritime Traffic monitoring & FMML monitoring):

Before or upon arrival at the observation point, typically the command deck, the Senior Observer must ensure that all required metadata are collected (Table 2.5) and that the relevant sections and fields on the datasheets (D1→D6, Annex I, and also available in the [ISPRA folder](#) "PRJ_FLT_Med Net") on the mobile app are correctly completed.

Tab 2.5 Metadata to be compiled prior to the start of all monitoring activities - Marine Megafauna/Maritime Traffic monitoring & FMML monitoring

| Data | Explanation |
|--|---|
| Transect code | Unique identifier for each survey (ID_survey or COD_Transect N). It must include the transect code (four letters, unique for each route) and the survey number (progressive for each fixed transect). Different ID_survey must be used for the outward and return journeys. If either the outward or return journey spans several days in the same direction, the ID_survey should be subdivided into sections (COD_effort = ID_survey + a, b, c, etc.) corresponding to each day (see Box 3.1) |
| Date | Date of survey |
| Ship name | Name of the ship |
| Observer names | Provide the list of the observers' names and specify the senior dedicated observer |
| Speed | Ship speed in knots (Kn) as indicated on the command screens, usually below the heading |
| Sea state | Beaufort sea state (D7) recorded |
| Cloud cover | Percentage of estimated cloud covering |
| Note: Any time that a new vessel is used to perform the survey, the height of the observation platform must be recorded and communicated to ISPRA coordinators. | |

Weather data

Weather data need to be compiled at the beginning of each survey and in case of any variation in weather conditions (e.g., sea state, visibility, wind, precipitation), according to the datasheet D3 for megafauna monitoring (Protocol 2.2.5.1) and datasheet D5 for FMML (Protocol 2.2.5.2). When the two monitoring activities are conducted simultaneously, it is essential that weather information is consistently recorded across both datasheets.

- *In case of variation*, mark the GPS position using the code MET and note the time and details (sea state, wind direction, visibility, cloud cover, etc.).
- *When visibility or sea state deteriorates* over the limits of ≤ Beaufort 3, and ≤ Beaufort 2 for FMML, stop all monitoring activities and *Mark* the GPS position with STOP and *Insert* the GPS code in the weather datasheet.
- *When conditions improve* and monitoring can restart: *Mark* the GPS position with START, *Insert* the GPS code in all related datasheets, and *Record* updated weather conditions before resuming observations.

Important: Animals sighted during a monitoring pause can be recorded but must be reported as "off effort" in the appropriate datasheet.

2.2.5.1 Protocol for Marine Megafauna and Maritime Traffic monitoring from large vessels

Standard line transect in passing mode for megafauna and point count transect for traffic

STANDARDIZED DATA SHEET: D1 – Cetaceans; D2 – Sea turtles and other sightings; D3 – Weather conditions; D4 – Maritime traffic, or a mobile app with all the required fields.

HUMAN RESOURCES: Minimum 

DURATION/TIMING OF OBSERVATION: Marine megafauna monitoring should be conducted continuously for the whole duration of the transect. Observers should rotate positions every 60-90 minutes, when monitoring is performed in the open air.

LIMITS: only when the sea state on the Beaufort scale is equal or less than 3.

LEVEL OF EXPERIENCE OF THE OBSERVERS: At least one Senior, maximum one in training for each survey.

a. Preparation

Following the instructions of the Senior DO, team members must:

- Position themselves at the two observation points located on opposite sides of the ship;
- Check and organize all required equipment (binoculars, range stick, datasheets, pencils, goniometer, etc.);
- Prepare the GPS/tablet device for recording all waypoints and keep it on the same side of the deck throughout the survey. If FMML monitoring is conducted, place the GPS on the command deck side used for litter monitoring and keep it there for the entire activity;
- Turn on the GPS and wait for the signal to be acquired. To speed up the process, it may help to go outside so the GPS can connect to satellites under a clear sky;
- Position one goniometer on each side, aligning the 0° mark with the ship's heading;
- With assistance from the command deck crew, observers must become familiar with reading the vessel's instruments to obtain information on heading, compass, speed, and true wind.

b. Beginning of monitoring effort

- Start recording a new track in Tablet/App or check that the GPS is set to automatically record the track at optimal resolution of 1 point every 10/20 seconds;
- Mark on the GPS/App the location at the beginning of the "on-effort" period;
- Record all data on weather conditions, vessel speed, and route associated with the beginning GPS code (Table 2.6, use D3 – Weather Conditions for reference);
- Set an alarm to be notified when to randomly collect traffic data in the absence of cetaceans (e.g., every 60 minutes);
- Record the first data on maritime traffic following the **Procedure for Maritime Traffic monitoring (point h)**.

Tab 2.6 - Data on weather conditions, vessel speed, and route associated with monitoring effort

| Data | Explanation |
|--|---|
| GPS code | The GPS alphanumeric code which is automatically generated by pressing MARK on the instrument to acquire a waypoint. |
| Time (local) | Local time of the port of departure. |
| Effort | Specify BEG at the beginning of the effort, STOP when the activity is temporarily suspended, START when the monitoring can be restarted after pausing, MET if any change in weather conditions occurs, and END when the monitoring activity ends (see also Table 3.1). |
| Sea State | Indicate the level of the Beaufort scale based on true wind speeds and sea state appearance (waves height, swell, etc., see D7 for reference). |
| True wind direction | The true wind direction is determined as where the wind is blowing from and can be read on the displays situated on the command bridge. Report it as a cardinal direction (e.g. SW, WSW, etc). |
| wind speed | Wind speed expressed in knots and can be read on the displays situated on the command bridge. |
| Rain | Indicate, in case of reduced visibility due to atmospheric precipitation, the type of rain: Mist, Fine, Drizzle . In case of heavy rain, monitoring activities should be interrupted. |
| Visibility | Report Optimus, Good, Mean, Scarce based on the visibility level. |
| Cloud cover | Percentage of estimated cloud covering. |
| Vessel route | Specify the ship's heading ; it can be read on the command screens, where it is usually indicated as HDG. Report the exact number without rounding. |
| Vessel speed | Ship speed in knots (Kn) is indicated as SPD on the command screens, usually below the heading. |
| NOTE: Data on weather conditions are ALWAYS collected at the beginning and end of monitoring, or when it is paused or restarted (STOP and START) for different reasons (i.e., rain, break). Furthermore, every change in weather conditions during the monitoring must be reported by marking the GPS location (code MET) and reporting related-information on the sheet. | |

c. What to do if weather conditions change during monitoring

Any variation in weather conditions (e.g., sea state, visibility, wind, precipitation) must be documented in the Weather Conditions datasheet (D3). Mark the GPS position using the code MET and note the time and details (sea state, wind direction, visibility, cloud cover, etc.).

- *When visibility or sea state deteriorates* over the limit of *Beaufort 3*, stop all monitoring activities, *Mark* the GPS position with STOP and *Insert* the GPS code in the weather datasheet.
- *When conditions improve* and monitoring can restart *Mark* the GPS position with START, *Insert* the GPS code in all related datasheets, and *Record* updated weather conditions before resuming observations.

Important: Animals sighted during a monitoring pause can be recorded but must be reported as “off effort” in the appropriate datasheet.

d. Observation Area

Each of the two observers positioned on the opposite sides of the vessel must continuously scan, with the naked eye, a 130° arc area ahead of the ship, ensuring scanning all the area from the ship to the horizon. The area behind the vessel is scanned only occasionally to avoid recounting sightings. Binoculars may be used to confirm a sighting, the species or occasionally to scan the area.

e. What to do in case of sightings

The observer who spots the animal immediately alerts the DO positioned on the GPS side to mark a waypoint. *Based on the position of the first animal sighted (= at initial detection)*, the dedicated observer must record:

- the *radial distance* measured using a range stick, binoculars with a reticle rangefinder, a ruler to measure the distance in centimetres or a clinometer to measure in grades, which can be successively converted into real distance in meters based on the DO's eye height above the sea level.
- the *angle between the sighting and the bow of the ferry* measured using the goniometer or, if the internal binocular compass is available, as the difference between the ship's heading and the sighting direction (Figure 2.2).

Important: Angle of sighting measurements must refer to the bow of the ferry, not the observer's position. Angle 0° applies only to animals sighted directly in front of the ship's bow. Provide the best estimate of the detection angle; do not always round to the nearest 5°; the swimming direction, instead, must refer to the geographic North and be recorded in cardinal direction.

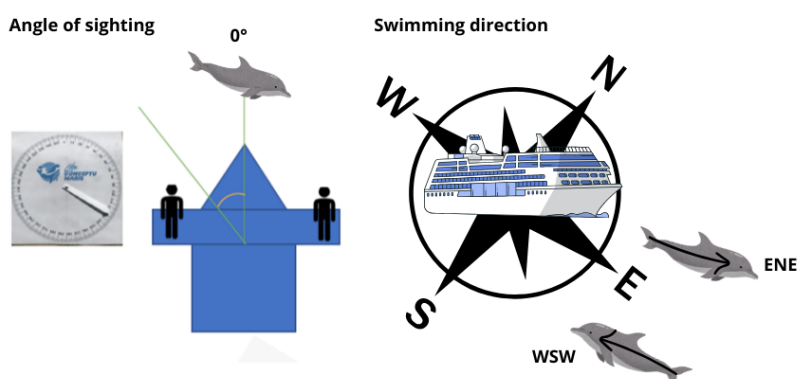


Fig 2.2 Angle of sightings measured with respect to the bow of the ferry (left), and swimming direction measured as the direction in which the animal is moving (right).

f. Cetaceans

The observer who first sighted the cetacean keeps watching it and, with the help of the secretary, collects all required information as detailed in Table 2.7. The use of a camera is strongly recommended to assist in species identification and group size estimation afterwards.

What to do in case of:

- *Mixed species.* If mixed species are sighted in a single group record it as a single sighting on the GPS. On the datasheet, report information separately for each species (using the same GPS code), specifying in the notes that it is an association of species.
- *Multiple groups.* If multiple groups of the same species are simultaneously recorded over a large area record it as a single sighting if assessed to be “sub-groups” (according to the definition of group), indicating the angle/distance of the first sub-group.
- *Collision or near collision.* Collect data on possible events of collision or near collision. A “near collision” is defined as a cetacean sighted at a minimum distance of 50 m in front of the bow and 25 m on the side, unaware of the approaching ship. Do not consider species that typically approach vessels (e.g., bow-riding dolphins). Record all aspects of the event before, during, and after, including behaviour, reactions, speed, etc., and measure the distance at the Closest Point of Approach (CPA) to the ferry.
- *Maritime traffic.* At each sighting, quantify the number of detectable vessels by naked eye following the procedure for *Maritime Traffic Monitoring* (point h).

Tab 2.7 - Type of data collected during sightings of cetaceans and sea turtles

| Data | Cetaceans | Sea turtles |
|--|---|---|
| GPS code | Progressive number on the GPS | |
| Local time | Local time | |
| Side of sighting | Left or Right | |
| Observer's name | Name of first observer | |
| Species | <p>Reported as the initials of the scientific name (e.g., <i>Sc</i>, <i>Tt</i>, etc.). Not identified species are registered using:</p> <p>US (Unidentified Species) followed by the indication of size at the best possible level:</p> <ul style="list-style-type: none"> • S=Small patterned "dolphin" species, • M=Medium cetacean species (LF large fin, SF small fin) • L=Large cetacean species (DD distinct dorsal, ID indistinct dorsal) | <p>Reported as the initials of the scientific name (e.g., <i>Cc</i>, <i>Cm</i>, etc.). For sea turtles alone if species identification cannot be confirmed, register the sighting as: Hard-shelled sea turtle, or Soft-shelled sea turtle.</p> |
| Group size | <p>Exact number of individuals or if it cannot be determined, record: Minimum, Maximum, Best estimate. If possible, compare the estimate made by different observers</p> | |
| N. of juveniles | <p>If juveniles are sighted, indicate the number; otherwise, leave it empty. Juveniles are primarily defined as individuals observed in close association with an adult and estimated to measure approximately one-third to one-half of the adult body length.</p> | |
| Linear distance from the vessel | <p>Distance between vessel and first location of first sighted animal (= distance at initial)</p> | |
| Angle between sighting and the vessel bow | <p>Angle between the bow of the vessel and the first location of first sighted animal</p> | |
| Swimming direction | <p>Indicate the angle of swim direction in cardinal directions with respect to the NORTH.</p> | |
| Response to the vessel | <p>Choose among the following options:</p> <ul style="list-style-type: none"> • Approaching, when the animal actively approaches the vessel. • Escape, when it moves away or quickly submerges. • Indifferent, when it appears unaware and maintains the same behaviour. | <p>Choose among the following options:</p> <ul style="list-style-type: none"> • Escape, when it moves away or quickly submerges. • Indifferent, when it appears unaware and maintains the same behaviour. |
| Behaviour | <p>Choose among the following behaviour: Traveling, Resting, Playing, Feeding Wild (i.e in the open water), Feeding Net (i.e. close to fishing gears), Mating, Unknown.</p> <p>In addition to the general behaviour, give as many details as possible: swim speed (slow, normal, fast, etc.), progress (straight, irregular, zig-zag) and whether the animal is maintaining the same direction. In the presence of boats at a distance <2Nm, take notes of any behaviour you can ascribe in response to these boats.</p> | <p>Choose among the following behaviour: Swimming, Resting, Breathing, Mating, Feeding, Unknown.</p> |
| Collision | <p>Choose between:</p> <ul style="list-style-type: none"> • Collision- physical contact occurred between the vessel and the cetacean. • Near collision-a cetacean sighted at a minimum distance of 50 m in front of the bow or 25 m on the side, unaware of the approaching ship. Do not consider species that typically approach vessels (e.g., bow-riding dolphins). | <p>Choose between:</p> <ul style="list-style-type: none"> • Likely collision (A turtle disappears under the bow and does not re-emerge behind the vessel) • Near collision (A turtle is caught in the vessel's vortex but remains floating on the side) |

| | | |
|---------------------------------------|---|--|
| Near collision additional data | <p>Additional data on NME should include: 1) the number of animals observed within the limits of NME (i.e., 25m at either side of the ferry or 50m in front). 2) behaviours (e.g., travel, rest/stationary, feeding, socializing, undetermined) occurring at first detection of animals, the term “emerging” (meaning: not seen before, or not monitored and suddenly pop-up at the surface in the vicinity of the vessel). 3) Additional information regarding the NME: a. If the animal has been re-sighted or not after the NME. B. If re-sighted – the animal has been seen injured (ship strike) or not injured and seems to behave identically (recovering) or differently than usual. c. Add comments to describe the whole event as it happens in detail from first detection to after the NME, to allow better post analysis and understanding.</p> <p>Data on Closest Point of Approach (CPA): 1) Distance and angle of observation at CPA. 2) Animal's behaviour. 3) Animal's reaction to the ferry (e.g., dive straight, turn abruptly, swim away as change of direction and/or accelerate, none/indifferent as no change, undetermined). 4) Space for comments in order to inform about the fact that the animal has been in contact with the hull or not, as the event might be a ship strike or not.</p> | |
| Interaction with litter | Indicate if the sighted animal is entrapped/entangled in marine debris/FAD or if there is any interaction with anthropogenic factors. | |
| Life stage | #For cetaceans compile 'N. of juveniles'# | <p><u>Only for <i>Caretta caretta</i> when the sighting occurs close to the vessel and the life stage can be reliably determined; otherwise, do not record.</u></p> |
| | | <p>Early juvenile (carapace with irregular and jagged margins, dark brown or reddish-brown, dorsal plates with spines (jagged crest), reduced head size, carapace size approximately < 40 cm).</p> <p>Late juvenile (jagged crest reduced or absent, carapace size between approximately 40 and 70 cm).</p> <p>Adult (large head size and well-developed musculature, approximately > 70 cm carapace size).</p> |
| Sex | | For large, adult sea turtles, indicate “male” only when a clearly visible, prominent tail extends well beyond the carapace. In all other cases it is unknown. |

g. Sea Turtles, Other Megafauna and Sea birds

The observer who first sights a sea turtle or another species of interest must *keep watching* it and, with the help of the secretary, collect all required information as detailed in Table 2.7 and Table 2.8. The use of a *camera* is strongly recommended to assist in species identification and group size estimation afterwards. For sea turtles, observers must be specifically trained to reliably assess size classes/life stages in the case of close sightings. Preferably, they are the same personnel rotating across marine litter monitoring, thus already familiar with measurement and size estimation at sea. Training includes calibration exercises using graduated rulers and basic trigonometric calculations, as well as reference objects of known dimensions. For sea turtles, collect data on possible events

of likely or near collision, where: *Likely collision*, is when a turtle disappears under the bow and does not re-emerge behind the vessel; *Near collision*, when turtle is caught in the vessel's vortex but remains floating on the side.

