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Challenges in conservation effort: the case of vulnerable vagrant species in a boundary-less threatened marine environment

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List of papers

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1. **Gregorietti, M**., Atzori, F., Carosso, L., Frau, F., Pellegrino, G., Sara, G., & Arcangeli, A. (2021). Cetacean presence and distribution in the central Mediterranean Sea and potential risks deriving from plastic pollution. *Marine Pollution Bulletin*, *173*, 112943.
2. Atzori, F., Carosso, L., Cadoni, N., Frau, F., Gutierrez, M. L. G., De Lucia, G. A., **Gregorietti, M**., Pellegrino, G., Liubartseva, S., Lecci, R., Coppini, G., Maffucci, F., Hochscheid, S. & Arcangeli, A. (2021). Loggerhead sea turtle, Caretta caretta, presence and its exposure to floating marine litter in the Sardinia Channel and the Strait of Sicily: Results from seven years of monitoring using ferry as platform of observation. *Mediterranean Marine Science*, *22*(2), 422-435.
3. David, L., Arcangeli, A., Tepsich, P., Di-Meglio, N., Roul, M., Campana, I., **Gregorietti, M.**, Moulins, A., Rosso, M., & Crosti, R. (2022). Computing ship strikes and near miss events of fin whales along the main ferry routes in the Pelagos Sanctuary and adjacent west area, in summer. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *32*(3), 442-456.
4. Arcangeli, A., Atzori, F., Azzolin, M., Babey, L., Campana, I., Carosso, L., Crosti, R., Garcia-Garin, O., **Gregorietti, M**., Orasi, A., Scuderi, A., Tepsich, P., Vighi, M. & David, L. (2023). Testing indicators for trend assessment of range and habitat of low-density cetacean species in the Mediterranean Sea. *Frontiers in Marine Science*, *10*, 1116829.
5. Scuderi A., Campana I., Gregorietti M., Martin Moreno E., Garcia Sanabria J. and Arcangeli A. (2024). Tying up loose ends together: Cetaceans, maritime traffic and spatial management tools in the Strait of Gibraltar. Aquatic Conservation: Marine and Freshwater Ecosystems. https://doi.org/10.1002/aqc.4066

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1. Arcangeli A., **Gregorietti M**., Pasanisi E., Santini E., Campana I. and Sarà G. Potential risk of floating marine litter to cetaceans and sea turtles: a review of spatial risk exposure assessments. *Oceanography and Marine Biology – An Annual Review* (in press, 2024)

To be submitted

A. **Gregorietti M**., Marchessaux G., Arcangeli A., Chevalier C. and Sarà G. Go with the flow: no cross-border policies for plastic pollution, no solution for marine conservation.
B. **Gregorietti M**., Di Bona G., Russi M., Sarà G. and Arcangeli A. Modeling maritime traffic exposure for marine megafauna: differentiated potential risk assessment for bottlenose dolphins in the Sicily Channel and surrounding waters.

Chapter 1

Introduction

General overview

Human footprint now pervades even the most remote corners of our planet and, in an era now defined as Anthropocene, human society emerges as one of the most important drivers of global change, able to quickly transform the Earth system (Ellis, 2015).

Nowadays, it is not possible to understand and forecast ecological processes not considering the also the human role in these (Barnosky et al., 2012; Ellis & Haff, 2009; Ellis & Ramankutty, 2008). Human presence and actions affect the distribution of species and habitats through a wide range of drivers and processes, ranging from climate change to biological invasions.

Globalization now implies that local anthropogenic impacts may actually be caused by activities or demands developed hundreds of miles away (Marques et al., 2019). The threats to the environment deriving from human activities are indeed increasingly recognized at global levels. For example, at the European level the EU Biodiversity Strategy for 2030 addresses the five main drivers of biodiversity loss (IPBES 2019) (e.g changes in lands and sea use, overexploitation, climate change, pollution and invasive alien species), sets out an enhanced governance framework to fill the remaining gaps, ensures the full implementation of EU legislation and pulls together all existing efforts. This strategy recognizes that legislation alone is not enough to assure the most reliable and effective protection and restoration of nature: citizens, businesses, social partners and research should be work together at local, national and global levels. Also, the United Nations 2030 Agenda for Sustainable Development has the goal of protecting the Planet from degradation by taking urgent actions to support the needs of the present and future generations. Systems and services of paramount importance for humankind are driven and provided by the world oceans, therefore how they are managed are crucial: Goal 14 “Life below water” intentions are the protection of marine and coastal ecosystems from pollution and acidification, as well as the sustainable use of ocean-based resources through international laws and measures.

Nowadays, the unceasing improvements of technologies supply the instruments for acquiring the high-quality data requested not only to guarantee a thorough knowledge of the pressure acting on species and ecosystems, but also to monitor progress, inform governance and assess alternative options for different decision-making processes (Chen et al., 2011; Mirtl et al., 2018).

Study aims and thesis outlines

The safeguard of biodiversity is one of the key objectives to be achieved to ensure the survival of our Planet. Nevertheless, in certain cases, it is still difficult to obtain the very basic information needed for conservation purposes, such as species presence and distribution through space and time. Missing but fundamental information is often also the real entity and diffusion of the anthropogenic threats to which species are vulnerable.

Moreover, while the marine environment is highly dynamic (Kavanaugh et al., 2016), until now in most of the cases protection areas are fixed and static, not considering shifting habitats or spatio-temporal changes in the distribution of anthropogenic threats and marine fauna. The situation become even more complicated if the conservation efforts regard highly mobile species, which are only partially protected by static management strategies (Dunn et al., 2016; Hazen et al., 2018).

Therefore, this study aims to face the problem of protection and conservation of highly vagrant species of marine megafauna, meaning cetaceans and marine turtles, from two of the most pervasive anthropogenic threats: marine litter and maritime traffic. Aim of the study was to investigate the up-to-date analytical tools to detect changes in species distribution and assess the risk of exposure of these group of species to these threats, ultimately discussing the effectiveness of static conservation measures (Figure 1).

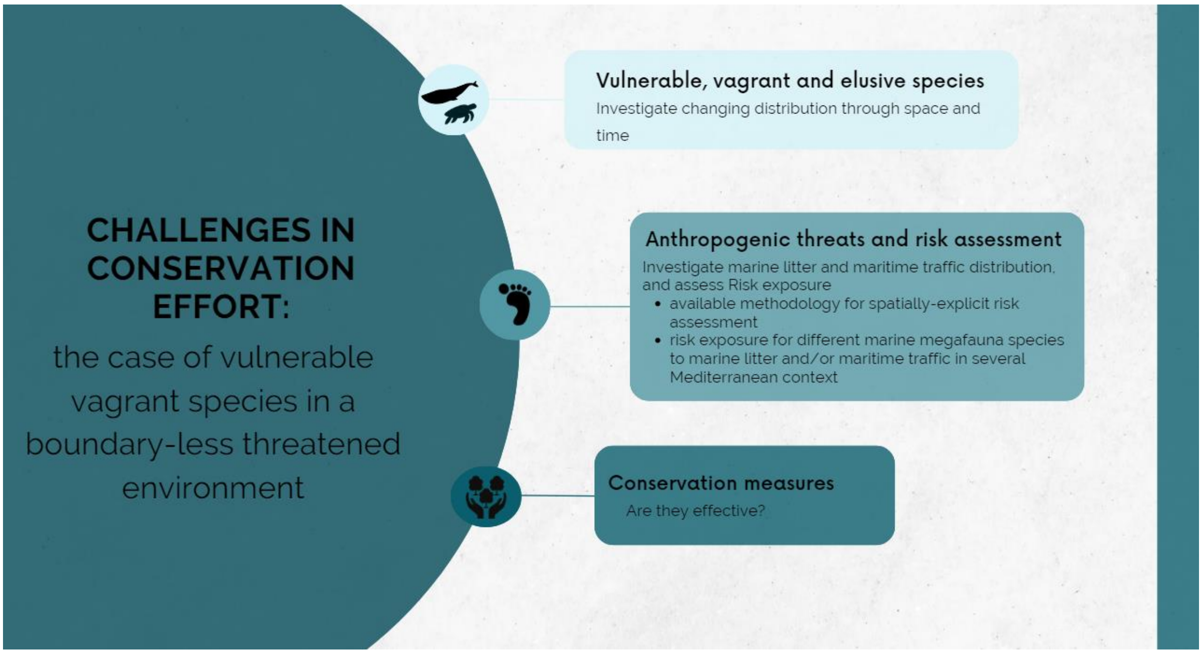

Figure 1 – Context of the thesis.

VULNERABLE, VAGRANT AND ELUSIVE SPECIES

Vagrant megafauna is the trophic top-level component of marine ecosystems playing important roles in transporting nutrients within and between habitats, connecting ecosystems with their long-distances migrations, consuming large amounts of biomass and modifying habitats through feeding, locomotion and mortality (Doughty et al., 2016; Hammerschlag et al., 2019). Nonetheless, habitat loss, ocean warming, pollution and human exploitation menaced them, causing a decline in their population and local extinction around the world (Estes et al., 2016; McCauley et al., 2015). Nowadays, according to the International Union for Conservation of Nature (IUCN), one-third of marine megafauna is a risk of extinction on the basis of species' rate of population decline and size, geographic distribution or rarity (IUCN, 2012). Therefore, efforts should be made to protect it with the best possible measures and regulations.

The sound basis on which to build them should consist of deep knowledge about the spatio-temporal distribution of vulnerable species or group of it. In this context, Species Distribution Models (SDMs) are a solid tool capable of I) identifying critical environmental variables for species/community (Droz et al., 2019); II) interpolating/extrapolating potential spatial distributions (McShea, 2014) from available species/community observations; III) provide possible past and future scenarios of species spatial distribution. These predictions, if treated as hypotheses and then tested with independent data (Lee-Yaw et al., 2022), can be used to plan conservation actions, to minimize the impacts of human development (Guisan et al., 2013), to identify the natural resources to be maintained and to assess the effects of environmental policies regarding the distribution of threatened/rare/invasive species (Charbonnel et al., 2023; Cianfrani et al., 2018; Esselman & Allan, 2011; Stirling et al., 2016). These models have the potential to become more and more accurate if they are fed by high resolution spatio-temporal data of environmental variables and human pressures. Currently, several platforms like Copernicus and EMODNet allow the free and open download of georeferenced information (from both satellites and in-situ sensors) about them at several scale of resolution, greatly expanding the analyses potential by the different users.

To achieve more refined predictions and to better understand and predict the current and future impacts of global change drivers on biodiversity, a close integration between remote sensing, modeling and *in situ* monitoring is now indispensable and of paramount importance.

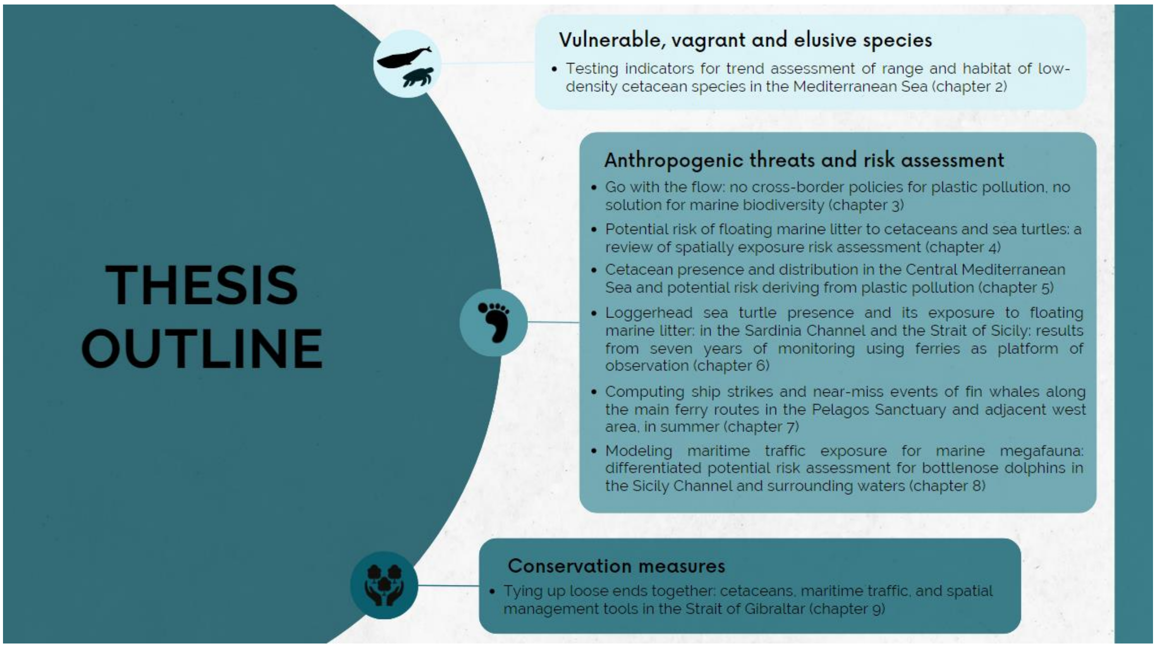

Figure 2 – The three main integrated topics developed in this thesis. The corresponding chapter is reported in brackets.

The first step of this thesis (**Chapter 2**) dealed with the spatio-temporal distribution of three vagrant, elusive and low-density species of cetaceans i.e. Risso's dolphin (*Grampus griseus*), long-finned pilot whale (*Globicephala melas*) and Cuvier's beaked whale (*Ziphius cavirostris*) (Fig. 2).

Using a 12-years dataset gathered in the field in the framework of the Fixed Line Transect Mediterranean Network (FLT Med Net), this study aimed to improve the knowledge on these species, evaluating potential approaches to support legislative requirements of the main European nature legislative framework. In particular, using the dataset collected during the third Habitat Directive six-years reporting cycle as a baseline, the study aimed to assess potential changes in the range and habitat of the three species over the subsequent periods (short-term trend) testing four potential indicators: 1) Observed Distributional Range, ODR: changes in the extent of ODR detected within the area covered by monitoring effort; 2) Ecological Potential Range, EPR: change in the extent of Ecological Potential Range predicted by means of SDM; 3) Range Pattern: percentage of overlap, and shifts of ODR and EPR between the two time periods; 4) ODR vs EPR: changes in the proportion of observed distributional range vs the ecological potential range between the two periods.

ANTHROPOGENIC THREATS AND RISK ASSESSMENT

Spatially evaluating hot spots of risk is of paramount importance for the conservation and management of marine megafauna (Nelms et al., 2016). Hazard areas for vulnerable species are where animals are likely to interact with the threat: studies highlighting these have spread only in recent years (Darmon et al., 2017; Matiddi et al., 2017; Soto-Navarro et al., 2021), because of they require comprehensive information about animals and threats spatial distribution which are often difficult to collect on large spatial scales (Darmon et al., 2017).

Usually, simulation-based approaches are mostly used (Schuyler et al., 2016; Wilcox et al., 2015), but observational, empirical and real data directly collected in field (e.g. ship or aerial surveys) provide valuable insights for the evaluation and location of sensitive zones (Darmon et al., 2017). Therefore, in the second step of this thesis, long-term datasets collected on the field (Fixed Line Transect Mediterranean Network, ISPRA) were used in integration with remote sensing information and modeling to evaluate the (potential) risks posed by two of the main threats menacing the overall marine biodiversity, with a focus on vagrant megafauna: (plastic) marine litter and maritime traffic.

Why marine litter is today considered the major threat for biodiversity?

Major environmental, social and economic problems in the world include marine litter (Derraik, 2002; Rochman et al., 2016; Thompson et al., 2004), defined as any waste originating from human activity and discarded, disposed or abandoned into a coastal or marine environment (UNEP 2009). It is estimated that between 4.8 and 12.7 million tons of land-based plastic waste enter marine ecosystems every year (Jambeck et al., 2015). To these, must be added the contribution of fishing, aquaculture, shipping and mining (Andrady, 2011; Kershaw, 2015).

Currently, it is estimated that about 60-80% of marine litter is made of plastic, constituting a global environmental problem (Eriksen et al., 2014; Van Sebille et al., 2015) primarily due to the longevity of plastic materials and their slow d deterioration rate. Moreover, approximately 50% of all plastics are less dense than water, and therefore they float over the sea surface (Geyer et al., 2017). Plastic objects often contain trapped air which enhance their buoyancy and windage, facilitating their spatial diffusion.

With no advances in the context of waste management, the overall plastics quantity potentially entering in the marine environment could increase by three times up to 2025 (Jambeck et al., 2015). Water masses movements and atmospheric agents action make it a threat without spatio-temporal boundaries (Figure 3) and, although it tends to accumulate mainly in densely populated coastal areas, bays and gulfs, at the mouth of rivers and in estuarine systems, scientific evidence demonstrate its presence even in remote areas such as the Poles (Suaria et al., 2020; Tirelli et al., 2020).
Five large accumulation areas of marine litters stable over time have formed in the world's oceans (Eriksen et al., 2014; Law et al., 2010; L. Lebreton et al., 2018); a separate case is represented by the Mediterranean Sea, as its hydro dynamism does not allow the formation of such structures (Mansui et al., 2015). However, given its peculiar characteristics, it is one of the marine areas most affected by marine litter issue (Mansui et al., 2015). In particular, plastic pollution, a category that is always the most represented regardless of the seasons, from the different areas of study and water column level considered is the major concern (Arcangeli et al., 2018; Chevalier et al., 2023; Cózar et al., 2015; Fossi et al., 2017; L. C. M. Lebreton et al., 2012; Scotti et al., 2021; Suaria et al., 2016; Suaria & Aliani, 2014). The Mediterranean is in fact a semi-closed and heavily urbanized basin, whose highly trafficked waters have been the site of a variety of human activities for centuries. Several and diversified are the plastics deleterious effects on marine ecosystems, biodiversity, economy and human heath (Figure 4). Considering its complex path in the environment and the different chemical-physical modifications it can encounter, litter can have many types of interactions with the systems it comes into contact with. Impacts on marine organisms has been described from the upper pelagic part of the water column to the deeper benthic habitats (Salerno et al. 2021; Berlino et al. 2023) and depend on the type and size of the objects as well as, of course, the species considered (Roman et al., 2021).

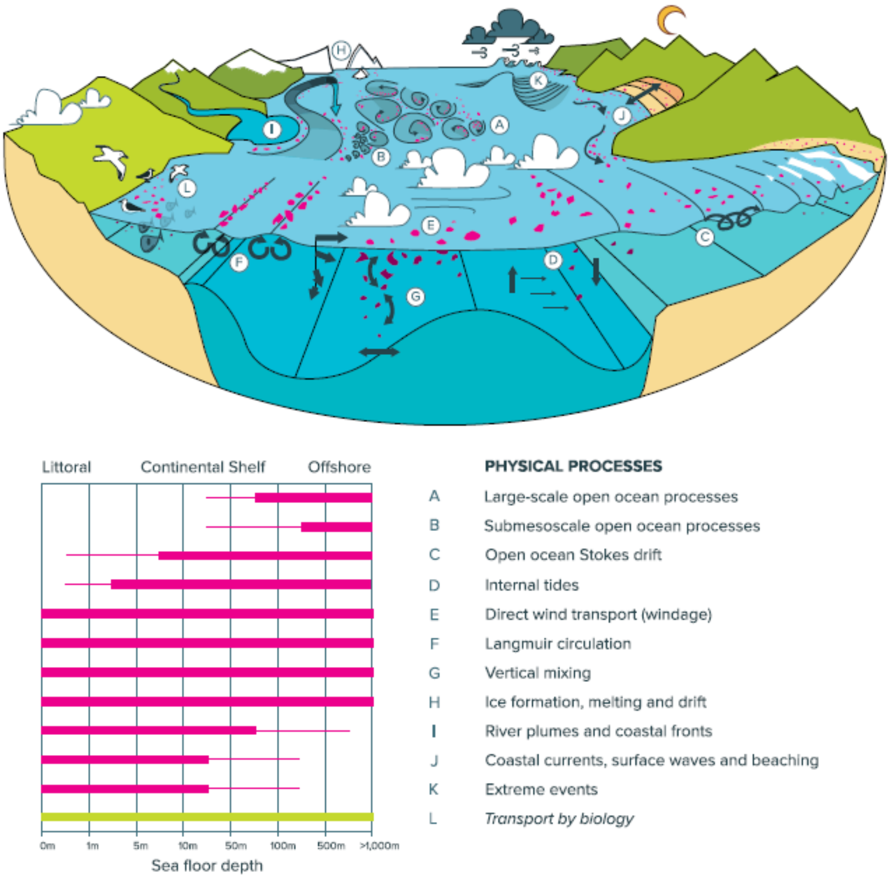

Figure 3 – Schematic of the physical processes that affect the transport of plastic (pink items) in the ocean (top panel). The table (lower panel) identifies in which regions different processes are important. Thick pink lines in the table mean that the process is among the most important in that water depth, while thin pink lines mean thet the process is only of secondary importance. Transport by organisms is not a physical process and therefore represented with a green line instead of a pink one (from Van Sebille et al., 2020).

Plastic marine litter has negatively impacted more than 1400 marine species, mainly through ingestion and entanglement phenomena (Kühn et al., 2015; Wilcox et al., 2015; Claro et al., 2019; Salerno et al., 2021; Berlino et al., 2021). Large-scale dispersion of organisms - and therefore also of alien species - can be facilitated by floating plastic litter (Aliani & Molcard, 2004; Rech et al., 2016), that can also promote the transport of toxic substances (Endo et al., 2005; Mato et al., 2001; Teuten & Reddy, 2007). Benthic habitats can be altered due to plastic accumulation (Consoli et al., 2020). Moreover, the fragmentation of different materials leads to the production of microparticles and toxic compounds that can accumulate through trophic nets, leading to bioaccumulation and biomagnification phenomena that particularly affect top predators and filtering species (Davison & Asch, 2011; Fossi et al., 2012, 2014; Wright et al., 2013).

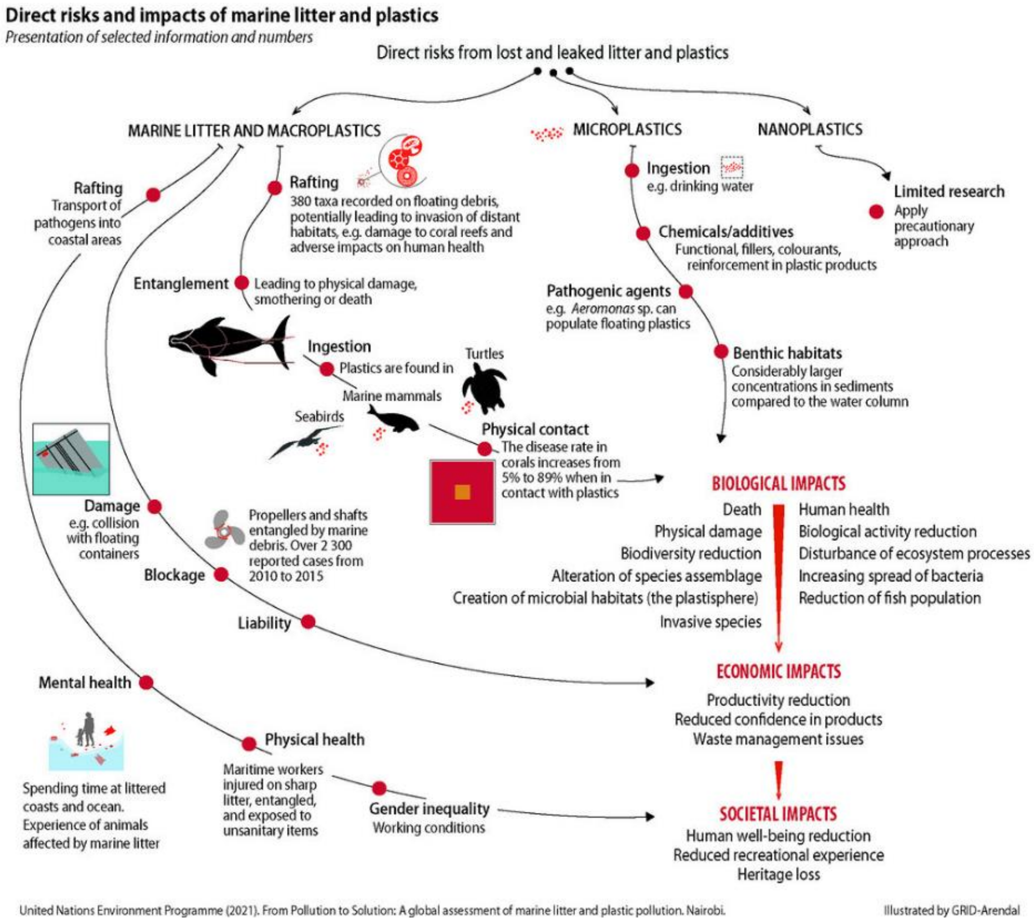

Figure 4 – Lethal and non-lethal effects of marine litter on organisms and impacts on ecosystems (from UNEP, 2021; illustration by GRID-Arendal).

Moreover, the enormous amount of plastic debris entering marine habitats every year cause a wide range of negative economic effects (Jambeck et al., 2015) Mouat et al., 2017): fisheries, aquaculture, navigation and tourism are just some of the factors negatively impacted by this threat.

Currently, most of the information about plastic dispersion are from Eulerian and Lagrangian numerical models (Eriksen et al., 2014; Guerrini et al., 2021; L. C. M. Lebreton et al., 2012; Maximenko et al., 2012; Mountford & Morales Maqueda, 2019) based mainly on virtual particles released from different sources. This is principally because, in general, observational and field data are sparse and difficult to analyze as a whole due to differences between collection protocols (Van Sebille et al., 2020).

Consequently, our understanding about plastic fluxes and pathways in the environment is still fragmentary (Van Sebille et al., 2020) even if several national, European and global action plans (OSPAR Regional Action Plan; G7/G20 Marine Litter Action Plan; Marine Strategy Framework Directive Descriptor #10; UNEA-4 2019) have as objectives marine litter monitoring, management and reduction. Tracking and understanding plastic litter movements and distribution, hence filling the data gaps, is of pivotal importance (Chassignet et al., 2021; Molina Jack et al., 2019), as it can be found in areas far away from its source and in general from human activities.

As the problem, the (potential) solutions to mitigate and manage plastic marine litter are widespread and complex and nonetheless, it still persists despite the existing efforts.

Chapter 3 aimed to characterize the spatio-temporal variability of plastic Floating Marine Macro Litter (FMML) dispersion modelling three years of consistent and long term fine-scale field data within the conceptual framework of "Risk" resulting from the combination of hazards and vulnerability. This concept was applied on plastic pollution in Marine Protected Areas (MPAs) and Fisheries Restricted Areas (FRAs), for defining their vulnerability to this threat (Fig. 2).

Considering the relevance of marine megafauna (e.g. cetaceans and sea turtles) as indicator to assess the risk deriving from marine litter pressure on marine ecosystems, **Chapter 4** focused on the review of the current scientific literature on spatial Risk Exposure Assessment (REA) related to the floating micro and macro marine litter in order to: I) identify, at global level, the main geographic areas where risk exposure studies were conducted; II) describe the typology of datasets currently available and the methodologies used for data collection on species and threats; III) investigate the approaches applied to carry out REA; and IV) highlight the main research findings and areas of high exposure risk to prioritize mitigation measures in the Mediterranean Sea. The main objective of this review was to identify key information gaps on REA, in order to highlight areas and topics that require further research (Fig. 2).

In **Chapter 5**, field observational data on cetaceans and marine litter over a 7-year time series were integrated to build a risk index over the different seasons. Moreover, the long-time dataset allowed the modeling of cetacean suitable habitat for the two most sighted species (striped dolphin *Stenella coeruleoalba* and bottlenose dolphin *Tursiops truncatus*), in an understudied and impacted area.

In **Chapter 6**, occurrence data on sea turtle *Caretta caretta* collected in the same area were analyzed to describe the presence and distribution of the species over a seven-year period, to characterize the exposure risk to floating marine macro litter and to understand the influence of upper layer currents on the distribution of both species and threat.

Maritime traffic: a not secondary threat for marine biodiversity

Maritime traffic, in its entirety and complexity, is one of the constantly increasing human activities in the marine environment since World War II. Over the last few decades, many efforts have been made to ease the burden on four-wheeled transport by sea (EC, 2004) and, at present, most of the world's trade uses maritime transport.

Despite being only 1% of the world's oceans, one of the busiest "motorways of the sea" is the Mediterranean Sea (Figure 5). About 30% of maritime traffic passes through this basin, and particularly the central-western part, where 80% of ports are located (Dobler, 2002).

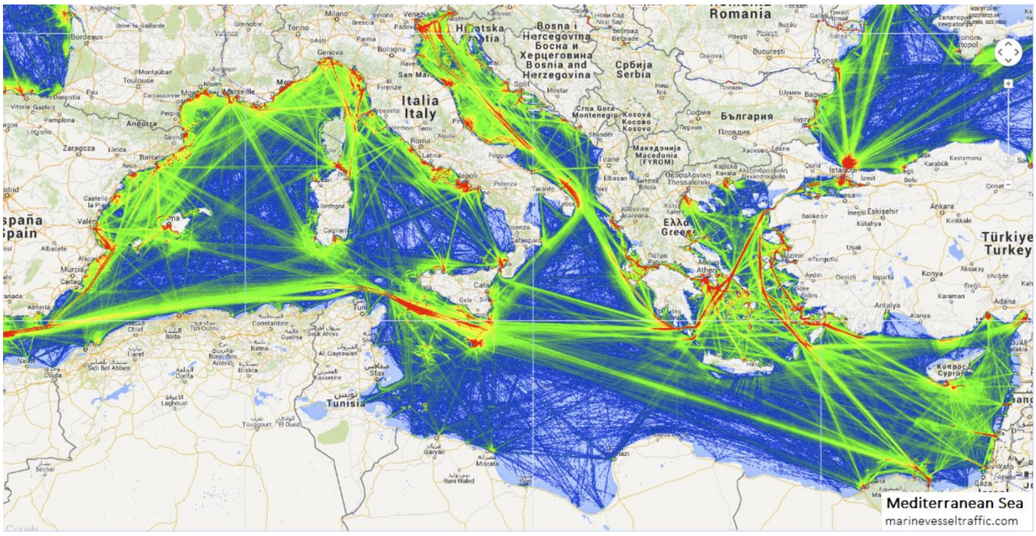

Figure 5 – Example of ship traffic density in the Mediterranean Sea (from marinevesseltraffic.com).

Most of the shipping consists mainly of cargos, tankers and merchant ships. These kinds of big vessels usually maintain regular routes and constant speeds throughout the year. In addition to these must be considered also the contribution of fishing, ferries, cruise ships and pleasure boats, that can be seasonal or limited to certain areas (David, 2002; Vaes et al., 2013).

In the last decades, an increase of the transit capacity (58%) and of the vessel size (30%) has been recorded and it is expected to continue to increase even more, looking at the growing trend in container port traffic development and the doubling of the Suez Canal (UNEP Mediterranean Action Plan 2017).

As a result, the concern for its potential impact on the marine environment and biodiversity grows too.

To date, there are different knowledge gaps about the negative consequences that maritime traffic can have on the environment, especially if coupled with other anthropogenic threats with whom can act in synergy (Jägerbrand et al., 2019).

Several international institutions reviewed the environmental impacts of maritime traffic (e.g. the European Commission, the European Union, the OSPAR Commission) but summaries on broad ecosystem assessment are still scarce in the scientific literature (Andersson et al., 2016; Walker et al., 2018), even if specific case studies can be found within specific impact areas (Bax et al., 2003; Neuparth et al., 2012; Peng et al., 2015; Yebra et al., 2004).

Although vessels must adhere to different standards of compliance (SOLAS, MARPOL 73/78), accidents, collisions and shipwrecks are the order of the day. To these, must be added also more "regular" sources of disturbance or stress (see Figure 6). For example, ballast waters and hull fouling can enhance the transfer of alien and non-indigenous species (Hulme, 2021). Maritime traffic is also responsible of underwater noise production (Duarte et al., 2021). In the last half-century, the low-frequency noise recorded along major shipping routes has increased by 32 times (Malakoff et al., 2010), being present also in many ocean regions even far away from the principal lanes due to long-range sound propagation underwater. The effects of this underwater noise on animals can range from single individual to population, comprehending temporal annoyance, behavioral changes, temporary or prolonged avoidance of an area (leading to changes in the usage of important feeding or breeding areas), protracted stress, hearing loss, barotrauma and ultimately death (Kight & Swaddle, 2011). Another form of pollution is, of course, air contamination (Firlag et al., 2018; Hassellöv et al., 2013): since the Nineties, shipping is in fact acknowledged as one of the main contributors of SO_x and NO_x to the atmosphere on local to global scales. Maritime traffic is also one of the contributors to (plastic) marine litter: whilst discharge at sea is forbidden from many years (MARPOL, 1998) losses or illegal behavior still occur.

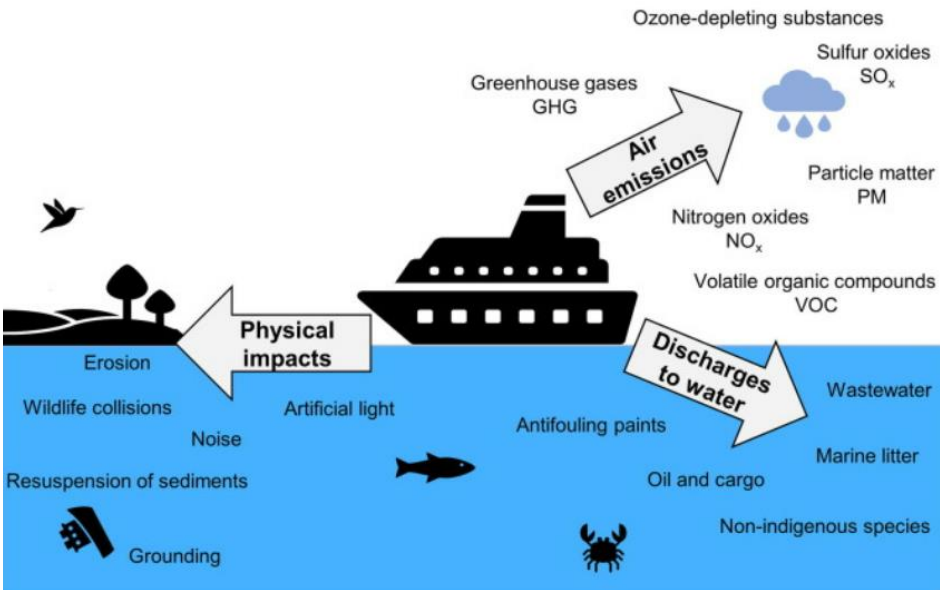

Figure 6 – Classification of main impacts of shipping on aquatic environments (from Jagerbrand et al., 2019).

Maritime traffic negative effects can act directly on single individuals, or indirectly by – for example – destroying a habitat. They can be also diversified spatially, being at local, regional or global scales. Nowadays, improved technology allows to have detailed information – in some cases in real-time – about the distribution of maritime traffic (see for examples AIS (obligatory for certain categories of shipping) and the density maps of maritime traffic available on EMODnet platform). This data should be increasingly considered and integrated with conservation and management measures to mitigate shipping for making it as "environmentally-friendly" as possible.

Within this context, **Chapter 7** investigated the fin whale (*Balaenoptera physalus*) risk of collision with vessels in the Pelagos marine mammals Sanctuary and adjacent areas. Long-term data were used for I) identifying the spatial and temporal occurrence of Near Miss Events (NME) of the species; II) quantifying NMEs occurring during summer in different parts of the Pelagos Sanctuary and the adjacent western area; III) understanding the context of NMEs through the analysis of the behaviors of animals; and IV) mapping the high-risk areas of exposure to ship strikes.

Chapter 8 combined maritime traffic data collected in the field with remote sensing information to investigate the potential impact through studying I) the influence of different type of vessel traffic on bottlenose dolphin presence along the routes; II) identify the spatial footprint of 11 different categories of maritime traffica and III) spatially represent and characterize the seasonal density of the 11 different categories of maritime traffic in the study area. The final goal of the study was to assess the risk of exposure of bottlenose dolphin to maritime traffic by building an index that considers the habitat preferences of the species and the spatial distribution of the threat.

CONSERVATION MEASURES

Identifying management policies that guarantee the protection of biodiversity while allowing the development of human activities is surely one of the biggest challenges of the modern world. In the last decade, Marine Spatial Planning (MSP) has become a useful tool to face it (Foley et al., 2010; White et al., 2012). Proper planning of marine space obviously requires the spatial identification of ascertained areas of exposure risk (Maritime Spatial Planning Directive, 2014/89/EU), as well as ecologically important areas for vulnerable species. Their spatially explicit contextualization is also one of the key Principles of the Integrated Monitoring and Assessment Program (principle 5, UNEP-MAP 2016).

In this perspective, **Chapter 9** aimed at evaluating the coexistence of cetacean vulnerable species and high levels of maritime traffic in a restricted and transboundary area (e.g. the Strait of Gibraltar), together with the existing mitigation and conservation measures. Their coherence was qualitatively discuss taking into account cetacean hot-spots, risk areas from maritime traffic and the recorded NMEs.

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Chapter 2

Testing indicators for trend assessment of range and habitat of low-density cetacean species in the Mediterranean Sea

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Testing indicators for trend assessment of range and habitat of low-density cetacean species in the Mediterranean Sea

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Introduction: Conservation of cetaceans is challenging due to their large-range, highly-dynamic nature. The EU Habitats Directive (HD) reports 78% of species in 'unknown' conservation status, and information on low-density/elusive species such *G.griseus, G.melas, Z.cavirostris* is the most scattered.

Methods: The FLT-Net programme has regularly collected year-round data along trans-border fixed-transects in the Mediterranean Sea since 2007. Nearly 7,500 cetacean sightings were recorded over 500,000 km of effort with 296 of less-common species. Comparing data across two HD 6-years periods (2013-2019/2008-2012), this study aimed at testing four potential indicators to assess range and habitat short-term trends of *G.griseus, G.melas, Z.cavirostris*: 1) change in Observed Distributional Range-ODR based on known occurrence, calculated through the Kernel smoother within the effort area; 2) change in Ecological Potential Range-EPR extent, predicted through Spatial Distribution Models; 3) Range Pattern, assessed as overlap and shift of core areas between periods; 4) changes in ODR vs EPR.

Results: Most ODR and EPR confirmed the persistence of known important sites, especially in the Western-Mediterranean. All species, however, exhibit changes in the distribution extent (contraction or expansion) and an offshore shift, possibly indicating exploitation of new areas or avoidance of more impacted ones.

Discussion: Results confirmed that the ODR could underestimate the real occupied range, as referring to the effort area only; it can be used to detect trends providing that the spatio-temporal effort scale is representative of species range. The EPR allows generalising species distribution outside the effort area, defining species' Habitat and the Occupied/Potential Range proportion. To investigate range-trends, EPR needs to be adjusted based also on the Occupied/Potential Range proportion since it could be larger than the occupied range in presence of limiting factors, or smaller, if anthropogenic pressures force the species outside the ecological niche.

Conclusion: Using complementary indicators proved valuable to evaluate the significance of changes. The concurrent analysis of more species with similar ecology was also critical to assess whether the detected changes are species-specific or representative of broader trends. The FLT-Net sampling strategy proved adequate for trend assessment in the Western-Mediterranean and Adriatic basins, while more transects are needed to characterize the Central-Mediterranean and Aegean-Levantine ecological variability.

KEYWORDS

monitoring, conservation, habitat modeling, Risso's dolphin, long-finned pilot whale, Cuvier's beaked whale, habitat directive 92/43/EEC, MSFD Descriptor 1

1 Introduction

The conservation of cetacean species is extremely challenging due to the large extent of their range and their highly dynamic migratory nature. The European Environmental Agency (EEA) Report (No 10/2020) states that "marine mammals (including cetaceans) are among the species with the highest proportion of unknown assessments (over 78%)". Data deficiency is mainly due to the fact that most cetacean species inhabit remote offshore areas which are more difficult to monitor due to logistical reasons linked to both the organisation of surveys and political barriers as coordinating effort in areas overcoming socio-political borders requires a functional international cooperation. Moreover, the high costs generally required for carrying out regular large-scale surveys limit the ability to gather sufficient information, especially on rare species.

1.1 Low-density cetacean species conservation status in the Mediterranean Sea

In the Mediterranean Sea, Risso's dolphin (*Grampus griseus, Gg*), long-finned pilot whale (*Globicephala melas, Gm*), and Cuvier' beaked whale (*Ziphius cavirostris, Zc*), are considered low-density elusive species. Their assessment status under the IUCN Red list of threatened species recently changed from 'Data Deficient' to, respectively, 'Endangered' (*Gg*, Lanfredi et al., 2021), and 'Vulnerable' (*Gm*, Gauffier and Verborgh, 2021; *Zc*, Cañadas and Notarbartolo di Sciara, 2018). A distinct subpopulation of long-finned pilot whales, limited to the Strait of Gibraltar area, and listed as 'Critically Endangered', was also identified during the last assessment (Verborgh and Gauffier, 2021). The three species are listed in Annex IV of the EU Habitats Directive (HD, Directive 92/43/EEC) as species requiring a special protection regime across their natural range, both within and outside the Natura 2000 sites, to enable their Favourable Conservation Status (FCS) to be maintained or, where appropriate, restored, in their natural range. The core areas of their habitat must be identified, designated as Sites of Community Importance, included in the Natura 2000 network, and managed in accordance with their ecological needs. Moreover, Member States must regularly report to the EU on their conservation status. Cetaceans are also a target species of Descriptor 1 (Biodiversity) of the Marine Strategy Framework Directive (MSFD, Directive 2008/56/EC), which aims at achieving a Good Environmental Status (GES) of EU marine waters by establishing a common approach and objectives for the prevention, protection and conservation of the marine environment. Thus, information about the preferred habitats of cetacean species and the early detection of potential changes in their distribution is essential to identify needed conservation measures.

1.2 Overview of approaches for assessing range and habitat trends

Despite the fact that the HD focuses on the conservation status of the species (i.e., the effects), and the MSFD on eliminating the causes (i.e., the threats) through mitigation measures that will restore the GES (Palialexis et al., 2019), the HD and MSFD have strong synergies. Under the MSFD, Member States are required to establish threshold values for each species through regional or sub-regional cooperation and, for species covered by the HD, these values shall be consistent with the Favourable Reference Values (FRV) established under the HD. Both HD and MSFD directives require reporting every six years equivalent parameters/criteria for the assessment of the species conservation status such as 'Range' (i.e., HD 'The *natural range* of the species is neither being *reduced* nor is likely to be reduced for the foreseeable future'; MSFD D1C4 'the *species distributional range* and, where relevant, the *pattern*, is in line with prevailing physiographic, geographic and climatic conditions') and 'Habitat' (i.e., HD 'There is, and will probably continue to be, a *sufficiently large habitat* to maintain its populations on a long-term basis'; MSFD D1C5 'The *habitat* for the species has the *necessary extent and condition* to support the different stages in the life history of the species'). Similarly, the EO1 assessment within the Barcelona Regional Sea Convention (UNEP-MAP, EO1) is based on the Common Indicators (CI) 3 ('*Species distributional range*') and 1 ('*Habitat distributional range*'). The IUCN Guidelines for the assessment of the conservation status of threatened species also foresee the assessment based on the criteria A2c ('A decline in *Area Of Occupancy*-AOO, *Extent Of Occurrence*-EOO and/or *habitat quality*') and B ('*Geographic range*'). Specifically, the AOO is defined as 'the area contained within the shortest continuous imaginary boundary that can be drawn to encompass all the *known, inferred or projected sites of present occurrence* of a taxon, excluding cases of vagrancy' (IUCN, 2001), where '*Projected sites'* are considered as the *sites spatially predicted* on the basis of habitat maps or models (*area of potential habitat*, also called *Extent of Suitable Habitat, ESH*). A suspected decline in the AOO could consequently be estimated based on the reduction of suitable habitat. In addition, also the Reporting Guidelines of the Habitats Directive (2017) suggest to evaluate the FRV as the AOO, or as the potential range in relation to available suitable habitat ('*Ecological potential'*, the potential extent of range considering physical and ecological conditions).

Within such legal requirements, Species Distribution Modelling (SDM) is a promising approach to support the assessment of cetacean species. Indeed, as long as the amount/quality of input data is reasonably adequate, SDM can be used to support regulatory decision-making for conservation, i.e., by informing on spatial prioritisation through the identification of biodiversity hotspots, important areas for vulnerable species, or valuable habitats, overcoming the problems related to coarse or incomplete knowledge (Franklin, 2010; Maiorano et al., 2019). Time series of comparable data with sufficient statistical power, coupled with standardised SDM analyses, can help identify changes from a reference period. A significant reduction in the extent or a shift of species geographical distribution can then be related to environmental variability, habitat conditions or changes in population size, or to the effect of anthropogenic pressures. Moreover, the comparison of the suitable habitat predicted through SDM with the distributional range observed indicate potential suitable areas that are not used by the species.

However, relevant indicators or threshold values for assessing species range and habitat have not yet been developed (Palialexis et al., 2019), and some recommendations were only recently provided through an international scientific cooperation to define indicators, assessment methods, and data requirements for the assessment of marine turtles under the MSFD (Girard et al., 2022). Moreover, despite an increasing research effort, a limited number of studies attempted so far to infer temporal changes in cetacean distributional range or habitat use, and the 'trend' criterion for these parameters/criteria is still considered 'unknown' for almost all cetacean species in the Mediterranean Sea (last HD report 2013-2018), likely due to the lack of comparable data and standard methodological approaches.

1.3 Aim of the study

The Fixed Line Transect monitoring Network (FLT Med Net) has been operating in the Mediterranean basin since 2007 collecting cetacean data along fixed trans-border transects regularly surveyed throughout the years. Using the dataset gathered across twelve years, this study aims to improve the knowledge on three low-density cetacean species of the Mediterranean basin Risso's dolphin (*Grampus griseus, Gg)*, long-finned pilot whale (*Globicephala melas, Gm)*, and Cuvier's beaked whale *(Ziphius cavirostris, Zc*), and evaluate potential approaches to support legislative requirements. In particular, using the dataset collected during the third HD six-years reporting cycle (2008-2012) as baseline, the study aims to assess potential changes in the range and habitat of the three species over the subsequent periods (short-term trend) testing four potential indicators: 1) Observed Distributional Range, ODR: changes in the extent of ODR detected within the area covered by monitoring effort; 2) Ecological Potential Range, EPR: change in the extent of Ecological Potential Range predicted by means of SDM; 3) Range Pattern: percentage of overlap, and shifts of ODR and EPR between the two time periods; 4) ODR vs EPR: changes in the proportion of observed distributional range vs the ecological potential range between the two periods. Overall, the study aims to test and evaluate such methodological approaches and indicators to contribute to the species assessment under the requirements of the main European nature legislative framework.

2 Material and methods

2.1 Study area

Cetacean monitoring was carried out from passenger ferries travelling along 11 trans-border transects, covering the Mediterranean Sea within the latitudes 43.6° N - 35.8° S and longitudes -5.5° E - 20.8° E, and connecting Italy, France, Spain, Greece, Tunisia and Morocco. These transects are included in the Fixed Line Transect Mediterranean Network (FLT Med Net, Arcangeli et al., 2019), and are representative of a large proportion of the Western-Mediterranean, the Adriatic Subregions, and two portion eastern and western of Ionian Sea in the Ionian-Central Mediterranean Subregion. Transects considered for the baseline period (2008-2012) covered the effort area shown in gridded grey in Figure 1. In the second period (2013-2019) monitoring was also extended to the area in light grey along the east Spanish coasts and Gibraltar Strait on Western Mediterranean, and in the Adriatic-eastern Ionian Sea.

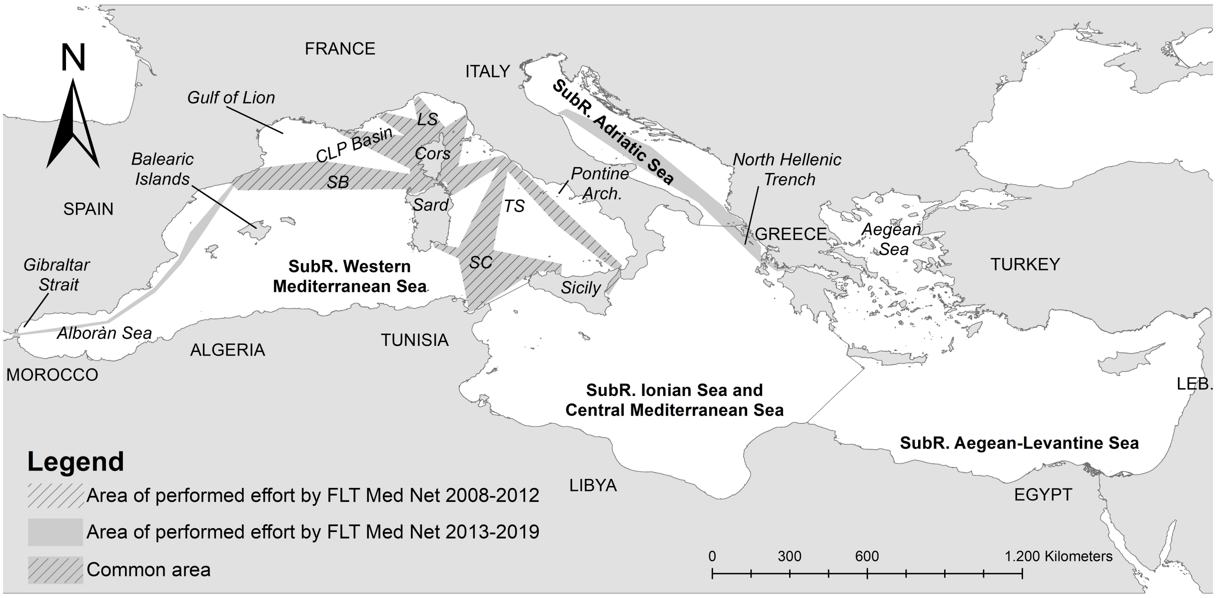

FIGURE 1
Study Area with the survey effort performed by the FLT Med Net during 2008-2012 (I baseline period, gridded grey only) and 2013-2019 (II period, plain grey). The four Mediterranean MSFD Subregions are shown in the figure: Western-Mediterranean (WMED), central-Mediterranean (Central MED), Adriatic, Aegean-Levantine Sea (downloaded from the European Environment Agency www.eea.europa.eu). LS, Ligurian Sea; CLP Basin, Corso-Ligurian-Provençal Basin; SB, Sardinian-Balearc Basin; TS, Tyrrhenian Sea; SC, Sardinian Channel.

2.2 Data collection

The monitoring activity was performed on a seasonal basis with at least three surveys per season along each sampling transect. Seasons were defined as winter (January to March), spring (April to June), summer (July to September) and autumn (October to December). Data on cetacean species were systematically collected following a standard protocol applied from large vessels (ISPRA, 2015) (FLT Net data, Supplementary Table 1). Ferries provided an observation point at 20–29 m above sea level and travelled at a mean speed in the range of 19–25 knots. Two experienced observers were positioned on the two sides of the command deck scanning both sides of the ship within an angle of 130° ahead in order to avoid re-counting the animals; observations were performed by naked eye and binoculars; binoculars and cameras were used to correctly identify the species and the number of animals. A dedicated GPS was used for automatically recording the survey track at the finest resolution, marking the beginning/ending points and the locations of cetacean sightings. Monitoring was carried out during daylight hours only in optimum weather conditions (≤3 on the Beaufort scale).

2.3 Data analysis

All the analyses performed for this study considered the sighting as the statistical unit, regardless of the number of animals within the sighted group. However, the mean group size was also examined to assess differences between the two periods. Data were analysed considering the different Mediterranean Subregions of the MSFD (https://www.eea.europa.eu/): Western Mediterranean (WMED), Ionian Sea and Central Mediterranean (Central MED), Adriatic, Aegean-Levantine Sea (Figure 1). As data were homogeneously collected within the same set of conditions, detection probabilities were assumed the same across all surveys and between the two survey periods.

2.3.1 Observed distributional range, ODR

As suggested by the HD Guidelines (DG ENV, 2017), the Kernel Density Estimator (KDE) was used to spatially generalize the distribution of the species occurrence and identify the extent and the core areas of species within the region covered by effort. After an initial testing, the KDE analysis was set with a resolution cell of 500 m and search radius of 50,000 m. The 95% isopleth was used to define the extent of ODR, calculated in km^2.

After calculating the area covered by the effort for each time-period (EffortArea), the proportion of species ODR inside the effort area was calculated per each Subregion and time-period. Then, the ODRs of the two periods were displayed and overlapped, and the temporal trend in the ODR extent was estimated as: Δ distribution = $[(ODR/EffortArea_{(2nd\ period)} - ODR/EffortArea_{(1st\ period)}) \times 100]$. Following the OSPAR indicators for seals (Palialexis et al., 2019), threshold values were defined as: if index > 10% = increase, if index < -10% = decrease, otherwise = no change.

2.3.2 Ecological potential range, EPR

The changes in the EPR between the two periods were assessed based on projected sites of species occurrence using spatially predicted sites based on the habitat map models (also called Extent of Suitable Habitat) (IUCN Guidelines, 2001; IUCN, 2022). The following criteria were applied: i) use of adequate spatial resolution for the species knowing their range in the Mediterranean Sea, key variables, and appropriate model validation; ii) validation of suitable maps with independent datasets not used to build models; iii) estimate of the proportion of suitable habitat likely occupied by the species (within the area of effort).

Maximum Entropy (MaxEnt version 3.3.3, http://www.cs.princeton.edu/~schapire/maxent/) was applied to model the relationships between environmental predictors and the occurrence records and to build the Suitable Habitat Maps for each of species over the two periods. MaxEnt was chosen as it provided more consistent results than the most common modelling approaches (Arcangeli and Orasi, in prep), and it is generally considered more appropriate than other SDM methods for low presence records or deep divers or elusive species where the probability of detection is unknown. MaxEnt is a machine learning method commonly used in systems with restricted information based on a probability distribution with maximum entropy (the most spread out closest to uniform) subject to known constraints (Phillips et al., 2006). MaxEnt generates a probability distribution of suitable habitats over pixels in the grid starting from a uniform distribution and repeatedly improving the fit to the data. Since MaxEnt accounts for sampling biases *via* correction features that consider area of sampling effort used to generate pseudo-absences points ('background points'), a bias file of effort was built using the Minimum Convex Polygon (MCP) around the surveyed sites (Figure 1). The model was built based on heterogeneously distributed effort in the Western-Mediterranean Sea and Adriatic-eastern Ionian region, largely representing the variability of the environmental parameters in these areas and adequate for the species distribution and their known ranges. The projection was performed at a Mediterranean basin-wide scale, and the outputs were successively tested for reliability. Two dataset were used: 1) the dataset obtained from the systematic long-term monitoring along the FLT routes including the effort track lines to build the background file and sightings as presence points; 2) sighting data gathered by ORCA NGO during cruises in the Mediterranean basin (2016-2018), ACCOBAMS Survey Initiative at Mediterranean scale (2018), and local scale data from Ketos-MareCamp organisations (Catania Gulf – east Sicilian Ionian coast) as independent dataset for the validation of the model results. The preparation of data for modelling included: 1) a Bias file (background file) built as Minimum Convex Polygon (MCP) around the tracklines of effort; 2) presence data per each species with information on Species, Longitudes, and Latitudes; 3) environmental variables prepared as raster files with same scale, extension and resolution. Nine key predictor variables, known to be relevant for the biology of the species (e.g. Fullard et al., 2000; Moors-Murphy, 2014; Breen et al., 2020; Dede et al., 2022), were included in the model (i.e., Depth, Standard Deviation of Depth, Distance from the coast, Distance from seamount, Distance from Canyon, Slope, Aspect North, Aspect South, mean chlorophyll-a concentration - Chl-a, mean Sea Surface Temperature - SST) and used as proxies of the factors that could affect species presence and distribution. Depth and canyons were obtained from the GEBCO portal (GEBCO Compilation Group, 2020) while vector layer of seamounts was obtained from Würtz and Rovere (2015). Standard deviation of depth was derived with the Zonal statistic tool in ArcGIS, and the rasters of the Euclidean distances from the nearest features were computed using the Distance tool after projecting all rasters using the Universal Transverse Mercator coordinate system. Slope was derived from Depth through Spatial analysis tool in ArcGIS. The aspect parameter was derived from depth through the Slope tool and converted into two linear components to be included in the analysis: Aspect Easting (sine of the aspect value) and Aspect Northing (cosine of the aspect value). SST (°C) and Chl-a (mg/m-3) Aqua-MODIS high-resolution data were downloaded from NASA satellite data (https://oceancolor.gsfc.nasa.gov) on 4-km-grid cells and clipped to the study area. Seasonal composite rasters based on daily data were averaged for each of the two periods using the 'Mosaic to new raster tool' in ArcGIS. For the MaxEnt modelling, all the environmental layers were prepared in order to match to the same extension and resolution. After a preliminary test to verify correlation among variables, the standard deviation of depth was excluded as correlated with slope.

MaxEnt was run splitting the dataset into two periods using 2008-2012 as a reference baseline for comparison to the more recent 2013-2019 period (almost corresponding to the third and fourth HD reporting cycles). The effort area was consistent between the two periods, except for the Adriatic-eastern Ionian region, the Barcelona-Tanger route and the Strait of Gibraltar route, which were only surveyed during the second period (light grey area in Figure 1). Thus, two bias files were used to define the area from which to extract the background points. For each period, distinct MaxEnt models were run using the same settings and set of variables. After preliminary runs with different setting parameters, default recommended feature classes (hinge, linear, quadratic) and regularisation parameters (i.e., = 1) were used with 10,000 background points and maximum iterations up to 500 to reach convergence at a threshold of 0.00001. Duplicates were removed to reduce problems of pseudo-replication and spatial autocorrelation of samples. Random seeds bootstrap replication type over 34% test samples (Efron and Tibshirani, 1997) and 100 iterations were used to obtain a summary output and response curves with statistical indication on standard deviation and error bars. A Jackknife test was conducted to obtain alternative estimates of the variable contribution to the MaxEnt run. The logistic format was used to improve model calibration, displaying output maps that better highlight the continuum of differences in the suitable maps produced, so that large differences in output values correspond better to large differences in suitability (Phillips and Dudík, 2008). As suggested by Pearson et al. (2007), more than 15 presence points were used for each model (Figure 2 left): 86 presence points were used for Gg (N_{1st} period = 27; N_{2nd} period = 59), 68 for Gm (N_{1st} period = 16; N_{2nd} period = 52), 142 for Zc (N_{1st} period = 27; N_{2nd} period = 115). The descriptive power of each model was evaluated by the Area Under the receiver operating characteristic Curve, a threshold-independent metric of overall accuracy (AUC; Thorne et al., 2012), and by the 'omission rate', i.e., the proportion of test localities falling outside the prediction. The AUC metric determines model discriminatory power by comparing model sensitivity (i.e., true positives) against model specificity (i.e., false positives). The

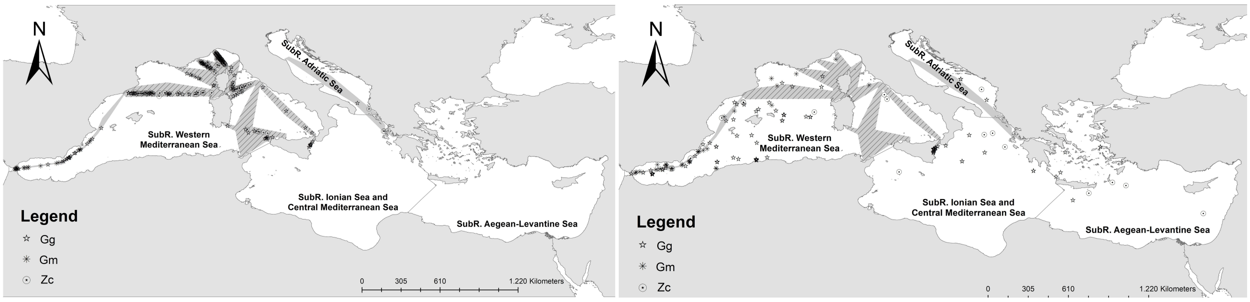

FIGURE 2
Dataset used for model building (left) and independent dataset used for validation (right).

AUC values range from 0 to 1, with values below 0.5 indicating worse model predictions than random, and values over 0.5 indicating improved model precision. The output maps were visually inspected by expert judgement to check for overfitting problems and the general reliability of results. The suitable output maps of the whole study period were first visualised as continuous colour scheme of suitable-unsuitable prediction and then reclassified in binary suitable-unsuitable predictions under three threshold scenarios (i.e., Minimum training presence logistic threshold, Equal training sensitivity and specificity logistic threshold, Maximum training sensitivity plus specificity logistic threshold). The three thresholds were chosen among the most commonly used by MaxEnt (e.g., Merow et al., 2013), considering the balance between the proportional predicted area (proportion of pixels that are predicted as suitable for the species) and the extrinsic omission rate (proportion of test localities that fall into pixels not predicted as suitable for the species). The best threshold method was then chosen based on expert considerations, after visual inspection of the suitable maps, in order to include the area that likely reflects the range of the species, knowing the biology and ecology of the species, the confirmed sites of occurrence, and the species dispersal capability. An independent dataset of sighting data coming from different research projects (Supplementary Table 2; Figure 2 right) was also used to validate the predictive ability of the resulting binary maps.

To calculate the extent of suitable area (Ecological Potential Range, EPR), the output binary suitable-unsuitable predictions rasters were converted into polygon layers including the highest suitable class for each species and period and were then used to measure the EPR in km^2. Then, the percentage difference in the EPR between periods was calculated for each species as: [($EPR_{(2nd\ period)}$ - $EPR_{(1st\ period)}$)/$EPR_{(1st\ period)}$)].

2.3.3 Range pattern

The trend in distributional pattern was calculated in terms of shift either in the surface or in the centre of gravity (centroid) of range areas (ODR, EPR), assessing the: a) overlapping area between the two periods (for the ODR considering only the common effort area between the two periods); b) percentage of overlapping area compared to the first period calculated as [(Overlapping area/Area 1st period)*100] and c) direction and magnitude of shift in the centroids of the range area between the two periods (calculated through the geometric spatial zonal statistic in GIS).

2.3.4 Observed distributional range vs ecological potential range, ODR/EPR

The proportion of the suitable habitat effectively occupied by the species (ODR vs EPR) was calculated for each period considering only the areas covered by the effort identified by the MaxEnt bias files. Within these areas, the extent of suitable habitats (Ecological Potential Range, EPR) was estimated in km^2. The percentage proportion of the predicted EPR occupied by the species (ODR) was calculated as: [(ODR/EPR) * 100], and differences between periods were computed as: [($\%_{(2nd\ period)}$ - $\%_{(1st\ period)}$)/$\%_{(1st\ period)}$)]

3 Results

During the twelve years between 2008 and 2019, the FLT Med Net covered almost 500,000 km of effort and recorded 296 sightings of *Gg* (86), *Gm* (68) and *Zc* (142). Group sizes of the species were not significantly different between the two periods, but they differed among species: *Gg* groups were composed by a mean of 5 individuals (5.7 ± 5.1 $SD_{1st\ period}$/4.7 ± 4.3 $SD_{2nd\ period}$), while *Gm* groups were generally larger (7.0 ± 9.5 $SD_{1st\ period}$/7.0 ± 6 $SD_{2nd\ period}$), and *Zc* smaller (mean group size of 1.67 ± 1.0 SD $_{1st\ period}$/ 1.87 ± 1.2 $SD_{2nd\ period}$).

3.1 Observed distributional range, ODR

The area covered by the effort was the largest in the WMED Subregion, while very limited in the Central MED during the first period (i.e., eastern Sicily), and increased during the second thanks to the inclusion of new Adriatic routes covering also the Northern Hellenic Trench (Figure 1). No effort was performed in the Aegean-Levantine Subregion (Table 1).

Between 10 to 37% of the effort area overlapped with the species observed range (ODR) in the WMED. In the Central MED instead,

TABLE 1 Distribution and extent (in km^2) of the area of effort per each Mediterranean Subregion, extent of observed species range calculated within the 95% KDE isopleth, and percentage of overlap between observed species range and effort area.

		WMED	Central MED	Adriatic	Aegean-Levantine Sea
Effort Area	**1 period**	191,658	1,579	NoEffort	NoEffort
	2 period	208,088	9,126	19,165	NoEffort
Observed Distributional Range (KDE, km^2)	**Gg_1**	38,415	1,568	NoEffort	NoEffort
	Gg_2	77,173	0,0	2,595	NoEffort
	Gm_1	19,664	0,0	NoEffort	NoEffort
	Gm_2	32,818	0,0	0,0	NoEffort
	Zc_1	29,169	0,0	NoEffort	NoEffort
	Zc_2	37,496	632	0,0	NoEffort
Observed Distributional Range vs Extent of Effort area (km^2)	**Gg_1**	20%	99%	NA	NA
	Gg_2	37%	0%	7%	NA
	Gm_1	10%	0%	NA	NA
	Gm_2	16%	0%	0%	NA
	Zc_1	15%	0%	NA	NA
	Zc_2	18%	2%	0%	NA

NA, Not Available.

99% of the effort area overlapped with Gg ODR during the first period (i.e., in the eastern Sicily), and a limited percentage with the ODR of Zc (2%) during the second period (i.e., in the Northern Hellenic Trench). In the Adriatic, 7% of the effort area intercepted the Gg ODR in the southern part.

ODR areas were mostly located in the northern part of the WMED Subregion for all the species (Figure 3) with ODR for *Gg* also located in the westernmost MED, the Tyrrhenian-Sardinian channel and the southern Adriatic, *Gm* in the westernmost MED, and *Zc* in the eastern Ionian (i.e., Northern Hellenic Trench). In the northern area, the ODR generally overlapped between the two periods, with a tendency to shift towards offshore in the Sardinian-Balearic basin for all the three species, and in the Ligurian Sea for *Gg* (Figure 3, left).

Considering only the common area of effort between the two periods, the trend calculated over the ODR extents revealed an expansion in all the three species with a significant delta index >10% for *Gg* (+16%).

3.2 Ecological potential range, EPR

Based on AUCs, validation data, and well-known sites of species presence, model outputs showed strong predictive skill at the basin wide scale. The ROC plots exhibited high average AUCs for both training and test datasets and small Standard Deviation and overfitting values for all models (Table 2), which indicates consistency and reliability. In general, performance of the prediction maps of the second period was higher compared to those of the first period when validated by the independent dataset collected during the same period. Performance was also higher for prediction maps for Gm2 (over 90% of correct prediction), while performance of *Gg* and *Zc* maps was fair-good in the WMED Subregion only (over 70% of correctly predicted sites).

In general, the areas of suitable habitats highlighted by the MaxEnt output maps were consistent with previous knowledge on the species (Figure 4) with the highest incongruence noted for the Gm_2 prediction in the Aegean-Levantine Subregion. Standard

FIGURE 3
Core areas highlighted by the 95% KDE isopleth within the area covered on effort during the two periods (in grey), used to define the Observed Distributional Range, ODR (Gg left, Gm centre, Zc right).

TABLE 2 MaxEnt Results for the first and second periods considered.

Species	#Training samples	#Test samples	AUC Train	AUC Test	AUC SD	*overfitting*	Minimum training presence logistic threshold	Equal training sensitivity and specificity logistic threshold	Maximum training sensitivity plus specificity logistic threshold
Gg_1	18	9	0.95	0.86	0.06	0.10	0.19	0.26	0.19
Gm_1	11	5	0.94	0.90	0.04	0.04	0.18	0.42	0.42
Zc_1	18	9	0.97	0.92	0.03	0.05	0.06	0.27	0.30
Gg_2	39	19	0.90	0.81	0.05	0.09	0.08	0.38	0.29
Gm_2	32	15	0.96	0.92	0.03	0.04	0.06	0.17	0.14
Zc_2	75	38	0.95	0.91	0.02	0.04	0.01	0.16	0.16

Deviations were generally low (<0.4), especially for the unsuitable areas. However, uncertainty was highest in general in the Aegean-Levantine Subregion and in the central and southern areas of the Central MED Subregion for the Gg_1 and Zc_2 outputs.

The 'Minimum training presence' threshold produced binary maps restricted to the most suitable habitat only excluding a large number of presence sights. The values identified through the 'Equal training sensitivity and specificity' and 'Maximum training sensitivity plus specificity' thresholds resulted similar (Table 2), but the first approach was chosen as being more conservative and was then used to define the EPR.

Some differences in the EPRs were found between the two periods (Table 3) in the WMED, where the EPR of *Gg* decreased by almost -7%, while Gm increased by 57% and Zc by 4%. Results for the other Subregions were not reliable as they were based on very small probability of presence in those areas (<5000 km^2).

In general, Distance from Canyon, Chl-a, and depth were the most important predictors for all the three species, followed by seamount distance and SST, but only for *Gm* and *Zc* (Table 4). Chl-a was the most important parameter for the definition of *Gg* habitats, either as percent contribution or permutation importance, in both periods, followed by canyon distance during the first period and depth during the second. Distance from Canyon was the most relevant parameter for *Gm* during the first period, while Chl-a strongly contributed during the second period, followed by the distance from seamounts. Chl-a and distance from canyon were the most significant parameters also for *Zc* during the first period, while depth and distance from seamounts were the parameters that most affected the distribution of the species during the second period.

3.3 Range pattern

In addition to the investigated changes in the extent of range areas, the analysis of spatial pattern revealed some shifts in the location of the main range areas. Indeed, the percentage of overlapping spanned 40-70% for ODR for the three species reaching the maximum overlap for *Zc*, and 30-50% for EPR. The location of overlapping areas for ODR (Figure 3) and EPR (Figure 5) showed the permanence over the time of some well-known areas for the three species.

In particular for *Gg*, some well-known areas of the WMED were predicted in both periods (e.g., Alboran Sea, Balearic Sea, Corso-Ligurian-Provençal basin, several spots in Tyrrhenian Sea including the Pontine Archipelago, and eastern Sicily). The offshore waters of the Gulf of Lion were no longer identified as the most suitable during recent years, while some new areas emerged (Figures 4, 5). A general reduction of suitable habitat was identified in the Pontine Archipelago and around the Sicilian coasts. Other widespread spots of potential suitable habitat appeared dispersed in the WMED from the recent model. Outside the more reliable area of the WMED, some suitable areas with higher uncertainty emerged in the eastern Mediterranean basin such as the southern Türkish, the northern Aegean during the more recent period and the coasts between Lebanon and Egypt.

Suitable *Gm* habitats were predicted in the WMED Subregion, in the Alboran Sea and along the continental shelf of Balearic, Gulf of Lion and the Corso-Ligurian-Provençal basin. A small area was highlighted in the Pontine Archipelago, and other patch areas were predicted around Sardinia Island. During the second period, new ODR areas were identified over the Alboran Sea and the Strait of Gibraltar due to the added effort in this region which intercepted the known important areas for the species identified by the large EPR. Outside the WMED, the large prediction stretching from the Aegean to Libya seems unreliable given the current knowledge on the species distribution.

Some well-known suitable areas were highlighted in both periods for *Zc* in the WMED such as the Alboran Sea, Ligurian Sea, northern Tyrrhenian Sea, and Balearic Sea. In the Central MED and Adriatic Subregions, the Hellenic Trench, northern Ionian Sea, and southern Adriatic Sea were predicted during the second period only with higher uncertainty.

A shift of centroids' core areas between the two periods was detected for the ODR and the EPR predicted over the WMED Subregion (Figure 6). The shift on EPR for the other Subregions or at all MED scale was not considered as based on a very limited predicted area in one or both periods (Table 3).

