European Union Network for the Implementation and Enforcement of Environmental Law

Monitoring large marine vertebrates along fixed transects from ferries and cargo vessels: data analysis to fill the requirements of the EU regulative environmental framework.

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Introduction to IMPEL

The European Union Network for the Implementation and Enforcement of Environmental Law (IMPEL) is an international non-profit association of the environmental authorities of the EU Member States, acceding and candidate countries of the European Union and EEA countries. The association is registered in Belgium and its legal seat is in Brussels, Belgium.

IMPEL was set up in 1992 as an informal Network of European regulators and authorities concerned with the implementation and enforcement of environmental law. The Network's objective is to create the necessary impetus in the European Community to make progress on ensuring a more effective application of environmental legislation. The core of the IMPEL activities concerns awareness raising, capacity building and exchange of information and experiences on implementation, enforcement and international enforcement collaboration as well as promoting and supporting the practicability and enforceability of European environmental legislation.

During the previous years, IMPEL has developed into a considerable, widely known organisation, being mentioned in a number of EU legislative and policy documents, e.g., the 7th Environment Action Programme and the Recommendation on Minimum Criteria for Environmental Inspections, and more recently in the General Union Environment Action Programme to 2030 and EU Action Plan: 'Towards Zero Pollution for Air, Water and Soil'.

The expertise and experience of the participants within IMPEL make the network uniquely qualified to work on both technical and regulatory aspects of EU environmental legislation.

Information on the IMPEL Network is also available through its website at: www.impel.eu

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Executive Summary

The existing environmental legislation for the protection of marine species operates at international, regional seas and European level; in general, this wide framework requires the monitoring of the status of the species and of their interactions with environmental and anthropogenic parameters for conservation purposes.

Large marine vertebrates, such as cetaceans and sea turtles, range across national boundaries and beyond national waters, therefore collaborative efforts are needed to respond these legislation requirements. In fact, the recent policies for the protection of marine ecosystems have directed efforts across national jurisdictions and through area-based management measures.

Systematic data collected from large vessels along fixed routes, such as those within the MTT project, are useful to monitor these species and this document highlights how they can be analysed to address the main policy requirements at European level.

Despite the differences in resolution and indicators applied by the various policies, the most important and common parameters relevant to large marine vertebrates (Population – Range – Habitat) can be deeply investigated using the robust information obtained from the long-term monitoring programmes involved in the MTT project. As well, long-term data series can be used to investigate the

trends of those parameters over time. Finally, this document also describes how data on potential threats that need to be addressed according to the EU legislative framework (i.e., maritime traffic, pollution by marine litter) are collected and analysed in order to identify priority conservation areas and seasons, and support effective mitigation actions towards marine species.
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1 Introduction and scope

Marine top predators play a crucial role for the conservation of the marine environment (Bowen, 1997). In particular, large marine vertebrates, such as cetaceans and sea turtles, are often referred as "keystone species'', due to their relevant ecological role in the environment they inhabit, “umbrella species” because of their significant influence on other species and ecosystems (Sergio et al, 2008), and are considered indicator species, being overly sensitive and responsive to variations in the health of the marine ecosystem and in their general habitat conditions (Wells et al, 2004; MacLeod et al, 2005; Williamson et al, 2021). These species have been chosen as targets for dedicated assessment and monitoring programs, as the measures implemented directly for their protection tend to act for the conservation of marine ecosystems in general (Sergio et al, 2008; Patricio et al, 2016; Teixeira et al, 2016), and a focus on these species can contribute to the implementation of the “ecosystems approach” in marine conservation (Parsons et al, 2010).

Despite the several studies produced on their ecology and conservation status, marine mammals and sea turtles are still relatively poorly known: they are wide-ranging and long-living elusive species that live a fully aquatic life. According to the assessments provided within the International Union for the Conservation of Nature (IUCN), several cetacean species are data deficient. The European Environmental Agency Report (Röschel et al, 2020, EEA, N. 10/2020) states that “marine mammals (including cetaceans) are among the species with the highest proportion of unknown assessments (over 78%)”, and most of the species considered within the EU Habitats Directive are assessed by means of ‘Expert opinion’ (Arcangeli et al, 2022). The lack of information to produce a proper conservation status assessment may lead to the lack of management actions for these species (Parsons, 2016). On the other hand, around 25% of all cetacean species and 85% of sea turtles species are considered critically endangered, endangered, or vulnerable (IUCN SSC, 2021).

The marine ecosystem is indeed affected by a growing number of threats, derived by the constant increase of human activities and pressures, which also affect the offshore habitats where most large marine vertebrates live (Evans et al, 2012; OSPAR, 2014; ORCA 2019). These interactions between species and anthropogenic pressures should be identified and quantified at basin scale in order to develop and implement successful conservation and management measures (McClellan et al, 2014; Arcangeli et al, 2019; David et al, 2022). As this information is also required by a wide legislative framework, large-scale and long-term data can be crucial to respond to current policy requests.

As reported by Palialexis et al (2019), survey-based assessments are suitable methods to infer the distribution of species and allow the concurrent monitoring of several species; ferries and large commercial vessels provide valuable platforms to carry out long-term monitoring programs across large geographic scales, throughout several seasons and years. For example, data collected in Atlantic North Sea from observers of the Rugvin Foundation were used by the Dutch Ministry of Agriculture, Nature and Food Quality, for government policy study carried out by Statistics Netherlands. The results included a trend analyses as well as information on how data collected by ferry surveys contribute, together with other Dutch cetacean monitoring projects, in the context of quality assurance of monitoring in the North Sea (Poot and Soldaat, 2022). The application of common standards for data collection within large-scale surveys is indeed leading to a growing consistency across European research bodies, as is the case of the "MTT - Marine Transborder Transect" project partners (Campana and Vighi, 2020). To date, many research papers have been published addressing the presence, abundance, distribution, habitat use and trends of large vertebrate species using data collected from fixed-route vessels, and have provided relevant information on species biodiversity, conservation status, as well as for some of the main threats faced by the species. These networks represent an important tool for tackling the scientific questions and priorities posed by the EU environmental legislation with respect to large marine vertebrates living in offshore waters.

1.1 Scope of the document

The key to carry out proper conservation science rests on the ability of policy and science to effectively interact to identify the questions of higher concern. However, the growing body of environmental law and the intrinsic differences among Member States, with different legal and administrative capacities, lead to a high level of non-compliance of environmental directives in the EU (Börzel and Buzogány, 2019). Clear scientific outputs are needed by the administrators to evaluate the efficiency of their management actions at local, regional, national and European level. The broader the level, the more complex the process of collating information is, given the higher number of countries involved and the different scales that need to be investigated. In fact, for mobile marine species, which range across national boundaries and beyond EU waters, a national focus is inappropriate, and collaborative efforts are needed (Murphy et al, 2019).

This document is intended to provide a description of the existing international regulative framework concerning the marine environment (Chapter 2), specifying the main requirements for what concerns large marine vertebrate species (Chapter 3), with the aim of highlighting how the data collected from large vessels on such species, the threats they face, and other relevant environmental parameters, can be used to address the main policy requirements at European level (Chapters 4 and 5).

2 The policy context

The European Commission considers cetacean and marine turtle species in several of its environmental policies. A large framework of the EU legislation for the protection of the species and/or marine biodiversity requires the monitoring of the status of the species and of their interactions with environmental and anthropogenic parameters for conservation purposes (for details see Campana and Vighi, 2020; Arcangeli et al, 2022). Large marine vertebrates can be effectively protected only by means of international cooperation, given their high mobility, the large-scale connectivity of the marine ecosystem and the oceanic dynamics. Indeed, wide approaches have been developed for a better understanding of this environment (Hoyt, 2011; Evans et al, 2012; Girard et al, 2022), and the recent policies for the protection of marine ecosystems have directed efforts across national jurisdictions and through area-based management measures (Murphy et al, 2019). However, there is still a limited communication between the scientific community, the policy makers and the general public, and more efforts are needed to support the application of scientific information into legislative and administrative actions (Pullin et al, 2009; Authier et al, 2018; Börzel and Buzogány, 2019).

2.1 Environmental regulative framework in European waters

This section describes the main policy framework concerning marine conservation and lists the most relevant stakeholders promoting the conservation of large marine vertebrates across European waters. International organisations and conventions are listed and briefly described, along with relevant EU directives. Table 1 summarises the main objectives, requirements and initiatives implemented within each of them, with a particular focus, where relevant, on the topics that can be addressed using data systematically collected by the European monitoring networks. A more extensive description can be found in Campana and Vighi (2020).

2.1.1 International Organisations

International Union for the Conservation of Nature (IUCN)

The key objective of IUCN is to share scientific knowledge through its conservation databases, such as the Red List of Threatened Species and the Database of Key Biodiversity Areas (KBAs, IUCN, 2015). The IUCN World Commission on Protected Areas (WCPA) Marine Vice Chair, and members of the IUCN Species Survival Commission (SSC) contributed, in 2013, to the creation of the Marine Mammal Protected Areas

Task Force (MMPATF), with the main objective to identify Important Marine Mammal Areas (IMMAs), defined as "*discrete portions of habitat, important to marine mammal species, that have the potential to be delineated and managed for conservation*", that will include marine mammals into existing conservation tools (i.e., marine protected areas, IUCN, 2016).

International Council for the Exploration of the Sea (ICES)

The goal of ICES is to advance and share scientific understanding of marine ecosystems and the services they provide, and to use this knowledge to generate state-of-the-art advice for management and sustainability goals.

International Whaling Commission (IWC)

Set up with the purpose of conserving whale stocks by applying specific regulatory measures (e.g., Revised Management Procedure), the IWC also undertakes extensive study and research on cetacean populations, through Conservation Management Plans (CMPs) and the definition of Critical Cetacean Habitat (CCH). Additionally, the IWC also collaborates with the IUCN and the International Maritime Organization (IMO) to develop mitigation measures on ship strikes (IWC, 2023).

2.1.2 International Conventions

The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES)

Established within an IUCN meeting, CITES aims at regulating wildlife trade and ensuring that it does not threaten their survival. Appendix I of the convention lists the species threatened with extinction, and includes several cetacean species and all sea turtle species.

The Convention on the Conservation of European Wildlife and Natural Habitats (BCCEW)

The BCCEW aims to conserve wild flora and fauna and their natural habitats, as well as to promote European cooperation in this field. All cetacean and sea turtle species are listed as Strictly Protected Fauna Species in Appendix II, or as species that can be exploited in Appendix III. The convention also designates Areas of Special Conservation Interest (ASCI). All parties submit biennial reports that provide a scientific assessment of the impact of the measures implemented to protect the species and habitats covered by the Convention.

The Convention on the Conservation of Migratory Species of Wild Animals (CMS)

The aim of this Convention is to provide a global platform to conserve terrestrial, aquatic and avian migratory species across their full range, throughout their habitats and migration routes; it "*brings together the States through which migratory animals pass, the Range States, and lays the legal foundation for internationally coordinated conservation measures throughout a migratory range*". Most cetacean and marine turtle species are highlighted as priority species (in danger of extinction in Appendix I, or in unfavourable conservation status in Appendix II). The CMS promoted the creation of two regional agreements for the conservation of cetaceans in European waters:

The Agreement on the Conservation of Small Cetaceans of the Baltic, North East Atlantic, Irish and North Seas (ASCOBANS)

ASCOBANS suggests conservation, research and management measures and recommends adequate surveillance schemes to the signatory countries (Parties), under the general conservation objective "*to allow populations to recover to and/or maintain 80% of carrying capacity in the long term*".

The Agreement for the Conservation of Cetaceans in the Black Sea, Mediterranean Sea and Atlantic contiguous waters (ACCOBAMS)

ACCOBAMS promotes and facilitates active regional cooperation at all levels, providing best expertise and standards, and recommends the implementation of monitoring activities and effective protection measures for cetacean species throughout its region. The overall objective of the recent ACCOBAMS Strategy (2019) is "*to improve the conservation status of cetaceans and of their habitats in the area of competence of the Agreement by 2030*".