Passenger birds, using the vessel as a resting platform, should be possibly noted in the monitoring notes, using photographic material to support species identification.

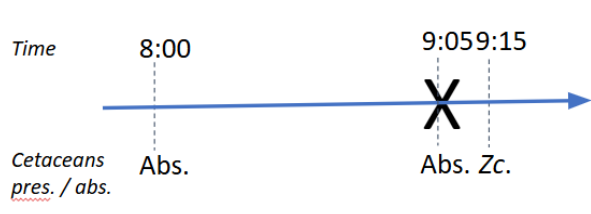
Tab 2.8 - Type of data collected during sightings of large fishes, jellyfish and sea birds

| Data | Osteichthyes | Chondrichthyes | Jellyfish | Shearwater (Yelkouan/Scopoli's) |
|--|---|----------------|-----------|---------------------------------|
| GPS code | Progressive number on the GPS | | | |
| Local time | Local time | | | |
| Side of sighting | Left or Right | | | |
| Observer's name | Name of first observer | | | |
| Species | Indicate the name of the identified species, or at least the name of the order. In case of not identifiable species, use N.D. | | | |
| Group size | For species usually observed in large groups, those are categorised into: Small (<10 individuals), Medium (10-100), Large (>100) | | | |
| If possible add information on: N. of juveniles, Linear distance from the vessel, Angle between sighting and the vessel bow, Swimming direction, Response to the vessel, Behaviour, Collision, Life stage, Interaction with litter (entanglement, sign of ingestion, etc). | | | | |

h. Maritime traffic monitoring

Scan sampling of maritime traffic is carried out by counting all vessels visible by eyesight all around the ferry up to the horizon. Vessels are divided based on their relative distance from the observation point into: *Close* (<2 nm from the observation platform), *Far* (>2 nm from the observation platform and up to the Horizon (the hull of the vessel has to be entirely visible, if not it is beyond the Horizon)) (Figure 2.1). Within these two categories vessels are then classified based on their size as: *Small* (<5m), *Medium* (between 5m and 20m, distinguished in: *Motor*, *Sailing*, *Fishing*), *Big* (>20m, such as cargos, tankers, passenger ships).

The scan for maritime traffic detection is performed: 1) *at the beginning and end of the survey effort*, 2) *each time a cetacean sighting occurs (record in presence of cetaceans)*, and 3) *at approximately every hour* (minimum distance of 10 NM or timelapse of 45 minutes) throughout the transects when animals are not sighted (**random record in absence of cetaceans**). **Important:** To avoid replication, a minimum interval of around 15 min is defined between presence/random records to avoid sampling twice the same vessels (*below 15 min rule*). In case of two very close consecutive cetacean sightings, information on maritime traffic must be collected only if detectable change in the composition of vessels occurred; in case of two close scan involving one rarer species (e.g., pilot, sperm or goose beaked whales, Risso's and Short-beaked Common dolphin), the traffic data to be kept is the one collected concurrently with that sighting, while the preview could be deleted afterwards.



Maritime traffic data collection sheet

| Transect CODE | | Date | Ship name | | | | | | | |
|---------------|------------------------------|--------------|-----------|--------------|---------|--|-------|--------|--------|--|
| GPS CODE | Cetacean Presence Absence | Time (local) | < 2 NM | | | | | > 2 NM | | |
| | | | Small | Medium | | | Big | Small | Med | |
| | | | < 5m | 5m < X < 20m | | | > 20m | < 5m | 5m < X | |
| | | | Motor | Sailing | Fishing | | | Motor | Sa | |
| X | Absence | 08:00 | X | | | | | | | |
| | Absence | 09:05 | | X | | | | | X | |
| | Presence | 09:15 | | X | | | | | | |

Fig 2.3 Maritime traffic scan: hourly random record in absence of cetaceans and 'below 15 minutes rule'

I. End of monitoring effort

At the end of the transect, or earlier in case of bad weather conditions, the Senior observer must close the monitoring activity by following these steps:

- Mark the GPS location of “End effort”;
- Stop the GPS track recording using the dedicated GPS device and save the track.

Before Landing, the team must:

- Review the datasheets carefully and check for missing or incomplete data;
- Verify that all equipment and tools have been recovered;
- Exchange photo material.

2.2.5.1 Protocol for FMML monitoring from large vessels

Fixed strip-width

STANDARDIZED DATA SHEET: D5 – Floating marine macro litter, D6 – Sea turtles and other sightings-on effort litter

HUMAN RESOURCES: Minimum 

TARGET: FMML items above 20 cm in size (to avoid missing smaller size that could not be detected).

DURATION/TIMING OF OBSERVATION: FMML monitoring should be performed constantly for the whole duration of the transect. Observers should rotate positions every 60 minutes.

LIMITS: only when the sea state on the Beaufort scale is equal or less than 2

LEVEL OF EXPERIENCE OF THE OBSERVERS: At least one senior DO

a. Preparation (FMML protocol)

Before the start of FMML monitoring, in addition to the general metadata specified in Table 2.5, the Observer Dedicated to the FMML completes the additional metadata specific to FMML activities (Table 2.9).

Tab 2.9 Additional metadata specific for FMML monitoring.

| Data | Explanation |
|-------------------------------------|--|
| Minimum litter size class | Minimum size class to be detected (20 -30 cm). |
| Strip width | Width in metres of the monitoring strip (50 m). |
| Height of observer eye | Height of the observer eye in metres above sea surface level (deck + observer height). |
| Position of observation | Report if the monitoring will be conducted on the side of the vessel (left or right) or on the front. |
| For observation on the side | Report the angle (in degrees) read at the clinometer to define the limit of the 50 m strip at the window. |
| For observation on the front | If observation is performed at the frontal window, indicate the width of the strip in cm and the distance in cm between the eyes of the observer and the window. |

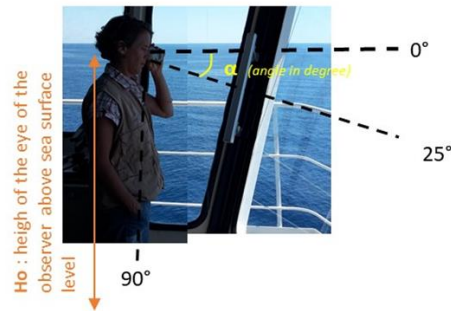
Following the instructions of the Senior DO, team members must:

- Identify the observation point on the vessel that offers the best visibility and the least sun glare on the sea surface;
- Prepare the GPS/Tablet device that will be used to record all waypoints during the survey. Note that this GPS will also be used for marine megafauna monitoring;
- Measure the width of the fixed observational strip (50 m) on the vessel window where FMML monitoring will be conducted (see Box 1).

BOX 1. How to measure the width and limit of the strip on the vessel window and marine litter size

For observation on the side:

The measurement is done using a clinometer and scotch/tape. Knowing the height of the eye of the observer above sea level H (deck + observer height) and having fixed the width of the strip (50 m), the angle between the monitored strip and the Horizon is obtained through trigonometry, applying one of the two the formula reported below and the value to be read at the clinometer.



$$\text{Angle (radians)} = \text{arccot} \frac{\text{width of the strip (m)}}{H \text{ (m)}}$$

$$\text{Angle (radians)} = \text{arctan} \frac{H \text{ (m)}}{\text{width of the strip (m)}}$$

The measured values need very often to be converted from radians to degrees before reading the clinometer. The upper limit of the strip (ex. here is 25°) is then marked with a scotch on the window's glass. Everything observed below the tape limit will be considered "in the strip".

For observation on the front:

The measurement is done using a clinometer, a measuring tape and scotch. Knowing the width of the strip required (50 m), the distance where to put the 2 scotch on the left and right (limit of the angle of natural view which is usually 60° for humans) on the frontal window, is obtained through the formula reported below. Distance between eyes and window is taken at the fixed place where the observer will stand to watch. The place where these measurements were done should also be marked, so the observer stands always at the same location.



$$\text{Distance between scotch (cm)} = \frac{\text{width of the strip (m)} \times \text{distance eye_glass (cm)}}{H \text{ (cm)}}$$

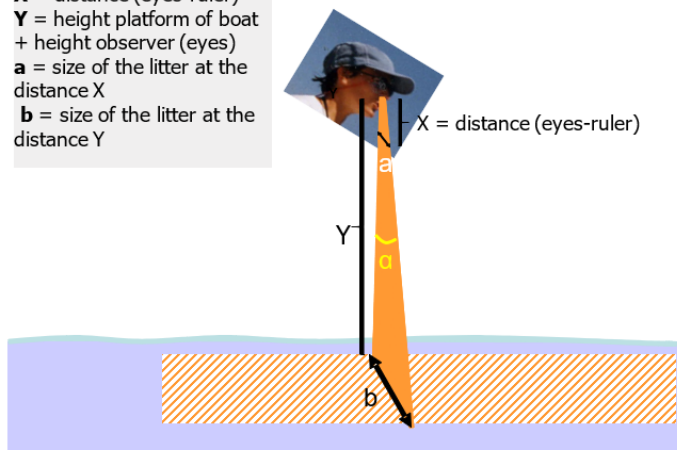
On the left picture: measuring the distance where to put the 2 scotch of the left and right limits (limit of the angle of natural view which is usually 60° for humans). On the right picture: measuring the distance of the eye window. The observer watch position is settled where the measurements were taken.

Marine litter size

The size measurement is done with a transparent ruler and a clinometer. The first step includes fixing a place near the observer where each item will necessarily pass through before passing away. The angle (α) at this place should be measured with the clinometer, so the distance to sea surface can be known. Each marine litter should be measured with the ruler when passing at this place to get the apparent size in cm (a). Then, knowing the distance eye-ruler (X), and the height of the observation Y (deck + observer height), the following formula is applied to get the real size of the object (b). The observer may also refer to standard sizes reported in the marine litter photo-catalogue (ANNEX VIII).

$$\text{Tang} (\alpha) = a/X = b/Y$$

X = distance (eyes-ruler)
Y = height platform of boat
 + height observer (eyes)
a = size of the litter at the
 distance X
b = size of the litter at the
 distance Y



The formula is used for measuring the real size of floating marine litter knowing the apparent size read on the ruler (source: ISPRA).

b. Beginning of monitoring effort (FMML protocol)

- Start recording a new track on the GPS;
- Mark the location on the GPS at the start of the 'on-effort' period (**BEG**). If FMML and macrofauna monitoring begin simultaneously, use the same GPS waypoint to indicate the beginning of effort.

c. Observation Area (FMML protocol)

Observations are conducted with the naked eye, using binoculars only to confirm litter sightings when necessary. Although the observational strip is defined at the side window, the observer should primarily focus forward to maximize sighting time and item detection. The observer should then shift sideways, back to the point used to set up the strip measurement, only to verify whether the object falls within the strip.

d. What to do in case of sightings (FMML protocol)

Each time the observer detects an item larger than 20 cm within the fixed 50 m-wide strip, they must record a waypoint on the GPS. Using a camera or binoculars, if necessary, the observer notices as much required information as possible (Table 2.10). Once the litter item has passed, its position, time, and GPS waypoint are recorded on the designated datasheet along with all item-related details. The observer is required to be familiar with the [Joint List of Litter Categories for marine macrolitter monitoring](#) (Fleet et al., 2021) and trained for the identification of litter size classes (see Box 1 for size measurements and D7 in Annex I for Marine Litter Photo catalogue). Training includes calibration exercises using graduated rulers and basic trigonometric calculations (Box 1), as well as reference objects of known dimensions.

Tab 2.10 - Type of data collected during FMML monitoring

| Data | Explanation |
|---------------------------------|--|
| GPS code | Progressive number of GPS code. |
| Buoyancy | Indicate the buoyancy of the observed items: Positive (+) when the item emerges whole or in part from the surface; negative (-) when it is completely submerged, absent (0) when it is aligned to the surface of the sea. |
| Material | The macro categories of the litter based on its composition: ArtificialPolymerMaterials, GlassCeramic, Proc.wood, PaperCartboard, Metal, TextileClothes, Rubber, Chemical, FoodWaste, NaturalMatter. |
| Use | Indicate the probable source of the item observed and the relative industry sector: Agriculture, Aquaculture, Food, Clothes, Sanitary, Cosmetic, Construction related, smoking related, Fishery related, Recreational related, Vehicle, Hunting, Undefined, Other. For shopping bags, the use is undefined (NN), while for a box of polystyrene it can be related, presumably, to the fishing industry (F). |
| Most common items | Indicate the most common items observed during the sighting- I.e. industrial packaging, shoppers, fish boxes, plastic bottles. |
| Specific item & note | In case of sightings that do not fall into any of the categories listed, the type of object is specified here by referring to the Joint List of Marine Litter Categories (Fleet et al., 2021) . Animal sighting occurring within the strip (e.g., Sea turtles) are also noted here, along with any additional information related to a change in weather conditions, ship speed, the observer (indicating initials of the new one), and the presence of Fishing aggregating devices (FADs) , usually single buoy or floating material) or windrows (for the aggregations of seafoam, seaweeds, plankton and natural debris that appear on the ocean surface) or Sea snot (Marine mucilage). In the case of a patch of litter , observers must also record its beginning and end points and the most common items. |
| Natural Matter | In the presence of organic material at the surface, such as logs or seaweed report in the corresponding column, indicating whether it is from " <i>sea-based sources</i> " (seaweed, carcasses of marine dead animals, etc.), or " <i>land-based sources</i> " (logs, plants, etc.). |
| Size | Specify the size category of the object starting from E=20-30 cm; F=30-50 cm; G=50-100 cm; H=100-200 cm; X>200 cm. In the case of objects < 20 cm, if items are clearly identifiable (i.e., smoking cigarettes, drinking can), they can be reported in the sheet. For some item categories, size can be reported to a standard size (refer to the Online Photo Catalogue of the Joint List of Litter Categories and D8). Measures can be taken through the ruler (see BOX 1 above for the method). |
| Color | Indicate the item appearance to light, whether it is O =Opaque, T =Transparent, or C =Coloured. If coloured specify the colour (White, Red, Blue, Green, Grey, Yellow, Brown, Black, Pink, Orange, Other). |
| State | State of the object: En =entire or Fr =Fragmented. FADs (Fishing Aggregating Devices), which are floating items used by fishers to aggregate fishes made of mixed materials, including plastics (i.e., plastic bottles, cork plates, inner tubes of car tires, polystyrene slabs, palm fronds), are also reported here. |

e. Sea Turtles and Other Megafauna (FMML protocol)

Marine turtles are considered a high-priority species, and systematic data on their presence are collected using the fixed-strip width methodology (strip transect) by the observer assigned to marine litter monitoring (while the other observers will use a line transect method for their sighting of sea turtles outside the FMML strip). The presence of jellyfish or other marine megafauna species of interest within the monitored strip must also be recorded.

Each time the observer detects a species of interest within the fixed 50 m-wide strip, they must record a waypoint on the GPS and try to collect all information specified in Table 2.7 *Type of data collected during sightings of cetaceans and sea turtles* and Table 2.8 *Type of data collected during sightings of large fishes, and jellyfish*. The use of a camera is strongly recommended to support species identification. Once the animal has passed, the observer must: report the GPS code on the FMML monitoring data sheet (D5), inserting in the field "Specific item & note" the species observed, compile the relative data sheet with all the information reported in Tab 2.7 (D6). **Important:** If FMML monitoring is conducted concurrently with standard line-transect marine megafauna monitoring, all data on sea turtles and other species may be recorded in the '*Sea Turtles and Other Species*

Sightings' datasheet of the fauna monitoring (D2). In such cases, indicate in the 'Notes' field that the animal was sighted **within the litter strip** to distinguish both datasets collected with different methods.

f. What to do when the side with the better visibility changes during the monitoring activity? (FMML protocol)

If the side with better visibility changes during the monitoring activity, the observer should switch to monitor the new side with better visibility as soon as it is safe and practical to do so.