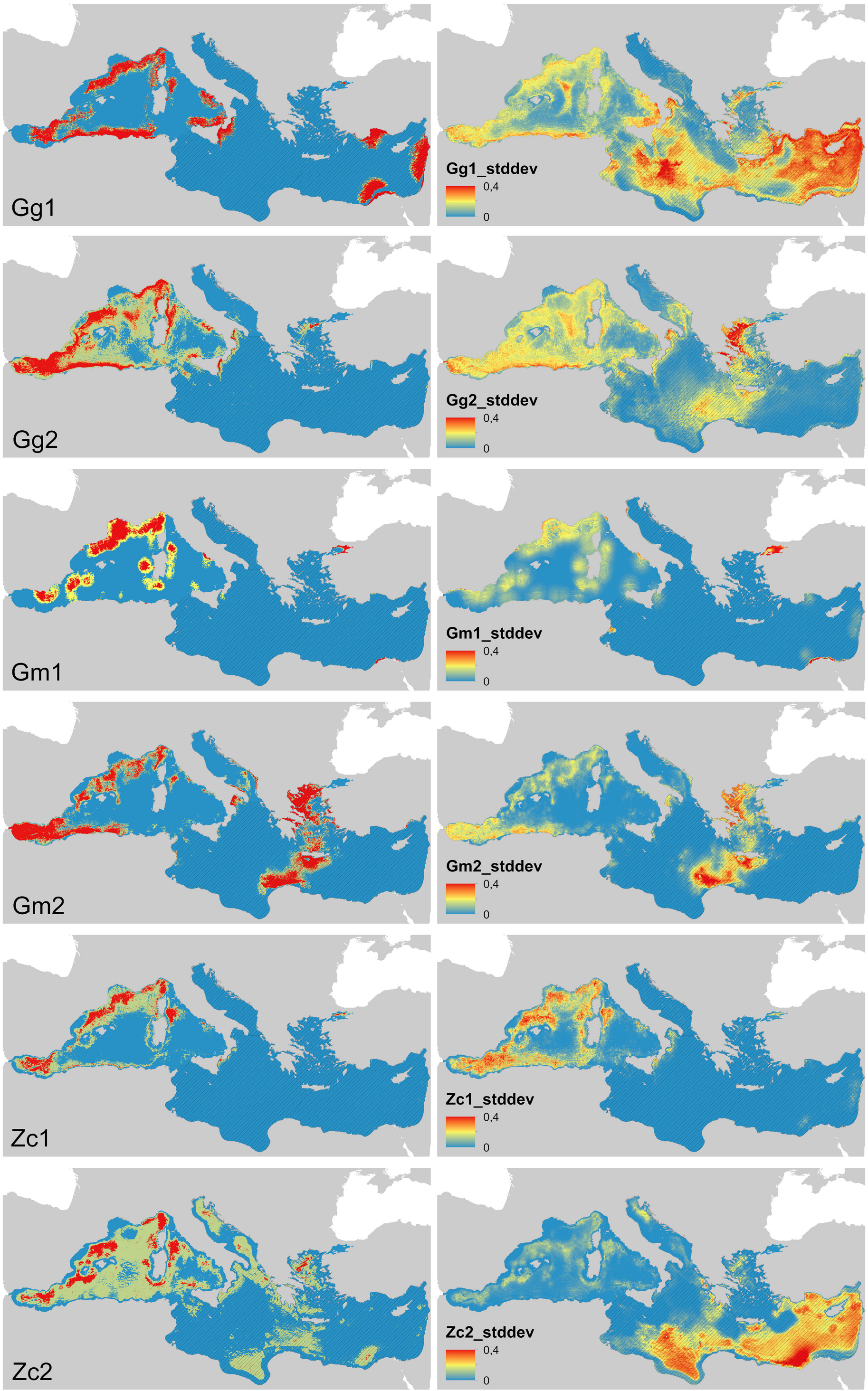

FIGURE 4
Output of the Suitable Habitats predicted based on 2008-2012 (Gg_1, Gm_1, Zc_1) and 2013-2019 (Gg_2, Gm_2, Zc_2) FLT Med Net data (left) with the relative Standard Deviation (right). The partition of suitable habitat is shown under three threshold scenarios defined by: 'Equal training sensitivity and specificity logistic threshold' (red), 'Minimum training presence logistic' and 'Maximum training sensitivity plus specificity logistic threshold' (values in Table 2). Blue colour displays the predicted unsuitable habitat. Striped lines identify the Subregions where the prediction must be considered with caution as based on limited or no effort.

TABLE 3 Extent area of potential range (EPR, km^2), based on Equal sensitivity plus sensitivity logistic threshold and percentage of change in the extent of suitable area (2008-2012: Gg_1, Gm_1, Zc_1; 2013-2019: Gg_2, Gm_2, Zc_2).

		WMED	Central MED	Adriatic	Aegean-Levantine Sea
Extent of Ecological Potential Range (km^2)	Gg_1	182,910	12,859	*0*	87,212
	Gg_2	170,028	*4,581*	*50*	*1,785*
	Gm_1	101,305	*20*	*0*	*1,275*
	Gm_2	159,226	48,888	*4,724*	88,960
	Zc_1	92,218	*591*	*0*	*0*
	Zc_2	96,136	*1,781*	*2,310*	5,879
% change	Gg_2/Gg_1	-7%	°	°	°
	Gm_2/Gm_1	57%	°	°	°
	Zc_2/Zc_1	4%	°	°	°

In Italic are indicates the very small extension of predicted suitable habitat (less than 5,000 km^2); ° not reliable results as based on very limited predicted area in one or both periods.

TABLE 4 Measures of environmental variables contribution to the ecological models for the target species.

	Gg_1		Gg_2		Gm_1		Gm_2		Zc_1		Zc_2	
	% cont.	Perm.	% cont.	Perm.	% cont.	Perm.	% cont.	Perm.	% cont.	Perm.	% cont.	Perm.
Aspect-E	8.6	6	11.3	3.9	3.2	0.9	3.6	1.6	8.5	1.9	2.9	3.3
Aspect-N	9.6	9.2	6.6	5.4	16.9	7.5	4.9	1.8	6.9	7.9	4.7	3.6
Canyon dist.	**23.1**	**20**	12.5	10.5	**45.9**	**73.6**	4.5	5.3	***20.8***	**43.9**	15.2	8.6
Chl-a	***17.4***	***29.5***	**24.1**	***25.8***	1.6	4	**38.4**	**43.5**	**25.7**	20.1	15.1	7.4
Dist. coast	6.1	3.3	7.2	7.1	2.7	6.4	11.1	4.2	4.6	4.6	3.7	6.2
Depth	13.5	7.8	***18.2***	**26.8**	2.8	1.2	13.4	15.2	***20.7***	8	**23.4**	**36.3**
Slope	11.1	3.4	6.1	3.3	2.7	0.9	3.3	2.2	7.6	6.3	3.3	1.6
Seamount dist.	4.8	10.3	9.7	13.4	1.8	2.2	***19.9***	***25.3***	4.8	6.5	***17.3***	11.7
SST	5.8	10.3	4.4	3.7	***22.4***	3.3	0.8	1	0.4	0.7	14.5	***21.3***

Percentage contribution (% cont) and permutation importance (Perm) derived from Maximum Entropy models. In dark and light grey respectively the first and second contributing variable.

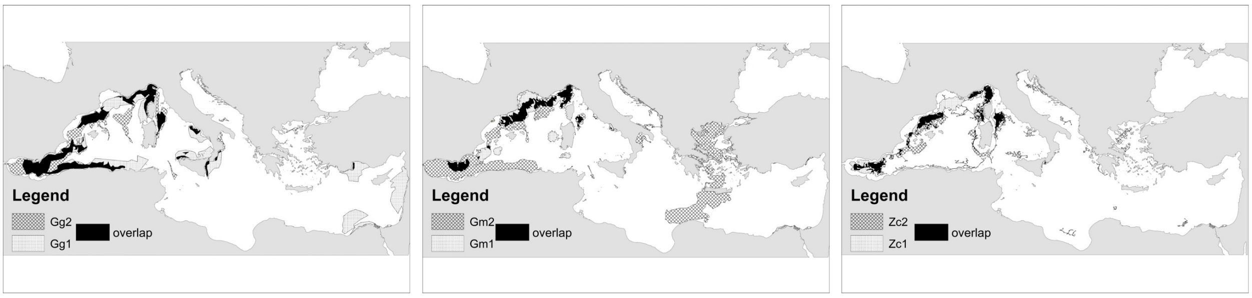

FIGURE 5
Overlap of EPRs over the two periods. Points EPR of the first period, strips EPR second period, and in black the overlapping areas.

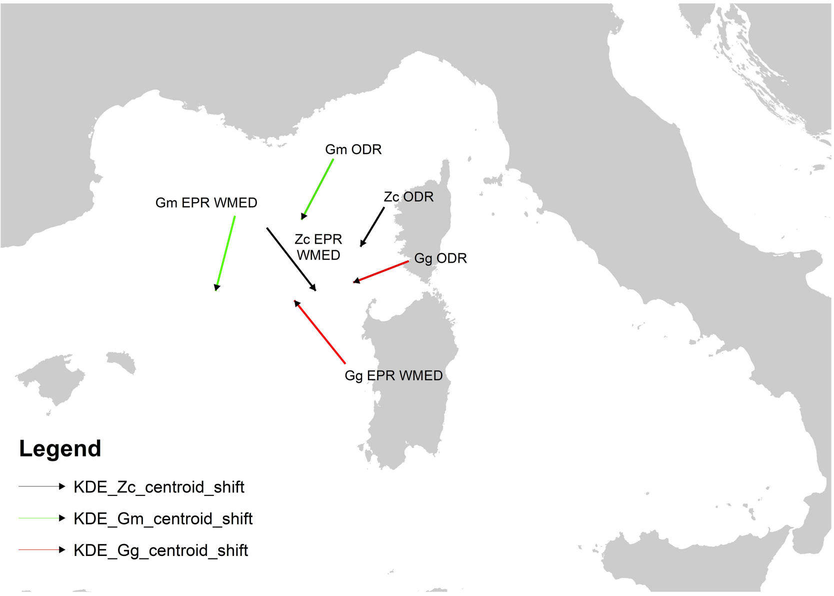

FIGURE 6
Direction and magnitude shift of the centroids of the distributional area respectively of ODR, and EPR WMED. Gg red, Gm green, Zc black lines.

3.4 Observed distribution range vs ecological potential range, ODR/EPR

Results showed that all the species regularly occur in almost the same areas or in a smaller proportion of their ecological potential habitat during both periods (ODR equal or smaller than EPR), with the only exception of Gg, whose ODR in the second period was larger than the EPR (Table 5, SM Figure 1). In the WMED, the proportion of suitable habitat effectively occupied by the species ranged between 62% for Gm_1 and 158% of Gg_2. No significant changes were detected in the proportion of occupied vs potential habitat over the two periods for the *Zc* (-1%), while for *Gg* and *Gm* increased this proportion by 59% and 46% respectively. Limited area was predicted for *Gg* and *Zc* in the Central MED, effectively occupied by the *Zc* by 50%, while the *Gg* was recorded largely outside the predicted potential area. *Gm* was never detected either in the surveyed areas of the Central MED or in the Adriatic Subregions. The spatial pattern of observed and predicted potential areas showed large overlap, but with some local differences (SM Figure 1). Both the areas of observed and predicted range of *Gg* in the northern part of the WMED expanded mainly towards offshore waters and stretched in patchy suitable areas in the centre. However, the shift in ODR detected in the more recent years in the western portion of the Corso-Ligurian-Provençal basin brought *Gg* outside predicted suitable areas. A contraction in suitable areas was instead detected in the south Tyrrhenian, where the species was no longer present, while new areas emerged in the Sardinian channel. A suitable area was confirmed in eastern Sicily in both periods. *Gm* observed range was almost similar across periods in the northern WMED, except for an enlargement towards offshore waters in the Sardinia-Balearic basin, which almost corresponded with the predicted potential range despite the latter being more scattered and fragmented during the more recent years. On the

TABLE 5 Percentage of the extent of Real Distribution (km^2, 95% KDE isopleth) over the Ecological Potential Range (km^2, based on Equal sensitivity plus sensitivity logistic threshold) calculated within the area performed on effort.

	WMED	Central MED	Adriatic	Aegean-Levantine Sea
Gg_1	99%	114%	NoEffort	NoEffort
Gg_2	158%	°	°	NoEffort
Gm_1	62%	°	NoEffort	NoEffort
Gm_2	90%	°	°	NoEffort
Zc_1	115%	°	NoEffort	NoEffort
Zc_2	112%	°	°	NoEffort

2008-2012: Gg_1, Gm_1, Zc_1; 2013-2019: Gg_2, Gm_2, Zc_2. ° not reliable results as based on very limited predicted area in one or both periods.

other side, a relevant area potentially suitable for *Gm* was revealed in both periods not overlapping any ODR in the central Tyrrhenian Sea. No noteworthy changes in observed and predicted range were detected for *Zc* in the northern part of WMED, while a new area emerged in the Sardinian channel both for the observed and predicted range.

4 Discussion

4.1 Sampling strategy

The sampling strategy of the FLT Med Net was set in order to homogeneously cover large portions of the Mediterranean basin, with regular monitoring of the sampled areas during all the seasons (Arcangeli et al., 2019). A recent study revealed that sampling designed along multiple fixed ferry routes detected more species and were able to recover known patterns in species richness and distribution at smaller sample sizes better than unconstrained sampling points (Boyse et al., 2023). Results of this study confirm that the sampling design of the FLT Med Net proved adequate for catching the known distribution of the species, providing high modelling performance, and allowing trends analysis even for rare or elusive cetacean species such as Risso's dolphin, long-finned pilot whale and Cuvier's beaked whale. This was particularly the case for the WMED Subregion, and especially during recent years when new monitored transects also covered the westernmost portion of the basin, the Alboran sea and the Strait of Gibraltar area (roughly 80% of WMED covered by the effort). In the Adriatic Subregion, the effort strategy resulted in coverage of almost the whole region although with still some uncertainty in the northernmost area, as also assessed by Zampollo et al. (2022). The Central MED was instead only represented by the effort in the eastern Sicilian coast and the Greek Ionian portion, and no effort was performed in the Aegean-Levantine Subregion, which leaves open opportunities for improvement. Indeed, an adequate proportion of the effort area intercepted the main distributional range and suitable habitats of *Gg*, *Gm* and *Zc* in the WMED Subregion (between 10-37% for the observed distributional range, over 46% of the predicted ecological range), and a more limited proportion in the Central MED and Adriatic Subregions, in correspondence with some known important areas for *Gg* (i.e., eastern Sicily. e.g., ACCOBAMS, 2021) and *Zc* (i.e., Northern Hellenic Trench, e.g., Frantzis et al., 2003). Therefore, in the WMED the sampling design of FLT Net proved to be adequate to intercept the ecological variability of the area, producing reliable results also outside the area of effort, whereas more transects are instead required to improve reliability in understudied Subregion (e.g., Central and Aegean-Levantine Subregions). Moreover, as the distributional range and habitat use of species varies seasonally, the seasonal based temporal resolution of sampling strategy allowed including the potential seasonal displacement of the species and thus the entire species range. The approach was also effective in terms of monitoring costs vs. acquired information, and these methods and indicators are suitable to be replicated across all seas.

4.2 Main findings on species distributional range and habitat

Most of the Observed Distributional Range (ODR) of the species highlighted by the Kernel analysis and the Ecological Potential Range (EPR) predicted on the basis of suitable habitat modelling were consistent with previous knowledge on the species, especially for the WMED Subregion, further confirming the importance of the north-western Mediterranean for *Gg*, *Gm* and *Zc* (ACCOBAMS, 2021). Consistency in these areas was also found across periods, with a general enlargement in the areas of distribution, and a shift towards more offshore areas in the Sardinian-Balearic basin for the three species, and in the Ligurian Sea for *Gg*. Outside the WMED, some known important areas for *Zc* such as the Ionian Sea and the deep Hellenic Trench were predicted, even if for a limited extent, during the second period only, when monitoring effort was added in the Adriatic-eastern Ionian region. Higher uncertainties or unreliable areas were revealed, as expected, in unsurveyed areas of the Central or the Aegean-Levantine Subregion.

Findings of this study on both ODR and EPR of Risso's dolphin (*Gg*) confirmed the permanence across the two investigated periods of some well-known important areas for the species in the WMED Subregion. The species is mostly found in the Western-Mediterranean Sea from the Alborán Sea, including deep offshore waters (Cañadas et al., 2002; Cañadas et al., 2005), to the south of the Provençal basin, with high values along the Algerian coast and the Balearic Islands (ACCOBAMS, 2021; Lanfredi et al., 2021). However, findings of this study no longer identified the offshore areas of the Gulf of Lion as most suitable during recent years, while highlighting new distributional areas in the offshore waters of the Sardinian-Balearic basin and Ligurian Sea. The species was considered favoured by the proximity of the continental slope, primarily in the north-western basin (Bearzi et al., 2011), with a very specialised niche and a habitat spatially restricted on the upper part of the continental slope (Praca and Gannier, 2008). A high fidelity for the Provencal continental slope, without strong seasonal pattern in abundance (Laran et al., 2010; Laran et al., 2017), and a transient use of the offshore area was also confirmed on a long-term basis between 1989-2012 by Labach et al. (2015). Nonetheless, during recent years *Gg* was sighted in more offshore environments than previously reported in literature (ACCOBAMS, 2021). This is also in line with the trend observed by Azzellino et al. (2016), who reported a significant decrease in *Gg* abundance between the early '90s and 2014 in coastal and continental slope areas of the Ligurian Sea, with stable occurrence in pelagic areas. The result was assumed as a loss of coastal group or a shift in animal distribution (Azzellino et al., 2016). Moreover, apart from the more defined sites, widespread spots of potential suitable habitats appeared dispersed in the WMED in the current study. A general reduction of suitable areas was also detected in the Pontine Archipelago, and around the Sicilian coasts and Ionian Sea, where only a portion of suitable habitat persisted eastern of Sicily and Taranto Gulfs where strong side fidelity was found by other studies (e.g., Monaco et al., 2016; Carlucci et al., 2020a; Cipriano et al., 2022). Relatively large groups of Risso's dolphins were reported further east in the southern Adriatic and Ionian Seas and the deep Hellenic Trench from ASI visual surveys, but no sightings were reported from acoustic surveys (ACCOBAMS, 2021) in line with the uneven prediction produced by this study. During the first period, some suitable areas emerged in correspondence of the Türkish Mediterranean, Palestinian and Israeli coasts consistent with the few contemporary reports (Öztürk et al., 2011; Kerem et al., 2012). The absence of effort in this area prevents any conclusion on whether or not the predicted reduction reflects a true species negative trend. The few encounters of *Gg* in mixed-species groups with striped dolphins and short-beaked common dolphins in the deep waters of the semi-closed Gulf of Corinth (e.g., Frantzis and Herzing, 2002; Frantzis et al., 2003), and for the unique stranding record in the 2012 in the Marmara Sea (Dede et al., 2013) appear to confirm the minor prediction in these areas.

Findings of this study confirmed some of the existing knowledge on the long-finned pilot whale (*Gm*). The species is known to be found almost exclusively in the WMED (Verborgh et al., 2016; ACCOBAMS, 2021) with a strong preference for deep pelagic waters. Relative higher densities were reported in the Strait of Gibraltar and Alboran Sea (Cañadas et al., 2005; De Stephanis et al., 2008) and lower in Balearic and Corso-Ligurian-Provençal Seas (Raga and Pantoja, 2004; Gómez de Segura et al., 2006; Azzellino et al., 2008; Praca and Gannier, 2008). The ACCOBAMS survey of 2018 (ACCOBAMS, 2021) also observed larger groups of *Gm* in the Alborán Sea, along the coast of Morocco and in the Gulf of Lion, and relatively smaller pods in the Ligurian Sea. The species was never recorded in the central Tyrrhenian Sea (Arcangeli et al., 2013; Arcangeli et al., 2017), but a stable pod has been recurrently sighted in the Pontine Archipelago since 1995 (Mussi et al., 2000). In accordance with the literature, the ODR in this study for *Gm* was exclusive of the WMED, but with a tendency to shift towards offshore waters during recent years, especially in the Sardinian-Balearic basin. Suitable habitats were also mostly predicted in the Alboran Sea and along the continental shelf of the Balearic Archipelago, Gulf of Lion and the Corso-Ligurian-Provençal basin with a similar shifting trend towards offshore as the Observed Range. Smaller areas were predicted in the Pontine Archipelago, supporting the stable presence reported by Mussi et al. (2000), and around Sardinia Island. In the Tyrrhenian Sea instead, a relevant potentially suitable area was highlighted during both periods, although no sightings have been reported either from this study or by literature (e.g. Arcangeli et al., 2017). Further investigation could be directed to determine whether anthropogenic activities or other pressures are operating there as limiting factors for the species. During the second period, a reliable enlargement of suitable habitat was predicted in the WMED Subregion, especially over the Alboran Sea and the Strait of Gibraltar, most likely as a result of the new added monitored transects representative of the westernmost part of the basin and intercepting the Strait of Gibraltar sub-population (Verborgh and Gauffier, 2021). A large Ecological Potential area stretching from Gibraltar towards the northern African coast was indeed predicted by this study in the second period, consistent with the ACCOBAMS (2021) sightings of large pods and by some reported strandings in Morocco (Bayed, 1996; Masski and De Stephanis, 2018), Algeria (Boutiba, 1994; Bouslah, 2012) and Northern Tunisia (Attia El Hili et al., 2010; Karaa et al., 2012). The species was never detected either in the Central MED and in the Adriatic Subregions, and no EPR was predicted here, while the large prediction stretching from the Aegean to Libya seems unreliable given the current knowledge on the species distribution.

Known habitats of **Cuvier's beaked whale** (*Zc*) were highlighted by the study in the WMED Subregion, while the south Adriatic and Hellenic Trench of the eastern Ionian Sea were only predicted during the second period likely due to the effort performed in those areas that allowed including some environmental features not considered by the environmental variability of the WMED effort area only. *Zc* is considered to inhabit both the western and eastern basins of the Mediterranean Sea (Podestà et al., 2016), and this species is mostly found in canyon areas in the Ionian Sea, the Hellenic Trench, the deep southern Adriatic Sea (Frantzis et al., 2003; Carlucci et al., 2020b), the central Tyrrhenian Sea (Gannier, 2015; Arcangeli et al., 2016), the Balearic and the Alborán Seas (Cañadas and Vázquez, 2014; Cañadas et al., 2018), and the Ligurian Sea (Moulins et al., 2007; Azzellino et al., 2008; Tepsich et al., 2014). The ACCOBAMS survey of 2018 confirmed the existing knowledge on the basin wide presence of the species and at the same time showed how Zc occur in relatively small patches at low densities (ACCOBAMS, 2021). In accordance with literature, this study highlighted the importance in particular in the WMED of the Alboran Sea, the central Tyrrhenian Sea and Ligurian Sea and also a permanent area of suitable habitat in correspondence with the Spanish-French continental slope coast and stretching offshore. However, despite being recognised by some studies (Raga and Pantoja, 2004; Gannier and Epinat, 2008; Praca and Gannier, 2008; Podestà et al., 2016; Arcangeli et al., 2017) and the records of the Accobams survey (ACCOBAMS, 2021), this latter area was not considered among the important areas for the species. This discrepancy could indicate either an underrepresentation of scientific literature or a minor occupancy of Ecological Potential habitat for the species.

4.3 Interpretation of trends

In general, the persistence over time of presence and suitable habitat of *Gg*, *Gm* and *Zc* in the WMED confirmed the importance of this Subregion for the species. However, the changes in the extent (whichever a contraction or expansion) and the shift highlighted on both the observed distribution and the suitable areas indicate changes in spatial distribution of the species across time periods (Table 6). This could be the result of exploitation of new potential suitable areas or an adaptation forced by existing pressures or changes in the distribution of habitat over time. In particular *Gg* enlarged the proportion of occupied area over the ecological potential by almost 50% distributing also outside the predicted suitable areas (i.e., in the Corso-Ligurian-Provençal basin). In addition, the new areas that emerged in the centre of the Sardinian Balearic basin or eastern Corsica coast, together with the contraction of the areas in the south Tyrrhenian Sea and around the Sicilian coasts, revealed changes that need further investigation. Moreover, results highlight a concurrent enlargement of the area of distribution of *Gm* and *Zc*, even if for a minor extent, that is not yet reported by other studies. If confirmed, this would be a signal of a general tendency towards a more dispersed distribution that surely deserves attention.

4.4 Methodological approach and indicators

The indicators here tested helped to describe the main consistencies or changes in short-term range trends between periods. Results highlighted the advantages and weaknesses of each indicator and of the approach tested.

The Observed Distributional Range (ODR) indicator has the advantage of preventing difference biases by data processing, analysis settings or approximations and is closely related to the real observed distribution of the species. On the other hand, results are only representative of the area where the effort is performed, introducing the need for specific planning of the sampling design of the data collection if used as representation of species distributional range. Spatially extensive surveys covering the whole range of species would deliver an adequate baseline for detecting ODR, but they are cost-expensive and may lack the temporal resolution needed to detect the natural species variability avoiding output linked to occasional or seasonal fluctuations. Continuous local scale surveys could provide long-term series but lose the spatial representativeness. Local and large scale surveys could be merged to increase the spatial representation of outputs providing that appropriate metric is used to match data collected with different methodologies. Time extensive large-scale monitoring data collected in sampled areas spatially representative of regional ecological conditions could represent a suitable balance and can be used as an index of the real species range. A prior assessment of the ecological variability representativeness of monitored transects is needed to avoid bias in underrepresented regions.

With regard to the methods to represent the distributional range, if compared to the species occurrence mapped in a 10 x10 km^2 grid as suggested by HD and MSFD, the Kernel density smoother proved to be a feasible tool to spatially generalize the distribution of species and define the area where the species is found. It is adaptable to the spatial scale (grain) and resolution of data through the adjustment of search radius and cell size resolution while still remaining relatively simple to apply. Moreover, when using high quality spatial data as those of this study, the use of KDE could be considered as more accurate than other coarser methods such as grid of occurrence or the Minimum Convex Polygon used by some EU Member States. Other approaches such as the Kriging could also apply to the same purpose and are worth exploring.

Finally, care must be taken when calculating the trend in the extent of ODR in cases when the monitored area changed between time periods. In this study, the trend was calculated as percentage of change of ODR vs Extent of Effort (i.e., it was normalised by the Effort), and the percentage of change didn't vary if considering the entire effort areas for each period or the common area only. However, the second approach was chosen as more conservative. Indeed, a change in the investigated area could produce a bias if, for example, an area completely outside, or, *vice versa*, in the core of the species range, is surveyed during one period only. Given the long-term monitoring required by the legislative framework at the large-range spatial scale needed for cetacean species, changes in the monitored areas over time could occur for example in the case of new organisations or countries joining an international effort. This aspect should be carefully considered, and the trend detected should be investigated with a conservative approach within the common effort area only.

The Ecological Predicted Range (EPR) based on sites of known occurrences and extrapolated through habitat maps models proved to be able to generalize the spatial distribution of the species also outside the area of effort providing meaningful outputs especially in the WMED Subregion where sampling was spatially representative of regional ecological conditions. Results of this study further confirm that sampling effort must be designed in order to assure representativeness of the regional ecological variability, and the SDM outputs in not surveyed regions (e.g., as in the case of the Aegean-Levantine basin in this study) should be taken with caution. In addition, predictions and extrapolations should be validated whenever possible by independent datasets as soon as new data become available. Results of this study indicate a general correspondence of trends detected in the Observed and Predicted Range both in terms of shifts (e.g., towards offshore areas in the Western-Mediterranean Subregion for all the species) and extent of areas (e.g., enlargement recorded for *Gm* in both ODR and EPR). These results confirm the potential for using the EPR to indirectly determine the AOO as suggested by the IUCN Guidelines (IUCN, 2001). However, some differences were also detected such as the new areas detected by the ODR in the Sardinia channel for *Gg* that

TABLE 6 Summary results on assessed trends for the WMED Subregion.

	Gg	Gm	Zc	
ODR Extent	↑	(↑)	(↑)	() not significant ↑ Positive ↘ Attention ↓ Negative ↔ Stable
EPR Extent	↔	↑	↔	
ODR Shift	↘	↘	↘	
EPR Shift	↘	↘	↘	
ODR/EPR	↑	↑	↔	
ODR > EPR	↘	↔	↔	

The term 'Attention' refers to situations, such as a shift in distribution or where the ODR is larger than the EPR, that could indicate a displacement of the species outside the suitable areas.

were not predicted by the EPR in the corresponding period. Thus, careful consideration is needed to correctly discriminate the meaning of the range predicted on the basis of SDM to investigate the species conservation status, as the Potential Range does not always correspond to the actual distributional range of the species. Output must be carefully validated and adjusted using the estimated proportion of ODR/EPR as suggested by IUCN (2001).

On the other hand, Suitable Habitat Maps can be directly used to define the extent, trend and pattern of the suitable habitats to answer the parameter/criteria 'Habitat' for the species (e.g., for HD and MSFD). By including information on the main ecological factors that drive their distribution, these models can also be used to investigate the "Habitat conditions" requirement if the pressures are added to the models.

Provided SDMs accurately reflect potential ranges, EPR can also be used to compare the Observed versus the Potential Range (IUCN, 2001; IUCN, 2022) as they indicate the area of occupied habitat and describe unoccupied habitats of suitable quality allowing the long-term survival of the species (DG ENV, 2017). If appropriate data are available, the comparison between the Observed and the Potential Range can also help to identify potential suitable areas that are not used by the species due to the influence of anthropogenic pressures or other limiting factors. Alternatively, EPR can also be used to determine if the species is

TABLE 7 Summary of limits/weaknesses of the indicators and approach tested, and recommendations.

	Limits/weaknesses	Recommendation
ODR	Results only representative of the effort area, can underestimate the real occupied range	Can be used as an index to detect trends given that there is a sufficient coverage of sampled range consistent over time.
	Spatial generalisation method (e.g., KDE) could better define the range that other coarser methods (e.g., grid, MCP) but needs to be fit to data.	Needs to be adjusted for spatial scale (grain) and resolution of data.
EPR	Potential bias linked to data processing	Test for the best SDM approach over the specific type of data/sampling strategy/species. Validate also by independent dataset
	Representativeness of prediction outside the surveyed region	Sampling design representative of regional ecological conditions. Extrapolation considered with caution and validated by independent dataset and as soon as new data become available.
	Could be larger than the occupied range or smaller by effect of anthropogenic pressures.	Investigate potential limiting factors. Adjust e.g., using the estimated proportion of ODR/EPR (IUCN, 2001).
	Not 'one-for-all' SDM approach.	SDM approaches set, tested, and chose for the dataset used through reliable validation process.
ODR & EPR	Potential bias linked to changes in monitored area if e.g., a core species area is surveyed during one period only.	Calculate trend within the common area of effort. Normalize ODR by the effort.
	The observed distribution can be driven by different ecological and anthropogenic factors.	Parallel use of complementary indicators.
Range Pattern	The extent of range could remain equivalent but shifted in different areas over time.	Contemporary investigation as either the trends in extent (surface range) and shifts (range pattern)
Six-year periods	May not be adequate for cetaceans: biological variability could be revealed under different time scales.	Test shorter periods (e.g., moving average) or longer time series.
Species	Higher uncertainty if trend is based on only one species per species group.	Synoptic analyses on more species with similar ecology could help assessing whether a detected modification refers to a single species or is likely representative of a more general change.
Sampling design	*Spatial resolution*:	
	Potential bias linked to underrepresentation of surveys.	Sampling design in order to be representative of species range and ecological conditions.
	Potential bias due to change in investigated areas e.g., if a species core area is surveyed in one period only.	Design of sampling to be representative of known species key areas (or take it into account during the assessment)
	Temporal resolution:	
	Potential bias due to species variability such e.g., seasonal-related displacements, intra-period occasional change in distribution, early-sign of climate-related changes.	Yearly or biennial surveys including all seasons or at least two seasons representative of main species migratory/displacement distribution.
	Difficulties in delivery homogenous data in the long term (e.g., monitoring programmes can vary in methods, timing, area investigated)	International coordination for the harmonisation of all the phases of the information chain.
		Cost-effective approach that can endure over time.
		Deal with uncertainty (e.g., enhance metrics able to deal with integrated heterogeneous data)

pushed outside of the preferred suitable habitat as a consequence of a pressure, change in the distribution of habitat or the exploitation of new resources. Trend in the ratio between Observed vs Potential range could then be used to correlate the detected changes with other environmental or anthropogenic parameters and/or assess the effectiveness of mitigation measures.

5 Conclusions

Our results highlighted the strengths and weaknesses of the analysed indicators and approach as summarised in Table 7. In general, the ODR based on known occurrence can underestimate the real occupied range and needs to be referred to the area of effort, but it can still be used as an index to detect trends. Conversely, the EPR could be larger than the occupied range in presence of limiting factors, either environmental or anthropological, or even smaller in the case of pressures that force the species outside the ecological niche so that careful validation of output is required. Therefore, the parallel use of complementary indicators, such as the Observed and Ecological Potential Range, may be preferable to using a single indicator to disclose the significance of a change.

Based on our results, we also recommend the contemporary investigation of the Range Pattern as either the trends in extent (surface range) and shifts (range pattern). In this study, for example, the enlargement of the Observed surface Range could have been interpreted as positive, but it was associated with a shift towards offshore less suitable or unsuitable areas which instead deserve attention. Moreover, synoptic analyses performed on more species with similar ecology are suggested to assess whether a detected modification refers to just a single species or is likely representative of a more general change.

This study tested and discussed the most common approaches for assessing six-year trends, as required by the HD and MSFD, on range and habitat of rare cetacean species using the longest dataset available at large scale in the Mediterranean Sea. It should be noted that the comparison between two six-year periods may not be adequate to highlight biological and ecological trends for such long-lived species as cetaceans. Biological variability could indeed be revealed under different time scales, and further investigation, such as a moving average of shorter periods or longer time series, might be necessary to confirm the usefulness of the six-year time frames required by the legislative framework or to propose more appropriate time periods.

Overall, our analyses also contribute to assess the most effective methods to evaluate the Range and Habitat indicators in compliance with the international legislative requirements of, among others, the HD, MSFD, and Barcelona Convention.

Data availability statement

The data analysed in this study were collected by several organisations participating in the FLT Med Net. Each organisation owns the data collected. Requests to access these datasets should be directed to the data owners listed in Supplementary Table 1.

Ethics statement

Ethical review and approval were not required for this study as the research was conducted solely by non-invasive collection of visual record from a passenger ferry. Animals were not approached by the vessel, and data were collected in passive mode approach.

Author contributions

AA conceptualisation of theoretical framework, design of methodology, performed formal analysis, writing and editing of the manuscript. AO Methodology for spatial distribution modelling. MA, LD, and PT contributed in the conceptualisation of theoretical framework; Data Curation; Writing - Review & Editing. IC, LB, OG-G, MG, AS, MV, and LC Data Curation, Writing - Review & Editing. RC contributed to the conceptualisation of the theoretical framework, Review & Editing. FA Help manage and supervise the project. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2023.1116829/full#supplementary-material

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Chapter 3

Go with the flow: no cross border policies for plastic pollution, no solution for biodiversity conservation

TO BE SUBMITTED

Abstract

Plastic production have increased greatly in the last fifty years, and by consequence enormous amounts of this material enter the marine environment. The Mediterranean Sea is one of the marine areas most affected by plastic litter, that represent one of the major threats for its variegated biodiversity. Aim of this study was then to characterise the spatio-temporal variability of plastic Floating Marine Macro Litter dispersion modelling 3 years of consistent fine-scale field data collected in the central Mediterranean Sea using the Java tool Ichthyop/Roms3D. The conceptual framework of risk assessment was then applied on plastic pollution in Marine Protected Areas (MPAs) and Fisheries Restricted Areas FRAs), to define their vulnerability to this threat.

After a year of travelling, the modelled particles covered a large portion of the Western and Central Mediterranean Sea. Almost all particles did not reach the seafloor, continuing to be suspended in the upper levels of the water column for at least the first 200 days. Twenty-eight MPAs from Italy, Malta and France, were involved in the plastic FMML particles flow especially during the summer season. Moreover, all the three FRAs located in the Sicilian Channel were at risk from plastic pollution with seasonal differences.

Applying the concept of risk, as well as using fine-scale field data, offer a valuable approach to assessing the implications of the vulnerability of MPAs and FRAs to plastic FMML. This study demonstrates the high potential of modelling techniques based on *in-situ* continuous field data and the crucial needs of considering different temporal and spatial scales when dealing with this kind of "no-border" heterogeneous threat such as FMML. Plastic pollution management requires a coordinated effort between Mediterranean countries to develop effective and harmonised strategies addressing the transboundary challenges posed by plastic pollution in MPAs and FRAs.

Keywords: Plastics, field-modelling data, Trans-boundary management

Introduction

Plastic production has increased enormously in the last 50 years, so much so as to define these times as "the age of Plastics" (Avio et al., 2017). By consequence, enormous amounts of this material enter the environment. Between 4.8 and 12.7 million tons of plastics enter the marine environment from land-based sources every year and, with no advances in the context of waste management, the overall plastics quantity could increase by 3 times up to 2025 (Jambeck et al., 2015). Even if its composition may vary, plastic debris is the most abundant fraction of marine litter, constituting a pervasive threat with no spatio-temporal borders (Eriksen et al., 2014; Van Sebille et al., 2015) primarily due to the longevity of plastic materials and their slowly deterioration rate (Andrady 2005). Moreover, approximately 50% of all plastics are less dense than water, and therefore they float over the sea surface (Geyer et al., 2017). Plastic objects often contain trapped air which enhance their buoyancy and windage, facilitating their spatial diffusion.

In the world's oceans there are five large and stable accumulation areas of marine litter (Eriksen et al., 2014; Lebreton et al., 2018; Law et al., 2010). A different case is represented by the Mediterranean Sea, as its hydrodynamics does not allow the formation and persistence of such aggregations (Mansui et al., 2015, 2020; Liubartseva et al., 2018; Cozar et al., 2015). However, given its particular characteristics, it is one of the marine areas most affected by marine litter problem (Mansui et al., 2015) and in particular by plastic pollution, a category that is always the most represented regardless of the seasons, the different geographic areas and the water column level considered (Lebreton et al., 2012; Cozar et al., 2015, Suaria et al., 2016; Suaria & Aliani, 2014; Fossi et al., 2017; Arcangeli et al., 2018; Scotti et al., 2021). The Mediterranean Sea is in fact a semi-closed and heavily urbanised basin, hosting on the coasts more than 500 million people whose highly busy waters have been the site of a variety of human activities for centuries.

At the same time, the Mediterranean Sea stands out as a biodiversity hotspot, host more than 8% of the world's biodiversity, with an exceptional wealth of marine species adapted to its unique environmental conditions. From *Posidonia oceanica* seagrass meadows to deeper coralligenous reefs, the Mediterranean Sea provides essential habitats for remarkable biological diversity, thanks to the presence of a large number of Marine Protected Areas (MPAs) and Fisheries Restricted Areas (FRAs). These areas are of fundamental importance as they supply ecosystem services, increase ecological and socioeconomic resilience and mitigate the effects of environmental threats on marine biodiversity, safeguarding it from pollution, habitat loss and resources overexploitation (Coll et al., 2010). Nevertheless, MPAs and FRAs are particularly endangered by plastic litter because it, unlike other anthropogenic stressors, can't be managed only locally and they often work as final sink.

To counter the problem of plastic pollution, multi-disciplinary studies integrating geographical, ecological and socio-economic aspects are essential to understand the scale of the impacts and design effective management strategies to reverse the alarming trend of plastic pollution in this unique and precious sea (Suaria et al., 2016). Plastics harmful effects on ecosystems and marine biodiversity are manifold and diverse. Considering its complex path in the environment and the different chemical-physical modifications it can encounter, there are many interactions it can have with the systems it comes into contact with. Impacts on marine biodiversity depend on the type and size of the objects as well as, of course, the species considered (Roman et al., 2021, Chevalier et al., 2023).

It is estimated that more than 1,400 marine species have been negatively impacted by plastic marine litter, mainly through ingestion and entanglement phenomena (Kühn et al., 2015; Wilcox et al., 2015; Claro et al., 2019; Peng et al., 2020). Benthic habitats can be altered due to plastic accumulation (Consoli et al., 2020). Floating plastic litter can facilitate the large-scale dispersion of organisms - and therefore also of alien species (Barnes 2002; Derraik 2002; Aliani & Molcard 2003; Rech et al., 2016; Sarà et al., 2018) - and promote the transport of toxic substances (Mato et al., 2001; Endo et al., 2005; Teuten et al., 2007). Finally, the fragmentation of different materials leads to the production of microparticles and toxic compounds that can accumulate through trophic nets, leading to bioaccumulation and biomagnification phenomena that particularly affect top predators and suspensivores (Davison and Asch 2011; Fossi et al., 2012, 2014; Wright et al., 2013).

Plastic marine litter monitoring, management and reduction are goals of several national and international legislations and action plans (OSPAR Regional Action Plan; G7/G20 Marine Litter Action Plan; MSFD descriptor #10; UNEA-4 2019). Nevertheless, our understanding of plastic fluxes, pathways and fate is still incomplete (Van Sebille et al., 2020). This may be due to the time delay between plastic fluxes through the water masses and their arrival in the study areas where most of the measurements are made (Lebreton et al., 2019), but also to biological, physical and chemical processes (e.g. ingestion, beaching, sedimentation, fragmentation) that must be taken into account to at least partially eliminate these discrepancies (Van Sebille et al., 2020).

Since plastic litter is found also in areas far away from human activities (Chiba et al., 2018), tracking its movements is of paramount importance (Chassignet et al., 2021). Plastics distribution and pathways across the oceans are mainly driven by currents, and at this moment the majority of information about them has been achieved via Eulerian and Lagrangian numerical models, or through an integration of them (Guerrini et al., 2021; Mountford et al., 2019; Van Sebille et al., 2015; Eriksen et al., 2014; Maximenko et al., 2012; Lebreton et al., 2012). This is mainly because generally observational and field data are sparse and difficult to analyse as a whole due to differences between collection protocols (Van Sebille et al., 2020).

Here, we characterise the spatio-temporal variability of plastic Floating Marine Macro Litter (FMML) dispersion modelling 3 years of consistent and long term fine-scale field data observed along the Palermo-Tunis route (FLT Med Monitoring Network, ISPRA) within the conceptual framework of "Risk" (Kumpulainen, 2006; Gilard, 2016), where it is defined as the result of damages caused by interactions between disruptive (probability that an hazardous event take place) and vulnerability factors (exposure of individuals to it, in a given time and place) according to the following formula:

Risk = hazard x vulnerability

In this study, we applied the Risk concept on plastic pollution in Marine Protected Areas (MPAs) and Fisheries Restricted Areas (FRAs), defining their vulnerability to this threat (Figure 1).

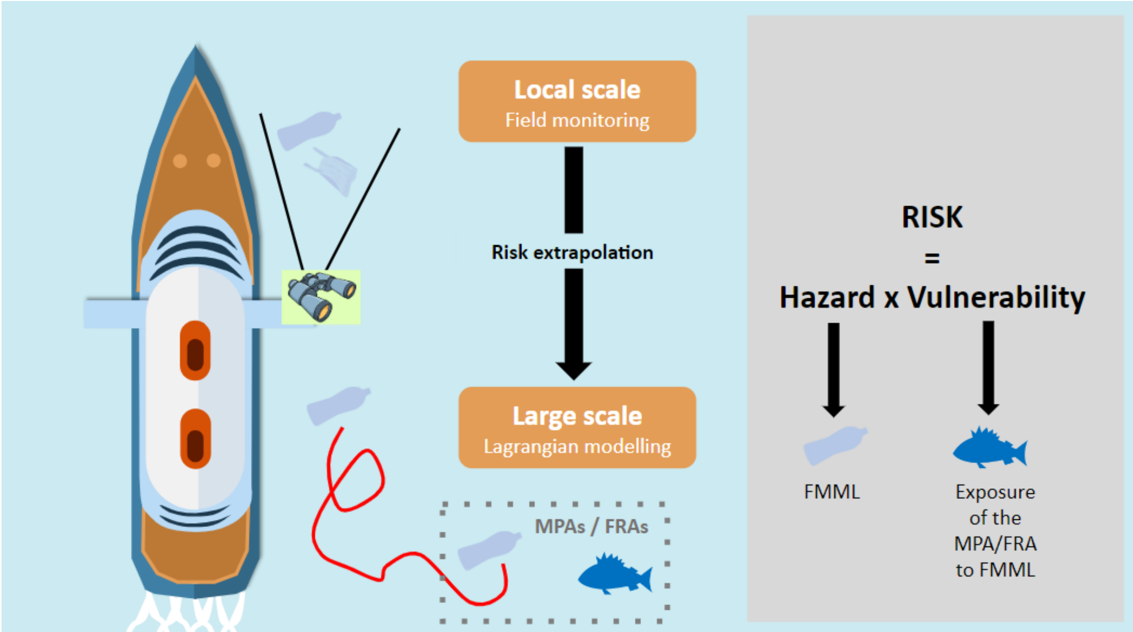

Figure 1: Conceptual diagram representing the framework of the risk assessment of plastic FMML at different scales, combining field monitoring and Lagrangian modelling.

Materials and Methods

Protocol for plastic FMML monitoring: define the real risk

In the conceptual framework of "Risk assessment", this study focused on FMML as the disruptive element (or hazard). On the other hand, "Vulnerability" is characterised by a greater or lesser exposure of MPAs and FRAs to the hazard (Figure 1). To start defining the real risk based on the FMML pollution, field observations were recorded during dedicated surveys connecting Palermo to Tunis performed all year round from 2017 to 2019 using passenger ferries as platforms of observation (Figure 2). Following a dedicated protocol (ISPRA, 2015b, Technical Annex II; Arcangeli et al., 2018) developed in the framework of the Fixed Line Transect Mediterranean Monitoring Network project (leaded by ISPRA since 2007), the plastic FMML monitoring (objects of size > 20 cm) was carried out by the side of the ferry with the best visibility and inside a fixed strip 50 m wide defined at the beginning of the activities (Thiel et al., 2003; Pyle et al., 2008; Arcangeli et al., 2018). For each plastic object, specific information was monitored as the type of object, colour, dimension and buoyancy, and GPS point recorded.

Tracking the plastics movement: define the vulnerability

To define the vulnerability of the Mediterranean MPAs, FRAs and seafloor substrates, plastic FMML trajectories were studied along both time and spatial scales using a Lagrangian model based on the field observations (Figure 2).

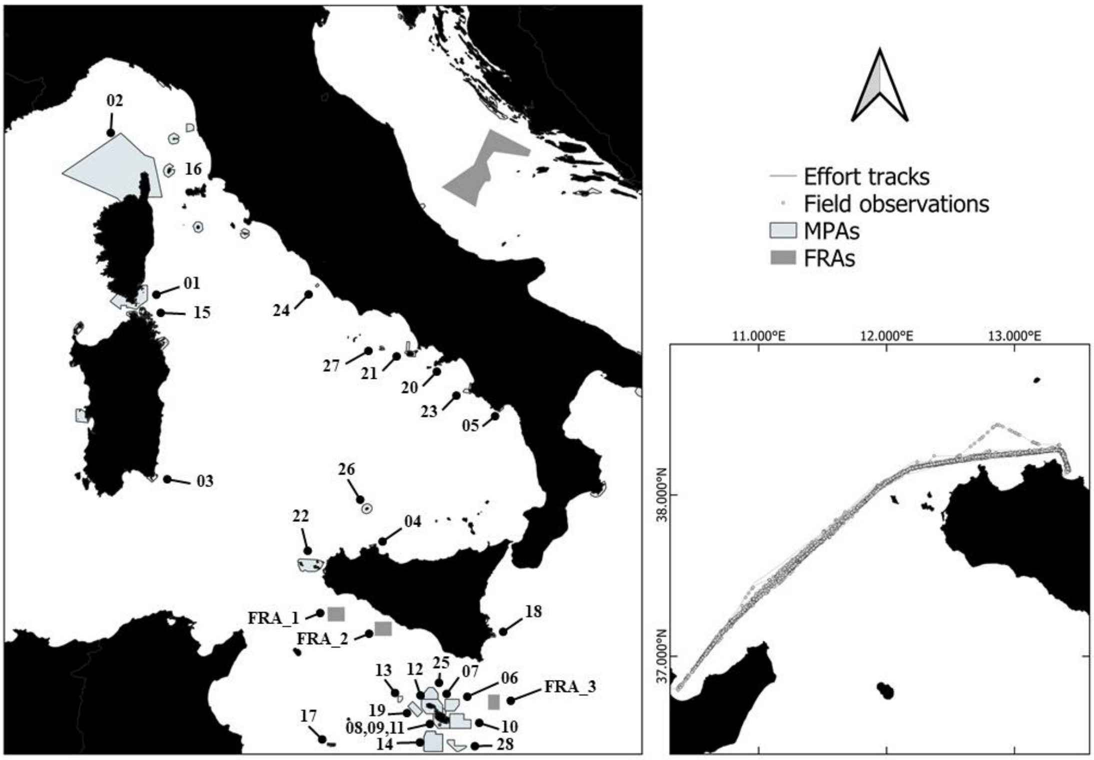

Figure 2: Left panel: Marine Protected and Fisheries Restricted Areas of the study area, alphanumeric codes indicate the MPAs and FRAs interested by the potential plastic presence: 01=Bouches de Bonifacio, 02=Cape Corse et Agriate, 03=Capo Carbonara MPA, 04=Capo Gallo e Isola delle Femmine MPA, 05=Costa degli Infreschi e della Masseta MPA, 06=Grigal, 07=Grigal ta' Malta, 08=Lapsi u ta' Fifla, 09=Lbic, 10=Lvant, 11=Madwar Fifla, 12=Madwar Ghawsex, 13=Majjistral, 14=Nofsinhar, 15=Parco Nazionale Arcipelago La Maddalena, 16=Parco Nazionale Arcipelago Toscano, 17=Pelagie MPA, 18=Plemmirio MPA, 19=Punent, 20=Punta Campanella MPA, 21=Regno di Nettuno MPA, 22=Riserva Naturale Marine Isole Egadi, 23=S. Maria di Castellabate MPA, 24=Secche di Tor Paterno MPA, 25=Tramuntana, 26=Ustica MPA, 27=Ventotene e S. Stefano MPA, 28=Xlokk, FRA_1=East of Adventure Bank, FRA_2=West of Gela Basin, FRA_3=East of Malta Bank. Right panel: monitoring effort and field observations used for modelling.

Hydrodynamic data were extracted from the Copernicus Marine Project database (https://marine.copernicus.eu, MEDSEA_MULTIYEAR_PHY_006_004) with a spatial resolution grid of 1/24° (~4.4 km) and 141 vertical levels with higher resolution at the surface. Particle tracking simulations to study plastic litter transportation were performed using the free Java tool Ichthyop/Roms3D (Version 3.3.3) (http://www.ichthyop.org/). Ichthyop is a Lagrangian particle tracking model initially created for studying ichthyoplankton dynamics (Deschepper et al., 2020; Martins et al., 2020), even though recently it was used also to model the fate and transport of invasive species (Marchessaux et al., 2020; 2023), solid particles from Wastewater Treatment Plants (Millet et al., 2018), microplastics (Frere et al., 2017; Collins et al., 2019), and applied to link propagules dispersion and population genetics (Reynes et al., 2021).

The modelling was performed over the three same years of the plastic FMML monitoring activities (e.g. 2017, 2018, 2019), setting for each particle a transport simulation of 365 days tracked every day. GPS positions of each plastic object observed on the field were integrated in Ichthyop, and the modelling started from the day and hour of observation on the field (for example the 28 January 2017 at 12:00). Virtual plastic particles behave as a Lagrangian drifter under the effect of horizontal and vertical advection, horizontal and vertical dispersion, and also a buoyancy force due to the difference between the particle and surrounding water density. In order to simulate a realistic scenario, the model was set to avoid a coastline barrier but to ensure the natural water movements and therefore the exchanges of particles between the sea and the shore. As in the data recorded during the monitoring at sea the object typology was specified, it was possible to associate to every particle its material and corresponding density. Then, in the software a medium buoyancy (density) value of 1.1 g/cm^3 was setted (Schwarz et al., 2019).

A total of 77 netcdf (.nc) format files (including 2,552 plastic objects) corresponding to each field monitoring day were produced at the end of simulations. For each particle the daily longitude, latitude, and depth were extracted using the software RStudio (version 4.0.5), package "RNetCDF" (Michna and Woods 2023). Trajectories (daily GPS positions) and distances (km) covered were also extracted. Trajectories data were uploaded in the software QGIS (version 3.22.4) and maps were produced for each field starting day. Distances covered were combined by seasons by year and boxplotted using the software SIGMAPLOT (version 12.5). To test the differences between years for each season, an ANOVA and Bonferroni post-hoc tests (significant difference = $p < 0.05$) were performed using R Studio (version 2021.09.0).

For each trajectory's ending point, the sinking depth was extracted and compared to the corresponding bathymetry in order to check if, after a year of modelling, the particles might reach the seafloor. The frequencies of plastics particles were calculated for each 10 m depth section from 0 to 1800 m depth to define the areas of concentration of plastics in the water column, and the plastics diffusion across time for 365 days for each year of simulation (e.g. 2017, 2018, 2019). Based on the results showing the highest frequencies in the first 40 m, plots for each year for the first 40 m depth were performed. The particles' depth frequencies across time and depth were plotted using the software OceanDataView (version 5.1.7; Schlitzer and Reiner, 2021).

Risk, hazard, vulnerability in MPAs and FRAs

To assess the potential pollution pressure by plastics in MPAs and FRAs, a risk index (%) was calculated following the equation:

$$Risk\ (R) = \frac{Nb\ time\ (hazard)}{Sum\ of\ events\ per\ area\ (vulnerability)}\ x\ 100$$

with "*Nb time*" corresponding to the number of times a plastic particle entered an area, and "*Sum of event per area*", the sum of the total particles observed in an area for each simulation starting day (corresponding to each field day).

The information needed to perform the calculation was obtained using the function "Count points in polygon" in QGIS. The MPAs and FRAs spatial layers used were obtained respectively from the EMODnet platform (https://emodnet.ec.europa.eu/en) and the GFCM Web Map.

MPAs and FRAs risk was averaged for each season (winter, spring, summer, autumn) and for the entire dataset. MPAs and FRAs Risk was plotted using the software SIGMAPLOT and mapped using the QGIS "heatmap" function. Moreover, it has been checked (i) what is the seabed substrate (EMODnet_Seabed_substrate_EUSeaMap) corresponding to each trajectories ending points, and (ii) if any trajectories ending point was situated inside an MPAs or FRAs.

Results

Overall, 2,552 plastic objects observed on the field were used as particles for the modelling exercise (Tab. 1, SM). After a year of travelling, the modelled particles starting from the Palermo-Tunis route area were able to move covering a large portion of the Western and Central Mediterranean Sea, from the Sicilian Strait heading north to the Ligurian Sea and south to the Sirte Gulf (Figure 3).

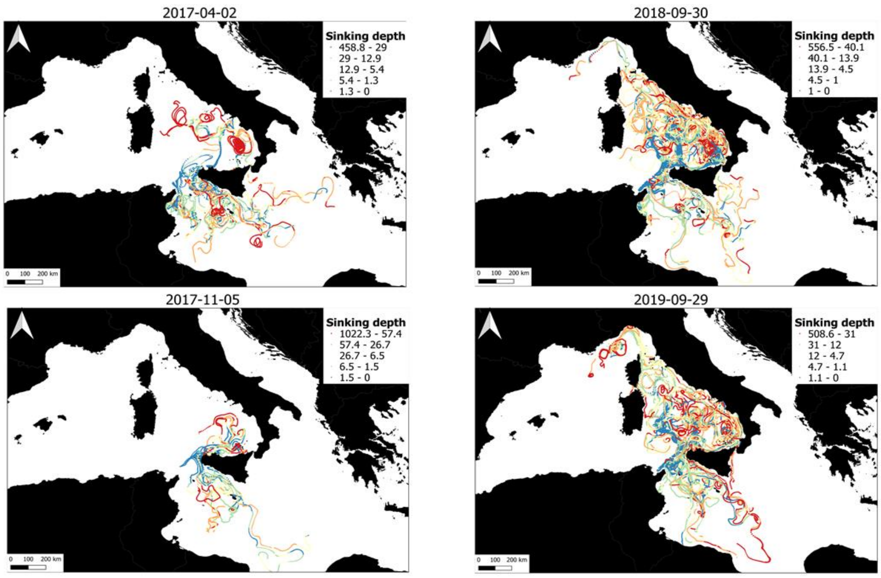

Figure 3: Trajectories examples showing the eastern (first)/ southern (third)/ northern and westernmost (second and fourth) points of the study areas reached by the particles.

Plastics covered long distances, overall between 200 and 800 km. The space crossed by the particles observed during winter time resulted to be statistically lower than the distances travelled in other seasons (ANOVA, Bonferroni post-hoc test, $p<0.05$) (Figure 4, panel B). Almost all of the plastic litter (99.9 ± 0.1 %) after a year of modelling did not reach the seafloor, continuing to be suspended in the water column (Figure 4, panel A).

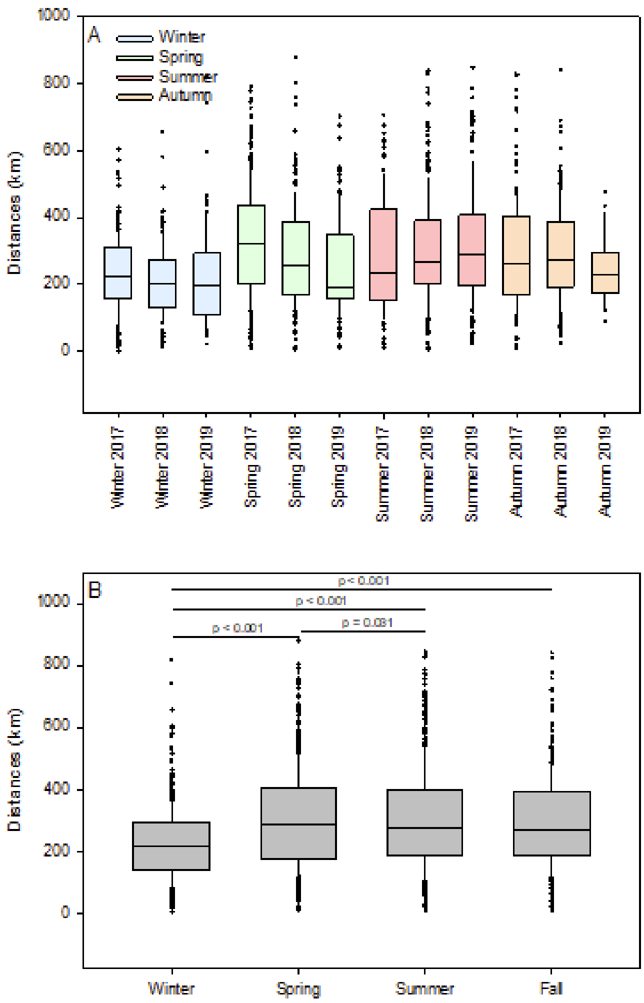

Figure 4: Distances covered by plastic particles (km) for the 3 years of study across the seasons.

For the 3 years of study, the same pattern of depths reached by the particles was observed, with few differences (Fig. 5, panel A). The maximum particle sinking depth reached 1800 m, but the majority of particles (75.0 ± 2.1 %) was concentrated in the first 200 meters of the water column for most of the year (please note that 200 m is usually the farther margin of the continental shelf, which develop from the coast up to 200 m worldwide). As observed on Figure 5, panel B, the particles were concentrated in the first 10 m depth showing the highest frequencies (65.9 ± 2.7 %) for about 200 days. After this time, particle diffusion was observed and distributed throughout the water column.

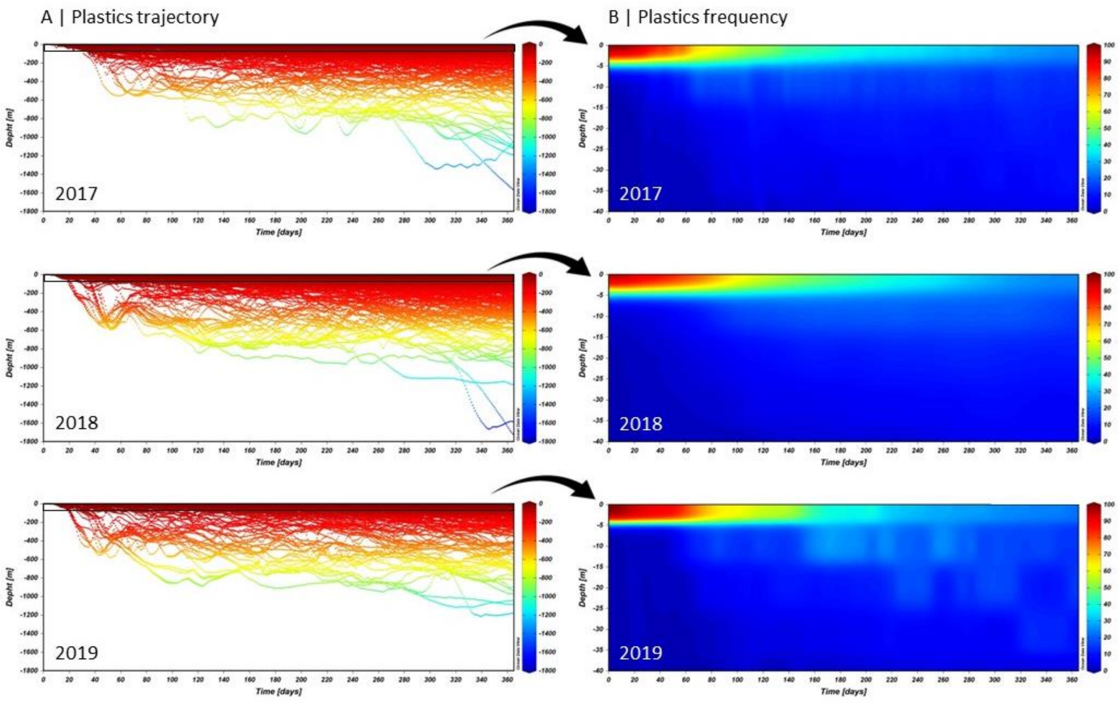

Figure 5: (A) Daily variations (days) of depth (m) for each particle along a year, the colour bar represents the sinking depth (m); (B) frequency (%) of particles according to the time and the depth for the first 40 m depth where the maximum frequencies were recorded, the colour bar represent the frequency (%). Results are presented for each year of study.

Twenty-eight Mediterranean MPAs from Italy, Malta and France, were involved in the plastic FMML particle flow (Figure 6; Table 2, SM) and this was translated into risk assessment showing seasonal differences in the % Risk and in the number of MPAs. The largest number of MPAs at risk was reported for the summer season, with Maltese MPAs showing the highest values of the index. Conversely, during the other seasons the Southern Tyrrhenian MPAs (Sicily, Italy) were the most potentially impacted (see Fig. 7 for the global average, and Fig. 1 of SM for the seasonal graphs and maps). After a year of modelling, 32 trajectory ending points were situated inside a MPA. The majority was located inside different Maltese MPAs, followed by Ustica MPA (Sicily, Italy).

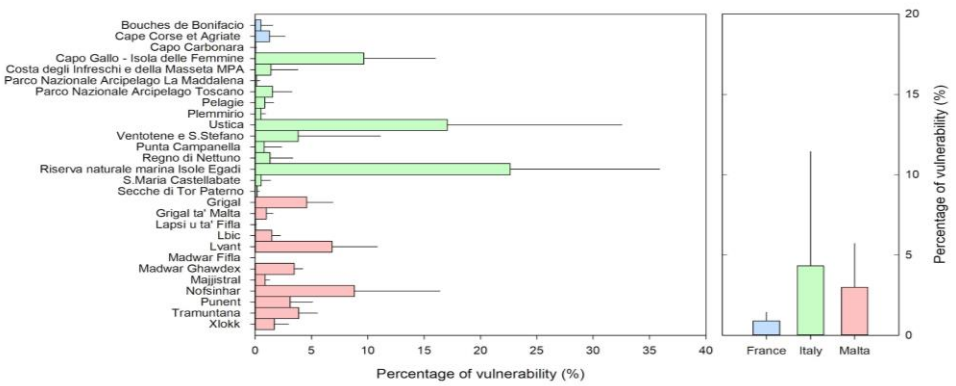

Figure 6: Index of Risk (global average) of Marine Protected Areas (on the left) and country averages (on the right). The colours represent the countries: blue = France; green = Italy; red = Malta.

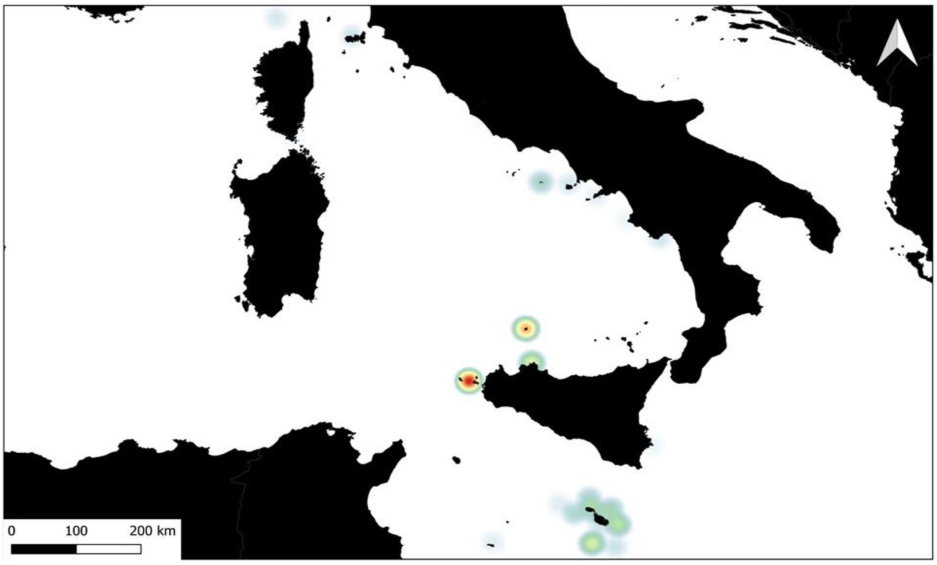

Figure 7: Heatmap (global average) of the risk index of Marine Protected Areas.

Through the four Mediterranean FRAs considered in this study, the three located in the Sicilian Channel were at major risk from plastic FMMLs with seasonal differences (Fig. 2, SM). For FRA_1 and FRA_2, winter and spring were the most at risk seasons, while autumn for FRA_3. After a year of modelling, 3 ending points were situated inside FRA_1, and one in FRA_2 and FRA_3. At the seafloor level, the largest ending points were found in correspondence of "Fine mud" and "Sandy mud" bottoms (Tab. 1).

Table 1 - EMODnet substrate types and correspective Risk index percentages.

Substrate types	**Risk index (%)**
Fine mud	40.6
Sandy mud	37.0
Sand	11.2
Muddy sand	9.4
Coarse and mixed sediment	0.9
Rock or other hard substrata	0.4
Posidonia oceanica meadows	0.4
Fine mud/Sandy mud/Muddy sand	0.1

Discussion

In situ measurement of macroplastic pollution is of crucial importance in understanding and mitigating the devastating impacts of this waste on aquatic ecosystems (Smith and Jones, 2020). Field surveys provide direct, location-specific data, enabling an accurate assessment of the scale of the problem. *In situ* measurement methods, such as collection nets and visual surveys, provide in fact detailed information on the spatial distribution of plastic FMML, their size, type and persistence in the environment (Smith & Jones, 2020; Chevalier et al., 2023). These data are essential for designing appropriate solutions, minimising impacts on local flora and fauna, guiding clean-up efforts and facilitating the implementation not only of targeted management strategies, but also the formulation of informed environmental policies.

After a year of modelling, plastic particles travelled for long distances throughout the Western-Central Mediterranean. Nevertheless, the space covered during winter-time resulted lower in comparison to the other seasons, indicating that the travelled distance can depend on the time of observation of each plastic particle (Chevalier et al., 2023). The central Mediterranean Sea and the Sicily Channel are highly dynamic areas, with processes that cover the full range of the spatio-temporal scale (Sorgente et al., 2011). In particular, the circulation is strongly influenced by mesoscale signals ranging from 3 to 10 days (Manzella et al., 1988). The superficial waters enter the Basin passing through the Gibraltar Strait and become warmer and saltier moving along the African coast, eastern ward. This Modified Atlantic Water (MAW) is then divided into 3 branches, one that flows along the Sicilian coast and two through the Sicilian Strait (Astraldi et al., 1999) with significant seasonal differences. The southern one reaches its maximum during late autumn (Astraldi et al., 1996), while the northern one is more abundant during summer and autumn. Such a kind of hydrodynamic variations caused by global factors can lead to different trajectories and resulting distances travelled, therefore making the whole process difficult to predict. Moreover, also the object's size can greatly determine the distance potentially travelled by plastics from their sources: the larger the debris, the longer the travelling distance (Fazey and Ryan, 2016). According to this extrapolated observation, the management of larger plastic debris can be more complicated (Hatzonikolakis et al., 2022).

Almost all the modelled plastic FMML particles, after a year of travelling, are still situated near the sea surface: only after 200 days they begin to sink. During this period of time, macroplastic objects are subjected to the bio-physico-chemical forces that enhance the formation of secondary microplastics, contextually increasing fouling colonisation inducing the process of sinking due to burdening (Fazey et al., 2016; Amaral-Zettler et al., 2021). Scientific literature underlines the abundant presence of microplastics at the sea surface, and especially within the neustonic habitat (Ryan et al., 2009). For example, in the case study of Chevalier et al. (2023) who model the distribution of microplastics in the water column of the bay of Marseille, microplastics concentration is higher at the surface layer and decreases exponentially towards the bottom. This distribution does vary with seasons, under the forces of wind and stratification. The presence and permanence of (micro and macro) plastics at the sea surface or in the few meters immediately below represent a great threat for all the organisms that live at this level of the water column or that reach it for specific purposes (e.g. breathing, feeding) (Davison and Asch 2011; Fossi et al., 2012, 2014; Wright et al., 2013; Abreo et al., 2023). More than 80% of the impacts generated by marine litter on marine species is caused by plastic items (CBD 2012) and, between plastic FMML, net fragments, ropes and lines, and various kinds of packaging items were identified as the objects most frequently associated with entanglement of marine fauna (Butterworth et al., 2016).

Applying the concept of risk offers a valuable approach to assessing the implications of the vulnerability of MPAs and FRAs to plastic FMML. Vulnerability in these sensitive ecosystems is linked to the sensitivity of marine habitats, local biodiversity and the economic dependence of the human communities that depend on them. The danger, meanwhile, is represented by the quantity, size and persistence of the FMML plastic present. Studies have shown that these areas, which are supposed to be ecological refugia (Ban et al., 2016), are also exposed to high levels of plastic pollution, compromising the health of marine ecosystems and the sustainability of fishing activities. For example, Johnson et al. (2019) highlighted the increased risk of mortality among marine fauna and the deleterious effects on fish stocks in areas where ecological vulnerability and the presence of macroplastics are higher. Soto-Navarro et al. (2021) reinforced this concept showing that the risk associated with marine litter pollution in MPAs is usually dependent on their location and it is site-specific. Due to their location in regions with high biodiversity, MPAs exhibit “naturally” high levels of exposure and vulnerability from plastic FMML making them very sensitive to this threat. All Mediterranean countries hosts at least one MPA with more than 55% of macroplastics originating from sources beyond their borders, emphasising the trans-boundary nature of this anthropogenic threat. From this study twenty-height Italian, French and Maltese MPAs resulted potentially at risk from the passages, within their boundaries, of plastic FMML (Hatzonikolakis et al., 2023). In our study, the highest number of MPAs at risk was found during the summer season because of a greater transportation of macroplastics by currents on long distances, with especially Maltese MPAs showing high levels of potential risk.