The Convention of Biological Diversity (CBD)

The CBD has three main objectives: the conservation of biological diversity; the sustainable use of the components of biological diversity; the fair and equitable sharing of the benefits arising out of the utilisation of genetic resources. To measure the progress toward the convention targets, and to assess the effectiveness of protected area designation, conservation, and marine resource management policy, Biodiversity Indicators (including cetaceans) have been adopted. Scientific criteria are applied for identifying ecologically or biologically significant marine areas (EBSAs) in need of protection.

The Law of the Sea Convention (UNCLOS)

UNCLOS establishes rules that govern all uses of the oceans and their resources. The convention states that countries shall protect and preserve the marine environment, including the areas beyond the limits of national jurisdiction (ABNJ), and set forth detailed measures to be taken, such as the definition of the exclusive economic zone (EEZ). Within the conservation of biological resources, cetaceans are considered in Annex I (highly migratory species). The concept of "*Conserve and sustainably use the oceans, seas and marine resources*" (i.e., ABNJ) has been included as goal 14 among the 17 Sustainable Development Goals (SDGs) adopted by world leaders, in the *UN Sustainable Development* commitment of States to develop an international legally binding instrument for a joint management of the ABNJ.

2.1.3 Regional Sea Conventions

Within the different regional seas surrounding Europe, the Regional Sea Conventions (RSCs) engage neighbouring countries for the conservation of their common marine environment. Their work areas cover maritime activities and pressures, as well as biodiversity and ecosystems' protection. The RSCs implement coordinated monitoring programmes in the regional sea basins, and perform joint assessments of the state of the environment. Four RSCs are active across EU waters:

The Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR)

OSPAR works under the umbrella of UNCLOS and is guided by the ecosystem approach to an integrated management of human activities in the marine environment. Its objective is to protect the maritime area against the adverse effects of human activities, to safeguard health and to conserve marine ecosystems and restore areas that have been adversely affected. Under the convention, Member States are required to undertake assessments of the quality status of the marine environment (Annex IV), and of the effects of the measures taken for conservation of the ecosystems and biological diversity under Annex V (including 7 cetacean species), at six-year intervals (Quality Status Report, QSR). To monitor environmental quality the Commission adopted a Coordinated Environmental Monitoring Programme (CEMP) aiming to deliver comparable data from across the OSPAR Maritime Area, which can be used in the assessments and for the coordinated implementation of other policies among Member States (HD, MSFD, ASCOBANS). Indicator M4 of the programme is related to marine mammals' abundance and distribution (OSPAR, 2014).

The Convention for Protection of the Mediterranean Sea against Pollution (Barcelona Convention, UNEP-MAP)

Since the adoption of the Mediterranean Action Plan (UNEP/MAP) for the protection of the marine environment and the sustainable development of the coastal areas of the Mediterranean, Parties are called to protect areas of particular natural or cultural value, through the establishment of Specially Protected Areas (SPAs) or Specially Protected Areas of Mediterranean Importance (SPAMIs), and to protect the threatened or endangered species of flora and fauna listed in the SPA/BD Protocol's Annexes. The conservation of marine turtles and cetaceans has been a priority for UNEP/MAP through the adoption of specific Action Plans for Conservation.

The Integrated Monitoring and Assessment Programme of the Mediterranean Sea and Coast and Related Assessment Criteria (IMAP) contains the principles for an integrated monitoring of biodiversity, non-indigenous species, pollution and marine litter, coast, and hydrography, and requires 6 years reporting periods, but recommends shorter reporting intervals to depict seasonal and inter-annual changes. The IMAP implementation is structured on 11 Ecological Objectives and 27 Common Indicators: under the Ecological Objective 1 ("*Biological diversity is maintained or enhanced*"), the abundance and occurrence of coastal and marine habitats and species (including marine mammals, seabirds, and marine reptiles) should be in line with prevailing physiographic, hydrographic, geographic and climatic conditions.

UNEP/MAP also promoted local agreements between Italy, France and Principality of Monaco, such as the ***RAMOGE*** on the protection of Mediterranean coastal waters, which promotes integrated coastal management and deals with issues such as pollution from land-based sources (including marine macro litter and general waste); and the ***Pelagos Sanctuary****,* a SPAMI that was established to ensure the proper state of conservation of marine mammal populations.

The Convention on the Protection of the Marine Environment in the Baltic Sea Area (Helsinki Convention, HELCOM)

HELCOM aims to protect the Baltic Sea from all sources of pollution from land, air and sea, as well as to preserve biological diversity and to promote the sustainable use of marine resources. The Baltic Sea Action Plan (BSAP), adopted by the HELCOM Contracting Parties in 2007 and updated in 2021, is the strategic programme of measures and actions that should be adopted to achieve good environmental status of the sea, ultimately leading to a Baltic Sea in a healthy state. HELCOM performs periodic holistic assessments (HOLAS) that give a comprehensive overview of the ecosystem health of an entire regional sea and assist the region's environmental managers and decision-makers, so that they can base their work on sound, up-to-date knowledge of the status of the sea.

The Convention for the Protection of the Black Sea (Bucharest Convention, Black Sea Commission, BSC)

The BSC is the intergovernmental body established in implementation of the Convention on the Protection of the Black Sea Against Pollution, its protocols and the Strategic Plan for the protection and Rehabilitation of the Black Sea (1996). Among others, it aims to elaborate criteria pertaining to the prevention, reduction and control of pollution of the marine environment of the Black Sea and to the elimination of the effects of pollution, as well as recommendations on measures to this effect, and to promote the adoption by the Contracting Parties of additional measures needed to protect the regional waters, and to that end receive, process and disseminate to the Contracting Parties relevant scientific, technical and statistical information and promote scientific and technical research.

2.1.4 European Directives

The Habitats Directive (HD)

The aim of the HD is "*to promote the maintenance of biodiversity, taking account of economic, social, cultural and regional requirements*" and to take action to maintain or restore natural habitats and species at favourable conservation status (FCS). Conservation status is defined within the HD as "*the sum of the influences acting on the species concerned that may affect the long-term distribution and abundance of its populations in the European territory of the Member State to which the Treaty applies*". This is considered favourable if the species is maintaining itself as a viable component of its natural habitats and if its abundance and distributional range are maintained and not reduced, and if there is a sufficiently large habitat to maintain its populations on a long-term basis. The parameters used to assess the conservation status of species are population size, natural range, habitat (extent and condition) and future prospects. The evaluation of conservation status is done by assessing separately each of the parameters with the aid of an evaluation matrix, and then combining these assessments to reach an overall assessment (DG Environment, 2017).

All cetaceans and sea turtle species are considered strictly protected under Annex IV of the HD, and 4 of these species are listed in Annex II, which includes species that require the designation of Sites of Community Importance (SCI) and that can justify their designation as Special Areas of Conservation (SACs), and thus part of the Natura 2000 network, as they are key sites that are used regularly by the species for life and reproduction.

Since time series data are necessary to detect changes in FCS, surveillance of the conservation status of the species of Annex II and IV is required under Article 11, which requires to determine the status and trend of the species across their range, and evaluate the impact of the conservation measures undertaken.

A report on the implementation of these measures and on the results of the surveillance is drawn by member states every 6 years (Article 17).

The Marine Strategy Framework Directive (MSFD)

The goal of the MSFD is to achieve or maintain a Good Environmental Status (GES) of the EU's marine waters, and follows an adaptive management approach so that it must be updated and reviewed every six years after implementation (not corresponding to the HD reporting period). This directive sets out 11 qualitative descriptors and a number of associated criteria, parameters and indicators for determining GES. Member States may choose which parameters and indicators to apply and set targets according to the background conditions relevant to each area.

The MSFD requires the adoption of specific and standardised methods for monitoring and assessing the achievement of GES, to ensure consistency and comparability among the assessments done by Member States, as well as the establishment of coherent and representative networks of MPAs, compatible with existing EU instruments (e.g., Natura 2000, OSPAR) and coordinated between countries. Descriptor 1 (D1, i.e., biological diversity is maintained), which requires the assessment of the abundance, range and habitat of species, that should be in line with predominant physiographic, geographical and climatological conditions (Article 11, Annex III), is particularly linked to the assessment processes under the HD. Cetaceans and marine turtles have been identified as functional groups of D1 and used for the assessment of GES in Italy, France, Spain, and also within other descriptors (D4, D8, D10, D11, Spitz et al, 2017; Authier et al, 2018).

The Maritime Spatial Planning Directive (MSPD)

The MSPD establishes a comprehensive framework to manage human activities, their multiple uses and interests in the maritime environment, and to minimise environmental impacts while reducing conflicts among users and supporting decision-makers. It "*works across borders and sectors to ensure human activities at sea take place in an efficient, safe and sustainable way*". Its target is to define the maritime spatial plans by 2021, with a minimum review period of 10 years, in the marine waters of each Member State (e.g., internal waters, territorial waters, EEZ). Monitoring strategies should be set for each basin to report at relevant intervals (e.g., 6 years). The MSP can help address several of the resolutions already set by regional agreements, such as to achieve a representative network of Marine Protected Areas (MPAs).

Table 1. Summary of the main characteristics of the international environmental regulative framework related to large marine vertebrates. Acronyms are explained in the main text.

	Objective	Methods/tools used for the assessment	Spatio-temporal scope of the	Conservation initiatives and instruments	Target Species

			assessment	implemented	
International Organisations					
IUCN	Assessment of the conservation status of species	Specialist groups Red List Assessments	2x2 km; 10 years / 3 generations	KBAs, IMMAs	Cetaceans, sea turtles, seabirds
ICES	Sharing of the scientific understanding of marine ecosystems and advice for management and sustainability goals	Working groups: on Joint Cetacean Data Programme- WGJCDP, Bycatch of Protected Species-WGBYC, Marine Mammal Ecology-WGMME. Framework: request formulation, knowledge synthesis, peer review and advice production	Statistical rectangles (30’ latitude x 1° longitude)		Cetaceans, sea turtles, seabirds
IWC	Global body responsible for management of whaling and conservation of whales	Conservation Management Plans; Revised Management Procedure		Sanctuaries, CCH, mitigation measures	Cetaceans (“stocks”)
International Conventions					
CITES	Regulation of wildlife trade	Licensing system Animal committee, standing committee, and Conference of the Parties	Every 2/3 years	-	Cetaceans, sea turtles, seabirds
BCCEW	Conservation of wild flora and fauna and their natural habitats	Assessment	2 years	ASCI	Cetaceans, sea turtles, seabirds

CMS	Protection of endangered migratory species across their full range	Monitoring activities	Migratory range Seasonal for migration	Regional agreements (ACCOBAMS, ASCOBANS)	Cetaceans, sea turtles, seabirds
CBD	Conservation and sustainable use of biological diversity	National reports Biodiversity indicators UN-Oceans Task Force	Every 3 years	EBSAs	Cetaceans, sea turtles, seabirds
UNCLOS	Establishment of rules that govern all uses of the oceans and their resources	Reports of the Secretary General UN-Oceans Task Force	Annually	ABNJ, EEZ Archipelagic States, Continental Shelf, High Sea, Islands, Enclosed or semi-enclosed seas	Cetaceans
Regional Sea Conventions					
OSPAR	Protection of the maritime area against the adverse effects of human activities, to safeguard health and to conserve marine ecosystems	Quality Status Report, Joint Assessment and Monitoring Programme	Regional sea 6 years	-	Cetaceans, sea turtles, seabirds
HELCOM	Protection of the Baltic Sea from all sources of pollution, and of biological diversity and promotion of the sustainable use of marine resources.	Holistic Assessments (HOLAS)	Regional sea 6 years		Cetaceans, seabirds
BSC	Prevention, reduction and control of pollution of the marine environment of the Black Sea, production of recommendations on measures to this effect, promotion of scientific and technical research	Black Sea Integrated Monitoring and Assessment Programme (BSIMAP)	Regional sea 5 years		Cetaceans, seabirds

UNEP/MAP	Protection of the marine environment and sustainable development of the coastal areas	Ecological Objectives, common indicators Integrated Monitoring and Assessment Programme	Regional sea 10x10 km (up to 50x50 km) 3-6 years	SPAMI (e.g., Pelagos Sanctuary)	Cetaceans, sea turtles, seabirds
European Directives					
HD	Achievement and maintenance of a "favourable" conservation status of the species	Parameters/indicators, Surveillance	National waters 10x10 km (up to 50x50 km) 6 years (12-24 years trends)	SCI – SAC – Natura 2000	Cetaceans, sea turtles, seabirds
MSFD	Achievement and maintenance of the Good Environmental Status of EU marine waters	Descriptors/criteria/parameters/elements; Monitoring	Marine Reporting Units (MRUs) 6 years	EEA marine assessment areas buffer zones-	Cetaceans, sea turtles
MSPD	Management of human activities and uses in the maritime environment	Monitoring	National waters 6 years	MPAs	Cetaceans, sea turtles

3 Policy requirements

The assessment of populations of pelagic marine species is extremely difficult and expensive, and to this aim, representative monitoring programmes should be established; the **spatial and temporal resolution** of the reporting required by the various policies differ according to the specific requirements of each one (Table 1). For example, the HD and the MSPD require the assessment in national waters, while other regulations require the assessment at regional or sub-regional scales (e.g., OSPAR, MSFD), thus showing different reporting resolutions (Figure 1, Girard et al, 2022). Despite this variability, consistency of reporting is recommended by the INSPIRE Directive (2007/2/EC), which requires that spatial data and services provided by different European sources are interoperable, to ensure that they are compatible and can be combined in a consistent manner across the EU. Concerning temporal scales, the minimum period recommended by the IUCN to assess population variations is 10 years (IUCN, 2019), but many regulations require large scale assessments at 6-years intervals; however, even cycles of the same duration are not always aligned (e.g., OSPAR, MSFD, HD), and shorter intervals can also be useful to depict seasonal variability (BCCEW, UNEP/MAP, 2017).