In that case, monitoring should be interrupted (**STOP**) before side-changing occurs and then restarted on the other side. Every change must be documented in the datasheet, specifying the time and position (GPS coordinates) when the switch occurred.

g. What to do if weather conditions change during monitoring (FMML protocol)

Similarly to mega and macrofauna monitoring any variation in weather conditions (e.g., sea state, visibility, wind, precipitation) must be documented in the Weather Conditions datasheet (D3). Mark the GPS position using the code MET and note the time and details (sea state, wind direction, visibility, cloud cover, etc.).

Monitoring must be stopped when visibility or sea state deteriorates beyond acceptable limits (e.g., Beaufort > 2 for FMML monitoring): *Mark* the GPS position with STOP, and *insert* the GPS code in all related datasheets (D5 – Floating marine macro litter, D6 – Sea turtles and other sightings-on effort litter). When conditions improve and monitoring can restart: *Mark* the GPS position with START, *Insert* the GPS code in all related datasheets, and *Record* updated weather conditions before resuming observations. If FMML monitoring is conducted concurrently with standard line-transect marine megafauna monitoring, all weather variations should be coherent between the two.

h. End of monitoring effort (FMML protocol)

At the end of the transect, or earlier in case of bad weather conditions, the Senior observer must close the monitoring activity by following these steps:

- Mark the GPS location of "End effort";
- Stop the GPS track recording using the dedicated GPS device and save the track.

Before Landing, the team must:

- Review the datasheets carefully and check for missing or incomplete data;
- Verify that all equipment and tools have been recovered.

To keep in mind

Data Integrity: Always use GPS waypoints (codes provided in the protocol) for all effort changes and sightings. Avoid manual entry of latitude/longitude unless the GPS is malfunctioning, to prevent transcription errors.

Communication: Effective communication between observers is essential to avoid double-counting the same group of animals. If possible, use walkie-talkies to minimize disturbance on the command deck.

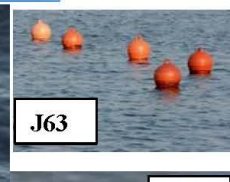
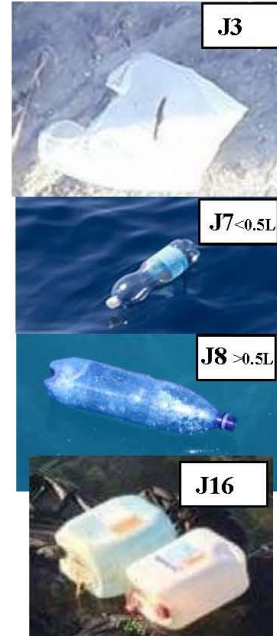
Conduct On Board: Maintain respectful behaviour towards the crew, remembering that you are guests. On the command deck, remain quiet, avoid loud noises during sightings, and never touch buttons or controls.

D7. BEAUFORT SCALE (source: Royal Meteorological Society)

| Wind Force | Description | Wind Speed | | Specifications | Probable Wave Height | | Sea State |
|------------|-----------------|-------------|--------------|--|----------------------|------------|-----------|
| | | <i>km/h</i> | <i>knots</i> | | <i>metres</i> | <i>Max</i> | |
| 0 | Calm | <1 | <1 | Smoke rises vertically. Sea like a mirror. | -- | -- | 0 |
| 1 | Light Air | 1-5 | 1-3 | Direction shown by smoke drift but not by wind vanes. Sea rippled. | 0.1 | 0.1 | 1 |
| 2 | Light Breeze | 6-11 | 4-6 | Wind felt on face; leaves rustle; wind vane moved by wind. Small wavelets on sea. | 0.2 | 0.3 | 2 |
| 3 | Gentle Breeze | 12-19 | 7-10 | Leaves and small twigs in constant motion; light flags extended. Large wavelets on sea. | 0.6 | 1.0 | 3 |
| 4 | Moderate Breeze | 20-28 | 11-16 | Raises dust and loose paper; small branches moved. Small waves, fairly frequent white horses. | 1.0 | 1.5 | 3-4 |
| 5 | Fresh Breeze | 29-38 | 17-21 | Small trees in leaf begin to sway; crested wavelets form on inland waters. Moderate waves, many white horses. | 2.0 | 2.5 | 4 |
| 6 | Strong Breeze | 38-49 | 22-27 | Large branches in motion; whistling heard in telegraph wires; umbrellas used with difficulty. Large waves, extensive foam crests. | 3.0 | 4 | 5 |
| 7 | Near Gale | 50-61 | 28-33 | Whole trees in motion; inconvenience felt when walking against the wind. Foam blown in streaks across the sea. | 4.0 | 5.5 | 5-6 |
| 8 | Gale | 62-74 | 34-40 | Twigs break off trees; generally, impedes progress. Wave crests begin to break into spindrift. | 5.5 | 7.5 | 6-7 |
| 9 | Strong Gale | 75-88 | 41-47 | Slight structural damage (chimney pots and slates removed). Wave crests topple over; spray affects visibility. | 7.0 | 10.0 | 7 |
| 10 | Storm | 89-102 | 48-55 | Seldom experienced inland; trees uprooted; considerable structural damage. Sea surface largely white. | 9.0 | 12.5 | 8 |
| 11 | Violent Storm | 103-117 | 56-63 | Very rarely experienced; accompanied by widespread damage. Medium-sized ships lost to view behind waves. Sea covered in white foam, visibility seriously affected. | 11.5 | 16.0 | 8 |
| 12 | Hurricane | 118+ | 64+ | Devastation. Air filled with foam and spray, very poor visibility. | 14+ | --- | 9 |

D8. MARINE LITTER PHOTOCATALOGUE to take on board for quick identification of J-code categories, refer to the most updated and complete version in the ISPRA Folder "PRJ_FLT_Med Net").

| TSG_ML General- Code | General Name | SIZE CLASS OF ENTIRE ITEM |
|---------------------------|--------------------------------------|---------------------------------|
| ARTIFICIAL POLYMER | | |
| J3 | Shopping bags | F |
| J7-J8 | Bottles | E - F |
| J16 | Jerry cans | E-F |
| J20-23 | Plastic caps and lids | ALL |
| J27 | Cigarettes butts and filters | B |
| J30 | Crisps packets/sweets wrappers | B-C-D |
| J31,G95 | Plastic sticks (lolly or cotton bud) | C |
| J33 (cups) | Tableware | B- C |
| J34 (cutlery and trays) | Tableware | D- E |
| J38 | Covers/packaging | ALL |
| J48 | Syntetic ropes | ALL |
| J51-56, J59 | Nets and lines | ALL |
| J18, J57 | Plastic boxes/crates | F |
| J58 | Polystyrene boxes | F |
| J63 | Buoys | E-F-G |
| J65 | Buckets | E |
| J96 | Sanitary towels/tampon | D-E |
| | | |



2.3 eDNA monitoring

This section provides technical instructions for the eDNA collection to monitor marine megafauna (cetaceans, sea turtles, and other macrofauna), along fixed routes using large vessels such as ferries or cargo ships as observation platforms. Environmental DNA sampling detects genetic traces shed by organisms into the environment, enabling identification of species presence without direct sighting, thus being able to survey the routes also during nighttime hours. It complements visual monitoring by capturing cryptic and low-density species and supports community-level biodiversity analysis. The methodology has been developed to allow sustainable, long-term monitoring of extensive offshore areas, generating comparable, integrative, high-quality data across different geographical and spatial scales.

2.3.1 Sampling design

The *spatial sampling design* is based on fixed transects along regular commercial vessel routes, enabling repeated, systematic monitoring over time. Transects are defined to capture key environmental and anthropogenic gradients (see paragraph 1.1 Identification of routes). The eDNA sampling occurs in Fixed Sampling Stations (FSSs), namely geographically fixed positions that remain constant across all survey cruises. The FSSs are selected based on: (i) biological significance, prioritising locations of known biological interest, as indicated by previous observational studies or existing literature; (ii) bathymetric features, considering target points indicative of significant habitat changes, such as the edge of the continental shelf; (iii) spatial and temporal distribution, positioning FSSs at roughly equidistant intervals (approximately 35-45 nautical miles) along the designated shipping lanes, and considering collection of samples during both day and night cycles. Additional samples are collected during opportunistic sightings of rare species to be adjusted depending on the relative frequency of the species in the investigated area (e.g., *Balaenoptera physalus*, *Physeter macrocephalus*, *Grampus griseus*, *Globicephala melas*, *Ziphius cavirostris*, *Delphinus delphis*).

Temporal coverage and frequency of survey: surveys should be conducted year-round, with repeated monitoring of the same transects, to capture seasonal and interannual variability and enable robust temporal trend analysis. The number of surveys conducted may vary depending on the route; however, the responsible research group or monitoring entity shall ensure the execution of one survey per season (Table 2.2). Exceptions are made for transects only available during a specific season. For the purposes of this protocol, a “season” is defined as a complete three-month period (e.g., Summer: from the beginning of July to the end of September - “meteorological season”).

By surveys in FSS along representative fixed transects year-round, the design provides robust, reproducible data for assessing species distribution, abundance, and exposure to anthropogenic pressures.

2.3.2 Team

The number of operators required for eDNA sampling and filtering on board may vary depending on factors such as route duration, number of FSSs and vessel characteristics. In general, the eDNA sampling team should consist of at least two people. The operators should be trained before embarking on : safety rules to be followed in the engine rooms and related DPIs; seawater collection for eDNA analysis; eDNA samples filtering; contamination risks and good practices to limit them. The operators are responsible for setting up the filtration ramp either in the engine room or in another dedicated space, coordinating with crew members, handling the samples and storing the filters. Having two operators on site allows for shifts working, and enhances safety, during nighttime sampling and filtering of multiple samples in series.

2.3.3 Equipment

The following table summarises the minimum set of tools required for conducting the eDNA collection and filtration activities on board.

Tab 2.11 – Equipment

| Category | Tool | Purpose |
|----------------------------|-------------------------------------|---|
| Seawater collection | BiB system (bags and support boxes) | Seawater collection and storage. |
| | Sterile gloved | Prevent the contamination of the seawater sample. |
| | Indelible markers | Mark the collection bags with the unique ID code and filter wrapping. |
| Seawater filtration | Filtration cylinders | Allows water filtration by containing the filtering membrane and collecting water from the BiB system. |
| | Sterile transparent bags | Used to isolate the filtration unit and to transport the cylinders. |
| | Vacuum Flasks (5 litres) | Collect filtrated seawater. The volume and number of the flasks depend on sampling effort and sample volume. To allow for 12L collection and filtration, several 5-litre flasks (from 2 to 4) are employed. |
| | Vacuum pump | Used to create the void and favour water passage through the filtering membranes. |
| | Filtering membranes | Collect and withhold the eDNA present in the sample. |
| | Sterile tweezers | Used to recover the filtering membrane from the cylinder. |
| | Bleach (10%) | Used to sterilise the collection station and filtration ramp. |
| | Connecting pipes | Used to connect the flask(s) to the vacuum pump. |
| | Aluminium foil | Wrap the filters after recovery and filter storage until further laboratory analysis. |
| | Filtration form | Collect filtration metadata. |
| | Parafilm | Isolate BiB system bags' opening; isolate the flasks' opening. |
| Filters transport | Ice packs and thermal bag | Allow for filter transportation at low temperatures. |

2.3.4 Getting Ready for Monitoring Activities

- In the laboratory: print and check the gear checklist, including all the items to be taken on board for both sample collection and sample filtration, illustrated in Table 1. Prepare the Bag-in-Box (BiB) sampling system in the laboratory before boarding, sealing the three openings with adhesive tape to avoid contamination.
- Coordination with crew members by sharing the sampling points (estimated coordinates of the FSSs) with the captain and crew. Note down the sampling time for each FSS. However, as the ferry never passes exactly on the same point, the exact coordinates of each seawater sample collection should be noted after the actual sampling.
- Settling up in the engine room: Before entering the engine room, wear the personal protective equipment (PPE) and sanitize the sampling/filtering area using a 10% bleach solution. Together with the crew, identify the water inlet with the minimum path for the in-taken water through the ship's pipes to minimize the contamination of the water sample. Once the most suitable point/inlet for collecting has been identified, ask the staff if the sample collection tap can be kept slightly open for the entire duration of the cruise to continuously rinse the pipe used for sample collection with "local" water. Dispose the seawater that flows incessantly from the tap in the bilge. If not, the dedicated tap should be open before collection to let the water run for at least 5 minutes before the actual seawater sample collection.

2.3.5 Procedures

2.3.5.1 Protocol for eDNA marine megafauna monitoring from large vessel

ASSOCIATED PROTOCOLS:

The procedure can be visualized step-by-step in the video article by Rota & Valsecchi (2025), published in the Journal of Visualized Experiments

STANDARDIZED DATA SHEET: The following is an example of a sampling and filtration data sheet, which can be integrated and cross-referenced with the visual observation team's activities

HUMAN RESOURCES: Minimum 

DURATION/TIMING OF ACTIVITY: seawater samples should be collected in correspondence of each Fixed Sampling Stations along the route, selected prior to embarking. Additional samples can be collected in co-occurrence with rare species sightings.

LIMITS: Sea-weather conditions to not affect eDNA sampling.

LEVEL OF EXPERIENCE OF THE OBSERVERS: At least one expert, maximum one in training for each survey.

a. Metadata Recording

Upon confirmation of the vessel's transit time for each sampling point (FSS), the eDNA team is to report to the engine room. The team will then initiate the sampling procedure and record all data as outlined in Table 2.12 in the provided form (Fig. 2.4).

| | | | | | | |
|---|--------|---------|-------|----------------|-----------|-----------|
|  | ROUTE: | CRUISE: | DATE: | SAMPLING TEAM: | FLT TEAM: | COMPLIER: |
|---|--------|---------|-------|----------------|-----------|-----------|

| SAMPLE* | DATE | HOUR | duration | GEO COORDINATES | | | | | | | | REF TO FLT SHEET | NOTES | |
|---|----------------|-----------|-----------|-----------------------------|--------------------------|--------------------------|--------------------------|-----------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| | | | | processed litres | total filtration time | processed litres | total filtration time | processed litres | total filtration time | | | | | |
| filtration on board <input type="checkbox"/> YES <input type="checkbox"/> NO | ____/____/____ | ____:____ | ____:____ | LAT | | | | LON | | | | | | |
| filters | | | | A) <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | B) <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| filtration on board <input type="checkbox"/> YES <input type="checkbox"/> NO | ____/____/____ | ____:____ | ____:____ | LAT | | | | LON | | | | | | |
| filters | | | | A) <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | B) <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| filtration on board <input type="checkbox"/> YES <input type="checkbox"/> NO | ____/____/____ | ____:____ | ____:____ | LAT | | | | LON | | | | | | |
| filters | | | | A) <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | B) <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| filtration on board <input type="checkbox"/> YES <input type="checkbox"/> NO | ____/____/____ | ____:____ | ____:____ | LAT | | | | LON | | | | | | |
| filters | | | | A) <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | B) <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| filtration on board <input type="checkbox"/> YES <input type="checkbox"/> NO | ____/____/____ | ____:____ | ____:____ | LAT | | | | LON | | | | | | |
| filters | | | | A) <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | B) <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| filtration on board <input type="checkbox"/> YES <input type="checkbox"/> NO | ____/____/____ | ____:____ | ____:____ | LAT | | | | LON | | | | | | |
| filters | | | | A) <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | B) <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| filtration on board <input type="checkbox"/> YES <input type="checkbox"/> NO | ____/____/____ | ____:____ | ____:____ | LAT | | | | LON | | | | | | |
| filters | | | | A) <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | B) <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| filtration on board <input type="checkbox"/> YES <input type="checkbox"/> NO | ____/____/____ | ____:____ | ____:____ | LAT | | | | LON | | | | | | |
| filters | | | | A) <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | B) <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| filtration on board <input type="checkbox"/> YES <input type="checkbox"/> NO | ____/____/____ | ____:____ | ____:____ | LAT | | | | LON | | | | | | |
| filters | | | | A) <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | B) <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| filtration on board <input type="checkbox"/> YES <input type="checkbox"/> NO | ____/____/____ | ____:____ | ____:____ | LAT | | | | LON | | | | | | |
| filters | | | | A) <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | B) <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |

* year-cruise-route-sample, e.g. 21-4UGA3 (for Fix Sampling Stations, where "3" is fix station n3); OR 21-4UGA35 (for Sighting Samples, where "3" is the 3rd sample collected during a sighting during cruise #4)

Fig. 2.4 Form for eDNA samples' metadata on collection and filtration

Tab 2.12 - Minimum general data to be collected before starting samples' collection

| Data | Explanation |
|----------------------|---|
| Transect code | The unique identifier for the effort code must include the current year, the transect code (which is unique for each route), the survey number (progressive). The transect code used by the visual monitoring team is also included in the metadata file as it may be different for the outward and inbound journeys. |
| Date | Date of activity |
| Hour | Hour of activity |
| Observer names | Provide a list of the operators' names. |
| FSSs collection time | FSSs coordinates are shared with the crew to estimate the sampling time based on vessel's speed and trajectory. |

b. Water sample collection (in the engine room)

To prepare for sampling collection, bring all necessary gear to the engine room around 15 min before the planned sampling time to sterilize the area where the BiBs are handled (using a 10% bleach solution). Use a permanent marker to prepare and label the BiB by writing on it: (i) the unique alphanumeric identification code of the sample on both sides of the BiB (important especially if the BiB are not processed straight away on board and are filtrated on land) (ii) the date; (iii) the exact time of starting filling the BiB. Remove the sealing film from the opening of the BiB and start filling it until completely full (about 13 L). When the bag is nearly full, seal the BiB with the lid, pressing it tightly until completely closed. Take note of the exact time when the sample collection is completed. Transfer the filled-in BiB to the storage room or to the filtration area (a dedicated space in the engine room itself or in a cabin).

c. Seawater sample filtration

Before filtration, assemble the filtering system and the filtration cylinder. Use a new cylinder for each station, a pair of sterile tweezers. Fill in the sample collection/filtration data log to keep track of the number of litres processed and to measure the filtering time: as soon as the vacuum pump is activated, start the timer to measure the filtering time. To reduce contamination risk with the surrounding environment, isolate the flow of water to be filtered from the surrounding environment by placing an insulating sleeve - plastic bag - between the tap and the filtering cylinder

To initiate filtration, activate the vacuum pump and filter up to 4 L of water for each filter. Keep the cylinder always full during filtration to avoid air getting into the system and slow down the filtration process. In this protocol, a membrane with a porosity of 0.45 µm proved the best solution, allowing to process large volumes of water. When the level of the filtered water inside the flask reaches the 4 L level mark, turn off the vacuum pump and stop the timer, reporting the filtering time value on the data log. Recover the first filter (filter A) from the cylinder, avoiding any possible damage to the filter. Fold the filter in half (with the side that retained the biological material folded on itself) and wrap it in aluminium foil, on which the sample number and the ID of the filter replicate should be marked. Mount a new filter (filter B) inside the filtration cylinder to process another 4 L sample. Empty the flask and start again with filtering the second replicate until 4 L are completely filtered. Recover filter B as explained and repeat the same steps for filter C.

After filtration, store the filters at -4° or -20° C until further laboratory processing.

To keep in mind

Protocol efficiency: the protocol described here allows for efficient filtration of a large amount of seawater (more than 10 liters) directly on board, either in the engine room itself or in a dedicated cabin. It is always desirable to filter seawater samples immediately after collection, to limit eDNA degradation and sample transportation.

Prepare the filtering station: To improve the efficiency of the filtration, you may: 1) use high-capacity vacuum flasks (from 2 L to 5 L flasks, depending on the volume to be withdrawn); 2) use multiple vacuum flasks arranged in series, a measure that allows the simultaneous filtration of multiple samples (bags).

Contamination limitation: To monitor the incidence of false positives (contamination), include in the filtering procedure, at least once per cruise, a "blank-bag", filtering in parallel with the samples a bag filled with drinking water taken from sealed bottles. This control sample does not require replicates and treat this as the rest of the samples and include it in the NGS run.

Conduct On Board: Maintain respectful behaviour toward the crew, remembering that you are guests. Both on the command deck when coordinating with the crew for the FSSs coordinates and in the engine room during sampling and filtering, remain quiet, avoid shouting during sightings, and never touch buttons or controls. Access to the engine room for eDNA collection personnel is conditional upon the use of mandatory Personal Protective Equipment (PPE), which includes a safety helmet, steel-toed safety shoes, and hearing protection.

Protocol Overlap: Sampling activities can be coordinated and synchronized with Stable Isotopes Analysis sampling.

2.4 SIA monitoring

This section provides step-by-step technical instructions for the systematic collection and processing of seawater samples for Stable Isotope Analysis (SIA) along fixed ferry routes. SIA of suspended particulate organic matter (POM) provides baseline isotopic values ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, C/N ratios) essential for understanding marine food web structure, ecosystem functioning, and trophic dynamics. The spatial distribution of stable isotope values, known as "isoscapes", reveals information on baseline biogeochemical conditions, nutrient sources and cycling, and trophic structure across different regions. Specifically, $\delta^{13}\text{C}$ isoscapes provide information on primary productivity patterns and carbon sources (offshore vs coastal, pelagic vs benthic), while $\delta^{15}\text{N}$ isoscapes reflect nutrient cycling, nitrogen sources (including upwelling, nitrogen fixation, and terrestrial inputs), and baseline trophic position. SIA data complements eDNA monitoring by providing quantitative ecological information on ecosystem functioning and energy flow.

This methodology enables long-term biogeochemical monitoring of extensive offshore areas and, through a harmonized data collection approach, ensures the production of comparable, high-quality isotopic data across different geographical and spatial scales. The instructions presented here have been developed and refined within the LIFE project Conceptu Maris.

2.4.1 Sampling design

SIA sampling is conducted simultaneously with eDNA monitoring at the same Fixed Sampling Stations (FSS) to enable direct spatial and temporal integration of molecular and isotopic data. This coordinated approach maximizes ferry transit efficiency while providing complementary datasets on community structure (eDNA) and ecosystem functioning (SIA).

Spatial sampling design

- FSS spaced 35-45 NM along ferry transects.
- Identical FSS positions as eDNA sampling for direct data integration.
- FSS numbered chronologically (by sampling order) rather than geographically to enable day/night paired sampling.
- Coverage of unmonitored Mediterranean marine sectors with known biological significance.

2.4.2 Equipment

SIA sampling requires a dedicated filtration system separate from eDNA equipment to prevent cross-contamination and accommodate different filter types and pressure requirements. The following table summarises the minimum set of tools and equipment required for conducting SIA monitoring activities.

Tab 2.13 Material for SIA monitoring

| Category | Tool | Purpose |
|-------------------|--------------------------|---|
| Data Recording | GPS (portable) | Record FSS coordinates and mark sampling positions (waypoints). |
| | Data Sheets/Clipboards | Record sample metadata: station, replicate, volume, time. |
| Sample Collection | Water splitter system | Simultaneous collection of 3 independent replicates. |
| | Clean 5L containers (9+) | Collect seawater (3 containers per station). |
| | Graduated cylinders (3L) | Measure exact volumes. |

| | | |
|---------------|-----------------------------------|--|
| Filtration | Vacuum pump + pressure gauge | Filter seawater at ≤ 300 mbar. |
| | Filtration apparatus + cylinders | Hold 47 mm filters during filtration. |
| | Pre-combusted GF/F filters (20+) | Retain POM (0.7 μ m, 450°C \times 4h pre-treatment). |
| | Sterile forceps + aluminium foil | Handle and wrap filters aseptically. |
| Sterilization | 10% bleach + distilled water | Sterilize areas and rinse equipment. |
| Safety | PPE (glasses, hearing protection) | Personal protection for engine room. |
| Storage | Freezer -20°C + coolers | Store samples frozen until lab processing. |

2.4.3 Getting Ready for Monitoring Activities

The number of personnel required for SIA sampling may vary depending on route duration and coordination with other monitoring activities (eDNA, visual surveys). Ideally, the SIA sample collection team should consist of a minimum of two to three dedicated personnel.

Team members are classified based on experience with aseptic sampling techniques, vacuum filtration operation, and SIA principles: senior, experienced, and in training. Each team must include at least one senior sampler, who is responsible for assessing and validating the experience level of other team members, and no more than one person in training.

During sampling, one person manages sample collection and labelling, another operates the filtration system, and a third (if available) handles filter processing and storage. This rotation ensures sustained attention and maintains high data quality throughout the cruise. The structured team composition enhances consistency, reduces contamination risk, and supports standardized data collection across different ferry routes.

The senior member of the team takes care of the following steps before starting with the sampling procedure:

- prepare and check all materials before departure
- secure identification documents/passport for international travel
- ensure that the GPS is fully charged, spare batteries are available. Set GPS to decimal degrees (3+ decimals), DATUM WGS84
- verify that GF/F filters are pre-combusted (450°C \times 4h) and individually wrapped
- test the vacuum pump (verify ≤ 300 mbar control)
- share FSS coordinates with the captain and eDNA team
- label containers by station before boarding (FSS1-A, FSS1-B, FSS1-C)
- conduct pre-departure briefing: review procedures, data logging, rotation schedule

Team members must pack appropriate clothing for engine room conditions and sufficient food/water for the entire trip.

Note: multidisciplinary monitoring requires coordination with the eDNA and visual survey teams to ensure synchronized sampling at FSSs.

2.4.4 Procedures

Tab 2.14 Activities procedures

| Step | Detailed Instructions |
|---|--|
| 1. Pre-boarding (48-72h before) | <p>Filter Pre-combustion:</p> <ul style="list-style-type: none"> • Place GF/F filters (47 mm) in ceramic/aluminium crucibles • Combust at 450°C × 4 hours • Cool in desiccator (2h minimum) • Wrap individually in foil using sterile forceps (edges only) • Label with combustion date, store in sealed container • Prepare 20-30% excess <p>Equipment Check:</p> <ul style="list-style-type: none"> • Assemble and test filtration system under vacuum • Verify pressure control at ≤300 mbar • Sterilize all reusable equipment (10% bleach, rinse with distilled water) • Test water splitter, pack organized containers • Print waterproof data logs, create inventory checklist |
| 2. Onboard Setup (Day 1) | <p>Initial Boarding:</p> <ul style="list-style-type: none"> • Transport equipment, conduct inventory check • Attend safety briefing, introduce to captain/engineer • Coordinate with eDNA team on schedule <p>Engine Room Setup:</p> <ul style="list-style-type: none"> • Don PPE (hearing protection, safety glasses, non-slip shoes) • Identify optimal water intake tap with engine staff • Test flow, check water quality (clear, no contamination) • Clean filtration area (10% bleach), set up equipment • Test vacuum pump in situ • Establish tap protocol: continuous flush or 5-min pre-flush • Mark tap clearly, photograph setup |
| 3. Sample Collection at FSS (30 min before) | <p>Preparation:</p> <ul style="list-style-type: none"> • Notify bridge of upcoming FSS • Move to engine room with 3 containers, splitter, labels, data sheets • Re-sterilize area (bleach spray) • Verify tap flow or flush 5 minutes • Prepare data log: FSS code, date, start time • Ready GPS, don fresh gloves <p>At FSS (GPS reached):</p> <ul style="list-style-type: none"> • Record time, GPS coordinates, sea state on data log • Label 3 containers: [FSS]-A, [FSS]-B, [FSS]-C + date + "SIA" + time • Attach splitter to tap, connect 3 containers • Open tap, fill simultaneously to 3L minimum • Monitor equal distribution • Alternative: fill sequentially within 5-min window • Measure exact volume (graduated cylinder), record • Cap, verify labels, note observations • Transport to filtration station |
| 4. Filtration (immediately after) | <p>Setup:</p> <ul style="list-style-type: none"> • Sterilize area (bleach), don fresh gloves • Unwrap pre-combusted GF/F filter with sterile forceps (edges only) • Place on base (no gaps/folds), assemble cylinder • Connect to vacuum flask, verify pump off • Fill data log: FSS, replicate (A/B/C), volume, start time <p>Active Filtration:</p> <ul style="list-style-type: none"> • Pour water from [FSS]-A into cylinder • Activate pump, adjust to ≤300 mbar (CRITICAL: never exceed) • Start timer • Maintain cylinder 2/3 full, avoid running dry • Typical: 3L in 15-45 min (varies with particulate load) • If filter clogs before 3L: NORMAL in productive waters |

| | |
|---------------------------------------|--|
| | <ul style="list-style-type: none"> • When clogged or 3L filtered: record exact volume + time • Rinse cylinder walls (~50 mL filtered water) through same filter • Turn off pump, disconnect <p>Recovery:</p> <ul style="list-style-type: none"> • Lift filter by edge (forceps) • Inspect: visible particulate (tan to dark brown) • Fold in half (filtered side inward - opposite eDNA) • Wrap in labeled foil: [FSS]-[replicate], date, volume • Seal edges, place in storage container • Transfer immediately to freezer -20°C • Record storage time • Repeat for replicates B and C (fresh filter each) |
| 5. Blank Control (once per cruise) | <p>Procedure:</p> <ul style="list-style-type: none"> • Select sealed drinking water (3L) • Label container: "BLANK-[Cruise]-[Date]" • Fill with 3L drinking water • Filter as regular sample (will be faster) • Wrap/label: "BLANK-[Cruise]-[Date]" • Store with samples • Single replicate only • Include in SIA run |
| 6. Between Stations | <p>Cleaning:</p> <ul style="list-style-type: none"> • Rinse cylinders with distilled water • Empty/rinse vacuum flasks • Wipe surfaces (bleach) • Replace gloves • Organize samples chronologically • Review data logs for completeness |
| 7. Laboratory (post-cruise) | <p>Preparation:</p> <ul style="list-style-type: none"> • Thaw samples at room temp (~30 min) • Unwrap foil carefully, transfer filter to petri dish <p>Differential Treatment:</p> <ul style="list-style-type: none"> • For $\delta^{13}\text{C}$: cover with 1N HCl, react 10-15 min, rinse with distilled water (3-5\times) until pH neutral • For $\delta^{15}\text{N}$: rinse only with distilled water (no acid) • If both: split filter in half (one HCl, one water) <p>Final Processing:</p> <ul style="list-style-type: none"> • Dry overnight at 60°C • Homogenize (ball mill) to fine powder • Weigh ~4 mg into tin capsule • Store in desiccator • Submit to SIA facility |

To keep in mind

Data Integrity: always use GPS waypoints for sampling stations. Record exact filtered volumes - critical for normalization when filters clog. Avoid manual coordinate entry unless GPS fails.

Communication: coordinate with the eDNA team for engine room access. Share metadata (GPS, SST, salinity, times) for integrated analyses.

Conduct On Board: respect crew, follow safety protocols, wear PPE in the engine room. Never touch equipment without permission.

Protocol Critical Points:

- Pre-combustion of GF/F filters (450°C × 4h) is MANDATORY
- NEVER exceed 300 mbar vacuum pressure
- Maintain -20°C cold chain continuously
- Record actual filtered volume when filters clog (normal in productive waters)
- Separate eDNA and SIA equipment strictly
- Include blank control in every analysis run to assess background contamination

3. Data management

The data management workflow adopted in the project ensures that all survey data are handled in a consistent, traceable, and interoperable manner. It is based on a standardised **Common Database structure**, developed to harmonize formats, predefined categories, relational tables, and coding systems across partners (LIFE Conceptu Maris, Milestone C1.1b and Deliverable E2.2).