Mediterranean biodiversity, in all its complexity and variety, is at risk by plastic marine litter. Accumulation of marine litter can be found at all level of the water column, (Barnes et al., 2009; Galgani et al., 1996; Galgani et al., 2000; Ryan et al., 2009; Schlining et al., 2013). Biota and habitats of all areas characterized by low hydrodynamism, are particular at risk by marine litter (Galgani et al., 1996; Pham et al., 2014; Schlining et al., 2013; Dameron et al., 2007; Kühn et al., 2015): here, the accumulation probability is higher due to the low intensity of the oceanographic forces that enhance its movement. Geomorphology can also affect the abundance of marine litter in the seafloor (Galgani et al., 1996; Galgani et al., 2000; Pham et al., 2014; Ramirez-Llodra et al., 2013; Watters et al., 2010). Here, as showed by diverse authors (Consoli et al., 2018; Oliveira et al.,

2015), the majority of marine litter is represented by lost or discharged fishery equipment (e.g. longlines) that can be entangled in rocky ledges damaging the complex aggregation of sessile fauna colonizing this habitat.
In our study, after a year of modelling, almost none of our plastic FMML particles reached the seafloor. Nevertheless, the majority of the trajectory's ending points correspond to fine mud and sandy mud substrates. Plastics in various forms, from macro to microplastics, infiltrate these habitats with harmful consequences. The physical entanglement of marine species in plastic debris, combined with the ingestion of plastic particles, directly threatens the survival of many marine organisms. In addition, plastic pollution can alter the chemical composition of marine environments, affecting water quality and disrupting essential ecological processes. Microplastics can penetrate deep into sediments, altering the chemical and physical composition of marine habitats (Phuong et al., 2021) and have been associated with adverse effects on benthic fauna, disrupting the reproductive cycles and health of marine organisms (Horn et al., 2020). The vulnerability of marine habitats to plastic pollution demands urgent attention, underlining the need for global initiatives to mitigate plastic litter and protect the complex balance of life within these vital ecosystems. Even if Mediterranean MPAs are regulated by multiple site-specific restriction measures that manage their protective status, they are still subjected to different anthropogenic threats. Considering the marine litter problem, studies demonstrate that in certain cases (e.g. MPAs in semi-enclosed gulfs) it can be successfully managed locally: for example, applying technologies that aim to reduce the amount of litter directly from its source (Gkanasos et al., 2021). Other protected areas, meaning the ones receiving plastic from different and distant sources, cannot obviously be managed locally (Hatzonikolakis et al., 2023). For these cases, it is important to consider the connectivity in the distribution of (micro and macro) plastics across the Mediterranean Sea based on field observations and monitoring.
Results has shown that it is important to consider several spatial and temporal scales. The first was to measure the real risk on the field at a small scale. The second was to show that the local plastic problem is actually a problem on a larger spatial scale, and especially on an international scale. Particularly in marine environments, currents play a key role in the distribution of plastics on local, regional and global scales, as well as on an international scale.
Plastic pollution management has to be transboundary, requiring a coordinated effort between Mediterranean countries. A thorough understanding of these factors would enable the design of appropriate management measures aimed at reducing vulnerability, mitigating hazards and ensuring the preservation of these crucial areas for marine biodiversity and fisheries-dependent communities. To achieve this, comprehensive studies addressing the transboundary nature of plastic pollution in the Mediterranean are essential. Longitudinal assessments of plastic transport and deposition patterns across borders would provide valuable insights into the regional dynamics of plastic pollution.
In this collaborative framework lies the Marine Strategy Framework Directive (MSFD), that in the descriptor #10 advocates the achievement of marine waters' Good Environmental Status (GES). This

will be reached through the development of monitoring plans that allow the evaluation of trends in the amount of marine litter, including analysis of its composition, spatial distribution, and where possible, source.

In 2020 (one year after the period considered for this study), with the support of several research institutes, Italy started the asked monitoring in different areas of the Mediterranean using the same protocol and sampling strategy applied here. This modelling approach employed can therefore be put into system using the existing datasets from different areas, obtaining increasingly solid results about marine litter spatio-temporal dispersion.

Other collaborative initiatives, such as those examining the efficacy of existing waste management practices and policies in different Mediterranean nations, could inform the development of harmonised strategies. Studies focusing on the socio-economic impacts of plastic pollution on coastal communities would contribute to the identification of vulnerable populations and the design of targeted interventions (Lebreton et al., 2017; Beaumont et al.; 2019). By fostering international cooperation and conducting interdisciplinary research, the Mediterranean countries can develop effective and harmonised strategies to address the transboundary challenges posed by plastic pollution in MPAs and FRAs.

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Supplementary materials

Tab. 1 - Number of plastic particles used for the modelling exercise subdivided for day/season/year.

Year	Season	Month/Day	N° drifters
2017	Winter	01/28	5
		01/29	86
		02/11	32
		02/12	42
		03/18	12
		03/19	41
	Spring	04/01	21
		04/02	46
		04/29	89
		04/30	74
		05/20	10
		05/21	12
		06/18	13
		06/24	63
		06/25	133
	Summer	07/01	17
		07/02	38
		09/30	57
	Autumn	10/01	116
		11/04	12
		11/05	24
		11/25	15
		11/26	5
2018	Winter	01/13	14
		01/14	42
		01/28	39
		02/10	13
		02/11	35
		03/10	20
		03/11	42

	Spring	04/07	33
		04/08	24
		04/21	35
		04/22	81
		05/05	11
		05/06	29
		05/19	12
		05/20	11
		06/23	8
		06/24	30
	Summer	07/07	17
		07/08	57
		07/21	4
		07/22	50
		09/29	31
		09/30	169
	Autumn	10/06	17
		10/07	50
		10/13	15
		10/14	32
		11/10	21
		11/11	71
		11/24	15
		11/25	5
		12/01	11
		12/02	37
2019	Winter	01/19	7
		01/20	11
		03/09	22
		03/10	25
		03/23	12
		03/24	25
	Spring	04/13	11

		04/14	18
		05/11	17
		05/12	5
		05/18	9
		06/08	25
		06/09	31
		06/22	9
		06/23	69
	Summer	09/21	10
		09/22	21
		09/28	57
		09/29	98
	Autumn	10/12	14
		10/13	12

Tab. 2 - MPAs potentially endangered by plastic FMML.

Code	**MPA name**
01	Bouches de Bonifacio
02	Cape Corse et Agriate
03	Capo Carbonara MPA
04	Capo Gallo e Isola delle Femmine MPA
05	Costa degli Infreschi e della Masseta MPA
06	Grigal
07	Grigal ta' Malta
08	Lapsi u ta' Fifla
09	Lbic
10	Lvant
11	Madwar Fifla
12	Madwar Ghawsex
13	Majjistral

14	Nofsinhar
15	Parco Nazionale Arcipelago La Maddalena
16	Parco Nazionale Arcipelago Toscano
17	Pelagie MPA
18	Plemmirio MPA
19	Punent
20	Punta Campanella MPA
21	Regno di Nettuno MPA
22	Riserva Naturale marina Isole Egadi
23	S. Maria di Castellabate MPA
24	Secche di Tor Paterno MPA
25	Tramuntana
26	Ustica MPA
27	Ventotene e S. Stefano MPA
28	Xlokk

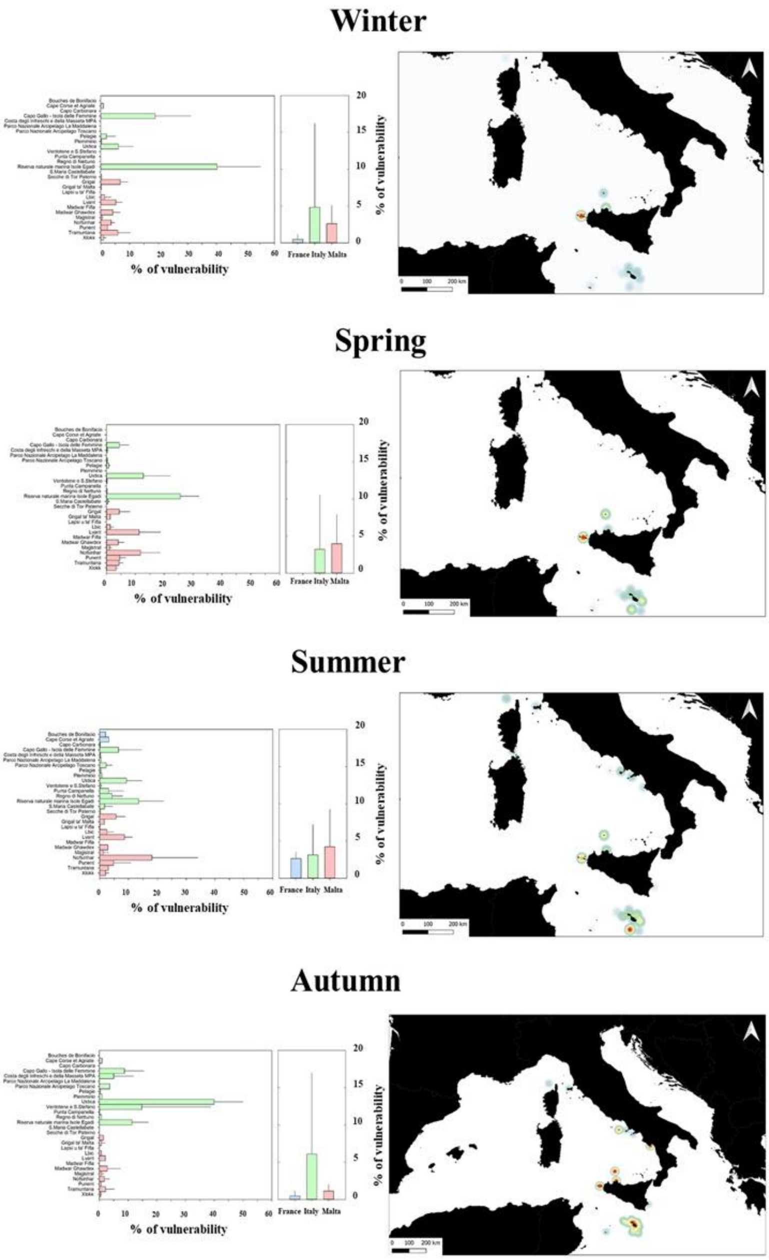

Fig. 1 - Seasonal percentages of vulnerability (left panel) and vulnerability heatmaps (right panel) for MPAs.

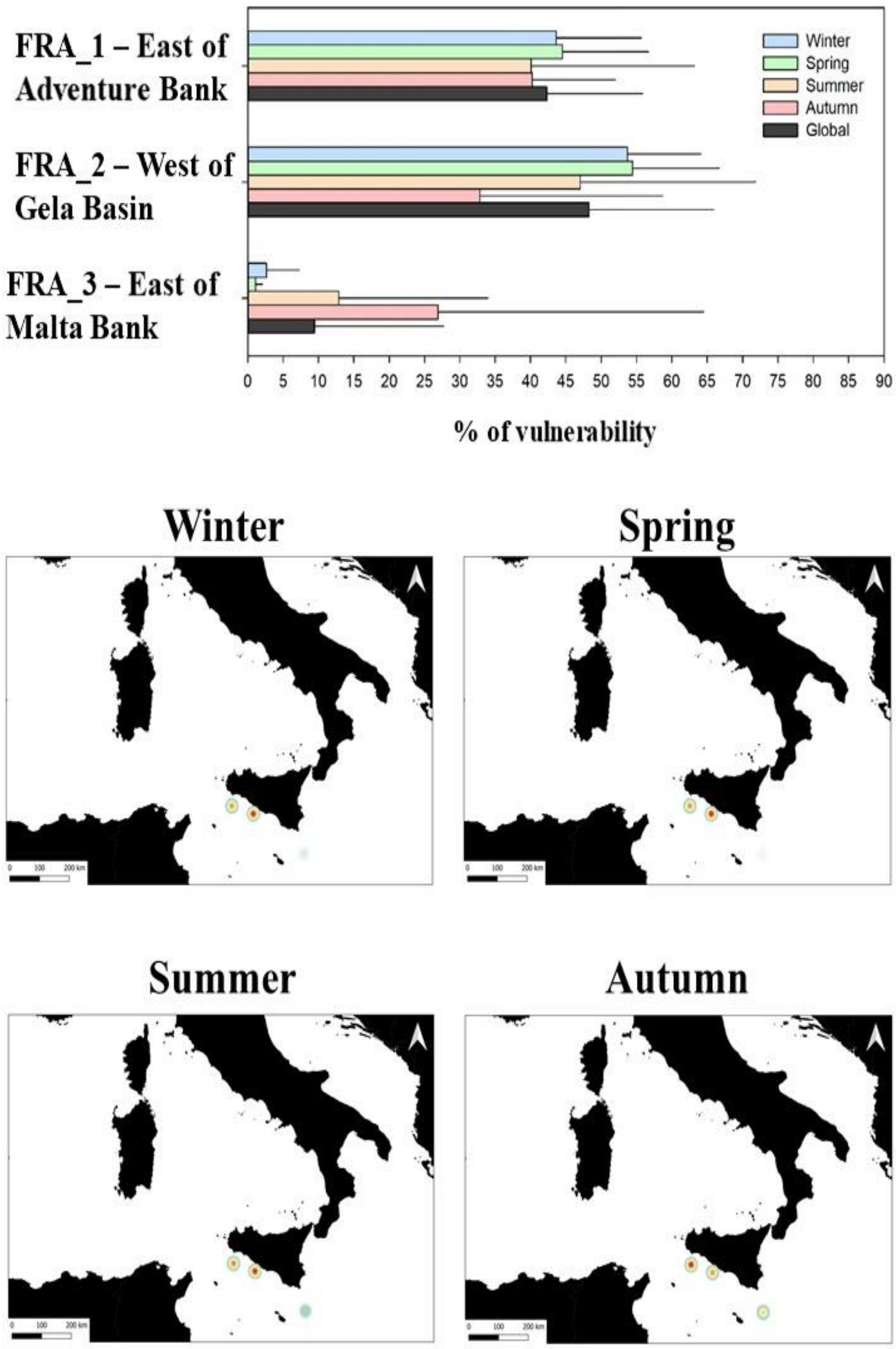

Fig. 2 - Seasonal percentages of vulnerability (left panel) and vulnerability heatmaps (right panel) for FRAs.

Chapter 4

Potential risk of floating marine litter to cetaceans and sea turtles: a review of spatial risk exposure assessments

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Abstract

Marine litter is a main threat for marine life, although the assessment of the associated risks has not yet been fully incorporated into area-based management tools. Floating litter is detrimental to cetaceans and sea turtles and thus, these organisms are considered an effective indicator of areas where litter accumulates. Increasing our ability to predict high exposure risk locations, i.e., where and when marine megafauna is exposed to the potential negative impacts of litter, is important for prioritizing smart-conservation planning and is an essential first step in characterizing the risk of real injury/damage. However, Risk Exposure Assessment (REA) is still underrepresented as a standardized procedure.

Here, a literature review framed the state-of-the-art of REA approaches for cetaceans and sea turtles from floating litter supporting the standardization of metrics and procedures. Of the 415 papers resulting from the literature search, the 23 selected (2011-2022) showed that 57% of the studies were conducted in the Western-Mediterranean Sea, evidencing inconsistent geographical applications. While a variety of REA methodological approaches revealed high informational heterogeneity, main limits and future recommendations were identified regarding raw data availability, information bias, geographical gaps, target species selection, lack of standard protocol needed to assess trends to evaluate the effectiveness of mitigation measures. Ultimately, the study showed that a spatial-contextual approach (possibly functional trait-based) is needed to effectively support long-term year-round monitoring programs, especially in still un-surveyed regions.

Introduction

Marine litter and main impact on species

Marine Litter (ML) is a major form of pollution in the marine environment, and is defined as any persistent, manufactured or processed solid material discarded, disposed of, or abandoned in the marine and coastal environment (UNEP 2009). ML, and in particular the accumulation of plastic debris, has been identified as a global problem alongside other key issues of our time, including climate change, ocean acidification and biodiversity loss (Sutherland et al. 2007, Moore 2015).

ML itself contributes to biodiversity loss affecting many free-swimming and sessile animals (Moore & Barco 2013). The complex path of ML, from the source to dispersal, fragmentation and accumulation, determines interactions with marine life at various levels, causing mostly negative effects (Coe & Rogers 1997). Its impacts on species vary, according to its type and size and depending on the organisms that encounter it (Vegter et al. 2014, Poeta et al. 2017, Bucci et al. 2020).

Species can be exposed to ML litter via a variety of exposure pathways, including entanglement, ingestion, chemical and biological transfer. Entanglement can cause direct harm (physical injuries) or death to individuals, as well as restricting natural movements, while affecting their ability to catch food, escape from predators and reproduce (Gregory 2009, Woods et al. 2019). Ingestion of ML by individuals can cause direct impact such as intestinal blockage, malnutrition and poisoning, or altering the ability to sense hunger, escape from predators and reproduce, potentially leading to severe suffering or death (Laist 1997, Derraik 2002, CBD 2012, Baulch & Perry 2014, Marn et al., 2020, López-Martínez et al. 2023). Ingestion of ML, especially microplastics, can also facilitate the transport of harmful chemicals into the organism potentially bioaccumulating along the trophic chain, and increasing the risk to apex predators or filter-feeding organisms (Cole et al. 2011, Davison & Ash 2011, Fossi et al. 2012, Wright et al. 2013, Ory et al. 2017, Berlino et al. 2021, Salerno et al. 2021, López-Martínez et al. 2023). Furthermore, ML can also act as a transport vector that facilitates the opportunistic dispersal of organisms, including alien species, alterying/modifying assemblages of species (Wilcox et al. 2013, Kühn et al. 2015, Werner et al. 2016, Claro et al. 2019).

Direct impacts of ML have been documented for more than 1400 species, including marine megafauna such as fishes, birds, sea turtles and mammals, with the primary causes being entanglement and ingestion (Wilcox et al. 2013, Kühn et al. 2015, Claro et al. 2019). In 2012, the Convention of Biological Diversity (CBD) revealed that all known species of sea turtles, about half of all species of marine mammals, and one-fifth of all species of seabirds were affected by entanglement or ingestion of ML, with about 15% of the total number of species affected being included in the IUCN Red List (CBD 2012). In 2016, a new assessment increased this figure to 23% (CBD 2016). As for entanglement, certain categories of ML may be much more prone to cause entanglement due to their shape, size, material: in 2016, the Joint Research Centre (JRC) report ascertained that 44 out of the 217 ML categories defined within the Marine Strategy Framework Directive (MSFD, 2008/56/EC) pose an elevated risk for entanglement, particularly fishing-related items, such as nets, traps and ropes (Werner et al. 2016) and that over 80% of the impacts have been associated with plastic items (CBD 2012). Among these, net fragments, ropes and lines (e.g., gill and trawl nets, lost or discarded line for pots and traps), monofilament lines, packaging bands, plastic circular rings and packaging such as multipack can rings were identified as the items most frequently associated with entanglement (Butterworth et al. 2012).

Floating ML (hereafter FML) is a major cause of entanglement and ingestion, especially macro FML (> 2.5 cm) which has previously been reported to negatively impact nearly 400 marine species (Wilcox et al. 2013, Gall & Thompson 2015, Kühn et al. 2015, Claro et al. 2019). FML is of particular concern for animals that are obligate air breathers such as cetaceans and sea turtles, which are bound to encounter these materials as they surface for air (Abreo et al. 2023). Moreover, according to the findings from the CBD, the global distribution of the FML can be an effective indicator of areas where litter accumulates, including seasonal trends, and where it can mostly affect vagrant pelagic megafauna such as cetaceans and sea turtles (CBD 2016, Poeta et al. 2017). The main EU marine policy Directive MSFD, in Annex I identifies ML as one of the eleven mandatory qualitative descriptors for assessing the Good Environmental Status of EU marine waters (GES, MSFD 2017, i.e., a state in which the seas remain healthy and productive and are used sustainably) and sets as overall provision for the GES that ‘composition, amount and distribution of litter and micro-litter on the coastline, in the surface layer of the water column (i.e., FML), and on the seabed, are at levels that do not cause harm to the coastal and marine environment' (MSFD primary criteria D10C1 and D10C2). According to these criteria, EU Member States should establish monitoring programmes and assess the extent of the pollution problem. Despite these, only a few member states have included FML in their monitoring strategies (Ruiz-Orejón et al. 2021, Vighi et al. 2022), and filling the data gaps in FML were recognised as a priority to improve our understanding of litter dynamics and distribution (Molina et al. 2019).

The risk assessment

In line with the legislative requirements, the MSFD task group on ML underlines the need to identify, quantify and prioritise the risks deriving from ML to ensure that there are no significant impacts on, or risks to, marine biodiversity (Werner et al. 2016). Although the traditional risk assessment framework is standardised to human health, it could provide a useful framework to analyse the potential harm of human activities on wildlife populations and ecosystems, and support decision-making processes that are faced with considerable uncertainty (Suter 2016). For the MSFD, cetaceans and sea turtles are considered relevant indicators to assess the risk of ML pressure on marine ecosystems, being representative components of marine ecosystems with key functional roles, sensitive to ML pressure, and present in wide-range marine areas (i.e., representative, relevant, present. European Commission 2017, Palialexis et al. 2022). The identification of risk areas, where marine fauna has an increased exposure to litter, is the first step for prioritising conservation measures to address the highest-risk settings (UNEP-MAP 2016). Unlike the 'traditional' risk assessment, where exposure is mostly given as ascertained, a fundamental part of the assessment of risk for highly mobile marine fauna from ML is focused on the identification of the pathways by which exposure might occur, and the organisms that are more likely to be impacted by the threat (e.g., Darmon et al. 2017, Matiddi et al. 2017, Compa et al. 2019, Soto-Navarro et al. 2021). The focus on spatial exposure risk is also in line with the precautionary principle, and is therefore considered to be adequate for wildlife conservation purposes. A spatially-explicit contextualization of the exposure risk is an essential component of the Maritime Spatial Planning Directive (MSPD, 2014/89/EU) where it has also been used to assess the risk from threats other than litter (e.g., maritime traffic, Pennino et al. 2017), and is one of the key Principles of the Integrated Monitoring and Assessment Program (principle 5, UNEP-MAP 2016).

Despite the growing concern of the adverse effects of ML on different marine species and the potential effects on marine ecosystems, the 'Risk Assessment' topic is still underrepresented within the European research effort (Maes et al. 2019). According to Maes et al. (2019), North Sea and Mediterranean Sea regions contributed more than other countries to ML research, and received more funds, while large gaps still exist in other areas and research related topics (e.g., 'Risk Assessment', 'Assessment Tools'). Most of the available information on ML and the risks it poses to the species is scattered widely across the literature (Soto-Navarro et al. 2021), and there is a pressing need to synthesise the information around concepts such as risk and vulnerability of species to ML.

Considering the relevance of FML and marine megafauna (i.e., cetaceans and sea turtles) as indicators to assess the risk deriving from ML pressure on marine ecosystems, this study was specifically focused to review the current scientific literature on spatial Risk Exposure Assessment (hereafter REA) related to the floating micro and macro ML in order to: (1) identify, at global level, the main geographic areas where risk exposure studies were conducted, (2) describe the typology of datasets currently available and the methodologies used for data collection on species and threats, (3) investigate the approaches applied to carry out REA, and (4) highlight the main research findings and areas of high exposure risk to prioritize mitigation measures in the Mediterranean Sea. Prior to these, a preliminary review on risk assessment terminology and framework approach was performed to contextualize the level and types of assessment methodologies reviewed (in 'Approach used'). The main objective of this review was to identify key information gaps on REA, in order to highlight areas and topics that require further research.

Methodology

Approach used

Risk is defined as '(exposure to) the possibility of loss, injury, or other adverse or unwelcome circumstance, a chance or situation involving such a possibility' (Oxford English Dictionary, OED 2005). Risk assessment is considered a technical supportive tool for decision making when faced with uncertainty (Suter 2016), and is mostly used in a variety of human-related sectors, such as engineering, medicine, wildfire management and environmental regulation (Guzik et al. 2020, Yu et al. 2020, Sakellariou et al. 2022). In the context of the environment, the terms 'environmental risk' and 'ecological risk' are often confused in the literature, the first being commonly used to describe risk to humans due to contaminants in the environment, but being also both generally used to refer to risks to nonhuman organisms, populations, and ecosystems (Suter 2016). In wildlife management, the conventional risk framework could provide a structured process to analyse the potential risks to wild species from human activities (Gormley et al. 2011). In the case of ML, for example, risk assessments can help identify priority mitigation actions by considering the complex sources, pathways, and consequences of ML on vulnerable species (i.e., cetaceans and sea turtles).

The process could be schematised in four main phases (Figure 1). The first phase is necessary to understand the source of the pressure, the pathways by which exposure might occur, and the receptors (vulnerable species). Considering that environmental threats can be spatially and temporally limited, the information about where (the spatial axis) and when (the temporal axis) vulnerable species may be exposed to the threat (Werner et al. 2016) must be well understood, identifying areas/time of exposure risk (Phase 2). Once a potential risk of exposure has been identified, the risk analysis focuses on the impact (how), investigating the probability of effects and the severity (nature and magnitude) of the impact from an action or a threat (Suter 2016) (Phase 3).

To evaluate the degree of impact, information is needed on how many individuals are affected (i.e., Specificity or "Potentially Affected Fraction" in Woods et al. 2019, Hoiberg et al. 2022) and on how severely these organisms are affected (i.e., Severity). Thus, in general, the risk of being impacted represents a combination of events, starting with the vulnerable organisms being exposed to the threat, and then eventually being affected by the threat at different levels, from movement restriction, ingestion, to injury or death (Gregory 2009). Exposure to threats does not always imply impact as the latter depends on the individual behaviour, the type of litter (e.g., Duncan et al. 2019, Bucci et al. 2020), and the nature of interactions between the two, so that only a fraction of all individuals potentially exposed to the threat may be affected.

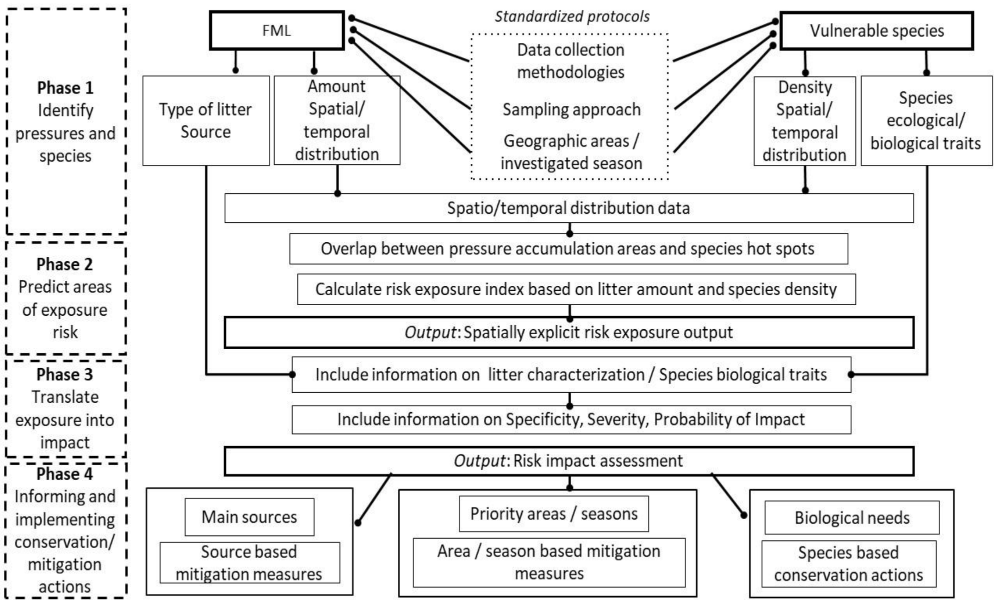

Fig. 1 – Risk assessment framework designed by the authors to analyse FML threat to marine vulnerable species, and support decision making processes (schematised and integrated based on the literature review).

The spatial risk exposure assessment focuses on the initial phase of potential risk (Phase 2) and does not require detailed information on sensitivity and specificity (Phase 3) that are difficult to collect at sea. The lack of these latter data would delay the assessment process hampering the possibility to intervene as soon as possible to mitigate the potential impact (Phase 4). Moreover, a standardised protocol for investigating the spatial risk exposure to marine wildlife from ML would provide a key technical support for risk assessment in line with the precautionary management approach. Consequently, the articles' search was focused on spatially-explicit risk exposure assessment (i.e., REA), excluding those studies not based on geospatial analysis, or based on toxicological risk (Table 1).

Literature review method

Review questions, criteria for inclusion

To formulate the search and to ensure an unbiased quality of the evidence synthesis, the following research questions and associated sub-questions were defined using the standard approach for research questions definition (i.e., Population, Exposure, Comparator, Output - PEC(O) framework, CEE 2022, Table 1):

- Considering studies conducted worldwide, which is the current state of the art for REA analyses evaluating the potential risk of exposure to FML for cetaceans or sea turtles at the global scale? In particular: 1) Which geographic regions have been studied so far? 2) What data sets on species and FML occurrences are currently available, and what are the methodologies used for data collection? 3) What are the main methodological approaches used for analysing data on species and threat, and to assess the exposure risk?
- At the local level for the Mediterranean Sea Region: 4) what are the main research findings and which areas have higher risk of exposure for cetaceans and sea turtles to FML?

We defined a common search strategy to answer all the questions. The key elements and the corresponding defined criteria are listed in Table 1. The definition of a priori criteria is necessary to ensure transparency and repeatability and to minimise bias (CEE 2022).

Table 1 - Eligibility criteria in relation to question key elements following the PEC(O) framework.

Question key elements	Eligibility criteria
Population (P): ● Cetacean and sea turtle occurrences	Included: All cetacean and sea turtle species occurring at the global scale and their at-sea spatial occurrences/abundances. Excluded: occurrences of nesting sites, bycatch and stranding events.
Exposure (E) ● Floating Marine Litter (FML)	Included: FML, irrespective of the object size, and their spatial occurrences/abundances. Excluded: beach and seafloor litter, ingested litter.
Comparators (C) ● Risk Exposure Assessment (REA) approaches	Included: spatially-explicit REA. Excluded: REA not based on geospatial analysis, or based on toxicological risk.

Literature search

We carried out an exhaustive systematic literature search using the SCOPUS and Web of Science databases. We curated specific search terms and synonyms according to the predefined research questions excluding terminology that was too broad (i.e., the use of the term “marine mammals" would have also included groups of species that were not the focus of our research). Therefore, the following search terms were used for each element of the PECO framework excepted for the "output" (O) parameter:

- Population: cetacean*, dolphin*, whale*, odontocet*, mysticet*, "sea turtle*", "marine turtle*" AND "marine", "ocean*", "sea".
- Exposure: macroplastic*, microplastic*, "plastic litter", "plastic debris", "marine litter", "marine debris", "anthropogenic litter", "ocean plastic*", "marine plastic*".
- Comparator: risk*, "risk assessment", "risk evaluation", "risk index", "risk exposure", threat*, impact*.

The terms within each category were combined with the Boolean operator "OR", while the categories were aggregated with "AND". The wildcard "*" was used to include singular/plural forms. The terms were entered for Abstract, Title and Keywords. No temporal and language restrictions were imposed. Each search was then refined by document types retaining primary literature (i.e., empirical and modelling studies) while choosing to exclude secondary literature (i.e., reviews, editorial material, early access) and grey literature (i.e., conference/proceeding papers) as the review was focused on relevant published, peer-reviewed research on the topic. The search was also refined by meso-topics, excluding those that were not related to marine science or ecology. A full list of search-related information (databases, URLs, subscribing institutions, search strings and filter options) can be found in Table S1 (Supplementary Materials). The search result was validated by testing it against a predetermined set of 10 relevant publications found in Google Scholar, resulting in a 100% sensitivity. The benchmarking set is provided in Table S2. The search was conducted in November 2022 resulting in a total of 550 articles. 135 duplicate articles in common between the two databases results were removed, leading to a total of 415 articles.

Screening

The screening process was conducted in two stages: Title & Abstract and Full Text Screening (Mangano & Sarà 2017, Mangano et al. 2017, CEE 2022). In the first stage, all articles were initially assessed by title and abstract, using the inclusion criteria listed in Table 1. To check for inter-rater reliability in the inclusion criteria, a subset of the Title & Abstract (10%) that emerged from the search was independently reviewed by two authors before the screening process began and the Cohen's Kappa test measured a perfect agreement (k=1, n=30). All full texts of the articles selected after this first step (n=45) were retrieved, read completely, and reviewed against the inclusion criteria (Figure 2). 22 full texts were excluded because they did not meet the specified criteria: Population (n=6), Exposure (n=6), Intervention (n=4) or because of the type of document (Reviews=6).

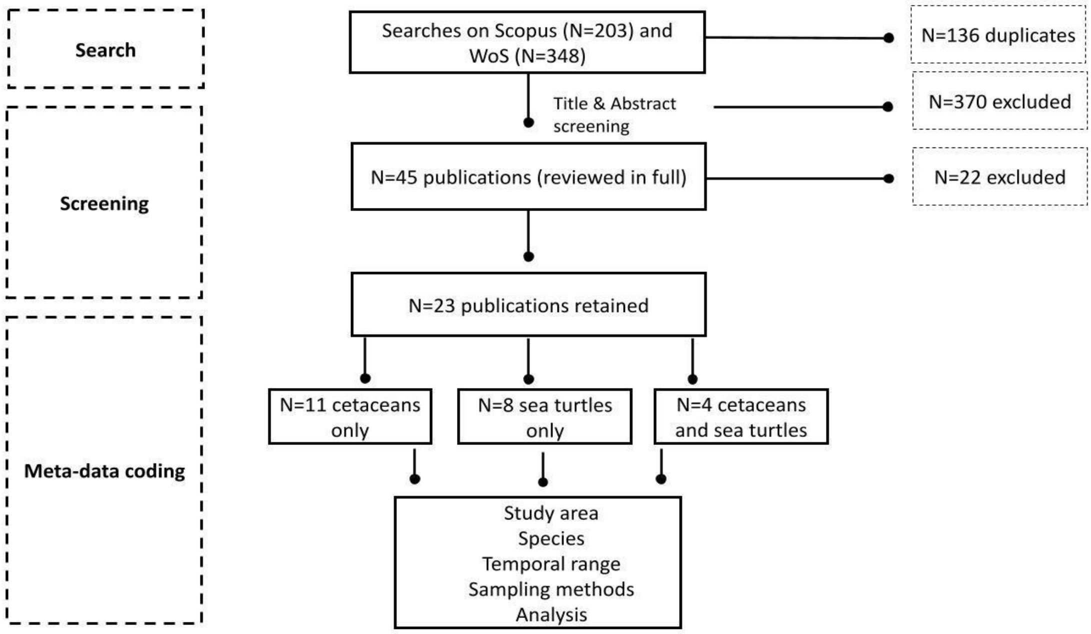

Fig. 2 - Flow chart outlining the systematic review process.

Meta-data extraction and coding

In order to systematically extract relevant descriptive information from the articles included in the evidence synthesis, a standard meta-data matrix was developed and agreed between the reviewers before the process of extraction began. Descriptive data were converted into a priori categories whenever possible. Studies were classified according to the following macro-categories: (1) Study (geographical) areas: indication of whether the study area is part of the Mediterranean Sea and the name of the Mediterranean sub-region (i.e., Western Mediterranean, Tyrrhenian & Sardinian-Sicilian Channels, Adriatic Sea, Central and Eastern Mediterranean, or overall Mediterranean Sea) or the non-Mediterranean area (i.e., Atlantic, Indian and Pacific Oceans or overall seas), (2) Species: cetaceans/sea turtles, indicating the name of all species studied, (3) Characteristics of FML: whether micro (<0.5 cm), meso (0.5-2.5 cm) or macro (>2.5 cm), (4) Sampling methods for data extraction/collection on species and FML and temporal range of data, (5) REA methodology: methods for species and FML data preparation (descriptive synthesis of analysis performed and inclusion in the macro-categories of occurrence points, gridded distribution, spatial generalisation of distribution, hotspot analysis, spatial distribution modelling), overlapping methods, presence/absence of a risk exposure index and its formula, whether or not the assessment considered multiple species, the temporal resolution of the REA (weekly, monthly, seasonal, annual), the number of seasons considered (1-4) and the temporal range, (6) Main results: indication of whether a risk exposure map, including seasonal maps, or no maps were produced and a description of the areas of potential high risk of exposure to FML evidenced by the 23 selected studies.

Narrative data synthesis

Data were narratively synthesised in four sections discussing key characteristics: the areas investigated; the type of datasets used on species and FML, and the methodology for the data collection; the methodology used for data analysis on species and FML and to perform the REA; the main findings from REA studies in the Mediterranean Sea. Given the limited number of resulting studies and the heterogeneous nature of the results, no statistical analysis was conducted. We provided tables and graphs to summarise data on included articles to support the narrative synthesis. As for the Mediterranean studies, areas at high risk of exposure to FML were grouped and presented in two maps, one for cetaceans and one for sea turtles. The areas were derived from the discussion sections of the 13 selected papers and from the risk exposure maps, when provided. As the REA approaches differed among studies, the synthesis maps should be considered qualitative descriptions only.

Results

The searches returned a total of 415 articles. Following the steps of inclusion/exclusion (outlined above) only 23 articles were retained and included in this review (Figure 3: 11 on cetaceans-only, 8 on sea turtles-only, and 4 articles on both cetaceans and sea turtles). The full list of retained articles is provided in Table S3. Analysing these 23 articles, it emerged that the risk exposure topic was considered only recently, with the first study being published in 2011 focusing on cetaceans (Williams et al. 2011), followed by another about sea turtles in 2013 (Wilcox et al. 2013, Figure 3); since 2016 risk assessment has been a constantly studied topic, with the highest number of papers published in 2022.

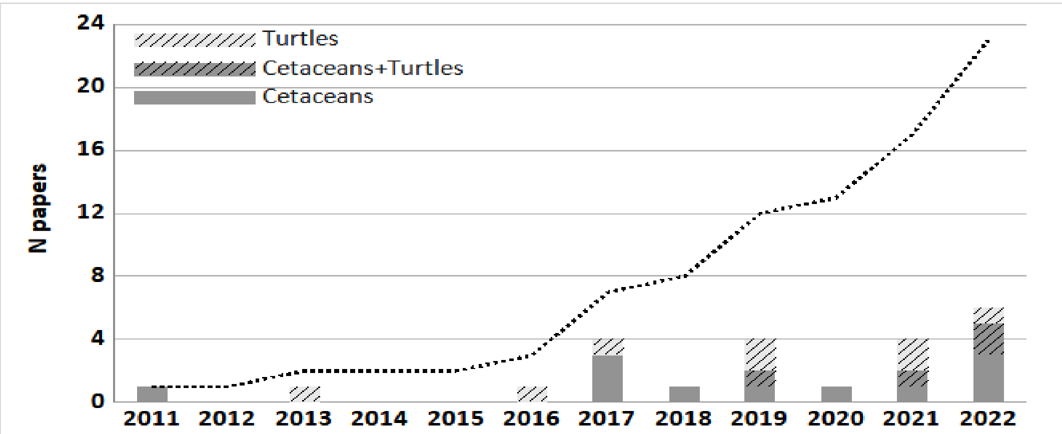

Fig. 3 - Temporal trend of papers about risk assessment used in this study (n=23), differentiated by species category. Dotted line represents the cumulative trend.

Geographic areas investigated

Most of the studies were conducted in the Mediterranean Sea (n=13, 57%) and Pacific Ocean (n=6, 26%), spanning Canada, California, Hawaii, Galapagos, China and Australia; two studies were from the Atlantic (Darmon et al. 2017, Sá et al. 2021) and only one from the Indian Ocean (Wilcox et al. 2013, Figure 4). Only two studies were carried out at a global scale (Schuyler et al. 2016, Hoiberg et al. 2022). In the Mediterranean Sea, the western sectors were the most studied (n=12, 92%), including the Pelagos Sanctuary, the Tyrrhenian Sea and the Sardinian-Sicilian Channels (Figure 4). Two studies were conducted in the Adriatic-Ionian (ADRION) sub-region (Arcangeli et al. 2019, Galli et al. 2022), and three considered the whole Mediterranean basin (Compa et al. 2019, Soto-Navarro et al. 2021, Almpanidou et al. 2022).

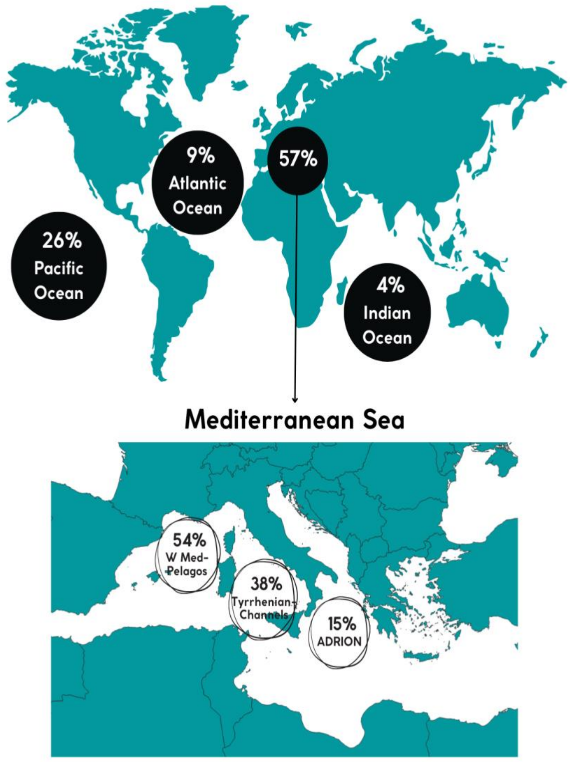

Fig. 4 – Global distribution of studies (upper panel) with a focus for the different regions of the Mediterranean Sea (bottom panel).

Datasets and methodology for data collection

Fin whales (*Balaenoptera physalus)*, bottlenose dolphins (*Tursiops truncatus)*, striped dolphins (*Stenella coeruleoalba)* and sperm whales (*Physeter macrocephalus*) were the most studied cetaceans among the 22 cetacean species investigated for REA, while loggerheads (*Caretta caretta)* were the most studied among the sea turtle species (Table S4). The use of data on multiple species was the most common approach (n=16), while 30% of the analysed articles focused on a single species.

Datasets on FML were mostly based on the macrolitter (>2.5 cm) with 15 papers (65%) examining only this category and another including the mesolitter (0.5-2.5 cm, Hoiberg et al. 2022). Two studies considered macro and micro-litter (Critchell et al. 2019, Zhang et al. 2020), while four (17%) examined only micro-litter data (<0.5 cm). Only one study analysed all size categories (i.e., Fossi et al. 2017).

More than 60% of risk exposure assessments used field data collected at sea on species (n=17) and litter (n=14): the most common methodology was performing visual observations from boat surveys (n=12, 52% for species; n=10, 43% for litter) and in one case, all data were collected from aerial surveys (Darmon et al. 2017). In three papers (13%), field data from telemetry were used to investigate species distribution, while five articles (22%) used data on the species retrieved from available collections or repositories (e.g., OBIS, Aquamaps). In four studies (17%) data on micro-litter were collected by manta samplings. Other studies based the risk assessment on data obtained from niche or distribution models for species (n=3, 13%) and hydrodynamic dispersion models for litter (n=8, 35%); four papers (17%) combined field data for the species coupled with a modelled distribution of litter.

Data were collected over different temporal ranges, from 10 days to 20 years. Seven publications (30%) considered all four seasons using data collected from one (Zhang et al. 2020) to seven years (Gregorietti et al. 2021); six studies (26%) were carried out during the summer season only, ranging from few days of surveys (Fossi et al. 2017, Galli et al. 2022) to nine years averaged (Guerrini et al. 2019). Three studies (13%) considered opposite seasons (autumn/winter and spring/summer, Darmon et al. 2017, Critchell et al. 2019, Campana et al. 2022), and one study used data collected during one month in spring (Jones et al. 2021). The remaining articles used data gathered in different periods or did not specify the temporal resolution.

Approaches for REA

Methods used to analyse data on cetacean and sea turtle species and floating litter

The reviewed articles reported on a variety of methods for analysing spatial data on species and FML, the results of which are then used as data inputs for REA. For the purposes of this study, the methods were grouped into five general categories of increasing complexity, ranging from simple visualisation of “occurrence points”, through “gridded distribution” and “spatial generalisation of distribution”, to “hotspot analysis” or advanced “spatial distribution modelling”. The first three are mutually inclusive, as the simple occurrence points (i.e., visualisation of georeferenced sighting points) can be used as input data to obtain quantitative information on gridded distribution (i.e., density/abundance of species/FML in grid cells), which in turn can be used to spatially generalise the distribution through smoothing factors (i.e., geometric features representing the area/s where the species/FML occur at different densities). For these first three categories, the methods were counted according to the higher degree of complexity. Further on, hotspot analysis can be performed on geometric features (points, lines or polygons) to infer the significance of hotspots (i.e., spatial clusters of high values), or spatial distribution modelling (SDM hereafter) can be applied to occurrence/density data of species/FML to infer distribution in unsurveyed areas. For the methods of the species distribution only, a sixth method category was included: "integrated multispecies analysis", which considers the cumulative occurrence of more than one cetacean/sea turtle species or biodiversity indices (e.g., species richness) as the starting data for REA.

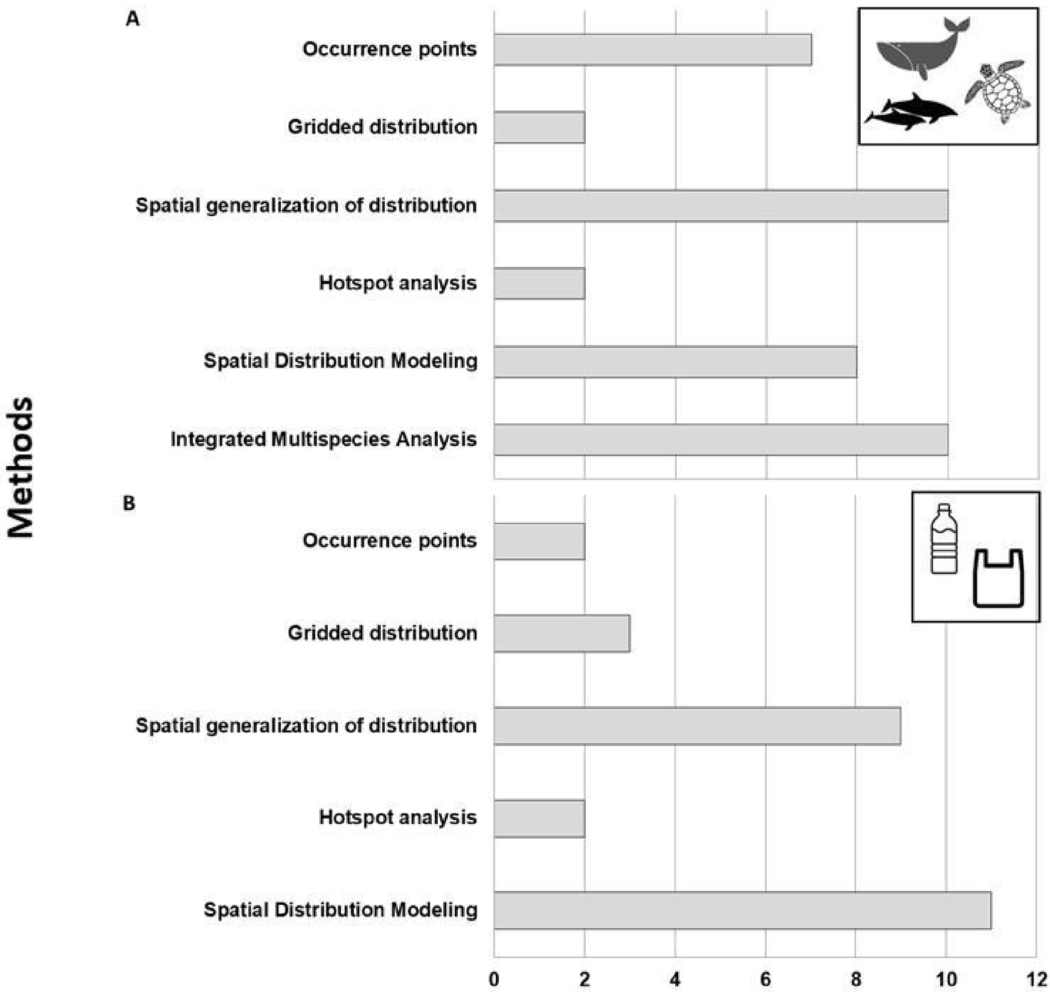

Fig. 5 – Methods used to analyse distribution data on cetacean and sea turtle species (A) and floating litter (B) prior to performing the REA; x-values refer to the number of articles.

Most of the studies used the same approach either for the analysis on species and on FML (i.e., occurrence points 4.3%, gridded distribution 8.7%, spatial generalisation 30.4%, hotspot analysis 8.7%, SDM 21.7%) while 35% of studies used different approaches on species and FML.

In particular, for the analysis of cetacean and sea turtle species distribution (Figure 5A, Table 2), seven studies (30%) used the occurrence points from visual sightings or telemetry data, while gridded distribution of species density was used in two studies (Wilcox et al. 2013, Currie et al. 2017). On the other hand, spatial generalisation of distribution was the predominant method used to determine the core area of the species distribution (n=10, 43%): within this category, eight studies used the Kernel density estimation technique based on species occurrence or abundance, while two studies used the area included in the Regional Management Units (RMU) as representative of higher sea turtle occurrences (Schuyler et al. 2016, Hoiberg et al. 2022). Hotspot analysis was conducted in two studies, in particular using the G* analysis (Getis & Ord 1992) to highlight the statistical significance of the different hotspots (Arcangeli et al. 2019, Gregorietti et al. 2021). SDM approaches were applied in eight studies (35%): for example, Generalized Additive Models (GAM) and density surface modelling were used to define areas of high presence of the target species, or the Feeding

Habitat Occurrence (FHO) model was applied to trace the potential fin whale feeding areas (Fossi et al. 2017, Guerrini et al. 2019).

For FML spatial analysis (Figure 5B, Table 2), only two studies used the occurrence locations of FML as data input for the REA (Jones et al. 2021, Kahane-Rapport et al. 2022). Three studies calculated the gridded distribution of FML density instead (13%), while nine articles (39%) used a spatial generalisation of the density distribution, smoothed through the Kernel function to define areas of higher pressure of FML. Two studies performed the hotspot analysis (i.e., G* analysis) to highlight the statistical significance of hotspots (Arcangeli et al. 2019, Gregorietti et al. 2021). Nevertheless, the majority of the studies (n=11, 48%) applied SDM approaches to define areas of high FML densities, mainly Lagrangian modelling.

Methods used for REA

The main principle in spatial REA is the identification of the areas of potential risk exposure for vulnerable species, by understanding the source of the pressure and the pathways by which exposure might occur. Two general approaches are commonly used for spatial REA: 1) the overlap of areas with a high density of both species and FML to identify areas with higher exposure; 2) quantification of REA through indices, calculated directly on spatially gridded data by combining species and FML occurrences/densities. Of the reviewed articles, four investigated the risk exposure by using the spatial overlap of hotspots of megafauna species and FML defining the percentage of seasonal overlap (Di-Méglio & Campana 2017, Campana et al. 2018) and describing it qualitatively (Fossi et al. 2017) or by assessing the proportion of the species' home range in areas of high ML densities (Critchell et al. 2019). The majority of the studies (n=19, 78%) estimated instead the extent of risk exposure using the risk index-based method. Ten studies aggregated the spatially gridded densities of FML and the vulnerable species (Williams et al. 2011, Schuyler et al. 2016, Currie et al. 2017, Arcangeli et al. 2019, Compa et al. 2019, Zhang et al. 2020, Atzori et al. 2021, Gregorietti et al. 2021, Sá et al. 2021) or their suitable habitat (Guerrini et al. 2019). Other studies integrated the risk index with other variables, such as species vulnerability, biological characteristics, or conservation status, to measure the degree of the impact (i.e., Specificity, Severity, Table 2). As for Specificity, Jones et al. (2021) considered a conservation value based on IUCN categories to calculate risk indices on sea turtles, one study calculated sensitivity values considering the presence of different marine species (Galli et al. 2022), and similarly another study considered a sensitivity index of cetacean species including biological features such as life stage (Campana et al. 2022). Other studies, calculated specific risk indices including ingestion probability (Compa et al. 2019, Soto-Navarro et al. 2021, Almpanidou et al. 2022, Kahane-Rapport et al. 2022) and turtles/cetaceans entanglement (Wilcox et al. 2013, Darmon et al. 2017, Hoiberg et al. 2022).

Table 2 – Summary of methodological approaches for data collection, methods used to analyse data on vulnerable species and floating marine litter (FML) and for Risk Exposure Assessment (REA).

#	Authors	Type of data collection		Methodological approaches for analysis		
		Species	FML	Species	FML	REA
1	Almpanidou et al. 2022	Telemetry	No data collection (from existing model)	SDM (ensemble modeling)	SDM (Lagrangian modeling)	• Threat risk x cell in turtle foraging grounds • Map
2	Arcangeli et al. 2019	Visual monitoring	Visual monitoring	Spatial generalisation of distribution; Hotspot analysis	Spatial generalisation of distribution; Hotspot analysis	• Aggregated raster of species and litter densities • Map
3	Atzori et al. 2021	Visual monitoring	Visual monitoring	Spatial generalisation of distribution; SDM (GAM)	Spatial generalisation of distribution	• Risk x cell (litter density x species abundance) • Map
4	Campana et al. 2018	Visual monitoring	Visual monitoring	Occurrence points	Spatial generalisation of distribution	• Overlap of species sightings and plastic density isopleth • Map
5	Campana et al. 2022	Visual monitoring	Visual monitoring	SDM (GAM); Integrated Multispecies Analysis	Spatial generalisation of distribution	• Biological traits • Sensitivity index x cell (litter density x species sensitivity) • Map
6	Compa et al. 2019	No data collection (databases)	No data collection (from existing model)	Occurrence points, Integrated Multispecies Analysis	SDM	• Litter density x binary/weighted species distribution • Biological traits • Ingestion probability estimation with GAM • Map

7	Critchell et al. 2019	Telemetry	No data collection (from existing model)	Occurrence points	SDM (Lagrangian modeling)	● Overlap of homerange % in the relative exposure categories (litter density)
8	Currie et al. 2017	Visual monitoring	Visual monitoring	Gridded distribution	Gridded distribution	● Risk x cell (litter density x species density) ● Map
9	Darmon et al. 2017	Visual monitoring	Visual monitoring	Spatial generalisation of distribution	Spatial generalisation of distribution	● Linear distance between turtles and litter ● Frequency of turtles surrounded by litter ● Mean number of surrounding litter x turtle occurrence for each distance class
10	Di-Meglio & Campana 2017	Visual monitoring	Visual monitoring	Spatial generalisation of distribution	Spatial generalisation of distribution	● Overlap of species sightings and plastic density isopleth ● Map
11	Fossi et al. 2017	Visual monitoring	Visual monitoring	SDM (FHO)	SDM (Lagrangian modeling)	● Qualitative overlap
12	Galli et al. 2022	Visual monitoring	Manta sampling	Spatial generalisation of distribution; Integrated Multispecies Analysis	Gridded distribution	● Risk index x cell (species sensitive scores x litter density) ● Map
13	Gregorietti et al. 2021	Visual monitoring	Visual monitoring	Spatial generalisation of distribution; Hotspot analysis; SDM (biomod)	Spatial generalisation of distribution; Hotspot analysis	● Risk index x cell (litter density x species abundance) ● Map

14	Guerrini et al. 2019	No data collection (databases)	No data collection (from existing model)	SDM (FHO)	SDM (Lagrangian modelling)	● Risk index x cell (litter density x species habitat) ● Map
15	Hoiberg et al. 2022	No data collection (databases)	No data collection (from existing model)	Spatial generalisation of distribution; Integrated Multispecies Analysis	SDM (Oceanographic dispersal modelling)	● Species sensitivity distribution (litter density x potential entanglement exposure areas) ● Potentially Affected Fraction of species (PAF) ● Map
16	Jones et al. 2021	No data collection (databases)	Manta sampling	Occurrence points; Integrated Multispecies Analysis	Occurrence points	● Biological traits ● Species distribution x Conservation scores x Severity
17	Kahane-Rapport et al. 2022	Telemetry	Manta sampling	Occurrence points	SDM	● Modelled litter ingestion rate
18	Sá et al. 2021	Visual monitoring	Visual monitoring	Spatial generalisation of distribution; Integrated Multispecies Analysis	Spatial generalisation of distribution	● Risk index x cell (litter density x species density) ● Map
19	Schuyler et al. 2016	No data collection (databases)	No data collection (drifting buoys)	Spatial generalisation of distribution; Integrated Multispecies Analysis	SDM (Lagrangian modeling)	● Risk index x cell (litter density in species RMU ● Map
20	Soto-Navarro et al. 2021	No data collection (databases)	No data collection (from existing model)	SDM; Integrated Multispecies Analysis	SDM (Lagrangian modeling)	● Risk index x cell (averaged litter density, vulnerability and species distribution) ● Map

21	Wilcox et al. 2013	Visual monitoring	No data collection (model of beached ghost nets)	Gridded distribution; Integrated Multispecies Analysis	SDM	● Risk index x cell (relative turtle density (caught) x ghost fishing effort) ● Map
22	Williams et al. 2011	Visual monitoring	Visual monitoring	Spatial generalisation of distribution; Integrated Multispecies Analysis	SDM (GAM)	● Risk index x cell (litter density x species density) ● Map
23	Zhang et al. 2020	Visual monitoring	Visual monitoring	Spatial generalisation of distribution	Spatial generalisation of distribution	● Risk index x cell (litter density x species density x coefficients) ● Map

Main findings from spatio-temporal risk exposure studies in the Mediterranean

The findings from the Mediterranean REA studies resulting from the literature search (n=13) were heterogeneous in terms of species, temporal resolution and provided maps. Areas reported as at higher-risk of exposure to FML were summarised and reported separately for cetaceans, sea turtles and marine biodiversity in general.

Cetaceans

The investigations carried out in the western Mediterranean basin revealed the main risk exposure areas during summer in the Liguro-Provençal basin, including the Pelagos Sanctuary (Figure 6). Di-Méglio and Campana (2017) highlighted monthly changes in risk exposure areas for cetacean species driven by a dynamic pattern of FML densities, with minimum values in the middle of the summer compared to other months (from May to September). FML resulted in overlapping by 50% with the known ranges for six cetacean species (fin, sperm and pilot whales, and Risso's, bottlenose and striped dolphins), indicating high potential of interaction, especially in the western part of the Pelagos Sanctuary. Fossi et al. (2017) showed a possible overlap of fin whale summer feeding habitats and areas of high microplastic concentration, which would increase the risk of ingestion, in the external part of both cyclonic and anticyclonic structures such as the Capraia gyre. As well, Guerrini et al. (2019) investigated fin whales' risk of exposure to microplastics via food ingestion during the summer season and found spatial and interannual variability of risk exposure patterns, with the highest values in the Central Ligurian Sea and along the Ligurian and Western Corsica coasts. At mid latitudes in the western Mediterranean Sea, Campana et al. (2018) provided seasonal data for fin whales, dolphins and squid eaters (sperm, Cuvier's beaked and pilot whales, and Risso's dolphins), showing the most evident overlaps between high density of FML and all cetacean species in the

Balearic Sea in all seasons, as also identified by Compa et al. (2019). During spring and summer, risk exposure areas for fin, sperm whales and dolphins were found in the Sardinian Sea and the coastal waters of the Bonifacio Strait (Campana et al. 2018, 2022). In the Bonifacio Strait a generally higher risk was also identified particularly for fin whales and striped dolphins in autumn-winter, due to the offshore accumulation of FML (Campana et al. 2022). In the southern Mediterranean basin, several areas have been identified at particular risk during all the seasons, as the African coast, the western central Med (Compa et al. 2019), the Sicilian Channel, where Gregorietti et al. (2021) identified the waters outside Palermo harbour along the coast to the Castellammare Gulf, the Egadi Islands and the Tunis Gulf as areas of risk exposure to plastic for striped and bottlenose dolphins throughout the year, albeit with seasonal variation of intensity. Other areas of exposure of cetacean species to FML have been described in the central Adriatic Sea and the Aegean Sea (Compa et al. 2019), while a specific area in the Ionian Sea has been highlighted during summer by Galli et al. (2022).

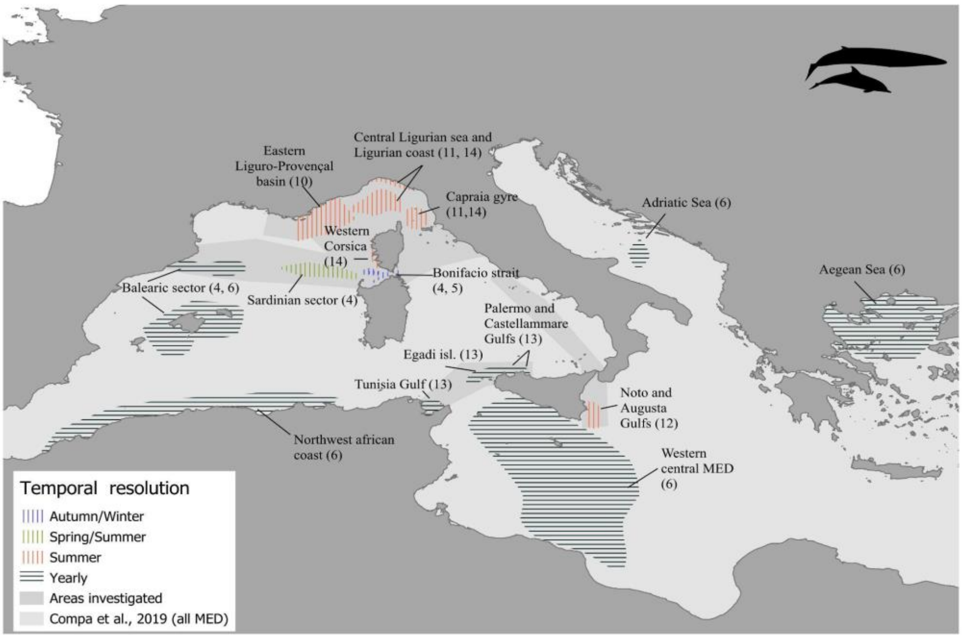

Fig. 6 – Synthesis map of annual/seasonal areas of higher risk of exposure to FML for different species of cetaceans emerged from the studies analysed (vertical lines coloured by seasonal resolution and black horizontal lines for yearly resolution); the numbers in brackets correspond to the studies to which each area refers, based on the numeration used in Table 2. For reference on the season and species investigated by the different studies, refer to the text. The areas investigated by the reviewed studies are indicated in grey in the figure; only Compa et al. 2019 (study N. 6) investigated the whole Mediterranean basin (light grey in figure).

Sea turtles

One of the major area of potential exposure risk to FML for sea turtles is reported by Almpanidou et al. (2022) in the foraging area enclosed within the Tunisia Plateau/Gulf of Sidra, with about 40% of its extent being characterised by medium up to very high levels of exposure (Figure 7). Seasonal differences have been highlighted in other parts of the basin, with larger risk areas detected during summer in the Sardinian-Balearic Sea (Darmon et al. 2017, Arcangeli et al. 2019) and Sardinian Channel (Arcangeli et al. 2019, Atzori et al. 2021) due to the increased density of FML and higher abundance of turtles (Figure 7). These authors in fact, investigating all seasons, suggested that species-specific foraging strategies, biological cycle, and oceanic features may condition the passive transport of debris, and to a degree sea turtles, explaining spatio-temporal variations in sensitive areas (Darmon et al. 2017, Arcangeli et al. 2019). In the middle of the Sardinian Channel, for example, the large South-Eastern Gyre appears to act as a trap or convergence area for both animals and FML (Atzori et al. 2021). During winter, Darmon et al. (2017) identified two major overlaps between areas of FML concentration and loggerhead turtles in the North of Corsica and between Sardinia and Balearic Islands, while Arcangeli et al. (2019) highlighted a high-risk exposure area for sea turtles in northern Corsica during spring. That study also confirmed the well-known area for loggerhead sea turtles in the Adriatic Sea, where high risk of exposure was recognised during spring as well. Finally, a localised area for microlitter exposure was described in the Ionian Sea during summer by Galli et al. (2022).

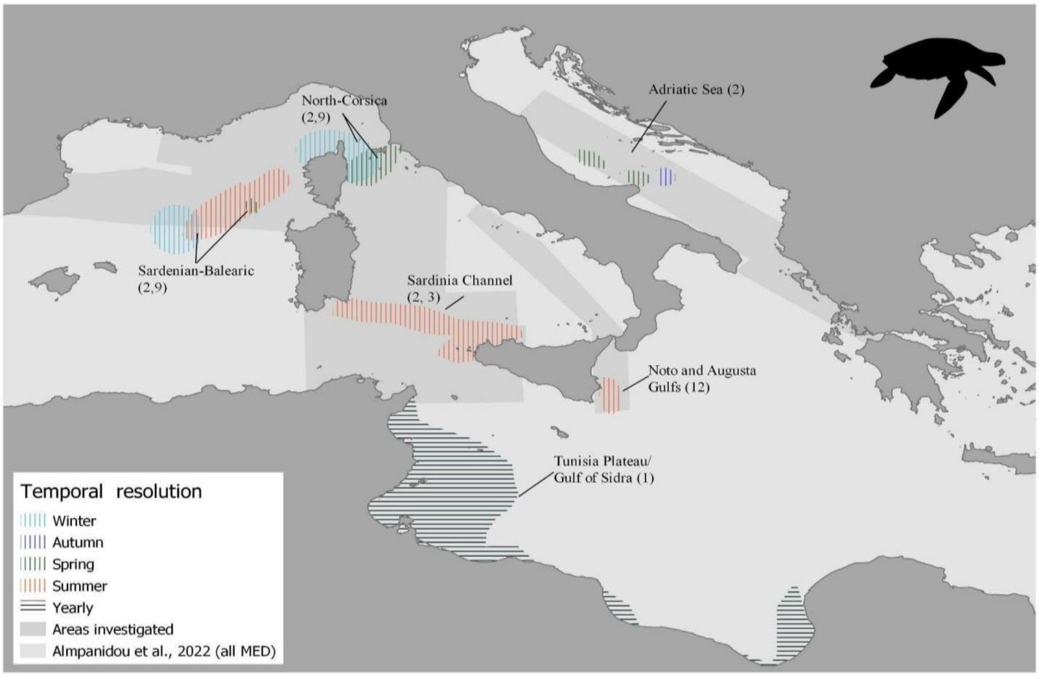

Fig. 7 – Synthesis map of annual/seasonal areas of higher risk of exposure to FML for sea turtles emerging from the analysed articles (vertical lines coloured by seasonal resolution and black horizontal lines for yearly resolution). The numbers in brackets correspond to the studies to which each area refers, based on the numeration used in Table 2. For reference on the season investigated by the different studies, refer to the text. The areas investigated by the reviewed studies are indicated in grey in the figure; only Almpanidou et al. 2022 (study N. 1) investigated the whole Mediterranean basin (light grey in figure).

Discussion

Despite the relevance of the topic, and the legislative requirements, this review revealed that using risk assessment to analyse the spatial risk exposure produced by FML on highly vulnerable cetaceans and sea turtles has only been considered as recently as 2011 and by a limited number of studies (23 articles). Among the global, EU and Regional legislative frameworks that address the threat posed by the anthropogenic pressures such as marine litter on marine ecosystems (e.g., CBD, Habitats Directive, MSFD, MSPD, UNEP-MAP Barcelona Convention), since 2008 the EU MSFD specifically requires member states to assess the risk for marine species by the ML threat, connecting the Descriptor 1 (i.e., Biodiversity) to the Descriptor 10 (i.e., Marine Litter) likely giving an effective impulse to the REA studies. However, despite the increase of recent research efforts, it appears that a large gap still remains in the literature dedicated to identifying critical areas for species, hampering the possibility to identify priorities for conservation, and successively effectiveness of mitigation measures.

By reviewing the available papers on spatial REA this study offered a first insight into the available methodological approaches that focus on the initial phases of potential risk assessment (Fig. 1) not requiring detailed information on true impact (Phase 3) that could be difficult to collect at sea. The information collected will potentially contribute to the standardisation of an approach in line with the precautionary principle.

Main studied areas

Most of the risk assessment studies were concentrated in European countries with few case studies carried out in other continents (Northern Australia, China, Hawaii, Canada). This could be due to the implementation of the EU MSFD that, since 2008, gives a direct input for integrating the effect of pressures in the assessment of the status of marine waters, and thus identifying mitigation measures according to the information gathered. Within the EU, results confirmed one of the findings of Maes et al. (2019) with most of the studies concentrated in the Mediterranean Sea, and only two in the Atlantic Ocean. Our review also showed that, within the Mediterranean basin, the western Mediterranean Sea was the most investigated area accounting for a higher number of studied species, likely due to the greater research effort in this subregion especially on cetaceans (e.g., Gnone et al. 2023).

Datasets

This review revealed that only a limited number of studies were able to use empirical data collected at sea on species and ML, and almost 40% of reviewed articles based the assessment on modelling data, especially for larger scale assessments. Most studies focused on common cetacean (i.e., fin whale, bottlenose, and striped dolphins) and sea turtle species (i.e., loggerhead turtle), reflecting the relatively larger availability of data, while little is dedicated to the other highly vulnerable cetaceans and sea turtle species. The limited availability of continuous data on abundance and distribution of species and on the amount, distribution, and composition of ML in representative geographical areas on a seasonal basis is an impediment for exhaustive REA, and likely the main causes for the limited number of articles addressing this topic. For FML in particular, the variability in FML occurrence within empirical data, leads to a reliance especially on modelled approaches. However, at a global level, it could also be the case that, despite several empirical or modelled datasets available for either species or for FML, they have not yet been used for assessing exposure risk (REA) (e.g., Miranda-Urbina et al. 2015). At EU, the EEA Report (2020) recognises that the ranges, population sizes and suitable habitat areas of cetacean species (apart from two Annex II species), remain unknown in most Member States, which is generally due to the lack of appropriate monitoring programmes. The review of reports produced by Member States on the MSFD Descriptor 1 (i.e., Biodiversity, Palialexis & Boschetti 2021) and Descriptor 10 (i.e., Marine Litter, Ruiz-Orejón et al. 2021) also recognised the lack of data on both, species and ML, and on the overlap between spatial distribution of human activities/pressures and species distribution.

Some large-scale synoptic visual data collection programs for FML and vulnerable species (e.g., cetaceans, sea turtles) exist in the Mediterranean basin (i.e., IMAP, UNEP-MAP Integrated Monitoring and Assessment Programme; ASI, Accobams Survey Initiative; FLT, Fixed Line Transect Mediterranean Monitoring Network). These make use of standardised protocols for data collection on cetaceans, marine turtles and/or on litter (e.g., Arcangeli et al. 2020), but are not yet comprehensive temporally or spatially, or in terms of the species considered. Telemetry is generally applied on sea turtles, and was indeed used by three of the investigated articles to infer the spatial pattern of the vulnerable species. Technological advances in satellite telemetry facilitated the increasing research effort and the identification of key neritic and oceanic areas, as for the example of loggerhead turtle in the Mediterranean Sea (Pasanisi et al. 2022). Telemetry could also provide useful information on demographic parameters (Girard et al. 2022) and biological traits for evaluating the probability of impact. Thus, despite some existing initiatives or techniques already available, there is still a large lack of coordination and integration. Indeed, the integration of existing datasets and initiatives that ensure continuous standardised data are collected on species and threats, should be a priority to guarantee that a solid baseline of information exists, allowing long-term assessment of the effectiveness of mitigation measures.

Approach for risk exposure assessment

This review confirmed, in line with Soto-Navarro et al. (2021), that methodologies for assessing spatial risk exposure vary according to the data available on the species and ML, their resolution and the study's specific scope. Generally, they make use of modelled or empirical data collected at sea on ML and sensitive species to assess the degree of overlap between the areas of species distribution and high pressure from ML. The choice of the investigated species is crucial for assessing the risk posed by marine litter on marine biodiversity and ecosystems. Most articles examined focused on a single taxonomic group, either sea turtles or cetaceans, and 30% examined a single species. The selection of species is likely mostly driven by the availability of data. Few articles calculated the risk of exposure across multiple taxa including polychaetes, crustaceans, gastropods, cephalopods, bivalves, fishes, sharks, other reptiles, pinnipeds and sea birds in addition to cetaceans and sea turtles, making use of large-scale data repositories trying to represent marine biodiversity in general (Compa et al. 2019, Soto-Navarro et al. 2021, Galli et al. 2022). Some nature and biodiversity regulatory frameworks (e.g., Habitats Directive, HD 92/43/EEC) are mostly species-focused and centred on the conservation of single threatened cetaceans and sea turtles, requiring the identification of specific priority needs. Instead, other more global or more recent legislation (e.g., CBD, MSFD, MSPD, UNEP-MAP Barcelona Regional Sea Convention) uses the ecosystem approach, which considers species to be functional components of the local biodiversity, driving ecosystem functions and processes, and the conservation effort is focused on preserving balanced and functioning ecological communities (Hartje et al. 2003, Shepherd 2004).

Cetaceans and sea turtles are generally considered valuable umbrella species (UNEP-MAP RAC/SPA 2010, Pace et al. 2015). They are good indicators of ecological processes, as their protection has been shown to have positive effects on community structure and ecosystem functioning (e.g., Pace et al. 2015, Pennino et al. 2017). They are among the species identified as target indicators for monitoring the impacts of ML (e.g., Fossi et al. 2017, 2020, Matiddi et al. 2017). The accurate selection of multiple species sensitive to the pressure but representative of different trophic guilds and different potential interaction with the threats could support scaling up the REA from the single species to the ecosystem level approach. To this end, a framework for a consistent and transparent selection of marine environment components (species) according to a set of scientific and practical criteria was recently suggested to identify the most representative species to be selected for REA (Palialexis et al. 2022).