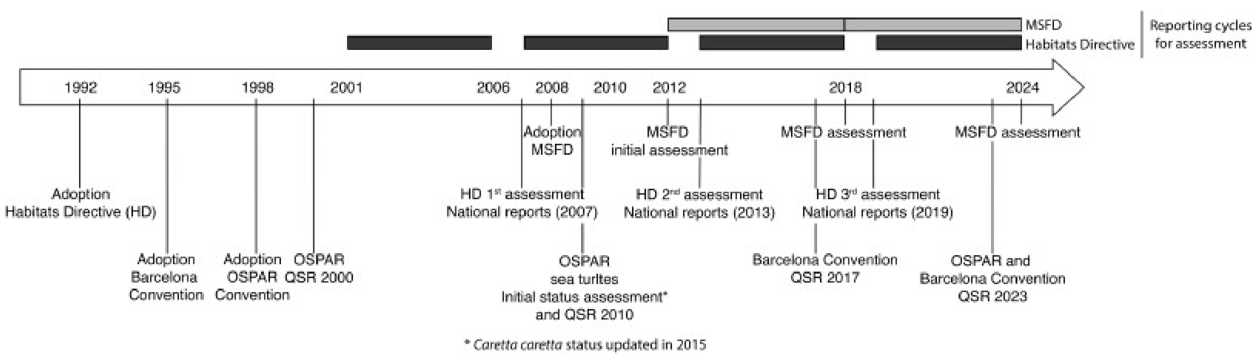

Figure 1. Timeline of reporting cycles and assessments under different Directives and Conventions operating in European waters (adapted from Girard et al, 2022).

Such a wide legislative framework, even if differing in the specific objectives and measures, often presents common assessment elements and makes use of similar baseline information (Teixeira et al, 2016). In fact, scopes and monitoring approaches defined under one legislative process can be supportive for others. For example, the objective of the MSFD is the achievement of the GES, which is a more detailed assessment of the FCS concept of the HD (Arcangeli et al, 2022), and consistency in methods and procedures to be applied between the two policies is explicitly referred for, so that Member States can report using the same formats and timescales (Palialexis et al, 2019). EU Directives have developed a number of criteria for the evaluation of the conservation status of the species and for the monitoring of its variations that also refer to widely used indicators and previous conventions/agreements (Teixeira et al, 2016; Girard et al, 2022). The most important and common parameters relevant to large marine vertebrates are described and compared in the next section.

3.1 Main attributes of the species: parameters/criteria and indicators

The approach for the assessment of species status among the EU environmental policies is similar, and considers various attributes of the species (parameters/criteria): in general, these are quite generic in the older policies, and become more detailed and with different levels of prioritization (primary-secondary) in the most recent ones. Indicators are used to measure the parameters/criteria, which are generally assessed separately and then aggregated at single species/species group level. Direct correspondences are identified among the attributes measured to assess the species status under different policies (see Teixeira et al, 2016; Girard et al, 2022); those that can be addressed for large marine vertebrates are summarised in Table 2 and compared in the following paragraphs.

Table 2. Biodiversity-related assessment within the main European environmental regulative framework: comparison of parameters/criteria relevant to the assessment of population, range and habitat of large marine vertebrates within the most relevant EU conventions and directives.

Parameters/ Criteria	**IUCN**	**CMS**	**OSPAR CEMP Theme BB2**	**IMAP Ecological Objective 1**	**HD**	**MSFD D1**
"Population"	Population size	Population distribution and abundance	M4b Population abundance	Common Indicator 4 Population abundance	Population Size, Population Trends and Favourable Reference Population	C2 Population abundance
		Population dynamic and viability	Population conditions (demography)	Common Indicator 5 Population demographic characteristics		C3 Population demographic characteristics

“Range”	Area and Extent of Occupancy	Species range, population distribution	M4b Geographical range and distribution	Common Indicator 3 Species distributional range	Surface of the Range, Trend in Surface of the Range, Favourable Reference Range	C4 Population distributional range and pattern
“Habitat”	Suitable habitat in the evaluation of the range and of its risk exposure	–	–	–	Area and quality of occupied habitat; available area of unoccupied habitat of suitable quality	C5 Habitat for the species

3.1.1 Population

Population refers to the total population in the biogeographical or marine region of interest; dynamic data indicate that the population is maintaining itself on a long-term basis as a viable component of its natural habitat (**Favourable Reference Population, FRP)**. Information on historic distribution/population and/or the best ‘expert judgement' may be employed to define FRP in the absence of other data. The estimate of population size has to be related to management units (e.g., IWC), particularly for wide ranging marine species; many assessments use statistically robust estimates obtained from regional Agreements or Conventions (ACCOBAMS, ASCOBANS, OSPAR), or from cooperative surveys between Member States that share the same population (i.e., NASS, Vikingsson et al, 2009; SCANS, Hammond et al, 2017; ACCOBAMS, Panigada et al, 2021).

Population Size is ‘favourable’ when it is not lower than its FRP. For mobile species such as marine mammals and sea turtles, the reporting unit for HD is either the number of individuals, considered as a range or best available single value, or the number of occupied 1x1 km grids (see Range) in density maps, with a similar approach to other regulations (DG Environment, 2017). For sea turtles, the estimate of population abundance is based on nesting females, but it needs to include observations at sea (Girard et al, 2022) and can be completed by modelling and/or extrapolation (Palialexis et al, 2019; Zampollo et al, 2022).

Detailed demographic characteristics are also required by OSPAR, IMAP, MSFD, but mainly for exploited species. For **Population trends**, the IUCN set a threshold in the observation of a 30% decline over 3 generations for a species (more than 5% in 10 years, IUCN 2019); the 3 generations period is however species-specific as the duration of a generation differs across species (44-72 years for odontocetes, 96 for sperm whale and 78 years for fin whales, IMAP EcAP). For sea turtles, data from a minimum of 10 years are optimal to detect trends in population abundance, but given the difficulty in obtaining such long-term datasets from at-sea observations, trends in abundance should be characterised over the 6-year reporting period (Girard et al, 2022) and estimates should be corrected using known behavioural data collected from tracking studies (Katsanevakis et al, 2012).

3.1.2 Distributional Range

The HD defines the Distributional Range as "*the outer limits of the overall area in which a species is found at present and it can be considered as an envelope within which areas actually occupied occur*" (DG Environment, 2017). It is a dynamic parameter that allows the assessment of the extent of the area within which any significant ecological variation of the species is included for a given biogeographical region and which is sufficiently large to allow the long-term survival of the species (**Favourable Reference Range, FRR).** The proportion of surveyed area where a species is detected defines the species occupancy (presence/absence). To estimate the quantity of occupied habitat for taxa with markedly different body sizes, mobility and home ranges, different spatial scales of measurement are required. For the IUCN, the Area and Extent of Occupancy have to be mapped in a 2x2 km grid, for a fine scale evaluation of the spatial extent of risk that a species can deal with (IUCN, 2019). For HD and IMAP reporting, the spatial distribution of a species is required in the form of a presence/absence map in a 10x10 km grid, but for highly mobile or migratory species that can occupy large territories during their life cycle (*sensu* CMS), reporting should be presented at biogeographical level (e.g., 50x50 km grids, UNEP/MAP, 2017). It should be noted that the width of a degree of longitude changes with the latitude, causing variation in the area of the grid cells, which calls for resolution grids measured in degrees when analysing data with large latitudinal extension (Wall, 2013; Rogan et al, 2017; Valente et al, 2019).

The **Surface of the range** is the total surface area (in km^2) of the current range within the biogeographical or marine region concerned, obtained through the spatial generalisation of the species distribution. This parameter should be calculated on the actual/predicted distribution using a standardised process to ensure repeatability of the range calculation in different reporting rounds, which involves: 1) creating an envelope around the distribution grids, using the procedure of "gap closure"; 2) excluding unsuitable areas (Arcangeli et al, 2022). For marine mammals and reptiles, the recommended maximum gap distance to calculate the surface of the range is 90 km. The distribution area is the sum of the areas of the cells where the species is present. For highly mobile species, the distributional range is derived from the large-scale surveys, modelling, extrapolation of expert opinion (DG Environment, 2017), which allow predictions on the probability of occurrence in areas where no data are available, and represents a low-cost alternative for monitoring abundance (Brereton et al, 2000; MacLeod et al, 2009; Katsanevakis et al, 2012; Palialexis et al, 2019).

Trends in Surface of the Range can be measured over a short-term (12 years) or long-term period (24 years) (DG Environment, 2017). Information on the direction of any change over the reported period should be provided as: stable, increasing, decreasing, uncertain, or unknown. According to the IUCN criteria, the threshold value is set as a 30% decline of the range over three generations for a species, which is however a variable temporal scale depending on the species considered (IUCN, 2019). For OSPAR, the threshold is set to a 10% of change in distribution detected over the 6-years assessment period (OSPAR, 2019). For sea turtles, as variations in distribution are particularly difficult to interpret given the many concurrent influencing factors, the MSFD suggests that GES is achieved when the distributional range remains stable between reporting cycles (Girard et al, 2022).

3.1.3 Habitat for the Species

The habitat of a species is an environment defined by specific abiotic or biotic factors, in which the species lives at any stage of its biological cycle (DG Environment, 2017). A special emphasis should be given to key habitats, which are those used during specific periods of the life cycle of species, such as for reproduction. Conservation measures should assure sufficiently large habitats to maintain populations on a long-term basis, thus also considering the different life stages of a species (e.g., Girard et al, 2022; Zampollo et al, 2022). The evaluation of this parameter is only included in the HD and MSFD, while the IUCN criteria recognise the suitable habitat in the evaluation of the range and of its risk exposure (IUCN, 2019). Three key elements are used to assess the habitat of a species: area, quality and spatial organisation, where quality is a continuous variable (from high to low) that refers to the resources available for the survival, reproduction and persistence of a population. The following indicators are required by the HD: **area and quality of occupied habitat**, to be evaluated as sufficient for the long-term survival of the species (Yes/No/Unknown); **available area of the unoccupied habitat of suitable quality** for the long-term survival of the species. For some highly mobile species, the actual habitat will often be equal to the range, so it might be difficult to precisely identify the area used, and the assessment should mainly focus on the 'habitat quality' (Palialexis et al, 2019). For marine species, since the Habitat quality is often difficult to assess, the information on Habitat is generally used to evaluate the Range of the species. Accordingly, the IUCN gives indications to assess Habitat for these species using predictions based on modelling and/or other extrapolation methodologies from a limited amount of data from detailed surveys.