The Conceptu Maris Common Database is hosted by the **National Biodiversity Network (NBB)**, <https://www.nnb.isprambiente.it/en>, which provides the centralised environment for long-term storage, maintenance, accessibility, and integration with the project's visualization platforms (GeoNode Web-GIS and Dashboard Stats); it allows, in addition, interoperability with other datasets repositories.

The Database is organised into **primary and secondary tables**, designed to accommodate both historical datasets and the new data collected under the new multidisciplinary protocol. This ensures a coherent framework that supports data harmonisation and long-term comparability.

Use of **standard templates, quality checks** and **multi-level validation** is essential to allow data collected by different teams to be aggregated without introducing bias, ensuring their suitability for spatial analyses, trend assessments, and multidisciplinary integration. These steps prevent structural mismatches, inconsistent coding, and inaccuracies that would compromise database integrity and interoperability.

The workflow distinguishes the responsibilities of **partner institutions** (data preparation, internal quality checks, formatting, and annual submission) from those of **ISPRA**, which include centralized validation, ingestion into the Common Database, and updating of the visualisation interfaces. This structured process guarantees the application of common standards across partners, ensures high data quality, and enables efficient use of the shared data infrastructure.

The workflow is summarised in **Figure 3.1**.

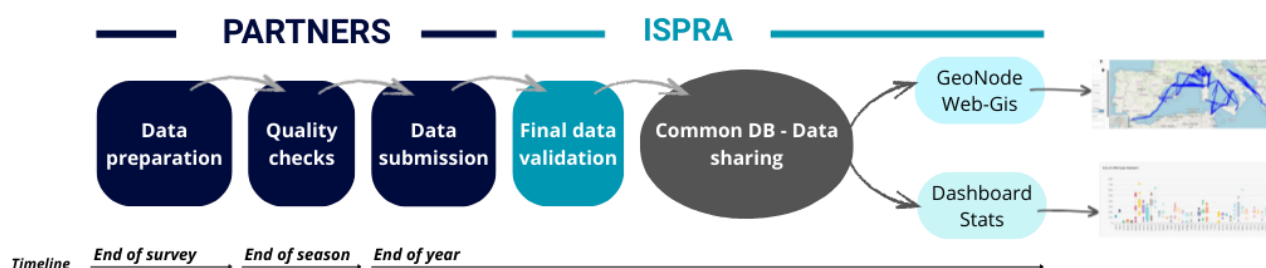


Figure 3.1. Workflow of the different steps, including timelines and responsibilities, for uploading and ensuring high-quality data into the standardised common centralised database.

3.1 Data preparation at the end of each survey (by Partners)

3.1.1 Archiving in Standard Data Format

At the end of the survey, raw data are prepared and archived using **standardised templates** (Excel) provided by ISPRA. The templates ensure that all information follows common rules for date/time formats, time zones, units of measurement, naming conventions, and taxonomic or behavioural

classifications. Because templates may be updated, partner institutions must always refer to the **latest version** present in the [ISPRA folder](#) ("PRJ_FLT_Med Net") and to the legend accompanying each file, which defines all predefined categories, field descriptions and column order.

For visual monitoring two different templates are used:

- one for mega- and macrofauna and maritime traffic (Table 3.1)
- one for floating marine macro litter (Table 3.2).

A key element common to all monitoring components is the **coding system based on ID_Survey and COD_Effort**, which must be assigned correctly to ensure consistency across datasets (**Box 3.1**).

BOX 3.1 - ASSIGNMENT OF COD_EFFORT

A key element common to all tables and monitoring components is the coding system based on ID_Survey and COD_Effort. These codes must be assigned consistently to ensure full compatibility across datasets.

The COD_Effort code is composed of:

4 letters (transect code) + 4 numbers (survey number) + section letter (if applicable).

- The **ID_Survey** corresponds to the transect code + survey number
- Section letter Indicates subdivisions of the same survey
- For marine litter, the code is extended by adding "Lit" before the section letter:

$$\text{COD_Effort} = \text{ID_Transect} + \text{N_Survey} + \text{Section}$$

CBAR_0129b
for megafauna

CBAR_0129_Litb
for marine litter

- Outbound and return journeys are counted as two separate surveys and must therefore receive different survey numbers.
- For surveys lasting more than one day (e.g., BTAN), section letters (a, b, c...) are added to distinguish consecutive days within the same survey number.
- In the Main Data summary, the ID_Survey (i.e., transect code + survey number) is reported as the statistical unit, while COD_Effort specifies the individual effort segments associated with that survey.

Data entry follows a standardised practical procedure (**Box 3.2**), which ensures consistency and traceability across surveys and must be applied while respecting all formatting rules and controlled vocabularies summarised in Tables 3.1 and 3.2.

| BOX 3.2 - PRACTICAL DATA ENTRY PROCEDURE | |
|--|---|
| 1. ↓ | Import of GPX track data: the procedure begins with the import of the full GPX track: latitude, longitude, UTC datetime and waypoint codes are copied into the corresponding columns of the Excel template. Automated formulas in the file are then used to extract the date, time and Time_effective (local time) from the original UTC timestamp. |
| 2. ↓ | Chronological ordering and verification: once imported, all records are sorted by date and time to confirm that the track points follow a correct chronological sequence and that the waypoints appear in the right order along the route. |
| 3. ↓ | Track cleaning: the track is then cleaned by removing all points outside the monitoring effort, including those recorded before BEG/START, those between a STOP and the next START, and those after the END waypoint. |
| 4. ↓ | Assignment of COD_EFFORT: each continuous effort segment is assigned a COD_EFFORT code. When a survey extends over more than one day, alphabetical sections (a, b, c...) are added to distinguish consecutive days. |
| 5. ↓ | Insertion of weather data: weather information is entered at BEG, MET, START, STOP and END waypoints, and then copied to all intermediate points until conditions change, always following the predefined categories included in the template. |
| 6. | Entry of biological and traffic observations: lastly, all sighting data (cetaceans, sea turtles, other species) along with maritime traffic or marine litter observations, are entered using the predefined codes, categories and formatting rules required for interoperability. |

Table 3.1. Fields and formatting: Data standard used for Visual Monitoring of Mega- and Macrofauna (Template: "DB_Macrofauna", available at the [ISPRA folder](#))

| Field | description | Format |
|---|---|------------------------------|
| "Main data" sheet | | |
| ID_Survey | The unique identifier of the survey, composed of the transect code (4 letters) followed by a progressive 4-digit survey number. It corresponds to the cod_effort without the final section (a/b/c). | text |
| O/R | O for on the way direction R for return. | text (predefined categories) |
| Date | Starting date. | data (yyyy-mm-dd) |
| N.days | Number of days required to complete the survey when the journey in one direction takes more than one day (e.g., long routes such as Barcelona-Tanger). | integer |
| Ship | Name of the Vessel (try to be consistent). | text |
| Obs1, Obs2, Obs3, Obs4, Obs5 | Name and Surname of observer, first the Senior then the others in order of decreasing experience. | text |
| Cetacean survey/Naval survey/Other species/Sea turtles/Sea birds/Litter_others | 0 to indicate no data were collected, 1 for monitoring not conducted systematically and 2 for systematic data collection for the specific information. | integer (predefined numbers) |
| eDNA | t if true and conducted f for false not conducted. | boolean (t or f) |
| "Track" sheet | | |
| Source | Origin of the GPS data (e.g., GPX file, ferry GPS system). | text |
| ns1: time2 | Original timestamp extracted from GPX, in UTC+0. | text |

| | | |
|---|---|------------------------------|
| COD_Effort | Unique identifier linking track points to the specific effort section (ID_Survey + _Lit + section). | text |
| Date | Date extracted from ns1: time2. | date (yyyy-mm-dd) |
| Time | Time extracted from ns1: time2 (UTC+0), without correction for local time. | time (hh:mm:ss) |
| Time effective | Local time of the departure port, which therefore takes solar/daylight saving time into account (UTC+01:00 or +02:00). Keep the local time of departure port, do not change even if crossing a timezone. | time (hh:mm:ss) |
| Lat | Latitude in decimal degrees (WGS84). | decimal, 7 decimal places |
| Lon | Longitude in decimal degrees (WGS84). | decimal, 7 decimal places |
| Speed (from GPS) | Speed of the ferry at that timestamp. | decimal, 1 decimal place |
| Route (from GPS) | Course direction recorded by the GPS. | integer number |
| Code GPS | Numeric ID of the GPS waypoint. | text |
| Cod points | Type of point recorded (BEG, START, MET, AVV, STOP, END, OS_SIGHT). | text (predefined categories) |
| Sea state | Beaufort sea state recorded during the point (maximum values is 3). | integer |
| Wind speed (knots) | Wind speed measured by observers. | decimal, 1 decimal place |
| Wind state | Wind intensity category according to beaufor scale. | integer |
| Wind direction | Cardinal wind direction (N, NNE, NE, ENE, E, ESE, SE, SSE, S, SSW, SW, WSW, W, WNW, NW, NNW). | text (predefined categories) |
| Rain | Rain conditions during the point. No, Mist, Fine, Drizzle . | text (predefined categories) |
| Visibility | Visibility category following protocol classes. Optimus, Good, Mean, Scarce . | text (predefined categories) |
| % cloud cover | Estimated cloud coverage (%). | integer |
| Note | Free-text note for track-associated information. | text |
| Speed_w | Ship speed recorded by observers (if different from GPS) in knots. | decimal, 1 decimal place |
| Route_w | Ship route recorded by observers. | integer |
| Ship side | Observer's position relative to the ship orientation. L for left, R for right, and Front for observations made from the front. | text (predefined categories) |
| Observer | Name of the observer who made the point/sighting. | text |
| Species (including association) | Indicates whether the sighting involves mixed-species groups (Association) or uncertain identification, for example: Balaenoptera sp., Delphinidae sp., or Association (mixed-species group). | text (predefined categories) |
| Species (best) | Best species identification. Full list in the Legend sheet (e.g., Z.cavirostris, G.melas, D.delphis ...etc) In case of Association duplicate the record and assign the different species of the mixed group. If an identified species is not listed, report it as Other and then specify it in the "note" column. | text (predefined categories) |
| Min_N | Minimum estimated number of individuals. | integer |
| Max_N | Maximum estimated number of individuals. | integer |
| Best | Best estimate of individuals (mandatory field). | integer |
| Juveniles_N | Number of juveniles, if present. | integer |
| Life stage (C. caretta only, ≤ 50 m) | Loggerhead turtle life stage: EJ (early juvenile), LJ (large juvenile), AD (adult). | text (predefined categories) |
| Sex (C. caretta only) | Sex: Male or Unknown . | text (predefined categories) |
| Distance (m) | Estimated distance from observer to first animal. | integer |
| Angle ° | Angle of bearing relative to ship bow. | integer (0–180) |
| Swim direction | Cardinal swim direction relative to North (N, NNE, NE, ENE, E, ESE, SE, SSE, S, SSW, SW, WSW, W, WNW, NW, NNW). Not compulsory for sea turtles. | text (predefined categories) |

| | | |
|-------------------------------------|--|------------------------------|
| Response to ship | Recorded animal behavioural response to the approaching vessel. Apr = approaching, Ind = indifferent, Esc = escaping. | text (predefined categories) |
| Behaviour | First observable behaviour according to predefined categories (Travel, Rest, Play, Feeding net, Feeding wild, Mating, Unknown, Swim, Breath, Other). Other not predefined behaviour can be reported as Other and specified in the notes column. | text (predefined categories) |
| Sup behaviour | Main surface behaviour. Text with predefined categories: Half leap, Full leap, Dorsal fin, Surfing, Blow, Breach, Spyhopping, Other. | text (predefined categories) |
| Note | Additional notes on the sighting (other species, behaviours, etc.). | text |
| Entangled | Whether entanglement was observed. | boolean (t or f) |
| Photo | Photographic material collected. | boolean (t or f) |
| Collision / likely collision | Collision or likely collision observed. | boolean (t or f) |
| Near collision | Near collision event observed. | boolean (t or f) |
| eDNA | eDNA sampling performed at the sighting. | boolean (t or f) |
| <2mn_small | Number of small vessels < 2 nautical miles. | integer |
| <2mn_mot_med | Number of Motor medium vessels < 2 nm. | integer |
| <2mn_sail_med | Number of Sailing medium vessels < 2 nm. | integer |
| <2mn_fish_med | Number of Fishing medium vessels < 2 nm. | integer |
| <2mn_big | Number of Large vessels < 2 nm. | integer |
| >2mn_small | Number of Small vessels > 2 nm. | integer |
| >2mn_mot_med | Number of Motor medium vessels > 2 nm. | integer |
| >2mn_sail_med | Number of Sailing medium vessels > 2 nm. | integer |
| >2mn_fish_med | Number of Fishing medium vessels > 2 nm. | integer |
| >2mn_big | Number of Large vessels > 2 nm. | integer |
| tot_small | Number of Total small vessels. | integer |
| tot_medium | Number of Total medium vessels. | integer |
| tot_big | Number of Total large vessels. | integer |
| Presence | Indicates whether the animal was Present or Absent during maritime traffic data collection. | text (predefined categories) |
| Duplicates | Indicates whether the maritime traffic record is a duplicate, i.e., collected too close in time to the previous one (<15 min). | boolean (t or f) |

Table 3.2. Fields and formatting: Data standard used for Visual Monitoring of Marine Litter (Template: “DB_Litter” available at the [ISPRA folder](#)).

| Field | Description | Format |
|-------------------------------------|--|-------------------|
| “Summary” Sheet | | |
| COD_Effort | Identifier of effort section (survey + Lit + section). es. CBAR_0129_Lita. | text |
| Date | Starting date of the survey. | date (yyyy-mm-dd) |
| Ship name | Name of the vessel (consistent naming across surveys). | text |
| SeaArea | Sea area where the survey was conducted (e.g., Ligurian, Tyrrhenian). | text |
| Segment | Section of the survey (a, b, c...). | text |
| Km on effort | Total kilometres on effort for the section, calculated in QGIS. | decimal |
| STRIP (mt) | Strip width in metres used for litter monitoring (50 m). | integer |
| Area on effort | Monitored area (automatic formula: km_on_effort × strip width, converted to km ²). | decimal |
| Item tot | Total number of >20 cm recorded objects (excluding Natural Matter and Chemical objects). | integer |
| Artificial polymer materials | Total count of artificial polymer/plastic items. | integer |
| Glass | Total count of glass items. | integer |
| Proc. wood | Total count of processed wood items. | integer |
| Metal | Total count of metal items. | integer |
| Textile | Total count of textile items. | integer |
| Paper | Total count of paper items. | integer |