Investigating the spatial overlap between hotspots of megafauna species and FML is the first step to identify areas of higher risk exposure. Few articles limited the analysis of risk exposure at this stage, or just qualitatively describing it, while most of the reviewed articles attempted to quantitatively estimate the magnitude of the exposure risk (78%). Moving from a qualitative approach towards more quantitative assessments of risk is surely required to better focus on the higher-risk contexts. The examined literature revealed that some progress is being made to quantify exposure intensity, but methodological approaches are still highly heterogeneous. Most of the studies used spatially-explicit risk indices over grid cells, quantifying the magnitude of risk exposure by multiplying density of litter and species. Some examples are also available of studies that accounted for biological features, which increase species vulnerability, such as integrating species sensitivity scores based on biological (e.g., Campana et al. 2022) or functional (e.g., Jones et al. 2021) traits. The inclusion of vulnerability and trait analyses (e.g., integrating information on life history, morphological and behavioural characteristics of species present in assemblages to indicate aspects of their ecological functioning) into the conservation frameworks would enable predictions to be made regarding organism responses to future environmental changes and allows broader conservation actions to be selected (Miatta et al. 2021). Ideally, the exposure risk index equation should include data on amounts of litter and species density, and parameters that account for species vulnerability (e.g., biological trait, species richness, presence of juvenile, etc.) and information on litter characterisation correlated with the probability of impact (e.g., type, size, material, shape, etc.).

Finally, the integration of multiple pressures for REA analysis is required specifically by the MSFD and MSPD, to account for their combined effects on marine species and habitats. Recently, in addition to North and Baltic Sea, also the western Mediterranean Sea and the Adriatic Sea were recognised among the areas with the most extensive combined pressure effects within the EU marine areas, being under severe anthropogenic pressure from pollution, habitat loss and increasing disturbance due to intensive fishery and coastal activities (Korpinen et al. 2019). A framework for recognising critical areas that integrates spatio-temporal information on sensitive species and multi-sectoral sources of potential impacts is still lacking (Maes et al. 2019). The MSP Directive is designed to manage multiple uses of the seas, ensuring efficiency and sustainability of human activities. Even if not directly targeting ML, the MSPD aims to sustainably manage marine activities recognised as potential sources of ML, both from land-based activities (coast-land interaction) or sea-based (e.g., aquaculture, fishing, maritime transport, tourism). The MSPD aims to implement a framework for consistent and evidence-based decision-making. As such, it could contribute to the identification of the spatial and temporal distribution of relevant existing and future activities, and to the sustainable development of these sectors to ensure the preservation of species and the protection of the environment.

Main research findings in the Mediterranean Sea

The available studies in the Mediterranean Sea were heterogeneous in terms of area, species studied, maps provided and temporal resolution. Nevertheless, findings were in most cases consistent across studies, with some high-risk exposure areas emerging from different authors, such as the Balearic Sea (Campana et al. 2018, Compa et al. 2019) and Pelagos Sanctuary (Fossi et al. 2017, Guerrini et al. 2019, Campana et al. 2022) for cetaceans, and the Sardinian-Balearic Sea, north Corsica (Darmon et al. 2017, Arcangeli et al. 2019) and the Sardinian Channel (Arcangeli et al. 2019, Atzori et al. 2021) for sea turtles. High spatio-temporal variability of FML exposure was found in all studies, either basin-wide (Arcangeli et al. 2019, Compa et al. 2019, Soto-Navarro et al. 2021, Almpanidou et al. 2022) or at regional or local scales (e.g., Di-Méglio & Campana 2017, Campana et al. 2018, Guerrini et al. 2019, Atzori et al. 2021, Galli et al. 2022), regardless of the species considered. These findings are in line with the known variability in the distribution of either the vulnerable species and the pressure. High seasonal and inter-annual abundance and dispersal variability is indeed known for cetaceans and sea turtle species (e.g., Arcangeli et al. 2017, Arcangeli et al. 2019, Zampollo et al.

2022). Also the accumulation/dispersion pattern of litter is highly variable in the Mediterranean basin, with a large transboundary dispersion and the absence of any formation of permanent large or local FML accumulations (i.e., Arcangeli et al. 2018, Liubartseva et al. 2018, Mansui et al. 2015, 2020, Macias et al. 2022). Marked seasonal and inter-annual variability with areas of FML accumulation, were recognised mainly during summer/autumn, but with lower values indicating the gradual formation/disintegration of accumulation pattern also seen in June, October, and November (Liubartseva et al. 2018). A spatial stratification for potential exposure was also identified between offshore and coastal areas (Compa et al. 2019, Arcangeli et al. 2020). In coastal areas, risk of exposure is considered to be mainly due to the marine fauna's proximity to sources of ML, such as estuaries, ports, touristic areas, that produce higher concentrations of FML (e.g., Compa et al. 2019, Gregorietti et al. 2021, Campana et al. 2022). In offshore waters, the main drivers of variability in risk exposure were recognised in the dynamic oceanographic factors that determine FML accumulation and, partially, influence the distribution of early-stage sea turtles (e.g., Darmon et al. 2017, Atzori et al. 2021) and the species active selection of foraging grounds in areas with a high accumulation of litter (e.g., Darmon et al. 2017, Fossi et al. 2017, Campana et al. 2022).

Knowledge gaps and research needs

Despite the difficulties in gathering the needed data at an adequate spatio-temporal resolution, and the lack of a standardised methodological approach, a growing number of studies have recently made an effort to support management decisions by identifying the highest risk context in which managers should prioritise mitigation measures or conservation efforts. However, high informational heterogeneity was recognised and several priorities to improve future research efforts can be found. With this scope, the following points outline some key aspects that must be considered to improve the ability to effectively assess cetacean' and sea turtle' risk of exposure to one of the main anthropogenic pressures, i.e., marine litter (Table 3).

- The risk exposure assessment process largely relies on the availability of empirical data, although there are few data sources available. The need for data is particularly necessary to account for the high variability on either the species and the pressure. The integration of different datasets has proved to be effective for sea turtle species (Palialexis & Boschetti 2021, Girard et al. 2022). Additionally, a combined effort of research and monitoring programmes, including those responding to the different directives/regional sea conventions, are able to enhance the availability of continuous data. In general, continuous long-term monitoring programmes at appropriate spatial and temporal scales are needed to provide realistic information, allow analysis of trends and support the development and design of important conservation and management measures to reduce the risk of exposure;

- In terms of temporal resolution, only a third of the reviewed papers considered all the seasons, while another third focussed on the summer season only. While present results underline that many studies are conducted during summertime, when still high risk can be found (e.g., Campana et al. 2018), seasonality plays a key role as the main driver of variability in species distribution and abundance (e.g., Arcangeli et al. 2017, Zampollo et al. 2022) as the composition and the source of litter change with the seasons (e.g., Mansui et al. 2015, Arcangeli et al. 2018). Thus a bias towards summer might seriously compromise the validity of some of the REAs, as it does not account for phenology of species that might reproduce or aggregate in sensitive habitats during all seasons, when the information is missing. Therefore, studies on seasonality are crucial in understanding how adaptive measures may be implemented in the right sector and areas at the right time;

- So far, attention has been given to the most common species (e.g., fin whale, bottlenose and striped dolphin, sperm whale, loggerhead turtle), however info is especially urgently needed for the least abundant species known to be affected by litter, such as the squid-eating species (e.g., sperm whale, Cuvier's beaked whale, López-Martínez et al. 2023) or other sea turtles (e.g., green turtle, Duncan et al. 2019). The inclusion of multi-species targets, effectively selected as representative of different potential harms and ecosystem compartments, and a trait-analysis (e.g., integrating information on life history, morphological and behavioural characteristics of species occurring in groups to reveal aspects of their ecological functioning), is the main path to support a smart-implementation of a functional and ecosystem based risk assessment approach, increasing the reliability of (bio-geo) distributional analysis and allowing prediction of organism responses to environmental changes;

- Few studies were conducted outside the EU countries. The Mediterranean basin was the most investigated among seas. However, large geographical gaps in risk assessment emerged also in the Mediterranean Sea, especially in the southern and Levantine basin and we need to increase our research effort to provide such information;

- Methodological approaches to assess the contextual presence between ML and animals from which to derive a robust assessment of risk exposure are still highly heterogeneous. There are, however, some examples for quantitatively investigating the magnitude of risk exposure, also incorporating biological traits to account for species vulnerability. Advancement towards a standardisation of approach for analysis, limiting informational heterogeneity and uncertainty degree, would enhance the possibility to assess risk trends over time and evaluate the effectiveness of mitigation measures;

- Lastly, while this review was specifically focussed on the first two phases of risk assessment (i.e., Risk Exposure Assessment), further input should be given to integrate data and analytical approaches that include information about the potential of items to be harmful, the biological-ecological variables that we expect having an influence on the species vulnerability (e.g., life stage), the rate of entanglement/ingestion (i.e., specificity), and the severity of the impact at the individual level.

Table 3 - Main knowledge gaps identified by the review for risk exposure assessment and recommendations for future improvement.

Knowledge gaps	Research needs
Scarcity of empirical data on ML and distribution of sensitive species	- Integration of available dataset. - Combined effort from research and monitoring programs. - Support continuous long-term monitoring programs.
Information biased towards the summer season	- Support year-round monitoring programs to include important areas for the species in all seasons and ensure adaptive measures targeting the right sector and area at the right time.
Lack of studies on less common sensitive species (e.g., squid-eating species)	- Enlarge the REA to other sensitive species. - Enhance multi-target studies targeting species representative of different potential harms and ecosystem compartments.
Lack of studies outside EU and in specific EU regions	- Promote REA studies where data are available, and enhance data collection in unsurveyed regions.
Lack of standard protocol for REA, mostly based on more qualitative analysis	- Advance towards a standardization of analytical approaches to assess trends in risk over time, and allow evaluation of the effectiveness of mitigation measures

Conclusion

Spatial risk exposure assessment provides a reliable framework, in line with the precautionary principle, to prioritise conservation and mitigation measures on the highest risk settings.

By summarising the methodological approaches and knowledge available to date on the indicative species of cetaceans and sea turtles, this review is a first step towards supporting the conceptualisation and standardisation of the spatial risk exposure approach that can be potentially extended to other species as well. While the review evidenced inconsistent geographical applications and high informational heterogeneity, main limits and future recommendations were identified regarding raw data availability, information bias, geographical gaps, target species selection, lack of standard protocol needed to assess trends to evaluate the effectiveness of mitigation measures.

Ultimately, the study showed that a spatial-contextual approach (possibly functional trait-based) is needed to effectively support long-term year-round monitoring programmes, especially in still un-surveyed regions. Given the urgent need to conserve marine biodiversity and ecosystem functioning, standardising REA to include potential threats for marine megafauna throughout all habitats and regions cannot be postponed.

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Zhang, D., Yu, W., Chen, B., Chen, H., Zhao, L., Wang, X., Wu, F., Hu, W. & Ma, Z. (2020). Risk assessment for marine plastic debris ingestion by Indo-Pacific humpback dolphins (*Sousa chinensis*) in Xiamen Bay, China. *China Environmental Science*

Supplementary materials

Table S1 – Search strings used in the literature search and related platform, URL, philtre options and subscriptions.

Platform	**URL**	**Subscription Institute**	**Search string**	**Search restriction**
SCOPUS	https://www.scopus.com	University of Palermo and University La Sapienza of Rome	TITLE-ABS-KEY (("macroplastic*" OR "microplastic" OR "plastic litter" OR "plastic debris" OR "marine litter" OR "marine debris" OR "anthropogenic litter" OR "ocean plastic*" OR "marine plastic*") AND ("cetacean*" OR "dolphin*" OR "*whale*" OR "odontocet*" OR "mysticet*" OR "sea turtle*" OR "marine turtle*") AND ("risk*" OR "risk assessment" OR "risk evaluation" OR "risk index" OR "risk exposure" OR "threat*" OR "impact*") AND ("marine" OR "ocean*" OR "sea"))	-no search restriction
Web of Science	https://www.webofscience.com	University of Palermo and University La Sapienza of Rome	TS = ((macroplastic* OR microplastic OR plastic litter OR plastic debris OR marine litter OR marine debris OR anthropogenic litter OR ocean plastic* OR marine plastic*) AND (cetacean* OR dolphin* OR *whale* OR odontocet* OR mysticet* OR sea turtle* OR marine turtle*) AND (risk* OR risk assessment OR risk evaluation OR risk index OR risk exposure OR threat* OR impact*) AND (marine OR ocean* OR sea))	-Refined by Document Types: Article or Book Chapters or Proceeding Paper.

Table S2 – DOIs of a predetermined set of relevant papers used to measure search accuracy.

1	https://doi.org/10.1016/j.dsr2.2016.07.005
2	https://doi.org/10.3354/esr00980
3	https://orcid.org/0000-0002-0279-5885
4	https://doi.org/10.1016/j.marpolbul.2015.07.012
5	https://doi.org/10.1016/j.marpolbul.2022.113550
6	https://doi.org/10.3389/fmars.2017.00167
7	https://doi.org/10.1016/j.envpol.2015.11.022
8	https://doi.org/10.1016/j.envpol.2019.113680
9	https://doi.org/10.1007/s12210-018-0680-0
10	https://doi.org/10.1016/j.marpolbul.2021.112943

Table S3 – Full list of the retained articles after screening.

Authors	Year	Title	Journal
Almpanidou et al.	2022	Foraging grounds of adult loggerhead sea turtles across the Mediterranean Sea: key sites and hotspots of risk	Biodiversity and Conservation
Arcangeli et al.	2019	Turtles on the trash track: Loggerhead turtles exposed to floating plastic in the Mediterranean Sea	Endangered Species Research
Atzori et al.	2021	Loggerhead Sea Turtle, Caretta Caretta, Presence and its Exposure to Floating Marine Litter in the Sardinia Channel and the Strait of Sicily: Results From Seven Years of Monitoring Using Ferry as Platform of Observation	Mediterranean Marine Science
Campana et al.	2018	Seasonal patterns of floating macro-litter across the Western Mediterranean Sea: a potential threat for cetacean species	Rendiconti Lincei. Scienze Fisiche e Naturali
Campana et al.	2022	Cetacean sensitivity and threats analysis to assess effectiveness of protection measures. An example of integrated approach for cetacean conservation in the Bonifacio Bouches	Biodiversity and Conservation
Compa et al.	2019	Risk assessment of plastic pollution on marine diversity in the Mediterranean Sea	Science of the total environment
Critchell et al.	2019	Predicting the exposure of coastal species to plastic pollution in a complex island archipelago	Environmental Pollution
Currie et al.	2017	Quantifying the risk that marine debris poses to cetaceans in coastal waters of the 4-island region of Maui	Marine Pollution Bulletin
Darmon et al.	2017	Risk assessment reveals high exposure of sea turtles to marine debris in French Mediterranean and metropolitan Atlantic waters	Deep Sea Research Part II: Topical Studies in Oceanography
Di-Méglio and Campana	2017	Floating macro-litter along the Mediterranean French coast: composition, density, distribution and overlap with cetaceans.	Marine Pollution Bulletin
Fossi et al.	2017	Plastic debris occurrence, convergence areas and fin whales feeding ground in the Mediterranean marine protected area Pelagos Sanctuary: A modeling approach	Frontiers in Marine Science
Gacutan et al.	2022	Mapping marine debris risk using expert elicitation, empirical data, and spatial modelling	Environmental science and policy

Authors	Year	Title	Journal
Galli et al.	2022	Microplastic abundance and biodiversity richness overlap: Identification of sensitive areas in the Western Ionian Sea.	Marine Pollution Bulletin
González et al.	2014	Young green turtles, Chelonia mydas, exposed to plastic in a frontal area of the SW Atlantic	Marine Pollution Bulletin
Gregorietti et al.	2021	Cetacean presence and distribution in the central Mediterranean Sea and potential risks deriving from plastic pollution	Marine Pollution Bulletin
Guerrini et al.	2019	Modeling plastics exposure for the marine biota: Risk maps for fin whales in the Pelagos Sanctuary (North-Western Mediterranean)	Frontiers in Marine Science
Hoiberg et al.	2022	Global distribution of potential impact hotspots for marine plastic debris entanglement	Ecological Indicators
Jones et al.	2021	Plastic contamination of a Galapagos Island (Ecuador) and the relative risks to native marine species	Science of the total environment
Kahane et al.	2022	Field measurements reveal exposure risk to microplastic ingestion by filter-feeding Megafauna	Nature Communication
Sá et al.	2021	Floating marine litter and their risks to cetaceans off Portugal	Marine Pollution Bulletin
Schuyler et al.	2016	Risk analysis reveals global hotspots for marine debris ingestion by sea turtles	Global Change Biology
Wilcox et al.	2013	Ghostnet impacts on globally threatened turtles, a spatial risk analysis for northern Australia	Conservation Letters
Williams et al.	2011	Marine mammals and debris in coastal waters of British Columbia, Canada	Marine Pollution Bulletin
Zhang et al.	2020	Risk assessment for marine plastic debris ingestion by Indo-Pacific humpback dolphins (Sousa chinensis) in Xiamen Bay, China	China Environmental Science

Tables S4 – Species considered, IUCN vulnerability status, areas investigated and corresponding number of papers. CR=Critically Endangered; EN=Endangered; Vu=Vulnerable; NT=Near Threatened; LC=Least Concern; DD=Data Deficient.

Cetacean families	Cetacean species	IUCN global status	IUCN Med status	N papers	Areas investigated	References
Delphinidae	*T. truncatus*	LC	LC	8	Pacific, Atlantic, Mediterranean	Campana et al. 2022, Hoiberg et al. 2022, Galli et al. 2022, Sá et al. 2021, Gregorietti et al. 2021, Campana et al. 2018, Currie et al. 2017, Di-
	S. coeruleoalba	LC	LC	6	Atlantic, Mediterranean	Galli et al. 2022, Campana et al. 2022, Sá et al. 2021, Gregorietti et al. 2021, Campana et al. 2018, Di-Méglio & Campana 2017
	G. griseus	LC	EN	4	Atlantic, Mediterranean	Sá et al. 2021, Compa et al. 2019, Campana et al. 2018, Di-Méglio & Campana 2017
	D. delphis	LC	EN	1	Atlantic	Sá et al. 2021
	S. attenuata	LC	-	1	Pacific	Currie et al. 2017
	S. longirostris	LC	-	1	Pacific	Currie et al. 2017
	S. frontalis	LC	-	1	Atlantic	Sá et al. 2021
	S. bredanensis	LC	-	1	Mediterranean	Galli et al. 2022
	O. orca	DD	-	1	Pacific	Williams et al. 2011

Cetacean families	Cetacean species	IUCN global status	IUCN Med status	N papers	Areas investigated	References
Delphinidae	*L. obliquidens*	LC	-	1	Pacific	Williams et al. 2011
	P. phocoena	LC	-	2	Pacific, Mediterranean	Compa et al. 2019, Williams et al. 2011
	P. dalli	LC	-	1	Pacific	Williams et al. 2011
	P. crassidiens	NT	-	1	Pacific	Currie et al. 2017
	S. chinensis	VU	-	1	Pacific	Zhang et al. 2020
	G. melas	VU	EN	3	Atlantic, Mediterranean	Sá et al. 2021, Campana et al. 2018, Di-Méglio & Campana 2017
Zphiidae	*Z. cavirostris*	VU	EN	4	Atlantic, Mediterranean	Galli et al. 2022, Campana et al. 2022, Sá et al. 2021, Campana et al. 2018
Physeteridae	*P. macrocephalus*	VU	EN	6	Atlantic, Mediterranean	Campana et al. 2022, Galli et al. 2022, Sá et al. 2021, Compa et al. 2018, Campana et al. 2018, Di-Méglio & Campana 2017
Kogidae	*K. sima*	LC	-	1	Atlantic	Sá et al. 2021

Cetacean families	Cetacean species	IUCN global status	IUCN Med status	N papers	Areas investigated	References
Balaenopteridae	*B. physalus*	VU	EN	8	Pacific, Atlantic, Mediterranean	Campana et al. 2022, Kahane-Rapport et al. 2022, Sá et al. 2021, Guerrini et al. 2019, Campana et al. 2018, Di-Méglio & Campana 2017, Fossi et al.
	B. musculus	EN	-	1	Pacific	Kahane-Rapport et al. 2022
	B. acutorostrata	LC	-	2	All world oceans	Williams et al. 2022, Hoiberg et al. 2022
	M. novaengliae	LC	-	3	Pacific	Williams et al. 2022, Hoiberg et al. 2022

Sea turtle families	Cetacean species	IUCN global status	IUCN Med status	N papers	Areas investigated	References
Cheloniidae	*C. caretta*	VU	LC	8	All world oceans, Mediterraneam	Almpanidou et al. 2022, Galli et al. 2022, Hoiberg et al. 2022, Atzori et al. 2021, Arcangeli et al. 2019, Compa et al. 2019, Darmon et al. 2017,
	C. mydas	EN	EN	4	All world oceans, Mediterranean	Jones et al. 2021, Darmon et al. 2017, Schuyler et al. 2016, Wilcox et al. 2013
	C. embricate	CR	-	4	All world oceans	Hoiberg et al. 2022, Jones et al. 2021, Schuyler et al. 2016, Wilcox et al. 2013
	N. depressor	DD	-	2	All world oceans	Schuyler et al. 2016, Wilcox et al. 2013
	L. olivacea	VU	-	3	All world oceans	Hoiberg et al. 2022, Schuyler et al. 2016, Wilcox et al. 2013
	L. kempii	CR	-	2	All world oceans	Hoiberg et al. 2022, Schuyler et al. 2016
Dermatochelydae	*D. coriacea*	VU	-	3	All world oceans, Mediterranean	Hoiberg et al. 2022, Darmon et al. 2017, Schuyler et al. 2016

Chapter 5

Cetacean presence and distribution in the Central Mediterranean Sea and potential risks deriving from plastic pollution

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Cetacean presence and distribution in the central Mediterranean Sea and potential risks deriving from plastic pollution

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ABSTRACT

The Sardinian and Sicilian Channels are considered hotspots of biodiversity and key ecological passages between Mediterranean sub-basins, but with significant knowledge gaps about marine mammal presence and potential threats they face.

Using data collected between 2013 and 2019 along fixed transects, inter and intra-annual cetacean index of abundance was assessed. Habitat suitability, seasonal hot spots, and risk exposure for plastic were performed using the Kernel analysis and the Biomod2 R-package.

661 sightings of 8 cetacean species were recorded, with bottlenose and striped dolphins as the most sighted species. The north-eastern pelagic sector, the coastal waters and areas near ridges resulted the most suitable habitats for these species. The risk analysis identified the Tunis, Palermo, and Castellammare gulfs and the Egadi Island as areas of particular risk of plastic exposure.

The study represents a great improvement for cetacean knowledge in this region and contributes to the development of effective conservation strategies.

1. Introduction

Planning decisions for species requiring special legal protections (Baker et al., 2021), such as vagrant large marine pelagic cetaceans, needs robust and transparent information at an appropriate and relevant spatial scale. Effective information dealing with how, where, and when animals use the environment is crucial for disentangling the effects of human impacts on the ecological traits of wild populations in order to address conservation strategies, design appropriate measures (Ceballos and Ehrlich, 2002), and above all, to increase understanding of dynamics at a landscape scale to maintain connectivity and environmental flows (Baker et al., 2021). Thus, data collection frameworks should encompass all possible aspects enhancing the ability to protect biodiversity, including the potential effects generated by anthropogenic impacts, such as litter especially of plastic origin, on distributional ranges and habitat preferences. Cetaceans are central components of the biodiversity in all oceans, often playing an apical trophic role in maintaining food web stability and ecosystem functioning, although they are vulnerable to a number of anthropogenic impacts (Dolman and Simmonds, 2010; Fossi et al., 2012; Lewison et al., 2014; Turvey et al., 2007; Bearzi, 2002) and suffer habitat fragmentation and loss (Simmonds and Nunny, 2002). This is particularly true in the Mediterranean Sea where, of the ten species regularly inhabiting the basin (di Sciara and Birkun, 2010), three are considered "Vulnerable" (fin whale *Balaenoptera physalus* - Bp, striped dolphin *Stenella coeruleoalba* - Sc, bottlenose dolphin *Tursiops truncatus* - Tt), two "Endangered" (common dolphin *Delphinus delphis* - Dd, sperm whale *Physeter macrocephalus* - Pm), four "Data deficient" (Risso's dolphin *Grampus griseus* - Gg, long-finned pilot whale *Globicephala melas* - Gm, killer whale *Orcinus orca* - Oo, Cuvier's beaked whale *Ziphius cavirostris* - Zc), and one "Not assessed" (rough-toothed dolphin *Steno bredanensis* - Sb) (IUCN, 2012). While the current regulations based on the Habitat Directive (Art.17) and the Marine Strategy Framework Directive (Art.11, Descriptor 1) consider monitoring actions of cetacean's distributional range, abundance and habitat of the species as crucial factors for designing effective conservation strategies, the collection of useful data for these purposes is complicated by cetacean biological and ecological features. The conservation status of cetacean species is indeed still considered data

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deficient for most taxa according to the last Habitats Directive Art. 17 Report (2013 - 2018) and the EEA Report (No 10/2020), mainly due to the fact that the species spend the majority of their life in remote offshore areas most difficult to monitor because of their extent, highly dynamic nature and the high costs involved in carrying out regular large scale surveys. Most of the information about cetacean abundance and distribution is reported mainly for the northern and western Mediterranean sectors and are concentrated to the summertime and on a few species (Panigada et al., 2011; Praca et al., 2009; Moulins et al., 2008; Laran and Gannier, 2008; Tepsich et al., 2020). Valuable information for conservation purposes is scant for other sectors, such as the Sardinian and Sicilian Channels (SSCC) (di Sciara and Birkun, 2010), where most studies are from coastal (Alessi et al., 2019; Papale et al., 2016; Naceur et al., 2004) and island waters of the southern Sicilian seas (Pulcini et al., 2014; Aïssi et al., 2008; Canese et al., 2006; Celona and Comparetto, 2006; La Manna et al., 2016) and from Maltese and Tunisian coastal waters (North-eastern coast of Tunisia) (Benmessaoud et al., 2012, 2013). Nevertheless, Mediterranean southern areas are crucial for connecting the population nuclei of cetaceans across the Mediterranean basins and maintaining meta-population dynamics. Thereby, the absence of effective information about the distribution and movement patterns of these priority species in these core zones of the distribution range in the Mediterranean Sea undermines the ability to protect marine biodiversity, not only locally but also at a Basin level, and weakens our ability to inform planning decisions (Baker et al., 2021). Indeed, the geographic area including Sicily, Sardinia, Malta, and Tunisia appears to be a key region for understanding of the exchanges between the Eastern and Western Mediterranean waters, because these zones of the Basin are characterized by peculiar environmental features. The Sicily Channel is a hotspot of biodiversity due to the hydrography and topographic features. A series of anti-cyclonic vortexes off the eastern coast of Tunisia and off Malta generates upwelling (Capodici et al., 2018) and increases the overall productivity making it among the most fished (and disturbed) zones in the Northern hemisphere (Falcini et al., 2020; Mangano et al., 2020). Due to its importance for biodiversity, the Sicily Channel has been identified as a priority for conservation (de Juan et al., 2012; Oceana, 2011) and declared as an Ecologically or Biologically Significant Area (EBSA) by the Convention on Biological Diversity (Bax et al., 2016). Nonetheless, the human impact in this area is getting stronger year by year reaching among the highest levels in the entire world. Trawling, shipping traffic, oil drilling, mining, recreational fishing tourism (Levi et al., 1998; FAO, G, 2016; Patruno, 2008) and aquaculture (Sarà et al., 2018; Giacoletti et al., 2021) are just the most striking examples of anthropogenic pressures that more or less directly may impair the wildlife in this area. Marine litter, in particular that of plastic origin, is abundant at exerting large detrimental effects on great pelagic species, such as fish, turtles, and above all cetacean species (Moore and Barco, 2013; Baulch and Perry, 2014; Gall and Thompson, 2015; Claro et al., 2020; Salerno et al., 2021). Giving its complex paths across the aquatic environment and the physical/chemical processes to which it may be subjected, the interactions with marine animals can be diverse and at various levels (Arcangeli et al., 2021). Recent studies confirmed that ingestion and entanglement are among the primary impacts of marine litter on marine species (Kühn et al., 2015; Wilcox et al., 2015; Claro et al., 2019); in particular, 13 of the 15 cetacean families interact with marine debris, and 81 of 123 of all marine mammal species appears to be involved in ingestion phenomena (Fossi et al., 2018; Kühn et al., 2015). This can cause the blockage of the digestive tract, suffocation or even starvation due to a false sense of satiety (Sheavly and Register, 2007; Roman et al., 2019). Entanglement, which was attested for almost the 30% of cetacean species (Fossi et al., 2018), can cause alterations in movements and buoyancy, preventing the animal from breathing, swimming, and feeding appropriately (Laist, 1997; Derraik, 2002; Jacobsen et al., 2010; De Stephanis et al., 2013; Moore et al., 2013). Above all, marine mammals' neck, flukes and flippers tend to get entangled in ghost or active fishing gears (Baulch and Perry, 2014; Moore et al., 2013). Moreover, plastic litter contain chemical additives like persistent organic pollutants (POPs) and heavy metals (Massos and Turner, 2017), many of which are neurotoxins or endocrine disruptors (Sussarellu et al., 2016). Therefore, its ingestion can start the process of bioaccumulation across all levels of the aquatic food web (Lavers et al., 2014; Bakir et al., 2016; Gutow et al., 2016), and of biomagnification, of particular concern when top predators like marine mammals are involved (Santana et al., 2017). Even if death can be caused by just one item of debris (Roman et al., 2019; Santos et al., 2015; Wilcox et al., 2018), not all of them contribute equally to mortality and the probability of ingesting a deadly item raises as more objects are ingested (Roman et al., 2019). In the case of marine megafauna and in particular of cetaceans, Roman et al., 2021 found that film-like plastic, plastic fragments, ropes/nets and fishing items are the most dangerous items among marine litter. Among the first category, plastic bags, sheets and packaging are the major cause of mortality for cetacean species (Panti et al., 2019).

The impact of marine litter on species is a combination of events that imply the exposure of the vulnerable animal to the threat, and then the different levels of impact from movement restriction to injury or death (Gregory, 2009). Being exposed to a pressure does not imply to be affected by it, depending by the individual behavior, the typology of litter item and the type of interaction between the two, so that only a fraction of all individuals potentially exposed to the threat is affected by it ("Potentially Affected Fraction" of Woods et al., 2019). Nevertheless, the identification of risk areas where marine fauna is mostly exposed to litter is the first step to prioritize conservation measures on the higher risk contexts (e.g. Darmon et al., 2017; Arcangeli et al., 2018; Campana et al., 2018; Fossi et al., 2017; Guerrini et al., 2019; Compa et al., 2019; Soto-Navarro et al., 2021).

The Mediterranean Sea is universally recognized as one of the most plastic polluted marine areas of the entire world (Lebreton et al., 2012; Cózar et al., 2015; Suaria et al., 2016). In the last decades, information has been collected about distribution, types, quantities and sources of marine debris in the Mediterranean waters (Suaria and Aliani, 2014; van der Hal et al., 2017; Schmidt et al., 2018). Simultaneously, scientists had tried to predict the faith of floating plastic litter through numerical modelling at basin and sub-basin scales, primarly implementing Lagrangian models of particle dispersion (Mansui et al., 2015, 2020; Maximenko et al., 2012; Liubartseva et al., 2016; Fossi et al., 2017; Palatinus et al., 2019), but this field is still in progress. The primary difficulty that lead to results that are different from model to model is the lack of accurate information about the sources and the amounts of litter discharged in the basin (Soto-Navarro et al., 2020). At present time, the models from Liubartseva et al., 2018, Soto-Navarro et al., 2020, and Guerrini et al., 2021 are the only ones that gave a realistic approximation of marine litter distribution for the entire Mediterranean Sea, taking into account different sources and considering respectively only floating litter or surface, neutrally buoyant and sinking particles, and floating microplastics. The detrimental effects of plastic on wildlife is so alarming that the scientific community is also trying to develop new methods to spectrally characterize the most common polymers and to quantify their spectral separability to determine those optimal band combinations to make plastics detectable through satellite imagery monitoring, so to help identifying the areas of accumulation of this threat (Corbari et al., 2020). However, at date, the most feasible way to identify marine litter accumulation in the large offshore Mediterranean areas still remain the collection of empirical data on floating marine litter at a seasonal temporal scale (Arcangeli et al., 2021).

With regards to SSCC area, studies reported the massive presence of litter entrapped in the seabed (Consoli et al., 2018a; Consoli et al., 2018b). Plastic is always the principal component of the anthropogenic litter recorded in the area (Suaria and Aliani, 2014; Arcangeli et al., 2018, 2019) and, even if the mean plastic density is lower with respect to other parts of the basin (Suaria and Aliani, 2014), plastic hotspots along the Tunisian coasts in the Sicily Channel, and in the gulf of Palermo are confirmed from both field surveys and models (Arcangeli et al., 2018, 2019; Liubartseva et al., 2018, Guerrini et al., 2021; Soto-Navarro et al., 2021, Atzori et al., 2021).

Such a "neglected" presence increases the alert level about the potential implications of plastic impact on biodiversity in general, and on cetaceans in particular. Spatial and temporal scales of data are crucial as the migratory nature of the species and the variability in litter distribution make the interaction largely dependent by seasonality. As a main consequence, to collect new information on how plastic may affect biodiversity at a relevant scale for conservation plays a crucial role when addressing decision planning. In doing so, here we integrated field observational data on cetaceans over a 7-year time series with plastic density obtained by field surveys to build a risk index over the different seasons. Moreover, the most important areas for cetacean species were investigated by modelling suitable habitat for the species. Species Distribution Models (SDMs) are valuable tools for drawing geographic distributional areas as a function of a suite of environmental variables (sensu Sarà et al., 2018), they are in fact a widely used tool to predict cetacean distribution and understand ecological precursors (Palacios et al., 2013; Gregr et al., 2013; Druon et al., 2012; La Manna et al., 2020). Here SDMs were used to predict suitable habitats for cetaceans in the whole area of the SSCC. The final goal of the study is to enhance the knowledge in this key area of the central Mediterranean Sea and produce information to address future conservation measures.

2. Methods

2.1. Study area

Cetaceans and marine litter were monitored in the SSCC (Fig. 1). Four trans-border transects covered this area from 2013 to 2019, connecting Palermo to Cagliari, Trapani and Tunis and Tunis to Civitavecchia. These routes cross both pelagic and coastal area, and pass close to two Marine Protected Areas (the Isole Egadi MPA, located off the north-western coast of Sicily, and the Capo Carbonara MPA, in the south-eastern part of Sardinia) and the Zembra and Zembretta National Park, located in the Gulf of Tunis.

2.2. Data collection

Surveys were performed using passenger ferries as platforms of observation, and data were collected following two different protocols defined by ISPRA (ISPRA, 2015a, Technical Annex I & ISPRA, 2015b, Technical Annex II) dedicated respectively to cetacean and floating marine litter. Of the four transects, two were carried out all year round (Palermo-Tunis PATU and Civitavecchia-Tunis TUCI) and two during the Summer season only (Cagliari-Palermo CAPA and Cagliari-Trapani CATRA), with a minimum of three surveys per season.

Experienced marine mammal observers were located on both sides of the ship's command bridge scanning within an angle of 130° ahead in order to avoid recounting animals. At the same time, one dedicated observer recorded data on floating marine macro litter using a standard protocol specifically developed for collecting data from ferries (Arcangeli et al., 2018) and conformed to the guidelines of the MSFD technical subgroup (Galgani et al., 2013). Observations were performed during daylight and only in good weather conditions (Beaufort scale $\leq$ 3 for cetacean and $\leq$2 for marine litter), monitoring the sea continuously by naked eye, and using binoculars (7 $\times$ 50 magnification) to confirm species identification, group size, or litter items type/material. The "on effort" track lines and each sighting, either of cetacean or litter, were recorded by two dedicated GPS and annotated on standard datasheets. For cetaceans, information about the distance and angle from the ship, species, number of individuals, direction of swimming, and surface behavior were recorded. Litter monitoring was carried out by the side of

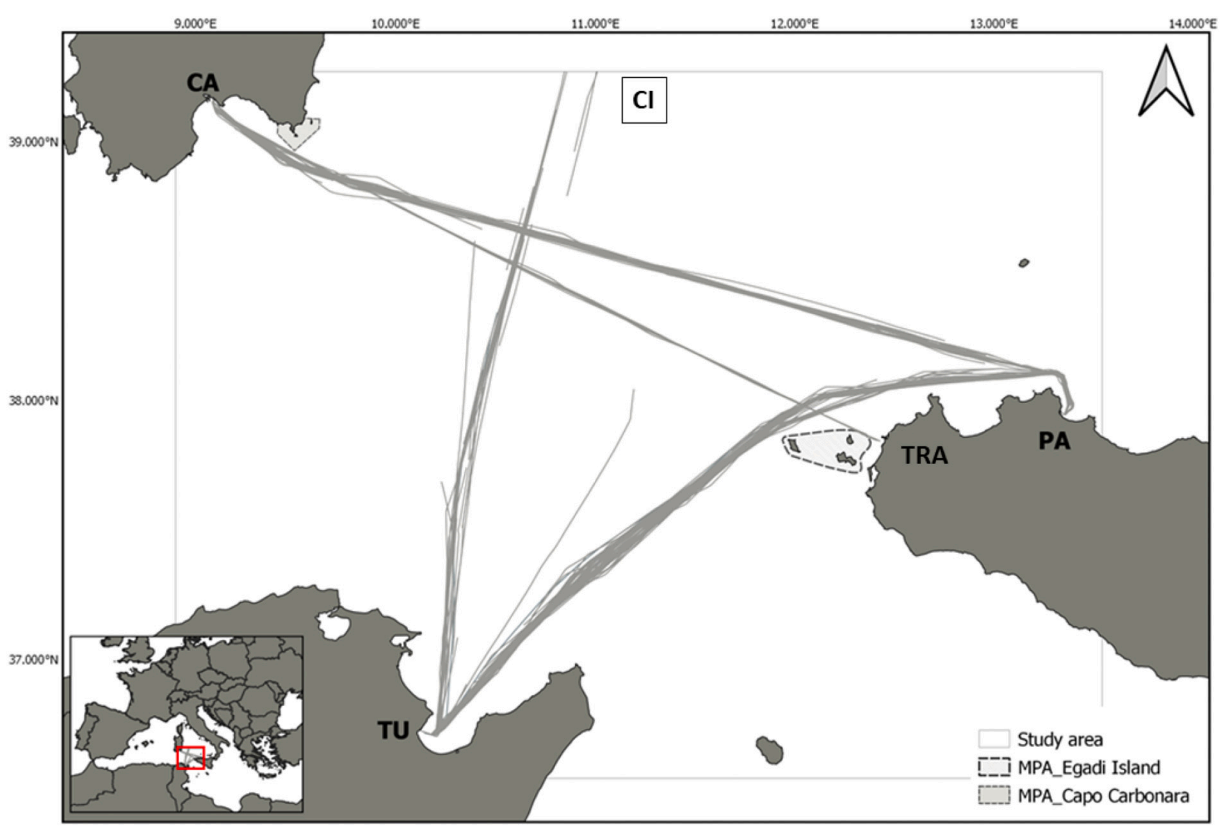

Fig. 1. Study area (in the box), with the Italian marine protected areas of the Egadi Island and Capo Carbonara. The effort performed along the surveyed transects (PATU, TUCI, CATRA and CAPA) between 2013 and 2019 is represented by the grey lines.

the ship's bridge with best visibility, and in the bow proximity in order to avoid the turbulence generated by the bow itself. Only items >20 cm and present in a fixed strip width (Thiel et al., 2003; Pyle et al., 2008) were recorded.

This strip was defined at the beginning of monitoring based on the sea state, glare, and ship's speed (Arcangeli et al., 2018). Litter characterization was based on the type of material (artificial polymer materials, processed wood, glass, paper, metal, textile, rubber, natural debris), and information about buoyancy, color, size, and state of the object (entire or fragment) were registered.

All the ferries used for monitoring belonged to the two categories "Passenger Ro-Ro Cargo ship" and "Ro-Pax passenger vessel", with a height of the command deck between 22 and 27 m above the sea level. The monitoring methodologies, both for cetaceans and marine litter, were consistent along all the study period.

2.3. Data analysis

For all statistical analyses, significant differences were investigated using the non-parametric Kruskal Wallis (KW) test and the post-hoc Mann-Whitney (MW) test with Bonferroni correction. Statistical analyses were performed using the software Past 4.1 (Hammer et al., 2001), while all the spatial analyses were carried out using the QGIS 2.14.21 software. The species habitat suitability was estimated using R 3.4.6.

2.3.1. Cetacean presence and distribution along the routes

The sighting rate (SPUE, Sightings Per Unit of Effort) was estimated per transect for each species and used as a proxy for cetacean abundance in order to compare changes over time. It was calculated as.

$$\mathrm{SPUE} = \frac{Number\ of\ sightings}{Km\ in\ good\ weather\ conditions} \times 10$$

Inter-annual analyses were performed considering all monitored transects for the Summer season, while intra-annual seasonal analyses were performed on the PATU and TUCI transects continuously monitored during all the seasons from 2014 to 2019.

To study the spatial distribution of the species, a grid of 5 × 5 km was overlapped onto the study area and, for each cell, the $\mathrm{SPUE_{cell}}$ was calculated as.

$$\mathrm{SPUE_{cell}} = \frac{Number\ of\ sightings\ per\ cell}{Km\ on\ effort\ per\ cell} \times 10$$

Only the cells with at least one track of effort were selected, and a minimum total effort per cell was set at 10 km (Arcangeli et al., 2017). The Average Nearest Neighbor analysis was preliminarily conducted in order to check if sightings distribution followed a clustered or random pattern. The Kernel Density Estimation (KDE) was then performed based on the $\mathrm{SPUE_{cell}}$ using a search radius of 20 km, to show the areas of highest probability of cetacean occurrence. The isopleths corresponding to the 80% of the total values of the entire region were then obtained to highlight the areas of highest species occurrence. In order to identify the statistically significant hotspot of cetacean species, the HotSpot Getis-ord G*Analysis was performed, using only the most significant values (>2.58) for displaying the hot clusters.

2.3.2. Habitat suitability modelling

With the aim of assessing the driving forces that define the habitat of the two most sighted species (Sc and Tt) and predicting their distribution for the entire study area, a habitat suitability analysis was carried out using the Ensemble Platform for Species Distribution Modelling "biomod2" package (Thuiller et al., 2016). This package runs consistently different single models on a presence/absence dataset and combines them into one ensemble model.

Only the Summer sightings from 2013 to 2019 of the two species were considered for the analysis. To avoid bias due to uneven effort, a minimum sampled effort value per cell was set to identify pseudo-absence cells ("absence" cells hereinafter). From the entire dataset (N tot cells = 1564) and for both species, only the cells where the sampling effort was greater than the median of 11 km were considered (N cells = 794). Sc presence cells were 23% of the total ($N = 185$), while for Tt they were only 4% ($N = 33$). Given the very unbalanced dataset for Tt, the Tt presence percentage was adjusted to that of Sc, sampling a number of 111 inferred absence cells from the 794 considered.

A set of eight topographic and oceanographic variables were associated with the dataset of presence/absence cells of Sc and Tt. These variables are those already known or considered as potential predictors of the species considered (Claro et al., 2020; Carlucci et al., 2016; Vassallo et al., 2018; Barragán-Barrera et al., 2019), and were: Sea Surface Temperature (SST, °C); Chlorophyll-a concentration (CHL-a, mg/m^{-3}); bathymetry (m); bathymetric slope (degrees); minimum distance from the nearest coastline, slopes, canyons, and ridges (km).

In order to obtain the most accurate CHL-a and SST seasonal means as possible, the raster files with the highest temporal resolution (8 days) and a spatial resolution of 4 × 4 km have been downloaded from NASA Ocean Color (http://oceancolor.gsfc.nasa.gov). Then, rasters were obtained by averaging each cell over time and calculating temporal standard deviation. Bathymetry values were extrapolated from the GEBCO raster file (GEBCO Compilation Group (2020) GEBCO 2020 Grid (doi: https://doi.org/10.5285/a29c5465-b138-234d-e053-6c86abc040b9), while bathymetric slope and minimum distance from the coastline raster files with a spatial resolution of 1 km were obtained from the MARSPEC dataset (Sbrocco and Barber, 2013). Vector layers of the geomorphic features, such as slopes, canyons, and ridges were obtained from the Blue Habitat dataset (Harris et al., 2014) and the rasters of the Euclidean distances from the nearest features were computed. Those rasters were matched to the same resolution of SST and CHL-a ones using the "raster" package in R. Moreover, before starting modelling, multicolinearity among explanatory variables was tested using VIF (Variance Inflaction Factors).

The influence of environmental predictors was initially investigated statistically comparing values of each variable in presence and absence cells, using Mann-Whitney U test to test for equal medians. Then, modelling analyses were performed using the R package biomod2 and GAM, GBM, GLM, RF, and MaxEnt models. For each model, a 10-fold cross validation with an 80-20 proportion for training set and test set was performed, obtaining 50 models for each species. Model performance was evaluated considering primarily AUC (Area Under the ROC Curve) but also TSS (True Skill Statistics), which combines the information of sensitivity and specificity. According to these metrics, and with the purpose of improving predictive power, biomod2 also creates an ensemble model whose performance was also evaluated and compared to other models. All resulting models were also visually inspected for detecting signs of overfitting. After obtaining the final models, variable importance was extracted in order to understand which were more useful for predicting the presence probability of the species. Finally, summary statistics of predictors were also observed in those points recording a presence probability higher than the 3rd quartile for Sc and higher than the threshold of 0.50 for Tt. With the assumption of stochastic independence between the presence of the two species, the probability to find both species (intersection) was also computed.

2.3.3. Floating plastic macro litter and cetacean risk assessment

In order to estimate the potential threat represented by plastic pollution on cetacean species, a seasonal case study considering only the annual transect PATU and the period 2016-2019 was carried out. Seasons were subdivided as follows: Winter (January-March), Spring (April-June), Summer (July-September), and Autumn (October-December).

First, the percentage composition of marine litter items belonging to the different material categories *per* season was calculated, as well as the correspondent total amount of objects detected *per* year. As the characterization of the artificial polymers fraction was the main objective, this portion of the marine litter dataset was used to identify the percentage and density of plastic item categories for each transect, season, and year as:

$$\text{Density} = \frac{\textit{Number of items observed}}{\textit{width of the observed strip x lenght of the surveyed transect.}}$$

Moreover, the most represented dimensional item categories were identified.

Using the Geoprocessing tools in QGIS, a buffer equivalent to the transect width was built around the effort tracks and intersected with the effort cells. Within each cell, the amount of plastic was calculated as

$$\text{Density}_{\text{cell}} = \frac{\textit{Number of plastic items observed}}{\textit{area}}$$

The average Nearest Neighbor analysis was performed to test if plastic litter distribution followed a cluster pattern, as well as the KDE based on the $\text{Density}_{\text{cell}}$ with a search radius of 30 km to show the areas of highest probability of litter occurrence along the routes in the different seasons (Arcangeli et al., 2018). The isopleths corresponding to the 80% of the total values of the entire region were then obtained to highlight the areas of highest litter occurrence. In order to identify the statistically significant hotspot the HotSpot Getis-ord G*Analysis was performed, using only the most significant values (>2.58) for displaying the hot clusters. To identify the areas of particular risk of cetacean exposure to plastic threat, the $\text{SPUE}_{\text{cell}}$ grids of the most sighted species were joined to the one of litter density using the Join attribute by location tool in QGIS.

A risk index was calculated as follows:

$$\text{Risk index} = \text{SPUE}_{\text{cell}}\ \textit{rank} \times \text{Density}_{\text{cell}}\ \textit{rank}$$

considering as ranks four intervals (0, 1, 2, 3) of both variables identified using the Jenks Natural breaks in QGIS, a data clustering method designed to determine the best arrangement of values into different classes according to the distribution of the data. Four different classes of risk were then identified: Null (white), Low (green), Medium (yellow), and High (red).

3. Results

3.1. Cetacean presence and spatial distribution along the routes

From 2013 to 2019, 207 surveys were conducted in the study area, for a total of about 50,000 km covered on effort and 1359 h of observation (Table 1).

During the study period, 661 sightings of cetaceans were recorded (Table 1), and eight of the cetacean species living permanently in the Mediterranean Sea were registered. Sc and Tt were the most sighted species, while Dd, Pm, Bp, and Gg were less frequently recorded, even if sighted almost every year. Gm and Zc were registered only occasionally. In particular, Pm and Zc were recorded in the Sardinian Channel only.

On an annual basis, considering only the Summer season and with all data pooled together (PATU, TUCI, CAPA and CATRA), the mean SPUE value for all cetacean species ranged between 0.020 ± 0.006 (2017) and 0.008 ± 0.002 (2013); no statistical differences were found between the survey years (KW, $p > 0.05$) (Fig. 2).

Stratifying per species, no statistical differences between years were founded for any of them (KW, $p > 0.05$), with the only exception of Sc which showed some significant variability among years (Fig. 1, Supporting Material first panel) mainly driven by variability in the Sardinian Channel (Fig. 1, Supporting Material second panel). Tt showed instead some significant interannual variability in the Sicilian Channel (Fig. 1, Supporting Material, third panel), with no records during the Summer of 2016 and 2017.

Seasonal analysis performed on the annual transects PATU and TUCI shows no differences in the mean SPUE values for all years and species pooled together, even if the highest value was found in Winter (0.03 ± 0.007) (KW, H = 0.43, $p > 0.05$). Stratifying data per species, Sc, Tt, Dd were recorded all year-round, while no record of Gg was registered during the Winter season. No significant differences between seasons were found in the SPUE values of each species (KW, $p > 0.05$).

Sightings of mixed groups were recorded. The most common association was between Sc and Dd ($N = 8$), recorded during Summer and Spring. Associations of Tt and Gg ($N = 3$) were recorded in Autumn and Spring. In two occasions, the associations Tt with Sc (Winter) and Gm with Gg (Summer) were observed.

Further analysis on the seasonal spatial distribution were performed considering only the two most sighted species in the study area, namely Sc and Tt. Considering only the Summer season and pooling data from all years and routes together, Sc and Tt sightings showed a statistically significant clustered pattern (Nearest neighbor index < 1). Sc had a spotted spatial distribution along the routes, and a significant Summer hot-spot (Gi* analysis > 2.85) was identified north of the Island of Marettimo (Egadi Island) (Fig. 2, Supporting Materials). Conversely, Tt hotspots were located only near the coasts, corresponding to Tunis, Cagliari, and Trapani harbors (Fig. 3, Supporting Materials).

Spatial analysis on the other seasons along the PATU-TUCI routes showed a clustered pattern in every season (Nearest neighbor index < 1). The Kernel analysis highlighted that the waters around Egadi Island, the Gulf of Tunis, and the NW Sicily coast were, along the routes, the areas with a higher probability of the presence of the two species. Sc presence was concentrated from the NW part of Sicily until Egadi Island in all seasons while, during Spring, a Sc hotspot was highlighted also in the Sardinian Channel. Tt presence was concentrated in the Gulf of Tunis in all seasons and in the water outside Palermo harbor during Spring.

Even if it was not possible to conduct detailed spatial analysis on the less sighted species, due to their low number, Bp, Pm and Zc were recorded only in the northern sector of the study area in the pelagic realm (Fig. 4, Supporting Material). Bp and Zc were recorded in water beyond 1000 m of depth, while Pm beyond 2000 m. Gg presence was recorded in the northern sector until Egadi Island, in which its sightings were positioned along the 600 m isobath. Dd sightings were distributed more homogenously along the transects; near Sardinia this species was recorded beyond 2000 m of depth, in the Sicily Channel was found within and beyond the continental platform; near the Egadi Island, such as Gg, followed the 600 m isobath and in the north-west of Sicily was

Table 1
Summary of the sampling effort, hours of observation, number of transects and of sightings in the considered study period.

Year	Km on effort	Hour of obs	N of transects	N of sightings							
				Pm	Bp	Gg	Gm	Tt	Zc	Sc	Dd
2013	2996.72	108	15	2				3		16	1
2014	7759.27	250	37	1				20		44	7
2015	6258.58	230	27	1	1			19		49	1
2016	4088.65	125	17	2	1	1	1	13		38	1
2017	8613.11	290	37	1	1	1		11		106	7
2018	11,436.16	329	46	1	1	5		21		111	3
2019	6975.53	204	28			2		20	1	69	2

(Pm = *Physeter macrocephalus*; Bp = *Balaenoptera physalus*; Gg = *Grampus griseus*; Gm = *Globicephala melas*; Tt = *Tursiops truncatus*; Zc = *Ziphius cavirostris*; Sc = *Stenella coeruleoalba*; Dd = *Delphinus delphis*)

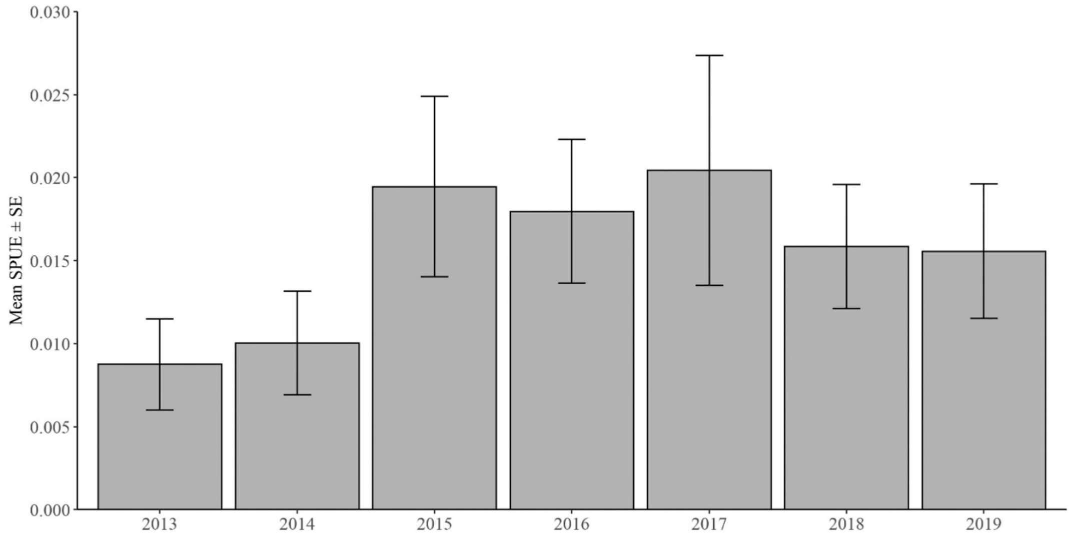

Fig. 2. Mean cumulative Sightings Per Unit of Effort (SPUE) values ± Standard Error (SE) for the Summer seasons in the SSCC for the years considered.

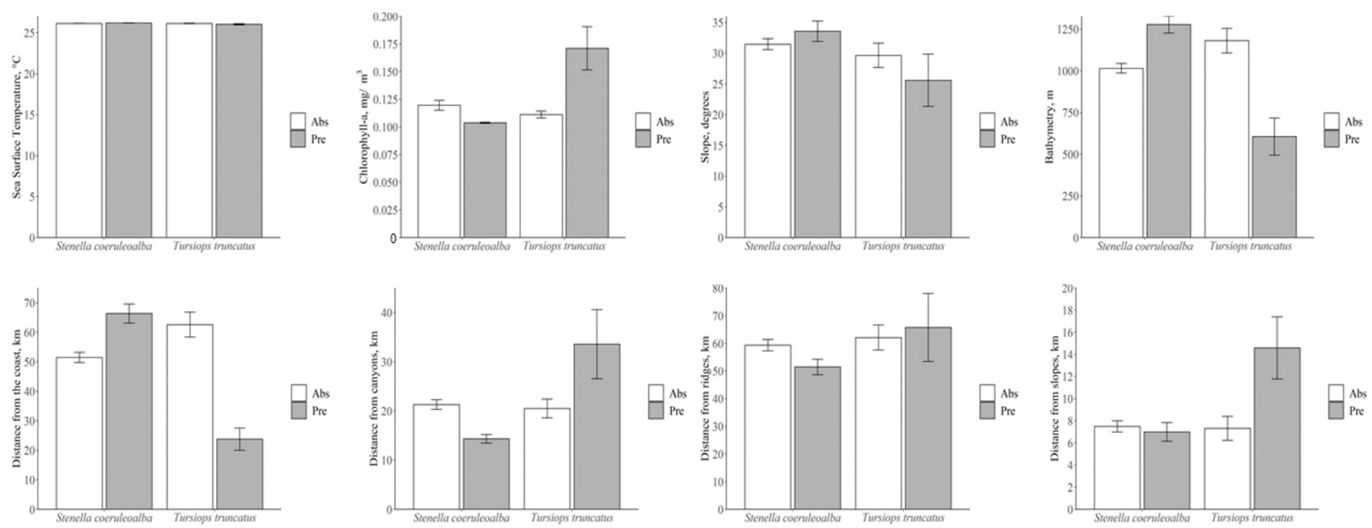

Fig. 3. Mean values of the environmental variables used to model Sc and Tt habitat suitability. White and grey columns represent respectively mean values for the pseudo-absence and presence cells.

located within 1000 m of depth.

3.2. Habitat suitability

The Habitat Suitability analyses showed for Sc a selection for areas furthest from the coast (MW, $p < 0.001$), with higher depths (MW, $p < 0.001$), nearest to canyons (MW, $p < 0.05$) and with water countersigned by lower mean CHL-a concentration (MW, $p < 0.001$). Most of the presence cells were in water with a depth > 1000 m, at a distance >40 km to the nearest coast (Fig. 3). Tt presence cells showed opposite features. These were indeed characterized by lower bathymetry (MW, $p < 0.001$) and distance from the coast (MW, $p < 0.001$) with respect to the pseudo-absence cells, and higher values of distance from slopes (MW, $p < 0.05$). Considering the environmental features of the presence cells only, the majority of them were characterized by bathymetry values either from 0 to 200 m or beyond 800 m within approximately 20, 60, 33, and 14 km of the nearest coast, ridge, canyon, and slope respectively (Fig. 3). No collinearity among variables was detected, and all VIF values were under 6. Best models results are shown in Table 2.

Sc single models, and in particular MaxEnt, had better performance with respect to the ensemble model with AUC = 0.65 (Table 2). Sc presence probability in the study area was mainly driven by bathymetry, distance to the nearest ridge, CHL-a concentration, and SST while bathymetric slope, distance to canyon, coast, and slope were less relevant in the determination of this species habitat. Sc presence probability was almost evenly distributed in the northern part of the study area, with higher values in its north-eastern sector in the south Tyrrhenian. Less suitable habitats were instead all the coastal areas, the shallow portions of the Sicily Channel, and in the small pelagic area south-east of Capo Carbonara MPA characterized by the absence of geomorphic features (Fig. 4).

For Tt, the ensemble model had excellent performance, with AUC = 0.95 (Table 2). For this species, the most important environmental variables shaping the habitat was distance from the coast, followed by distance from slope and ridge and bathymetric slope. SST, CHL-a, bathymetry and distance from canyon were instead less relevant. Tt higher presence probability was found in the coastal areas of Tunisia and Sicily, in the Cagliari gulf and corresponding to the Carbonara ridge (Sardinia), in the Adventure Bank, around Egadi Island, and Ustica's coastal areas, ridge and bank (Sicily). Less suitable habitat was instead represented by

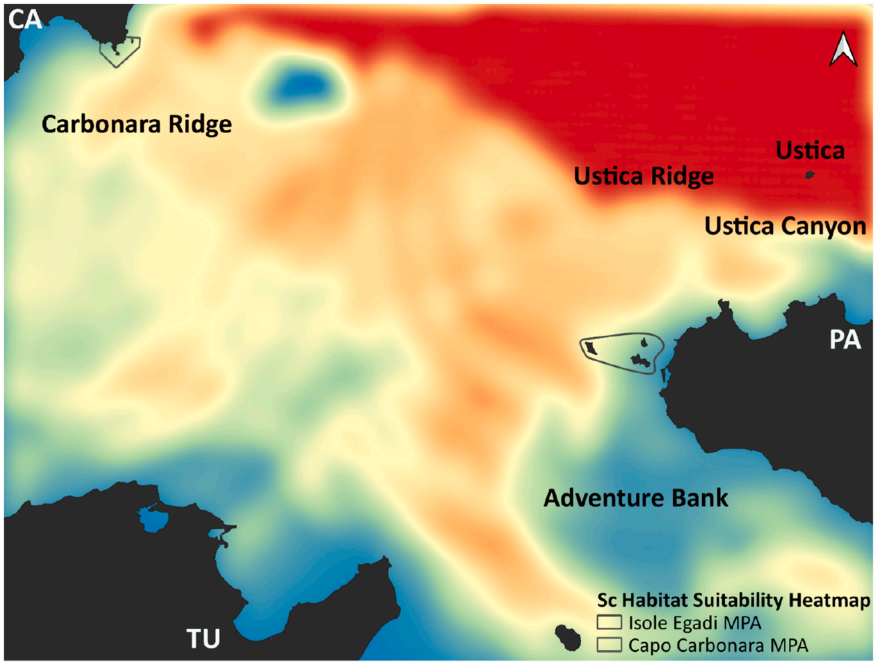

Fig. 4. MaxEnt of *Stenella coeruleoalba* probability of occurrence in the study area for the Summer season.

Table 2
Biomod2 best models results for Sc and Tt.

Species	Model	AUC	TSS	Sensitivity	Specificity
S. coeruleoalba	MaxEnt	0.65	0.29	94.59	34.95
T. truncatus	ensemble	0.94	0.82	93.93	88.28

(AUC = Area Under the ROC Curve; TSS = True Skill Statistics (sensitivity+-specificity-1)).

the central part of the study area (Fig. 5).

Fig. 6 showed the portions of the study area where it is more likely to find both species, resulting from the intersection between the two species presence probability: the waters of the north-eastern and of the north-western sectors, respectively around Ustica Island and near Sardinia, together with Castellammare Gulf in Sicily. Those areas are characterized by high bathymetry values (mean value >500 m) and by the presence of several geomorphic features, including slopes, ridges, and canyons. Moreover, the entire Ustica MPA (Sicily) and a portion of the Capo Carbonara MPA (Sardinia) fall in the detected portions of the study area.

3.3. Floating plastic and risk assessment

The marine litter monitoring was carried out from 2015 to 2019

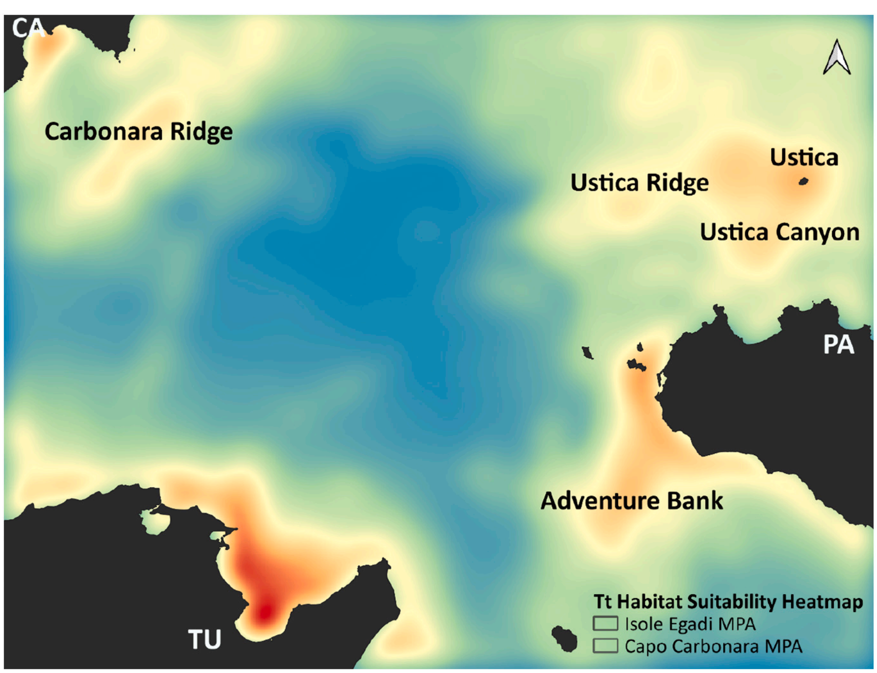

Fig. 5. Ensemble model of *Tursiops truncatus* probability of occurrence in the study area for the Summer season.

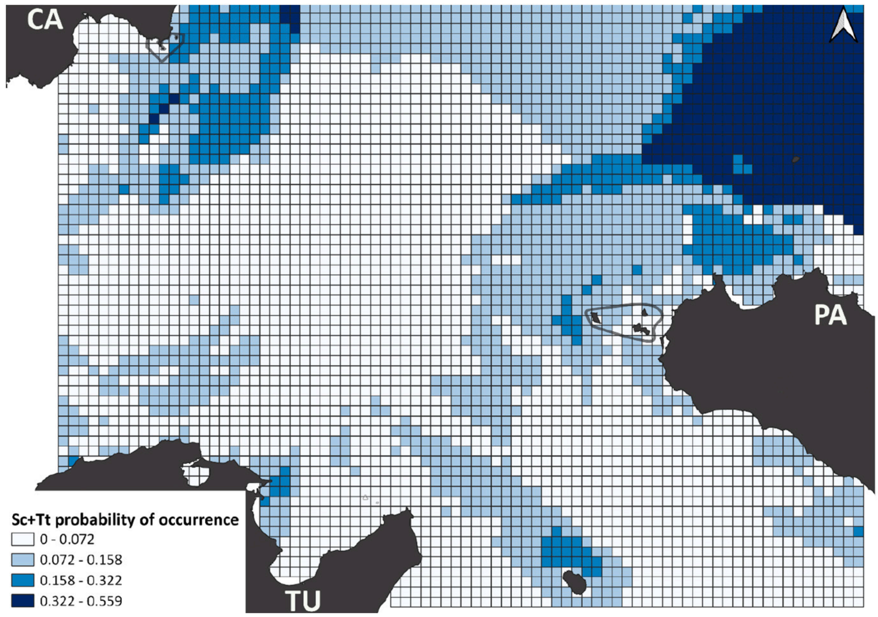

Fig. 6. Sc + Tt concurrent probability of occurrence in the study area for the summer season. Grey contours represent the Capo Carbonara (Sardinia) and Isole Egadi (Sicily) MPAs.

along the PATU and CAPA routes. For the seasonal risk evaluation, only the data recorded along the PATU transects were characterized and analyzed. During the years of monitoring (2016 - 2019), almost 19,600 km of effort have been traveled, and 3572 marine litter items were recorded (Table 1, Supporting Materials).

Of these, 84% was composed by artificial polymer materials. Plastic was the principal recorded fraction in all years and seasons, representing always more than the 75% of the total amount of litter recorded (Fig. 7). Plastic density in 2018 is significantly lower than 2017 and 2019 (MW, $p < 0.05$). No differences were found between seasons (KW, $p > 0.05$).

Among the artificial polymer materials, the most recorded subcategories were shopping bags ($N = 645$, 22%), plastic sheets ($N = 460$, 16%), bottles ($N = 425$, 14%), buoys ($N = 234$, 7%), and polystyrene boxes ($N = 213$, 7%), followed by tableware, nets and lines, jerry cans, buckets, and plastic boxes. Density values do not differ between seasons with the only exception for that of the buoys and of the beach and coastal amenities, higher during summer and autumn, and summer respectively (MW, $p < 0.05$) (Fig. 5, Supporting Materials).

The second most abundant observed fraction was the organic material, followed by paper and processed wood. Rubber, glass, metal and textile were instead the less present (Table 3 and Fig. 7). No significant seasonal differences in their density values were found with the exception of the paper category, higher in summer with respect to winter or autumn (MW, $p < 0.05$).

Fig. 7. Marine litter categories percentage composition in the different seasons.

The Nearest Neighbor Analysis for artificial polymer materials sightings showed that they had clustered patterns in all seasons (Nearest neighbor index <1). Areas with higher density values based on Kernel analysis and validated by the Gi* analysis changed slightly as seasons proceed. During Winter, plastic accumulation was concentrated in the water outside Palermo harbor while, during Spring, it expanded a little toward the west. Over Summer, in addition to the hotspot localized in the Carini Gulf, another area with high plastic density values is found in the Tunis Gulf. These two hotspots lasted until Autumn (Fig. 8).

The risk analysis identified the waters outside Palermo harbor until Castellammare Gulf and the Egadi Island as the areas of particular risk for Sc of exposure to plastic threats in almost all seasons (cells colored in yellow and red in Fig. 9). During Winter and Spring, even the Tunis Gulf became a potentially dangerous area for this species. For Tt, one of the area of major risk was located outside Palermo harbor. During Spring and Autumn, the Egadi Island became an area of particular risk of exposure, while higher risk values were detected during Winter and summer near the Tunis Gulf (cells colored in yellow and red in Fig. 10).

4. Discussion

An effective management of wildlife populations requires robust evidence of species distribution and their threats. In the general framework of knowledge of cetacean spatial distribution in the Mediterranean Sea, the SSCC are still areas with scarce information about species distribution and habitat preferences (di Sciara and Birkun, 2010), and with scant evidence about the potential sources of risk generated by plastic pollution. The present study helped fill these gaps of knowledge. The 7-years of monitoring revealed a constant presence in the SSCC of at least 8 cetacean species (Sc, Tt, Dd, Bp, Pm, Zc, Gm, and Gg) regularly observed throughout the whole study period. These findings allowed us to derive that these species showed high fidelity for the area at least during the summer season. Moreover, the seasonal analysis performed in the Sicilian Channel confirmed the presence of at least four of these species (Sc, Tt, Dd, and Gg) almost all-year round, with only the last one absent during Winters. During the study period, various sightings of mixed groups were recorded. The most common association found in this study (Sc + Dd and Tt + Gg) were documented also in other areas of the world, like the Gulf of Corinth (Frantzis and Herzing, 2002), the Alboran Sea (García et al., 2000), and off southern California (Bacon

Table 3
Seasonal and yearly characterization of the recorded marine litter categories.

Material	Seasons								Years							
	Winter		Spring		Summer		Autumn		2016		2017		2018		2019	
	N obj	%	N obj	%	N obj	%	N obj	%	N obj	%	N obj	%	N obj	%	N obj	%
Artificial polymer materials	543	**75.21**	989	**81.00**	663	**87.47**	735	**84.39**	307	**90.56**	1002	**82.33**	1086	**79.44**	535	**82.43**
Organic	114	**15.79**	124	**10.16**	42	**5.54**	56	**6.43**	15	**4.42**	134	**11.01**	142	**10.39**	45	**6.93**
Paper	25	**3.46**	52	**4.26**	26	**3.43**	34	**3.90**	7	**2.06**	39	**3.20**	58	**4.24**	33	**5.08**
Rubber	6	**0.83**	6	**0.49**	2	**0.26**	1	**0.11**			4	**0.33**	4	**0.29**	7	**1.08**
Glass	4	**0.55**	16	**1.31**	2	**0.26**	4	**0.46**			6	**0.49**	14	**1.02**	6	**0.92**
Metal	4	**0.55**	9	**0.74**	5	**0.66**	4	**0.46**	2	**0.59**	7	**0.58**	10	**0.73**	3	**0.46**
Processed wood	18	**2.49**	20	**1.64**	14	**1.85**	26	**2.99**	8	**2.36**	21	**1.73**	34	**2.49**	15	**2.31**
Textile	8	**1.11**	5	**0.41**	4	**0.53**	11	**1.26**			4	**0.33**	19	**1.39**	5	**0.77**

Bold numbers in the table represent the percentage of marine litter items belonging to a particular category over the total number of items collected.