3.1.4 Future Prospects/Trends

"Future prospects" is an indicator that is only explicitly present in the HD and should be interpreted as a measure of a directional change and future condition of one of the above parameters over time, in the next 12/24 years (2/4 reporting cycles) for detecting short-term and long-term trends, respectively (DG Environment, 2017). The IUCN indicates 10 years as a suitable interval for the short-term trend assessment (IUCN, 2019; Palialexis et al, 2019; Girard et al, 2022). Surveillance is required in important marine areas (Natura 2000, IMMAs, EBSAs, ABNJ) and it is essential to provide long-term datasets within conservation legislation, to detect patterns of species presence and distribution and evaluate the efficiency of protection measures (Pullin et al, 2009; Patricio et al, 2016; IUCN, 2019; Palialexis et al, 2019). Within the HD, Future prospects can be 'good', 'poor', 'bad' or 'unknown' and should derive from the balance between species needs, response to the changing environment, and effects of conservation measures. In the context of species-habitat studies, the rate of change is often more relevant than the absolute value of the investigated parameters (Katsanevakis et al, 2012).

4 Data analyses to comply with the EU requirements: examples from the MTT network

Systematic visual observations from non-dedicated platforms are among the preferred methods to monitor marine species and contribute to determining populations abundance and range, as well as habitat preference; in addition, monitoring trends over time is also possible given the repeatability of the surveys (Berrow et al, 2012).

To prepare this chapter and provide a detailed overview of the analyses performed, the scientific production derived by the monitoring networks using ferries and cargos (i.e., ORCA, FLT MED NET, CETUS) was considered (see Campana and Vighi, 2020); since standardised and systematic protocols are used for data collection within the networks, data provided in the different publications were considered uniform. More recent literature was additionally searched in Google Scholar and Pubmed, using the keywords “transect monitoring”, “large vessels/ships of opportunity”, “cetacean/sea turtle/large vertebrates”, and the analytical process carried out in publications that specifically addressed the policy framework was assessed. As a result, 105 documents were reviewed, of which 79 international and national research papers, 25 technical reports and one Master thesis. The majority came from the FLT MED NET and ORCA networks, but literature from other research groups in the Mediterranean Sea, Atlantic or Pacific Oceans was also included. Most of the documents dealt with a great variety of cetaceans species (93%), while 15% with sea turtles, mainly *Caretta caretta*. Some documents considered both taxa. From each document, information about the target species, the policy addressed, the preliminary analyses, the spatio-temporal resolution, the methodologies applied with respect to the Population/Range/Habitat parameters, and the presence of analyses on threats was extracted.

4.1 Preliminary analyses

Data exploration is performed prior to any deeper investigation to evaluate the representativeness and robustness of the datasets, to detect possible biases and ensure data homogeneity. Data exploration can include all or some of the following steps:

The dataset can be **stratified** by year and/or season: seasons are usually divided into winter (January to March), spring (April to June), summer (July to September) and autumn (October to December), but in some cases only two opposite seasons can be analysed (autumn-winter/spring-summer, Campana et al, 2022; Pace et al, 2022). This can be done at the beginning of the analyses, to verify the number of records available, or further on during the process. Data can be also split geographically according to the marine region (e.g., Arcangeli et al, 2017, 2023a; Tepsich et al, 2020), habitat type/bathymetry (e.g., Lopez et al, 2004; Brereton 2005; Kiszka et al, 2007; Gnone et al, 2023).

Only **on-effort** tracks are considered, with sea state <4 Beaufort, for equal probabilityof observing cetaceans (see details on protocols, Campana and Vighi, 2020); in cases of low detectable species, such as *Z. cavirostris, P. phocoena*, sea turtles, only lower sea state (<3) can be used for analyses (Eguchi et al 2007; Bouveroux et al, 2020; Robbins et al, 2022).

The total **length** of each on-effort transect is computed. For each monitored route in a specific area, each trip from port to port, considered as an independent transect, is the **statistical unit**. For outbound/return trips performed within the same day, spatial and temporal **auto-correlation** should be assessed: Spearman's rank correlation test can be applied to abundance estimates computed from transects of the same area and sampled within the same day or consecutive days (Arcangeli et al, 2013, 2014, 2016; Morgado et al, 2017); based on results, one transect is randomly selected among the two available on the same daily route (Tepsich et al, 2020; Arcangeli et al, 2021).

In order to avoid biases due to poorly surveyed areas/seasons, a **minimum sampling effort** criterion has to be set, by investigating the relationship between encounter rate and survey effort (Brereton et al, 2003; Correia et al, 2020). Preliminary exploratory data analysis can be done by plotting effort with encounter rate and Z score values, finding the limit of effort when the variance of the encounter rate does not appreciably change (Arcangeli et al, 2016, 2017). Based on previous results, transects are discarded from the analysis when shorter than 10 km (Arcangeli et al, 2021; Atzori et al, 2021). When dealing with groups of transects, the maximum length recorded for a single transect in a group is used to set a threshold value for assessing transect representativeness: Tepsich et al (2020) selected only the transects of at least the 30% of the maximum length within transect groups.

When analysing the effort within grid cells, the minimum effort to consider a cell surveyed can be set by using a quantile analysis (e.g., 1st quantile, Robbins et al, 2022), while some authors defined insufficient effort when the 10%-20% of the cell size is not covered (Azzolin et al, 2020; Grossi et al, 2021; Ham et al, 2021); in other cases, the minimum effort is chosen consistently with the cell size (1 km in 1x1 km grid, Correia et al, 2015; Bouveroux et al, 2020; 10 km in 5x5 km grid, Arcangeli et al, 2017, 2019; Gregorietti et al, 2021; diagonal of the cell, Gnone et al, 2023) or with the objective of the study (50 km in 1x1 km grid, Arcangeli et al, 2016; 5 km in 5x5 km grid, Campana et al, 2022; 100 m in 10x10 km, Arcangeli et al, 2022).

Similarly, the **minimum number of sightings** can be defined, according to the analyses to be performed. Some authors chose 10-15 (Campana et al, 2015, 2017; Pace et al, 2019), other 30-40 (Kiszka et al, 2007; Correia et al, 2020, 2021) as the minimum number of sightings that would allow for statistical comparisons and significance; when working on grids, other authors chose to retain a cell if the sighting-buffer zone covers ≥20% of its area (Grossi et al, 2021; Ham et al, 2021).

To inspect the community **composition**, sightings frequencies are computed, representing the proportion of each species sightings of the total species records in the studied area (e.g., MacLeod et al, 2005; Wall et al, 2006, 2013; Leeney et al, 2012; Aissi et al, 2015; Pace et al, 2019; Valente et al, 2019; Correia et al, 2020; Sá et al, 2021; Gnone et al, 2023; Scuderi et al, 2024).

Species richness is another value that can be calculated within data exploration. It indicates the total number of species detected, which can be compared among areas/periods: it can be expressed as the total number of species per sampling unit (Brereton et al, 1999; Wall et al, 2006; Leeney et al, 2012; Vella, 2013; Correia et al, 2020; David et al, 2020) or by commonly used indices, such as the Shannon-Weaver or Simpson's diversity index (Brereton et al, 2003; Aissi et al, 2015; Arcangeli et al, 2017; Matear et al, 2019; Campana et al, 2022; Gnone et al, 2023). A specific Biodiversity index was created by McClellan et al (2014) by summing umbrella groups of megafauna. The number of sightings and number of species observed in relation to the sampling effort can be also modelled (e.g., through Generalised Additive Models (GAMs), Correia et al, 2020; or Generalised Linear Models (GLMs), Gnone et al, 2023).

To evaluate the effect of **observation conditions** on species detection, platform characteristics have been tested with a GLM including cruise speed and deck height (Arcangeli et al, 2014b; Cominelli et al, 2016), which showed the influence of the height of the ship over the sighting distance of cetaceans. Lopez et al (2004) investigated the effects of boat type and speed on sightings rates through different statistical approaches, while Monestiez et al (2006) assessed the correlation between the number of fin whale sightings and the number of observers aboard or the type of observation platform used (ferry, sailing, fishing). Similarly, Northridge et al (1995) adopted a modelling approach to generate estimates of relative detectability for cetacean species in different sea states, while Reid et al (2003) modelled sightings rates as a function of several covariates including sea state using GAMs, and estimated appropriate correction factors for each sea state category. Given these effects, some authors divide sighting and effort data into segments with uniform observation conditions, according to the study area and target species (Forney, 2000 (2 km); Williams et al, 2006 (2 NM); JNCC, 2015 (10 km); Cominelli et al, 2016; ORCA, 2019, 2021, 2023 (7 km); Robbins et al, 2020 (5 km)).

4.2 Analyses for "Population"

The simultaneous collection of effort and sightings data enable the calculation of standardised estimates for each species at various spatial/temporal scales, providing a tool for long-term monitoring. Information on population attributes can be provided by the indicator **abundance**, through the use of an index or distance sampling method. This indicator can be analysed by year/season/area, to test spatio-temporal differences prior to pooling data. Composite indices (i.e., aggregation of indicators) can be developed as a way of describing the change in species populations at country level (Brereton et al, 2003).

4.2.1 Abundance indices

The **Encounter Rate (ER)** and/or the **Sightings per Unit of Effort (SPUE)** are used as indices of abundance for the taxa/single species, and represent the number of presences recorded during the observation effort. According to the scale of the study area and the species investigated, they can be expressed as:

- *N sightings/ km effort* (*10, *100) (MacLeod et al, 2005; Monestiez et al, 2006; Kiszka et al, 2007; Brereton et al, 2012; Aissi et al, 2015; Arcangeli et al, 2014b, 2016, 2017, 2021; Morgado et al, 2017; Azzolin et al, 2020; David et al, 2020; Tepsich et al, 2020; Robbins et al, 2020, 2022; Gregorietti et al, 2021; Ham et al, 2021; Campana et al, 2022; Falk Lindberg and Lindqvist, 2022; Zampollo et al, 2022; Gnone et al, 2023; Scuderi et al, 2024);

- *N sightings/ NM effort* (*10, *100) (Correia et al, 2015, 2019);

- *N animals/ km effort (*100, *1000)* (Northridge et al, 1995; Brereton et al, 1999; Lopez et al, 2004; Brereton et al, 2005, 2009; Eguchi et al, 2007; Cominelli et al, 2016; Di Méglio et al, 2018; Arcangeli et al, 2019; Bouveroux et al, 2020);

- *N animals/ NM effort (*100, *1000)* (Johannessen et al, 2022);

- *N sightings/ hour effort* (Arcangeli et al, 2013; Santoro et al, 2015); or days (Herrera et al, 2021) when the effort was not continuous;

- *N animals/ hour effort* (Reid et al, 2003; Southall et al, 2005; Wall et al, 2006; Wall, 2013), more appropriate than length when including observations from different sources, short survey lines, or stationary points (e.g., fishing effort, Braun-McNeill and Epperly, 2002; Girard et al, 2022).

Specific **Occurrence Indices** (proportions of the total ER) can be calculated by dividing the ER relative to each sub-region by the overall ER calculated for the entire study area at the same temporal scale (yearly/monthly, Morgado et al, 2017).

The effects of weather conditions (sea state, cloud cover, wind speed and direction) on the abundance index have been considered by applying GLMs (Cominelli et al, 2016). In order to get sampling units representing a continuous period of effort under the same meteorological conditions, a single transect can be divided into several sampling units (Cominelli et al, 2016).

Sighting rates have also been modelled as a function of several covariates including sea state using GAMs (Reid et al, 2003). Smoothed functions relating sea state to sightings rate were generated for eight cetacean species or species groups and used to adjust survey effort within each sea state category by an appropriate correction factor (Reid et al, 2003). Survey effort in higher sea states was down-weighted compared with effort in low sea states.