| | | |
|--|--|---|
| Rubber | Total count of rubber items. | integer |
| Food waste | Total count of food waste items. | integer |
| NaturalMatter | Total count of NaturalMatter items. | integer |
| Chemical | Total count of Chemical items. | integer |
| Density of Item Tot | Density of total items: Item_tot / monitored area (km ²). | decimal |
| "Track" sheet | | |
| Source | Origin of GPS or data source. | text |
| Lat | Latitude (WGS84). | decimal (7 decimals) |
| Lon | Longitude (WGS84). | decimal (7 decimals) |
| ns1_time2 | Original timestamp from GPX (UTC+0). | text |
| Date | Date extracted from timestamp. | date (yyyy-mm-dd) |
| Month | Month of survey. | integer (1-12) |
| Year | Year of survey. | integer (4 digits) |
| Time_2 | Time extracted from timestamp (UTC+0). | time (hh:mm:ss) |
| Time_effective | Local time corrected for the timezone of the departure port. | time (hh:mm:ss) |
| COD_Effort | Identifier of effort section (survey + section, es. CBAR_0129_Lita). | text |
| ID_Survey | Unique survey ID (transect code + progressive number, es. CBAR_0129_Lit). | text |
| ID_Transect | Transect code (4 letters, es. CBAR). | text |
| Survey_N | Progressive survey number, es. 0129. | integer |
| Section | Effort section (a, b, c...). | text |
| Cod_GPS | GPS waypoint code. | integer |
| Cod points | Point category (BEG, END, START, STOP, MET, LIT, OS_SIGHT, TURTLE, FAD, OTH). | text (predefined categories) |
| Source1 | Additional data source if applicable (Land Sea, Indetermined (default)). | text (predefined categories) |
| Sector | Sector of observation (Fishing related, Aquaculture Food, Sanitary, Cosmetic, Agriculture, Clothes, Construction related, Smok. related, Recreation related, Vehicles, Hunting, Undefined). | text (predefined categories) |
| Buoyancy | Positive, Negative, Neutral. | text (predefined categories) |
| Material | Material category (ArtificialPolymerMaterials, GlassCeramic, Proc.wood, Metal, TextileClothes, PaperCartboard, Rubber, Chemical, FoodWaste, NaturalMatter). | text (predefined categories) |
| General name | General litter item General name according to Joint List (and additional sheet "Legend"). | text (predefined categories) |
| Size | Size class of object. | text (predefined categories) |
| Colors | Dominant colors of objects (White, Transparent, Colored Red, Blue, Green, Grey, Yellow, Brown, Black, Pink, Orange, Other). | text (predefined categories) |
| Object state | If the object is Fragment or Entire . | text (predefined categories) |
| Note | Additional notes. | text |
| Sea state | Beaufort scale (0, 1, 2, 3). | integer |
| Strip | Strip width activated. Should be always set to 50 (m). | integer |
| Mean speed | Ship speed averaged over interval. | decimal (1 decimal) |
| Cloud cover | % cloud coverage. | integer (0-100) |
| J-CODE | Code from the Joint List of Marine Litter categories. | text (predefined codes: refer to the additional sheet "Legend") |
| SUP_FG | Indicates whether the item falls within the SUP (Single Use Plastic) or FG (Fishing Gear) categories, as defined in the Joint List for the corresponding J-code; otherwise, the field is left empty . | text (predefined codes: refer to the additional sheet "Legend") |
| Ship side | Observer position (L, R, Front). | text (predefined categories) |
| Observer | Name of observer. | text |
| Hour | Hour of observation (local). Can stay empty. | time (hh:mm:ss) |
| Species (including association) | . | text (predefined categories) |

| | | |
|-------------------------|--|---|
| Species (best) | Best species identification. Full list in the Legend sheet (e.g., <i>Caretta caretta</i> , <i>Mola mola</i> ...etc) In case of Association duplicate the record and assign the different species of the mixed group. If an identified species is not listed, report it as Other and then specify it in the "note" column. | text (predefined categories) |
| N_min | Minimum individuals estimated. | integer |
| N_max | Maximum individuals estimated. | integer |
| N_best | Best estimate of individuals. | integer |
| Juveniles_N | Number of juveniles observed. | integer |
| Distance m | Estimated distance to sighting. | integer |
| Angle ° | Bearing angle relative to bow (0–180). | integer |
| Swim direction | Cardinal swim direction relative to North (N, NNE, NE, ENE, E, ESE, SE, SSE, S, SSW, SW, WSW, W, WNW, NW, NNW). Not compulsory for sea turtles. | text (predefined categories: N, NNE, NE...etc.) |
| Response to ship | Recorded animal behavioural response to the approaching vessel. Format: text (predefined categories: Apr = approaching, Ind = indifferent, Esc = escaping). | text (predefined categories) |
| Behaviour | First observable behaviour according to predefined categories (Travel, Rest, Play, Feeding net, Feeding wild, Mating, Unknown, Swim, Breath, Other). Other not predefined behaviour can be reported as Other and specified in the note's column. | text (predefined categories) |
| Surface behavior | Main surface behaviour. Text with predefined categories: Half leap, Full leap, Dorsal fin, Surfing, Blow, Breach, Spyhopping, Other . | text (predefined categories) |
| Other | Additional notes. | text |
| Photo | Photographic material collected. | boolean (t or f) |
| Collision | Collision or likely collision observed. | boolean (t or f) |
| Near collision | Near collision event indicated. | boolean (t or f) |

3.2 Quality checks at the end of each season (by Partners)

At the end of each season (every 3 months) and again before the annual submission, partners perform a quality control process to ensure that datasets are complete and coherent.

3.2.1 Completeness, category compliance and internal consistency

Partners conduct a combined review of completeness, formatting and internal coherence. This includes verifying that:

- **All fields are complete**, and all predefined *mandatory fields* (ID_Survey, COD_Effort, Date, Time, Lat, Lon, COD_Point) contain valid entries with correct formatting.
- **COD_Effort codes and sections** are assigned correctly and consistently across all tables.
- Controlled vocabularies match exactly the **predefined categories** provided in the template legends (see also Tables 3.1 and 3.2);
- Numeric fields contain a **single value** (no intervals such as "3–5");
- **Units of measurement** are correctly applied, particularly distances, which must always be expressed in **metres**;
- Entries are **biologically and operationally coherent** (e.g., no impossible behaviours or conditions);
- Timestamps fall within the correct **effort section**;
- Coordinates do not show **implausible jumps** or positions outside the expected sea area; distance, angle and sea-state values fall within protocol-defined ranges;
- Strip transect values match the declared monitoring mode;

- Combinations of **Material**, **Buoyancy**, and **Object state** are coherent;
- Duplicate records are identified and removed.

3.2.2 GIS inspection of geographical data

Tracklines and associated observations are visually inspected in GIS (QGIS or ArcGIS) to verify their spatial accuracy and coherence. This step ensures that all geographical information is consistent with vessel navigation and with the declared monitoring effort.

The checks include verifying that:

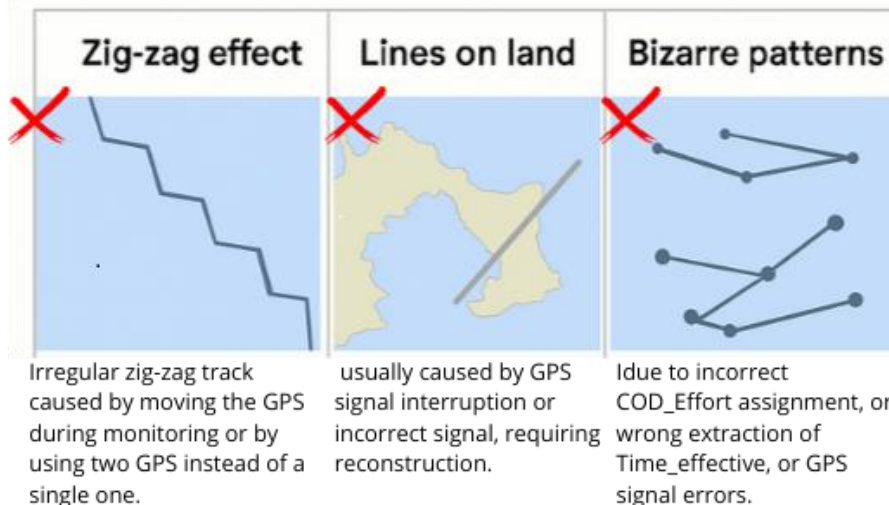
- there are no GPS-related errors, duplicated points, or incorrect waypoint sequences;
- vessel speeds are realistic and do not show abrupt or impossible accelerations or course changes;
- no clusters of points fall far from the transect line;
- there are no time gaps incompatible with the expected navigation speed.

Additional checks can be performed using formulas listed in **Box 3.3**. Spatial outliers are corrected when possible (e.g., adjusting coordinates or timestamps) or removed if they do not meet the quality criteria. Most issues occur at **waypoints**; in these cases, the recommended approach is to copy all the information of the problematic waypoint to the closest valid point in time.

BOX 3.3 - LIST OF RECOMMENDED GEOGRAPHICAL CHECKS:

- **Visual inspection** (by zooming to a sufficient resolution and moving along the tracklines)
- Check on the **track length** (field calculator -> "\$length/1000"
- Check on the **travelling speed** (field calculator -> "(\$length/1000)/1.852)/(hour ("end" - "begin"))"
- Divide in grid cells (e.g., 1x1 or 5x5Km) and calculate the length of the effort that must not exceed the length of the diagonal
- Check on the **bearing** (field calculator -> "(atan((xat(-1)-xat(0))/(yat(-1)-yat(0)))) * 180/3.14159 + (180 * (((yat(-1)-yat(0)) < 0) + (((xat(-1)-xat(0)) < 0 AND (yat(-1) - yat(0)) > 0)*2)))"

Examples of common errors:



3.3 Data submission at the end of the year (by Partners)

3.3.1 Exporting data into CSV format

At the end of the year, once all data have been validated, including the complete set of quality checks and the geographical verification, partners export the macrofauna datasets into **UTF-8 CSV files** structured according to the Common Database requirements. All columns must follow the correct formatting, naming conventions, predefined categories, and field order specified in the official templates. The export must comply with the structure of the main CSV tables (Provider, Main, Track, Weather, Cet, Turtle, OS, Naval, Cet_off, OS_off). Partners must use the official CSV templates available in the dedicated ISPRA folder ([Link](#)).

A recommended workflow is to start from the Excel master template, where all tables are organised in separate sheets. Information is first copied from the Excel files used for data storage (e.g., Macrofauna template), ensuring that each sheet is completely and correctly formatted. Each sheet is then exported as an individual CSV file using UTF-8 encoding.

After exporting, an additional check must be carried out on the Track CSV to remove any duplicated track points, as duplicates may prevent correct database ingestion and interfere with automated validation procedures.

For Marine Litter data, partners may submit the annual Excel file in the official DB_Litter format, provided that all fields respect the required structure, formatting, and predefined categories.

3.3.2. Checking for unmatched data

Before uploading, a final verification is performed to identify any **unmatched records**, ensuring full internal consistency between CSV track files and all related tables. This check relies on the combined use of COD_Effort and datetime as unique keys, confirming that every sighting, litter observation, or associated entry matches a valid track point to the second. These consistency checks can be carried out using different tools, such as R, OpenRefine, or equivalent data-cleaning software.

3.3.3 Uploading the finalised data to the library

Once all checks are completed, the final CSV files are uploaded by partners to the centralised **SinaNET library**, in their dedicated partner-specific directory (accessible by password at this [link](https://groupware.sinanet.isprambiente.it/life-conceptu-maris/login), <https://groupware.sinanet.isprambiente.it/life-conceptu-maris/login>). Files must follow the agreed naming convention (e.g. Nametable_TransectCode_Year.csv) to allow clear versioning and facilitate automated ingestion into the Common Database. Partners access the shared library via the dedicated link and, after uploading their files, **notify ISPRA by email** to confirm that the data have been successfully uploaded and are ready for centralised validation and ingestion.

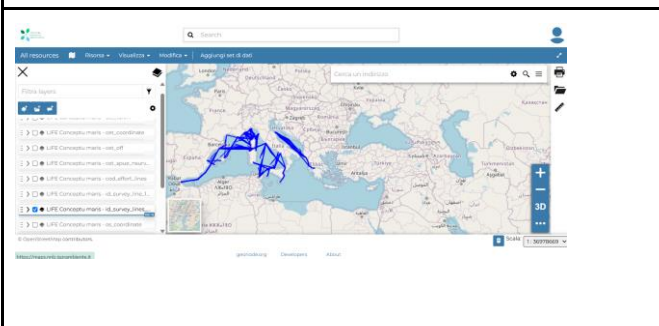
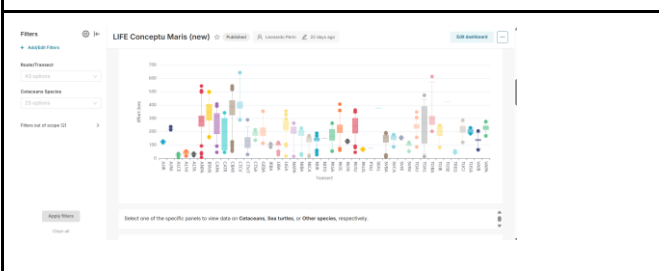
3.4 Final data validation (by ISPRA)

Data validation at ISPRA is a multi-step process designed to ensure the accuracy, consistency, and reliability of all submitted datasets before they are stored and used for analysis. This rigorous procedure safeguards data integrity and supports precise analyses, reporting, and decision-making. This phase include:

- **CSV manual consistency check.** All CSV files are visually examined by ISPRA staff to verify their structural correctness, the presence of mandatory fields, alignment with the Common Database schema, and full consistency between track, sightings, and litter tables, as well as the geographical correctness of the data;
- **CSV upload into the Common DB and automated validation.** Once verified, the finalized CSV files are uploaded and ingested into the Common Database, where automated checks confirm coherence across tables and detect potential formatting inconsistencies;
- **Creation of secondary tables.** After successful ingestion, ISPRA generates and updates the secondary and derived tables used for summary statistics, effort calculations, spatial aggregation and dashboard visualizations, ensuring that validated datasets are fully integrated and ready for visualisation.

3.5 Data sharing

Once validated and correctly uploaded, ISPRA integrates the new data into the Common Database and updates the related visualization and analysis tools. The datasets then become accessible through two main interfaces that provide harmonized and validated information for internal consultation, analysis, reporting, and collaborative work across the project: the GeoNode Web-Gis interface and the Dashboard Stats Interface, accessible by password (BOX 3.4).

| BOX 3.4 - VISUALISATION INTERFACES | |
|---|--|
| <p>GeoNode Web-GIS (Link, https://maps.nnb.isprambiente.it/catalogue/#/)</p>  | <p>The GeoNode Web-GIS platform offers a spatial visualization environment where tracklines, sightings, and litter observations can be viewed, filtered, queried and exported as georeferenced layers, ensuring rapid inspection and comparison of survey data across years and transects.</p> |
| <p>Dashboard Stats interface (Link, http://superset.isprambiente.it/)</p>  | <p>The Dashboard Stats interface provides a complementary analytical view, allowing interactive exploration of aggregated metrics, temporal trends, species occurrence, litter density and effort-standardized indicators, with options for filtering and exporting summary outputs for reporting and further analysis</p> |

4. Data Analysis

The analytical phase transforms the harmonised datasets collected across the monitoring programme into robust ecological indicators supporting the assessment of the conservation status of CEPTU species. The procedures described in this section provide a coherent and standardised framework for deriving the indicators required under the EU Habitats Directive (92/43/EEC) and the Marine Strategy Framework Directive (2008/56/EC). Although these directives specify the assessment elements, they do not prescribe the analytical methods.

The methodologies proposed here therefore translate policy requirements into practical, scientifically robust, and reproducible analyses that can be consistently applied across species, regions, seasons and reporting periods. Through harmonised workflows, the analyses quantify spatial distribution, population abundance, habitat preferences, and exposure to major anthropogenic pressures. Each indicator is computed using dedicated and validated methods, described in the following sections. A final synthesis of the analytical approaches proposed for the indicators described in Box 4.1.

4.1 Population

When dealing with species conservation, one of the key parameters that needs to be assessed is population size. Ideally, the real abundance of a given population shall be assessed at a defined temporal interval, to determine the trend over the years. When dealing with 'rare' species, inhabiting and moving in very wide areas, such as cetaceans, reliable abundance estimations are very difficult, if not impossible, to obtain. When an effective, replicable and bias-controlled survey design is available, including determined protocols for data collection, it is possible to overcome the issue of absolute abundance with the computation of an **Abundance Index**. An Abundance Index is a parameter that considers the number of observation events of a species in relation to the effectively surveyed area. The sampling design and data collection protocols described in the Data collection (Chapter 2) allow for an almost 'ready to apply' data analysis for the estimation of abundance index over different temporal and spatial scales. To compute Abundance Index using data collected from ferries, the following analyses shall be done, as detailed in Table 4.1:

- **Computation of ESW** (see tab 4.1) for each species/group of species, each different type of platform and considering weather conditions. ESW can be computed using the R package Rdistance.
- **Definition of observation events:** it is possible to use sightings (not considering the number of individuals in each sighting) or individuals (considering the real number of individuals observed, or a best estimation in case of large groups). It is always better to analyse both and see whether differences occur (e.g., even species whose individuals are usually sighted alone might aggregate during particular years; number of encountered groups might not vary but the size of the groups might vary)
- **Check for autocorrelation in the dataset**, especially if routes are monitored along the outbound/return trips within the same day or week. A random selection of either outbound/return is advised if a correlation is found. **Important:** correlation shall be checked separately for each species, as it might depend on the species ecology.