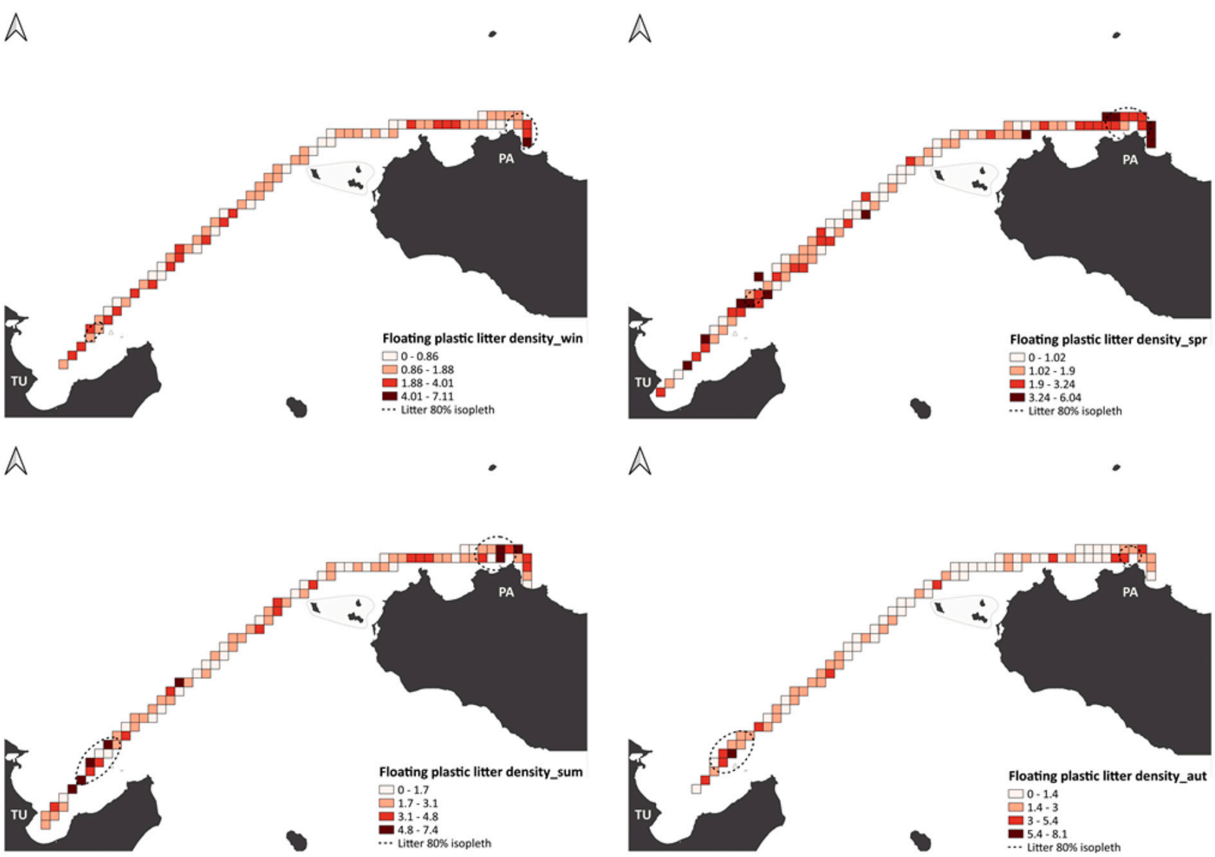

Fig. 8. Cumulative floating plastic litter density$_{cell}$ during the 4 seasons. Dotted line represent the 80% isopleth.

et al., 2017).

In the study area, the Odontocetes Sc and Tt were the most sighted species considering both annual and seasonal sighting data, even if variation in the abundance index values were found for both species. Both Sc and Tt showed a clustered pattern along the routes, despite having different seasonal distribution. Our data and models confirmed what we know about the habitat preferences of these two species: Sc seasonal hotspots were mainly linked to submarine canyons (Carlucci et al., 2018; Kenney and Winn, 1987; Mussi and Miragliuolo, 2003), while those of Tt were mostly detected in shallow waters (Benmessaoud et al., 2012; Alessi et al., 2019; di Sciara, 2002). This may be related to the species feeding habits, preying mostly on benthic and demersal fishes (Blanco et al., 2001; Santos et al., 2001).

Between the less common species, Bp was the recorded in the study area almost exclusively in the north-western pelagic sectors, in accordance with previous study (Aïssi et al., 2008; Canese et al., 2006; Celona and Comparetto, 2006). Deep and offshore waters are in fact the usual favorite habitat of Bp, found mainly in the western and central portion of the Mediterranean Sea; nevertheless, this species can occur in slope and coastal waters depending on the distribution of its prey (di Sciara, 2002; Panigada et al., 2005, 2008). In general, however, the use of this area as passage way for the seasonal latitudinal movement in the western Mediterranean basin was documented by different studies (e.g. Marini et al., 1996; Canese et al., 2006; Panigada et al., 2017) and findings of our study are in line with a relative low permanence of the species in this areas.

Dd was recorded in both coastal and pelagic habitats, as expected giving the mainly epipelagic and mesopelagic fish prey species (Silva and Sequeira, 1996; Ohizumi et al., 1998; Neumann and Orams, 2003). This species, once widespread and abundant in the Mediterranean Sea, has suffered a dramatic decline in the last decades (Bearzi et al., 2003). Indeed, it disappeared from wide portions of the basin even if, to date, it

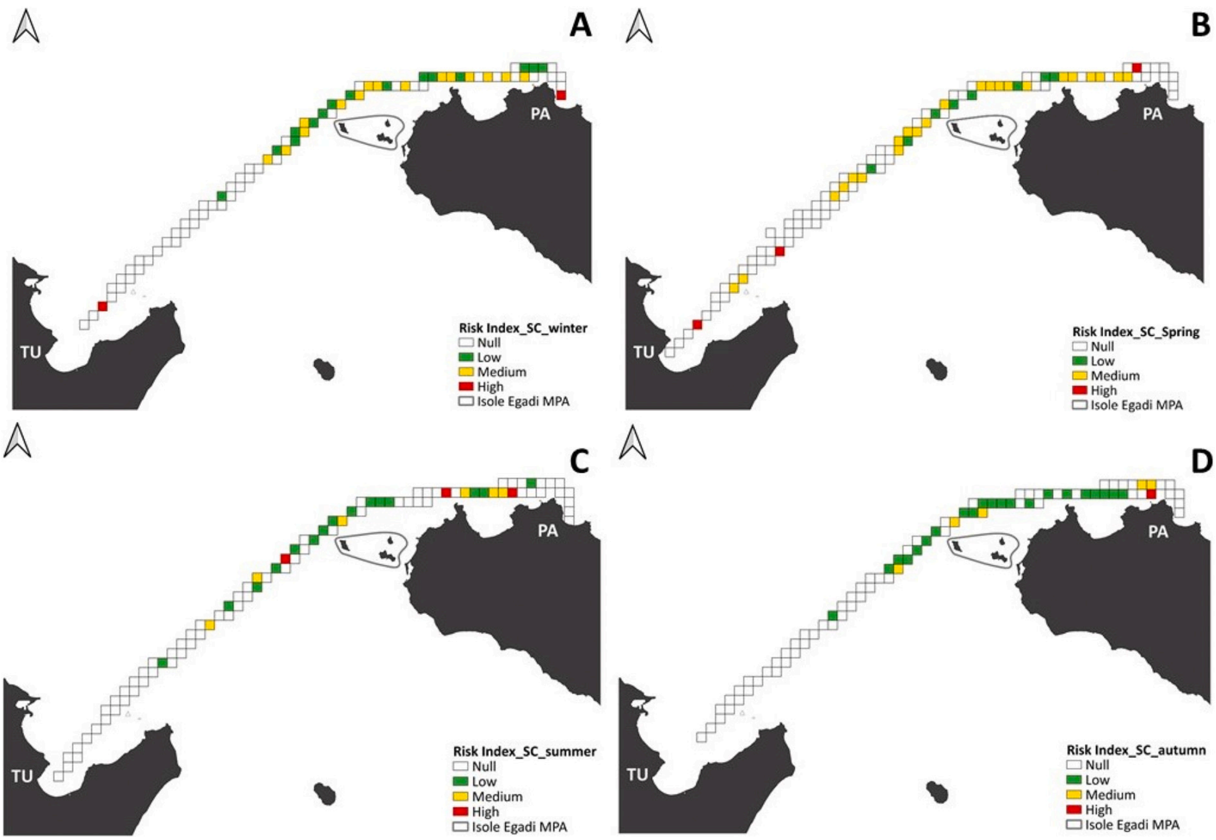

Fig. 9. Sc Risk Index per cell along the PATU route for A) Winter, B) Spring, C) Summer and D) Autumn. The four different levels of potential exposure to plastic (Null (white), Low (green), Medium (yellow) and High (red)) are obtained multiplying four classes of the SPUE $_{cell}$ values with the correspondent classes of Plastic Density $_{cell}$. The grey line identifies the Isole Egadi MPA. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

is more common in isolated clusters mostly in the westernmost portion of the basin, including the ones recorded in the Sicily Channel. Coastal groups of Dd can occasionally share their habitat with Tt, while the pelagic ones with Sc (Bearzi et al., 2003). This particular association occurred mostly at the Mediterranean northernmost latitudes (Cañadas and Hammond, 2008; Frantzis and Herzing, 2002; Pace et al., 2015; Arcangeli et al., 2017), where Dd is less abundant and cannot form single species schools. In the southern Tyrrhenian basin instead it is more present (Pace et al., 2015), and associations are less recorded (Santoro et al., 2015). Along the studied transects, in fact, the majority of Dd sightings were of single-species groups ($N = 14$), with eight Dd + Sc associations.

Gg was mainly recorded around the Egadi waters along the 600 m isobath, confirming the typical pelagic behavior reported for this species, usually sighted in deep areas between 500 and 2000 m, mainly over steep shelf slopes and submarine canyons (Azzellino et al., 2008, 2012, 2016; David and Di-Méglio, 2012). Similarly, Pm was mainly found near the underwater canyons south of Capo Carbonara, the typical habitat of its favorite preys, the cephalopods (Pace et al., 2018, 2019; Claro et al., 2020; Pirotta et al., 2020). Zc was seen halfway between Sardinia and Sicily, an area previously identified by the models of Cañadas et al. (2018) as suitable for this species. In the Mediterranean Sea, Gm is found most exclusively in its western portion (Boisseau et al., 2010; di Sciara and Birkun, 2010; Verborgh et al., 2016), with very sporadic records around the isle of Malta (Metzger et al., 2015; Environment and Resources Authority (ERA), 2020). In this framework, and although it was a single sighting, the record of Gm close to the canyon system of the Egadi Island (but in relatively shallow waters, 262 m) add new information about this species occasional presence.

4.1. Habitat suitability

The best prediction of performance was displayed by Tt model, with distance from the coast as the most important contributing variable, in line with the typical coastal habitat of the species. Nevertheless, in the northern sector of the study area, Tt appears to explore deeper sea sites far from the coasts and close to ridges and canyons. Ridges are continuous submarine mountain chains, and together with isolated sea mountains can be hotspots of biodiversity and can affect the productivity of offshore ecosystems, as well as the distribution of top predators and hence of Tt (Shank, 2010; Greene et al., 1992; Vetter et al., 2010; Morato et al., 2010; Fiori et al., 2015; Cañadas et al., 2002). Another factor that could lead Tt outside its preferred habitat can be the disturbance due to the increased coastal marine traffic in the study area during the summer season (Haviland-Howell et al., 2007; Marley et al., 2017; Nowacek et al., 2001).

Sc in general prefers areas characterized by high deep values; the pelagic environment is in fact the favorite habitat of the species throughout the Mediterranean Sea (Forcada et al., 1994; Gannier, 2005; di Sciara et al., 1993; Carlucci et al., 2016). Within these areas, Sc presence probability appeared to be driven by the distance from the nearest ridge, likely for the same reasons as Tt. Also SST appears to drive Sc spatial distribution, and indeed, in this study, the species showed preference for surface temperatures between 25 and 27 °C, as reported in the ADRION region (Azzolin et al., 2020). However, in other regions of the Mediterranean Sea like the Ligurian Sea, Sc shows a preference for lower range of SST between 22 and 24 °C, probably due to latitudinal differences (Panigada et al., 2008).

The intersection analysis between Tt and Sc more suitable summer habitats showed an overlap, when probably Tt exploit Sc traditional

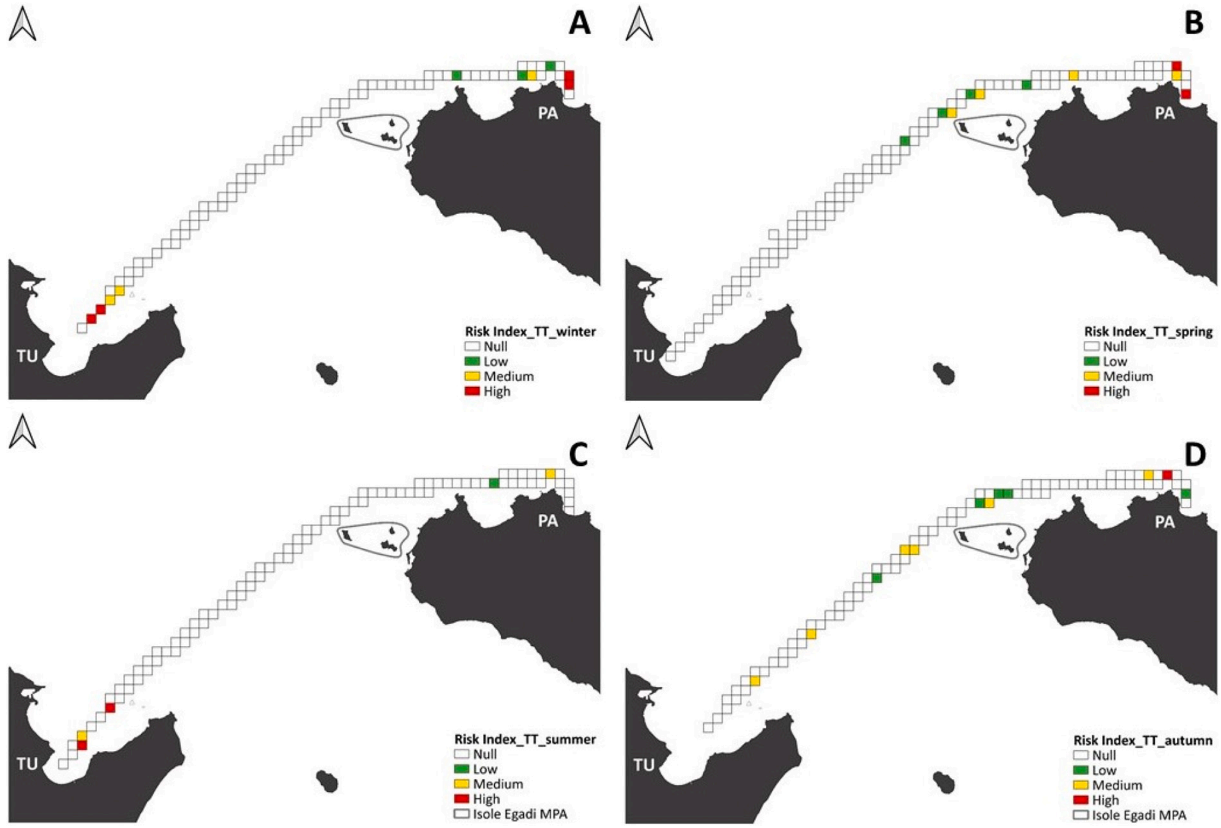

Fig. 10. Tt Risk Index per cell along the PATU route for A) Winter, B) Spring, C) Summer and D) Autumn. The four different levels of potential exposure to plastic (Null (white), Low (green), Medium (yellow) and High (red)) are obtained multiplying four classes of the SPUE $_{cell}$ values with the correspondent classes of Plastic Density $_{cell}$. The grey line identifies the Isole Egadi MPA. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

habitat for feeding purposes. Tt excursions from coastal to pelagic areas is also documented in the western Mediterranean Sea (Gnone et al., 2011; Arcangeli et al., 2017), and it is well known that this species is an opportunistic feeder that can vary its diet according to the availability of the most abundant and catchable prey (Klinowska, 1991). A small difference in prey preference may be enough to support the feeding requirements of more than one species, allowing sympatric dolphins to coexist (Hoelzel et al., 1998); otherwise, competition for the same prey may arise.

4.1.1. Model rationale and limitation

Despite several discussions within the scientific community regarding the predictive power and stationarity of SDMs, single-species distribution models have been and will continue to be invaluable tools for conservation applications (Baker et al., 2021). Nonetheless, there are many potential sources of bias that we need to control to fix the reliability of the modelling effort. For example, SDMs often rely on the collection of both real presence and absence data (Brotons et al., 2004). In the study cases with vagrant and elusive species, such as cetaceans, to get reliable absence data is complicated by the mobility and wide home range that makes it difficult to spot them on the water surface (availability bias). Although mistake rate decreases with observational effort (Barbet-Massin et al., 2012), the correct attribution to the "true" absences (where animals are actually not present) and "false" absences (where animals are present but undetected) is however difficult and the analysis may be impaired by a certain uncertainty degree that should be quantified before to interpret results (Hall, 2000; Martin et al., 2005). There are a number of statistical adaptations to reduce this inherent uncertainty. The random selection of a number of cells, for example, is used to establish where no presence was recorded equal to the number of presence cells (Azzellino et al., 2012; Carlucci et al., 2016; Vassallo et al., 2018) or almost three times higher (Smith, 2010; Arcangeli et al., 2016) or incorporating the survey effort in the definition of absences (Phillips et al., 2009; Gu and Swihart, 2004). In cetacean studies, true absences are usually not available and thus, for the present study, we generated inferred absence data as the cells with the highest survey effort where animals were not detected, and selected among them a number almost three times higher than that of the presence cells. This definition of inferred absence data assumed that the selected cells were close to the real absence data, since they were surveyed several times without the species being detected.

We are aware that, having considered only the environmental features of the study area, our modelling results represent the purely potential suitable habitats of the species, not considering the influence that human activity could have on their presence and distribution. Moreover, in this study only summer suitable habitats were modelled. The other seasons were excluded from the analysis due to the limited number of sightings, not sufficient to adequately sample the study area.

4.2. Marine litter and risk assessment

The marine litter monitoring carried out along one of the analyzed transects underlined that plastic was the most abundant fraction in all years and seasons considered. Those results are in line with the previous field studies in the area (Suaria and Aliani, 2014; Arcangeli et al., 2018). In particular, the most recorded plastic objects were shopping bags, plastic sheets, bottles, buoys and polystyrene boxes, and the majority of these items was smaller or equal to 50 cm. Even if few studies mentioned the specific object ingested, these kind of items (especially plastic bags and sheets) are the ones that could cause cetacean fatal gastric obstructions (Alexiadou et al., 2019; Roman et al., 2021).

Some of the most important Italian fisheries exploit the Sicily Channel area, and this is probably the cause of the high occurrence, in all seasons, of abandoned buoys and polystyrene boxes. This can explain also the seasonal presence of FADs (Fish Attractive Devices), traditionally used in the southern Mediterranean waters to attract pelagic fishes. This kind of floating objects could be very dangerous for marine megafauna, that could be trapped in their ropes and then have serious problems of movements.

The semi enclosed seas like the Mediterranean Sea had particularly high concentrations of marine debris (Lebreton et al., 2012; Cózar et al., 2015), and plastic accumulation is known to occur in different areas. Nevertheless, no evidence of big and stable "garbage patches" are known for the Mediterranean, and plastic accumulates but then distributes with currents through mesoscale processes (Mansui et al., 2015; Liubartseva et al., 2018; Arcangeli et al., 2018). In this study, the only statistically significant detected plastic accumulation area that lasted during all seasons was localized near the gulfs of Palermo and Carini (Sicily), whereas the one in the Tunis gulf appears during the Summer and Autumn only. Those results are consistent with the study of Liubartseva et al., 2018, that classified the gulfs of Palermo and Tunis between the areas with higher sea surface plastic density. Also Suaria and Aliani, 2014 found the highest anthropogenic litter density along the North-Western African coasts.

Those same areas were identified as the ones of major risk for both cetacean species considered, together with the waters around Egadi Island and the Castellammare Gulf for Sc. The region of the Sicily Channel, and the Tunisian and Sicilian coasts were already identified by the models of Soto-Navarro et al., 2021 and Compa et al., 2019 as areas of medium-high potential risk of plastic ingestion in general for pelagic species and in particular for marine mammals.

4.3. Conclusion and implication for cetacean conservation

The study area provides a migratory corridor and nursing and foraging grounds for 8 species of cetaceans. The coastal waters of Kelibia (northeast Tunisia) are recently classified as IMMA (Important Marine Mammals Area), because they support a resident subpopulation of Tt that consistently occupy the area and appears to have long term fidelity. Moreover, the Marine Mammal Protected Area Task Force individuates two more Areas of Interest (AoI): the Egadi Island (Sicily) and the Bay of Bizerte (Tunisia). Those AoI are considered to be of interest for potential marine mammal conservation, requiring enhanced effort for monitoring those species, and may be future candidates in becoming IMMAs.

This study corroborates the hypothesis about the importance of the waters near the Egadi Island MPA for cetacean species. Furthermore, the Tunis gulf, in addition to the Bay of Bizerte and Kelibia, were added as areas of particular interest for Tt. Moreover, the outcome of the study emphasizes the relevance of the northern sector of the study area, in particular near the Carbonara and Ustica Ridges, as aggregation zones of multiple marine mammal species at least during the Summer season. Further analysis, to be conducted throughout the years, is needed to investigate if this condition is maintained.

Despite the growing concern of the adverse effect of marine litter and potential effects on ecosystems, the 'Risk Assessment' topic is still underrepresented (Maes et al., 2019). The identification of risk areas where marine fauna is mostly exposed to litter is the first step to prioritize conservation measures on the higher risk contexts. However, to predict the areas where the animals are most likely to be affected by the risk linked to marine litter is challenging as the needed data on spatio-temporal distribution of the pressure and the vulnerable species are difficult to collect. Most of the animals vulnerable to entanglement or ingestion are highly migratory (e.g. seabirds, sea turtles, and marine mammals) and tend to be scattered across marine areas. On the other and, in the Mediterranean Sea there are no permanent structure able to retain floating items in the long-term (Mansui et al., 2015; Zambianchi et al., 2017; Liubartseva et al., 2018; Mansui et al., 2020) so that the hazard debris is scattered over broad areas, with high seasonal variability both in the amount and composition of items (Darmon et al., 2017; Fossi et al., 2017; Arcangeli et al., 2018; Campana et al., 2018). As a consequence, the interactions between the vulnerable species and the pressure is possible almost anywhere in the species range, but with different intensity depending on areas and seasons. By building a spatially explicit risk index based on plastic density value and vulnerable species encounter rate this study individuated area/season at higher exposure risk for cetacean in the SSCC, taking in consideration also the presence of the most harmful items.

Moreover, integrating species distribution information into marine spatial planning (both inside and outside MPAs) is essential for understanding the risk represented by anthropogenic activities impacting cetacean populations (Azzellino et al., 2012; Cañadas et al., 2005). Results of this study can contribute to design strategies whose ultimate purpose is to protect cetacean species, such as implementing regulations for marine traffic or reducing the impact of fishing activities in the more important areas and seasons for the species, or even individuating new areas to protect. This study is the first to model the potential suitable habitat of the two most abundant cetacean species in the SSCC, hence representing a great improvement for cetacean knowledge in this region.

CRediT authorship contribution statement

M. Gregorietti: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing. **F. Atzori:** Validation, Investigation, Resources, Writing – review & editing. **L. Carosso:** Formal analysis, Investigation, Data curation, Writing – review & editing. **F. Frau:** Investigation, Data curation, Writing – review & editing. **G. Pellegrino:** Investigation, Data curation, Writing – review & editing. **G. Sarà:** Resources, Writing – review & editing, Supervision, Project administration. **A. Arcangeli:** Conceptualization, Methodology, Validation, Resources, Data curation, Writing – original draft, Writing – review & editing, Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2021.112943.

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Chapter 6

Loggerhead sea turtle, *Caretta caretta*, presence and its exposure to floating marine litter in the Sardinia Channel and the Strait of Sicily: results from seven years of monitoring using ferries as platforms of observation

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Loggerhead sea turtle, *Caretta caretta,* presence and its exposure to floating marine litter in the Sardinia Channel and the Strait of Sicily: results from seven years of monitoring using ferry as platform of observation

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Abstract

The loggerhead turtle is the most common sea turtle species in the Mediterranean Sea. Despite relevant research efforts, information about its distribution is still scarce, particularly in the open sea where they may be exposed to different threats, among which marine litter is of great concern.

Here we investigated the distribution of loggerhead turtles and floating marine macro litter (FMML) in the Sardinia Channel and Strait of Sicily, a key area of the central Mediterranean Sea, by using 7 years of data collected by experienced observers aboard passenger ferries along commercial routes. The high-risk exposure areas were identified and the influence of upper layer currents on turtle and FMML distribution was evaluated. Overall, loggerhead sighting rates were higher than those reported from other Mediterranean routes, but the distribution of turtles within the study area was clearly heterogeneous and influenced by the surface circulation pattern. Summer sighting rates were significantly higher in the Sardinia Channel with respect to the Strait of Sicily. Analysis of the co-occurrence of FMML and loggerhead turtles identified a priority risk area in the central Sardinian channel where the large South-Eastern Sardinia Gyre acts as a trap for both animals and FMML. This study corroborates the effectiveness of using passenger ferries as platforms of observation to conduct systematic surveys of sea turtles and floating macro litter in offshore areas. Results highlighted the importance of the Sardinia Channel and Strait of Sicily for the loggerhead turtle and the areas of greater risk of exposure to the marine litter threat.

Keywords: Loggerhead sea turtle; *Caretta caretta*; distribution; Abundance; marine litter; Sicily and Sardinia channels; sea surface currents; risk exposure; monitoring.

Introduction

Although there are only seven existent species in the world, sea turtles are not a relictual group. These animals have been extremely successful, adapting to the changing environmental conditions over the last 120 million years (Motani, 2009). They exhibit some extraordinary adaptations to an aquatic existence, possess a surprising diversity of life history traits, are important components of marine ecosystems, and occupy unique ecological niches (Renous *et al.*, 2000; Wallace *et al.*, 2010; Hochscheid *et al.*, 2007; Maffucci *et al.*, 2013; Wyneken J. *et* *al.*, 2013,). However, sea turtles are extremely vulnerable to mankind. Fishery bycatch, habitat destruction, marine pollution, and climate change pose constant threats to the survival of these species and have led to their inclusion on most lists of vulnerable or endangered species worldwide (Hamann *et al.*, 2010). Over the last few decades, the impact of marine litter, i.e., man-made waste in the marine environment, on sea turtles has become a major concern (Nelms *et al.*, 2016; Galgani *et al.*, 2019; Claro *et al.*, 2019).

The loggerhead turtle, *Caretta caretta* (Linnaeus, 1758), is the most abundant sea turtle species in the Mediterranean Sea (Casale & Margaritoulis, 2010). Nesting occurs mostly in the warmer eastern basin, but the number of nests documented in the Western Mediterranean has significantly increased over the last decade (Maffucci *et al.*, 2016; Carreras *et al.*, 2018). Older juveniles and adults are found throughout the Mediterranean offshore and coastal waters with abundances that vary across regions and seasons (Bolten, 2003; Casale *et al.*, 2018). On Mediterranean foraging grounds, individuals from local nesting beaches mix with juveniles from the Atlantic RMUs (Regional Management Units) that enter this basin trough Gibraltar and disperse aided by the prevailing surface currents towards both the Western and Eastern Mediterranean Sea (Clusa *et al.*, 2013). Several studies showed that significant inter-basin exchange occurs regularly through the Strait of Sicily, the Strait of Messina, the Strait of Otranto, and the Sardinia Channel (Casale *et al.*, 2018 and reference therein). Juvenile and adult loggerhead turtles are accomplished swimmers, but their movements in the open sea are often associated with mesoscale oceanographic features that concentrate prey and may create temporary foraging hot spots that can be opportunistically used (Bentivegna *et al.*, 2007). Despite being a very well researched species, information about the actual distribution and seasonality of loggerhead turtle presence in the Mediterranean oceanic areas are still scarce and mostly based on bycatch data, satellite telemetry, and mark-release-recapture studies (Casale *et al.*, 2018 and references therein).

Following more than 30 years of conservation efforts, in 2015 the Mediterranean loggerhead sub population was listed as Least Concern by the International Union for Conservation of Nature (IUCN) Red List of threatened species (Casale & Tucker, 2015), a result that is completely dependent upon the maintenance of all ongoing conservation activities. Among the many anthropogenic threats, marine litter is an important stressor (Casale & Margaritoulis, 2010; Gall & Thompson, 2015; Galgani *et al.,* 2019; Claro *et al.,* 2019). Entanglement in derelict nets, traps, strapping bands, or plastic bags are regularly reported and may cause serious injuries leading to maiming, amputation, altered buoyancy, and restricted movements which prevent the turtle from behaving normally and may lead to the death of the individual (Duncan *et al.*, 2017). Ingestion of marine litter is also very common, due to the species' generalist feeding strategy, and can cause gastro-intestinal obstruction, internal injuries, a false sense of satiation, and potential absorption of xenobiotics; Lazar & Gračan, 2011; Casale *et al.*, 2016; Nelms *et al.,* 2016; Matiddi *et al.*, 2017). Regular plastic consumption has been one of the reasons for choosing the loggerhead turtle as an indicator species for monitoring the amount and composition of litter ingested by biota in the Mediterranean Sea within the Marine Strategy Framework Directive (MSFD 2008/56/EC, Descriptor 10 C3; Matiddi *et al.*, 2017). Finally, marine litter may cause the degradation of key habitats and produce wider ecosystem effects which may have strong implications for loggerhead turtle survival (Nelms *et al.,* 2016).

The Mediterranean Sea is one of the areas with the highest concentrations of marine litter worldwide due to its limited sea water exchanges with other oceans, heavy coastal anthropization, intense maritime traffic, and multiple significant inputs from rivers that cross highly urbanized areas (Suaria & Aliani, 2014). Every year, millions of tonnes of litter, mostly plastics (Barboza *et al.*, 2019), end up in the sea mainly through storms, water runoff, recreational activities along the coasts, or by being dumped directly from ships (Jambeck *et al.*, 2015; Galgani *et al.*, 2019). Depending on density and composition, after entering the sea, marine litter items can float at the surface for variable periods of time until they sink to the ocean floor, are degraded, fractionated, or washed ashore (Galgani *et al.*, 2019; Miladinova *et al.*, 2020). The distribution of this floating marine litter is shaped by prevailing winds and surface ocean currents that may carry items very far away from their sources and create transient accumulation areas corresponding to convergent zones, sea water fronts, and eddies (Galgani *et al.*, 2015). These areas are also highly productive and may act as temporary foraging hotspots for loggerhead turtles, which increases the probability of exposure to floating marine litter and hence to correlated threats (Nelms *et al.*, 2016). Nevertheless, empirical data on the spatio-temporal overlap between loggerhead turtles and floating litter in the Mediterranean Sea are still scarce (Casale *et al.*, 2018) mostly because of the high costs involved in at-sea surveys using dedicated observation platforms (Arcangeli *et al.*, 2019).

Since 2013, the Fixed Line Transect Mediterranean Network (FLT Med Net, ISPRA) is gathering systematic data on sea turtles and floating marine macro-litter (> 20 cm, FMML hereafter) distribution along specific trans-border routes in the Mediterranean Sea, using regular passenger ferries as observation platforms. The method proved to be effective for long term monitoring on pelagic species (i.e. cetaceans, sea turtle) and the evaluation of potential threats (i.e. marine litter, maritime traffic) (e.g. Tepsich *et al.*, 2020; Arcangeli *et al.*, 2017; Campana *et al.*, 2015; Pennino *et al.*, 2017). A first synoptic analysis conducted in the Western Mediterranean and Adriatic Sea showed a significant seasonal and regional variability of FMML abundance, distribution, and composition (Arcangeli *et al.*, 2019). This study highlighted also the existence of a previously unreported zone of high loggerhead turtle presence in the Sardinia-Sicilian Channels (SSCC), the southern triangle between Sardinia, Tunisia, and Sicily which is coherent with the recent finding of an important oceanic foraging areas for juveniles and adult sized turtles in the southern Tyrrhenian Sea (Blasi & Mattei, 2017; Luschi *et al.*, 2018; Chimienti *et al.*, 2020). Individuals from this area have been observed to switch to neritic foraging when crossing the Strait of Sicily and reaching the Tunisian Plateau, one of the most important Mediterranean neritic habitats (Chimienti *et al.*, 2020). Overall, the SSCC exhibited the highest loggerhead turtle encounter rates among the surveyed routes in the Western Mediterranean and Adriatic Sea and a strong seasonality in the risk of exposure to floating marine litter (Arcangeli *et al.*, 2019). However, this synoptic study considered the SSCC as a whole, without taking into consideration the complexity of the surface circulation patterns (Sorgente *et al.*, 2011; Pinardi *et al.*, 2015) that may affect loggerhead turtle and floating marine macro litter distribution in the area whose understanding requires finer scale investigations.

In this study, we analysed the data collected along three trans-border transects covering the Sardinian Channel and the Strait of Sicily with the aim to: 1) describe the presence and distribution of loggerhead turtles in this key study area of the central Mediterranean sea over a seven year period (2013-2019); 2) characterize the exposure risk to FMML; 3) understand the influence of the high resolution upper layer currents provided by the E.U. Copernicus Marine Service on the distribution of loggerhead turtles and FMML items in the study area.

Material and Methods

Study area

Data were collected along 3 trans-border transects covering the Sardinia-Sicilian Channels (SSCC), in the area between Sardinia, Sicily and Tunisia (Fig. 1). The study area is defined as a key region for understanding the exchanges between the Eastern and Western Mediterranean basins through the Strait of Sicily and between the Algerian Basin and Tyrrhenian Sea through the Sardinia Channel (Astraldi *et al.*, 1998; Onken *et al.*, 2003).

The Strait of Sicily is a geomorphologically complex area, characterized by several sea mountains composed of sedimentary and volcanic rocks (Civile *et al.*, 2016). There is no universally accepted definition of this important strait that covers a great part of the Central Mediterranean and corresponds to the westernmost part of the subarea 2.2 of the FAO (Food and Agriculture Organization) area 37 (Fig. 1). The topography affects the currents resulting in substantial upwelling, which increases the overall productivity and makes the strait one of the most important biodiversity hotspots in the Mediterranean basin (Di Lorenzo *et al.*, 2018).

Contrary to the Strait of Sicily, which does not reach high depths, the Sardinian Channel extends to a depth of 2,500 m and represents an important passage for marine species, such as large cetaceans, sea turtles, and seabirds (Coll *et al.*, 2010; Arcangeli *et al.*, 2019). In addition, the area acquires greater ecological value due to the presence of two marine protected areas (MPAs) at the two

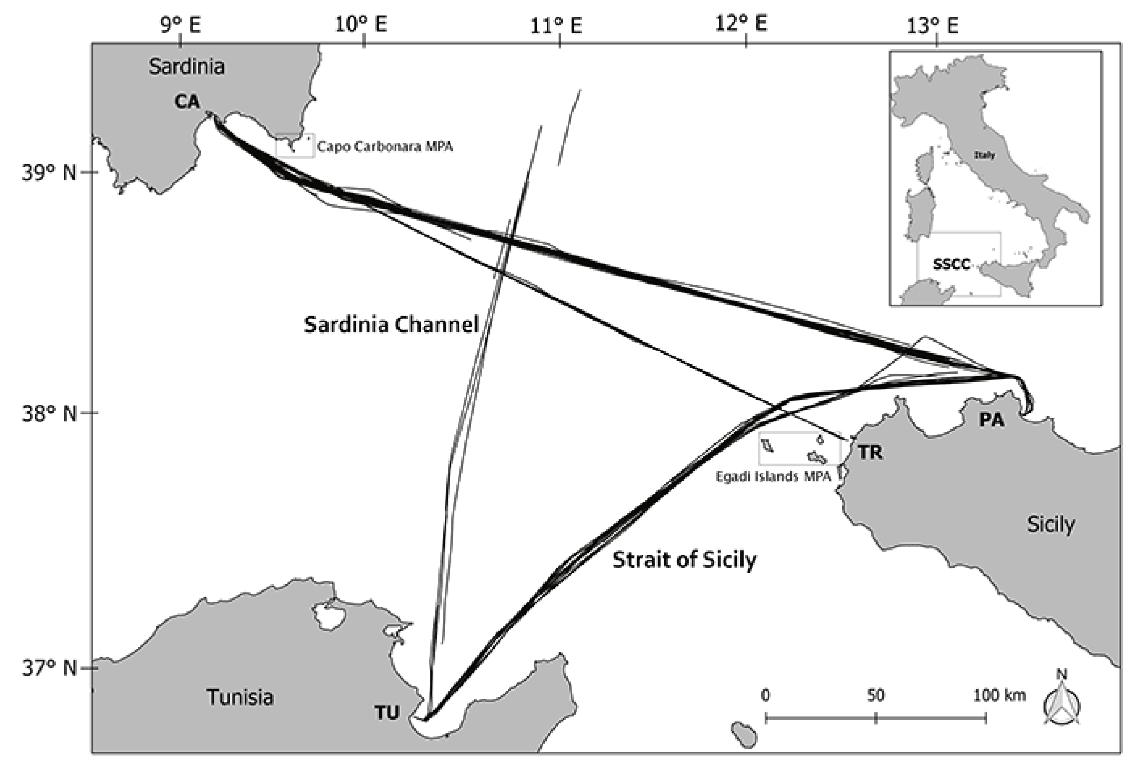

***Fig. 1**:* Study area in the Sardinia-Sicilian Channels (SSCC) (top right box) and effort performed along the surveyed transects (black lines).

borders of the Sardinia Channel: Capo Carbonara MPA (south-eastern coast of Sardinia) and the Egadi Islands MPA (north-western coast of Sicily).

Data collection on sea turtle and marine litter data

Data were collected using ferries as observation platforms, allowing easy repeated sampling along the same transects in a cost-effective way. The monitored transects were Cagliari-Trapani (CA-TR) and Cagliari-Palermo (CA-PA), linking Sardinia and Sicily Islands; Palermo-Tunis (PA-TU), linking Sicily and Tunisia; Tunis-Civitavecchia (TU-CV), considering the part of the transect falling within the selected area only (SSCC) (Fig. 1). Data was collected during the summer period along the CA-PA and CA-TR transects, and over all the seasons (Winter: January to March; Spring: April to June; Summer: July to September; Autumn: October to December) along the PA-TU and TU-CV transects. Two standard protocols were used for collecting data on cetaceans, sea turtles, other pelagic species, and maritime traffic (ISPRA, 2015a), and for collecting data on FMML over 20 cm size (ISPRA, 2015b; Arcangeli *et al.*, 2020). Sea turtle data were collected from 2013 to 2019, while FMML data were collected from 2016 to 2019.

The monitoring was carried out in good weather conditions (≤ 3 on the Beaufort scale for sea turtles and ≤ 2 for marine litter protocols). Two dedicated GPS devices, one for each subject of monitoring, were used to record the position of detected animals or litter and the track line of effort at the finest resolution. The observations were made by naked eye, using binoculars and cameras, when necessary, to confirm species and group size, or litter composition. The monitoring was carried out by experienced dedicated observers, which were specifically trained based on the protocols for both FMML and macro fauna data collection from ferries. This type of platform provided an observation point at 20–25 m high, travelling at a mean speed of 20 Knots. Two observers, one for each side of the command deck, monitored the macro fauna by scanning the sea from the bow (considered to be 0°) to a maximum of 130° in order to avoid recounting animals. At the moment of sighting, information about animal position, data on angle and distance from the ferry, animal orientation, and size classes were recorded. Animal orientation was considered as the direction identified by the head of the animal at the moment of sighting. Data on sea turtle size (i.e. approximate straight carapace length) was recorded whenever visible from the surface along the Cagliari–Palermo route by experienced observers considering three arbitrarily defined size classes: small juveniles (20-35 cm), juveniles (35-70 cm), and adults (>70 cm). The measures were undertaken using the protocol developed for measuring the size classes for marine litter over the 20 cm length (Arcangeli *et al.*, 2020).

For the FMML monitoring, an additional dedicated observer was positioned on the side of the ferry with better visibility, scanning a fixed strip of 50 m width to detect all FMML items greater than 20 cm (see Arcangeli *et al.*, 2020, for details). Litter items were identified and categorized by size classes, material (Artificial polymer material, Glass, Processed wood, Metal, Textile, Paper, Rubber, Natural debris), and general names, according to the MSFD master list (Galgani *et al.*, 2013).

Data analysis

Sea turtle abundance, distribution, and size

Based on the availability of the data and the type of analysis carried out, different routes and/or time intervals were selected. An abundance index was calculated as Sighting Per Unit of Effort [SPUE= (N / km) × 10] ± SE (Standard Error), where "N" was the number of animals sighted and ''km'' was the distance travelled in effort in good weather conditions. SPUE trends over the study period were analyzed using the summer data alone and dividing the surveyed ferry routes in two groups corresponding approximately to the Sardinia Channel and the Strait of Sicily. Given that the PA-TU route crosses both areas, we used the coordinates of the western most part of the Island of Marettimo, Egadi archipelago, to split the transect in two parts, with the northern falling into the Sardinia Channel (PA-TU SC) and the southern belonging to the Strait of Sicily (PA-TU SS).

Seasonality in SPUE was investigated for the Strait of Sicily only, for which monitoring data were available all year round from 2014 to 2019.

Statistical differences were investigated using the non-parametric Kruskal-Wallis (KW) test with the Bonferroni correction, and the post hoc pairwise comparison of Mann-Whitney (MW) U-test testing the hypothesis of equal medians among samples. All analyses were performed using the software Past 2.17c (Hammer *et al.*, 2001).

Sea turtle spatial distribution was examined by overlapping a grid of 5x5 km cells to the study area and by calculating the SPUE within each cell, using each cell as the statistical unit. The SPUE cell was calculated as: [(number of animals sighted per cell / km of effort within each cell) × 10]. To account for uneven effort, sea turtle analysis was performed only on cells with a minimum sample effort of 10 km. SPUE per cell was calculated over the entire study area, and the Kernel Density Estimation (KDE) was performed based on the SPUE cell, using a search radius of 30 km to show areas of highest probabilities of sea turtle occurrence and identify potential hotspot areas (Arcangeli *et al.*, 2017). Hotspots were then graphically represented by the 75% isopleths.

To verify the influence of the year and the spatial position on the abundance of sea turtles, and highlight potential significant patterns, Generalized Additive Models (GAMs) were performed using the number of individuals per cell as the response variable and considering year, km on effort and the cell's position as the predictor variables. GAM, extension of generalized linear model, is a non-parametric regression technique not restricted by linear relationships and is flexible regarding the statistical distribution of the data (Murase *et al.*, 2009; 2014), that allows non-normal residuals as well as a general links between predictors and the response variable (Chebana *et al.*, 2014). In addition, GAMs use a smooth function to link the dependent variable to the predictors, whose purpose is to highlight significant patterns. Based on the data structure, two families, Zero-inflated Poisson and Negative Binomial, and different values of k were tested. The Explained Deviance (analogous to variance in a linear regression), adjusted r2, AIC and GCV scores were used to validate the models and identify the best model settings. Model analyses were performed using the "mgcv" package of RStudio, version 1.2.5042.

Upper layer circulation modelling and influence on sea turtles

In line with Tintoré *et al.* (2019), the ocean circulation in the area has been represented by the 2013 – 2019 reanalysis subset extracted from the Copernicus Marine Environment Monitoring Service (CMEMS) product (Simoncelli *et al.*, 2019). Along with the other hydrodynamic variables, the service provides daily ocean current data with a horizontal resolution of 1/16° (ca. 6.5 km) over the 72 unevenly distributed vertical levels. The multivariate data assimilation is maintained for vertical profiles of temperature and salinity, and along-track satellite observations of the sea surface height. The upper layer kinematics in the SSCC area (Fig. 1) is computed by vertically averaging the currents over the four model levels of ~ 1.5 m, 4.6 m, 8.0 m and 11.6 m.

Sea turtle orientation data were explored considering the two investigated channel's data separately. In the sea stretch between Sardinia and Sicily, two routes, CA-TR (2013) and CA-PA (2014-2019), were independently analysed. In the Strait of Sicily, only data from the PATU route (2014-2019) were analysed.

Upper layer currents were calculated for each point of the turtle detection by means of bilinear interpolation of the model dataset described above. After that, the currents were averaged along the three transects: CA-TR, CA-PA, and PA-TU, and normalized by the number of observations per each transect using a percentage scale to make them comparable for further analysis.

Floating marine litter and risk exposure

Floating Marine Macro Litter was recorded per material category, but considering that the artificial polymer items are a major threat to sea turtles (93% of the total litter items), the plastic component was used to perform the risk exposure analysis. The FMML and risk analyses were performed using the summer dataset from 2016 to 2019 along the CA-PA and PA-TU routes.

The amount of plastic was normalized by accounting for the effort and calculated as density $[(D) = n / (w \times l)]$ being "n" the number of items recorded, "w" the width of the monitored strip and "l" the length of the surveyed transect (in km) (Matsumura & Nasu, 1997; Thiel *et al.*, 2003; Shiomoto & Kameda, 2005).

Spatial distribution of plastic densities was analysed using QGis 2.14.21. The study area was divided into a 5×5 km grid cell, and only the cells crossed by at least 10 km effort were selected from the entire grid for the analysis in order to account for bias due to uneven effort. A buffer was built around each surveyed track corresponding to the value of the transect width. The buffered tracks were then associated within the intersected cells and pooled together. The total surveyed area, the number of plastic items and the density values were calculated per each cell (Arcangeli *et al.*, 2018). The kernel density estimation was then performed based on the density value per cell using a 30 km radius, and the isopleths on 75% of the total values obtained by KDE of plastic density were created. To highlight the areas of overlap between the concentration of litter and high densities of sea turtles, a KDE analysis was performed based on the SPUE cell values of sea turtles using the same time period as the litter data (2016–2019).

The high-risk exposure areas, where sea turtles are most likely to encounter plastic items (Darmon *et al.*, 2017), were identified by considering only the cells where both effort data on plastics and loggerhead turtles were available. The litter density values and turtle SPUE values were used to calculate an exposure risk index: considering that the exposure changes depending on both the occurrence of the sensitive species and the concentration of the threat (i.e., plastic items), the risk exposure index was calculated by multiplying the litter density value and sea turtle SPUE per cell. For representation, the obtained values were equally divided into four categories of "no exposure", when the sea turtles and/or plastic items were not present, "low," "medium," and "high risk."

Results

Sea turtle abundance, distribution, and size

From 2013 to 2019, a total of 47,564 km were travelled on effort in standard conditions during 205 surveys over the entire study area, and 1,392 loggerhead sea turtle sightings were recorded, with a mean SPUE of 0.23 ± 0.025 SE animals per 10 km of effort.

The majority of these surveys were conducted during the summer period (n = 99, total km travelled on effort = 23,794.76 km) when 1,144 sightings were recorded for a mean SPUE of 0.38 ± 0.05 SE. The distribution of loggerhead turtles within the SSCC area during summer appears to be heterogeneous, with a higher presence in the Sardinia Channel with respect to the Strait of Sicily (average SPUE 0.39 ± 0.05 and 0.17 ± 0.05 SE respectively, KW, $p < 0.05$). No statistically significant inter-annual difference was detected among SPUE summer averages in either of these areas (KW, $p > 0.05$, Fig. 2), with the highest sighting rates recorded in 2015 and 2017 in the Sardinia Channel (SPUE index of 0.60 ± 0.21 SE and 0.49 ± 0.14 SE respectively). The absence of inter-annual differences was confirmed also by the analysis of the yearly average SPUEs available for the Strait of Sicily (KW, $p > 0.05$).

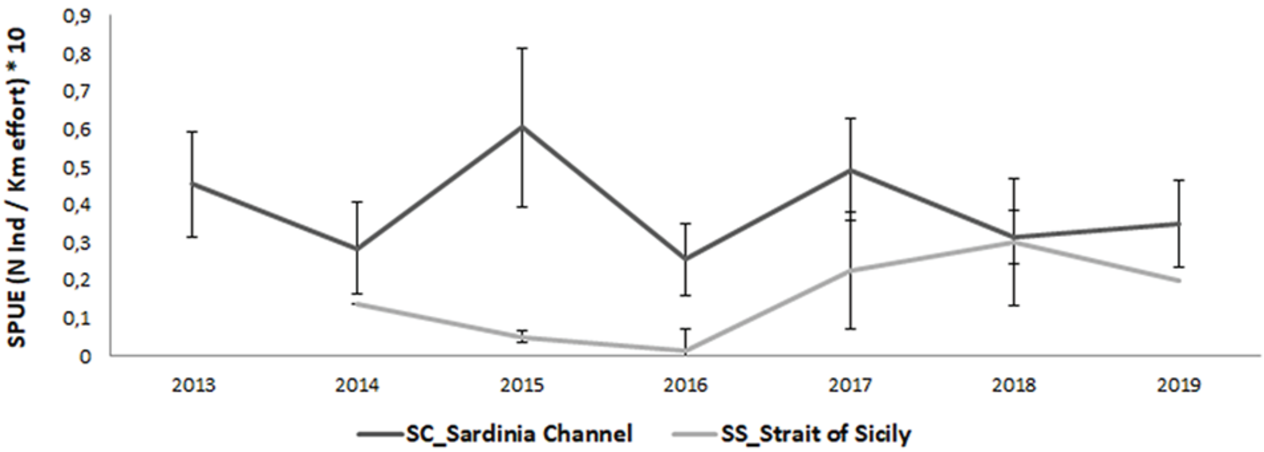

Fig. 2: Sea turtle summer sightings per unit effort (SPUE) recorded during the years (N = 1,144 individuals; 23,794,8 km of effort) in the SC_Sardinia Channel and SS_Strait of Sicily. Error bars represent SE.

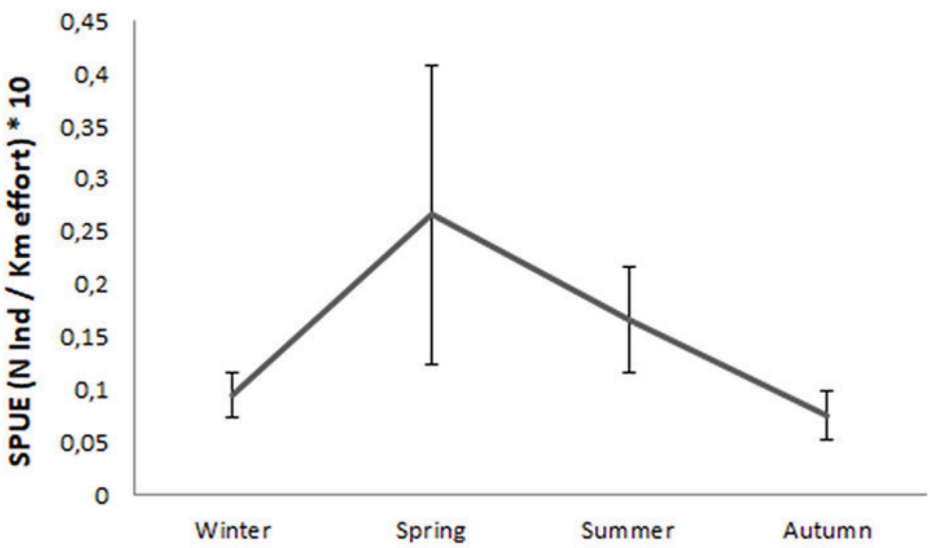

Fig. 3: Seasonal variability of sea turtles detected along the Sicilian Strait.

Intra-annual analysis in the Strait of Sicily showed that the higher sighting rate was recorded during spring (0.27 ± 0.14 SE) compared to summer, winter, and autumn (0.17 ± 0.05 SE, 0.10 ± 0.02 SE and 0.08 ± 0.02 SE, respectively) (Fig. 3), although no significant differences between seasons were detected (KW, $p>0.05$).

The spatial analysis showed a patchy distribution of sea turtles, where cells with higher sighting values were particularly concentrated in the area of the Sardinia Channel and towards Sicily, in the stretch of sea around the Egadi MPA (Fig. 4).

The GAMs were performed considering a total of 851 cells. The chosen model predicted 64.6% (deviance explained) of the variation in space and time of the loggerhead turtle abundance. The shapes of the functional forms for selected covariates illustrated that the number of sea turtles did not change over the years, with only a slight increase in abundance during 2015 and 2017. Results on distribution, using the latitude and longitude as covariates, confirmed the importance of the central area in the Sardinian channel.

A total number of 294 loggerhead turtles were categorized in the three arbitrary size classes along the CAPA route during the summer season from 2017 to 2019: the majority of the individuals were juveniles (56%) followed by adults (26%) and small juveniles (18%).

Model-based upper-layer circulation and sea turtle orientation

As shown in Figure 5a, the upper layer patterns were found to be in line with the basin-scale circulation patterns described by e.g., Malanotte-Rizzoli *et al.* (1997), Sorgente *et al.* (2011), and Pinardi *et al.* (2015).

More specifically, the study area is controlled by the Atlantic waters moving eastward as the Algerian Current (AC) that splits in two branches caused by a topographic effect after crossing the Sardinia Channel. The first branch penetrates into the Tyrrhenian Sea as the Bifur-

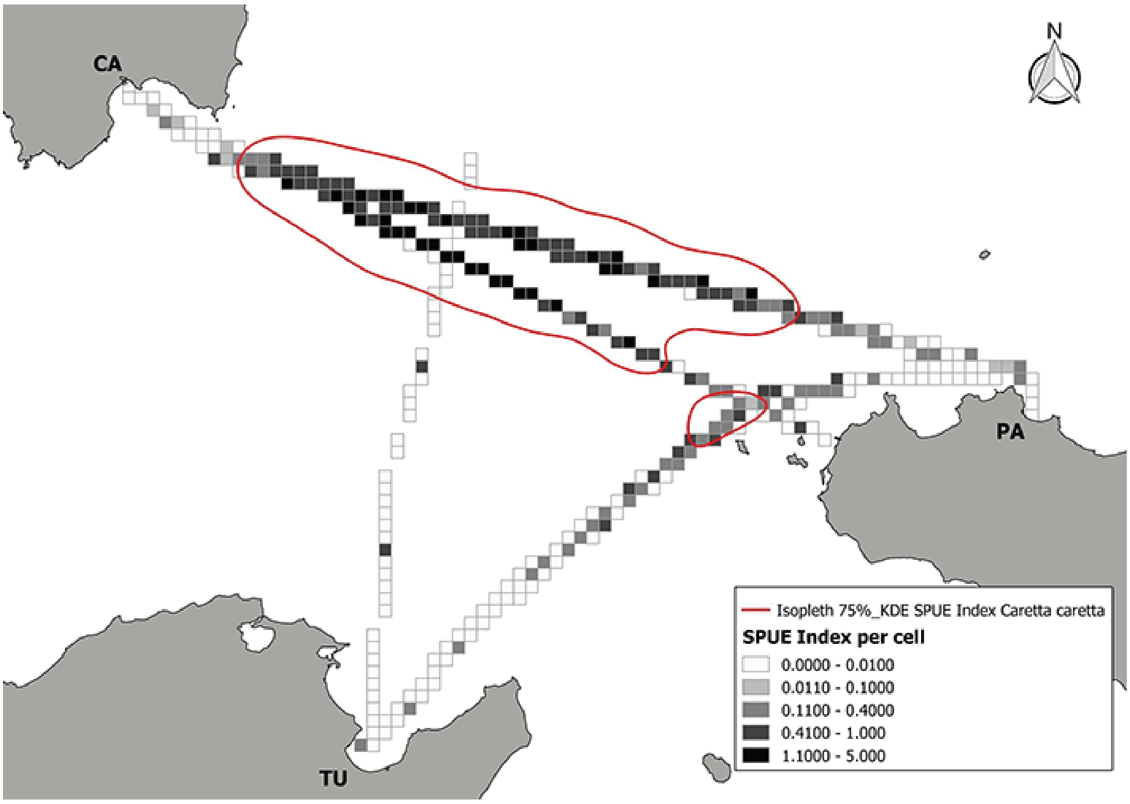

Fig. 4: Spatial distribution of the abundance index (Sightings Per Unit Effort, SPUE) of sea turtles in the monitored areas. The 75% isopleths show the hot spot area of loggerhead turtle identified by the kernel analysis (kernel density estimation; KDE) based on the index of abundance. All data are pooled together.

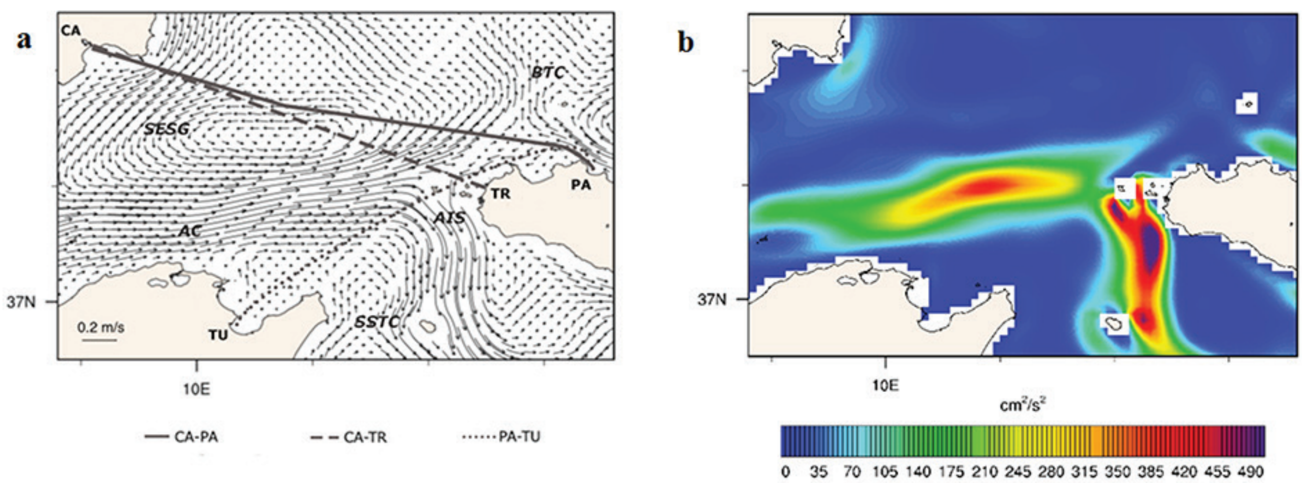

Fig. 5 a: Model upper layer currents averaged over the days of observations with surveyed transects in the Sardinia-Sicilian Channel area (SSCC). The averaged 2013–2019 model currents reveal the Algerian Current (AC), Bifurcation Tyrrhenian Current (BTC), Atlantic-Ionian Stream (AIS), Strait of Sicily Tunisian Current (SSTC) and South-Eastern Sardinia Gyre (SESG); **b:** model-based mean kinetic energy per unit mass (MKE) in cm^2/s^2 averaged over the days of observations.

cation Tyrrhenian Current (BTC) that flows along the northern coast of Sicily. The second one moves eastward representing a two-jet structure that embraces the Atlantic-Ionian Stream (AIS), mainly flowing eastward along the southern coast of Sicily, and the Strait of Sicily Tunisian Current (SSTC) running south-eastward over the Tunisian continental slope. The central part of the area is under the influence of the south-eastern Sardinia Gyre (SESG), a wind curl driven cyclonic gyre, whose diameter varies between 200 km and 300 km (Sorgente *et al.*, 2011).

The model currents averaged over the days of observations (Fig. 5b) indicate the main basin-scale circulation patterns including the Algerian Current (AC), Bifurcation Tyrrhenian Current (BTC), Atlantic-Ionian Stream (AIS), Strait of Sicily Tunisian Current (SSTC), and South-Eastern Sardinia Gyre (SESG). The time-specific differences from the overall 2013–2019 averaged map (Fig. 5a) include a seasonal intensification of the Algerian Current that allows the development of the coastal currents which flow northward in the Gulf of Tunis and along the southern shore of the Gulf of Hammamet; the north-eastern recirculation related to the summer upwelling on the Adventure Bank (western Sicilian shelf); the westward coastal current in the Gulf of Castellammare; as well as amplification of the South-Eastern Sardinia Gyre. Additionally, the kinetic energy of mean flow per unit mass (MKE) was calculated to quantify the energy levels involved in the upper layer of circulation: $MKE_{ij}=1/2meant[u_{ij}^2(t)+v_{ij}^2(t)]$, where meant is the time average over the days of observations; $u_{ij}(t)$ and $v_{ij}(t)$ are the zonal and meridional components of the velocity field at the (i, j) grid point, respectively. As shown in Figure 5b (right), all the observational transects (CA-PA, CA-TR, and PA-TU) were mainly located in the MKE interval of ~ 60–80 cm^2/s^2 which is typical of the summer season, with the exception of the transect parts crossing the high kinetic zones such as the PA-TU segment south-west of the Egadi Islands.

Considering their geographical location and the different ecological characteristics, the orientation data of loggerhead turtles were analysed separately for the two areas. In the area between Sardinia and Sicily, a total of 726 turtles were considered from 2013 to 2019: 143 turtles from CA-TR data (2013) and 583 from CA-PA data (2014-2019). Regarding the CA-TR route, the results showed that turtles were orientated predominantly towards the northwest (~39%) and secondly, towards the southwest (~21%). The upper layer currents along this transect were directed mainly to the southwest (~50%), which was associated with a wide western branch of the South-Eastern Sardinia Gyre, that controlled the water flow over nearly half of the CA-TR transect. Contributions of the northeast transport (~29%) was caused by a relatively narrow jet of the Bifurcation Tyrrhenian Current (Fig. 6a) on the Sicilian edge of the transect. Along the CA-PA route, the turtles mainly oriented towards the west (~37%), while the north, east, and south sectors contributed almost equal percentages (Fig. 6b). Like the aforementioned transect, the currents indicated a dominance of the southwest transport of ~45% on the Sardinian part of the route with a ~35% deflection to the west due to a northern branch of the South-Eastern Sardinia Gyre that influenced the Sicilian edge of the transect. As Figure 6c shows, the distribution of the turtles' spatial orientation along the PA-TU transect (N=195, 2014-2019) was almost isotropic with a slight elongation from southwest (21%) to northeast (19%). Each of the north, northwest, and west directions contributed ~ 9÷11%. Symmetrically, each of the south, southeast, and east directions contributed ~ 8÷12%. The upper layer currents mostly indicated the eastward transport (~56%) caused by the Algerian Current in the Tunisian part of the transect and the northeast direction (~22%), when the PA-TU route was collinear to the Bifurcation Tyrrhenian Current.

Floating marine litter and risk exposure

During the marine litter monitoring in the two channels, almost 10,800 km were travelled on effort covering a total area of 694 km^2. The total number of plastic items recorded was 2,756, with an average density that did not differ significantly over the years (KW, $p>0.05$). The KDE performed on plastic distribution, showed areas of litter accumulation localised near the Cagliari, Palermo, and Tunis ports, and generally high-density values along the Sardinia Channel, to the west of the Egadi Islands and in the southern portion of the Strait of Sicily (black dotted line in Fig. 7). Sea turtle hotspots highlighted by the KDE analysis, were also localised in the middle of the Sardinia Channel and western Egadi Islands (red line in Fig. 7). The overlap between the 75% KDE isopleths of plastic and sea turtles highlighted overlapping areas in the central Sardinia channel and west of the Egadi MPA (Fig. 7). The risk exposure index analysis confirmed highest risk values in the middle of the Sardinia channel, in localized spots near the Ports of Cagliari and Palermo, and in the portion of sea west of the Egadi Islands (Fig.7).

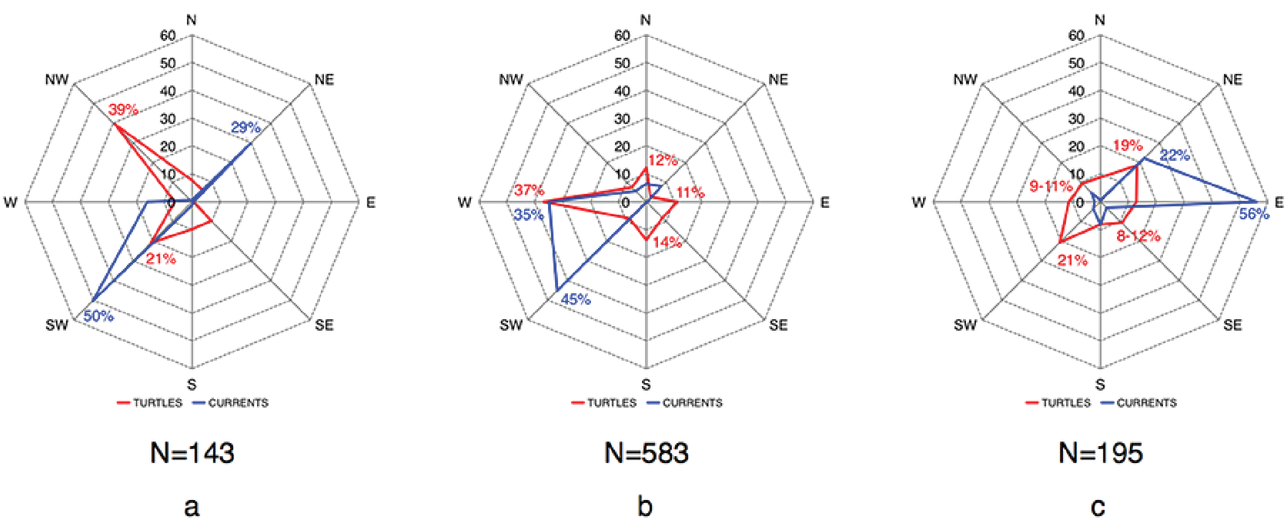

Fig. 6: R-charts represent the average frequency in directions (%) for turtles (in red) and currents (in blue) along the routes of CA-TR 2013 (a), CA-PA 2014–2019 (b), and PA-TU 2014–2019 (c). N. represents the number of observed turtles.

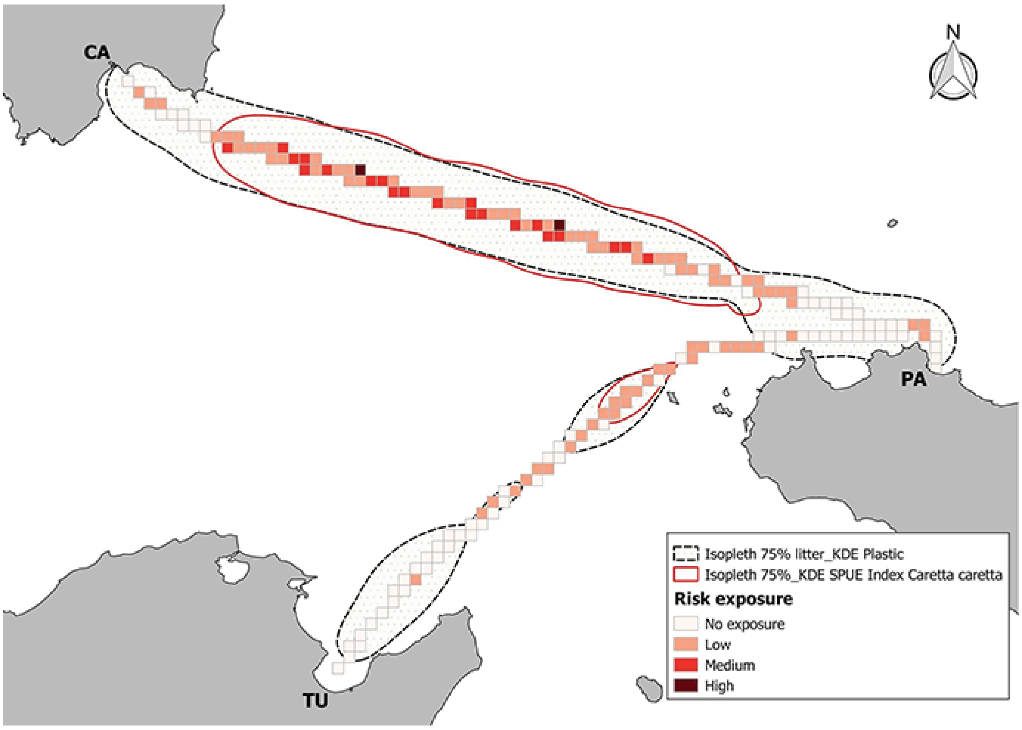

Fig. 7: Isopleths corresponding to 75% of the total values obtained by KDE of plastic density (dotted line and area) and KDE of loggerhead turtle SPUE Index (continuous line). The risk exposure Index is represented by cells of increasing colour gradient.

Discussion

Effective management of marine migratory species, such as the loggerhead turtle, is a complex endeavour. These highly mobile animals use several ontogenetic habitats throughout their life, segregated by hundreds or even thousands of kilometers, where they may be exposed to different threats. Understanding the spatio-temporal overlap between species distribution and anthropogenic stressors is essential for tackling threats in the right place at the right time, but it may be complex or logistically prohibitive, particularly in the highly dynamic and vast open sea (Arcangeli *et al.*, 2019). Surveys on dedicated research platforms are considered the most reliable approach for collecting empirical data on sea turtle distribution and abundance over vast regions, but they are generally unaffordable to carry out regularly (Lauriano *et al.*, 2011; Casale *et al.*, 2018; Arcangeli *et al.*, 2019). The use of the so-called platforms of opportunity, such as passenger ferries or cruise vessels, is a cost-effective complementary approach that can help monitor marine megafauna on a long-term basis (Kiszka *et al.*, 2007; Viddi *et al.*, 2010; Leeney *et al.*, 2012; Boer *et al.*, 2018). Although the routes are not designed by the researchers, which poses some constraints on the statistical analysis, relating animal sightings to effort can be used to infer relative abundance indices and to monitor temporal or spatial variation of a species' presence (Viddi *et al.*, 2010; Arcangeli *et al.*, 2019).

Here we analysed data from seven consecutive years (2013-2019) of sea turtle monitoring along commercial ferry routes in the Sardinia Channel and Strait of Sicily, which play a crucial role in the circulation of the whole Mediterranean Sea. This region is a high-energy site that dynamically modulates the exchanges between the Mediterranean sub-basins. In fact, the Strait of Sicily provides the direct interface between the Eastern and Western Mediterranean, while the Sardinia Channel allows the water exchanges to occur between the Algerian sub-basin and the Tyrrhenian Sea (Astraldi *et al.*, 1998). The Tyrrhenian-Sicily-Sardinia area reveals the basin-scale semi-permanent circulation and mesoscale phenomena associated with ocean eddies, meandering currents, fronts, and upwellings, that are induced by the wind, topography, or instabilities in large-scale circulation (Onken *et al.*, 2003; Béranger *et al.*, 2004). The particular geomorphological and hydrodynamic characteristics make the region extremely interesting from an ecological point of view, and a key area for understanding loggerhead sea turtle displacement in the Mediterranean Sea.

Sea turtle abundance, distribution and size

Overall mean sighting rate was comparable to that reported for this area in the first synoptic survey of loggerhead turtle distribution in the Western Mediterranean, Ionian, and Adriatic Seas (Arcangeli *et al.*, 2019). This is not surprising considering that the two studies share a large portion of the dataset. However, our finer scale analysis revealed a clear heterogeneity in loggerhead turtle distribution within the study area. The conspicuously high mean SPUE observed in the SSCC was mainly a result of the higher summer encounter rates observed in the Sardinian Channel with respect to the Strait of Sicily. However, this comparison is based on summer data only, whereas seasons may affect loggerhead turtle presence differently in these two areas. Among the many factors that influence species presence and habitat use, seasonality plays a key role as the main driver of biological and ecological processes. Detailed analysis on seasonality was performed for the Strait of Sicily, where the effort was consistent during all seasons. The Strait is an important area used by sea turtles for feeding purposes and as an inter-basin exchange passage. Indeed, it seemed that encounter rates in the Strait of Sicily were higher during spring than during other seasons, confirming observations recorded by Arcangeli *et al.* (2019), albeit it must be acknowledged that these results were not statistically significant. To date there is no clear evidence in the published literature that supports a seasonal use of the Strait of Sicily. Bentivegna (2002) reported seasonal migrations at the beginning of autumn from the Western to the eastern Mediterranean, but three out of four turtles used the Strait of Messina and only one took the route via the Strait of Sicily. A more recent study by Chimienti *et al.* (2020) revealed that four out of eleven large juvenile turtles tracked in the Tyrrhenian Sea left this basin during summer and travelled through the Strait of Sicily, while others remained in the circulation system of the Tyrrhenian Sea including the Sardinian Channel area. Simulation of hatchling dispersal from the southwest Italian coast showed that most of the small turtles were retrained in the surface circulation of the Tyrrhenian Sea and eventually moving north into the Ligurian Sea, while only a small percentage leaked through the Strait of Sicily into the Eastern Mediterranean (Maffucci *et al.*, 2016).

Westward migrations through the Strait of Sicily by turtles coming from the Ionian Sea are instead far less documented, although they certainly occur, as was indirectly shown through mixed stock analysis, which attributed large proportions of turtles found in the Tyrrhenian Sea to Eastern Mediterranean rookeries (Maffucci *et al.*, 2006; Maffucci *et al.*, 2013; Karaa *et al.*, 2016). However, it seems that loggerhead turtles move into the western basin when they are an older age, because the probability that hatchlings drift with ocean currents into the western Mediterranean is low (Casale & Mariani, 2014; Hays *et al.*, 2010), whereas the distance from major rookeries to the western Mediterranean is less than the migration ceiling (maximum migration distance: 2150 km) known for the species (Hays & Scott, 2013). In fact, a few single individuals have been tracked into the Western Mediterranean during post-nesting migration, yet the majority of adult females remain in the Eastern Mediterranean and show high fidelity to neritic foraging grounds (Broderick *et al.*, 2007; Stokes *et al.*, 2015; Snape *et al.*, 2016). Also, turtles foraging on the nearby Tunisian shelf area and in the deeper waters of the southern Strait of Sicily (Casale *et al.*, 2008) could move into the northern part of the strait and contribute to the presences observed on the Sicily-Tunisian ferry line. Therefore, summing up this information, there is no clear evidence of seasonal use of the Strait of Sicily, but rather a constant year-round presence of loggerhead turtles.