4.2.2 Abundance – Distance sampling

Species density (D) is computed as the number of animals/sightings over the surveyed area (e.g., grid cells, Lopez et al, 2004). The observation protocols from large vessels assure that sightings are recorded by applying a line transect method, in which the angle and radial distance are measured to transform the linear transect into a strip transect and to compute the perpendicular distances required to apply the **Distance Sampling** (DS) analysis (Buckland et al, 2001). To take into account possible biases for imperfect detection, the Effective Strip Width (ESW) is calculated, which ensures to respect the assumption of a 100% probability of sightings within the strip and allows setting the total width of each transect for each species, and excluding all sightings above the maximum detectability distance (Williams et al, 2006; Cominelli et al, 2016; Morgado et al, 2017; Robbins et al, 2020; Tepsich et al, 2020; Arcangeli et al, 2021; Sá et al, 2021; David et al, 2022; Johannessen et al, 2022). In some cases, a specific buffer is applied to each monitored track and to each sighting to consider the effective distance of species detectability (Ham et al, 2021; Grossi et al, 2021).
When considering multiple observation platforms, ESW is computed separately for each type of platform, classified according the height of the main deck or speed (see Arcangeli et al, 2014a; Cominelli et al, 2016; Cañadas et al, 2018; Leonard & Øien, 2020; Robbins et al, 2020; Tepsich et al, 2020; Arcangeli et al, 2021).
Detection functions can be modelled (mostly with GAMs) to explore the relationship between animal presence and/or abundance and environmental covariates, considering the entire survey effort or portions of the transects (e.g., Williams et al, 2006 (2 NM); JNCC, 2015 (10 km); Cañadas et al, 2018; Robbins et al, 2020 (5 km)); and results can be included in a model-based Density Surface Modelling (Brereton et al, 2009; Robbins et al, 2020; Johannessen et al, 2022). Different detection functions can be tested, with 0 or 1 adjustment, according to the considered species: Half normal (Williams et al, 2006; Eguchi et al, 2007; Arcangeli et al, 2022), Uniform and Hazard rate (Tepsich et al, 2020; Robbins et al, 2020; Arcangeli et al, 2021; Johannessen et al, 2022). The choice of the optimal detection function is based on the AIC value, QQ plot and Cross Validation. After fitting the detection function with the number of individuals and perpendicular distance from the transect, the density of species along the transect is calculated, as:
*D = N/2ESW L*100,* where:
N is the number of individuals observed;
2ESW is the total width of the transect;
L is the length of the transect (km).

Density can be calculated for the total dataset and stratified for years/seasons/areas for comparison (Eguchi et al, 2007; Tepsich et al, 2020; Arcangeli et al, 2021, 2022; Sá et al, 2021); a randomization method can be applied to compute uncertainty around estimated densities and determine a confidence interval (Eguchi et al, 2007).
Due to data specificity on the loggerhead turtle *Caretta caretta*, a two-step method was used to perform model selection between distance sampling models in the face of overdispersion (Howe et al, 2019), using the following equation:
$QAIC = -2 (\log L(\vartheta) / \hat{c}) +2K$, where:
QAIC is the adjusted version of AIC,
log L is the log likelihood value,
"theta" is a vector of maximum likelihood parameter estimates,
K is the number of parameters in the current model.
When covariates on platform type and weather conditions were added to the models, the best fitting function to assess effective strip width (ESW) resulted:
ESW = Pa * w (with Pa = probability of detection of a turtle, w = strip width), including also the mean group size (s) (see Arcangeli et al, 2022; David et al, 2023).
Considering the effect of weather conditions (sea state, cloud cover, wind speed and direction) on species detectability, and thus on the density estimate, A Multiple Covariate Distance Sampling can be applied to the density index (MCDS, Cotté et al, 2009; Cominelli et al, 2016). In order to get sampling units that represent a continuous period of effort under the same meteorological conditions, a single transect can be divided into several portions (Cominelli et al, 2016).
Another method to estimate density is based on modelling the distances between detections, also called waiting distances, because in areas of high density the waiting distance between sightings is short (Cotté et al, 2009). The density surface is then obtained by calculating the inverse of the waiting area, defined as twice the effective strip half-width, times the waiting distance (Cotté et al, 2009).

4.2.3 Trends

Changes in population abundance can be evaluated by considering the variations of both indicators, ER and density, between two 6-years periods (Vella and Vella, 2015; Leonard & Øien, 2020; Tepsich et al, 2020; Arcangeli et al, 2021, 2022). Despite the limitations of providing information on large areas, the index of abundance, as defined by the HD, is a good indicator for identifying trends (Berrow et al, 2012). However, within this report we observed that only a few studies consider the specific interval required by the policies, while most of them provide general temporal comparisons about species abundance/density, even if using a long-term data series (>10 years, Northridge et al, 1995; Eguchi et al, 2007; Brereton et al, 2009; Lambert et al, 2011; Di Méglio et al, 2018; Valente et al, 2019; Robbins et al, 2020; Grossi et al, 2021; Herrera et al, 2021; Poot and Soldaat, 2022; Gnone et al, 2023). In other cases, short periods have been compared over 20 years, providing insights into general population changes (Arcangeli et al, 2013, 2016).

Short/long-term population trends have been also assessed through linear modelling (Poisson regression) on relative abundance by Brereton et al (2003), who also suggested the use of **Power analysis** to assess the sensitivity of monitoring data and to identify improvements in the survey design required to detect significant levels of change within reporting cycles (Brereton et al, 2009).

Tepsich et al (2020) applied GAMs on species density to inspect the effect of the year in describing trends; and in the analysis of time series a **moving average** can be used on the computed yearly densities (Arcangeli et al, 2022). This method provides a smoothed analysis of the observed data, less prone to 'random' or punctual variations (e.g., anomalous years). Preliminary tests are run in order to define the direction and width of the moving average, that can be computed as centred, left aligned, or right aligned. For the analysis of trends over the 6 years-period (HD and MFSD), a moving average left centred is applied, and then two different widths for the moving window are tested (2- and 3-years window). As a consequence, yearly densities are transformed into 'moved' Densities, as for each year the observed density is averaged with the density observed during the previous years. Trends are then computed by considering the two reference periods separately, and the statistical significance of the regression line is used as a proxy for trend significance (Arcangeli et al, 2022).

Comments

Despite large scale programmes performed over decades provide robust population estimates (Víkingsson et al, 2009; Hammond et al, 2017), surveys carried out with higher frequency (yearly-monthly) allow excluding uncertainties due to year-to-year variations and better characterising spatial distribution (Kiszka et al, 2007; Arcangeli et al, 2016; Azzolin et al, 2020; Tepsich et al, 2020), detecting low density or less detectable species (Williams et al, 2002; Bouveroux et al, 2020; Robbins et al, 2020; Arcangeli et al, 2023a). The index of abundance doesn't provide an absolute estimate of population size (Palialexis et al, 2019) but can be used to assess trends, evaluate differences between consecutive periods, and, through the development of models, can be converted into an estimated number of individuals for specific marine regions (Arcangeli et al, 2022). The estimated densities derived from these monitoring activities show a decrease in the confidence intervals as the number of surveys included in the analysis increases (Brereton et al, 2009), while Power analysis can be useful to assess the sensitivity of current monitoring and to identify improvements in the survey effort (Brereton et al, 2003; ACCOBAMS, 2022).

Distance sampling applied to data collected from large vessels is effectively used to estimate abundance of animals at sea, especially if used in combination with aerial surveys (Panigada et al, 2021; Girard et al, 2022) and if the availability and the perception bias are taken into account (Cañadas et al, 2018; Katsanevakis et al, 2012).

The moving average method performed better in smoothing peak events within the time series, but could still detect the interannual variability. Specifically, the 3-years moving average method proved to reduce the limitations posed by the analysing of a dataset with insufficient data. The detection of patterns also within each 6-years reference period (i.e., Cominelli et al, 2016; Morgado et al, 2017; Correia et al, 2019, 2020; Gregorietti et al, 2021; Atzori et al, 2021) could support the interpretation of trends between reference periods (Tepsich et al, 2020; Arcangeli et al, 2022).

Finally, indicators can be aggregated to identify changes in species populations at national level (Brereton et al, 2003), as shown by the combined use of density and range indicators to describe species abundance (Arcangeli et al, 2022).

4.3 Analyses for "Range"

4.3.1 Observed distributional range

Monitoring surveys ideally should be based on species-specific design criteria that optimise sampling within all habitats relevant to each species throughout its entire range (Forney, 2000). The systematic monitoring over large sea areas, despite not covering the entire range of a species, is however considered excellent in providing information about the distribution of the species and its trends (Berrow et al, 2012). For some species that occupy different habitats throughout the year and their life cycle, distributional range estimations should be seasonal and life stage-specific (Girard et al, 2022; Zampollo et al, 2022).

The easiest way to report the spatial presence of a species population is to map the distribution of sightings, i.e., their **occurrence**, over the studied area (Brereton et al, 2001; Wall et al, 2006; Cotté et al, 2009; Lambert et al, 2011; Arcangeli et al, 2013; Vella et al, 2013; McClellan et al. 2014; Santoro et al, 2015; Cominelli et al, 2016; Leonard and Øien, 2020; Herrera et al, 2021; Sá et al, 2021; Johannessen et al, 2022). Some authors defined a buffer around each sighting to take into account the effective distance of species detectability (Ham et al, 2021; Grossi et al, 2021).

Spatial reporting can be done over a **grid**, whose size can vary according to the study area, the species and the objective of the study:

- *small grid cells* (1x1 km) are applied to investigate habitat preference at fine scale (Lambert et al, 2011; Arcangeli et al, 2013, 2016; Correia et al, 2015; Azzolin et al, 2020; Bouveroux et al, 2020; Grossi et al, 2021; Ham et al, 2021), and are compliant with the recent INSPIRE Directive requirements for spatial information (2007/2/EC);
- *4x4 km* grid cells, or similar, are used for coherence with the resolution provided by remote sensing data (McClellan et al, 2014; Lambert et al, 2014; Pennino et al, 2017; Zampollo et al, 2022);
- *HD reporting scale* is used as a portion (5x5 km grid cells)(Williams et al, 2006; Leeney et al, 2012; JNCC, 2015; Morgado et al, 2017; Arcangeli et al, 2017, 2019, 2021; Robbins et al, 2020; Atzori et al, 2021; Gregorietti et al, 2021; Sà et al, 2021; Campana et al, 2022; Scuderi et al, 2024) or entire cells of 10x10 km (Brereton et al, 2012; Arcangeli et al, 2014a; Matear et al, 2019; David et al, 2020, 2022; Arcangeli et al, 2022); a multiple-steps process that takes into account effort and sightings made on different grid cells has been used from 1 km to 5 km grid cells (Ham et al, 2021; Grossi et al, 2021) and from 10 km to 50 km grid cells (Arcangeli et al, 2022);
- *larger grid cells* (14x14 km, 20x20 km, ICES grid cells, 50x50 km, 100x100 km) can be used for biogeographical scale studies and reports (Northridge et al, 1995; Brereton et al, 1999, 2003; Reid et al, 2003; Kiszka et al, 2007; MacLeod et al, 2009; Wall, 2013; Aissi et al, 2015; Correia et al, 2020; ORCA, 2019, 2021, 2023; Gnone et al, 2023) or to reduce the noise due to low number of sightings in some areas resulting in a map with high spatial variability

(Arcangeli et al, 2022; Cañadas et al, 2018; Valente et al, 2019); for example, the reporting of some cetacean species under the HD in UK waters considers a 50 km grid (UK Article 17 Habitats Directive Report 2019, available at: https://jncc.gov.uk/our-work/article-17-habitats-directive-report-2019/) but it would be difficult then to include such information in policy reports requiring finer scales (DG Environment, 2017; IUCN, 2019).