Once the Abundance index has been computed separately for each survey, it can be used to understand the yearly relative abundance of the species (as a measure of the overall mean and standard deviation). When looking for trends, a 2-years left rolling mean can help smoothing out peaks due to anomalous years, evidencing the underlying pattern.

For the purposes of HD and MSFD, trends shall be understood considering the defined reporting periods. Non-parametric tests (i.e., Kruskal-Wallis and post-hoc Dunn test) can be used to assess differences among reporting periods.

Tab 4.1 – Suggested metrics for the assessment of the Abundance parameter.

| Parameter Component | Indicator/Metric | Calculation Method |
|-------------------------------------|--|---|
| Abundance Index | Density of animals (D) | $D = N / (L \times 2ESW)$, where N is total sighted animals, L is total transect length, and ESW is Effective Strip Width |
| Sighting Count Density Index | D_{count} | $D_{count} = (count / 2ESW \times L) \times 100$ (based on number of sightings) |
| Individual Sum Density Index | D_{sum} | $D_{sum} = (count / 2ESW \times L) \times 100$ (based on number of individual) |
| Effective Strip Width (ESW) | Estimated using a detection function fitted to perpendicular sighting distances (x). Multiple-covariate methods are applied, accounting for factors like species, ferry type (height of command deck), sea state, and wind speed | ESW is the perpendicular distance where animals detected at greater distances equal those missed at lesser distances |
| Trend Analysis | Rolling Mean (moving average) | Used to smooth interannual variability, particularly for fin whales. Density for a year is computed considering data from the year before (e.g., two-year rolling mean) |
| Reporting Periods | Trend assessment periods | Data is analyzed based on HD reporting cycles: 2008–2012, 2013–2018, and 2019–2024, etc. |

4.2 Range

Assessing species range is essential for assessing species conservation status and is best achieved by combining complementary indicators that capture both the extent and spatial dynamics of distributions. The range metrics suggested to assess the Conservation Status of CEPTU species are based on the EU HD and IUCN Guidelines, refined by the LIFE project Conceptu Maris using long-term FLT Med Net data. These include **Extent of Occurrence (EOO)**, **Area of Occupancy (AOO)**, and the **Observed Distributional Range (ODR)** derived from KDEs, alongside the **Ecological Potential Range (EPR)** from SDMs. Used together, these indicators provide a comprehensive picture of range changes, distinguishing between expansion, contraction, and spatial shifts. The **ODR** reflects the area actually occupied and must be interpreted relative to the survey effort (EffortArea) to avoid underestimation. The **EPR**, derived from SDMs, identifies the extent of suitable habitat based on environmental variables but may overestimate range if limiting factors displace the species. Assessing **range patterns** is therefore essential, as changes may involve not only variations in total extent but also spatial shifts; for example, an increase in surface area may coincide with a shift toward less suitable offshore regions. When integrated with population and habitat metrics, they enable evaluation of whether a species' range remains stable or increasing and above its Favourable Reference Range. Suggested metrics are summarized in Table 4.2.

Tab 4.2 – Suggested metrics for the assessment of the Range parameter

| Parameter Component | Indicator/Metric | Calculation Method |
|---|---|---|
| Range Extent (Observed) | Extent of Occurrence (EOO) | Calculated as the entire area the species is known to exist by drawing the smallest possible continuous boundary (Minimum Convex Polygon - MCP) around all observed presence sites, while excluding vagrancy sites. |
| Range Extent (Observed) | Area of Occupancy (AOO) | Determined by applying a grid system (the European 10x10 km UTM grid, ETRS89-LAEA projection) to the map and counting the number of occupied cells. |
| Range Extent (Observed) Distributional Range - ODR | Kernel Density Estimator (KDE) Area | Calculated using KDE to spatially generalize the species distribution. This approach uses a resolution cell of 500 m and a search radius of 50,000 m (50 km). The extent of the ODR (km ²) is defined by the 95% isopleth. KDE generation uses Sighting per Unit Effort (SPUE) values for common species, and presence points for less common species (e.g., <i>Grampus griseus</i> , <i>Ziphius cavirostris</i> , <i>Physeter macrocephalus</i>). |
| Range Extent (Ecological Potential Range - EPR) | Extent of Suitable Habitat (km ²) | Derived from outputs of Species Distribution Models (SDMs), primarily implemented using the MaxEnt algorithm. The model output (continuous suitability map) is classified (e.g., into 12 classes). The EPR is delineated using the Jenks natural breaks threshold that separates the second and third classes, representing the extent of suitable area. |
| Range Trend (Observed) | Change in ODR Extent (Δ distribution) | Compares the proportion of species ODR inside the Effort Area between two consecutive HD reporting periods. Calculation: $\Delta \text{ distribution} = [(ODR/EffortArea(2nd \text{ period}) - ODR/EffortArea(1st \text{ period})) \times 100]$ |
| Range Trend (Potential) | Change in EPR Extent | Measures the percentage difference in suitable area predicted by SDMs between two HD reporting periods. Calculation: $[(EPR(2nd \text{ period}) - EPR(1st \text{ period})) / EPR(1st \text{ period})]$ |
| Range Trend (Pattern) | Range Pattern (Overlap and Shift) | Assessed in terms of: a) Overlapping area between ODRs/EPRs of two periods (for ODR, only considering the common effort area). b) Percentage of overlapping area compared to the first period: $[(Overlapping \text{ area}/Area \text{ 1st period}) \times 100]$. c) Direction and magnitude of shift in the centroids of the range area (calculated using geometric spatial zonal statistics in GIS). |
| Habitat Occupancy | ODR vs. EPR (Proportion of Occupied Suitable Habitat) | Calculates the proportion of the predicted suitable habitat (EPR) that is effectively occupied by the species (ODR). Calculation: Proportion occupied (%) = $[(ODR/EPR) \times 100]$. Trend is assessed by calculating differences between periods |

4.3 Habitat

The assessment of the "*Habitat for the species*" parameter is a fundamental requirement of the HD. Since the ecological niche or habitat quality of highly mobile species like CEPTU species in pelagic waters often remains largely unknown or poorly resolved, the analytical framework relies mainly on SDMs to identify the ecological driver of species distribution.

The overarching utility of the suggested Habitat assessment framework is to move beyond basic species presence mapping toward a detailed, ecologically informed understanding necessary for robust conservation planning and mandated HD reporting. The use of SDMs is essential for exploring the spatio-temporal distribution patterns and identifying the environmental conditions that define a species' realized niche in offshore habitats, and in the areas where direct monitoring is scarce. In general, the preference for the 5 km spatial resolution compared to the EEA HD 10x10 grid provides superior generalization capacity, stronger environmental signal integration, and

higher spatial precision for subsequent management analyses. Temporal stratification, especially across seasonal and HD reporting periods, is crucial for capturing dynamic shifts in habitat suitability that may be linked to environmental or climate-related changes. Furthermore, the framework intentionally integrates innovative data sources: eDNA provides complementary validation for SDMs and enhances distribution assessment by capturing nocturnal or elusive species, helping to correct underestimations of species range. Details for the application of the suggested methodological framework for the assessment of the Habitat parameter is provided in Table 4.3.

Tab 4.3 – Methodological framework suggested for the ‘Habitat for the species’ parameter

| Parameter Component | Indicator/Metric | Calculation Method |
|---|---|--|
| Core Methodological Tool | Species Distribution Models (SDMs) (Suggested Algorithm: MaxEnt) | SDMs are implemented using the MaxEnt Java software and the logistic output format to generate continuous habitat suitability maps (scale 0 to 1). |
| Data Input and Resolution | Input Data: Species presence data; Predictors: Extensive set of static (e.g., bathymetry, distance to canyons) and dynamic (e.g., SST, salinity, EKE) environmental variables | All environmental layers are averaged across temporal resolutions (seasonal/reporting periods) and spatially aligned/resampled to a 5 km cell size (EPSG:3035). A bias file is incorporated to guide the selection of background points, accounting for uneven survey effort. |
| Model settings (apply only for Maxent) | Maximum 5000 iterations, 10,000 background points, logistic output format, 100 bootstrap replicates, Jackknife test for variable importance, and use of a bias file to correct for sampling effort. | Models can be run using the Java MaxEnt software, using species CSV files containing species, X, Y coordinates, together with environmental raster variables prepared at the appropriate temporal resolution and projected in EPSG:3035, provided in .asc format. |
| Suitable Area Extent (EPR) | Ecological Potential Range (EPR) (Extent of Suitable Habitat in km ²) | Derived from the continuous SDM output. |
| Core Habitat Area | Core Zone (Highest predicted suitability) | Defined by threshold like Maximum Test Sensitivity plus Specificity Logistic Threshold (suggested) computed by the MaxEnt software OR using the Jenks natural breaks threshold separating the second and third classes (after excluding zero values) to delineate core and extended suitable area. |
| Seasonal Assessment | Seasonal Patterns in Habitat Use | SDMs are developed using temporally stratified datasets corresponding to the four seasons: Winter, Spring, Summer, and Autumn. |
| Temporal Assessment | Trend Assessment (Changes in extent and spatial shift) | SDMs are developed separately for the HD reporting periods (e.g., 2008, 2012, 2013–2018, 2019–2024). Comparison of total predicted suitable areas across periods quantifies habitat trend. |
| Model Validation (Internal) | e.g., Area Under the Curve (AUC), Boyce Index (CBI) | Calculated across all model replicates (100 bootstraps replicates) using 70-80% dataset for Training and 30-20% for Testing. |
| Model Validation (External) | AUC, Sensitivity, Specificity, F1-score | Assessed by testing model predictions against independent datasets (e.g., sightings from other programmes, eDNA presence records) not used in model training. |
| Integration of Novel Data | eDNA presence data | eDNA presence records are compiled and spatially filtered to be used primarily for the independent external validation of SDM outputs ot to incorporate prey data into habitat models, improving ecological realism. |

4.4 Conservation Priority Index

Identifying key areas for specific taxa ensures efficient use of limited conservation resources by protecting sites critical for biodiversity persistence, life-history functions, and recovery of threatened species. Priority sites are recognized through frameworks such as Ecologically or Biologically Significant Areas (EBSAs), Key Biodiversity Areas (KBAs), Important Marine Mammal Areas (IMMAs), and Important Shark and Ray Areas (ISRAs), which assess ecological value, vulnerability, and life-history requirements (Becker et al., 2025; Corrigan et al., 2014; Tetley et al., 2022; IUCN, 2016). EBSAs, KBAs, and IMMAs identify sites based on criteria such as uniqueness, life-history importance, presence of threatened species, aggregations, and key life-cycle activities (Corrigan et al., 2014; Johnson et al., 2018; Tetley et al., 2022; Becker et al., 2025).

Building on this framework and making use of the available high-resolution data on species' biological traits, which allow comparisons across space and seasons, LIFE Conceptu Maris and FLT Med Net developed a multi-criteria index to identify and prioritize conservation areas for CEPTU species in the Mediterranean Sea.

The Conservation Priority Index (CPI) is a tool designed to provide a standardized and objective framework to support flexible and adaptive management strategies, an essential approach for wide-ranging migratory species whose habitats are dynamic and increasingly influenced by climate change. By integrating multiple criteria, the index helps policymakers make more efficient and informed decisions, targeting conservation priorities across time and space.

Tab 4.4 – Ecological Priority Criteria (Indicators) for calculating Conservation Priority Index

| Indicator/Ecological Priority Criteria | Ecological Significance | Criteria and Thresholds Score 1 if: | Metric |
|--|--|--|--|
| Species Richness | Assesses community structure and biodiversity health (number of species) | Higher than 50% of the number of Mediterranean population species are present | $N_s = \frac{\text{Number of species in area}}{\text{Total Mediterranean population species}}$ |
| Diversity (Shannon-H Index) | Reflects how evenly individuals are distributed within the community | Over half the Shannon maximum value (based on sightings) | $H_s = \frac{H_{\text{area}}}{H_{\text{max}}}$ |
| Dominance (Dominance_D Index) | Highlights if a few species heavily dominate the community | Below half the Dominance value (based on sightings) | $D_s = 1 - \frac{D_{\text{area}}}{D_{\text{max}}}$ |
| Abundance of sensitive species (SPUE) | Acts as a proxy for relative abundance, density, and habitat use, standardizing sightings by effort | Higher than the mean SPUE recorded across all areas together (by season) | $ER_s = \frac{ER_{\text{area}}}{\text{mean ER across all areas}}$ if >1, else 0 |
| Rarity (R) | Measures the conservation value of areas that are essential for the survival of rarer Mediterranean species (i.e., <i>G.griseus</i> , <i>G.melas</i> , <i>Z.cavirostris</i> , <i>P.macrocephalus</i>) | Species is present | If present 1, else 0 |
| Mean Group Size (GS) | Reflects population density, social structure, and behavioural responses to the environment | Higher than the mean Group Size recorded across all areas together (by season) | $GS_s = \frac{GS_{\text{area}}}{\text{mean GS across all areas}}$ if >1, else 0 |
| Juvenile Presence | Acts as a proxy for identifying breeding or nursery grounds, critical for assessing recruitment areas | Score assigned if over the percentage of total sightings with juvenile presence across all areas and seasons | $J_s = \frac{\text{juvenile sightings \% in area}}{\text{max juvenile sightings \% across areas}}$ |
| Range-restricted species | Measures the conservation value of areas that are essential for the survival of range-restricted species | Score assigned if the species is present in fewer than 5 areas (considering all seasons) | $R_s = \begin{cases} 1 & \text{if species present in } <5 \text{ areas} \\ 0 & \text{otherwise} \end{cases}$ |
| Summing scores: | | | CPI = \sum Indicator Scores |
| NOTE: Average or sum the indicator scores across seasons to account for temporal variation. | | | |

The rationale for developing this composite index is that relying on a single conservation metric, such as species richness or abundance, can lead to a fragmented understanding of an area's importance. The Conservation Priority Index addresses this by combining multiple ecological indicators into a single, holistic assessment.

4.5 Threat analysis and Risk Exposure Assessment

The risk exposure assessment integrates three main data layers, **Floating Marine Macro Litter** (FMML), **Species Encounter Rate** (ER), and **Maritime Traffic** (MT), to identify areas where cetaceans and turtles are most exposed to human pressures.

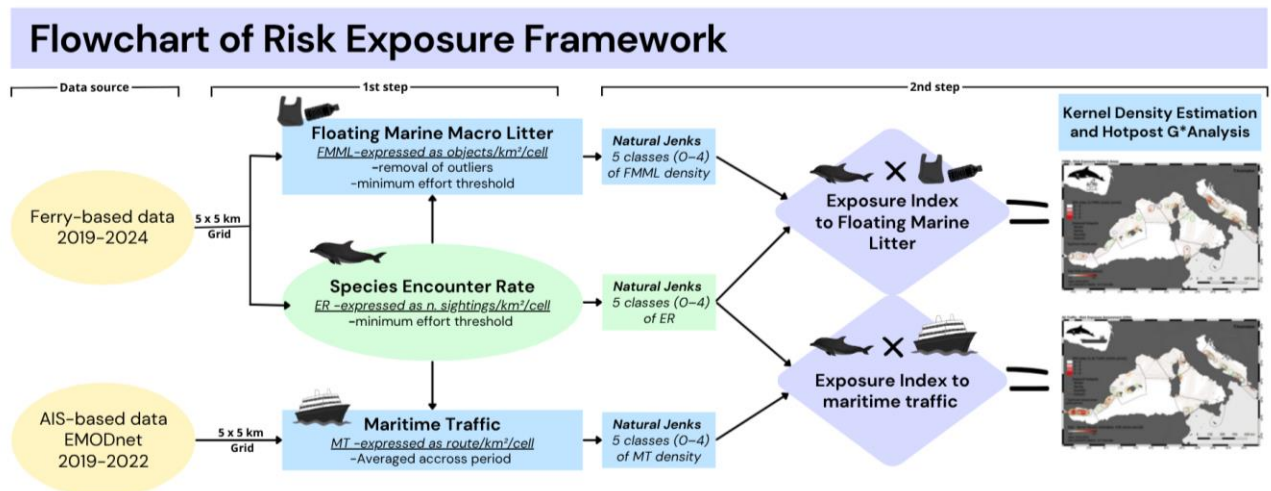


Fig 4.1 - Workflow for threat and risk exposure analysis

4.5.1 Data source

Floating Marine Macro Litter (FMML) (≥ 20 cm) and species sighting data collected through ferry-based monitoring using a fixed-width strip transect methodology. Maritime traffic density (AIS-based) obtained from EMODnet as monthly route density layers for the same period of FMML monitoring. All datasets need to be harmonized and aggregated into the 5×5 km EEA (European Environmental Agency) standard grid.