The distribution of loggerhead turtles along the Sicily-Tunisia line, concentrating more in the northern sector of the transect, is most certainly driven by the strong Algerian current which transports turtles from both the Western Mediterranean and those moving with the South-Eastern Sardinia Gyre (see Fig. 5a and b). The surface current patterns also identify the waters off western Sicily, including the Egadi Islands, as a highly dynamic area, which may constantly receive turtles that passively drift with the Algerian current and continue either northwards with the Bifurcation Tyrrhenian Current or southwards with the Atlantic-Ionian Stream. The association of turtle distribution with surface current patterns has been widely documented, particularly for juvenile oceanic specimens foraging in the Mediterranean which passively drift within a basin broadly favourable for developing loggerhead turtles (Cardona & Hays, 2018; Clusa *et al.*, 2013).

The prevailing surface currents at the time of observations were also likely responsible for the high turtle encounter rates in the Sardinia Channel area. Here, turtles foraging in the Tyrrhenian Sea and those coming from the Algerian Sea meet in the South-Eastern Sardinia Gyre, as some satellite tracking studies have shown (Chimienti *et al.*, 2020; Eckert *et al.*, 2008) and probably find good foraging opportunities. Actually, turtle presence in the SSCC is higher than could have been expected from these satellite tracking studies, where only a few individuals wandered into this area. The SSCC thus gains a previously unrecognized importance as an area that aggregates oceanic foraging loggerhead turtles and has a great potential for revealing trends in turtle abundance if integrated into long-term monitoring programs such as the FLT Med Net.

Based on the experience gained in identifying the size of floating litter items, the observers also estimated turtle sizes during monitoring and revealed a rough distribution of size classes in the SSCC. As could have been expected, the majority of individuals were juveniles in the typical size range of oceanic stage loggerhead turtles, but a quarter of the sighted turtles were also adult sized. This is an interesting result, since adult turtles mostly prefer neritic foraging areas, and there are no nearby known rookeries that would explain the presence of adult turtles passing through during their reproductive migrations. It would be worth examining the state of maturity and sex ratio of these large turtles. However, loggerhead turtles are also known for their foraging plasticity, and many subpopulations, including the Mediterranean, have reported that even adult turtles continue to forage in the water column, either opportunistically or over a longer term (Hatase *et al.*, 2002; Hawkes *et al.*, 2006; Casale *et al.*, 2008). Therefore, the observed proportion of adult-sized turtles may in fact be representative of the foraging aggregation in the western Mediterranean pelagic habitats (Luschi *et al.*, 2013; Chimienti *et al.*, 2020).

Judging the size of smaller objects and turtles from almost 20 m above the sea level platform is challenging, and the proportion of small juveniles observed here could have been underestimated, while post-hatchling and yearling turtles could have been present but passed by entirely unnoticed. As already discussed above, the area surveyed here is unlikely to host the youngest individuals of the

Mediterranean loggerhead subpopulation, so that the bias in the sampling method may be negligible. Nonetheless, the methods for assessing turtle size from moving observation platforms could be refined, so that the proportion of small juveniles can be estimated more accurately. This would be particularly useful for future monitoring programmes in this area, because a recent increase in loggerhead nesting activity in the Western Mediterranean, that has been attributed to climate warming, may lead to the development of new nursery areas in this basin (Maffucci *et al.*, 2016; Abalo-Morla *et al.*, 2018).

Upper layer currents did not appear to have a clear influence on loggerhead sea turtle orientation. In the Strait of Sicily, an area with very high kinetic energy and strong directed currents, turtles were almost isotropic (Fig. 5a and b). On the other side, in the Sardinia Channel the overall westward orientation of the individuals was in a certain agreement with the prevailing current system. It must be acknowledged that we could not associate information about turtles' orientation with those on their behaviour at time of spotting in our analysis and this may have affected the results. Further data are required in order to understand the relevance of collecting information about turtle orientation during at sea surveys from passenger ferries.

Floating litter and risk assessment

The observed plastic KDEs at the finer scale of this study are found to be partially consistent with the model patterns of sea surface plastic concentration (Liubartseva *et al.*, 2018), which were simulated statistically based on the upper layer circulation and static distribution of marine litter sources. In fact, the observations indicate the highest densities in the middle of the Sardinia Channel, and medium accumulation values outside the Ports of Cagliari and Palermo, and the Gulf of Tunis, while the model showed the local maximum of plastic in Tunis, followed by the Port of Palermo and then by the area close to Cagliari. Moreover, the Capo Carbonara MPA located in the NW part of the CA-PA and CA-TR transects is found to be one of the least polluted sites in the study area according to the model. The reason for such inconsistency might be due to the absence of seasonality in the model sources of plastic. Indeed, during peak tourist season, marine litter increases by up to 40% in the Mediterranean (Dalberg Advisors, 2019), which calls for seasonal re-distribution among the model sources of plastic over the basin. Modeling results might be also improved by using Lagrangian drifters that statistically characterize the upper layer kinematics (Poulain & Zambianchi, 2007, Poulain *et al.*, 2009).

By contemporaneously collecting *in situ* data on vulnerable species and floating plastic, this method identified areas and seasons in which sea turtles are most exposed to this hazard, which is essential information when delineating priority mitigation measures (Darmon *et al.*, 2017; Arcangeli *et al.*, 2019).

The central Sardinian channel is a priority risk area, at least during the summer season. The large South-Eastern Sardinia Gyre appears to act as a trap for both the animals and floating plastics, increasing the local exposure risk for sea turtles. Other risk areas were identified, as expected, near the main ports of Cagliari and Palermo, but there were not low or "no risk" detected in the Tunis Gulf. Despite the seasonal intensification of the Algerian Current over the days of observations, a plastic accumulation area was identified by the kernel analysis outside the Gulf of Tunis, proving that local input is likely a more significant driver than oceanographic circulation alone, especially during peak tourist season. Nevertheless, given the low number of sea turtles recorded in that area, the risk was found to be low or absent. On the contrary, along the western side of the Egadi MPA, corresponding to the Algerian Current bifurcation, the high presence of sea turtles was found to have a significant, albeit low, risk index despite the high kinetic energy recorded in the upper layer flow. Our results showed that the spatio-temporal overlap between sea turtles and floating macro litter distributions is influenced by a variety of factors related to the biological cycle of species, sources of littering, and the variable distribution of both, for which sea surface circulation plays a major role, and that the analysis of empirical data is essential for validating and further refining model previsions.

Conclusion

Well identified indicators must be developed to evaluate the conservation status of species and their habitats, as well as the effectiveness of conservation measures. Given the long and complex life cycle of the loggerhead sea turtle, and the wide distributional range, long term studies are needed to gather the required information, and in particular, to fill the knowledge gaps on the distribution and abundance in the vast open sea. Linking information about species presence to the main threats to which they are potentially exposed is also essential for identifying those critical habitats where mitigation measurements must be urgently enforced. Our results proved, once again, that the use of passenger ferries as platforms for observation can be a useful and effective method to gather information on loggerhead sea turtles and marine litter in remote offshore areas and to conduct systematic long term surveys over the vast and highly dynamic pelagic realm.

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Chapter 7

Computing ship strikes and near miss events of fin whales along the main ferry routes in the Pelagos Sanctuary and adjacent west area, in summer

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RESEARCH ARTICLE

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Computing ship strikes and near miss events of fin whales along the main ferry routes in the Pelagos Sanctuary and adjacent west area, in summer

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Abstract

1. The Mediterranean Sea is a high-density maritime traffic area, particularly in the Pelagos Sanctuary. Ship strikes pose a substantial threat to fin whales (*Balaenoptera physalus*) according to reports from the IUCN, the IWC, the ACCOBAMS and the EU Habitats Directive.
2. Near miss events (NMEs) were collected, as a proxy indicator of ship strikes for fin whales, along the main ferry routes crossing the Pelagos Sanctuary and adjacent western waters during 'summertime' (April to October). The 'Fixed Line Transect Med Network' carries out systematic surveys from ferries and collects data according to the 'linear transect' method. From 2008 to 2019, 13 different ferry routes were surveyed with 238,499 km monitored.
3. Of the 2,775 fin whales encountered, 43 individuals were involved in NMEs (1.55% of the sightings). NMEs occur over the great majority of the routes monitored with enough effort and were correlated with the density index of fin whales.
4. High-risk areas for NMEs were identified in the central and deeper parts of the north-western Mediterranean Sea and in some sections of the northern Tyrrhenian Sea.
5. Of all NMEs, the majority of whales (63.4%) surfaced in front of the vessel (<50 m), leaving no time for the crew to manoeuvre the vessel. The others were travelling (26.8%) or resting (9.7%) without any noticeable reaction at the vessel.
6. The speed of the ferries seems to play a role in the occurrence of the NME, as this parameter is significantly different (t-test, $P = 0.002$) for NMEs compared to all fin whale sightings, whereas month and hour of day were not.
7. Quantifying NMEs based on real-time observation with observers on board, could be used as a feasible and efficient way to limit collisions, raising awareness by the crew, and testing or evaluating other potential tools that can help mitigate this threat.

KEYWORDS
assessment, cetacean, conservation, fin whale, Mediterranean Sea, monitoring, protected species, relative abundance, ship strikes, threat

1 | INTRODUCTION

Ship strikes are considered a main threat for cetaceans that involves mainly large species (Laist et al., 2001) due to their slower mobility and longer periods spent on the surface, compared to small species such as dolphins (Ritter, 2012). Among the 11 cetacean species at risk by collisions worldwide (Jensen & Silber, 2003; van Waerebeek et al., 2007), the fin whale (*Balaenoptera physalus*) is most frequently struck by vessels, followed by the humpback whale (*Megaptera novaeangliae*), the right whale (*Eubalaena glacialis*) and the sperm whale (*Physeter macrocephalus*) (van Waerebeek & Leaper, 2008; Winkler et al., 2019). The Mediterranean Sea is the second most significant place in the world where ship strikes occur (Winkler et al., 2019).

The Mediterranean Sea is a high-density maritime traffic area, and maritime transport in the area is increasing in terms of both average ship size and ship traffic frequency (Piante & Ody, 2015). Within the Mediterranean basin, shipping is particularly heavy in the Pelagos Sanctuary, with many tourist destinations spread along the coasts (Folegot et al., 2015; Coomber et al., 2016). This area is protected by the Agreement signed by France, Italy and Monaco in 1999 in order to conserve marine mammals, and it became a Specially Protected Area of Mediterranean Importance (SPAMI) in 2001 (Notarbartolo-di-Sciara et al., 2008).

At the same time, the north-western Mediterranean Sea represents a key area for Mediterranean fin whales, and results of studies based on genetics, contaminants, satellite tags and acoustics have shown the existence of a Mediterranean population distinct from North Atlantic populations (Bérubé et al., 1998; Bentaleb et al., 2011; Cotte et al., 2011; Castellote, Clark & Lammers, 2012a; Panigada et al., 2015; Gauffier et al., 2020; Tardy et al., 2020).

Fin whales appear to be fairly widespread across the Mediterranean region during winter, whereas in summer, the favourable feeding habitat is dramatically reduced, mostly concentrating in the western Ligurian Sea and offshore from the Gulf of Lion (Forcada et al., 1996; Geijer, Notarbartolo di Sciara & Panigada, 2016; Notarbartolo-di-Sciara et al., 2016; Morgado et al., 2017), which is the reason why the north-western Mediterranean Sea has been endorsed through the process of identifying Important Marine Mammal Areas (IUCN, 2017). The species is known to follow seasonal patterns of productivity, concentrating in high Mediterranean latitudes during the summer before dispersing/migrating across the basin during the winter; with peaks of occurrence recorded during spring and autumn in medium latitudes in the Sardinian and Tyrrhenian Sea by Arcangeli, Campana & Bologna (2017). The conservation status of this species in the Mediterranean Sea is assessed as 'Endangered' in the IUCN Red List of threatened species™ (Panigada, Gauffier & Notarbartolo di Sciara, 2021). Threats faced by this species are linked to the high pressure of human activities, such as ship strikes (David, 2002; Panigada et al., 2006; Di-Méglio et al., 2010; Panigada & Leaper, 2010; Di-Meglio, David & Monestiez, 2018), chemical pollution (Tapie et al., 2012; Fossi et al., 2016), physical disturbance (Jahoda et al., 2003), underwater noise (Castellote, Clark & Lammers, 2012b), by-catch in pelagic driftnets (Reeves & Notarbartolo di Sciara, 2006) and floating plastic (Campana et al., 2018).

Collisions can pose a substantial threat to fin whales according to the IUCN Red List for the Mediterranean Regional assessment (Notarbartolo di Sciara et al., 2012; Panigada & Notarbartolo di Sciara, 2012; Panigada, Gauffier & Notarbartolo di Sciara, 2021); whereas the Italian report of the status of the species and habitat protected by the EC Habitats Directive considers 'death or injury by collision' of high importance for fin whale (Genovesi et al., 2014). In the report for the evaluation of marine mammals for the Marine Strategy Framework Directive for France (Spitz, Peltier & Authier, 2018), ship strike is recognized as an anthropogenic pressure for large cetaceans and is evaluated, when possible, in the French sub-regions; among these, the Mediterranean sub-region presents the most numerous cases of ship strikes with large cetaceans (fin whales and sperm whales).

Indeed, the north-western Mediterranean Sea, including the Pelagos Sanctuary and adjacent western waters, is a high-risk area for collisions given the high occurrence of both maritime traffic and sensitive species. According to Panigada et al. (2006), 82% of the known lethal collisions for fin whales are listed within or in waters adjacent to the Sanctuary, and the mortality rate due to known collisions is 3.25 times higher there than in the basin as a whole.

One of the most important factors contributing to collision risks seems to be the spatial overlap of the zones of presence of cetaceans and the zones of intense traffic (e.g. Mayol et al., 2007; Panigada & Leaper, 2010). With a higher density of cetaceans during the summer season in the region coinciding with the peaks of vessel intensity, the scale of the risk is season-dependent (Druon et al., 2012; Campana et al., 2017).

There have been several studies that have investigated fin whale collisions in the north-western Mediterranean Sea. These studies aimed to identify high risk areas based on habitat modelling and maritime traffic overlap (David, Alleaume & Guinet, 2011; Vaes & Druon, 2013; Di-Meglio, David & Monestiez, 2018), the number of stranded animals killed by ship strikes (Peltier et al., 2019), the proportion of live animals bearing scars of ship strikes (Panigada et al., 2006; Di-Méglio et al., 2010) and the theoretical number of animals potentially in front of a vessel (Panigada et al., 2009; David, Alleaume & Guinet, 2011).

An important factor influencing the probability of ship strikes is the types of vessels present in the area. In the Mediterranean Sea, in 40 known cases of collision for which the type of ship is indicated, the majority (62%) involved ferries or passenger vessels, while cargo vessels (container-ship, methane carrier etc.) and high-speed crafts contributed 15% and 10%, respectively (Panigada et al., 2006). At the same time, passenger vessels and cargo ships are the two main types of vessel contributing to the overall spatial distribution of shipping in the Pelagos Sanctuary (Coomber et al., 2016). Moreover, the total distance travelled by ferries inside the Pelagos Sanctuary is greater than that travelled by any other type of vessel, resulting in ferries being most involved in ship strikes in the study area (David, Alleaume

& Guinet, 2011; Folegot et al., 2015). Finally, it has been shown through model studies that vessel speed and size influence both the frequency and severity of ship strikes: large whales may be critically injured at a speed of 10–14 knots, with near 100% mortality at speeds greater than 20 knots when the mass of the vessel significantly exceeds the whale (Laist et al., 2001; Vanderlaan & Taggart, 2007). It has been estimated that a 10-knot speed limit would result in approximately 5- and 4-fold reductions in blue whale and humpback whale mortality, respectively, off the US west coast (Rockwood et al., 2020).

Given the different approaches used in the studies dealing with ship strikes, the number of ship strikes remains uncertain especially because it cannot be determined from direct records. Thus, the best approach is to use a proxy. Richardson et al. (2011) defined that 'surprise encounters' and near misses can be used as proxies of ship strikes (rough proxy of potentially injurious interactions with whales). These authors used the term 'surprise encounter' to describe when a whale is detected for the first time at a distance ≤300 m from the vessel. They used the term 'near miss' to describe those surprise encounters that occurred off the bow of a moving vessel, and at a distance ≤80 m. No official or consensual definition exists yet on the distance at which to consider a near miss event (NME) (Ritter et al., 2016).

The aim of this study was to collect data on NMEs, as a proxy indicator of ship strikes for fin whales along the main ferry shipping routes in the Pelagos Sanctuary and in the adjacent western area during the summer period, a period characterized by the overlap of high intensities in maritime traffic and cetacean occurrence.

The use of ferries as observation platforms to collect data on cetacean presence and distribution allows systematic surveys at sea throughout the year to be carried out at a lower cost. In the Mediterranean Sea, the Fixed Line Transect Mediterranean Monitoring Network (FLT) started data collection in 2007 following an adaptation of the standard line transect protocol (Arcangeli, 2010). In 10 years, the network, coordinated by ISPRA (Istituto per la Protezione e la Ricerca Ambientale, Italy) grew to include 22 shipping lines. The network was initially created to monitor cetacean populations but expanded its activities to data collection of mega-fauna occurrence (Arcangeli et al., 2019), maritime traffic (Campana et al., 2015), marine litter (Arcangeli et al., 2018) and NMEs.

Data collected from the network are used in this study to: (i) identify the spatial and temporal occurrence of NMEs of fin whales; (ii) quantify NMEs occurring during summer in different parts of the Pelagos Sanctuary and the adjacent western area; (iii) understand the context of NMEs through the analysis of the behaviours of animals; and (iv) map the high-risk areas of exposure to ship strikes.

2 | MATERIALS AND METHODS

2.1 | Data collection

Between 2008 to 2019, data were collected on-board ferries along 13 different ferry routes (the name links the departure and arrival ports) in the north-western Mediterranean Sea. Among the surveyed routes, 12 cross the Pelagos Sanctuary (Table 1). The Toulon–Alcudia route is entirely off the western border of the Pelagos Sanctuary and the Civitavecchia–Barcelona (CBAR) route was split in two parts: one inside the Pelagos Sanctuary (CBAR) and the other, which is on the western side outside of the sanctuary, was renamed as CBAR (W) (Figure 1).

Most of the routes run almost perpendicular to the coastline and any expected density gradient, and at right angles to the direction of whale long-distance movements as suggested by Evans & Hammond (2004). On the ferries 'Fixed Line Transect' surveys (Arcangeli, 2010; Arcangeli, Marini & Crosti, 2012b) involved regular and repeated sampling of a predefined section of the route with continuous on-effort in good weather conditions. A minimum of three samples per route per season were collected.

TABLE 1 Name and responsibility for the ferry routes

Name of the routes	Years	Owner of data	Ferry company
Civitavecchia–Barcelona (CBAR; CBAR W)	2012–2019	ISPRA/Accademia del Leviatano	Grimaldi Lines
Livorno–Bastia (LIBA)	2009–2019	Univ. Pisa/Accademia del Leviatano/ISPRA	Corsica and Sardinia ferries
Livorno Golfo Aranci (LIGA)	2012–2019	Univ. Pisa/ISPRA	Corsica and Sardinia ferries
Savona–Bastia (SABA)	2008–2018	CIMA research Foundation	Corsica and Sardinia ferries
Savona–Calvi (SCA)	2013–2015		
Savona–Ile Rousse (SIR)	2015–2018		
Nice–Calvi (NICA)	2009–2016		
Nice–Ile Rousse (NIR)	2009–2018		
Nice–Bastia (NIBA)	2017–2018		
Toulon–Ajaccio (TAJ)	2011–2019	EcoOcéan Institut	Corsica and Sardinia ferries
Toulon–Alcudia (TAL)	2018–2019		
Toulon–Ile Rousse (TIR)	2018		
Toulon–Bastia (TBA)	2018–2019		

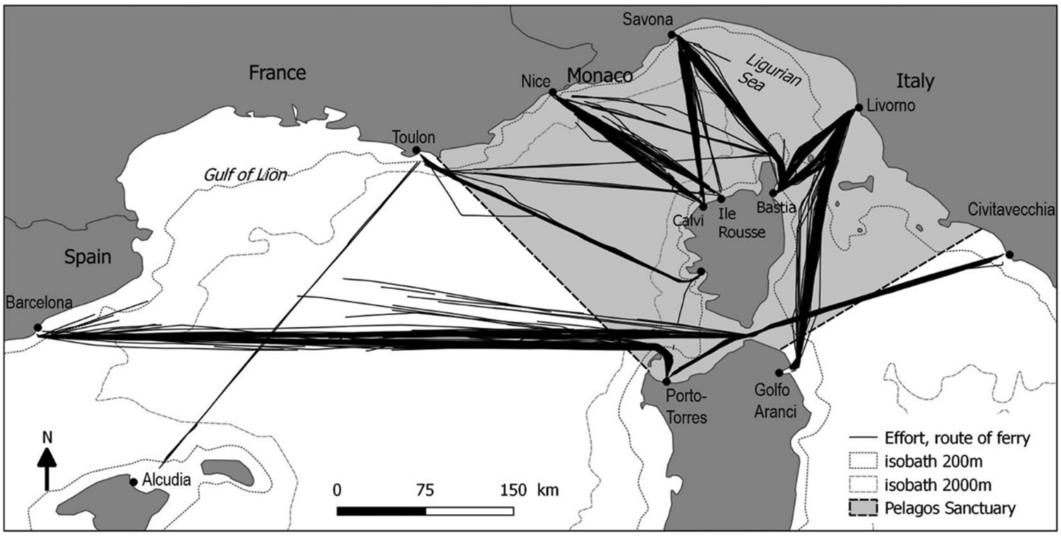


FIGURE 1 The ferry routes run by the Fixed Line Transect Med network in the north-western Mediterranean Sea (2008–2019), within the Pelagos Sanctuary and in an area west of the Sanctuary (offshore the Gulf of Lion)

The data were collected according to the 'linear transect' method described by Buckland et al. (2001) in a 'passing mode': rigorous and continuous observation was carried out by 2–4 dedicated and expert marine mammal observers (MMOs) located on both sides of the ferry command deck, scanning mostly by naked eye 130° forward. The ferries used to collect the data varied in size and speed (Cominelli et al., 2016), but were within a speed range of 18–25 knots and observational platform height of 15–28 m. Tepsich et al. (2020) classified the ferry into three types depending on their height: type I included vessels from 12 to 15 m height, type II from 20 to 24 m, and type III more than 25 m. Globally, Tepsich et al. (2020) found that height is correlated with the speed (Pearson test, $P < 0.0001$), so that higher and bigger ferries travel faster, with the following means: 17.3 knots (SE 1) for type I, 22.8 knots (SE 3.3) for type II and 24.3 knots (SE 2.6) for type III. A ferry type is not specific to one route and the fleet could run different routes within the year. The speed of the vessel throughout the transect was instead constant. The track of the ferry along the transect was continuously recorded by a handheld GPS (date, time, latitude, longitude). Wind conditions and sea state were ≤3 on the Beaufort scale. Binoculars and sometimes photographs were used to confirm sightings and to assess species and group size. For each sighting of cetaceans, the species, number of individuals, presence of juveniles, behaviour, distance and angle from the ferry, and direction of swim were recorded.

The definition of an NME within the FLT network protocol was established considering the characteristics of the vessels used, also in accordance with Ritter et al. (2016), as "when an animal seen in an area located 50 m in front of the vessel's bow and 25 m on the side (Figure 2) and the animal shows no attractive behaviour for the ship's bow, but seems rather unaware of the approaching vessel".

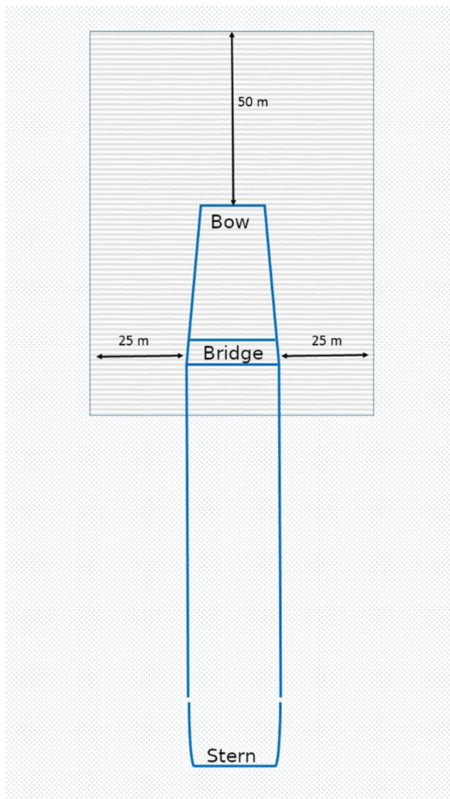


FIGURE 2 Delimitation of the area where a near miss event can occur, according to the definition of near miss event given by the Fixed Line Transect network protocol

2.2 | Behaviour

At the first detection of all encounters of fin whales, the distance of the animal from the ferry was measured with the help of reticulated binoculars, clinometer or range stick. Then initial behaviour was determined, based on the first sight, as one of the four following categories: resting (when the animal is motionless in the water); travelling (when the animal is moving heading in a specific constant direction, leaving surface 'footprints' regularly); feeding (when animal is moving within a small area and heading and speed are varying); and the newly created category emerging close to the ferry (when animal just surfaces to the side (Figure 2) in the vicinity of the ferry in the near miss range). Simultaneously, the heading of the animal/group was noted, if possible. Then, in case fin whales were along the shipping lane within the range of an NME, the minimum distance between the ferry and the whale was also recorded. As the ferry passes fast, any close encounter happens quickly and often the reaction of the animal is also quick and very clear. Consequently, the reaction of the animal to the passage of the ferry was recorded as one of the following categories: dive straight away rapidly, turn abruptly, swim away faster, none. Finally, it was also noted if the vessel took action to avoid the whale or not.

2.3 | Analysis

For each transect, the prospecting effort ***d*** (as kilometres prospected 'on-effort'), the number of sightings ***n***, the number of whales ***m***, and the number of whales involved in the NME ***nme*** were calculated, as well as the mean size of groups E(s) as m_i/n. Density index (DI), expressed in whales per km^2 was then calculated for each transect based on the equation by Buckland et al. (2001):

$$\mathbf{DI} = n * E(s)/(d * (ESW * 2))$$

For each transect, **ESW** (effective strip width), the efficient width of detection, was taken from Tepsich et al. (2020), who analysed the same dataset and obtained an ESW for the three types of ferry based on their height: 1,235 m for type I, 1,415 m for type II, and 1,143 m for type III.

In the same way, a density index for NME (DNME) was calculated as:

$$\mathbf{DNME} = NME/(d * (ESW * 2))$$

Given the fact that NME are in general rare events, the distance in km travelled on effort prior to detecting an NME was calculated for each of the monitored routes, and a correlation between NMEs and effort was performed using the Pearson's correlation coefficient test. Then, the test was used to investigate potential correlation between NME events and whale DIs, first considering all investigated routes and then deleting the routes with insufficient effort.

Kruskal–Wallis and Dunn's tests were used to compare the results of DI and DNME over the different lines, globally and by pairs.

The Mann–Whitney–Wilcoxon test was used to investigate differences of mean among the variables of month, hour and speed of vessel for the two datasets, with one including only the NME cases and one including all the other fin whales' encounters.

For the spatial analysis, the whole database was integrated under Qgis 2.4.13 Essen to map the distribution of survey effort and sightings. The spatial analyses were carried out on the INSPIRE grid with a cell resolution of 10 ≤ 10 km (www.eea.europa.eu/data). For each cell, the prospecting effort ***d***, the number of whales ***m***, and the number of whales involved in the NME ***nme*** were calculated. These parameters were then used to compute encounter rate (ER_i) = **m/d** and NME rate = ***nme/d*** per cell. A kernel analysis (Kernel smoother, Hengl et al., 2009) was then performed under QGis with the plug-in 'heatmap' in order to highlight hot-spot areas of NME along the ferry routes. Input data were NME rate per cell, including 715 cells with information and a radius of 30 km (defined by QGis based on the data).

3 | RESULTS

3.1 | Indexes of density

From 2008 to 2019, surveys were performed along the 13 ferry routes (CBAR is split into two parts), for a total of 238,499 km monitored (Table 2). A total of 2,775 fin whales were encountered, of which 43 individuals were involved in an NME (Figure 3).

During the summertime, fin whales were seen on all monitored routes (Figure 3). There was a significant difference in the density index of whale sightings between ferry routes (Kruskal–Wallis, $P <$ 0.0001). Based on the Dunn's test and comparing routes by pairs, groups of routes were obtained with the highest DIs occurring along routes crossing the northern part of the study area (Figure 4): Toulon–Ile Rousse (28.5×10^{-3} whales/km^2) and then along Savona–Calvi, Nice–Calvi, Nice–Ile Rousse, Savona–Ile Rousse and Nice–Bastia (between 14.4 and 11.1×10^{-3} whales/km^2). The lowest DIs were obtained on the lines sampling the eastern and southern part of the Sanctuary, namely Livorno–Bastia, Livorno–Golfo Aranci/Olbia, and the part of the CBAR within the Pelagos Sanctuary (from 0.3 to 1.6×10^{-3} whales/km^2).

Considering the index of DNME, this differed significantly between routes (Kruskal–Wallis, $P < 0.0001$), with some grouping emerging by Dunn's test results comparing routes by pairs. The highest densities of NME (Figure 5) were along the Nice–Ile Rousse and Toulon–Ajaccio routes (0.3×10^{-3} NME/km^2) whilst 0.1 to 0.05×10^{-3} NME/km^2 occurred along the Nice–Calvi, Savona–Calvi and CBAR (W) routes. Finally, along the nine other routes, NMEs were rare or never reported.

As NMEs are rare events, they may be correlated to the effort. A minimum of effort of around 1,123 km is necessary in order to witness an NME in the study area (Table 3). With this rough threshold of effort needed, it appears that the routes of Savona–Ile Rousse, Toulon–Ile Rousse and Toulon–Bastia were insufficiently covered to witness an NME (effort <740 km), and Toulon–Alcudia and Nice–Bastia were also close to the required threshold of effort (effort

TABLE 2 Per route: Number of surveys, km on effort, number of sightings, individuals and near miss events for fin whales (2008–2019) during summer (April to October) and calculated index of density of whales (DI) and near miss events (DNME) as an average of all transects done over the route

	Data collected					Calculated index of density			
Route units	Surveys No.	Effort km	Sight. No.	Ind. No.	NME No.	Mean DI	SE of DI	Mean DNME *(ind/km²)* × 10^{-3}	SE of DNME
Civitavecchia–Barcelona (E)	255	30,055	98	133	4	1.6	5.2	0.05	0.52
Civitavecchia–Barcelona (W)	283	55,599	456	661	12	5.2	11.1	0.08	0.47
Livorno–Bastia	318	32,411	19	24	0	0.3	1.3	0	0
Livorno–Golfo Aranci	138	30,170	43	52	2	0.6	1.2	0.02	0.16
Nice–Bastia	7	1,289	27	40	0	11.1	11.3	0	0
Nice–Calvi	136	18,576	484	627	5	13.4	14.6	0.10	0.51
Nice–Ile Rousse	65	7,861	216	298	7	12.9	17.1	0.30	1.04
Savona–Bastia	287	39,083	236	312	1	2.8	5.3	0.01	0.17
Savona–Calvi	49	7,459	190	278	1	14.4	15.6	0.05	0.36
Savona–Ile Rousse	3	476	12	14	0	11.9	1.5	0	0
Toulon–Ajaccio	58	13,083	195	259	11	8.1	8.0	0.31	0.71
Toulon–Alcudia	3	1,191	17	23	0	6.8	4.3	0	0
Toulon–Bastia	2	510	5	5	0	3.5	0.05	0	0
Toulon–Ile Rousse	4	737	28	49	0	28.5	30.6	0	0

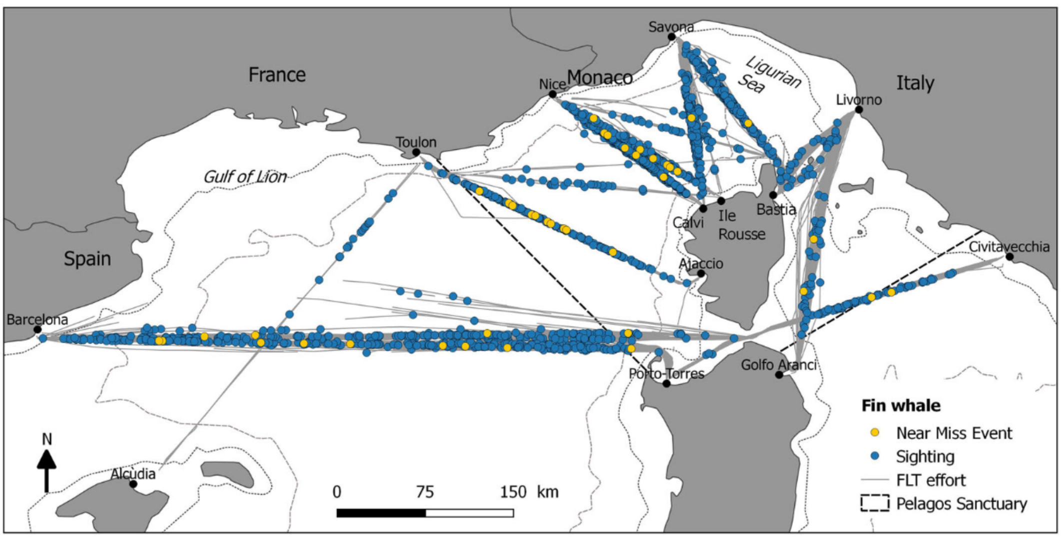


FIGURE 3 Sightings of fin whales and near miss events observed by the Fixed Line Transect network in the north-western Mediterranean Sea (April–October 2008–2019)

1,190–1,290 km). On the other hand, the route between Livorno–Bastia reported no NME despite the huge effort, and with only rare sightings of fin whale on this route it is likely that this is correct.

Considering the DNME, results were not correlated with the amount of effort, nor with the density of whales (DI) over each route (Pearson's test, $P = 0.65$ and 0.21 respectively, Figure 6), but after removing the five routes with insufficient effort to detect an NME, a correlation appeared between DI and DNME over the eight remaining routes (Pearson's test, $P = 0.011$).

3.2 | Mapping of NMEs hot-spots or high-risk areas

As the NME rate is highly correlated with DNME (Pearson test, $P < 0.0001$), the first parameter was used for mapping.

The kernel interpolation of NME rates per cell produced a map of high-risk areas to ship strikes within the north-western Mediterranean Sea (Figure 7), along surveyed routes. Areas with a high level of NME rates or ‘hot spots’ appear in the deep northern portion between

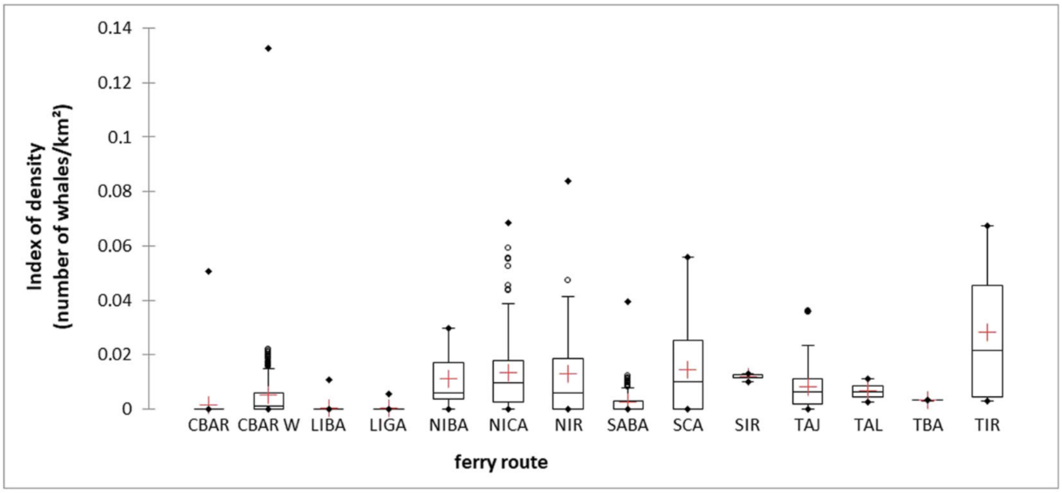


FIGURE 4 Boxplot of the index of density of fin whales on different ferry routes in the north-western Mediterranean Sea (April–October 2008–2019). Red crosses indicate the mean DI values, and small circles outliers. See Table 1 of definitions of route abbreviations

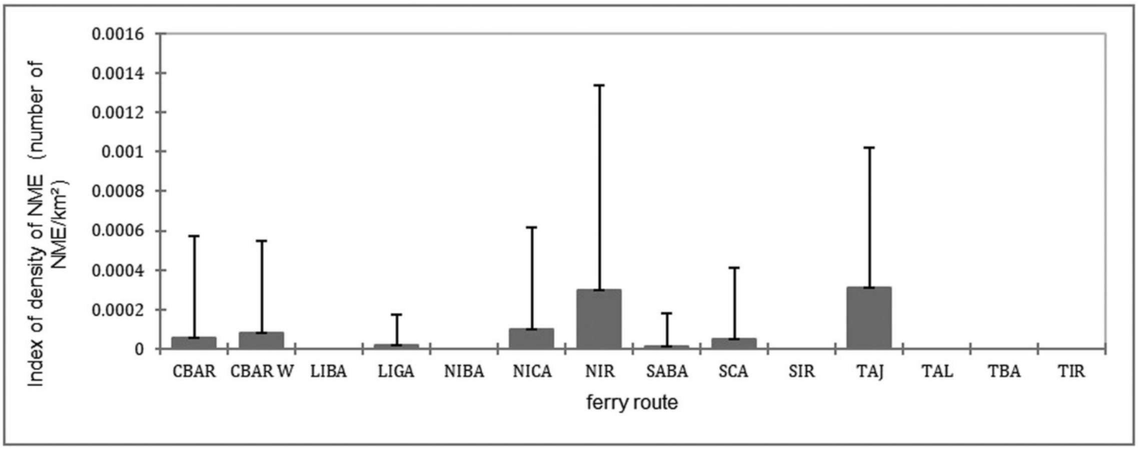


FIGURE 5 Mean and standard-error of the index of density of near miss events (NME) of fin whales calculated for each of the routes of the Fixed Line Transect network in the north-western Mediterranean Sea during summer (2008–2019). See Table 1 of definitions of route abbreviations

TABLE 3 Effort, number of near miss events (NMEs) in total, and distance (effort, km) to be surveyed prior to encountering one NME

Route	Effort (km)	NME	Number of km for 1 NME
Civitavecchia–Barcelona	30,055	4	7,514
Civitavecchia–Barcelona (W)	55,599	12	4,633
Livorno–Bastia	32,411	0	NA
Livorno– Golfo Aranci	30,170	2	15,085
Nice–Bastia	**1,289**	**0**	NA
Nice–Calvi	18,576	5	3,715
Nice–Ile Rousse	7,861	7	1,123
Savona–Bastia	39,083	1	39,083
Savona–Calvi	7,459	1	7,459
Savona–Ile Rousse	**476**	**0**	NA
Toulon–Ajaccio	13,083	11	1,189
Toulon–Alcudia	**1,191**	**0**	NA
Toulon–Bastia	**510**	**0**	NA
Toulon–Ile Rousse	**737**	**0**	NA

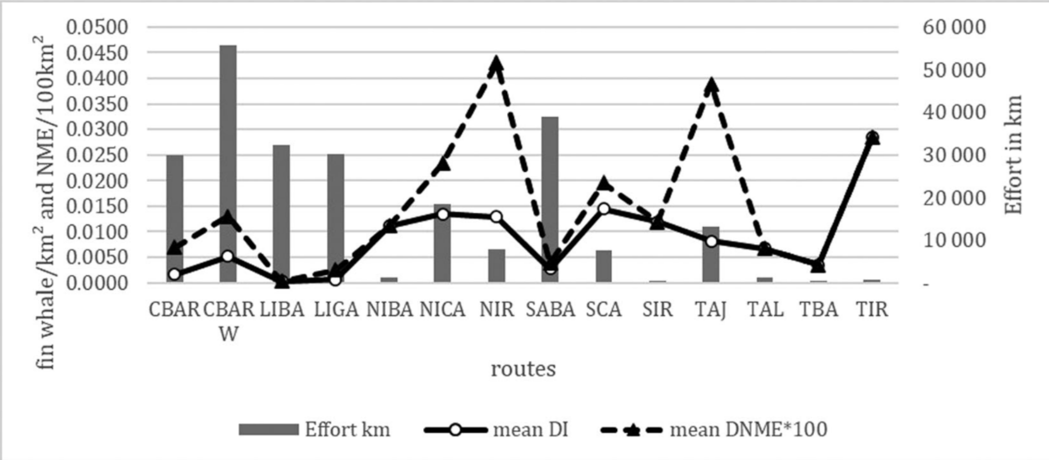


FIGURE 6 Effort, density index of fin whales (DI), density index of near miss events of fin whales (DNME) calculated for each of the routes of the Fixed Line Transect network in the north-western Mediterranean Sea (April–October 2008–2019). See Table 1 of definitions of route abbreviations

Toulon and Ajaccio, and offshore Calvi (crossed by several routes). Areas with a medium level of NME rates appear offshore Nice, and all along CBAR (W). In the south east of the Sanctuary, some areas with low level or 'low spots' were reported. Overall, high risk areas are in the deep part of the Sanctuary, mostly on its western side, as well as being widespread within the western waters adjacent to the Sanctuary.

3.3 | Behaviours of NME

On the total NMEs for which the behaviour was recorded (N = 41), the majority of animals were simply emerging (26 cases, 63.4%) within the vicinity of the ferry's hull (Figure 8) and 10% were seen stationary (resting) on the route of the ferry before the ferry got very close. Considering the two stationary cases and the 11 travelling cases, two of them were seen up to 1.2 km and 850 m away, respectively, and the others from 350 m to 100 m; all ended as an NME (<50 m).

Among the whales emerging, 69% dived straight away as an avoidance reaction to the approaching vessel. The majority of travelling or resting whales either did not show any reaction to the ferry even if the animal and vessel came close (nine of 13 NMEs, 69%), or they turned sharply to avoid the vessel (23%).

The variables of month, hour and speed of vessel for an NME were compared to those for all the other fin whale encounters. It appears that there are no significant differences for both data sets for hour and month (Mann–Whitney, $P = 0.40$ and 0.92 respectively). Speed, however, was significantly different (Mann–Whitney, $P = 0.008$, Figure 9), and was higher for NMEs with a mean of 22.5 knots (SE 3.8) compared to 20.5 knots for other sightings of fin whales (SE 4.1). Sightings occurred at the lowest speed when the ferry was leaving the harbour (5 knots), whereas NMEs were witnessed from 16.6 knots only.

Calculating the mean of the speed of ferry for each behaviour types for the NMEs, showed that for whales presenting a behaviour of 'emerging' the mean speed of the ferry is 22.9 knots (SE = 3.8 and N = 26); 20.4 knots for the behaviour 'travelling' (SE = 4.2 and N = 10) and 23.0 knots for 'resting' (SE = 1.2 and N = 3). However, the differences of speed between behaviour types are not significant (Mann–Whitney, $P = 0.250$ between emerging and travelling, and $P = 0.600$ between travelling and resting),

4 | DISCUSSION

4.1 | Indexes of density of fin whales and NMEs

The results from the fin whale spatio-temporal index of density (DI) are consistent with previous knowledge in this area of the Mediterranean Sea (Notarbartolo-di-Sciara et al., 2016). During summer, fin whales were abundant in the whole north-western Mediterranean Sea, with highest abundances in the Ligurian–Corsican–Provençal part of the Sanctuary (Notarbartolo-di-Sciara et al., 2003; Morgado et al., 2017). This is also the area where most of the NMEs occurred. In the eastern part of the Sanctuary (between Italy and Corsica) fewer whales were sighted and fewer NMEs occurred compared to the western part of the Sanctuary. Moreover, on the shelf between Italy and Corsica (LIBA), almost no sightings occurred and no NMEs were observed. Overall, the correlation between NMEs and whale sightings occur when there is sufficient effort to detect the rare event of NME. Other parameters, such as the behaviour of the animal, and probably the characteristics of the ferry, namely speed, also influence NME occurrence. Indeed, speed seems to be one of main parameters playing a role in an NME (Richardson et al., 2011). In this study, there were no NMEs recorded when ferries travelled at speeds of less than 16.6 knots, whereas first sightings of fin whales were made at a ferry speed of

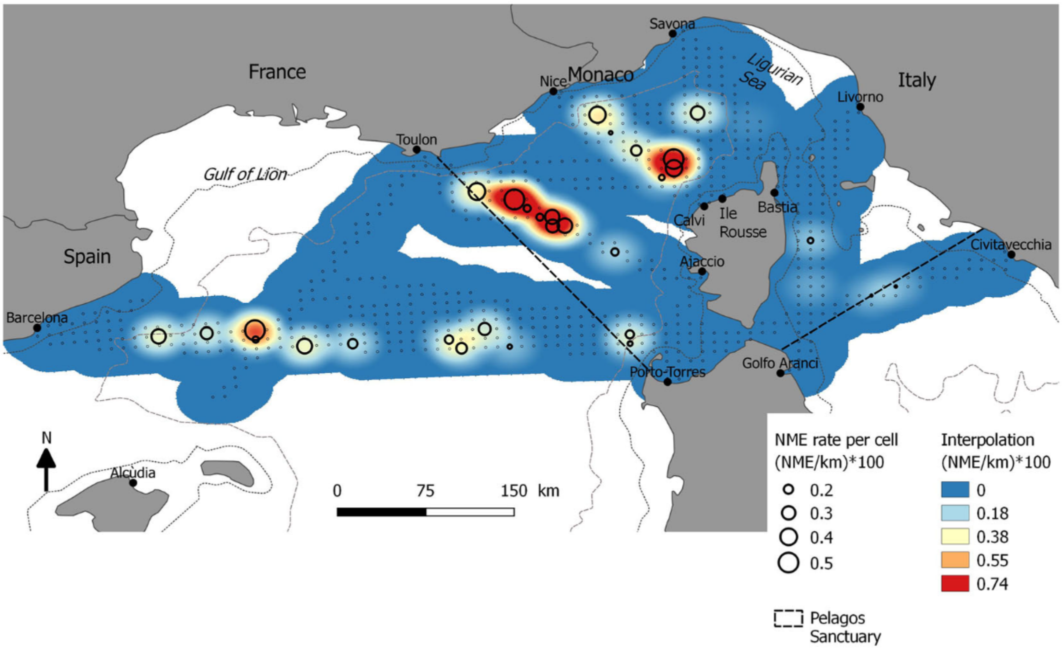
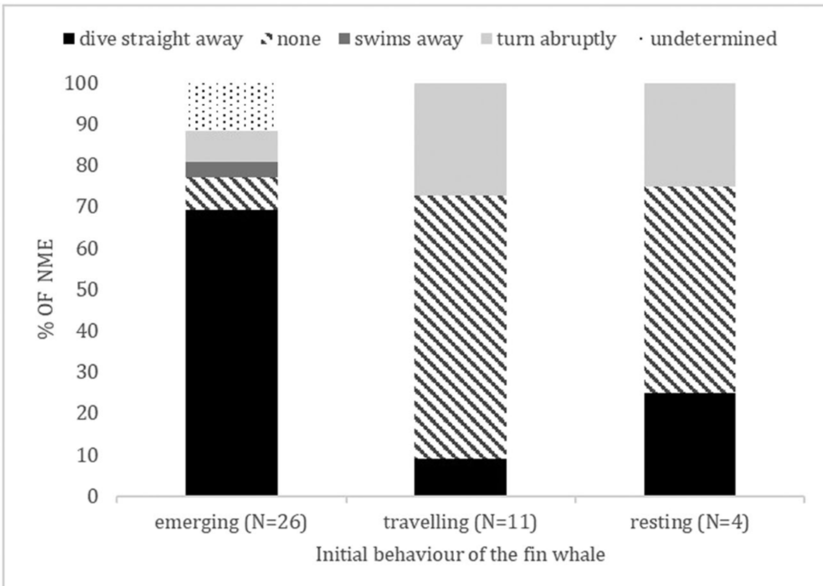


FIGURE 7 Kernel interpolation of near miss events per 100 km per cell of 10 × 10 km, observed by the Fixed Line Transect network in the north-western Mediterranean Sea (April–October, 2008–2019). Small black dots represent effort without near miss event (NME)

FIGURE 8 Reaction of whales to the proximity of the ferry in a near miss event (NME), as a function of three different initial behaviours



5 knots. However, the proportion of effort carried out at low speeds is generally small and mainly close to harbours (e.g. Bay of Ajaccio or Toulon, continental shelf in front of Civitavecchia), which are areas less suitable for fin whales and therefore this result should be taken with caution.

For this study, the FLT dataset over 10 years recorded no ship strike with a fin whale. Because this study is only based on visual observation during the daylight hours, it is possible that the risk of strike might be at least the same or even higher during the night, depending on the whale's behaviour and dive pattern (Calambokidis et al., 2019; Keen et al., 2019). It will depend also on ship traffic patterns at night, and indeed, David & Di-Méglio (2010) stressed that ferries travelling at night are more numerous than during daylight, increasing the risk of whale exposure to vessels. Moreover, the animal's vision is less effective at night and acoustic propagation is likely to be different as well. Furthermore, we cannot be sure that all animals involved in an NME seen diving to avoid the vessel were not injured, even if the MMOs scanned for a while behind the vessel after an NME occurred. So, the FLT may underestimate the real number of ship strike cases. By contrast, through the examination of stranded animals, the mortality rate due to collisions may be over-estimated due to collisions occurring with already dead animals or when

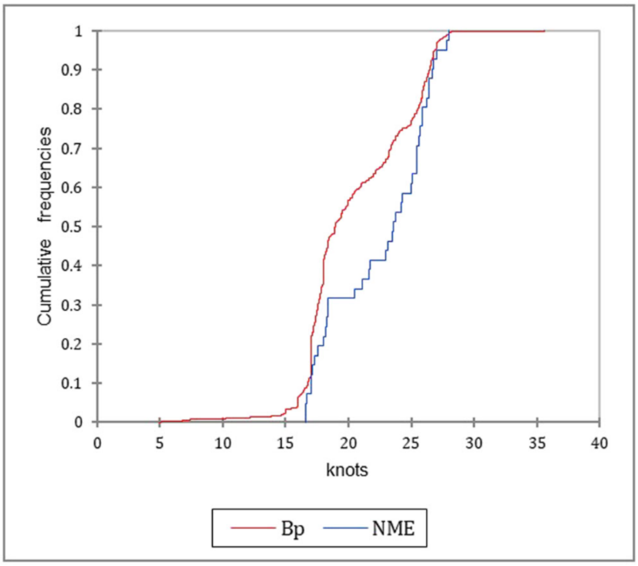


FIGURE 9 Cumulative frequencies of sightings of near miss events (NME) and all other fin whales (*Bp*) at increasing speed

sickness may make animals more susceptible to strikes (Laist et al., 2001). Wrong estimations of ship strike events could be also due to the fact that investigations of the presence of large haematomas, which is an indicator of being alive at the moment of the strike, on dead bodies is non-systematic in the Mediterranean (Giorda et al., 2017). So, the real impact of ship strikes on the population of whales is still not precise.

Of the 2,775 fin whales encountered between April and October in the north-western Mediterranean Sea, 43 individuals were involved in NMEs (1.55% of the sightings). Within the Pelagos Sanctuary, this percentage is 1.47%. The latest estimation of the number of fin whales in summer in the Pelagos Sanctuary was 1,128 animals (561–2,415) and 2,500 (1,472-4,310) for the north-western Mediterranean Sea (Laran et al., 2016). Based on those estimates and the calculated percentage of NMEs, each year 16.6 individuals (8.2–35.5) could experience an NME with a ferry between April and October in the Pelagos Sanctuary and 38.7 individuals (22.8–66.8) within the north-western Mediterranean Sea. Therefore, across 10 years the FLT may have witnessed 11% of the potential NMEs in the whole north-western Mediterranean Sea, and almost 19% of those potentially occurring in the Pelagos Sanctuary.

David, Alleaume & Guinet, (2011) estimated that 118 fin whales were theoretically at risk of suffering a strike with a classical ferry hull, each summer (July–August) in the Pelagos Sanctuary. The mean number of NMEs per km of ferry travel in the Pelagos Sanctuary was calculated as 0.17×10^{-5} NME/km for this study, whereas David, Alleaume & Guinet (2011) calculated a much higher value at 9.5×10^{-5} NME/km. This is probably due to the calculation used by these latter authors, being based on the fact that neither the animals nor the vessels reacted when face-to-face and suggests that animals may effectively avoid vessels in practice, while vessels rarely avoid animals.

Existing reviews of ship strike cases in the Mediterranean Sea revealed an average of 1.51 fin whales were killed per year between 1972–2001 and 1.14 between 2001 and 2009 (Di-Méglio et al., 2010). The updated © IWC Ship Strike database (2020), from 1972 to 2019 recorded 58 'definite cases', or 72 'definite, probable, and possible cases' of ship strikes for fin whales in the Mediterranean Sea involving all types of vessels. This equates to a mean of 1.2–1.5 fin whale strikes per year. Furthermore, according to the rough localization of the events from this IWC database, ship strikes in the Ligurian and Balearic areas, corresponding roughly to the north-western Mediterranean Sea, reach a mean of 0.75 to 1 animal/year for all types of vessels. Considering the amount of effort made by the FLT in the north-western Mediterranean Sea (238,499 km) in the summer period aboard ferries, we could conclude that one NME occurs for every 5,546 km travelled and that strikes are a rare event, or that MMOs aboard ferries were able to avert some strikes from occurring.

4.2 | Defining high-risk areas

The hot-spots of high risk that emerged from this study are, in general, in line with other existing theoretical maps of potential high-risk areas of exposure dealing with all ferries in the Pelagos Sanctuary (David & Di-Méglio, 2010; Folegot et al., 2015) and in the western Mediterranean Sea (David, Alleaume & Guinet, 2011; Vaes & Druon, 2013), although some medium and low spots were less similar. The differences in spatial scales, mapping representation and data input for maritime traffic limits the possibility of comparison between the different studies. The high-risk areas obtained according to the real observations of NMEs from the FLT help to validate the theoretical maps of published hotspots, but further analysis should include the comparison between theoretical maps and maps based on real *in situ* data using the same spatial–temporal resolution and same ferry routes. Extending the FLT over other ferry routes would greatly help to improve coverage and result in more comprehensive mapping of high-risk areas. Mapping high-risk areas based on real data, including other areas and seasons, will be useful in the processes led by several organizations (IWC-IUCN-ACCOBAMS, 2019), namely the IWC ship strikes working group, the IUCN Marine Mammal Protected Area Task Force and the ACCOBAMS Cetacean Critical Habitat process, to identify where, when, and which relevant area-based or threat-based mitigation measures.

4.3 | Behaviour and mitigation measures

This study enhances understanding of why ship strikes occur, such as because animals are resting or engaged in dive feeding and have just emerged from such dives (Panigada et al., 1999). The fact that animals can emerge just in front of a vessel or travel without changing route because they seem not to have noticed it (Nowacek, Johnson & Tyack, 2004; Panigada et al., 2009), can also be due to the acoustic properties of the stratified water limiting their ability to hear the approaching vessel (Gannier & Marty, 2015) or low hearing capacities because of masking by ambient noise or damaged ears (Richardson et al., 1995). Last, they may not be aware that an arriving vessel might be a danger, or their ability to avoid an approaching ship is limited (McKenna et al., 2015).

In a large number of ship strike cases, it was not possible to detect the whale early enough to manoeuvre and avoid it due to the fact that it was emerging from depth just in front of the ship, or was resting subsurface, so not visible from the bridge. Ritter (2012) also recorded a prevalence of resting (25%) or emerging whales (35%) in the 20 cases of ship strikes with sailing vessels mostly in the Atlantic, of mostly humpback and sperm whales. Even when animals were seen slightly further away it was often not possible to take evasive action as only one blow was seen, which was not sufficient to be able to determine the behaviour or heading of the whale or to predict the next surfacing and thus give the master time to react. A ferry travelling at a speed of 20 knots covers 617 m/min, and at a speed of 28 knots it covers 864 m, thus a whale 350 m ahead has only about 30 s to react at 20 knots, and around 24 s at 28 knots. In case of travelling whales seen beyond 350 m on a collision course, an MMO operating on the bridge could alert the master, and if the conditions (speed, weather, traffic, crew) allow for a change of course or speed, vessels could better avoid strikes and even NME (Panigada et al., 2009). As an example, on the Toulon–Ajaccio line, on four out of 10 occasions, fin whales were seen from 150 to 800 m in front of the vessel, and three times the behaviour and heading of the animal could be determined and the master was able to alter the course of the ferry resulting in an NME as opposed to a possible strike.

It is widely recognized that speed plays an important role in ship strikes: speed is related to the probability of encounters (Currie et al., 2015), to encounter distance, and more importantly, to lethality (Vanderlaan & Taggart, 2007; Gende et al., 2011). All these studies point to similar speed thresholds of 11–13 knots (5.7–6.7 m/s). In the case of the fin whales with ferries travelling at speeds of between 12 and 28 knots the threshold appears to be around 16.6 knots. This is perhaps also linked to the fact that the fin whale swims faster compared to some other species, such as the right whale and humpback whale, where ship strikes have been studied.

Looking at the behaviour of whales in response to ships, they seem to respond more to medium sized boats than to ferries (Campana et al., 2015). It is possible that they perceive large ferries travelling in straight lines as less dangerous than smaller boats that are less predictable, but when a strike occurs, it is more likely to be fatal the bigger and the faster the vessel is (Laist et al., 2001; Silber, Slutsky & Bettridge, 2010). Also, NMEs are probably very stressful for animals as shown by their startle reaction to avoid the vessel.

5 | CONCLUSION

Near miss events occurred with ferries on almost all routes crossing the north-western Mediterranean Sea between April and October. Qualified observers on board are useful for collecting real-time data in order to increase understanding of the number, locality, species, and context of such events. As NMEs or strikes stay often unnoticed by the crew on a large vessel, the existing IWC ship strike database suffers from missing data or imprecision.

Strengthening the collection of data aboard the ferries would greatly improve the knowledge on NME and ship strikes, including raising coverage on routes that thus far have received insufficient attention, and extending the network beyond the Pelagos Sanctuary in the western part of the north-western Mediterranean Sea, the Spanish whale migration corridor and the Alboran Sea. As the FLT collects data when possible, throughout the year, an analysis should be done when sufficient data are available for the winter season (November to March) for fin whale and also on both seasons for sperm whales, as NMEs occur all year round for both species (Di-Méglio et al., 2010; Folegot et al., 2015; Di-Meglio, David & Monestiez, 2018). This extension in coverage will help also to map observed high-risk areas of ship strikes for fin and sperm whale and improve global or dynamic modelled maps to help predict such events.

Ultimately, quantifying NMEs based on real-time observations with observers on board, could be used as a feasible and efficient way to limit collisions, raising awareness of the crew, and testing or evaluating other potential tools that can help mitigate the risk of ship strikes. The permanent collection of data from ferries would *per se* also help as a surveillance system under the EU Habitats and Marine Strategy Framework Directives and the IMAP EcAp process under the Barcelona Convention.

Currently all relevant data relate to daylight hours, and it is crucial to understand the diel pattern of fin whale dives in the Mediterranean Sea to better estimate exposure not only during daylight hours but also during the night. In other parts of the world, Keen et al. (2019) and Calambokidis et al. (2019) from studies using suction cup tags have estimated that the risk to fin whales of a ship strike was higher at night than during the day. Following those tagged whales, it could be interesting to record how they react at a very close range to approaching vessels, as did McKenna et al. (2015), and the results could also be useful for assessing the energy needs of a startle or avoidance reaction by the animal.

To reduce the risk of strikes, consideration is needed at the scale of the north-western Mediterranean Sea, and also at the scale of when a vessel and an animal are in close proximity. In the case of the Mediterranean Sea the IWC ship strike working group (Panigada & Leaper, 2010; Cates et al., 2017) have indicated that mitigation measures aimed at keeping vessels away from whales, such as the major re-routing of ferries, is not feasible as fin whales are widespread over a large area. Instead, permanent or seasonal speed restriction zones over the whole at-risk region, based on the north-western IMMA, the upcoming ship strike new Cetacean Critical Habitat and the high-risk exposure maps from this study and others, could be the better choice (IWC-IUCN-ACCOBAMS, 2019). It is clear that reducing vessel speeds to below 13 knots could be a good option to reduce NME rates, distance of sighting and lethal strikes (Vanderlaan &

Taggart, 2007; Silber, Slutsky & Bettridge, 2010; Laist, Knowlton & Pendleton, 2014; Constantine et al., 2015; Van der Hoop et al., 2015). A survey conducted on ferry passengers crossing the Pelagos Sanctuary showed that more than 75% of passengers would prefer to choose a company that reduced ferry speed if this would reduce the risk of ship strikes (Arcangeli, Bonaventura & Calicchia, 2012a).

Besides, an avoidance manoeuvre with real-time alerting tools appears best suited. Marine mammal observers aboard vessels, operating during daylight and good weather conditions, seem useful for that purpose, and with the willingness of the crew, altering course is the easiest and fastest option when an animal is detected. Another option is to decrease speed when approaching areas of high animal concentration or a system of real-time notification to all vessels of the presence of whales in an area through a system like 'whale alert', REPCET or through the AIS. Tepsich et al. (2020) using almost the same FLT dataset as in this study, showed that the abundance of fin whales over consecutive transits within a day on the same ferry route in the Pelagos Sanctuary were highly correlated, meaning that animals stayed in the vicinity for at least 1 day. Sharing information on almost real-time whale localization can be very useful across a day, but it seems that, in the Mediterranean Sea, even with such information of a nearby whale, the vessels equipped with a system do not take precautionary action such as temporary speed reduction nor alteration of course (Folegot, Gallou & Ody, 2019). Raising awareness or training programmes for vessel crews, within a global pro-active avoidance system (Gende et al., 2019) could improve the efficacy of such mitigation.

In general, including representatives of the maritime industry in defining potential mitigation measures, integrating the different potential mitigation measures reviewed here, and the adoption of a voluntary code of conduct by maritime companies could be the best choice for the implementation of measures to reduce ship strikes.

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CONFLICT OF INTEREST

None.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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Chapter 8

Modeling maritime traffic exposure for marine megafauna: differentiated potential risk assessment for bottlenose dolphins in the Sicily Channel and surrounding waters

TO BE SUBMITTED

Abstract

Successful protection and management practices to ensure the sustainable coexistence of marine biodiversity and human activities needs to be based on sound scientific knowledge able to inform either at the large processes scale and at the local level to which the measures act.

In this framework, using fine-scale field data systematically collected along fixed transects in a highly busy area in the central Mediterranean Sea (FLT Med Monitoring Network project, ISPRA), the habitat preference of the *vulnerable* and of priority importance species *Tursiops truncatus* (i.e bottlenose dolphin) was evaluated on a seasonal basis with the MaxEnt algorithm. The influence of several categories of maritime traffic on the species presence was assessed along the surveyed routes comparing two different dataset recorded in absence and presence of the species. Moreover, seasonal densities and spatial footprint of 11 vessel categories were estimated for the whole study area using remote sensing data acquired from the EMODNET platform. Then, a spatial explicit risk assessment was carried out combining information on species and vessels.

MaxEnt analyses allowed creating seasonal distribution models with good predictive capability, showing that distance from sea mountains and coasts were the most relevant environmental variables shaping the species habitat. The Egadi Island and the Tunis gulf were detected as the most suitable areas, and seasonal remarkable differences were found for the southern coast of Sicily and the lowest part of the Sicilian Channel. The number of vessels recorded along the routes was always significantly lower in presence of the species, especially for the 'Big' and 'Fishing' categories. Maritime traffic densities, and likewise the spatial footprint, changed over time for each considered categories.

The spatial distribution of the risk of exposure to maritime traffic varied according to its categories, determining different potential impacts in the different sectors of the marine environment over time. Bottlenose dolphin presence, unlike in other Mediterranean areas, appeared to be highly conditioned by all categories of maritime traffic. Understanding the complex interaction and variability of situations between bottlenose dolphin and this multi-faceted source of disturbance is of paramount importance for managing its exposure and mitigating the consequent responses.

Keywords

Maritime traffic, risk assessment, bottlenose dolphin, Sicilian Channel

Introduction

Comprehending the habitat preferences of vulnerable species is of paramount importance to plan effective conservation measures. Mapping this information together with the anthropogenic pressures and obtaining a spatially explicit risk assessment represent the starting point of the Marine Spatial Planning (MSP) (Metcalfe et al., 2018). This is one of the key tools in the Ecosystem Based

Management (EBS) framework, an approach for delivering sustainable development in the marine environment (Douvere, 2008). Together, they aim to minimize environmental impacts and to reduce potential conflicts among sea users (Crowder et al., 2006).

In this framework, Species Distribution Models (SDMs) are an essential and widely used tool to study the distribution of the species. They provide a useful analytical method for obtaining spatially explicit information about the suitability of a certain environment for the species of interest (Guisan et al., 2013; Sarà et al., 2018) and to investigate the environmental and anthropogenic factors affecting its distribution (Elith et al., 2006). This information is pivotal to understand which areas constitute the species important habitats and to identify where conflicts with human activities could develop (Guisan et al., 2013).

Marine mammals are essential component of the marine biodiversity, as they play the key role of apical predators. As umbrella and keystone species, their protection has strong effects on the structure and functioning of marine communities (Foley et al., 2010) also attracting other species (Sergio et al., 2004; Ritchie and Johnson, 2009), leading to increase biodiversity and improve ecosystem services (Sergio et al., 2006). In fact, protecting cetacean species is one of the main goal of several national and international regulations as the Habitats Directive (Annexes II and IV), and underpin their preferred habitats should be of priority importance for MSP (Hooker et al., 2011).

Cetaceans specific life-history traits, like late sexual maturity and low reproductive rate, make them particularly susceptible to human threats (Passadore et al., 2018).

Even if considered a safe form of good transportation, maritime traffic is one of the main stressor for cetacean species (Bearzi, 2002; Coomber et al., 2016; Crosti et al., 2017).

The maritime traffic threats that could directly or indirectly affect cetacean species are diverse (Abdulla & Linden, 2008). For example, activities connected with oil extraction and transportation can affect cetaceans' health (Piante & Ody 2015). Discharged marine litter, in particular fishing gears and plastic (Pham et al., 2014), can cause their death mainly through ingestion and entanglement (de Stephanis et al., 2013). Underwater noise from shipping, seismic surveys for oil and gas exploration, and naval sonar can potentially affect cetaceans in different ways and levels, interfering with their communication, excluding them from their habitat (Castellote et al., 2012; Kavanagh et al., 2019), or even causing direct mortality (Frantzis 1998; Jepson et al., 2013; Notarbartolo di Sciara et al., 2014; Podestà et al., 2016). Vessels speed and size are positive related to the risk of collisions (Laist et al., 2001; Silber et al., 2010), reported mainly for large whales but also affecting smaller cetacean species all over the world (Panigada et al., 2006; Carrillo and Ritter, 2010; Geijer and Jones, 2015). All cetaceans result in danger from naval traffic due to the heterogeneity and spatial distribution of vessel categories, with a footprint that involves not only the coastal environment but also the pelagic realm.

One of the busiest waterways in the world is the Mediterranean Sea, even if it covers only the 1% of the world's oceans. The 30% of the naval traffic pass through this basin, where the 80% of the ports are located in its western and central part (Dobler, 2002; LMIU, 2008; Vaes and Druon, 2013). From the mid-1990s to the mid-2000s an increase of the transit capacity (58%) and of the vessel size (30%) has been recorded, and it is expected to continue to increase as a consequence of the growing trend in container port traffic development and the doubling of the Suez Canal (UNEP Mediterranean Action Plan 2017). As a result, the concern for its potential impact on the marine fauna grows too (Notarbartolo di Sciara, 2010; Geijer and Jones, 2015).

Several studies have yet analysed the interaction between cetaceans and maritime traffic in the Mediterranean Sea. In the north-western part of the basin, a multispecies avoidance response has been recorded (Campana et al., 2015). Ship strikes, as well as near miss events, have been reported in several Mediterranean regions like the French coasts (Peltier et al., 2019), the Hellenic Trench (Frantzis et al., 2019) and in the Pelagos Sanctuary (di Meglio et al., 2018; David et al., 2022).

Maritime traffic densities (Coomber et al., 2016; David, 2002; Vaes and Druon, 2013) and cetacean preferred habitats (Arcangeli et al., 2016; Gannier, 2002; Panigada et al., 2011) within the Mediterranean Sea change with seasons, hence the effects of this threat can be variable over time. Therefore, understand how cetacean habitats overlaps with maritime traffic distributions throughout the years is crucial for improving future decision-making regarding the zoning of the marine space, especially in poorly studied areas.

In this work we evaluated the habitat preference of the Habitats Directive priority species common bottlenose dolphin (*Tursiops truncatus*) with the purpose of understand its relationship with maritime traffic in a highly busy environment, the Sicily Channel and surrounding waters in the central Mediterranean Sea. The Mediterranean populations of this species was listed as “Vulnerable” since 2010 (Bearzi et al., 2012); nevertheless, in the recent ACCOBAMS report (2021) a down-listing to “Least concern” is proposed (Natoli et al., 2021). Either way, due to their widely distribution, mostly but not limited to the continental shelf, bottlenose dolphins are exposed to a number of pressures, many of which related – directly or indirectly – to maritime traffic (Liret, 2001; La Manna et al., 2010, 2013; Rako et al., 2013; Bas et al., 2017c).

Using fine-scale field data collected in a highly busy area through systematic surveys on marine mammals and naval traffic (FLT Mediterranean Monitoring Network, ISPRA), the study firstly aimed at identifying bottlenose dolphin seasonal habitat preferences. Secondly, the potential impact of maritime traffic was evaluated through: a) the influence of different type of vessel traffic on bottlenose dolphin presence during monitoring surveys; b) the spatial footprint of 11 different categories of maritime traffic and their temporal variability in the study area. Finally, the risk of exposure of bottlenose dolphin to maritime traffic was assessed by building an index that considered the habitat preference of the species and the spatial distribution of the threat.

Material and Methods

Study area

Bottlenose dolphins and maritime traffic were monitored in the western Sicilian waters and in the Sicily Channel (Fig. 1). Two trans-border transects crossed this area from 2013 to 2019 linking Palermo and Civitavecchia to Tunis and passing near three Marine Protected Areas (MPAs): the Capo Gallo and Isola delle Femmine MPA, situated just outside Palermo; the Egadi Island MPA, located off the western coast of Sicily, and the Zembra and Zembretta MPA in the Tunis Gulf. In the north-eastern sector of the study area insist also the Ustica MPA, and in the coastal waters of Cape Bon (North-east Tunisia) is located the Kelibia Important Marine Mammal Area (IMMA), that support a resident population of vulnerable Mediterranean bottlenose dolphins. Moreover, the Egadi Island are proposed as "Area of Interest" (AOI) by the Marine Mammal Protected Areas Task Force, in the framework of marine mammal conservation in the Mediterranean region.

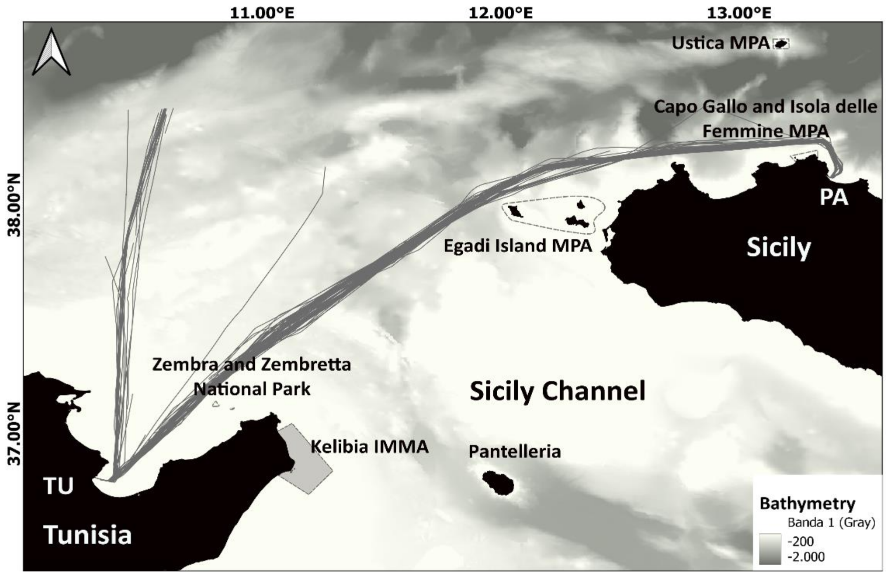

Fig.1 – Study area and monitored routes along the years.