Distribution is analysed as the number grid cells with sightings within the surveyed cells: the binary presence-absence of the sighting (i.e., **occupancy**) is computed only for cells covered by effort. A distribution grid is created considering all the cells with presence (MacLeod et al, 2009; Brereton et al, 2009; Arcangeli et al, 2022, 2023a). The occupancy rate is calculated for each species/area/period, as:
Number of occupied cells/Number of cells with effort (MacLeod et al, 2009; Arcangeli et al, 2021).
Occupancy can be used to measure two components of a species status. Individual distribution can be used to verify changes in fine-scale spatial distribution over time (MacLeod et al, 2009; Brereton et al, 2009). Occupancy has been found to relate to species abundance, and it has been used to monitor changes in the conservation status of a number of organisms (Holt et al, 2002). Occupancy models can be fitted with different software, used as a low-cost surrogate of abundance (Katsanevakis et al, 2012).
When considering species groups or different taxa, the distribution of **species richness** can be an important tool to identify biodiversity hotspots, and propose marine conservation measures, such as IMMAs (Brereton et al, 2012; Arcangeli et al, 2014a; IUCN, 2016; Matear et al, 2019; Campana et al, 2022; Gnone et al, 2023).
Sighting counts can be converted to **density** for mapping purposes by dividing the number of sightings by the area of each cell (Southall et al, 2005; Williams et al, 2006). A more detailed description of species occurrence can be provided by reporting the spatial distribution of the standardised values of abundance indices (ER or SPUE) or Density (from distance sampling) over these grid cells, by considering the amount of effort within each cell, and investigating spatio-temporal differences (Northridge et al, 1995; Reid et al, 2003; Lopez et al, 2004; MacLeod et al, 2005; Monestiez et al, 2006; Kiszka et al, 2007; Brereton et al, 2012; Wall, 2013; Aissi et al, 2015; Correia et al, 2015, 2019, 2020; Arcangeli et al, 2019; ORCA, 2019, 2021, 2023; Robbins et al, 2020; Arcangeli et al, 2021; Gregorietti et al, 2021; David et al, 2022; Zampollo et al, 2022; Scuderi et al, 2024). The Occurrence Index (Morgado et al, 2017) can also be reported in each cell.
A **minimum effort** can be set in the cells according to the resolution (see preliminary analyses): for example, 1x1 km cells covered with less than 100 m effort (Arcangeli et al, 2022, 2023a), or those with less than the 1st quantile value (Robbins et al, 2022), are discarded.

4.3.2 Spatial generalisation of range

A spatial generalisation of the range can be calculated from the distribution maps of presence/absence or abundance of a species. According to the HD guidelines (DG Environment, 2017), the surface range of a population is first calculated by creating an envelope around the presence/absence distribution grids, using the "gap closure" procedure (gap = 90 Km), which creates a **Minimum Convex Polygon** (MCP). Unsuitable areas, such as terrestrial ones, are then excluded from the envelope (Arcangeli et al, 2022). In some cases, the current range of the species can be obtained based on the literature and experts' collective knowledge (e.g., MacLeod, 2009; Reeves et al, 2013) or by extrapolation from a limited amount of data for reporting purposes (e.g., UK Article 17 Habitats Directive Report 2019, available at: https://jncc.gov.uk/our-work/article-17-habitats-directive-report-2019/).

Other methods can provide a better definition of the observed distribution than the coarse methods based on the occupancy grid or MCP, but these might be more complex to apply, since adjustments might be required for spatial scale and data resolution (DG Environment, 2017).

A **Kernel Density Estimator** (KDE, Hengl et al, 2009), based on the abundance index calculated for each cell, can be used to spatially identify the extent and core areas within the area covered by the effort (real species distribution, Girard et al, 2022). KDE analyses can be set with varying cell resolution and search radius depending on the areas and species investigated. For example, 95 % isopleths are used to define the extent (km²) of occupied area (i.e., Observed Distributional Range, Arcangeli et al, 2022, 2023a; Gnone et al, 2023), while 70-80 % isopleths usually identify core area locations (Arcangeli et al, 2014b, 2016; Pace et al, 2019; Arcangeli et al, 2021; Atzori et al, 2021; Gregorietti et al, 2021). Lower thresholds can be chosen for specific investigation purposes (50 %, Sá et al, 2021).

The **Poisson Kriging** is another approach based on ER cell values. This is a multiple process that includes the exploratory statistical analyses of the data, the modelling of the variograms, the creation of the surface, and possibly the exploration of the surface variance. Kriging can generate prediction surfaces and surfaces that describe the prediction quality of the model. A specific geostatistical kriging was developed by Monestiez et al (2006) to consider the counting variability under the Poisson distribution, better fit the data, and allow the continuous interpolation (even where the effort is null) of values. The Kriging system was applied to interpolate ER, which can be mapped with an associated map of variance to estimate the distribution and surface of the range of the target species (Di Méglio et al, 2018; Arcangeli et al, 2022; David et al, 2023). In a second step, the kriging can be processed for different periods, so that only cells with effort in common across different periods are considered. Kriged maps based on ER can be transformed in **maps of densities** using ESW and group size, which are used as indicators of abundance. Cells above the threshold of 90 percentile of density are considered as “occupied” and are used for range calculation.

To identify the locations of statistically significant **hotspots** and “coldspots” for the species, spatial gridded records are tested to highlight whether data showed random or clustered patterns using the Average Nearest Neighbour and the Morans I index, and data with clustered patterns are investigated by Getis-Ord Gi* analysis (Getis and Ord 1992; Arcangeli et al, 2017, 2019; Gregorietti et al, 2021). To define hotspots for a species, an <u>aggregation index</u> can be computed as the number of sightings/animals, standardised by the mean and by the standard deviation of that sub-region (Morgado et al, 2017); a cell is classified as a “Hotspot” whenever the computed index is higher than its mean value by two standard deviations. A similar method is based on normalised SPUE values in the cells, which are considered a hotspot if their SPUE value exceeds the yearly mean by 1 standard deviation (Grossi et al, 2021).

The **Ecological potential range**, i.e., the potential area that a species can occupy, can be estimated from the location of sightings through modelling approaches, by considering several predictors that are usually linked to habitat characteristics and defining the Extent of Suitable Habitat (IUCN, 2019) (Knowlton et al, 2002; Druon et al, 2012; Lambert et al, 2014; Correia et al, 2019, 2020; Valente et al, 2019; Azzolin et al, 2020; Bouveroux et al, 2020; Atzori et al, 2021). For details see Habitat for the Species (section 4.4).

4.3.3 Trends

In species-habitat studies, the rate at which a change occurs is often more relevant than the absolute value of the occupancy state (Katsanevakis et al, 2012). The proportion of change in the observed distributional range and its potential spatial shift have to be investigated between reporting cycles, taking the life stage of the species into account (Girard et al, 2022). As for the Population parameters, only few of the considered studies investigated the specific range variations over the 12-24 years intervals specifically required by the policy framework (Leonard & Øien, 2020; Arcangeli et al, 2021, 2023a). Trends in distribution can be assessed by visually comparing maps of sightings frequencies, ER or densities (Leonard & Øien, 2020; Arcangeli et al, 2021), and calculating the percentage of changes and the patterns over time (Arcangeli et al, 2023a). Other studies reported these comparisons over 20 years, but considered a three-years dataset for each period (Arcangeli et al, 2013, 2016).

Some studies report the spatio-temporal **persistence** of yearly hotspots (i.e., species abundance) over a long period, by computing an Hotspot Index for each cell, to take the number of times each cell was considered as Hotspot (into account JNCC, 2015; Grossi et al, 2021); the index varies between 0, which indicates persistent absence, and 1, which indicates cells with persistent species presence (Grossi et al, 2021), and can be used to inform the development of protected areas (JNCC, 2015).

Given the relationship between occupancy and abundance, changes in occupancy are to be used to infer changes in abundance (Brereton et al, 2009, 2012) and the direction of range change (e.g., due to climate change, MacLeod, 2009).

Range trend values can be estimated by the assessment of the species <u>occurrence</u> over a defined period, by calculating the percentage difference in the number of cells occupied between two investigated periods within the same geographical unit (MacLeod et al, 2009; Lambert et al, 2014; Arcangeli et al, 2021), as:

[(occurrence period 2 – occurrence period 1) / occurrence period 1] (Arcangeli et al, 2021, 2023a);

or as the difference in the occurrence referred to the area surveyed in each period:

*[(occurrence period 2/total grid cells period 2)– (occurrence period 1/total grid cells period 1)]*100* (OSPAR, 2019; Arcangeli et al, 2022, 2023a).

Shifts of distribution can also be calculated as indicated by OSPAR (Arcangeli et al, 2022):

2(number of same cells occupied during periods 1 and 2) / (occurrence period 1+occurrence period 2).

Shifts of range can concern either the surface or the centre of gravity (centroid) of range areas, and can be assessed as the percentage of overlap between two periods within a common effort area; the percentage of overlapping area compared to the first period:

*[(Overlapping area/area period 1)*100]*;

or as the direction and magnitude of shift in the centroids of the range area (Arcangeli et al, 2023a).

Further investigation can be done by comparing the spatial generalised distribution, based on sighting densities/abundance (see paragraph 4.3.2); visual overlap can provide information in terms of changes in the location of distributional range (Arcangeli et al, 2013, 2014b, 2016; Di Méglio et al, 2018; Arcangeli et al, 2021, 2022), while variations in the surface area is computed as difference in extensions and percentage differences over the two investigated periods:

[(area period 2 – area period 1) / area period 1] (Arcangeli et al, 2023a).

Based on the cells with common effort between two reference periods, a mask can be created for kriging results, extended with a buffer of half the size of a cell based on the lowest variance (25 km buffer), to show and compare the surface obtained only within the common sampled area (Arcangeli et al, 2022; David et al, 2023).

Comments

Distribution maps are related to the Population parameter, so they are often used and assessed together. A good way to investigate GES is to mix two criteria, for example obtaining maps of densities of observed animals.
Some studies tested the "HD range tool" and concluded that it is inappropriate for cetaceans (and probably sea turtles); spatial interpolation is useful to allow inferring the presence of the species at larger scales and provides better insights (e.g., Kernel, Kriging). A reduction of the grid's cell size and "search radius" for the Kernel has still to be tested (Arcangeli et al, 2022). The Kriging method has the advantages to take the spatial heterogeneity of effort into account and allows inferring the spatial structure, the covariance and the variogram, helping the estimate of spatial distribution of densities over less known areas (Arcangeli et al, 2022). This method integrates the densities over the entire domain of interest and obtains estimates of the total abundance and standard deviation (Bellier et al, 2013). This will help integrate some covariables (bathymetry, distance to the coast) in order to calculate a spatial "drift", which will improve the robustness of trends predictions. This method is increasingly recognised and has been improved with the SPDE-INLABRu method, a model that includes the spatial effect (as for kriging) and the effect of covariables, mainly the static ones, such as bathymetry and distance to the coast (David et al, 2023).
Occupancy is also a good indicator for range that can be used to infer changes in abundance (Brereton et al, 2009). As for other methods, its weakness may relate with the effort, since analyses should not be performed at a basin scale, but rather within subareas according to the data. Occupancy and Observed Distributional Range are good indicators to detect trends provided the coverage of sampled range is consistent over time (Brereton et al, 2009; Arcangeli et al, 2023a), as it is the case for the networks involved in the MTT project. Indeed, for assessing trends, the comparison is possible andmeaningful only over common areas between both periods,

which calls for a monitoring strategy that ensures the coverage of the same areas at large scale (e.g., 50x50 km grid; David & De Jesus, 2023). The extent of range could be equal among periods, but shifted in different areas (e.g., as an effect of pressures), so that the contemporary investigation of the trends in extent (surface range) and shifts (range pattern) is recommended (Arcangeli et al, 2022, 2023a). As for the Population parameter, also studies conducted within the 6-years period (Wall, 2013; Arcangeli et al, 2014b; Morgado et al, 2017; Correia et al, 2020; Ham et al, 2021) can be useful to better interpret the trends.
Finally, the assessment of spatial changes in distribution within a study area can be used to examine whether these changes are related to variations in habitat use or in the environmental conditions (MacLeod, 2009; Brereton et al, 2009).