4.5.2 Threat analysis (1st step)

In this first step, the spatial density of each pressure (FMML and MT) and the species encounter rate (ER) are estimated on the 5×5 km EEA standard grid. Analyses can be conducted both seasonally and across the entire multi-year period.

- **FMML density** is calculated as the number of items per sampled km^2 within each 5×5 km grid cell. A buffer equivalent to the strip width is applied to each survey transect, intersected with grid cells to compute for each cell: 1) the total sampled area; 2) the number of floating litter items ≥ 20 cm; 3) the density for each cell calculated as $D = n \cdot \text{objects} / \text{total sampled area}$. A minimum effort threshold of 0.25 km^2 per cell is used. Then, eventual residual outliers are removed using the upper-whisker threshold ($Q3 + 1.5 \times \text{IQR}$).
- **Species Encounter Rate (ER)** is expressed as the number of sightings of the species per km per cell, applying a minimum effort threshold of 5 km per cell.

- **Maritime Traffic (MT)** is calculated as the average route density (route/km² per cell) across the AIS time series.

4.5.3 Risk Exposure Assessment - REA (2nd step)

Each of the three layers measured at the previous step (FMML, ER, MT) are classified into **five classes (0–4)** using Natural Jenks optimization. Then the **Risk Exposure indices** are calculated as:

- **Risk Exposure to Marine Litter = ER × FMML**
- **Risk Exposure to Maritime Traffic = ER × MT**

Values range from **0 (no exposure)** to **16 (maximum exposure)**.

Cumulative exposure layers can be produced by summing species-specific indices for groups of species (e.g., all cetaceans, low-density cetacean species, sea turtles, cetaceans and sea turtles combined).

To identify statistically significant hotspots of exposure two levels of analysis can be performed:

- **Kernel Density Estimation (KDE)**, weighted by exposure index, is applied to grid centroids using a 50 km search radius (bandwidth), a 500 m output resolution, and a quartic kernel function. The 90th percentiles isopleths are taken to delineate areas of highest exposure;
- **Getis-Ord Gi*** hotspots are computed with a 25 km neighbourhood to detect clusters of high exposure ($G_i^* > 1.96$).

The resulting maps highlight areas where species presence consistently overlaps with marine litter or maritime traffic pressure, providing a spatial basis for risk prioritization.

4.6 Near Miss Events

Encounter Rates of NME (number of NME.km⁻¹; ER_NME) are calculated as the numbers of NME events over the effort in km per cell (e.g., EEA 10x10 km grid cell). Those values are then used for interpolation using the Kernel Density Estimation (KDE) method (Qgis software via the “Heat Map” module). This method interpolates the known values to estimate the unknown values in other neighboring cells within a smoothing radius ‘Rb’ as:

$$Rb = 0.9 * \left(\frac{sd}{\sqrt{\ln(2)}} * Dm \right) * n^{-0.2}$$

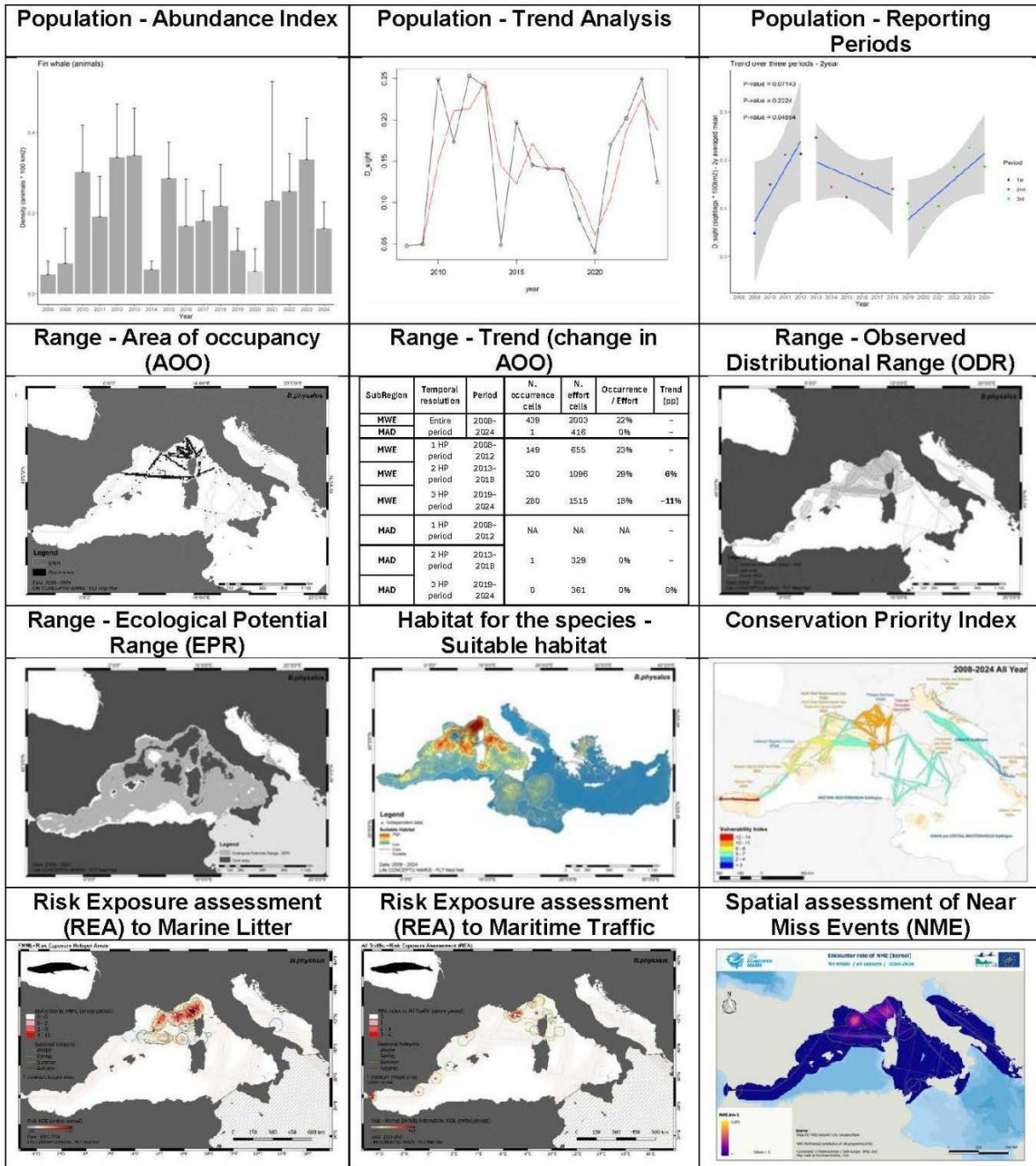
Where:

- Rb= Core bandwidth radius (in m)
- Sd= Standard distance (a single summary measure of the distribution of features around their geometric mean center)
- Dm= Median distance to mean center (distance of each point of observation relative to the barycenter of the points cloud)
- N=number of observations (=number of cells with effort)

The weight assigned to each centroid point of the cell used for the KDE analysis is the ER_NME. Sd and Dm are calculated using the “Spatial point pattern analysis” processing tool in QGis. The

isopleths highlighting the areas with ER_NME positive values, obtained by the interpolation, are defined as values > one fifth (1/5) of the mean, the rest are values near zero.

BOX 4.1 - SUMMARY OF INDICATORS



5. Integrating Data into the Decision Support System (DSS)

The systematic monitoring described in Chapters 2-4 generates a considerable amount of high-resolution data. To transform this data into information that can be immediately used for management and spatial planning, the LIFE project Conceptu Maris has developed a Decision Support System (DSS). The DSS is a digital tool that integrates *in-situ* data with open-source data (oceanographic, model-based, maritime traffic) and the results of statistical analyses (Chapter 4). It provides a synoptic and interactive cartographic representation. The goal is to allow managers and decision-makers to overlay key areas for species (suitable habitats, priority areas) with current conservation strategies (Natura 2000, IMMAs, SPAMIs, Particularly Sensitive Sea Areas, Exclusive Economic Zones, MSFD subregions) and anthropogenic pressures (Maritime Traffic, Marine Litter). This makes it possible to identify potential protection gaps and define targeted improvement actions.

The DSS is publicly accessible at the following link: <https://conceptumaris-dss.nnb.isprambiente.it/>

The DSS is directly connected to the **ISPRA Common Database** hosted by the **National Biodiversity Network** (NBB, <https://www.nnb.isprambiente.it/en>), which serves as the central, authoritative repository for all project data following the procedures described in Chapter 3. All *in-situ* data (visual observations, eDNA samples, SIA samples, FMML records, maritime traffic data) must first be uploaded to the Common Database following the procedures in Sections 3.1-3.4. Only after successful validation and ingestion by ISPRA (Section 3.4) will the data become accessible for visualization within the DSS. This direct connection ensures that the DSS always visualizes the most up-to-date and validated data, preventing duplication and guaranteeing full alignment across the system.

In addition to project-collected data, the DSS integrates key open-source datasets from European services to provide a comprehensive oceanographic and anthropogenic context. These include:

- **Copernicus Marine Service data:** Model-based and satellite observations including sea temperature, salinity, currents, sea level, mixed layer depth, chlorophyll, and primary production (Mediterranean Sea Physics and Biogeochemistry Analysis and Forecast products).
- **European Marine Observation and Data Network (EMODnet) data:** Human Activities route density maps (AIS-based traffic data for cargo, fishing, passenger, tanker and other vessels).

5.1 Requesting Data Integration into the DSS

To request the integration of new datasets into the DSS, users must first complete the registration process on the DSS portal. Registration requires creating an account by providing a valid Username/Email and Password. Once registered, users must send a formal request via email to info@conceptumaris-dss.nnb.isprambiente.it, specifying the datasets they wish to integrate and including all relevant metadata as described in Section 5.2. The request will be reviewed by the DSS management team, and upon approval, the data will be processed and made available for visualization within the system.

5.2 Criteria for Data Integration into the DSS

To ensure interoperability and the correctness of spatial analyses within the DSS, all data must comply with the standardized procedures for data management (Chapter 3) and the methodological requirements for subsequent analysis (Chapter 4). The following criteria summarize the key requirements; detailed procedures for data preparation, formatting, and validation are provided in Chapter 3.

- **Compliance with Common Database Standards**

Format and Structure: All data must be prepared using the standardized templates provided by ISPRA and exported according to the specifications detailed in Sections 3.1 and 3.3.

CSV Data: Tabular data must be exported as CSV files following the official CSV templates structure (Section 3.3.1). This applies to all observation data (sightings, tracks, weather, traffic, litter) that will be ingested into the Common Database before visualization in the DSS.

Vector Data: Geospatial data such as tracklines, sighting points, survey areas, polygon layers (e.g., ODR, EOO, AOO, MPA boundaries), and isopleth contours must be provided in standard open formats such as GeoJSON, Shapefile (zipped), or as services (WMS/WFS). Vector data must include complete attribute tables following the Common Database structure.

Raster Data: Gridded data such as SDM habitat suitability maps, density surfaces, CPI layers, and risk analysis outputs must be provided in formats such as GeoTIFF or NetCDF, complete with metadata describing the variable, unit of measurement, and time interval. For dynamic oceanographic data, integration via WMS services with temporal support is recommended.

Coding System: The unique identifiers ID_Survey and COD_Effort must be assigned correctly following the rules defined in Box 3.1.

Controlled Vocabularies: All fields must use the predefined categories listed in the template legends (see Tables 3.1, 3.2, 2.12, 2.14). No free text is allowed in categorical fields.

Validation: Data must have passed the quality checks (Section 3.2) and final validation by ISPRA (Section 3.4) before integration into the DSS.

- **Georeferencing and Reference System**

Georeferencing Requirement: Every data point must be associated with precise geographic coordinates recorded using a GPS device, following the procedures in Box 3.2. For area data (model outputs, derived products), the geometry must be uniquely defined.

Unified Coordinate System: All geographic data must be provided in the WGS84 reference system (EPSG:4326) with coordinates in decimal degrees and more than 3 decimal places (Section 2.2.4). For raster data and analysis grids, it is recommended to also provide versions resampled to the standard EEA 5 km x 5 km grid (EPSG:3035) to ensure comparability with European datasets and the outputs described in Chapter 4.

- **Metadata Standardization**

Each layer loaded into the DSS must be accompanied by metadata describing:

Source: Partner who produced the data.

Description: What the data represents, the target species, and a brief summary of the methodology used (referencing the relevant section of this guideline).

Temporal Coverage: The reference period (e.g., "2008-2024," "Summer 2023," "HD reporting period 2019-2024"), following the seasonal definitions in Chapter 2.

Spatial Resolution: The native resolution of the data (e.g., point data, 5 km grid).

Access and Rights: Usage restrictions and license. Data accessibility follows the procedures in Section 3.5.

Quality Indicators: Accuracy metrics as defined in Chapter 4 (e.g., AUC for SDM models, effort thresholds, outlier removal criteria).

- **Data Derived from Models and Analyses**

Population Indicators: Abundance Index, density maps, and trend analysis outputs must follow the methodologies in Section 4.1. These should be provided as raster grids (density surfaces) or vector points (sighting locations).

Range Indicators: Extent of Occurrence (EOO), Area of Occupancy (AOO), Observed Distributional Range (ODR) from KDE, and Ecological Potential Range (EPR) from SDMs must follow Section 4.2. These should be provided as vector polygons with associated attribute data.

Habitat Indicators: Species Distribution Model outputs must follow Section 4.3, including seasonal and HD reporting period aggregations. These must be provided as continuous raster layers (GeoTIFF) with values 0-1, accompanied by standard deviation layers for uncertainty.

Conservation Priority Index (CPI): Composite index maps and individual indicator layers must follow the methodology in Section 4.4. These should be provided as raster grids following the 5 km EEA standard.

Threat and Risk Exposure Layers: Floating Marine Macro Litter density, maritime traffic density, Risk Exposure indices (REA), and Near Miss Event (NME) kernel densities must follow Sections 4.5 and 4.6. These should be provided as raster grids (density surfaces) or vector polygons (hotspot isopleths).

5.3 Using the DSS to Identify Conservation Gaps

The DSS is designed as a decision-support tool. Its use involves:

- **Synoptic Visualization:** The interface allows users to navigate the map, turn layers on/off, and view data at different temporal scales. The dual-map comparison feature is particularly useful for comparative analysis.
- **Overlay Analysis and Gap Identification:** The user can access the DSS portal and overlay biological layers (e.g., CPI, ODR, suitable habitat maps from SDMs) with legislative layers (Natura 2000, IMMAs, SPAMIs, Particularly Sensitive Sea Areas, Exclusive Economic Zones, MSFD subregions). By overlaying these layers, it is possible to visually identify important areas for conservation that fall outside the boundaries of current protected areas. This is the identification of a gap.
- **Risk Analysis and Priority Definition:** To refine priorities, the user can add threat layers (maritime traffic density, FMML density) and risk exposure layers (REA, Near Miss Event hotspots). The overlay of a high conservation priority area with a high-risk area identifies a **conflict hotspot**. This provides an objective scientific basis for proposing targeted mitigation measures, such as establishing traffic restriction zones, modifying shipping routes, or implementing dynamic management measures.

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- **Producing Outputs for Reporting:** The DSS allows users to export the generated maps as georeferenced images. These maps can be used directly in official reports (e.g., Art. 17 reports under the Habitats Directive, MSFD initial assessments) to visualize conservation status, pressures, and management proposals, bridging the gap between scientific data collection and the implementation of effective conservation policies.

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