Data collection

Both bottlenose dolphin occurrences and maritime traffic data were recorded during surveys conducted on board of passenger ferries, following a protocol designed by ISPRA (ISPRA, 2020, Technical Annex I). Surveys were carried out from 2013 to 2019 all year round, with a minimum of three surveys per season.

Trained marine mammal observers were located on both side of the ship's command bridge scanning an angle of 130° ahead. Information about the maritime traffic presented in the surveyed transect were collected during bottlenose dolphin sightings ("Presence" dataset) and approximately every hour in random points in their absence ("Absence" dataset). Maritime traffic was subdivided into 2 macro-categories (< 2 nm and > 2 nm from the ferry), each of which consisted of 3 sub-categories:

Small (< 5 m), Medium (motor, sail or fishing with dimension between 5 and 20 m), and Big (> 20 m) vessels.

Observation were performed during daylight and only in good weather conditions (Beaufort scale ≤ 3), monitoring continuously the sea by naked eye, using binoculars (7x50 magnification) to confirm the species identification, group size or naval traffic category. For every bottlenose dolphin sighting, information about the size of the group, the surface behaviour, the response to the ship, the direction of swimming, the distance and angle from the ship were recorded.

The "on effort" track lines and each sighting, either of cetacean or maritime traffic, were recorded by a dedicated GPS and annotated on standard datasheets.

Data Analysis

All statistical analyses discussed later were performed using the software Past 4.1 (Hammer et al., 2001). The non-parametric Kruskal-Wallis (KW) test and the post-hoc Mann-Whitney (MW) test with Bonferroni correction were used to test for significant differences. The spatial analyses were carried out using the R 3.4.6 and the QGIS 3.22.4 softwares, while the habitat suitability models were executed using MaxEnt software.

Species habitat suitability

With the purpose of determining what are the environmental variables characterizing bottlenose dolphin habitat and forecast the species potential distribution for the entire study area, MaxEnt software was used. It was specifically developed for presence-only data, and it is one of the most used algorithms for SDMs (Phillips et al., 2006), as it has higher predictive capabilities when compared to other algorithms (Elith et al., 2006) especially when the number of presences used is low (Baldwin, 2009; Giannini et al., 2013), as it is in this case.

Seven explanatory variables were associated to the bottlenose dolphin occurrence dataset. Those factors were chosen because previously considered predictive of the habitat of this species (Barragán-Barrera et al., 2019; Carlucci et al., 2016, 2018). They can be divided into:

- Topographical variables (not subjected to seasonal changes): Bathymetry [m], Seabed slope [degrees], Minimum Distance from the nearest coast, canyon and seamount [km].
- Oceanographic variables (change dynamically with seasons): Sea Surface Temperature [SST, C°], CHL-a concentration [mg m^{-3}].

The CHL-a and SST raster files with an 8-days temporal resolution and a 4x4 km spatial resolution have been downloaded from NASA Ocean Color (http://oceancolor.gsfc.nasa.gov) and averaged on a seasonal basis.

Bathymetry values were obtained from the GEBCO raster file (GEBCO Compilation Group (2020) GEBCO 2020 Grid (doi:10.5285/a29c5465-b138-234d-e053-6c86abc040b9); bathymetric slope and minimum distance from the coastline 1x1 km raster files were acquired from the MARSPEC dataset

(Sbrocco and Barber, 2013), while the vector layers of the geomorphic features from the Blue Habitat dataset (Harris et al., 2014). The rasters of the Euclidean distances from the nearest features were computed, and all were matched to the same resolution of SST and CHL-a ones and averaged for the two considered periods using the "raster" package in R.

In order to obtain the best setting for MaxEnt the R package "ENMeval" was used. This package is able to generate - on the basis of species occurrences and predictive variables - a number of possible setting from which to choose the one with the ΔAIC equal to zero. In particular, the results related to the feature type [Linear (L), Quadratic (Q), Product (P), Threshold (T), Hinge (H)] and the values of the regularization multiplier (rm) were used. Regulation multiplier is very similar to the AIC used for the model comparison (Burnham and Anderson, 2002). It is set by default for each feature class (Merow, 2013) but can be multiply by a user-specified constant to produce more or less complex models (Anderson and Gonzalez 2011; Elith et al., 2011). Substantially, regularization multiplier reduces over-fitting of the model (Merow, 2013) and it ranges from 1 to 5.

The package also generated a bias file, meaning a function that allows correcting uneven distributions of the sampling effort to be included in the software (Phillips et al., 2006).

The models performance was evaluated using the AUC (Area Under the roc Curve), which defines the discriminatory power of the model by comparing its sensitivity (i.e True Positive) with 1-specificity (i.e False Positive) (Phillips et al., 2006).The AUC values ranged between 0 and 1: values ≤ 0.5 indicate that the model performs better than random (Phillips and Dudik, 2008), while values between 0.6 and 0.9 expresses that it fits well (Breen et al., 2016). Moreover, a Jackknife test was used for evaluating the possibility of excluding of some of the predictive variables to obtain a final model with improved predictive capabilities and with less background noise.

Finally, the presence probability obtained from the model was converted to continuous in binary using the threshold value "*maximum training sensitivity plus specificity*" (Cloglog threshold) generated as output from MaxEnt (Sahri et al., 2020) and the extension of the suitable areas was calculated for both study periods.

The hazard – Maritime Traffic

Maritime traffic characterization along the monitored routes The classification of maritime traffic along the surveyed transects was carried out starting from the information recorded in the "Naval traffic sheet". In order to verify variation with the time of the year, it was characterized on seasonal basis: Winter (January, February, March); Spring (April, May, June); Summer (July, August, September) and Autumn (October, November, December) (Campana et al., 2017, 2018).

With the purpose of evaluating the relationship between the presence of maritime traffic and bottlenose dolphins, the records belonging to the "Absence Dataset" were compared to those of "Presence Dataset" using the two samples Komogorov-Smirnov test (KS) to test the null hypothesis that the number of vessels does not differ between them (Campana et al., 2017, 2018).

Moreover, the average percentage difference (D) between the number of recorded vessels in presence and absence of sighting was reported as:

$$D = \frac{N\ average\ vessels\ in\ presence - N\ average\ vessels\ in\ absence}{N\ average\ vessels\ in\ absence} \text{ x } 100$$

Maritime traffic characterization in the study area The vessel density (total number of hours per square km) rasters for all months between 2017 and 2019 were downloaded from the EMODnet platform (https://emodnet.eu/en), splitted on a seasonal basis (Autumn-Winter: October to March, while Spring-Summer: April to September) and then averaged through the three years period. These vessel density maps were generated starting from the vessel position extracted from the Automatic Information System (AIS), which is a satellite device developed to track and monitor vessel movements for different purposes, such as avoiding collisions and aiding navigation. According to the International Maritime Organization (IMO, 2000) all ships of ≥ 300 gross tonnage engaged on international voyages, cargo ships of ≥ 500 gross tonnages not engaged on international voyages and all passenger ships independently of size require this tracking system. In addition, starting from May 2014, the EU requires its placement on all fishing vessels ≥ 15 m (Council regulation (EC) No 1224/2009).

All EMODnet available density rasters of maritime traffic categories (Fishing, Service, Dredging or Underwater operations, Sailing, Pleasure craft, High-speed craft, Tug and Towing, Passenger, Cargo, Tanker, Military and Law enforcement) were used in order to assess and discriminate the potential risk represented by the different vessel categories. A total of 22 vessel density raster maps (11 vessel categories for 2 seasonal periods) for the whole study area were produced.

To describe the general spatial footprint determined by the different vessel categories on the study area, it was first computed the proportion between the area of the cells observing any value of vessel density and the total area of study (Eigaard et al., 2017). Afterward, to take into consideration the animal response to traffic, a potential footprint of impact based on the empirical relation between marine traffic and sightings found in section 2.4.1 was estimated. A threshold was firstly set equal to the lower vessel density value associated to no sightings; then, the proportion between the area of the cells observing a value higher than the percentage of marine traffic associated to no sighting (see SM1 and SM Tab. 1) and the total area of study was computed. The latter indicator could express the extent of the area where absence of cetacean species can occur with higher probability due to vessel traffic.

Risk of exposure assessment

A spatial explicit risk assessment was carried out throughout the study area in order to identify the areas of particular risk of bottlenose dolphin exposure to maritime traffic threat, considering the different EMODnet vessel categories.

Outputs from habitat suitability and vessel density maps were converted into four categories, 0 (Null); 1 (Low); 2 (Medium) and 3 (High), expressing with a qualitative value the presence probability of the species and the intensity of the naval traffic, respectively.

For habitat preference the four categories were obtained using the "*maximum training sensitivity plus specificity*" threshold value of the presence probabilities to define the lower extreme of the higher rank, and the other three dividing equally the remaining values.

Maritime traffic density was categorized using the natural breaks (Jenks) classification on the distribution of the average values between the two seasonal periods of all vessel categories selected. This classification approach uses an algorithm that optimizes the arrangement of a set of values into natural classes, minimizing the average deviation from the class mean and maximizing the deviation from the means of the other groups.

The Jenks classification was applied to the seasonal density mean within the overall Mediterranean basin ("Med" classification). This was decided to account the whole spatial distribution of maritime traffic at a bigger scale and to compare it to the actual distribution inside the study area.

A risk of exposure index was computed by the product between the resulting categorized maps following the rules described on Tab. 1, for each time period considered.

Tab.1 – Risk of exposure index possible values.

Presence probability / Vessel density	Null (0)	Low (1)	Medium (2)	High (3)
Null (0)	Null (0)	Null (0)	Null (0)	Null (0)
Low (1)	Null (0)	Low (1)	Low-Medium (2)	Medium (3)
Medium (2)	Null (0)	Low-Medium (2)	Medium (4)	Medium-High (6)
High (3)	Null (0)	Medium (3)	Medium-High (6)	High (9)

Results

From 2013 to 2019, 135 surveys were conducted in the study area covering 30674 km on effort and reporting 79 bottlenose dolphin sightings (Tab. 2).

Tab.2 – Sampling effort, hours of observation, number of transects surveyed and number of bottlenose sightings in the study area on a seasonal basis.

Season	Km on effort	Obs. hours on effort	N transects	N sightings
Winter	5406.67	886.4	26	21
Spring	10752.66	1303.2	44	24

Summer	7128.50	994	30	10
Autumn	7386.85	1053.2	35	24

Species habitat suitability

For the habitat modelling, N = 49 and N = 35 bottlenose dolphin occurrence points were used, respectively for the first and the second seasonal periods.

The best MaxEnt setting generated from ENMvalue package suggested to use for both periods feature types Linear and Quadratic, and rm = 1. No correlation was found among variables, so the two models were generated using the entire set of predictive variables. The final AUC values were 0.893 for the first period, and 0.910 for the second.

For both models, the most important variables in determining bottlenose dolphin habitat were Distance from sea mountain (respectively 41.4% for Aut-Win and 21.8% for Spr-Sum) and from the coast (31.5% and 58.3%). A minor role was played by Bathymetry in both periods (12.3% and 5.9%), followed by Slope (8.2%) and Distance from canyon (4.4%) in Aut-Win, and by CHL-a (5.8%) in Spr-Sum.

In the Aut-Win model, the highest presence probability was found, in general in all the coastal environment, with particularly high values around the Egadi Island and in the Tunis gulf (Fig. 2, first panel). The Spr-Sum model underlined the same areas as well, but remarkable differences were found for the southern coast of Sicily and the lowest part of the Sicilian Channel, characterized by lower presence probability values (Fig. 2, second panel). In both models, the whole north-western part of the study area showed the lowest suitability. Moreover, low values were found also for the central part of the Sicily Channel for both periods.

The threshold values used to transform the presence probability from continuous to binary were 0.47 and 0.53 for Aut-Win and Spr-Sum respectively. The resulted suitable areas resulted more extended in the Aut-Win period (19,113.94 km^2) compared to the Spr-Sum one (16,567.13 km^2), accounting respectively for the 26% and 23% of the total study area.

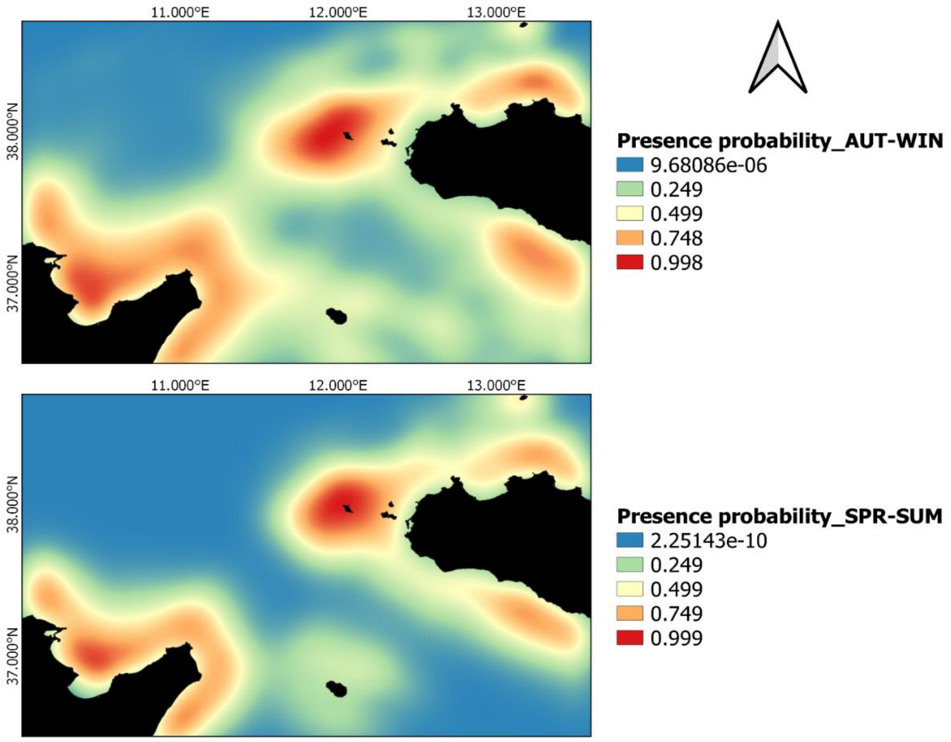

Fig. 2 – Autumn-Winter (upper panel) and Spring-Summer (lower panel) bottlenose dolphin probability of occurrence in the study area according to the Habitat Suitability models.

The hazard – Maritime Traffic

Maritime traffic characterization along the monitored routes During the seven years of monitoring 2499 vessels were reported in the "absence" dataset. The highest and lowest numbers were found in Spring (N = 805) and Winter (N = 359) seasons respectively; however, no inter-seasonal differences were statistically significant (KW test, H = 5.39; $p > 0.05$).

Differences in vessel categories were identified. Big was always the most recorded category, followed by Fishing (MW test, $p < 0.05$) while the least represented was Small, recorded in each season significantly lower than the others (MW test, $p < 0.001$). No significant inter-seasonal differences were found within each of these categories (KW test, H = 0.05; $p < 0.05$). Motor and Sailing were instead lower during Winter than during Spring and Summer (MW test, $p < 0.05$).

Influence of maritime traffic on dolphin presence In presence of the species, the number of recorded vessels was always significantly lower than in absence of sightings (-87%, KS test, $p < 0.001$). The same result was obtained by stratifying for the different seasons and for the various vessel categories (KS test, $p < 0.001$) (Fig. 3).

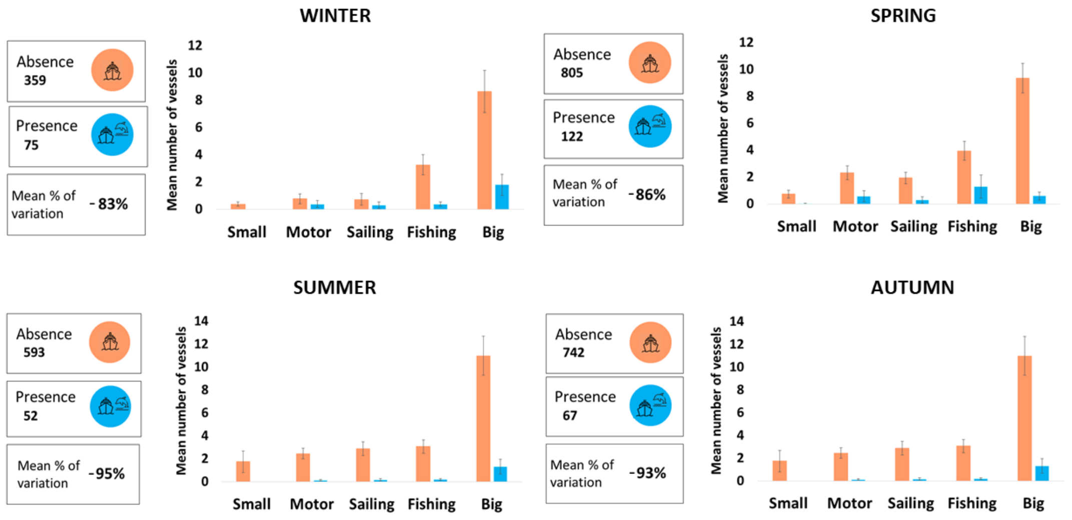

Fig. 3 – Mean number of vessels ± standard error in presence and absence of bottlenose dolphins during the seasons. In the squares, the total number of vessels in the two conditions and the mean % of variation.

In fact, in almost all seasons and categories, the % difference between the number of vessels in absence vs. the number of vessels in presence of the species was between -73% and -100%; the lowest values were recorded for motor boats and sailing in Winter (-52% and -58%) and for fishing vessels in Spring (-68%).

Maritime traffic characterization in the study area Cargo and Fishing were the categories with higher mean density values in both considered periods, followed by Tanker and Passenger, for the whole study area. Almost all categories increased their densities during Spring and Summer (Tab. 3).

Tab.3 – EMODnet vessel categories used for the analyses. Vessel density (hours in navigation/km²) mean, maximum, and Standard Deviation (SD) values of respectively Aut-Win and Spr-Sum are represented, in reference to the study area.

	EMODnet Vessel Category	**Aut-Win**			**Spr-Sum**		
		Mean	SD	Max	Mean	SD	Max
1	Fishing	2.62	127.34	28528.66	4.25	154.23	33653.17
2	Service	0.07	10.46	2373.02	0.05	6.96	1572.22
3	Dredging or underwater operations	0.02	0.54	74.65	0.014	0.52	81.90
4	Sailing	0.41	27.44	4050.36	0.93	31.67	3964.16
5	Pleasure craft	0.35	31.48	5117.63	0.78	36.49	5270.22
6	High-speed craft	0.11	6.29	1120.12	0.18	9.17	1390.89
7	Tug and towing	0.24	16.37	3127.52	0.23	11.75	1838.32
8	Passenger	0.65	34.76	5542.45	0.90	41.98	7470.91

9	Cargo	2.70	36.75	7231.24	3.01	36.95	7263.83
10	Tanker	1.07	7.20	1190.87	1.22	6.38	921.72
11	Military and law enforcement	0.07	6.61	1156.20	0.09	8.41	1471.24

Despite the heterogeneity of vessel categories and seasonality, the Sicily Channel was characterized by high mean maritime traffic density values. The areas most influenced by the presence of big vessels (Cargo, Tanker, Passenger, High-speed vessel) were limited to the main motorways crossing the study areas, while Fishing, Sailing and Pleasure crafts were more heterogeneously distributed, in particular during the Spr-Sum period. Other specific categories, like Service and Military and law enforcements, were concentrated in limited portion of the study area such as the Tunis and Palermo harbours (SM, Fig. 1-2).

This spatial arrangement was confirmed by the percentages obtained from the maritime traffic footprint analysis (Tab. 4).

Fishing, Cargo, and Tanker footprints remained high in both considered periods even if Fishing showed a noteworthy increase during the Spr-Sum. A similar increase during the latter period was registered also for Sailing and Pleasure craft. High-speed craft showed the lowest footprint percentage in both periods, together with Service in Aut-Win, and Dredging or underwater operation in Spr-Sum.

Footprints estimated through the empirical threshold value were lower compared to the one considering the whole maritime traffic distribution but with a similar seasonal change in their values among the vessel categories. The lower values compared to the overall footprints are related to the presence of areas where vessel density was similar to areas of sightings of cetacean species during the monitoring survey. This reduction is more noticeable for vessel categories such as Fishing, Pleasure craft, Cargo and Tanker.

Tab. 4 – Footprints (expressed as % of surface covered) of the different maritime traffic categories for the Aut-Win and Spr-Sum periods, calculated without (white columns) and with (light grey columns) the correspondent thresholds.

	EMODnet Vessel Category	Aut-Win		Spr-Sum	
1	Fishing	73.40	64.90	93.67	75.46
2	Service	19.72	14.20	34.11	28.66
3	Dredging or underwater operations	25.38	21.20	28.83	26.24
4	Sailing	67.73	66.38	98.55	98.55
5	Pleasure craft	65.47	47.18	96.41	80.93

6	High-speed craft	15.69	13.10	29.67	27.00
7	Tug and towing	63.65	53.18	74.87	68.14
8	Passenger	76.24	63.68	81.38	74.05
9	Cargo	97.04	81.16	95.84	87.26
10	Tanker	91.89	76.79	91.32	83.11
11	Military and law enforcement	27.75	23.17	37.77	34.37

Risk of exposure assessment

The habitat suitability ranks, as well as the four Jenks classes corresponding to the classification of maritime traffic, are shown in the SM (Tab. 2).

The risk of exposure to maritime traffic was detected in almost all the studied areas except for the north-western sector, with different distribution due to the different vessel categories. In more coastal water, the risk of exposure resulted mostly associated with Sailing, Pleasure crafts, High-Speed crafts as well as Service, Dredging or Underwater operations, and Military and Law enforcement. Cargo and Tanker mostly determined a wider risk in offshore waters, while the risk associated to Passenger vessel was specifically limited to the fixed travelled routes. Dredging or Underwater operations, Tug and Towing and Fishing associated risk was detected either in coastal and offshore waters. The exposure areas were in general more spread out during Aut-Win (Fig. 4) while more concentrated during the Spr-Sum season (Fig. 5).

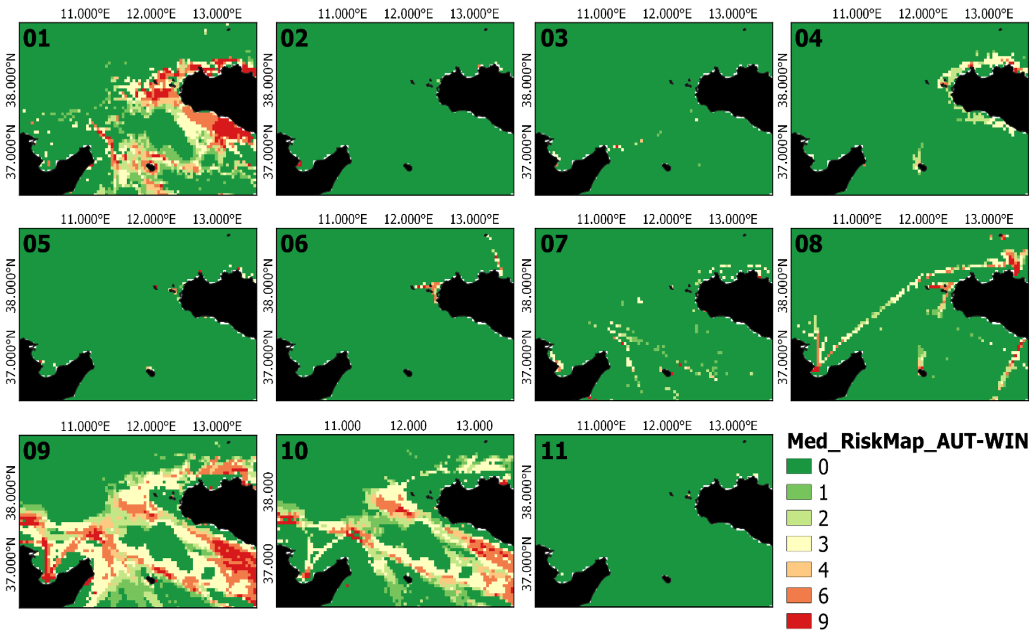

Fig. 4 – Risk Index maps for the Aut-Win period (01=Fishing; 02=Service; 03=Dredging or Underwater operations; 04=Sailing; 05=Pleasure crafts; 06=High-Speed crafts; 07=Tug and Towing; 08=Passenger; 09=Cargo; 10=Tanker; 11=Military and Law enforcement) calculated using the naval traffic maximum density values of the entire Mediterranean Sea.

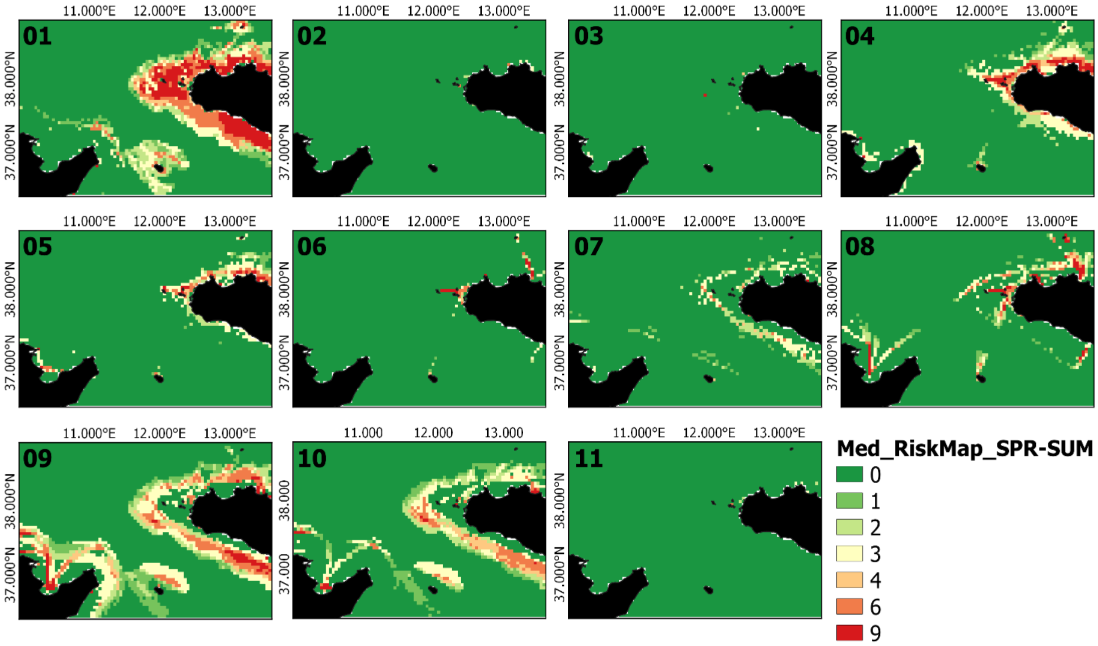

Fig. 5 – Risk Index maps for the Spr-Sum period (01=Fishing; 02=Service; 03=Dredging or Underwater operations; 04=Sailing; 05=Pleasure crafts; 06=High-Speed crafts; 07=Tug and Towing; 08=Passenger; 09=Cargo; 10=Tanker; 11=Military and Law enforcement) calculated using the naval traffic maximum density values of the entire Mediterranean Sea.

Fishing, Cargo and Tanker showed the higher area percentage characterized by high values of risk (i.e 4, 6 and 9) (Fig. 6). Almost no differences between seasons were recorded for the first category, while for Cargo and Tanker the area percentage was higher during Aut-Win.

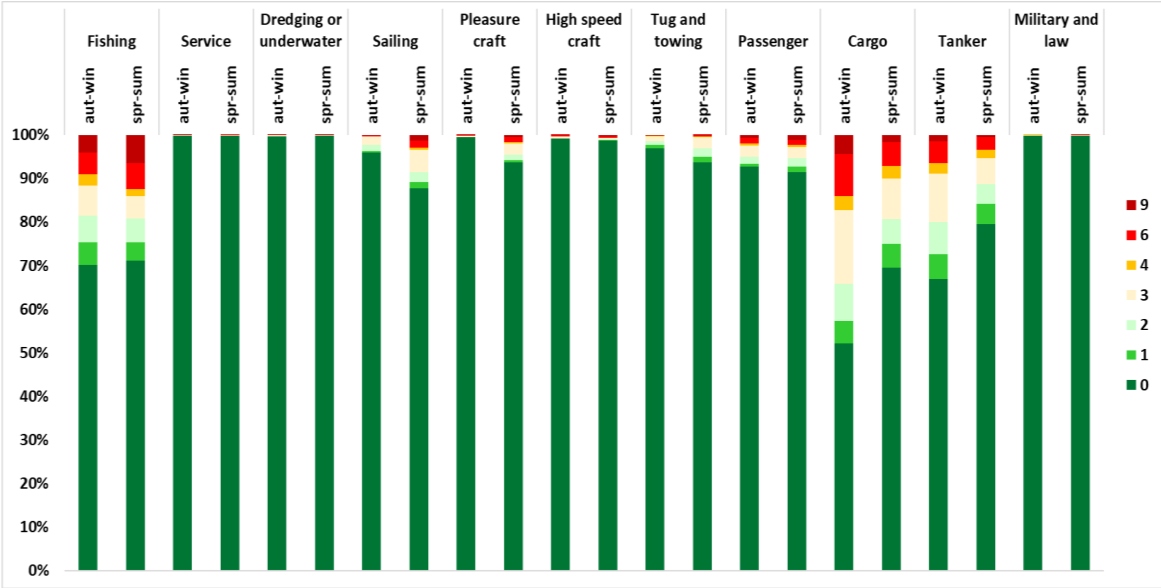

Fig. 6 – Risk of exposure index values and correspondent % occupied area for each vessel category in the two study period considered.

Discussion

Species habitat suitability

Findings of the study highlighted the permanence of bottlenose dolphin along the monitored transects throughout all seasons, although with a slight decrease during the summer period.

Small-scale presence, distribution and population dynamics of bottlenose dolphins in the Sicily Channel were reported from several studies. In the north-western side of the Channel they form an open population with low site fidelity, with females with calves staying in the area longer than other individuals (Papale et al., 2017). Bottlenose dolphin long-term presence is also recorded in the coastal waters of Lampedusa, which represent an important natural habitat where feeding, social activities and mating regularly occur (Pace et al., 1999; Pulcini et al., 2004). In the area, also association with aquaculture cages has been registered (Pace et al., 2012; Pulcini et al., 2004), together with exposition to boat traffic and related noise (La Manna et al., 2010, 2013). Bottlenose dolphins are regularly present also in the coastal water of north-eastern Tunisia, with different degrees of residency (Benmessaoud et al., 2012, 2013) and around the isle of Malta, where the species prefers deep and offshore waters.

The habitat suitability models showed good predictive ability for both periods considered. Bottlenose dolphin presence probability was mostly influenced by the distances from seamount and coast, followed by bathymetry. Those results are in line with the habitat of this species, mostly found within its typical environment, the continental platform, but also capable to explore deeper water, likely for feeding purposes. Sea mountains are in fact hotspot of biodiversity who can attract top predators like bottlenose dolphins (Shank, 2010; Greene et al., 1992; Vetter et al., 2010; Morato et al., 2010; Fiori et al., 2015; Cañadas et al., 2002).

In both periods, the north-western sector of the study area resulted the less suitable: it is characterized by the highest absolute depth values, resulting in an environment probably too distant from the habitat preferences of the species. Also, the central portion of the Sicily Channel showed low levels of suitability.

The hazard – Maritime traffic

The maritime traffic monitoring along the transects has allowed to record approximately 2500 vessels in absence of the species, without substantial differences between the different seasons. Large size vessels ("Big") were always the most represented reflecting the importance of the Sicily Channel as a "Motorways of the Sea" connecting the western to the eastern Mediterranean Sea as alternative to land transportation (EC, 2004; Patruno, 2008). Fishing vessels were the second macrocategory in importance. Fisheries are indeed one of the most traditionally practiced sectors along the Sicilian/Tunisian coast and in the Sicily Channel, with more than 1000 operating vessels for demersal fisheries only (FAO, The State of Mediterranean and Black Sea Fisheries). Seasonal variations were registered, as expected, for Sailing and Motor boats, recorded mostly during Spring and Summer than in Winter, as the study area encloses some of the most popular Sicilian tourist destinations, which is most likely the cause of the increased observations of these two categories.

Overall, the number of vessels recorded was always significantly lower during bottlenose dolphin sightings than in the random location in their absence. The percentage difference between the two cases was greater than 90% in almost all seasons and categories. A minor difference – although still significant – was recorded for the Motor category during Winter and for Fishing during Summer. This strong response towards the presence of maritime traffic is of particular interest because, on the contrary of the other cetacean species, bottlenose dolphins are known to be more adaptable to this kind of threat (Bearzi et al., 2009). Being a mainly coastal species, it is surely more accustomed to the presence of maritime traffic (Campana et al., 2015). Nevertheless, unquestionably ships presence do affect bottlenose dolphins that often put in place diverse behavioural responses while not moving away from their preferred environment (Nowacek et al., 2001; Bejder et al., 2006b; Arcangeli and Crosti, 2009; Papale et al., 2012; La Manna et al., 2013). Results of this study confirm the plasticity of this species that, unlike in other Mediterranean areas (Campana et al., 2015), is likely forced to put in place also an avoiding behaviour in this highly trafficked area of the Mediterranean Sea. In fact, regardless of the season considered, bottlenose dolphin presence appears to be conditioned by all categories of maritime traffic, even by those that in other Mediterranean areas had relatively low influence on cetacean species (e.g. Pleasure boats, Campana et al., 2018) or from which this species is usually attracted (e.g. Fishing boats, Scuderi et al., in press).

Both maritime traffic footprint estimates well represent the seasonal variability of this anthropogenic threat. In both periods, the more prevalent categories were Cargo, Tanker, Passenger and Fishing and, seasonally, Sailing and Pleasure crafts. Those results are not surprising considering the wide

distribution of those categories related to important economic sectors insisting in the study area (transportation, fisheries, and tourism-related activities).
Passenger, Sailing, High-speed and Pleasure crafts the density increases and the footprint become larger in the Spr-Sum season, in particular in the Sicily Channel and along the Sicilian northern coasts. With the improvement of the environmental conditions and the advent of the summer season these kind of transportation increases and become more frequent, especially towards the major tourist destinations, also in other sectors of the Mediterranean Sea (Notarbartolo di Sciara, 2010; David et al., 2011; Vaes and Druon, 2013).

Risk of exposure assessment

Despite acknowledged as a ubiquitous threat to the conservation of marine megafauna (Jarvela Rosenberger et al., 2017; Rolland et al., 2012; Williams and O'Hara, 2010), shipping is rarely actively managed.
Vessels are the most widespread source of noise, generating sounds over longer periods of time and larger areas (Tyack, 2008). The exposure of cetaceans to chronic sources of underwater noise from shipping has been related to non-lethal effects like behavioural disruption (Wisniewska et al., 2016), communication (Holt et al., 2009, 2011) and echolocation masking (Veirs et al., 2016), while the exposition to acute sources of noise (deriving from high-power echosounders, airguns, pile driving, navy sonar) can cause hearing loss and increases in stress (Bailey et al., 2010; Erbe et al., 2019). Even if Odontocetes like bottlenose dolphins use higher frequency for signaling and have lower sensitivity to low-frequency sounds with respect to Mysticetes, recent findings suggest that their sensitivity to shipping noise have been underestimated (Dyndo et al., 2015; Aguilar Soto et al., 2006). The continuous growing of maritime traffic density, the increasing vessels mean travel speed and size had augmented the number of collision events between them and cetaceans over the years, and ship-strikes are now considered one of the major threat to them (Cates et al., 2017). Most fatal injuries regard large cetaceans, and are caused by large motorized vessels (> 80 m) moving at > 14 knots (Laist et al., 2001). Nevertheless, some cetaceans have been known to recover even from severe injuries (Dwyer et al., 2014).
Commercial fisheries often interact with vulnerable, non-target species of megafauna like sharks, sea turtles, and marine mammals (Werner et al., 2015; Lucchetti et al., 2017; Erguden et al., 2022). Among the notable interactions, the most significant is the one with cetaceans: they are seen like potential competitors for the same resources and conflictual relationships are reported throughout the world. For the Mediterranean Sea, those are mainly between small-scale fisheries and bottlenose dolphins (Diaz-Lopez, 2006; Brotons et al., 2008; Rocklin et al., 2009), that can either be directly (e.g. injury or death) or indirectly (changes in feeding behaviour and distribution). On the other hand, fishermen activities can be damaged and disturbed due to their depredation.
Dolphin coastal communities like the ones of bottlenose dolphins can be significantly impacted by boat traffic (Van Waerebeek et al., 2007). However, the interactions between the presence of boats,

their noise and their behaviour around animals, and the consequent risk perception by them, are not always straightforward (Pirrotta et al., 2015; Ellison et al., 2012). Single individuals can be able of compensating, for example for missed foraging opportunities, but this capacity is currently not well known and an increasing number of interaction could compromise their wellbeing.

With shipping constantly increasing, our study provides useful information about bottlenose dolphins exposure to this threat, underlining the areas and seasons of increased risk according to the different categories of maritime traffic. Understanding the complex interaction and variability of situations between bottlenose dolphin and this multi-faceted source of disturbance is of paramount importance for managing its exposure and mitigating the consequent responses.

Anyway, it is important to highlight that both species preferred habitat and disturbance are under spatio-temporal changes: any conservation or management measures, to be effective, must take this into account to ensure the protection of the species. For example, Maxwell et al. (2020) stated that only area-based management tools without fixed boundaries are the only option to sufficiently protect wide-ranging species like the one considered in this study. This kind of "adaptive" management can now be achieved thanks to the development of science and technology, and boundaries can be defined in different ways (Maxwell et al., 2015). In this framework, it is important to underline that, as adaptive management measures could be required during specific periods and in specific areas, restrictions in human activities can be instituted for shorter amount of time (Dunn et al., 2016).

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Supplementaty materials

Supplementary S1

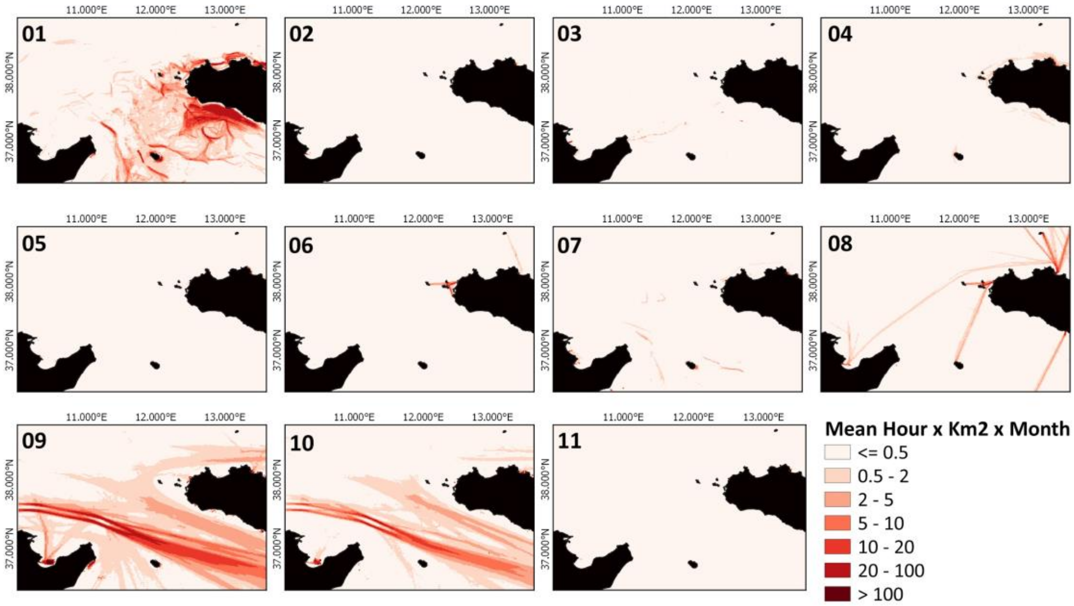
The threshold values (as number of hours per square km) used for describing the extent of vessel density inside the study area that could cause a reduction of presence of cetacean sighting was extracted from the % difference computed between the number of vessels in absence vs. the number of vessels in presence of the species as described in section 2.1. This difference assigned to every vessel sub-category (Small, Motor vessel, Sail, Fishing, Big) was first averaged between the two seasonal selected period (Autumn-Winter and Spring-Summer) then the results were assigned to the vessel categories present in the EMODnet vessel density rasters with the following criteria (Tab. 1):

- Fishing was linked to Fishing
- Service and Pleasure craft were linked to Motor vessel
- Dredging or Underwater operations, High-speed craft, Tug and Towing, Passenger, Cargo, Tanker, Military and Law enforcement were linked to Big
- Sailing was linked to Sail

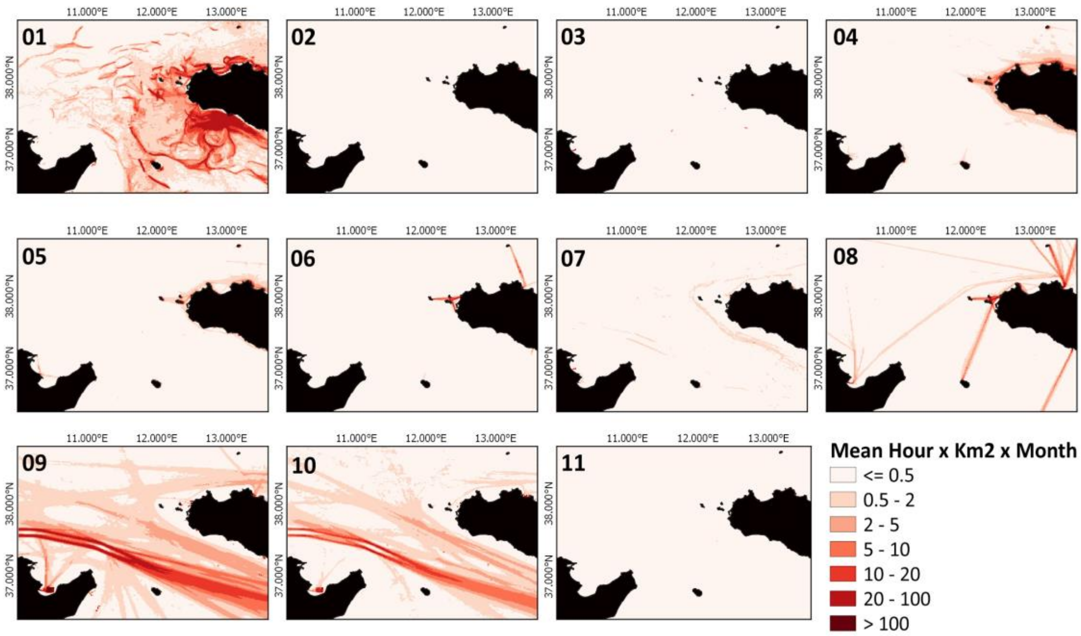
Since the empirical study results express the percentage as the reduction of vessel traffic in presence of the cetacean species, the complementary difference (100-%difference) was computed in order to express the percentage of vessel traffic above which the absence of the species manifests. This complementary difference was then applied as percentile to the different vessel categories density raster distribution (excluding the cell equals to 0 since they represent no disturbance) to find the seasonal thresholds for the footprint estimation (Tab. 1).

SM Tab.1 - Percentage and complementary difference between the mean number of vessels in presence vs. in absence of bottlenose dolphins for the different maritime traffic categories and two seasonal periods. The thresholds used for the seasonal footprint estimates are also indicated.

Vessel category	**Aut-Win**			**Spr-Sum**		
	% difference	**Comp. difference**	**Threshold**	**% difference**	**Comp. difference**	**Threshold**
Sailing	98	2	0.02	100	0	0
Motor	72	28	0.28	84	16	0.16
Fishing	86	14	0.14	80.5	19.5	0.195
Big	83.5	16.5	0.165	91	9	0.09



SM Fig. 1 - Maritime traffic mean density maps for the Aut-Win period (01=Fishing; 02=Service; 03=Dredging or Underwater operations; 04=Sailing; 05=Pleasure crafts; 06=High-Speed crafts; 07=Tug and Towing; 08=Passenger; 09=Cargo; 10=Tanker; 11=Military and Law enforcement).



SM Fig. 2 - Maritime traffic mean density maps for the Spri-Sum period (01=Fishing; 02=Service; 03=Dredging or Underwater operations; 04=Sailing; 05=Pleasure crafts; 06=High-Speed crafts; 07=Tug and Towing; 08=Passenger; 09=Cargo; 10=Tanker; 11=Military and Law enforcement).

SM Tab. 2 – The four Jenks classes for the dataset of vessel density values ("Med" classification) and habitat suitability periods used for the construction of the potential risk index.

Variable		**Jenks class**			
		0	1	2	3
Vessel density	**Med**	0 - 0.275	>0.275 - 1.033	>1.033 - 2.447	>2.447 - Inf (Max 4.815)
Habitat suitability	**Aut-Win**	0 - 0.158	>0.158 - 0.315	>0.315 - 0.474	> 0.474 - Inf (Max 0.998)
	Spr-Sum	0 - 0.176	>0.176 - 0.350	>0.350 - 0.528	>0.528 - Inf (Max 0.999)

Chapter 9

Tying up loose ends together: cetaceans, maritime traffic and spatial management tools in the Strait of Gibraltar

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RESEARCH ARTICLE

WILEY

Tying up loose ends together: Cetaceans, maritime traffic and spatial management tools in the Strait of Gibraltar

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Abstract

1. The transboundary area of the Strait of Gibraltar is home to seven protected cetacean species that are threatened by high intensity of maritime traffic. More comprehensive knowledge of cetaceans and maritime traffic is required, together with analyses of legislations, strategies and policies.
2. This study quantitatively investigates cetacean distribution and maritime traffic intensity and, for the species of community interest bottlenose dolphin, habitat suitability. Results are qualitatively discussed considering the overlap of cetacean hot spots with different maritime activities and the consistency of spatial conservation management measures in force.
3. The Fixed Line Transect Mediterranean Monitoring Network protocols were followed for 59 visual surveys using ferries as observation platforms for monitoring cetaceans and maritime traffic. Surveys were carried out along the transects between Algeciras and Ceuta and between Algeciras and Tanger-Med, in 2018 and 2019. 264 cetacean sightings, including seven different species and four near-miss collision events (involving pilot, sperm and fin whales), were reported.
4. Monitoring cetaceans from ferries in the Strait provided insights into cetacean distribution and maritime traffic, enabling the identification of cetacean hot spots, suitable habitats and maritime traffic high-risk zones.
5. A transboundary management effort is required, together with an adaptive approach for protecting highly mobile species such as cetaceans. Proposals include a long-term cetacean monitoring program carried out by dedicated observers on board ferries as a cost-effective methodology and mandatory training for crew members, to increase cetacean knowledge and reduce collision risk.
6. The designation of an international temporal or, in some zones, permanent speed reduction area (i.e., Cetacean Critical Navigation Zone, with a maximum speed of 13 knots) and of a micro-sanctuary with a seasonal no-take zone in the Bay between Algeciras and Gibraltar, together with international surveillance, are recommended measures for the enhancement of conservation efforts in the Strait.

KEYWORDS
Atlantic Ocean, conservation, dolphin, ferry, governance, integrated management, marine mammal, Mediterranean Sea, monitoring, whale

1 | INTRODUCTION

Based on the IUCN Red List of Threatened Species, the Strait of Gibraltar (henceforth the Strait) hosts seven protected 'cetacean' species: these are the Endangered short-beaked common dolphin (hereafter common dolphin), *Delphinus delphis* (Bearzi et al., 2021); the Critically Endangered populations of long-finned pilot whale (henceforward pilot whale), *Globicephala melas* (Verborgh & Gauffier, 2021) and killer whales, *Orcinus orca* (henceforth orca) (Esteban & Foote, 2019); and the Endangered Mediterranean subpopulations of the fin whale, *Balaenoptera physalus* (Panigada et al., 2021) and sperm whales, *Physeter macrocephalus* (Pirotta et al., 2021). The Mediterranean subpopulations of the striped and bottlenose dolphins, currently listed as 'Least Concern' (*Stenella coeruleoalba* and *Tursiops truncatus*) (Lauriano, 2021; Natoli et al., 2021), also inhabit the waters of the Strait (Espada Ruíz et al., 2018; Tenan et al., 2020), with the latter being a priority species listed in Annex II of the Habitats Directive (92/43/CEE, HD) for which Special Areas of Conservation are required. Species such as pilot whales and orcas are among the most studied in the Strait, and a specific Conservation Plan for the Critically Endangered orcas was designed in 2017 (Boletín Oficial del Estado, 2017; Cañadas et al., 2005; Cañadas, 2008; de Stephanis et al., 2008; de Stephanis et al., 2008; de Stephanis et al., 2008; de Stephanis et al., 2014; Esteban et al., 2014, 2016; Giménez et al., 2018, 2018). There are still knowledge gaps regarding the seasonal distribution and habitat use of cetaceans inhabiting the Strait, probably due to the difficulties in conducting year-round surveys. These gaps could have an impact on the effectiveness of management measures for the conservation of protected species.

As well as hosting a high number of cetacean species, the Strait is also an area of high 'maritime traffic density', with an average of 116,128 vessels transiting the Strait every year (data provided by *Tarifa Tráfico VTS of Salvamento Marítimo*, http://www.salvamentomaritimo.es, for the years 2018 and 2019). Injuries of anthropogenic origin were detected in all of the seven cetacean species regularly occurring in the area (Herr et al., 2020). The high intensity of recreational fishing and whale watching activities have probably harmed the population of common dolphins inhabiting the Bay between Algeciras and Gibraltar (henceforth referred to as Bay) (Olaya-Ponzone et al., 2020), while ferry traffic was negatively correlated to the annual apparent survival of the local bottlenose dolphin population (Tenan et al., 2020). Moreover, ship strikes are considered to be one of the main threats affecting fin and sperm whales (Grossi et al., 2021), and evidence of past collision events on the fin whale population crossing the Strait has been reported (Gauffier et al., 2018).

The Strait of Gibraltar (Figure 1a) is a transborder marine area connecting the Mediterranean Sea and the Atlantic Ocean. In this area, the coastal countries Spain, Morocco and Gibraltar have ratified 'international conventions' to protect cetaceans, including the International Convention for the Regulation of Whaling (ICRW), the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), the Bern Convention (BCCEW) and the Bonn Convention (CMS). Other protection tools include the 'spatial management measures' in force in the Strait. For instance, UNESCO (United Nations Educational Scientific and Cultural Organisation) established the Intercontinental Biosphere Reserve that includes both Spanish and Moroccan marine and terrestrial habitats. Other spatial protection measures are in place under the Bird and Habitats Directives, the Ramsar Convention and local governments (Gibraltar, Spain, Morocco) (see Supporting Information S1 for further details). The entire Strait was also designated as an Important Marine Mammals Area (IMMA) by the International Union for the Conservation of Nature (IUCN) IMMA task force, and as a Cetacean Critical Habitat (CCH) by ACCOBAMS, resulting in a threat management approach used to combine human activities and cetaceans distribution (ACCOBAMS, 2017).

'Monitoring cetaceans using ferries as a platform' of opportunity is an environmentally sustainable and cost-effective program that takes advantage of the vessels already sailing in the area. A current long-term program, consistent over space and time and repeatable all year round, is being conducted over large geographic areas in the Mediterranean basin (Arcangeli et al., 2019, 2021; David et al., 2022) and allows for the detection of eventual changes in the distribution of the different species over time (Arcangeli et al., 2013; Arcangeli et al., 2023). Data collected on cetaceans from ferries also allow for the investigation into their relationship with environmental parameters (Arcangeli et al., 2013; Arcangeli et al., 2023) and pressures, such as maritime traffic (Campana et al., 2015, 2017, 2022) or floating marine macro litter (Arcangeli et al., 2020; Gregorietti et al., 2021).

Specifically, this program could contribute to the monitoring, assessment and reporting of the status and trends of cetaceans as required by the Habitats Directive (HD) and the Marine Strategy Framework Directive and could also provide valuable information for the evaluation of local protection measures (Arcangeli et al., 2021). Moreover, data collected by the program could assist in the establishment of the Nature 2000 ecological network of protected areas HD, as well as in the regulation of maritime areas adjacent to coastal areas, in turn contributing to the sustainable development of maritime transport (as required in the Marine Spatial Planning Directive).

Likewise, the dedicated observers (DOs) that are part of the program play an important role in spotting marine mammals and

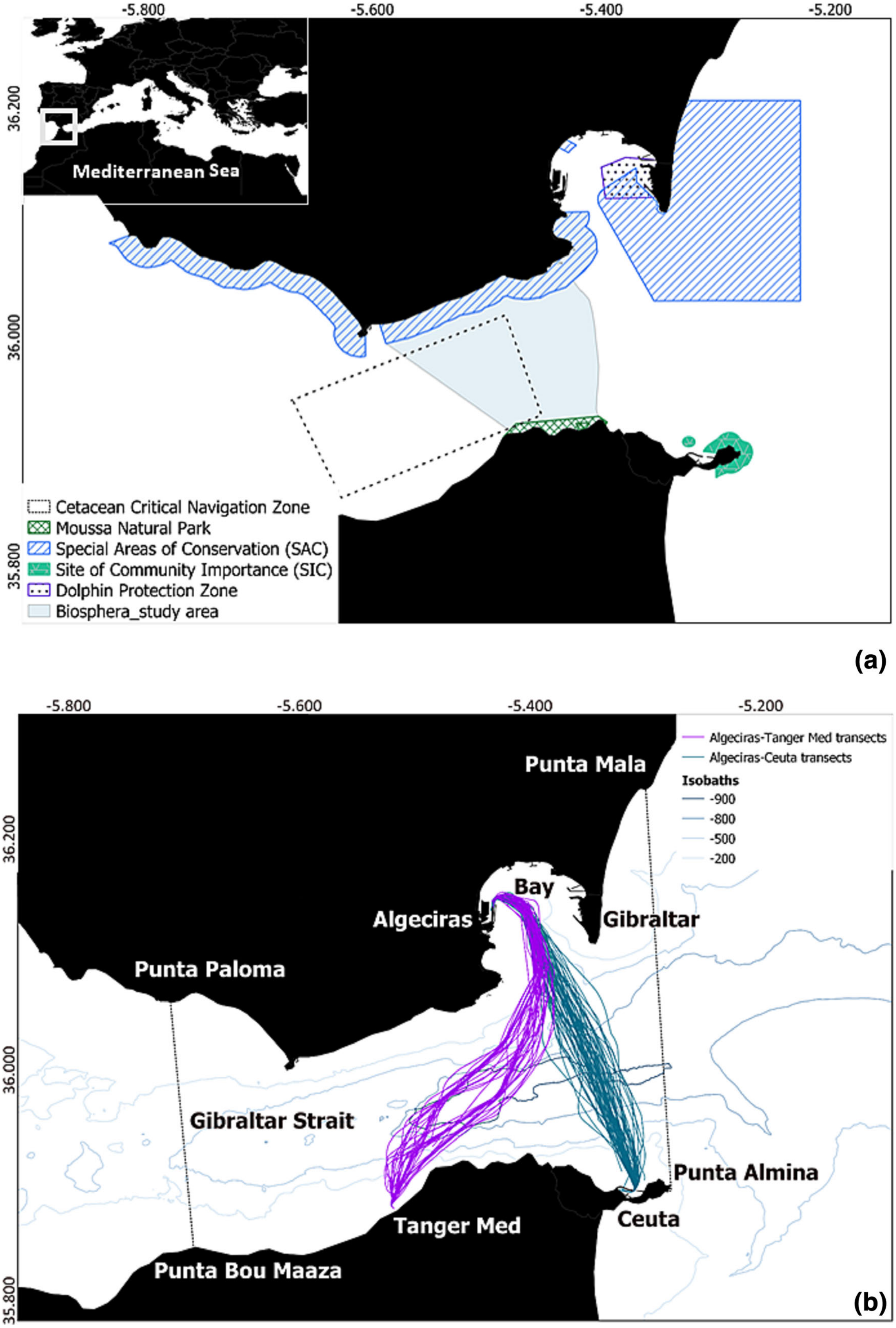

FIGURE 1 Maps of the area of study. Image (a) is a map of the Strait with spatial management tools in force for the protection and conservation of nature georeferenced. Image (b) shows a map of the Strait of Gibraltar highlighting the transects Algeciras-Ceuta (Spain) and Algeciras-Tanger Med (Spain-Morocco) in blue and purple respectively. The sub-area of study includes the area from Punta Paloma (Spanish coast) and Punta Bou Maaza (Moroccan coast) in the West and Punta Mala (Spain) and Punta Almina (Morocco) in the East.

reducing the risk of collision (Weinrich et al., 2010), by detecting rare events such as collisions or near collisions (David et al., 2022) and, contemporaneously, raising awareness of sea life conservation among the crew.

Considering the co-existence of protected species of cetaceans with the high level of maritime traffic in the Strait, along with the complex management framework of this transboundary area, this study aims to:

i. contribute to the understanding of cetacean seasonal presence and distribution by identifying hot spots in the area,
ii. study the intensity of maritime traffic and identify risk areas in the marine zone directly monitored from the ferries,
iii. provide insights into the habitat use by the species of community interest bottlenose dolphin (Annex II, Habitats Directive) applying a model on a wider area of the Strait of Gibraltar and
iv. qualitatively discuss the coherence of the marine conservation and mitigation measures already in force for the protection of cetaceans in the Strait of Gibraltar, taking into account cetacean hot spots, identified risk areas and recorded near miss events (NMEs) of collision.

2 | MATERIALS AND METHODS

2.1 | Study area

The Strait that separates Africa from Europe is the only passage between the Mediterranean Sea and the Atlantic Ocean and includes the waters between −5°W and −6°W (Figure 1a). Approximately 60 km long and 20 km wide, the Strait is characterized by deep waters reaching 1000 m in its eastern part, and shallow waters in its western part that are less than 300 m deep in some points. A sub-area of the Strait was considered for the investigation of seasonal distribution, habitat use and the relationship between cetaceans and maritime traffic. In Figure 1b, the borders of the designated sub-area are marked as a line from Punta Paloma (Spain) to Punta Bou Maaza (Morocco) in the West and a line from Punta Mala (Spain) and Punta Almina (Morocco) in the East (Figure 1b). This sub-area was selected due to monitoring coverage (sea transects' tracks in Figure 1b) and due to the similarity of the sea bottom, in terms of both bathymetry and slope (Figure 1b).

2.2 | Method for data collection

Monitoring data were gathered thanks to the support of the Baleària Foundation (http://fundaciobalearia.org), using the ferries Poeta López Anglada, Passió per Formentara and AMMAN as platforms of observation along the transects Algeciras-Ceuta (Spain, ALCE) and Algeciras-Tanger Med (Spain-Morocco, ALTA). Data collection followed the standard monitoring protocol of the Fixed Line Transect Mediterranean monitoring Network (FLT Med Net), an international project that has been coordinated by ISPRA (Italian Institute for Environmental Protection and Research) since 2007 (https://www.isprambiente.gov.it/en/archive/news-and-other-events/ispra-news/2021/10/fixed-line-transect-mediterranean-monitoring-network-flt-med-net). Under this monitoring program, data collection was carried out systematically along 16 cross-border transects throughout the Mediterranean, using scheduled ferry lines (https://www.isprambiente.gov.it/en/activities/biodiversity/flt-mediterranean-monitoring-network-marine-species-and-threats).

During the monitoring surveys, a minimum of two expert DOs were located on the main bridge of the ferries, scanning both sides of the vessel (from 0° to 130°) to record data on cetacean sightings and maritime traffic. Binoculars and cameras were used by the DOs to maximize cetacean monitoring capabilities. The distances between the DOs on the main bridge and the sea level were 21 m on board the ferry Poeta López Anglada, 11 m on the Passió per Formentara and 11.8 m on the AMMAN. Data were collected under all weather conditions, notwithstanding only data collected in good conditions (Beaufort ≤ 3) were used for analysis. Whenever possible, five surveys per summer (July–September), autumn (October–December), winter (January–March) and spring (April–June) were conducted for each transect, with a minimum of one monthly survey per transect, except in August when the unavailability of the ferry company meant that no survey could be carried out. Data collection depended on the sea state, weather conditions and availability of the ferry company. Surveys were carried out from January 2018 until December 2019. The monitoring project was interrupted due to restrictions caused by the COVID-19 pandemic, which led to delays in the use of the collected data.

Data collected on cetaceans included records about species, group composition and behaviour and the distance between the animals and the platform. The speed and the route of the ferries were also noted. Scan sampling to count all visible vessels around the ferry (range of vessel detection in condition of good visibility is approximately 20 km according to Campana et al., 2017) was performed each time a cetacean sighting occurred (presence dataset) and in the absence of cetaceans at a minimum interval of 15 min from a presence location. Given the short length of the transects (≈1 h), the sampling in the absence of cetacean sightings was conducted at least once for each survey (cetaceans absence or pseudo-absence dataset). Only vessels sailing, that is, not anchored, were classified; either as small (smaller than 5 m), medium (between 5 and 20 m, and grouped under motor, sailing or fishing) or big (longer than 20 m, such as cargos, tankers, passenger ships). Vessel presence data were also obtained from the AIS system of the ferry. Maritime traffic data were compared with data provided by the Spanish governmental agency *Salvamento Marítimo* (SM). SM works to increase the safety of maritime traffic by monitoring and facilitating the movement of traffic in the Traffic Separation Devices of Tarifa. In addition, NMEs of collisions were documented and described quantitatively and qualitatively. NMEs were defined when the animal was located 50 m in front of the bow or 25 m from the side of the vessel and was displaying neither approaching behaviour nor signs of evasion (David et al., 2022).

2.3 | Methods for data analysis

2.3.1 | Species composition and seasonal distribution

All cetacean records were firstly stratified per year and season. All records were investigated using GIS software (the Free and Open Source QGIS 3.10 and 3.22, 2020) in order to calculate the relative length of the survey tracks within the study area, along with the number of cetacean sightings for each transect. Each survey transect was used as a replicate for temporal comparisons.

The diversity of cetacean species was investigated for each transect and season as species presence and as percentage composition (i.e., number of sightings of a species relative to the total number of sightings of all species). Relative abundance was expressed as abundance index Sightings Per Unit of Effort (SPUE) and calculated as the number of sightings per km travelled on effort in good conditions (Beaufort ≤ 3) within each transect. SPUE was computed seasonally for all cetacean species and compared between the two monitored transects and the two investigated years using the Mann–Whitney (MW) paired test. To test specific seasonal differences, the Kruskall–Wallis test (KW) for multiple samples (four seasons) was used, with the Bonferroni correction used for multiple comparisons and the MW for post hoc comparison between the two pairs. All results with a p-value of <0.05 were considered statistically significant. All these tests were performed using PAST 2.17 software (Hammer et al., 2001).

For the spatial analysis, the study area was divided on a grid cell basis of 5 × 5 km to analyse and compare the spatial distribution of all records in the four seasons. For each cell, the total effort for cetacean monitoring and the number of cetacean sightings for each species were combined, in order to calculate the SPUE value in each cell. Value results were very low; hence, SPUE values were multiplied by 10 to allow differences to be perceived more easily. To reduce outliers, only cells with more than 10 km covered on effort were considered (Arcangeli et al., 2017; Zuur et al., 2010).

2.3.2 | Important areas for bottlenose dolphins (habitat suitability model)

To identify the important areas for the species of community interest bottlenose dolphin whose conservation requires the designation of special areas of conservation (SAC), a species distribution modelling (SDM) was used to characterize suitable habitats and predict the core areas of distribution in the whole Strait. MaxEnt was used, as it is considered the most adequate method when absence data are not certain and when the number of presence records is low (Phillips et al., 2006). As suggested by Pearson (2007), more than the minimum number of 15 presence records (i.e., 22 presence records of bottlenose dolphins) were used to perform the modelling. MaxEnt is a machine learning method commonly used in systems with restricted information based on a probability distribution with maximum entropy (the most spread out, closest to uniform), subject to known constraints (Phillips et al., 2006). MaxEnt accounts for sampling biases via correction features that consider the area of sampling effort used to generate pseudo-absence points or 'background points'. In line with the recommendations of literature (Elith et al., 2011), a bias file of effort was built using the minimum convex polygon (MCP) around the surveyed sites, and as a comparison with the species presence sites, 10,000 'background samples' were randomly selected within this area. After preliminary runs with different setting parameters, default recommended feature classes (hinge, linear, quadratic) and regularization parameters (i.e., =1) were used, with maximum iterations up to 500 to reach convergence at a threshold of 0.00001. Four explanatory variables, selected between the factors already considered predictive of bottlenose dolphin habitat, were used in the model: bathymetry (m), bathymetric slope derived by bathymetry (degrees), distance from the nearest coast calculated in relation to a standard coastline shapefile (m) and mean sea surface temperature (SST, °C). Bathymetry values were obtained from the GEBCO raster file (GEBCO Compilation Group, 2020), while SST raster files were downloaded from NASA Ocean Colour (http://oceancolor.gsfc.nasa.gov) and averaged for the entire period (2018–2019). No correlation was found among variables; therefore, none has been removed. A Jackknife test was conducted to obtain alternative estimates of the variable contribution to the MaxEnt run. Starting from the species occurrences and the predictive variables, the R package 'ENMeval' was used to obtain the bias file, a function that permits the correction of irregular distribution of the sampling effort (Phillips et al., 2006).

2.3.3 | Maritime traffic and cetaceans

The office of *Tarifa Tráfico VTS* of SM provided data on the vessels identified crossing the Strait of Gibraltar or the entrance/exit points of the Spanish ports in the years 2018 and 2019. A preliminary analysis was performed to compare the seasonal pattern of total maritime traffic of the two investigated years obtained from SM using the two sample tests Kolmogorov–Smirnov (KS) and MW. Information on vessel types sampled along the monitored transects was then used to characterize seasonal composition in maritime traffic and provide an indicator of real-time vessel abundance, to be compared with seasonal pattern obtained from SM (Supporting Information S2).

To verify the influence of year, season and transect on traffic intensity, comparisons were performed with non-parametric statistics of a two-way PERMANOVA, a geometric partitioning of variation across a multivariate dataset, defined in the space of a chosen dissimilarity measure (Anderson, 2017), in this case, Bray-Curtis. Subsequently, seasonal differences in traffic intensity were computed by aggregating the data of the two transects and using the KW test with the Bonferroni correction and the MW for post hoc comparison between the two groups.

To study the relationship between maritime traffic and cetacean sightings, all records of the presence and absence datasets (map of records in Supporting Information S3) were compared to test the null hypothesis that the number of ships does not differ between them. The two datasets (with at least 10 records) were statistically compared using the KS test, and the mean percentage difference between the number of vessels recorded in the sighting locations (Npres) and those recorded randomly in the absence of sightings (Nabs) was reported as: [(Npres − Nabs)/Nabs] * 100 (Campana et al., 2017). The analysis was performed on all maritime traffic and single vessel categories, pooling all seasons and sightings of all species together, later sorting by season. Finally, investigation at the species level was carried out for the most sighted species: common dolphin, striped dolphin, bottlenose dolphin and pilot whale. To study the distribution and intensity of maritime traffic in the area, the total number of vessels counted in the presence and absence of cetacean sightings was linked to the grid cells and the mean value was calculated for each season; the same was also done for the five categories of vessels. To identify the overlapping of cetacean hotspots (identified by the SPUE) and risk areas (with high traffic intensity) (Section 3.1), the Kernel density estimate was used to identify areas of higher intensity of maritime traffic, by weighting the analysis on the mean number of vessels and considering a radius of 10 km. This radius was chosen as it can be representative of the potential effects of vessel presence in the environment such as noise and pollution, and it is in accordance with the 5-km spatial resolution applied. The 70% isopleths were used to define areas of major vessel density (Campana et al., 2022), and they could be compared with the cells of higher SPUE. In this way, it was possible to highlight the potential risk areas for cetacean species, first by considering total traffic and all the species together, then by specifying the overlap for each species in each season.

The NMEs of collision (Section 3.2.2; Supporting Information S4) were quantitatively described, calculating the percentage of occurrence per all species, and per each species involved, in each transect and considering data collected in both transects. A qualitative description of the cetaceans' behaviour before and after the NME and of ferry navigation (e.g., speed and course) involved in the NMEs was also provided. NMEs have been georeferenced and discussed considering the spatial management measures in force.

2.3.4 | Spatial protection measures

The marine spatial protection measures in force in the Strait of Gibraltar were mapped and then overlapped with cetacean hot spots (area with higher SPUE value) and maritime traffic high-risk zones.

For each spatial management measure in force, the presence or absence of a management plan was highlighted and practical measures to protect cetaceans were extrapolated by the management plans when present (Supporting Information S1).

3 | RESULTS

Effort data including both transects were represented as a total time of 115 h and 24 min spent and/or a distance travelled of 2927.17 km on effort in good weather conditions (Beaufort ≤ 3). A total of 264 cetacean sightings were recorded, including seven identified species (Table 1).

TABLE 1 Number of sightings per species in both transects Algeciras-Ceuta (ALCE) and Algeciras-Tanger Med (ALTA), total time spent and km travelled on effort (from January 2018 to December 2019).

Transect	ALCE	ALTA	TOTAL
No. transects	33	26	59
Species sighted			
D. delphis	53	25	78
S. coeruleoalba	25	15	43
T. truncatus	11	11	22
G. melas	6**	13	19**
B. physalus	4	4*	8*
P. macrocephalus	1*	5	6*
O. orca	0	2	2
Unidentified species (U.S.) Small	40	46	86
U.S. Medium	0	2	2
U.S. Large	0	1	1
Tot. of sightings	140***	124*	264****
Time on effort (hh:mm)	57:44:00	57:40:00	115:24:00
Km on effort	1548.66	1378.51	2927.17

Note: No. of * = No. of NMEs.

3.1 | Species composition, seasonal distribution and habitat use

The species composition of the six species: striped, common and bottlenose dolphins and pilot, sperm and fin whales, was similar between the two monitored fixed transects. In contrast, orcas were only sighted twice along the ALTA transect. The most common species in both transects was the common dolphin, followed by the striped and bottlenose dolphin and the pilot whale. Other less frequent species were the fin and sperm whale.

For all species, no significant differences in SPUE values were detected among the two investigated transects (MW ALTA-ALCE, $p > 0.05$). Similarly, when the data were then pooled, and cetacean sighting rates were compared among the two investigated years, no differences emerged, except in the case of the fin whale, which was significantly more frequent in 2019 (MW 2018–2019, $p < 0.01$). For the majority of the species studied, there were no significant differences in the seasonal SPUE, except for the striped and common dolphins for which the summer sighting rate was the highest (KW $p = 0.016$). Species distribution also presented seasonal differences for these two species (Figures 2 and 3): the common dolphin was concentrated in the Bay from spring to autumn, while the striped dolphin showed central southern distribution during winter and spring, as well as being present in the Bay between Gibraltar and Algeciras during summer and autumn.

The bottlenose dolphin was seen in the Strait from summer to winter, with a high concentration of the species evident in the Bay in spring (Figure 4). Pilot whales, however, were mainly observed in the central part of the study area in all seasons (Figure 5), as were fin and sperm whales (Supporting Information S5 and S6).

The SDM performed on the bottlenose dolphin proved to perform well, with a final AUC value of 0.82. The most important variable determining the species' habitat was bathymetry (65.3%), followed by minimum distance from the coast (19.7%) and bathymetric slope (14.8%). In general, the species' potential preferred habitat extended over the outer limit of the continental shelf, excluding the southern areas where the continental slope is close to the coast, and into the very central part of the study area in correspondence with the western steep limit of the deepest part of the Strait (Figure 6).