4.4 Analyses for “Habitat for the species”

4.4.1 Suitable habitat – ecological potential range

Data of wide-ranging marine species may be limited to just a set of presence values or extrapolated distribution. To analyse the habitat used by the species, which can be variable according to seasons or life stage (Girard et al, 2022), the mean values of the **environmental variables** (topographic and oceanographic) of the monitored tracks/cells with and without sightings can be compared (Knowlton et al, 2002; Brereton et al, 2005; Wall et al, 2006; Kiszka et al, 2007; Vella, 2010; Wall, 2013; Arcangeli et al, 2016; Azzolin et al, 2020), and the relationship between species abundance and habitat features can be investigated(Lopez et al, 2004; Arcangeli et al, 2014b; Vella and Vella, 2015; Herrera et al, 2021). The assessment of the occupied niche can also be carried out using an outlying mean index (OMI) analysis, an ordination technique designed to ‘seek combinations of environmental variables that maximize the average species marginality used by a species and the mean habitat conditions of the sampling domain’ (Karasiewicz et al, 2017; Zampollo et al, 2022).
Where no data are available, different modelling techniques, chosen according to the specific type of data/sampling strategy/target species, can be applied to predict the probability of occurrence of the species and thus define their **Ecological Potential Range.**
Species Distribution Models (SDM, environmental envelopes, or ecological niche models) can be defined as statistical and/or analytical algorithms that predict the actual or potential distribution of a species, based on field observations and auxiliary maps of environmental variables (Hengl et al, 2009). These techniques allow defining the preferred habitat features for the species (area of potential range or Extent of Suitable Habitat, ESH) and providing estimates of uncertainty (standard errors; confidence intervals) that have to be critically reported, analysed and interpreted. Models, in fact, can be useful to provide information on the areas potentially used by the species, and thus also support the definition of the parameter Range, but don’t inform about the quality of the occupied habitat, which is required by the HD.
There are static or dynamic features (or “species distribution factors”, Correia et al, 2020) that characterise the environment where a species lives. In SDM, the most commonly used **variables** are:

topographic factors: longitude, latitude, depth, slope, aspect east, aspect south, distance from nearest coast, a given bathymetry, canyon, sea mountains, ports, seabed sediment or any other topographic feature;
oceanographic variables: mean Chlorophyll-a concentration, mean Sea Surface Temperature, currents. Also year/season can be included as dynamic variables. These variables can be selected after testing for multicollinearity problems through correlation analyses and estimates of the variance inflation factor (VIF) (Correia et al, 2015; Arcangeli et al, 2017; Pennino et al, 2017; Correia et al, 2019, 2020, 2021; Gregorietti et al, 2021; Grossi et al, 2021; Campana et al, 2022; Robbins et al, 2022; Arcangeli et al, 2023a). When comparing different areas, the variability of the environmental features can be checked for a better setting and interpretation of the models (for example with PCA, Correia et al, 2020; Zampollo et al, 2022).

The construction of SDMs is based on methods that differ according to the type of data available for the target species.

Models based on "presence/absence records":
The repeated sampling over the same routes allows the collection of presence and 'absence' data, defined after setting a minimum effort threshold (Arcangeli et al, 2013, 2016, 2017); considering the sightings of other species as absence points of the studied species (Lambert et al, 2011; Valente et al, 2019); or considering equidistant "pseudo-absence" points along the surveyed tracks (Forney, 2000; Correia et al, 2021). Models applied to these data include: GLMs (Forney, 2000; Arcangeli et al, 2013; Bouverox et al, 2020; Arcangeli et al, 2022, 2023a), GAMs (Forney, 2000; Williams et al, 2006; Cotté et al, 2009; Correia et al, 2015; JNCC, 2015; Arcangeli et al, 2016, 2017; Correia et al, 2019; ORCA, 2019; Valente et al, 2019; Correia et al, 2020, 2021; Azzolin et al, 2020; Ham et al, 2021; Grossi et al, 2021; Robbins et al, 2022; Campana et al, 2022), GAM Negative Binomial, GAM tweedy (Arcangeli et al, *in prep*), Logistic regression, Neural Networks, ordination and classification methods (Bioclimatic Envelope, Lambert et al, 2011, 2014), Bayesian models (Pennino et al, 2017), Density surface modelling (corrected for uncertain detection via distance sampling methods, JNCC, 2015; Robbins et al, 2020; Johannessen et al, 2022).

Models based on "occurrence-only records" (or presence-background data):
These methods allow the use of different data sources without accounting for the observation effort, so maximizing the usefulness of scattered biological data, that do not homogeneously cover the entire range, or when the absence of the species may not be reliable (Pace et al, 2019; Arcangeli et al, *in prep*). The methods generate pseudo-absences points ('background points') around the surveyed areas. The most used models of this type are: Maximum Entropy method (McClellan et al, 2014; Pace et al, 2019; Azzolin et al, 2020; Ham et al, 2021; Zampollo et al, 2022; Arcangeli et al, 2023a; Scuderi et al, 2024), Ecological-Niche Factor Analysis, Random Forest (Gregorietti et al, 2021; Arcangeli et al, *in prep*), Genetic Algorithm for Rule-Set Prediction, Multinomial Logit models, Regression-kriging method (Monestiez et al, 2006). In some cases, abundance indices have been used instead of occurrence data, to allow the inclusion of the information on the sighting effort in the modelling, reducing possible sampling biases (Monestiez et al, 2006; Azzolin et al, 2020; Zampollo et al, 2022; David et al, 2023).

The use a **complementary approach** based on two or more types of models allows using the entire dataset and integrating of the results, to provide a robust habitat characterisation and interpretation of habitat preferences (Correia et al, 2021; Ham et al, 2021). The Ensemble Platform for Species Distribution Modelling ("biomod2" package) allows building a wide set of models, comparing them and grouping the best ones into a single combined model (Gregorietti et al, 2021; Arcangeli et al, 2023a). The use of an independent dataset is recommended to validate of the output models, and evaluate the representativeness of predictions outside the surveyed region (Cotté et al, 2009; Lambert et al, 2011; Druon et al, 2012; Arcangeli et al, 2023a); when an independent dataset is not available, the original dataset can be split in two halves, one used for testing and the other for training purposes (Arcangeli et al, 2022). The cross-validation approach is also applied, in which data from one period reciprocally serve to test the predictive power of the other period's best model (Forney, 2000; Arcangeli et al, 2016). For the **selection** of the best performing models, different methods are used according to the modelling approach and type of data used. The most common measures of predictive performance are: area under the receiver operating characteristic curve (AUC); continuous Boyce Index (only presence data); percentage of the explained deviance, the Akaike Information Criterion (AIC), Cross-Validation, accuracy score, Log Loss.

SDMs produce **maps** with continuous values that indicate the habitat suitability on a scale from low to high (e.g., Pennino et al, 2017). In some cases, a specific prediction area can be defined, where the major effort occurred (Williams et al, 2006; Pace et al, 2019, 2022). To calculate the ESH area, a threshold can be set to distinguish different levels of suitability (e.g., 60 %-100 %, medium-high suitability, Zampollo et al, 2021; Campana et al, 2022), and the results can be visually inspected by experts. Within MaxEnt, the best threshold method is selected (Equal training sensitivity, specificity logistic threshold). The output binary suitable-unsuitable prediction rasters can be converted into polygon layers that include the highest suitable class for each species/period, and where the extent of the ESH in km² can be measured (Campana et al, 2022; Arcangeli et al, 2022, 2023a;). This value can be compared between periods/areas or overlapped with existing protected areas (Campana et al, 2022; Scuderi et al, 2024).

To compare the suitable habitat actually occupied by the species (observed distribution ranges vs ecological potential range) to the available habitat in the study area, a quantile analysis (boxplot) can be performed, through a set of equidistant points along the effort tracks (Forney, 2000; Correia et al, 2015, 2019, 2020) or across the study area (Arcangeli et al, 2016; Azzolin et al, 2020), where the environmental variables are extracted; some authors randomly generated sampling points in the different levels of the spatial generalisation of presence (McClellan et al, 2014). Considering only the areas covered by the effort, the extent of suitable habitats is estimated in km² and the percentage proportion of the area actually occupied against the area of the modelled suitable habitat is calculated as:

*[(area occupied) / (area suitable)]*100* (Arcangeli et al, 2023a).

4.4.2 Trends

Trends in habitat for the species have been evaluated by comparing suitability models computed for different periods (Arcangeli et al, 2016) or by combining predictions from habitat niche with climatic niche models (Lambert et al, 2011, 2014) or other risk factors (Grossi et al, 2021). Change in the potential range can be evaluated using the percentage of changes in the extension and overlap of the Suitable Habitat between the two periods (Girard et al, 2022; Arcangeli et al, 2023a). If the effort area is not consistent between the two periods, two distinct models, using the same settings and set of variables, should be run for each investigated period.

The change in the extent of suitable habitat is calculated as:

[(ESH period 2 – ESH period 1) / ESH period 1] (Arcangeli et al, 2023a).

The extent of area of overlap of the Suitable Habitat between the two periods can be also calculated.

Differences in the proportion of the modelled suitable habitat occupied by the species is calculated between periods as:

[(%period 2 - %period 1) / % period 1] (Arcangeli et al, 2023a).

Comments

The species distribution modelling identifies the environmental variables that support higher densities of the target species, thereby providing information on physical and biological factors essential to their life and reproduction, thus for their conservation (Druon et al, 2012; Azzolin et al, 2020; JNCC, 2015). Environmental variability can affect species trend analyses, especially when they are sampled on fixed routes that don't include their entire range: this results in apparent fluctuations in local abundance, difficult to separate from true trends (Forney, 2000). Sampling design should be representative of the species range and known key areas (Arcangeli et al, 2023a; Forney, 2000), which is not always true when using non-dedicated platforms; however, it may be possible to model the effects of habitat variability analytically, partitioning changes in apparent abundance into components that can and cannot be explained by environmental changes (Forney, 2000). Data systematically collected from large vessels provide good datasets that can fit different types of models to define the habitat use of the species and its variations (Berrow et al, 2012), and how temporal aspects of habitat preferences affect cetacean abundance and distribution (Brereton et al, 2009). Given the nature of species-environment relationships, in fact, models should be constructed with multi-year data reducing unexplained variability and increasing power to detect trends (Forney, 2000).

Among the tested algorithms that employ presence/absence data, GAM is the most used and it appeared the best (Arcangeli et al, 2022, in prep). Among the methods for presence-only data, MaxEnt has a good power in predicting habitat niches and it can be used to measure the ESH, allowing comparison among periods (Arcangeli et al, 2022, 2023a). To date, many policy reports define "unknown" or "with insufficient data" the trends for the Habitat parameter, meant as the extent of suitable habitat, indicating the need for increasing the application of the described methodologies to the datasets of these monitoring programmes.

A good way to investigate GES for the MSFD Descriptors or HD Parameters is using several indicators for the same parameters; similarly, mixing two criteria, for example by comparing the Observed Distributional Range (Range) versus the Ecological Potential Range (Habitat; Arcangeli et al, 2023a), helps the interpretation of the species conservation status for each parameter (Arcangeli et al, 2022).

5 Threats and other collateral data collected within the MTT network

Several human activities, such as coastal urbanisation, tourism, shipping and fisheries, are acting as stressors for the marine environment by favouring biological invasions, producing acoustic or chemical pollution and waste discharge that can directly physically damage habitats and marine species and spoil the regular functionality of natural ecosystems (Halpern et al, 2008). These stressors are also mentioned in the environmental policy framework (e.g., IWC, IUCN, HD), with some specific objectives defined by the most recent directives (Spitz et al, 2017; MSFD, MSPD).

Indeed, the effective management of marine species requires knowledge of the temporal and spatial extent of co-occurrence of specific anthropogenic pressures with the species, in order to identify priority conservation areas and seasons, and mitigate threats in the adequate place and time. Risk assessment studies are generally based on the observed/predicted distributions of target species and threats, but for species that have a complex life cycle and use a variety of habitats, such as marine large vertebrates, dynamic assessments are required (Arcangeli et al, *in press*).

Data derived from systematic monitoring programmes are useful to obtain information about some anthropogenic pressures, such as maritime traffic and pollution by marine litter, contributing in this sense to fulfil some requirements of current EU policies; in the following sections, the different approaches applied to describe and quantify the variable effect of these threats on marine species, using data collected within the networks of the MTT project, are briefly reported.

5.1 Maritime traffic

The presence of vessels can produce different effects on marine species, spanning from the short- and long- term alteration of their behaviour and distribution, to acoustic disturbance, injuries or death caused by collisions with large ships or fast motor boats. These effects can be particularly evident in high traffic areas or seasons, according to the species characteristics. To investigate the potential effect of maritime traffic on the species abundance, some studies included vessel traffic in the predictive models for whale distribution (JNCC, 2015; Falk Lindberg & Erika, 2022).

Behavioural responses to vessels presence can be evaluated by dedicated observations (e.g., Falk Lindberg & Erika, 2022) that cannot be easily carried out from large vessels travelling along fixed routes. However, these monitoring programmes can provide information about the distribution and abundance of large vertebrates in areas of intense shipping, such as the Bay of Biscay or the NW

Mediterranean Sea, which are useful to identify potential areas and seasons at high risk. The spatial overlapping of zones of presence of species and traffic has indeed been recognised as one of the most important factors determining risks for the species.