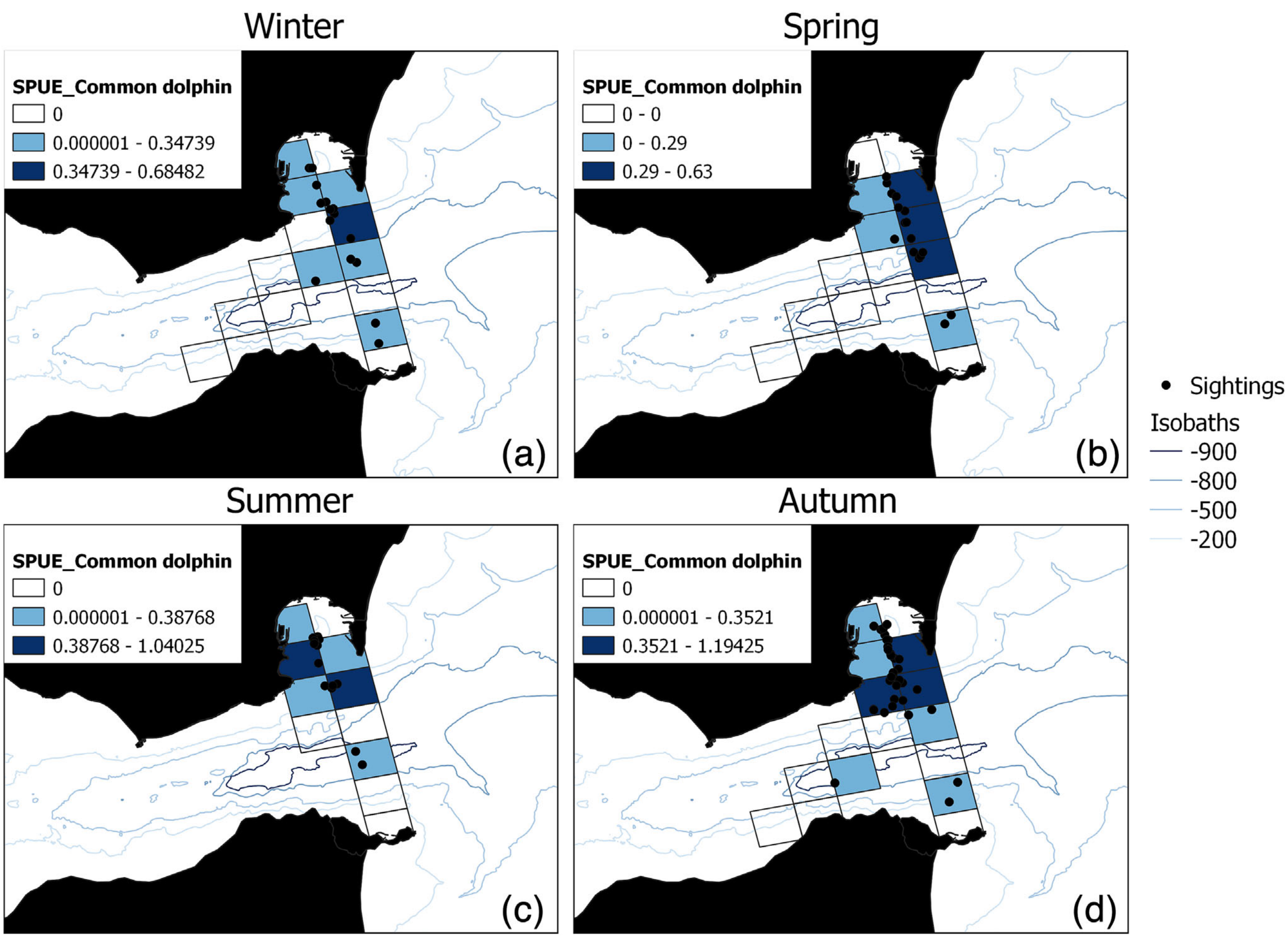

FIGURE 2 Maps of relative abundance of short-beaked common dolphin expressed as Sightings Per Unit of Effort (SPUE), per winter (January–March, map a), spring (April–June, map b), summer (July–September, map c) and autumn (October–December, map d).

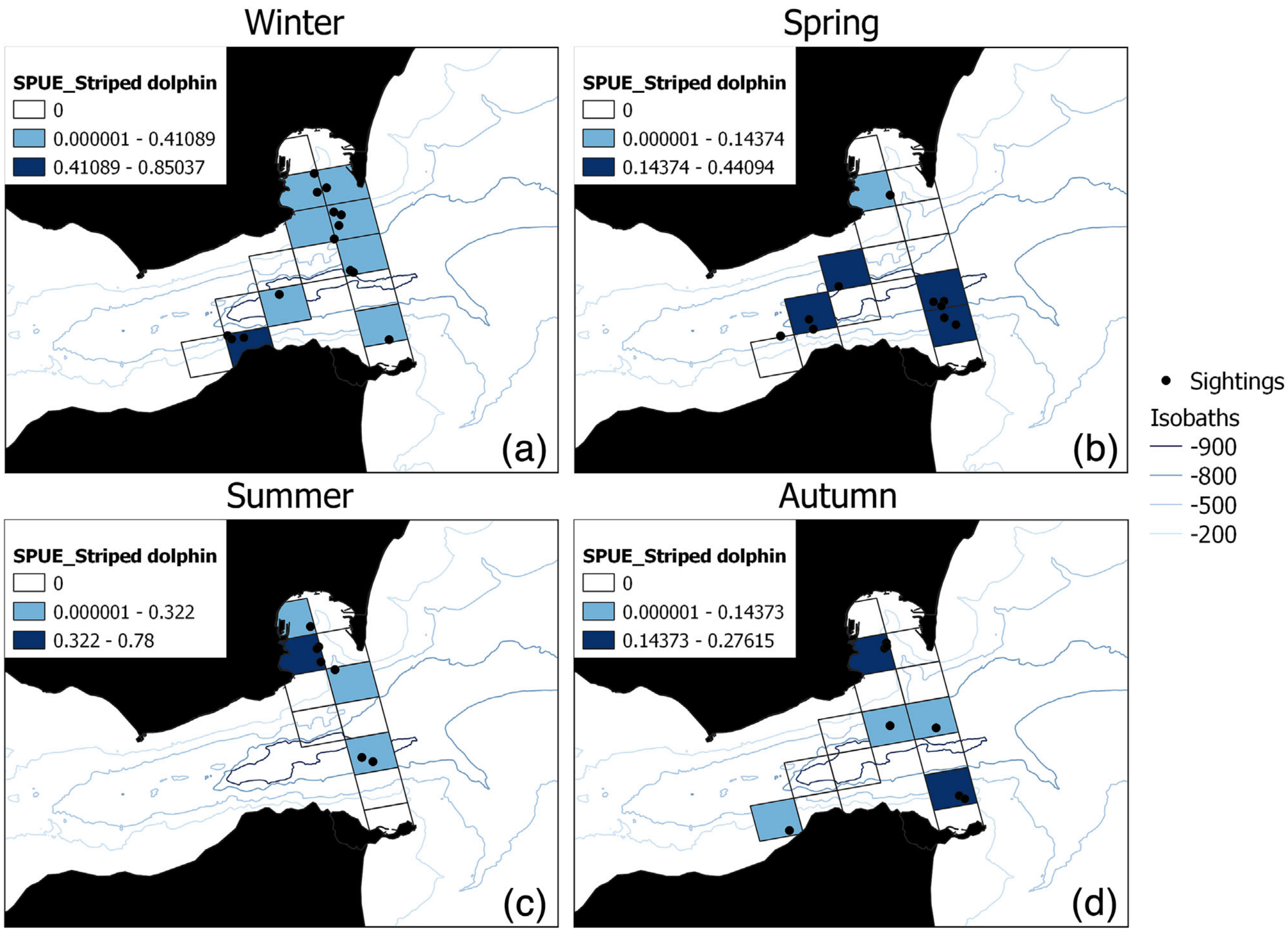

FIGURE 3 Maps of relative abundance of striped dolphin expressed as Sightings Per Unit of Effort (SPUE), per winter (January–March, map a), spring (April–June, map b), summer (July–September, map c) and autumn (October–December, map d).

3.2 | Maritime traffic and cetaceans

Between 2018 and 2019, no differences were observed in the total monthly number of vessels reported by *Tarifa Tráfico* (Supporting Information S2; KS and MW, $p > 0.05$), whereas there was a significant seasonal difference (KW $p < 0.001$), as the mean amount of maritime traffic reported during the summer was significantly higher than during spring and winter (MW $p = 0.03$).

Using the data on vessels recorded along the monitored transect (i.e., presence and absence datasets), the total number of vessels observed in ALTA did not show significant seasonal variations (KW $p > 0.05$; Figure 7a). However, no summer data were available for this transect, and 80% of all traffic over all the seasons was represented by big ships (>20 m).

In the ALCE transect however, a significant difference between seasons was found, with fewer vessels during the summer than in the other seasons (KW $p = 0.000$; Figure 7b). No differences were observed between the two transects when comparing maritime traffic intensity during spring and winter, while traffic intensity was higher in ALTA than in ALCE during autumn (MW $p = 0.034$; Figure 7). In fact, PERMANOVA analysis revealed no variation in the traffic intensity in relation to year and transect ($p > 0.05$) but did reveal a significant seasonal effect ($p < 0.02$). Therefore, all the subsequent analyses were performed by pooling together all data and maintaining separation on a seasonal basis. The presence of big ships (>20 m) prevailed over other vessel types in all seasons, representing more than 75% of all traffic (Figure 8), a proportion that only decreased to 63% during summer (ALCE only). The medium-sized categories of sailing and motor boats represented 20% of traffic in all seasons, with an increase during summer, during which time fishing boats were not represented at all (Figure 8). Additionally, small boats reached the highest proportion during summer (5%), while fishing boats reached their highest proportion during winter (4%) (Figure 8).

The different vessel categories showed uneven distribution in the study area, with high densities of big, sailing and fishing boats over wider areas when compared with the other categories (Supporting Information S7), which were generally distributed in the central part of the Strait during winter and spring, while being more dispersed from north to south during the other seasons (Figures 8, 9 and 10). During summer, all categories showed high densities within the Bay (Figures 8, 9 and 10).

When considering all seasons and all cetaceans encountered, a similar number of vessels was observed in both the ‘presence and

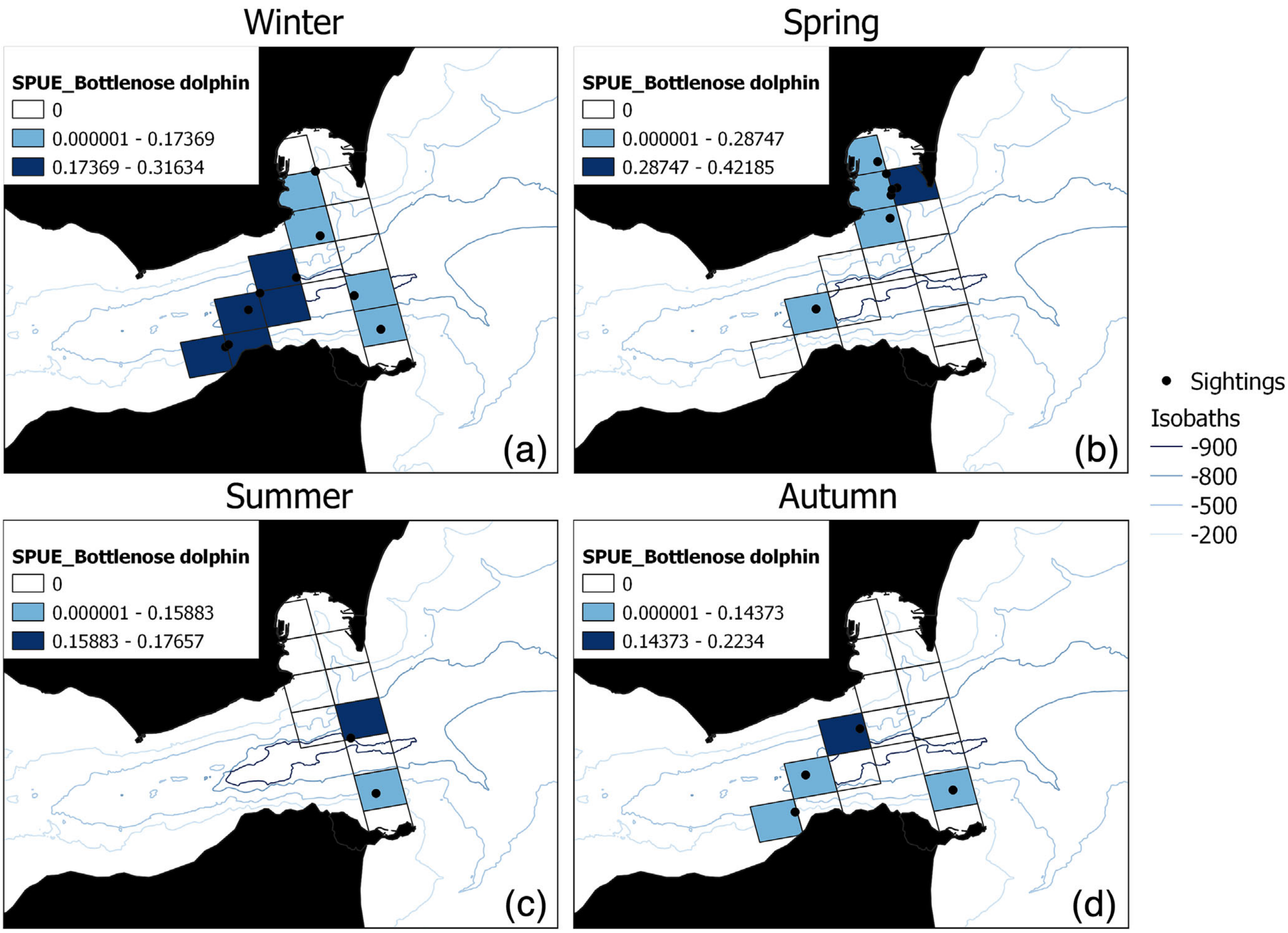

FIGURE 4 Maps of relative abundance of bottlenose dolphin expressed as Sightings Per Unit of Effort (SPUE), per winter (January–March, map a), spring (April–June, map b), summer (July–September, map c) and autumn (October–December, map d).

absence of cetaceans' (KS $p > 0.05$). Significant differences were revealed, however, in the vessel types (KS $p < 0.03$), with a higher number of small, motor and fishing boats in the presence of cetaceans (77%, 76% and 371% respectively) and a lower number of sailing and big ships (Table 2a).

During autumn and winter, a significantly higher number of small and fishing vessels were recorded in the presence of cetacean sightings (KS $p < 0.01$), with a greater number of sailing vessels also recorded when cetaceans were sighted in winter (KS $p = 0.008$). During spring however, a 29% decrease in the number of total vessels in the presence of cetacean sightings, when compared with the absence, was observed, driven by the categories sailing ships and big ships (KS $p < 0.01$; see Table 2a). On the contrary, fishing boats were only observed when cetacean sightings occurred, and during summer, no significant differences were detected (KS $p > 0.05$; Table 2a).

In terms of the investigated species, for common, striped and bottlenose dolphins, a higher presence of small and fishing boats and a minor presence of big ships in the location of sightings was evident (KS $p < 0.05$; Table 2b). Additionally, during sighting of common dolphin, the number of motorboats was 80% higher than in the absence of sightings (Table 2b). On the other hand, the pilot whale was observed in locations with a lower presence of small, motor and sailing boats, but with significantly higher presence of fishing vessels (KS $p < 0.05$; Table 2b).

3.2.1 | Overlapping cetacean hot spots, maritime traffic high-risk areas and spatial protection measures

By overlapping the cells with higher SPUEs with the seasonal isopleths of all vessel categories, areas of potential risk for cetacean species could be highlighted. Species were grouped according to size (i.e., small, medium and large cetaceans), species association (i.e., bottlenose dolphin with pilot whale) and according to the potential risk of interaction with ships (e.g., fin and sperm whales). Common and striped dolphins were grouped as small cetaceans and were the species that were more frequent in the Bay (Elejabeitia et al., 2012; Olaya-Ponzone et al., 2022). The pilot whales were grouped with the bottlenose dolphins, as they were often sighted associated in the Strait waters (Andréu et al., 2008). The fin, sperm and killer whales were grouped due to their large size, due to their vulnerability to collisions (David et al., 2022; Di-Méglio et al., 2018;

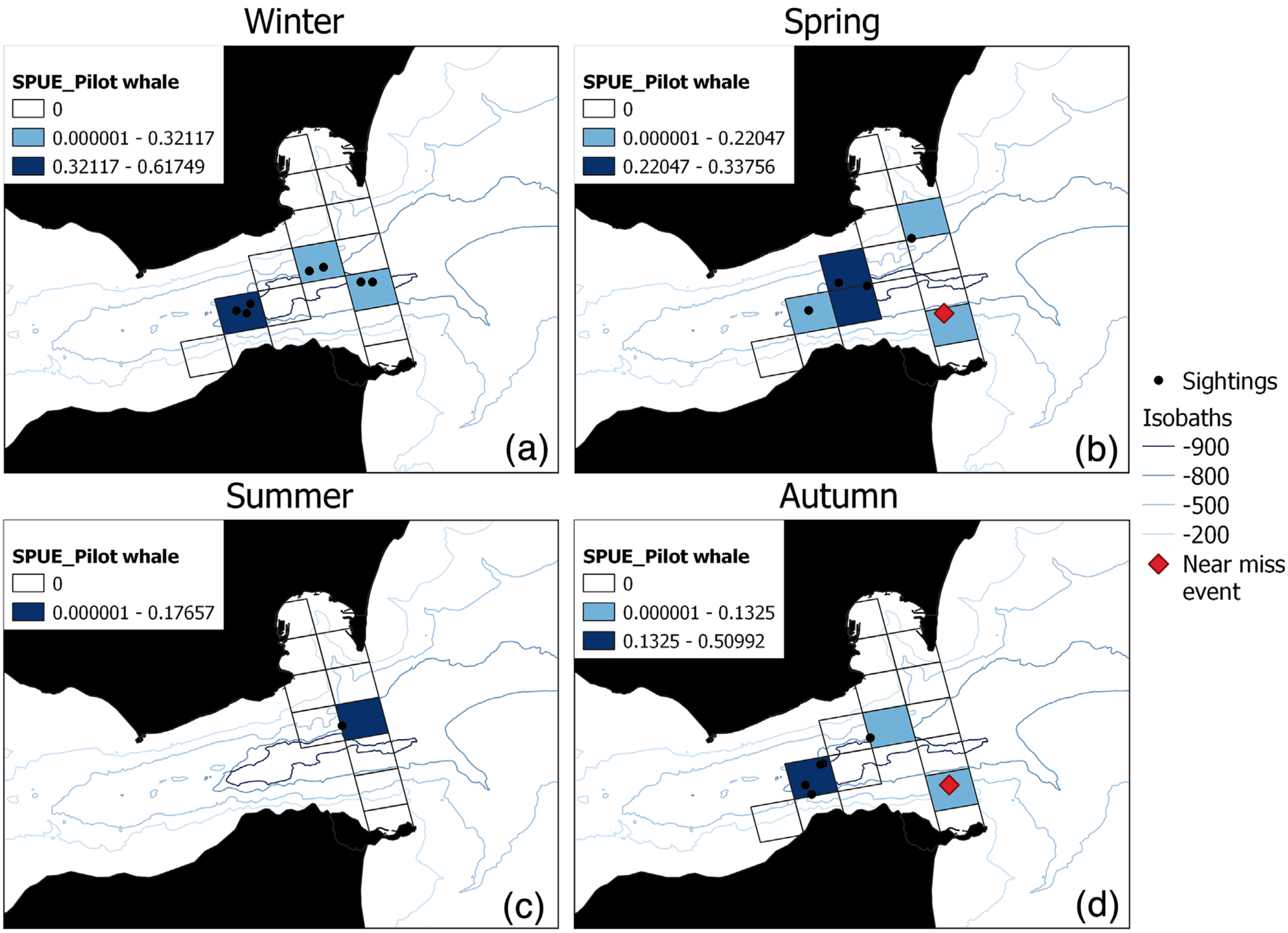

FIGURE 5 Maps of relative abundance of long-finned pilot whale expressed as Sightings Per Unit of Effort (SPUE), per winter (January–March, map a), spring (April–June, map b), summer (July–September, map c) and autumn (October–December, map d). Near miss events of collision are georeferenced and represented with a red rhombus (maps b and d).

Gauffier et al., 2018; Williams & O'Hara, 2010) and due to their low number of sightings.

Putting all the species sightings together, cetacean risk areas were highlighted in the Bay and in the central-eastern part of the sub-area due to sailing and motor boats, the central-southern area due to fishing boats and the edge of the whole Bay due to small boats. The risk zone for big vessels covered almost the entire area in which cetaceans were sighted (Supporting Information S7).

Common and striped dolphins were frequently recorded close to the Bay during spring, summer and autumn, resulting in a high overlap with all vessel categories. In contrast, during winter, their occurrence was limited to areas with lower traffic density (Figure 9).

On the other hand, pilot whales and bottlenose dolphins presented major overlap during winter and autumn in the central part of the Strait, where all vessel categories showed high densities. During summer, the overlap was mainly with big ships (Figure 10).

The central part of the Bay was identified as the most important area for common, striped and bottlenose dolphins. This only partially overlaps with the spatial management measures of the Dolphin Protection Zone and the SAC-ES6120032 Estrecho oriental (further details in Supporting Information S1).

For large whales, the areas of higher overlap were highlighted in the central part of the Strait during winter and spring with all types of vessels and in the southern waters of the study area during autumn with big ships (Figure 11).

Results show that the central-southern and eastern parts of the sub-area of study are also important for the bottlenose dolphin, as well as for the pilot, fin and sperm whales. These areas only partially overlay with the Intercontinental Mediterranean Biosphere Reserve and the Cetacean Critical Navigation Zone (Figure 1).

3.2.2 | Near miss events of collision

Pilot (10.53% of NMEs per species considering both transects), fin (12.50%) and sperm (16.67%) whales were the species involved in NMEs of collision, three of which happened in the transect ALCE (2.14% of NMEs for all species) and one in ALTA (0.81%). The

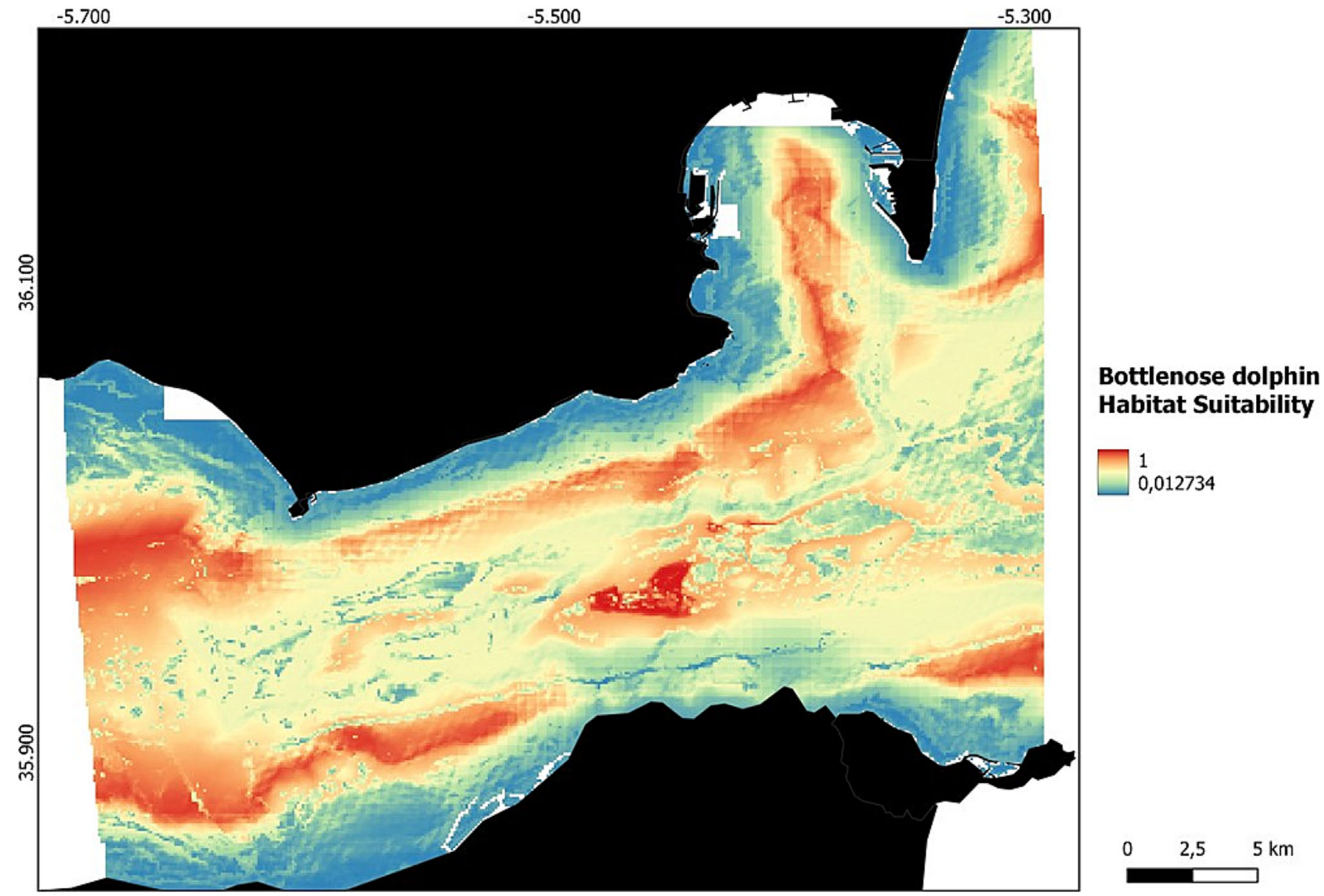

FIGURE 6 Map of the habitat suitability for the bottlenose dolphin in the Strait of Gibraltar.

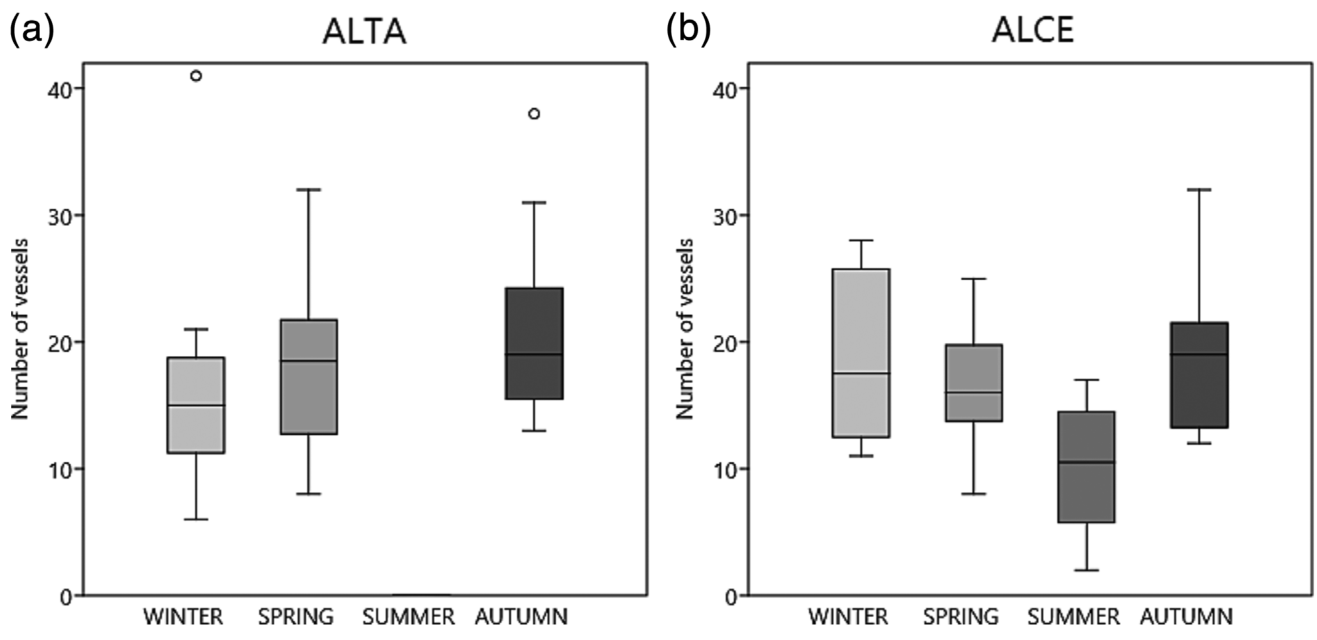

FIGURE 7 Seasonal boxplots representing the total number of vessels recorded along the fixed transects Algeciras-Tanger Med, ALTA (a), and Algeciras-Ceuta, ALCE (b).

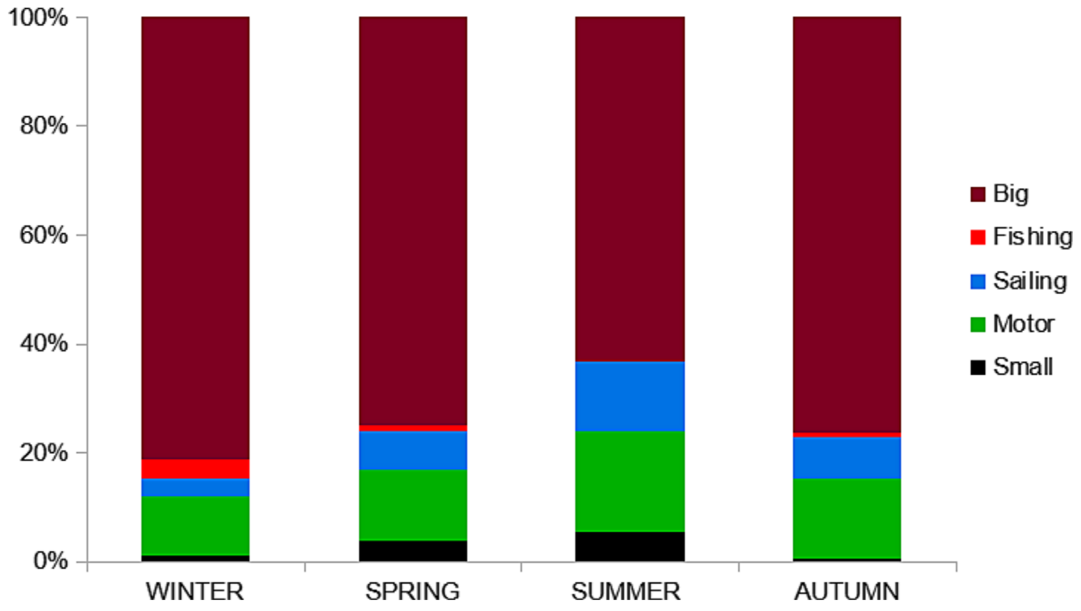

FIGURE 8 Bar chart of the seasonal composition of maritime traffic in the investigated area (ALCE-ALTA joined transects), data collected along the fixed line transect.

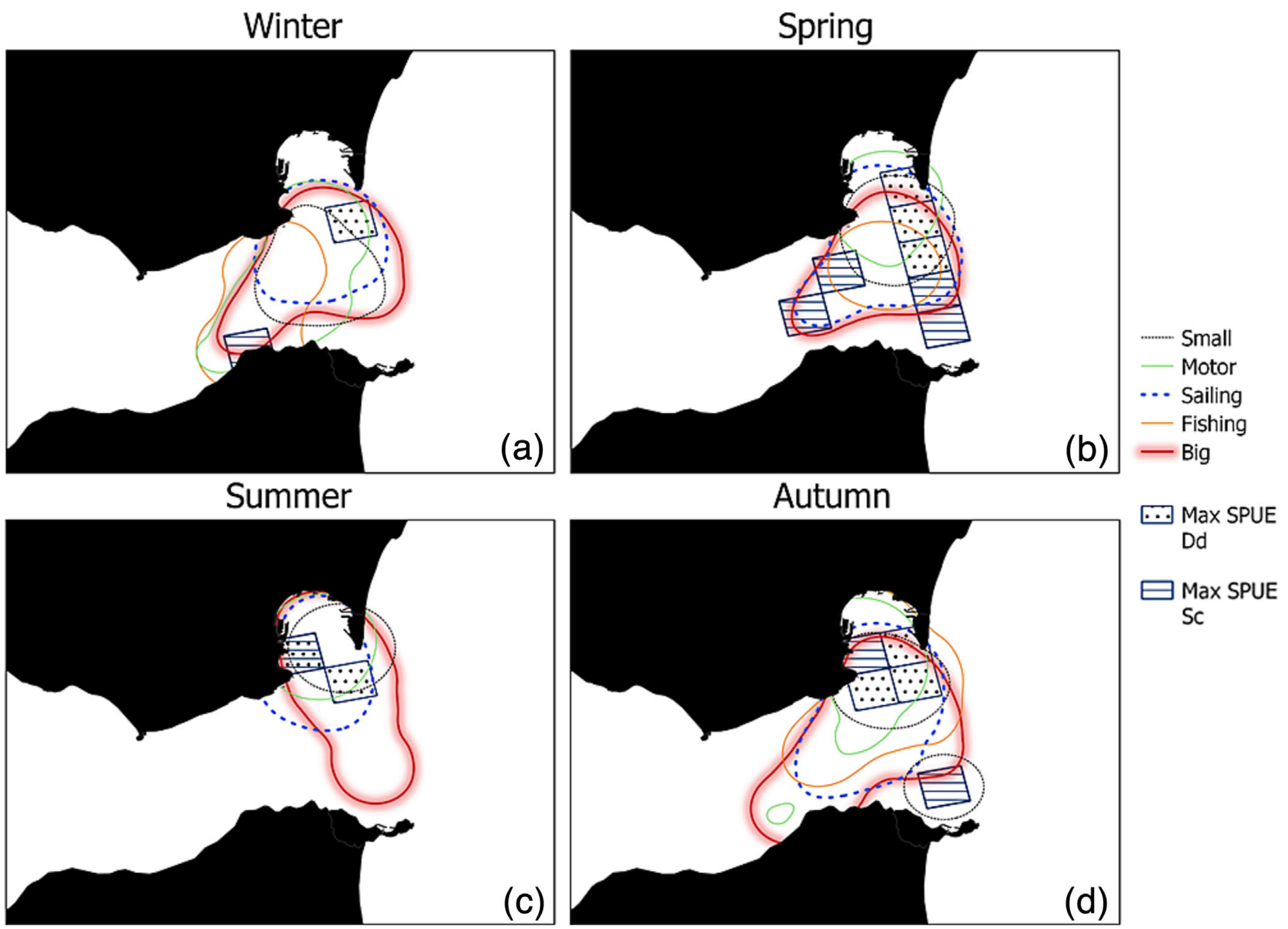

FIGURE 9 Maps of the Strait of Gibraltar highlighted the relative abundance of common (Dd) and striped (Sc) dolphins expressed as Sightings Per Unit of Effort (SPUE) and with marked isopleths of the different types of vessels. Lines are the 70% isopleths used to define areas of major vessel density for each type.

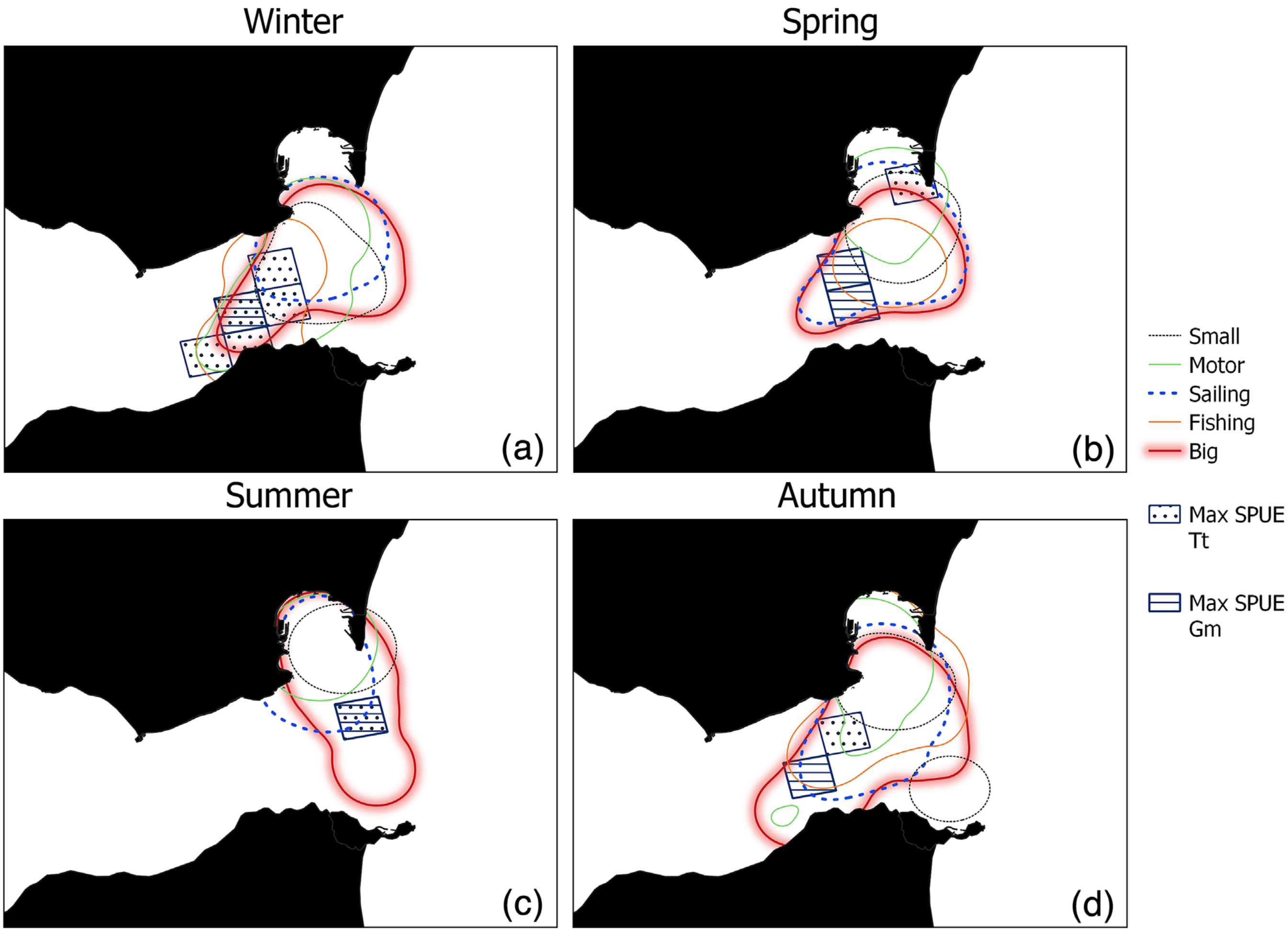

FIGURE 10 Maps of the Strait of Gibraltar highlighting the higher relative abundance of bottlenose dolphin (Tt) and pilot whale (Gm) expressed as Sightings Per Unit of Effort (SPUE), and with marked isopleths of the different types of vessels. In summer, the cell with higher SPUE is the same for both species. Lines are the 70% isopleths used to define areas of major vessel density for each type.

visibility state (measured as optimum, good, mean or scarce) during the NMEs was mean except for the case of the sperm whale in which case it was good. The sea state was mainly 2 (3 cases of 4, on a scale from 0 to 10), and rain was never present (Figure 5 and Supporting Information S4, S5 and S6). No cetaceans involved in NMEs displayed signals of approaching or evasion at a distance of 50 m from the bow or 25 m from the side of the vessel.

Pilot whale NMEs (Figure 5 and Supporting Information S4) occurred twice in 2019 along the ALCE transect (33.33%) and are represented by codes GmNME1 and GmNME2. GmNME1 happened in June and involved a pod of 18 individuals with two juveniles that were at 60 m and at 0° (in front of the bow) from the ferry at the beginning of the sighting. There were 12 additional vessels in the area, and the sea state was 3. GmNME2 was in October, where three adult pilot whales and one juvenile were slowly travelling northwest when they were spotted early at 500 m and at 0° from the platform. At the moment of the NME, there were 22 other vessels in the area, of which 20 were bigger than 20 m.

On 8 June 2018, an individual sperm whale travelling during the NME (100% for the species in ALCE) was at first spotted at 400 m and at 70° on the starboard side from the ferry (Supporting Information S4 and S6). There were only two other big ships in a 2 nautical mile radius from the ferry.

The last NME was reported along the ALTA transect in May and involved an adult and a juvenile fin whale travelling (25% for the species in ALTA). The fin whales were at 1900 m at the beginning of the sighting and 30° to port from the platform (Supporting Information S4 and S5). Thirteen other vessels were in the area.

4 | DISCUSSIONS ON CETACEAN DISTRIBUTION, MARITIME TRAFFIC AND SPATIAL MANAGEMENT TOOLS

The 'use of a non-dedicated platform to monitor large marine areas' is a cost-effective method that allows for the long-term investigation of cetacean species and the pressures they face, such as maritime traffic (Campana et al., 2017). In the present study, data collected from ferries travelling across the Strait of Gibraltar provided useful insights into the species' seasonal presence along with the characterization of

TABLE 2 Differences in the number of vessels (total and single categories) counted in the presence and absence of all cetacean species sightings (a) and most common species (b) in the surveyed transects percentage difference and Kolmogorov–Smirnov test results.

a

All species	Vessel type	All seasons	Seasons: Autumn	Winter	Spring	Summer
Presence		135	46	42	35	12
Absence		67	21	18	20	8
% Difference	Total	−2%**	−7%	22%	−29%**	1%
	Small	77%***	37%***	NA***	9%	500%
	Motor	76%*	69%	135%	57%	28%
	Sailing	−14%*	3%	20%**	34%*	−44%
	Fishing	371%***	357%***	226%**	NA**	NA
	Big	−12%**	−18%	8%	−40%**	−4%

b

	Vessel type	Common dolphins	Striped dolphin	Bottlenose dolphin	Pilot whale
Presence		38	23	14	17
Absence		67	67	67	67
% Difference	Total	2%	−8%	−2%	15%
	Small	316%**	4%***	3%***	−72%***
	Motor	82%*	86%	211%	−25%*
	Sailing	−1%	−20%	2%	−16%*
	Fishing	47%***	143%***	1017%***	1214%***
	Big	−11%	−18%*	−31%*	15%

Note: NA = all zero absence data, that is different from no data.
*$p < 0.05$.
**$p \leq 0.01$.
***$p \leq 0.001$.

maritime traffic and are sources of critical information in the evaluation of existing spatial management tools.

This study corroborates the 'presence of the seven cetacean species inhabiting the Strait's waters, all of which show a persistent presence over the investigated period. Only the fin whale presented an increase in the second year, which may be due to its highly dynamic seasonal behaviour (Geijer et al., 2016). Furthermore, the number of fin whale sightings per year in the Strait has shown a high degree of variability in the past (between 7 and 29, data from 1999 to 2014; Gauffier et al., 2018). For the most sighted species, the common and striped dolphin, seasonal variations in abundance were also documented by this study, as was reported by Espada Ruíz et al. (2018).

Although the data collected dates from 2018–2019, the Strait continues to be an area of intense maritime traffic (www.marinetraffic.com). 'Maritime traffic intensity' was quite high throughout the year, with an increase during summer and with variations in composition, in accordance with that which has been reported in other areas (Campana et al., 2017; Coomber et al., 2016). The results of sampling data coincided with the general information obtained from *Tarifa Tráfico* but included additional specifications concerning smaller types of vessels and their maritime activities, confirming the reliability of the visual sampling protocol. The investigation of different vessel types, which can affect species in different ways (Grossi et al., 2021; Herr et al., 2020; Tenan et al., 2020), is therefore important when planning effective management measures.

It was possible to highlight the main areas of overlap, where interaction is most likely to occur thanks to the spatial analysis of 'cetaceans and maritime traffic'. Results show that the study area is dominated by a high level of maritime traffic intensity, especially in its central part, due to big ships transiting along the Traffic Separation Scheme of the Strait of Gibraltar. Meanwhile, areas with a higher frequency of cetacean sightings were identified in the Bay and the central-southern part of the Strait, close to the main ports. Considering all cetaceans, the difference in vessel abundance was positive for small, motor and fishing boats, which likely indicates a real overlap between traffic and cetacean presence. This difference could also be driven by a possible positive/approaching behaviour between human activities and some species, such as fishing during autumn and winter (sometimes also represented by small boats) or whale watching (motorboats). These differences were confirmed in autumn, winter and spring when fishing vessels were even observed only in the presence of cetaceans. Conversely, the lower number of sailing and big ships in the presence of cetaceans can be related to the effect of

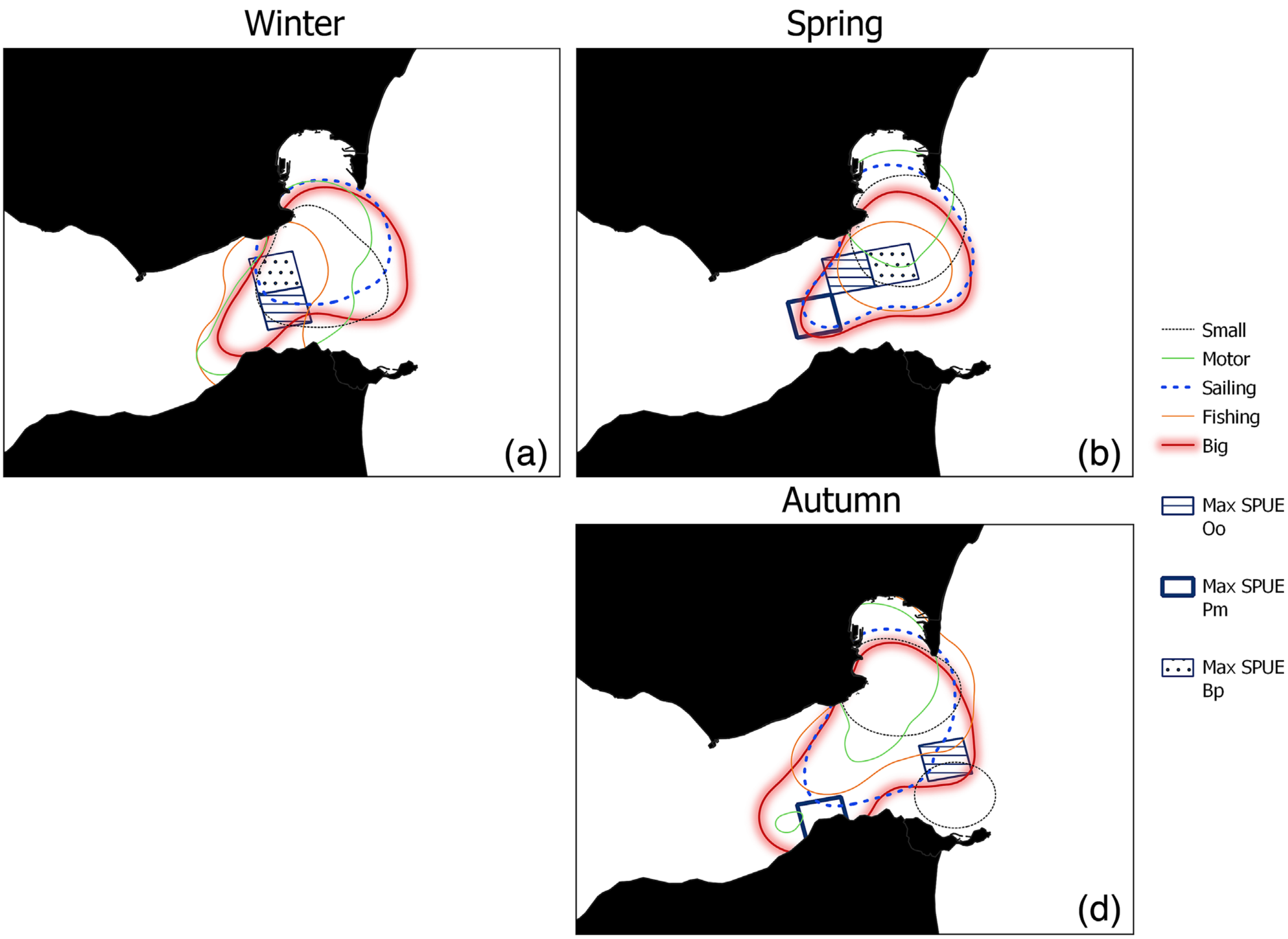

FIGURE 11 Maps of the Strait of Gibraltar highlighting the higher relative abundance of fin (Bp), sperm (Pm) and killer (Oo) whales expressed as Sightings Per Unit of Effort (SPUE) and with marked isopleths of the different types of vessels. Due to the absence of sightings, the summer is not represented. Lines are the 70% isopleths used to define areas of major vessel density for each type.

traffic on the animal's avoidance behaviour or to the independent spatial segregation of cetacean and vessel observations (Campana et al., 2017). During summer, however, no relationship was found between maritime traffic and cetacean presence, although few data were collected during this season. This result is probably a consequence of the actual co-presence of species and vessels in the season of major abundance of both, which has also been reported in other studies identifying potential areas of increased risk (Campana et al., 2015; Pennino et al., 2017).

The current study confirms that the central part of the Bay represents an important area for 'common dolphins' (Olaya-Ponzone et al., 2022), especially from April to December, with a peak in presence during summer. This coincides with the peak in the presence of mothers with calves pods (Espada Ruíz et al., 2018) that are more vulnerable and prone to changes in behaviour (Castro et al., 2022). A higher presence of small boats and fishing boats, as well as motor boats, was observed during the sightings, which is consistent with existing literature (Espada Ruíz et al., 2018; Olaya-Ponzone et al., 2020, 2022). In fact, Espada Ruíz and colleagues also described a co-presence of different types of vessels during sightings, 43% of which were whale watching boats, 29% recreational boats (that could encompass our categories of motor and small boats) and were Atlantic bluefin tuna fishing boats (Espada Ruíz et al., 2018). Common dolphins are frequently used as signs to find aggregations of bluefin tuna, explaining the higher presence of fishing boats recorded by this study during the sightings. Despite the Dolphin Protection Zone (Government of Gibraltar, 2018) partially covering the hot spot of the Endangered *Delphinus delphis*, documented injuries such as propeller strike were correlated to the maritime activities previously listed (Olaya-Ponzone et al., 2020).

Even though 'striped dolphins' have previously been spotted in mixed groups with common dolphins (Olaya-Ponzone et al., 2022), the former were observed as having a wider distribution throughout the Strait. This result could coincide with the spatial separation of the core areas of distribution of the two species (Giménez et al., 2017). Both species showed signs of injuries of anthropogenic origin (Herr et al., 2020; Olaya-Ponzone et al., 2020), and in our study, a higher presence of small and fishing boats was observed during sightings of these species. A positive association with these types of vessels has also been reported in Sardinian waters by Pennino and colleagues (Pennino et al., 2016).

'Bottlenose dolphins' were sighted in the Strait from July to March, using the same central part of the Bay from April to June as the common dolphin, although spatial segregation among species was observed in the Bay (whale watching operators' personal communication) and in Galicia (North Spain) (Methion & Díaz López, 2021). *Tursiops truncatus* is listed in the EU Habitat Directive (its transpositions Spanish R.D. 1997/1995 and British Conservation Natural Habitats &c. Regulations 1994) as a species of special interest whose conservation requires the designation of a SAC. It is also included in Annex IV as a species of community interest requiring strict protection. The Strait includes three SACs and one site of community importance (SIC) (Supporting Information S1). Even so, it is notable to observe that all of the bottlenose sightings in this study, as well as those of other studies (de Stephanis, Cornulier, et al., 2008), only partially overlap with some of the protection areas in force in the Strait (Figure 1b). The Intercontinental Mediterranean Biosphere Reserve covers the highly suitable habitat in the central part of the Strait but incorporates neither cetacean protection measures nor management plans. The SAC-ES6120032 and the Dolphin Protection Zone, which establish measures directed to the protection of dolphins, partly include the central part of the Bay, which is a highly suitable habitat area for the bottlenose dolphin. The bottlenose population of the Strait showed evidence of anthropogenic injuries (Herr et al., 2020), and their apparent annual survival probability was negatively correlated with ferry traffic (Tenan et al., 2020). In addition to these points, as the Strait's population is spatially segregated from the adjacent population in the Gulf of Cádiz (Giménez, Louis, et al., 2018), an adjustment of the conservation management tools applied in the Strait is necessary, independent of any measures being carried out in other areas. As was observed for striped and common dolphins, there was a strong presence of small and fishing boats during the sightings of bottlenose dolphins.

Considering the similarity in the results for the three dolphin species mentioned above, it can be assumed that, in addition to the common dolphin (Espada Ruíz et al., 2018), bottlenose and striped dolphins could be used as indicators of fish aggregation by fishermen. The results of this study strongly support the need to improve protection measures for these species, especially during the summer when a higher presence of all types of vessels has been observed in the Bay during cetacean sightings, and when higher levels of traffic are reported in the whole Strait. Conservation efforts for common, striped and bottlenose dolphins could be positively supported by the designation of a specific micro-sanctuary that could include a temporal no-take/more restricted-use zone inside the Bay from April to September. Similarly, a coordinated effort among Gibraltarian and Spanish patrolling forces and the enforcement of surveillance could also contribute to the protection of the species.

Results of this study further confirm the presence of the resident Critically Endangered 'long-finned pilot whale' in the central-eastern part of the Strait, as reported in other literature (Cañadas, 2008; Cañadas et al., 2005; de Stephanis et al., 2014; de Stephanis, Cornulier, et al., 2008; de Stephanis, García-Tíscar, et al., 2008; de Stephanis, Verborgh, et al., 2008). The species is known to be afflicted by different injuries of anthropogenic origin, such as collisions or entanglement in fishing lines or hooks (Herr et al., 2020; Verborgh et al., 2016), which corresponds to the association observed with fishing boats (+1214%). This study also reported two NMEs of collision in the southern eastern part of the Strait, where traffic of big ships is heavy and where no specific spatial management measures (such as speed reduction) have been implemented.

Due to the reduced number of sightings of 'fin, sperm and killer whales', a description of their distribution and seasonal presence was not possible. Nevertheless, all of these species were sighted in the central-southern part of the Strait while NMEs involving fin and sperm whales took place in locations overlapping the Traffic Separation Scheme of the Strait of Gibraltar and within the area of major presence of big ships (e.g., containers, bulk cargos and cruise). It has been reported that a portion of this area deemed important for these species was designated as a precaution zone known as a 'Cetacean Critical Navigation Zone', as named in the Marine Spatial Plan of the Strait and the Alborán Sea by the Spanish Environmental Ministry (https://www.miteco.gob.es/es/costas/temas/proteccion-medio-marino/estrategias-marinas/demarcacion-estrecho-alboran/). In this zone, speed must be restricted to 13 knots in order to avoid collisions with whales, and a good lookout should be maintained between April and August (National Geospatial Intelligence Agency, 2022), in particular for the sperm whales (Silber et al., 2012). Despite these mitigation measures, the percentages of NMEs with fin (12.5% in both transects, 25% in ALTA) and sperm (16.6% in both and 100% in ALCE) whales were found to be quite high compared with the low number of sightings. This could be due to the fact the measures are not mandatory and/or to the lack of surveillance, and further investigation is surely needed in order for the suggestion of possible improvements in the effectiveness of the measures put in place. The presence of DOs on board and the training of crew members (Gende et al., 2019) could be applied as effective measures for reducing the risk of collision. Considering that which has been previously discussed for pilot, sperm and killer whales, the 'Cetacean Critical Navigation Zone' should be extended to the east and the currently recommended reduction in speed to 13 knots should be changed from being a recommendation to being mandatory for all vessels.

All three states that line the Strait (i.e., Morocco, Spain and Gibraltar) are signatories to the conventions ICRW, CITES, BCCEW and CMS, which aim to conserve and protect endangered species, including cetaceans, as well as their habitats. Despite this, to the best of our knowledge, there is currently no 'common international management plan' for the waters of the Strait that focuses on conserving cetaceans. The importance of the Strait for cetaceans is confirmed by the designation of the IMMA that crosses it, and the criticality of the area was highlighted when it was named a CCH. Although important tools, the presence of the SAC and SIC may not be sufficient to conserve highly mobile species such as cetaceans (Dwyer et al., 2020). The temporality and variability of cetacean presence must be considered when managing the space (Wilson et al., 2004). For instance, seasonal and dynamic regional shipping plans, including mandatory speed reductions and/or rerouting, have been adopted in portions of the Salish Sea (https://www.pac.dfo-mpo.gc.ca/fm-gp/mammals-mammiferes/whales-baleines/srkw-measures-mesures-ers-eng.html#maps) and the North Atlantic (https://www.fisheries.noaa.gov/national/endangered-species-conservation/reducing-vessel-strikes-north-atlantic-right-whales) to protect southern resident killer whales and North Atlantic right whales (NARW). In the case of NARW, the effectiveness of this approach was proved with a reduction of mortality due to ship strike events (Laist et al., 2014). Recently, a temporal-spatial management tool was used in the Strait to reduce negative interaction among orcas and vessels. Navigation limitations from the Gulf of Cádiz to Tarifa were in full force to increase vessel safety between 8 August and 22 September 2021 (Ministerio de Transporte Movilidad y Agenda Urbana, 2021a, 2021b), https://www.orcaiberica.org. This supports the idea that a temporal plan, including speed restriction and a no-take zone inside a micro-sanctuary, could be designed to protect the cetaceans of the Strait.

4.1 | Implications for conservation

It may be possible to enhance the impact of global conservation by prioritizing conservation interventions according to their 'cost-effectiveness' (Pienkowski et al., 2021).

Results on how the different maritime sectors use the space in the Strait, together with the identification of cetacean hot spots, could help in the development of effective and conservation-oriented management measures.

More specifically, the central part of the Bay between Algeciras and Gibraltar is an important area for common, striped and bottlenose dolphins, particularly from April to September. The designation of a specific 'micro-sanctuary' in this part of the Bay is suggested to mitigate anthropogenic impacts (e.g., injuries and sub-lethal stressors). A micro-sanctuary could include additional temporal restricted-use zones that, together with enforcement, surveillance and international coordination among patrolling forces, could optimize conservation efforts for these three species of dolphins.

The presence of pilot, fin, sperm and killer whales coupled with the NMEs reported in the central-southern-eastern parts of the Strait calls for the implementation of specific spatial management measures such as speed limit reductions. The area known as the 'Cetacean Critical Navigation Zone' should be extended to the East, and inside this area, the reduction in speed to 13 knots should be mandatory for all vessels to reduce the risk of collision.

5 | CONCLUSIONS AND RECOMMENDATIONS

Monitoring cetaceans using ferries as a platform provides significant insights into cetacean distribution and maritime traffic, essential knowledge for improving the cost-effectiveness of marine area managements.

Despite the Strait of Gibraltar being widely recognized as an important area for the diversity of highly protected and mobile species, the spatial management tools in force currently only partially cover cetacean hot spots and are static tools. In addition, the transboundary area of the Strait of Gibraltar does not have a respective transboundary management effort. It is time for the Spanish, Gibraltarian and Moroccan States to move from conservation intentions such as international agreements and conventions, to conservation actions such as transboundary zones with a mandatory reduction in vessel speed.

The current study suggests:

- that cetacean monitoring using ferries as a platform should cover all seasons, including the summer, in order to improve understanding of the distribution patterns of highly mobile cetaceans,
- that the presence of cetacean DOs on board ferries should be supported by local administrations beyond private nautical companies and environmental NGOs,
- that mandatory training for bridge officers and other ferry crew members, alongside the presence of DOs on board, would significantly reduce the risk of collision,
- the designation of an international temporal, or in some zones, permanent speed reduction area (i.e., Cetacean Critical Navigation Zone) and
- the designation of a micro-sanctuary with a seasonal no-take zone (in the Bay between Algeciras and Gibraltar).

Finally, following the presence of this long-term monitoring program carried out throughout the Mediterranean Sea, the method employed in this study, which combined cetaceans' SPUE, maritime traffic and spatial management tools analysis, may apply to other sensitive areas.

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CONFLICT OF INTEREST STATEMENT

The authors have nothing to disclose.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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Chapter 10

Discussion and final considerations

The central theme of this dissertation revolves around the challenge of safeguarding marine megafauna, particularly vagrant species, in an ever-changing and threatened marine environment. In a highly dynamic marine environment, those species – which often cover vast distances for several bio-ecological purposes – face many anthropogenic threats which also change in time and space. Furthermore, essential conservation information such as their spatial and temporal presence and distribution, is often lacking.

It is now evident that to gain a better understanding of the current and future impacts of anthropogenic threats on vulnerable species, it is imperative to combine traditional research methods with advanced techniques such as remote sensing and modelling.

In this study, all field-related information was obtained from a long-standing monitoring project known as the "Fixed Line Transect Mediterranean Monitoring Network" coordinated by ISPRA since 2008. This project collects georeferenced data on marine fauna, maritime traffic and marine litter using ferries as observation platforms and consistent protocols. These data allow researchers to better comprehend the distribution of these factors over time and space, as well as to identify areas and seasons particularly at risk for the monitored species, including cetaceans and sea turtles.

While **Chapter 1** describes the thesis' structure, in **Chapter 2** long-term occurrence data were utilized to assess the short-term trends in range and habitat for three low-density cetacean species (Pilot whale, Cuvier's beaked whale and Risso's dolphin) across two 6-years periods mandated by the Habitat Directive. Using tools such as the Kernel smoother and MaxEnt model, along with environmental data from satellite sources, the Observed Distributional Range (ODR) and the Ecological Potential Range (EPR) of the species were estimated. Additionally, the Range Pattern (overlap between core areas) and the ODR/EPR ratio (proportion of suitable habitat effectively occupied by the species) were calculated. Results confirmed the Western Mediterranean as a crucial area for the three species, but changes in in their distribution and suitable areas over time highlighted shifts that warrant further investigations.

In particular, Risso's dolphin expanded its occupied area beyond the ecological potential by almost 50%, venturing outside the predicted suitable areas. These findings raise questions about the factors driving these changes, such as the exploitation of new areas, adaptation to existing pressures, or shifts in habitat distribution. The study emphasized the importance of considering multiple complementary indicators rather than relying solely on a single metric to assess the significance of changes in species distribution. Additionally, it recommended investigating both range extent and shifts and conducting synoptic analyses across multiple species with similar ecology to determine if observed changes are species-specific or indicative of broader ecological shifts.

Chapter 3 integrated long-term marine litter data with remote sensing information on surface currents to model the spatio-temporal dispersion of plastic Floating Marine Macro Litter (FMML) throughout a year. The study highlighted the extensive travel of plastic particles across the Western

and Central Mediterranean Sea. Nevertheless, these particles trajectories and distances travelled were influenced by hydrodynamic variations, especially in the Sicily Channel. Almost all the modelled plastic FMML particles, after a year of travelling, are still situated near the sea surface, posing a significant threat for all the organisms that live at this level of the water column or that reach it for specific purposes (e.g. breathing, feeding).
The concept of risk was applied to assess the vulnerability of Marine Protected Areas (MPAs) and Fisheries Restricted Areas (FRAs) to plastic FMML. Results revealed that twenty-height Italian, French and Maltese MPAs resulted potentially at risk due to the passage of plastic FMML within their boundaries. The summer season exhibited the highest potential risk, primarily due to increased macroplastics transport by currents over long distances. Notably, Maltese MPAs showed particularly high levels of potential risk during this season. This study underscored the need of considering various spatial and temporal scales when assessing the threat of plastic pollution, and called for transboundary efforts among Mediterranean countries for conducing comprehensive studies addressing the boundary-less nature of this threat.
Marine megafauna (e.g. cetaceans and sea turtles) is considered a relevant indicator to assess the risk deriving from marine litter pressure on marine ecosystems. Therefore, in **Chapter 4** the current scientific literature on spatial REA was reviewed for identifying key information gaps on this subject and for highlighting areas and topics that required further research. The review highlighted that only a limited number of studies have addressed this topic, mainly in European countries and the Mediterranean Sea, with recent increases due to national and international regulations such as the EU Marine Strategy Framework Directive.
Gaps remain in assessing spatial risk for less common and vulnerable species, indicating a need for more continuous and long-term data on species and threats. Moreover, even existing large-scale data collection programs lack comprehensiveness and coordination, hindering the establishment of a solid information baseline for long-term assessments. The review emphasized the necessity of a spatial-contextual approach to support year-round monitoring programs and standardization in REA.

Chapter 5 and 6 focused on the Sicilian and the Sardinian Channels, which are relatively understudied areas regarding species distribution, habitat preferences and amount of plastic pollution.
In particular, **Chapter 5** considered 7 years of field data on cetaceans occurrence, revealing that least 8 out of the 9 cetacean species regularly present in the Mediterranean were observed throughout the whole study period, revealing high fidelity for the examined area at least during summer. The most observed species were striped and bottlenose dolphins, with habitat modeling indicating a generally good fit with their known habitat preferences. However, the more coastal species (e.g. bottlenose dolphin) appeared to explore also deeper areas close to ridge and canyons possibly attracted by rich food sources or influenced by maritime traffic disturbances. The study also highlighted areas with potential changes in distribution, emphasizing the need for further investigation. The marine litter monitoring carried out along one of the analyzed transects underlined that plastic was the most

abundant fraction in all years and seasons considered. In particular, the most recorded objects were shopping bags, plastic sheets, bottles, buoys and polystyrene boxes, items are the ones that could cause cetacean fatal gastric obstructions. Plastic accumulation areas were identified near the gulfs of Palermo and Carini in Sicily, as well as the Tunis Gulf seasonally. These areas coincided with high-risk regions for cetaceans, including bottlenose and striped dolphins.

Results confirmed the importance of some areas already identified as important for cetaceans. Moreover, others (e.g. the Tunis gulf, and the ridges near Ustica (Sicily) and Capo Carbonara (Sardinia)) were found to be of interest: further analysis, to be conducted throughout the years, is needed to investigate if this condition is maintained. This study is the first to model the potential suitable habitat of the two most abundant cetacean species in the area of the Sardinian and Sicilian channels, hence representing a great improvement for cetacean knowledge in this region.

In **Chapter 6** was evaluated the distribution of the migratory species loggerhead turtle and its spatio-temporal overlap with marine litter density, in order to characterize the exposure risk in the study area during the summer season. Results highlighted the importance of both Sardinian and Sicilian Channels for the species, more present in the Sicilian Channel in spring and in the Sardinian during summer. Turtles' distribution was associated with surface current patterns, with animals likely passively drifting with currents.

The surface current patterns also identify the waters off western Sicily, including the Egadi Islands, as a highly dynamic area which may constantly receive turtles that passively drift with the Algerian current and continue either northwards with the Bifurcation Tyrrhenian Current or southwards with the Atlantic-Ionian Stream.

Results also showed that loggerhead turtle presence in the study area was actually higher than could have been expected from satellite data collected in other studies. Hence, this particular region gained a previously unrecognized importance as an area that aggregates oceanic foraging loggerhead turtles. The majority of the observed individuals were juveniles, in the typical size range of the oceanic stage, but a quarter of the total was also recognized as adult sized: this is an interesting result, since adult turtles mostly prefer neritic foraging areas. The central Sardinian Channel was identified as a priority risk area, at least during the summer season: the South-Eastern Sardinia Gyre appeared in fact to act as a trap for both animals and floating plastics, increasing the local exposure risk for sea turtles.

Moving to the another anthropogenic stressor considered in this dissertation, in **Chapter 7** the risk of ship-strikes on fin whales in the Pelagos Sanctuary was evaluated during the summer season. Results aligned with the existing literature, highlighting high whale abundance in the Ligurian-Corsican-Provençal part of the Sanctuary. Here, also the majority of near-miss events were registered. Near-miss events recorded emphasizes the importance of assessing and mitigating ship strike risks. The study suggested real-time alerting tools and the collection of data from ferries as potential solutions to limit collisions.

Large cetaceans like whales are not the only at risk by maritime traffic. In **Chapter 8**, was examined the influence of different categories of shipping on bottlenose dolphin presence and, using differentiated remote sensing data on vessel density over the whole area of the Sicily Channel and North-Western Sicily, to assess the risk of exposure of the species to this threat.

Results of this study showed that large-size vessels were always the most represented category, reflecting the importance of the Sicily Channel as one of the "motorways of the sea" od the Mediterranean. Fishing vessels were the second macrocategory in importance, as fisheries is indeed one of the most traditionally practiced sectors along the Sicilian/Tunisian coast and in the Sicily Channel.

In general, the number of vessels recorded was always significantly lower during bottlenose dolphin sightings than in the random location in their absence. This strong response towards the presence of maritime traffic is of particular interest because, on the contrary of other cetacean species, bottlenose dolphins are known to be more adaptable to this kind of threat. Results of this study confirm the plasticity of this species that, unlike in other Mediterranean areas, is likely forced to put in place also an avoiding behaviour in this highly trafficked area. In fact, regardless of the season considered, bottlenose dolphin presence appears to be conditioned by all categories of maritime traffic, even by those that in other Mediterranean areas had relatively low influence on cetacean species or from which this species is usually attracted.

With shipping constantly increasing, our study provides useful information about bottlenose dolphins exposure to this threat. Understanding the complex interaction and variability of situations between bottlenose dolphin and this multi-faceted source of disturbance is of paramount importance for managing its exposure and mitigating the consequent responses.

To evaluate the coexistence of cetacean vulnerable species and high level of maritime traffic of a trans-border area, **Chapter 9** took into consideration the case of the Strait of Gibraltar. Here, were evaluated also the existent mitigation and conservation measures in order to discuss their coherence. The study corroborates the presence of 7 cetacean species inhabiting the Strait's waters, all of which show a consistent presence over the investigated period. Results show that the study area is dominated by a high level of maritime traffic intensity, especially in its central part, due to big ships transiting along the Traffic Separation Scheme of the Strait of Gibraltar. Meanwhile, areas with a higher frequency of cetacean sightings were identified in the Bay and the central-southern part of the Strait, close to the main ports.

Positive correlations between cetacean sightings and certain vessel categories suggested potential behavioral interactions. The study called for adjustments to conservation management tools in the Strait to protect cetaceans effectively, as only a limited number of sightings were recorded within the protection areas in force in the Strait, while others had not specific cetacean protection measures.

All three states that line the Strait (i.e. Morocco, Spain and Gibraltar) are signatories of several conventions aimed to conserve and protect endangered species, including cetaceans, as well as their habitats. Despite this, to the best of our knowledge, there is currently no common international management plan for the waters of the Strait that focuses on conserving cetaceans.

In conclusion, this dissertation delves into the intricate realm of marine conservation, with a focus on safeguarding the vulnerable and elusive marine megafauna in a constantly changing and threatened environment. The research presented here underscores the urgency of our commitment to understanding, monitoring and mitigating the myriad of anthropogenic threats that imperil these remarkable creatures.
Throughout this dissertation, we have seen the value of continuous, comprehensive and long-term monitoring efforts. Such data collection not only provides us with fundamental information about common and rare species, but also unveils the nuanced spatiotemporal dynamics of their distributions. By integrating traditional research methods with cutting-edge technologies like remote sensing and modeling, we have gained deeper insights into the ever-evolving interactions between marine megafauna and their environment.
Our findings emphasize the pressing need for transboundary cooperation among Mediterranean countries. Marine pollution and maritime traffic are international concerns that demand coordinated efforts, harmonized strategies and effective management measures. The establishment of mobile protected areas, tailored to the movements of species and informed by real-time data, emerges as a promising approach to adapt to the dynamic nature of these threats.
We have also recognized the significance of considering not only the well-studied regions but also the understudied areas of the Mediterranean. These uncharted waters harbor essential insights into species distribution, habitat preferences and threats that require further investigation. We have seen the importance of addressing the risks posed by plastic pollution and maritime traffic, highlighting the necessity of adaptive and region-specific conservation measures.
This work finally advocates for a dynamic and proactive approach to marine conservation. As our understanding of marine ecosystems and species dynamics evolves, so must our strategies and interventions. By embracing the principles of continuous monitoring, transboundary collaboration and adaptable conservation measures, we can strive to protect marine megafauna and ensure the vitality of our oceans for generations to come.

In summary, the dissertation emphasized the importance of continuous and comprehensive monitoring, integrating data from various sources and considering the dynamic nature of species distribution and threats. It recommended the adoption of mobile protected areas to adapt to species' movements and the development of tailored conservation strategies based on real-time data. Collaboration among countries and the coordination of efforts were highlighted as crucial for effective marine conservation.

In short, key findings/suggested measures include:

- **Continuous Monitoring:** results gathered through this PhD research effort emphasizes the importance of consistent long-term monitoring, particularly in understudied Mediterranean areas, to gather essential data on species distribution and impacts.
- **Anthropogenic Threats:** data stresses the need to accurately assess threats to vulnerable species at various spatial and temporal scales. Integration of field data with modeling and new technologies is vital.
- **Dynamic Management Strategies:** outcomes advocate for mobile protected areas and real-time protection strategies, aligning with the dynamic nature of marine species and their threats.
- **Conservation Implications:** results suggest designing tailored strategies for cetacean species protection, considering maritime traffic and fishing activities and establishing micro-sanctuaries and specific conservation zones.
- **Adaptive and dynamic conservation strategies**: results suggest to increase our understanding on how apply the adaptive and dynamic conservation strategies at large scale above all addressing the transboundary nature of threats and the dynamic distribution of marine species.