To obtain spatial distribution of shipping density, some studies used data from an automatic identification system (AIS)(Reeves et al, 2013; Ham et al, 2021) or Lloyd's Intelligence Maritime Unit (LMIU)(Di Méglio et al, 2018). Data on maritime traffic composition and intensity are also visually collected on board large vessels with a specific protocol by the FLT MED NET (Campana and Vighi, 2020; Arcangeli et al, 2022), which provides synoptic information with the species that can be used to verify the effect of **vessels presence** on the observation of the species. Traffic intensity is compared in the presence of animals' sightings to random locations as:

(N presence- N absence) / N absence

where N is the number of vessels counted in the presence and in the absence of sightings (random locations) along the surveyed transects. Campana et al (2015, 2017) reported that maritime traffic was generally lower in areas of cetacean sightings, which likely indicated a different use of space by animals and shipping; where no differences emerged, this could suggest an actual overlap of the species with shipping, which can still result in potential risk, so that further investigation in these areas can be planned (Campana et al, 2015; Scuderi et al, 2024). This analysis can be stratified per species, seasons, type of vessels, marine regions, in order to highlight specific relationships and significant variations.

Traffic intensity can also be analysed through **interpolation** methods, such as KDE, which describe the spatial distribution of the general shipping or for specific vessel types recorded by the visual monitoring protocols. Some authors spatially compared the obtained high traffic areas, defined by a threshold of the 70% isopleths, with species observations or distribution models (Campana et al, 2022; Scuderi et al, 2024). SDM can be also compared with maritime traffic distribution models (Pennino et al, 2017; Di-Méglio et al, 2018; Ham et al, 2021); in some cases, geographic predictors have been used in both modelling approaches and direct comparison between the resulting distributions allowed identifying potential overlapping areas (Pennino et al, 2017).

Given the known risk of **ship strike** for large whales, likely due to their reduced mobility and longer periods spent at the surface between dives (Laist et al, 2001), there is increased interest in quantifying "dangerous" encounters between ships and whales that do not result in a collision as a proxy for actual strikes (i.e., *Near Miss Events*, NME, David et al, 2022; ORCA, 2023; Scuderi et al, 2024). Direct observations have been conducted from large vessels in different marine regions, such as Alaska, Bay of Biscay (ORCA, 2023), NW Mediterranean (David et al, 2022), Strait of Gibraltar (Scuderi et al, 2024): in this way, important behavioural information can be provided to understand how whales perceive and react to large ships and to identify at what point the whale's behaviour changes in relation to the vessel, defining the 'critical zone' (ORCA, 2023). David et al (2022) computed an NME rate and density index per cell, evidencing the areas of higher probability for ship strike occurrence. A collision risk index was instead calculated by Ham et al (2021), by multiplying the probability of whale occurrence predicted by modelling and the ferry density obtained from the AIS data processing. Similarly, a collision rate was estimated by Di Méglio et al (2018), by considering also the trajectories, distance travelled and the number of passages for each ship category in each cell.

From a conservation perspective, the reduction of the speed of vessels (IWC, 2023) is an effective measure to reduce the risk of ship strikes that can be proposed in areas where no spatial separation is possible (Scuderi et al, 2024).

5.2 Marine litter

The complex path of marine litter, from the source to dispersal, through fragmentation and accumulation processes, determines interactions with marine life at various levels, which cause detrimental effects, mostly dependent on the litter type and size and on the organism affected. Several types of litter are present in the marine environment, but plastic items are widely recognised as the most widespread, endangering organisms from all trophic chain levels, mostly through ingestion and entanglement (CBD, 2012). Risk-exposure studies identify the areas with high potential for interactions where marine litter accumulations overlap with the presence of target marine species (Arcangeli et al, *in press*).

Information on marine litter amount and distribution, and on its potential impacts on marine organisms, are required to assess GES within Descriptor 10 of the MSFD. Monitoring of floating marine macro litter (FMML) has been implemented within existing research programmes using large vessels as platforms of observation, namely by the FLT MED NET. FMML monitoring is carried out synoptically with marine species observations and allows the detection of areas of concurrent presence of litter and vulnerable species.

The quantification of the FMML is obtained by applying two main approaches: the FLT MED NET applies a standardised protocol based on the MSFD Guidelines, which defines a **strip transect**, in which litter density can be computed as:

*Number of items/(Transect length*Width of the strip)*

(Campana et al, 2018; Arcangeli et al, 2019; Gregorietti et al, 2021; Atzori et al, 2021; Campana et al, 2022).

Other authors apply the **distance sampling** method, in order to obtain the correct items density over the monitored area (Sá et al, 2021).

In all cases, litter densities can be standardised over grid cells and generalised through **KDE**. High density areas, where the overlap with target species presence is computed, are defined by setting a threshold according to the study area and target species (70-90% isopleths) or considering different levels of density (10%, 50% and 70%, Sá et al, 2021). Some authors also applied specific analyses to evidence the hotspots of marine litter, such as G* analysis (Getis and Ord, 1992), or Average Nearest Neighbor analysis (Arcangeli et al, 2019; Gregorietti et al, 2021).

To evaluate the **overlap** of high accumulation of marine litter with the target species, the percentage of sightings falling within high density areas can be considered (Campana et al, 2018; Arcangeli et al, 2019). Conversely, the number of floating items falling within a buffered area around the species sightings can be counted, in order to highlight the percentage of animals exposed to plastic and the number of items surrounding the individuals (Arcangeli et al, 2019). In many cases, the KDE is also calculated for the species, reporting the overlap with marine litter as a percentage of the species generalised distribution (Arcangeli et al, 2019; Atzori et al, 2021; Gregorietti et al, 2021; Sá et al, 2021).

A more detailed analysis can be performed by calculating **risk indices** that combine densities of sensitive species (as density or ER) and threat (density), on a grid basis. The product of the species and the litter densities, weighed by the survey effort, provides a layer that represents co-occurrence and potential risk (Arcangeli et al, 2019; Sá et al, 2021; Atzori et al, 2021; Gregorietti et al, 2021); areas of interaction risk can be estimated through inverse distance weighted interpolation (Sá et al, 2021). The same approach has been applied by Campana et al (2022) by considering both marine litter and maritime traffic in the risk index.

5.3 Climate change

Among the potential threats caused by the increased anthropization, climate change is certainly the most global one, but with yet less predictable effects. Indeed, climate change not only involves the increase of temperatures, both of the atmosphere and the oceans, but also a raise in the oceanic level, extreme weather events, heat waves, drought, and other phenomena that could produce a number of impacts over marine species, and marine biodiversity in general, which are difficult to foresee.

It has been demonstrated that climate change may affect the composition and structure of ecological communities, also in the marine environment, where one of the measurable effects is the gradual replacement of cold-water species by warmer water ones (e.g., MacLeod et al, 2005). Such shifts in the composition and structure of communities, which also affect cetacean and sea turtle species, could be assessed by comparing their sightings frequencies and relative abundances from previous studies (MacLeod et al, 2005) or within a long-term monitoring effort of the same marine area (e.g., MacLeod et al, 2009), such that carried out within the MTT project (e.g., Arcangeli et al, 2013).

Cetacean species may also respond to the increase in water temperature by changing their range. Knowledge of the climatic preferences and other aspects of the ecology of species, such as their trophic ecology, allows producing predictions of how individual species would react to the climate change effects. In his review of the potential implications of climate change on cetacean populations, MacLeod (2009) predicted that changes in water temperature resulting from global climate change may affect the ranges of 88% of cetaceans, with unfavourable conservation implications for the conservation of 47% of the species, and leading to the risk of extinction at least one geographically isolated population of 21% of the species. The consequences of these changes are difficult to predict, but may include competitive interactions, in which new species exclude existing species from some or all of their current range and/or preferred habitats; the introduction of novel pathogens and/or parasites caused by the novel mixing of species, or of previously isolated populations of the same species, loss of genetic uniqueness of previously isolated populations (MacLeod, 2009).

In this scenario, the long-term assessment of the habitat and range of cetacean and sea turtle populations, other than to respond to the EU environmental regulation requirements, is of extreme importance to determine the potential impacts of climate change. This information, coupled with data on the trends of sea surface temperature and other environmental parameters, can be used to feed prediction models to assess the potential impact on populations under different climate change scenarios, and identify potential areas and species at higher risk due to their geographic characteristics and/or sensitivity to the increase of temperature. Long term monitoring programmes such that developed within the MTT project are able to provide extremely useful data on the species distribution, range and habitat use, and on the evolution of environmental parameters that could determine their modification over time, in relation to climate change effects or in response to a combination of other anthropogenic stressors.

5.4 Environmental DNA data and other environmental samples

The network of commercial vessels travelling along fixed routes that serves as platform for the visual observations of large marine fauna within the MTT project also offers the opportunity to collect other information and/or samples that could be relevant to feed EU environmental directives.

Environmental parameters

During navigation, samples of the water collected for the engine cooling system can be easily collected by dedicated researchers placed in the engine room for research purposes. Chemo-physical analyses can be performed to assess, e.g., the salinity and temperature of the water, along with its chemical composition. This information is of relevance, among others, for some of the MSFD Descriptors, such as D7 (hydrological conditions), which considers salinity, temperature, presence of nutrients, among the parameters relevant to the assessment of GES. On a longer term, the analysis of variations or trend in the measured environmental parameters could be used to support climate change studies.

Stable Isotope Analysis (SIA)

Similarly, samples of water could also be used to analyse the stable Isotopes of C, N or O, or those of other elements. This technique allows providing a baseline of the trophic web (the so-called *isoscape*, Graham et al, 2009), which is then useful to assess the diet, feeding ecology, movements and migration patterns of larger organisms, including cetaceans and sea turtles. This information, in turn, would also complement the data provided by visual surveys for the assessment of population range and habitat parameters within the relevant EU directives. On a longer term, the assessment of variations in stable isotope values, and consequently of isoscapes, could provide useful information to feed climate change models and assess its impacts on marine trophic webs.

eDNA sampling

Sophisticated molecular investigation techniques such as the Next Generation Sequencing allow sequencing different DNAs within a single environmental sample. Thanks to these developments, the analysis of marine environmental DNA (eDNA) has become a widespread technique, which has been proposed as an alternative approach to monitor the status of biodiversity and its variations over time (Valsecchi, 2021), and could complement the information provided by the "traditional" visual surveys and analyses to feed the requirements of the EU directives. The same samples of water collected, as described above, to assess environmental parameters and perform SIA, could also be used for eDNA analyses, to perform systematic surveys on marine biodiversity that cope with the cost constraints of using dedicated vessels, and allow assessing species composition also during night hours, when visual surveys cannot be performed. Rare species, such as the monk seal (*M. monachus*) can be detected by this technique long before they are visually observed (Valsecchi et al, 2021), and seasonal or long-term variations of species composition could be assessed through repeated sampling (Boldrocchi et al, 2023). Pilot studies carried out within the FLT MED NET demonstrated that commercial ships can be used as platforms for eDNA marine sampling. Systematic eDNA samplings over regular transects contribute to the assessment of marine biodiversity (Valsecchi, 2021), and provide useful information regarding the range of populations and other parameters required for environmental and species assessment within relevant EU legislation.

6 Final remarks

In this report, the available literature produced by the different networks involved in the MTT project was considered to address the data analyses performed to respond to the relevant EU environmental regulative framework for the conservation of large marine vertebrates. The review pointed out to some general highlights:

- ✓ Data obtained from systematic surveys from large vessels can be effectively used to respond to policy requirements on the Parameters “population”, “range”, “habitat”;
- ✓ Systematic surveys are relevant to investigate fine-scale temporal variations and produce consistent datasets over long time periods;
- ✓ To date, even if long-term data series are available, they are not sufficiently used to investigate trends, as required by EU directives;
- ✓ The use of these long-term datasets can be successfully combined with other sources of data, such as the collection of environmental parameters (temperature, salinity, etc), or of samples for environmental DNA analyses, to produce more complete and robust information on the required parameters;
- ✓ The use of further indicators is suggested to improve the definition and interpretation of parameters and trends;
- ✓ Long-term monitoring programmes such as that implemented within the MTT project can also provide detailed information on the threats that need to be addressed as required by the EU legislative framework (i.e., maritime traffic, pollution by marine litter).

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